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Effects of Short Chain Fatty Acids and Total Parenteral Nutrition on Intestinal Gene Expression

by

Laurie Drozdowski

A thesis submitted to the Faculty of Graduate Studies and Research in partial fufillment of the requirements for the degree Master of Science

in

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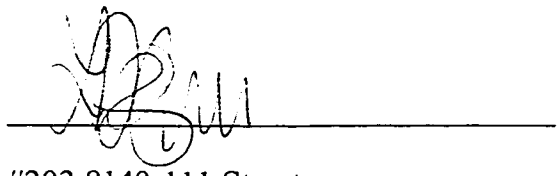

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *Effects of Short Chain Fatty Acids and Total Parenteral Nutrition on Intestinal Gene Expression* submitted by *Laurie Ann Drozdowski* in partial fufillment of the requirements for the degree *of Masters of Science in Nutrition and Metabolism.*

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ABSTRACT

The supplementation of total parenteral nutrition (TPN) formulas with short chain fatty acids (SCFAs) increases glucose uptake and the expression of glucose transporters following intestinal resection. While several signals may be involved in intestinal adaptation, increases in proglucagon and early response genes mRNA levels are seen in non-resected animals receiving SCFA supplemented TPN. While the effects of a mixture of SCFAs are well documented, the relative contribution of the individual SCFAs is unknown. Butyrate is of interest as it is a preferred fuel of colonocytes, with documented effects on cellular proliferation and gene expression. Accordingly, this study was undertaken to determine the relative role of butyrate in initiating an adaptive response in non-resected rats receiving TPN. Animals received standard TPN for 66 hours, followed by 6 hours of either a) standard TPN, b) TPN supplemented with a mixture of SCFAs (60 mM total), c) TPN supplemented with butyrate (9 mM). An oral control group was fed an elemental diet, similar in macronutrient content to the TPN, such that all animals received the same amount of energy daily. SCFAs increased ileal GLUT2 mRNA expression compared to the orally fed group. SCFAs also increased proglucagon mRNA expression as compared to the TPN group. The ileal protein abundance of SGLT1 was reduced by both TPN and butyrate compared to the orally fed group. No changes in $Na^{+}K^{+-}$ATPase or early response gene expression were found in this study. In conclusion, 9 mM butyrate was not able to mimic the effects of a 60 mM mixture of SCFAs on GLUT2 and proglucagon expression.

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LIST OF ABBREVIATIONS

BBM	brush border membrane of enterocyte
BLM	basolateral membrane of enterocyte
BLOTTO	Bovine Lacto Transfer Technique Optimizer
cDNA	complimentary deoxyribonucleic acid
GALT	gastrointestinal associated lymphoid tissue
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
gGLI	glucagon like immunoreactivity
GLP-1	glucagon-like peptide 1
GLP-2	glucagon-like peptide 2
GLUT2	sodium independent glucose transporter
GLUT5	sodium independent fructose transporter
HRP	horseradish peroxidase
IgA	immunoglobulin A
IGF-1	insulin-like growth factor 1
IAP	intestinal alkaline phosphatase
Km	Michaelis affinity constant
mRNA	messenger RNA
MSBR	massive small bowel resection
Na^+K^+-ATPase	sodium, potassium-adenosine triphosphatase
NIDDM	non-insulin dependent diabetes mellitus
Pd	passive permeability coefficient
PKA	protein kinase A

PKC	protein kinase C
PPAR	peroxisome proliferator-activator receptor
PPRE	peroxisome proliferator response element
RNA	ribonucleic acid
RS1	regulatory subunit 1
SCFA	short chain fatty acids
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	standard error of the mean
SGLT1	sodium dependent glucose transporter
STZ	streptozotocin
TPN	total parenteral nutrition
TTBS	Tween Tris buffered saline
Vmax	maximal transport rate
YAMC	young adult mouse cells

1) INTRODUCTION

Total parenteral nutrition (TPN) is used to provide nutritional support to patients who are unable to absorb nutrients through the gastrointestinal tract. Many side effects have been associated with intravenous feeding including mucosal atrophy (Goldstein et al, 1985), increases in intestinal permeability (Buchman et al, 1995) and reductions in amino acid and glucose transport (Inoue, 1993). Researchers have looked at the possibility of supplementing TPN formulas with nutrients that are trophic to the gastrointestinal tract.

Intestinal adaptation is the process by which the intestine adapts to physiological or pathological changes. Functional alterations in the uptake of nutrients may occur in response to changes in the composition of nutrients delivered (Thomson et al., 1989; Thomson et al., 1994; Ferraris et al., 1989). Similarly, the parenteral delivery of nutrients results in changes in intestinal morphology and function (Buchman et al., 1995; Inoue et al., 1993). Changes in the maximal rate of glucose transport, and the expression and abundance of glucose transporters have been seen in several models of intestinal adaptation (Thomson, 1986; Thomson and Rajotte, 1983; Burant et al., 1994).

Short chain fatty acids (SCFA's) are the products of fiber fermentation in the colon. The major products of this fermentation are butyrate, acetate and propionate. Dietary fiber has been established as a trophic factor to both the small intestine and colon

(Jacobs et al, 1993). More specifically, highly fermentable fiber has been shown to increase intestinal glucose uptake and the expression of glucose transporters in rats (Reimer et al, 1997) and dogs (Massimino et al, 1998). Similarly, supplementing TPN formulas with a mixture of SCFA's for 3 to 7 days has been shown to increase the glucose uptake and the expression of glucose transporters following massive small bowel resection (Tappenden et al., 1997). While several signals may be involved in intestinal adaptation, increases in both proglucagon and early response gene mRNA have been found following only 6 hours of treatment with SCFA supplemented TPN (Tappenden et al., 1998).

While the effects of a SCFA mixture on intestinal adaptation is well established, the relative role of each of the SCFA's is unknown. Butyrate, in particular, is of interest due to the observation that it is the preferred fuel for colonocytes (Clausen, 1994). In vivo studies demonstrate positive effects of butyrate on colonic mucosal growth (Kripke et al, 1989), crypt cell proliferation and early response gene expression (Velasquez et al, 1996).

The objective of this study was to determine the relative role of butyrate, as compared to a mixture of SCFA's, in initiating an intestinal adaptive response in a rat model of TPN feeding. The effect of 6 hour treatments with standard TPN, TPN supplemented with SCFA's, or TPN supplemented with butyrate alone, on the expression of glucose transporters, proglucagon and early response genes was examined.

2) LITERATURE REVIEW

2.1) TOTAL PARENTERAL NUTRITION

Total parenteral nutrition (TPN) plays an important role in the nutritional management of patients unable to absorb nutrients enterally. Unfortunately, the absence of luminal nutrients has been associated with negative effects including mucosal atrophy (Goldstein et al., 1985), decreased enzymatic activity (Guedon et al., 1986), impaired immune function (Alverdy et al, 1988), and increases in intestinal permeability (Li et al., 1994).

Bacterial translocation is defined as the passage of both viable and nonviable bacteria and their products across the intestinal barrier to extraintestinal sites, including the liver, kidney, spleen and blood. The translocated bacteria may serve as triggers to initiate, perpetuate or exacerbate the septic state and thereby promote multi organ failure. Parenteral feeding is associated with increases in intestinal permeability, (Li et al., 1994) which may result in bacterial translocation (Alverdy et al., 1988). This translocation is thought to be a potential initiator of multi organ failure (Marshall et al., 1993) and may increase the patient's risk of death from sepsis (Alexander et al., 1990).

The most dramatic and rapid morphological and functional changes have been seen in rodent models of TPN (Levine et al., 1974, Johnson et al., 1975 and Hosada et al., 1989). Results from piglet experiments are in general agreement with the mucosal

atrophy and reductions in brush border membrane (BBM) dissacharidase activity seen in rats (Goldstein et al., 1985).

Clinically significant findings from TPN have been reported in human studies. Buchman et al. (1995) found decreases in the intestinal mucosal thickness, as a result of decreased villus height, following 14 days of TPN. Increases in urinary lactulose-mannitol ratios, as a measure of intestinal permeability, were also seen in these TPN dependent patients. In a study using subjects with chronic pancreatitis, Groos (1996) found that the use of TPN resulted in decreases in villus height, accompanied by a remodeling of the intestinal mucosal surface pattern, thereby decreasing the absorptive surface area. Mild villus atrophy, as well as reductions in BBM disaccharidase activity and [^{3}H] thymidine incorporation, have also been found in children dependent on long-term TPN (Rossi et al., 1993). Inoue (1993) prepared BBM vesicles from jejunal and ileal segments taken from patients receiving either TPN or regular oral diets. A rapid mixing/filtration technique was used to determine the effect of TPN on the vesicle uptake of various nutrients. Functional decreases in BBM amino acid and glucose transport following one week of TPN were found. This suggests that even in the absence of the major structural changes that are seen in rodents, significant functional alterations may be occurring in humans receiving TPN.

The negative effects of TPN, coupled with the high costs of maintaining patients on intravenous nutrition, have led investigators to study ways of optimizing current TPN formulas. In an attempt to maintain intestinal structure and function, and to facilitate the

transition from TPN to enteral feeding, researchers have looked at supplementing TPN with nutrients or growth factors that are trophic to the intestine. Several of these will be discussed.

Glutamine is considered to be a conditionally essential amino acid, and it is the preferred fuel for enterocytes (Duee et al., 1995). While the *de novo* synthesis of glutamine is generally sufficient, during periods of prolonged catabolism both plasma and intracellular glutamine may be depleted (Parry-Billings, 1992).

Glutamine is absent from current TPN formulas, largely due to its instability during storage. Researchers have looked at the effects of the more stable glutamine-containing dipeptides such as L-alanine-L-glutamine or glycyl-L-glutamine in TPN formulas. Schroder et al. (1995) found that supplementation of TPN with the L-alanine-L-glutamine dipeptide reversed the intestinal villus atrophy, the decreased enzymes, and the reduced absorption that was seen in TPN fed rats. Similarly, Van der Hulst et al. (1993) used enteral lactulose and mannitol administration to show that glutamine prevented the deterioration in intestinal permeability commonly seen with TPN. Histological examination also showed that glutamine supplementation preserved a normal mucosal structure. Glutamine has also been shown to have beneficial effects on gastrointestinal immune function. Gastrointestinal associated lymphoid tissue (GALT) atrophy and IgA depression, associated with the use of TPN, were reversed with glutamine administration in mice (Li et al, 1997).

Both insulin like growth factor 1 (IGF-1) and growth hormone (GH) are regulators of tissue growth. IGF's are small homologous peptides related to insulin by structure and function (Herington, 1991). GH regulates circulating concentrations of IGF-1 and local expression of IGF-1 in a number of tissues including the small bowel (Lund, 1994). Receptors for both IGF-1 and GH are found throughout the GI tract (Lobie et al., 1990 , Ziegler et al., 1995), suggesting a role in intestinal growth and maturation.

Subcutaneous administration of IGF-1 selectively increased gut mass, villus height and crypt depth in orally-fed rats (Steeb et al., 1994). More recently, Alexander and Carey (1999) studied the effect of orally administered IGF-1 on small intestinal structure and function. They found increases in sodium-dependent nutrient absorption, independent of changes in mucosal mass or surface area. The effect of enteral IGF-1 on parenterally fed piglets was investigated by Park et al. (1999). IGF-1 augmented intestinal morphology and disaccharidase activity over that observed with partial enteral nutrition alone. While the role of GH is less clear, anabolic effects on gut structure (Schulman et al., 1992) and intestinal ion transport (Guarino et al., 1995) have been reported.

Using an intravenous model, Peterson et al. (1996) demonstrated that IGF-1 attenuated the TPN induced changes in ion transport and gut structure seen in rats. Further work by this group showed that while GH is known to induce IGF-1 synthesis in the liver, GH alone did not stimulate intestinal growth or normalize changes in epithelial function seen with TPN.

Ketone bodies are oxidized by most tissues and are energy sources for gastrointestinal tissues (Roediger et al., 1982). Unlike glucose, maintaining narrow ranges of blood concentrations of ketones is not critical and, therefore, it may be advantageous to consider giving ketone bodies as alternatives to glucose in TPN formulas. Researchers have looked at the effect of intravenous ketones on gastrointestinal structure. Kripke et al. (1988) showed that when monoacetin was substituted for a portion of the glucose in TPN, jejunal and colonic atrophy was inhibited. Nagayama et al. (1990) demonstrated that ketones were better than glucose in supporting colonic healing following anastomosis.

2.2) GLUCOSE TRANSPORT

The kinetics of glucose transport is characterized by three parameters: maximal transport rate (Vmax), the Michaelis-Menton affinity constant (Km) and the passive permeability coefficient (Pd). Carrier mediated transport is saturable and predominantly regulated by changes in Vmax (Karasov and Diamond, 1983). Variations in Vmax reflect alterations in either the quantity or activity of the transporters. Nonspecific mechanisms that may affect uptake include changes in the: 1) surface area, 2) electrochemical gradient for Na+, 3) plasma membrane lipid composition and 4) ratio of transporting to non-transporting cells. Specific mechanisms that may influence uptake include changes in the: 1) transporter turnover, 2) affinity constant and 3) site density of the transporters (Ferraris and Diamond, 1997).

The Km represents the concentration of the solute at ½ Vmax. Failure to correct for the effective resistance of the intestinal unstirred water layer (UWL) leads to an overestimation of Km and an underestimation of Pd (Thomson et al., 1979, 1980, 1981, 1987).

While carrier-mediated transport is responsible for the vast majority of glucose transport, passive permeation does occur. A study conducted by Madara and Pappenheimer (1987) suggested that the presence of D-glucose in hamster enterocytes caused contraction of the cell leading to dilation of the tight juctions, leading to an increase in paracellular glucose transport. A subsequent study using human jejunam

tissue by Fine and colleagues (1993) did not find increases in tight junction permeability induced by sodium dependent glucose transport. As a result of these findings the authors concluded that in humans, paracellular transport is trivial. Most recently, Lane et al. (1999) studied this phenomenon in unanesthetized dogs, and found that at physiological concentrations of D-glucose, L-glucose absorption accounted for only 4-7% of total glucose absorption.

The sodium dependent glucose transporter (SGLT1) in the BBM is responsible for the transport of glucose and galactose from the intestinal lumen into the enterocyte (Wright et al., 1994). This transporter is competitively inhibited by phloridzin. The absorption of fructose across the BBM is a sodium-independent process mediated by the facilitative transporter, GLUT5 (Thorens et al., 1996). The transport of hexoses out of the enterocyte across the basolateral membrane (BLM) occurs via the facilitative GLUT2 transporter (Thorens et al., 1996). GLUT2 is competitively inhibited by cytochalasin B. The BLM $Na^{+}K^{+}$-ATPase establishes and maintains the sodium gradient required for the BBM sodium-dependent transport (Freeman et al., 1993).

SGLT-1 was the first intestinal transporter to be cloned by novel expression cloning techniques (Wright et al, 1993). SGLT-1 transports glucose and galactose from the lumen into the enterocyte (Semenza et al., 1984; Stevens et al, 1984; Wright et al., 1993). SGLT-1 has a molecular weight of 73 kDa and is found on the enterocyte BBM. It is thought to be an asymmetrical protein, as phloridzin inhibits its function only from the luminal side (Karasov and Diamond, 1983).

SGLT-1, as a functional co-transporter, is a homotetramer, consisting of a catalytic subunits and regulatory subunits (RS1), each with weights of 70 kDa (Veyhl, 1992). There are two potential sites for glycosylation at positions 248 and 306 and post translational processing adds 15kDa to SGLT-1 (Hediger et al., 1989). Dietary or hormonal manipulations may induce cellular events that modulate transcriptional and posttranscriptional processing of the RS1 protein. Insertion of the RS1 regulatory subunit may also be involved in inducing transporter activity (ref?).

The Na^+ gradient required for SGLT-1 maximal activity is maintained by the Na^+K^+-ATPase (Horisberger et al., 1991). When sodium is absent, the transporter is negatively charged. When sodium binds to SGLT1, conformational changes occur and expose the glucose-binding site, allowing the binding and transport of the substrate (Peerce and Wright, 1984). SGLT-1 has recently been discovered to be a water pump, transporting 210 water molecules in conjunction with 2 sodium and 1 glucose molecule (Meinild et al., 1998).

In humans, SGLT-1 expression is greater in the proximal small intestine, and parallels the proximal-to-distal gradient of glucose absorption (Hopfer et al., 1987). The onset of SGLT-1 expression in the rabbit small intestine occurs at the crypt villus junction (Hwang et al., 1991). Rat studies show that SGLT1 activity increases along the length of the villus, with protein and mRNA levels remaining relatively constant (Smith et al., 1992). Because of the disassociation between mRNA levels and activity along the

crypt-villus axis, positional factors are thought to influence post-transcriptional control of SGLT-1 (Smith et al., 1992).

Studies using weaning lambs suggest a role for post-transcriptional regulation of SGLT1. Shirazi-Beechey et al., (1991) found that while mRNA levels fell with weaning, the decrease was not parallel to changes in protein or activity levels. Ishikawa and colleagues (1997) postulate that the identification of consensus sites for phosphorylation suggest that control of glucose transport may be modulated by phosphorylation of the transporter. Similarly, Wright et al., (1997) studied protein kinase A (PKA) and protein kinase C (PKC) regulation of SGLT-1. While PKC was found to reduce the maximal transport rate (Vmax) by 60%, PKA increased this rate by 30%. These changes in Vmax were accompanied by proportional changes in the number of cotransporters, and were found to be independent of consensus sites for SGLT-1. Recent work by Vayro and Silverman (1999) suggests that PKC regulates glucose transport through a direct effect on the transporter. In this study, COS-7 cells expressing recombinant NH2-terminal *myc*-tagged rabbit SGLT-1 were treated with a PKC agonist. The decreases in sugar uptake seen with this treatment were reversed when treated with a PKC inhibitor. Despite the changes in uptake, measurement of high-affinity Na^+-dependent phloridzin binding revealed no differences in the number of cell surface transporters. The investigators conclude that PKC modulates glucose uptake by reducing the transporter turnover rate.

GLUT2 is the basolateral facilitative transporter that transports glucose, galactose, mannose and fructose (Venkatramen et al., 1988; Venkatramen et al., 1991; Thorens et

al., 1988; Thorens et al., 1990; Burant and Bell, 1992; Cheeseman, 1993). While GLUT2 does transport fructose, it does so with 6 fold less affinity when compared to GLUT5 (Colville et al., 1993).

GLUT2 is a 61 kDa low affinity, high capacity transporter. Because the kinetics are not rate-limiting, sugar uptake increases with increasing concentration of substrate (Mueckler et al., 1990). GLUT2 is expressed along the villus, and is absent from the crypt.

GLUT2 is subject to developmental regulation and appears in late gestation (Davidson et al., 1992). While little is known about the regulation of GLUT2 expression in the intestine, glucose metabolism is required for induction of the GLUT2 gene in the liver, and this effect is transcriptionally regulated (Rencurel et al., 1996).

GLUT5 is the 47 kDa sodium independent facilitative BBM fructose transporter (Burant, 1991). Maximal protein expression is seen in the upper villus and maximal mRNA expression in the mid villus (Parent, 1992). Expression is greatest in the proximal small intestine, in differentiated villus cells (Miyamoto et al., 1994).

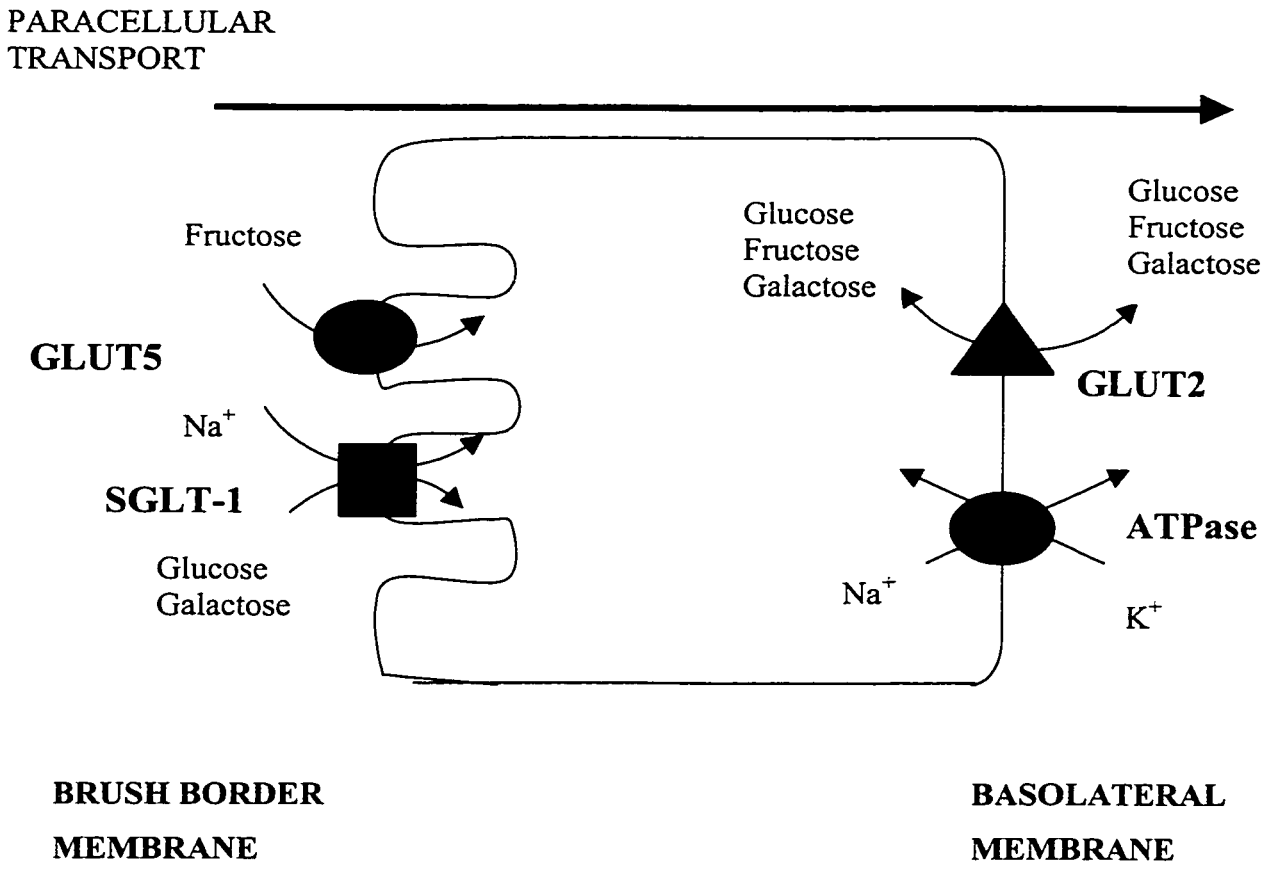
GLUT5 mRNA expression is subject to diurnal variations (Castello et al., 1995). Twelve-fold increases are seen at the end of the light cycle as compared to the beginning. Protein levels also varied throughout the day, but were found to be out of phase with mRNA fluctuations.

The expression of GLUT5 is influenced by cAMP levels. Brot-Laroche et al. (1992) found increased levels of mRNA in Caco2 cells that were treated with forskolin, a cAMP agonist.

The Na^+K^+-ATPase maintains the Na^+ and K^+ gradient across the cell membrane (Horisberger et al., 1991). The transport of 2 K^+ molecules into the cell, and 3 Na^+ molecules out of the cell maintains the gradient necessary for sodium dependent transport. The Na^+K^+-ATPase is a heterodimer, consisting of a 110 kDa α catalytic subunit along with a highly glycosylated 55 kDa β subunit (Fambrough et al., 1994). In the adult small intestine, only $\alpha_1$and β_1 are expressed (Wild et al., 1994).

Increases in $\alpha_1$and β_1 mRNA levels, along the crypt villus axis, parallel increases in Na^+K^+-ATPase activity (Wild et al., 1992). In streptozotocin-induced diabetes mellitus, however, regional alterations in mRNA and protein expression suggest post-transcriptional mechanisms of control of Na^+K^+-ATPase (Wild et al., 1999). Post-transcriptional events may be associated with assembling the subunits and targeting newly synthesized molecules to the BLM (Fambrough et al., 1994).

Figure 1. Model of enterocyte hexose transport.



2.3) INTESTINAL ADAPTATION

The intestine is capable of modulating the quantity of nutrients absorbed in response to pathological changes or alterations in nutritional demands, and thus affect the welfare of the animal. This process is referred to as intestinal adaptation, and it occurs in response to various pathological states including diabetes (Thomson, 1980,1981,1983; Thomson and Rajotte, 1983), intestinal resection (Gleeson et al., 1972; Thomson, 1986b), and external abdominal radiation and chronic alcohol consumption (Thomson 1983,1984). Adaptations also occur in response to physiological changes such as pregnancy (Musacchia and Hartner, 1970), lactation (Cripps and Williams, 1975) and as a result of aging (Thomson et al., 1986). In most cases, adaptation is beneficial and improves the animal's nutritional status. For example, following massive small bowel resection (MSBR), the remnant intestine compensates by increasing nutrient uptake and thereby minimizing malabsorption (Thomson et al.,1986b). In contrast, the increases in hexose uptake that occur with uncontrolled diabetes may exacerbate prevailing hyperglycemia (Burant et al., 1994).

Both morphological and functional changes occur with intestinal adaptation (Thomson et al., 1989). Early studies demonstrated that following MSBR, the remnant gut becomes markedly hyperplastic with increased villus height and crypt depth (Dowling, 1967). More recently, both crypt cell production rate and enterocyte turnover time have been shown to change in several models of intestinal adaptation (Thomson et al., 1994). While morphological changes may be associated with alterations in nutrient

transport, the two processes appear to be independently controlled. Work by O'Connor and colleagues (1999) showed that following an 80% resection, the mass of the remnant intestine increased 5-fold within a one week period, to 50-70% of its pre-resection level. Despite this increase, mass-specific glucose uptake was reduced, restoring uptake to only 33% of control values.

Membrane cholesterol content, phospholipid and fatty acid composition can be modified in mammalian cells (Spector and Yorek, 1985). Alterations in the lipid composition of the BBM (Thomson et al, 1987) and changes in membrane fluidity (Meddings, 1988b) occur with intestinal adaptation, and may influence the activity of membrane bound transporters. Indeed, passive lipid permeability and carrier-mediated D-glucose uptake are influenced by changes in membrane fluidity (Brasitus et al., 1989; Meddings, 1988a; Meddings and Thiesen, 1989; Meddings et al., 1990). While these changes in the composition of membranes may influence transport processes, they do not fully explain the changes in nutrient transport seen with intestinal adaptation (Keelan, PhD thesis).

Functional changes in carrier-mediated nutrient transport and passive transport occur in several models of adaptation. The increases in sugar and amino acid uptake following MSBR result from alterations in the value of the maximal transport rate (Vmax), as opposed to changes in the Michaelis constant (Km) (Thomson, 1986b). Increased Vmax suggests either an increase in the transport activity of the enterocytes or an increase in the number of transporting enterocytes. Using a resection model Sigalet

and Martin (1998) found that functional measures of adaptation were paralleled by an increase in SGLT1 mRNA.

Resection also selectively affects the passive uptake of lipids, with effects differing depending on chain length and degree of unsaturation (Thomson et al., 1986b). The changes in uptake seen in this study could not be attributed to changes in mucosal surface area or changes in the effective resistance of the unstirred water layer.

Both carrier mediated and passive transport increase with diabetes (Thomson and Rajotte, 1983a,b; Fedorak et al, 1987). This increase in nutrient transport cannot be solely explained by enterocyte hyperplasia and intestinal hypertrophy (Thomson and Wild, 1997). Increased transport activity represents a response to a perceived state of tissue starvation and is accompanied by increases in the abundance of SGLT1 and GLUT2 protein and mRNA levels (Burant et al., 1994). *In situ* hybridization results suggest that these increases are due to the premature expression of hexose transporters along the crypt-villus axis (Burant et al., 1994). Similarly, Fedorak et al. (1987) used [3H]-phlorizin binding to show increases in SGLT1 transporters in the lower villus and crypt region of enterocytes. The increases in these regions were associated with increases in transport activity to a greater extent than the villus tip region. This phenomenon is referred to as the "recruitment" of transporters to the more distal portions of the crypt villus axis.

Increases in Na^+K^+-ATPase α_1 and β_1 isoform protein, corresponding mRNA, and levels of transcription are also increased in the jejunum and ileum of the chronically diabetic rat (Wild et al., 1999). Discrepancies between mRNA levels and corresponding protein values, lead the authors to speculate that transcriptional and posttranscriptional mechanisms may lead to the precocious expression of genes in immature enterocytes at the level of the crypt or lower villus. The increased Na^+K^+-ATPase activity seen in diabetes may result in an increased Na^+ gradient across the BBM and, as a result, enhanced glucose uptake.

While previous work on resected animals have found reductions in Na^+K^+-ATPase mRNA in response to SCFA supplemented TPN as compared to standard TPN (Tappenden et al., 1997), no changes were observed in the non-resected animals (Tappenden et al., 1998).

Adaptation may also result in reductions in nutrient uptake in response to physiological or pathological changes. Chronic ethanol consumption and external abdominal irradiation result in decreases in carrier-mediated and passive transport (Thomson et al., 1983c,1984). With aging, sugar transport per unit intestinal tissue declines with age, however, because of the mild hyperplasia that occurs, intestinal uptake remains relatively unchanged from that seen in younger animals (Ferraris and Diamond, 1997).

2.4) NUTRITIONAL REGULATION OF INTESTINAL ADAPTATION

Altering the composition of the nutrients that the gastrointestinal tract is exposed to can affect small intestinal structure and function. Adaptations in nutrient transport help to maintain a safety margin among intake and requirements (Ferraris and Diamond, 1989). Intestinal function is modified by dietary lipids (Thomson et al.,1986a, Thomson et al.,1987a, Thomson et al., 1989). Intestinal transport may be modified by the presence of cholesterol (Keelan et al., 1994), by altering the ratio of saturated to polyunsaturated fat in the diet (Keelan et al., 1990) and by altering the ratio of n6 to n3 in the diet (Thomson et al.,1994). Changes in the Vmax of sugar transporters were shown to be responsible for the changes in sugar uptake that result from altering dietary lipids (Thomson et al.,1986a).

Several studies have characterized the effects of dietary lipids early in life. Alterations in the fatty acid content of maternal diets during gestation produce changes in the ontogeny of intestinal nutrient transport in suckling offspring (Perin et al., 1997). A study by Jarocka-Cyrta et al. (1998) showed that the ontogeny of the intestine is critically influenced by the diet of the mother during both gestation and the nursing period. The same alterations in dietary lipid resulted in adaptation of intestinal transport in postweaning rats (Perin et al., 1999).

The mechanism by which fatty acids alter nutrient transport is not fully established. Although dietary fatty acids modulate BBM fatty acid composition (Keelan

et al., 1990), adaptive changes in transport are not fully explained by alterations in membrane lipids. Polyunsaturated fatty acids regulate gene transcription (Clarke and Jump, 1993). This may occur due to effects on the activity of transcription factors (Clarke and Jump, 1994). Fatty acids also activate the peroxisome proliferator-activator receptor (PPAR), which may mediate effects on gene expression (VandenHeuvel, 1999). The PPAR controls gene expression by interacting with specific DNA response elements called peroxisome proliferator response elements (PPRE) located upstream of responsive genes (Tugwood et al, 1992). The genes regulated by the PPAR include those involved in fatty acid metabolism (reviewed in Schoonjans et al., 1997) and cell cycle control (Ledwith et al., 1993, 1996, 1997; Rokos and Ledwith, 1997).

Scharrer (1972) demonstrated a specific adaptation of active amino acid transport to the level of dietary protein. Feeding a high protein diet increases the Vmax for the uptake of amino acids, particularly those that are considered essential (ref? Diamond et al early 90's). The ability of a single amino acid to affect the gastrointestinal tract has been demonstrated by Schroder et al.(1995), who showed that supplementing TPN formulas with glutamine reversed villus atrophy, decreased enzymes and reduced absorption resulting from TPN in rats. In contrast, parenteral arginine supplementation in rats with massive SBR leads to a slowing of intestinal adaptation, indicated by reduced glutamine uptake and protein synthesis (Welters et al., 1999).

Work by Ferraris and Diamond (1989) demonstrates specific regulation of intestinal nutrient transporters by their dietary substrates. Adaptation to increased

carbohydrate levels in the diet result in increased glucose transport capacity in both the BBM (Diamond and Karasov, 1984) and the BLM (Cheeseman and Harley, 1991). Increases in the maximal rate of glucose transport (Vmax) were seen, while the carrier's affinity (Km) for glucose did not change (Diamond and Karasov, 1984). While a high glucose diet stimulates glucose transport activity and increases levels of SGLT1 and GLUT2 mRNAs in rat jejunum, GLUT5 mRNA was only increased by fructose (Miyamoto et al., 1993). Similarly, Shu et al. (1997) confirmed that dietary fructose enhances intestinal fructose uptake and GLUT5 expression. In addition, precocious introduction of dietary fructose selectively increases the expression of GLUT5 during the midweaning period.

Ferraris and Diamond (1992) postulated that the signal for the up-regulation or down-regulation of BBM nutrient transport is perceived in the crypt, where future transport capabilities of the enterocyte are irreversibly programmed. Switching from a high carbohydrate to low carbohydrate diet produces a 2-3 day lag in the decline of glucose transport. This lag corresponds to the average enterocyte lifetime, suggesting that enterocyte migration times are responsible for this delay. Indeed, phloridzin binding studies show that changes in transporter site density first appear in the crypts, and gradually migrate up to the villus tip.

In contrast to brush border nutrient transport, studies have shown a rapid component to changes in basolateral nutrient transport. Cheeseman and Maenz (1989) found increases in basolateral transport, independent of cytochalasin B binding, within 30

minutes of the induction of hyperglycemia in rats. The results of this study suggest that basolateral D-glucose transport is regulated by a modulation of membrane bound carriers and changes in the carrier site density.

Dietary fiber is trophic to the small intestine and colon (Jacobs and Lupton, 1984; Jacobs et al., 1993). Goodlad et al. (1989) showed increases in proliferation associated with ingestion of fermentable fiber. In contrast, fermentable fiber fed to germ free animals had no effect on proliferation, suggesting a role for SCFAs, the products of the bacterial fermentation of fiber, in eliciting the proliferative response. More recent work by Reimer et al. (1997) showed that physiological levels of fermentable rhubarb fiber modulate intestinal glucose uptake in rats. Similarly, Massimino et al.(1998) found changes in villus height, jejunal glucose transport and GLUT2 protein abundance in dogs fed a highly fermentable fiber.

Short chain fatty acids (SCFAs) are the products of the microbial fermentation of fiber in the colon. The major end products of this fermentation are acetate, propionate and butyrate, which account for 83% of the SCFAs produced (Cummings et al., 1987). These products are produced in a nearly constant ratio of 60:25:15, with a total concentration of 100 mmol/L (Rechkemmer et al., 1988). Human studies show that ~50-60 grams of carbohydrate are fermented daily, yielding 0.5-0.6 moles of SCFAs with a total energy value of 14-180 kcal (Hoverstad 1986, McNeil 1984). Using an *in vitro* fermentation system, in conjunction with the human ileostomy model, McBurney et al. (1988) found SCFAs to account for 3-13% of metabolizeable energy.

As a result of the pH of the colonic lumen, greater than 90% of the SCFAs are present as dissociated anions, representing the major anion in the colonic lumen (Engelhart and Reckhemmer, 1983). Perfusion studies show increases in colonic Na^+, Cl^-, $NH3^+$, Ca^+, Mg^+ and water absorption, as well as HCO_3^- and K^+ secretion, in response to SCFA absorption (Binder and Mehta, 1989, McNeil et al., 1979, Ruppin et al., 1980). Fluid and electrolyte fluxes may be affected by the generation of CO_2 from butyrate oxidation (Roediger, 1982) or as a result of butyrate induced up-regulation of Na^+-H^+ transporter and Na^+K^+-ATPase mRNA (Bishop et al., 1992 and Chehab et al., 1987). While the rates of transport of the three major SCFAs in the proximal colon were found to be similar, absorption rates in the distal colon increased with increasing chain length (Ronnau, 1989).

SCFAs may be absorbed via three mechanisms:

(1) concentration dependent passive diffusion of the ionized or unionized acid.

(2) paracellular diffusion through "leaky spots" (Engelhart and Reckhemmer, 1983, Binder et al., 1997).

(3) carrier mediated transport via a HCO_3^-/SCFA antiport system, independent of Cl^-/$HCO3^-$ exchange and Na^+ transport (Harig et al., 1996).

Vesicle studies have previously demonstrated the presence of this carrier-mediated system in human ileum (Harig et al., 1991) and rat colon (Mascolo et al., 1991).

The β oxidation of SCFAs meets 80% of the energy requirements of the colonic epithilium (Barnard et al., 1997). SCFAs are utilized by the intestinal epithilial cells or

are transported to hepatic or peripheral tissues to be metabolized (Bugaut, 1987). Portal concentrations of SCFAs are 4-10 times higher than circulating levels, suggesting a substantial clearance function for the liver (Hoverstad, 1986, Dankert et al., 1981). Propionate is largely metabolized in the liver, and due to its odd numbered chain length, is used as a gluconeogenic substrate (Bergman, 1990). Acetate accounts for 95% of SCFAs in peripheral blood (Skutches et al., 1979) and is largely metabolized in peripheral tissues such as adipose and muscle. Butyrate appears to play an important role in colonic mucosal metabolism. In isolated human colonocytes, greater than 70% of oxygen consumption was attributed to butyrate oxidation (Roediger, 1980). The importance of butyrate as an energy source is emphasized by the observation that of the SCFAs, butyrate has the lowest Michaelis constant (Km), indicating that it is more readily oxidized by rat colonocytes. (Clausen and Mortensen, 1994). When considering the interaction of various colonic substrates, the colonocytes utilized fuels in the order of butyrate>acetoacetate>glutamine>glucose (Roediger, 1982). A constitutive preference for butyrate was found in neonatal and adult rat colon epithilial cells (Krishnan and Ramakrishna, 1998).

There is evidence of competition between intestinal fuels. Clausen and Mortensen (1994) found butyrate oxidation to be competitively inhibited by propionate, but only slightly inhibited by acetate. Butyrate was found to competitively inhibit the oxidation of both acetate and propionate, while inhibiting glucose oxidation non-competitively.

The SCFAs alter colonic mass and in vivo proliferation rates (Jacobs and Lupton, 1984, Goodlad et al., 1987). Both intracolonic and intraperitoneal injections of SCFAs stimulate mucosal proliferation in the jejunum and ileum of normal rats (Sakata et al., 1984, Kripke et al., 1989). A study by Koruda et al. (1988) investigated the effect of supplementing TPN formulas with SCFAs on adaptation following massive small bowel resection. Significantly greater ileal and jejunal mucosal weights, protein, RNA and DNA contents were seen compared to the standard TPN group. Further work by this group demonstrated that both intravenous and intracecal infusions of SCFAs significantly reduced mucosal atrophy associated with TPN in non-resected animals (Koruda et al.,1990).

More recent work by Tappenden et al. (1996) using a model of TPN and intestinal resection, showed similar positive effects on intestinal morphology, as well as increases in proglucagon and ornithine decarboxylase mRNA. This suggests a mechanism by which SCFAs influence adaptation. Effects on functional aspects of adaptation were also seen, including increases in ileal glucose uptake, as well as upregulation of GLUT2 mRNA expression, demonstrating the ability of SCFAs to influence both nutrient transport and gene expression (Tappenden et al., 1997).

Work with non-resected animals showed SCFAs to have similar trophic effects (Tappenden et al., 1998). Changes in morphological parameters, as well as proglucagon and basolateral glucose transporter expression, suggest a role for SCFAs in adaptation of the non-resected intestine. SCFA treatment for as little as 6 hours produced changes in

glucose transporter and proglucagon and oncogene mRNA expression (Tappenden et al., 1998).

While most *in vivo* studies have looked at mixtures of the SCFAs, Kripke et al. (1989) compared the effects of intracolonic infusions of saline, butyrate (20 mM) and a SCFA mixture (125mM). Both the SCFA mixture and butyrate alone stimulated colonic mucosal growth. Further increases in butyrate concentrations had no additional effect, suggesting that butyrate is the primary colonotrophic factor, with physiological intraluminal levels being optimal with respect to colonic epithilial proliferation.

Incubating human colonic mucosal biopsies with butyrate results in increased proliferation rates (Scheppach et al., 1992). An *in vivo* study by Velasquez et al. (1996) looked at the effect of intraluminal butyrate on crypt cell proliferation. Butyrate was found to increase crypt base proliferation, but decrease crypt surface proliferation in the rat colon. This effect is consistent with the role of butyrate as a protective agent against carcinogenesis, because a shift in the proliferative zone from base to surface of the crypt is considered a predictor of cancer risk. The study also demonstrated that the effects may be mediated by changes in the expression of the early response genes, *c-myc* and *c-jun*, which are known to regulate cell proliferation and differentiation.

Linseisen and Wolfram (1997) examined the effect of TPN supplemented with a structured triglyceride containing butyric acid on gut integrity in traumatized animals. No significant differences in ileal and colonic morphological parameters were observed

despite a theoretically increased supply of systemic butyrate. While the investigator speculates that a certain ratio of SCFAs may be necessary, favorable results have been seen with parenteral triacetin administration (Karlstad et al., 1992).

Sodium butyrate exerts a wide variety of biological effects. Early studies showed n-butyrate to induce terminal differentiation in a variety of cell lines (Leder and Leder, 1975). Researchers have investigated the cellular mechanisms by which butyrate modulates gene expression. Butyrate inhibits histone deacetylase, leading to histone hyperacetylation (Kruh et al., 1994). This results in a relaxing of the chromatin structure, making DNA more accessible to transcription factors (Perrin et al., 1994). Work by Siavoshian et al. (2000) compared the effects of butyrate and trichostatin A, an inhibitor of histone deacetylase. Trichostatin A mimicked the effects of butyrate on specific protein expression, as well as cell proliferation and apoptosis. Butyrate's effects on proliferation and apoptosis have also been linked to changes in protein kinase A (PKA) isozyme-dependent signal transduction. Incubating young adult mouse cells (YAMC) with 1 mmol/L of butyrate reduced PKA type I/II activity ratio (Aukema et al., 1997). Similarly, butyrate has been found to affect protein kinase C (PKC) activity in LIM1215 colon cancer cells (Rickard et al., 2000). A 50% reduction in cellular PKC activity was found in butyrate treated cells. This effect was not due to a reduction in the synthesis of the PKC protein, leading the authors to speculate that butyrate enhances the degradation of this enzyme. Interestingly, the inhibition of β oxidation did not alter butyrate's ability to alter PKC activity. This observation suggests that PKC regulation is not mediated by a metabolic by-product of butyrate or due to the supply of ATP to the cell.

Inan et al. (2000) examined the effect of butyrate on NF- κB activity, gene expression, and protein abundance in a human colonic epithelial cell line. Exposure to buyrate eliminated constitutive NF- κB, p50 dimer activity. This change did not correlate with changes in IκB levels. Effects on NF- κB, p50 activity may occur as a result of butyrate's role as a deacetylase inhibitor. This notion is supported by the observation that p50 binding is also reduced by the selective deacetylase inhibitor trichostatin A.

Recently, a link has been made between triiodothyronine, butyrate, and histone hyperacetylation in regard to enterocyte specific gene expression. The induction of intestinal alkaline phosphatase (IAP) occurs when Caco-2 cells, transfected with the 5′ flanking region of the human IAP gene, are co-incubated with triiodothyronine and butyrate (Meng et al., 1999).

2.5) EARLY RESPONSE GENES

Early response genes are cell cycle related genes involved in cellular proliferation. Since the expression of these genes is both rapid and transient, they are often referred to as "early response genes". Both the *fos* and *jun* genes encode for DNA binding proteins. Leucine zipper dimerization of these proteins results in the formation of the AP1 transcription factor (DeGroot et al., 1990). The early induction of AP-1 binding activity suggests a role for these proteins in the differentiation of the Caco-2 intestinal cell line (Ding et al., 1999). The subsequent regulation of several other genes involved in growth and differentiation is thought to occur as a result of the binding of this factor. The ability of polyamines to regulate cell growth may be partially achieved through the modulation of positive and negative *Jun*/AP-1 activities in the intestinal mucosa (Patel and Wang, 1999).

c-myc encodes for a nuclear phosphoprotein which also acts as a transcription factor, regulating cell cycle progression and programmed cell death, or apoptosis (Gu et al., 1994). Upon mitogen or serum stimulation, a rapid and transient burst in both *c-myc* mRNA and protein as cells enter the G1 phase, followed by a slow decline to low levels in proliferating cells (Campisi et al., 1984; Kelly et al., 1983). Recently, a cellular binding partner, named *Max*, has been identified. The *Myc-Max* heterodimer has been shown to regulate gene transcription (reviewed in Luscher and Larsson, 1998).

More recent studies, using the short chain fatty acid butyrate, demonstrate it's unique ability to exert opposite effects on cellular proliferation and differentiation in normal vs. cancer cells. As a result of these paradoxical effects, a large amount of research has focused on the effect of butyrate on cellular proliferation and early response gene expression.

Using neoplastic cells *in vitro*, several investigators have demonstrated the ability of sodium butyrate to affect early response gene expression. Nishina et al. (1993) used embryonal carcinoma cells to show that butyrate elicits rapid, reversible effects including the induction of the *jun* gene as well as other markers of differentiation. Regulation of *c-fos* expression by sodium butyrate has been demonstrated using fibroblasts (Muller et al, 1984), colon carcinoma cells (Souleimani and Asselin, 1993) and leukemic cells (Rabizadeh et al., 1993). A marked effect on AP-1 dependent gene transcription was found when a colon carcinoma cell line was exposed to butyrate (Glinghammar et al., 1999).

In contrast to the inductions seen with the *fos* and *jun* genes, sodium butyrate was found to decrease *c-myc* expression (Rottleb et al., 1995). Barnard et al. (1993) concluded that because blocking protein synthesis abolished this effect, butyrate may induce the synthesis of a protein with a negative effect on *c-myc* mRNA abundance.

While the effects on transformed cell lines are well documented, less is known about the in vivo effects on normal gastrointestinal cells. Holt et al. (1991) examined

oncogene expression following starvation and refeeding using an in vivo rat model. Increases in *fos* and *jun* mRNA was seen following refeeding, demonstrating the importance of nutrients in the stimulation of gastrointestinal proliferation. The ability of short chain fatty acids (SCFAs) to influence the in vivo expression of early response genes in the gastrointestinal tract has been studied. Velasquez et al. (1996) found that intraluminal butyrate instillations in normal rats resulted in increases in colonic *c-jun* but not *c-fos* protein abundance. These changes were associated with decreases in crypt surface proliferation and indexes of premalignant hyperproliferation. Work done using a model of total parenteral nutrition in rats by Tappenden et al. (1998) showed increased expression of *c-myc, c-fos and c-jun* mRNA following 6-12 hours of intravenous formulas supplemented with a mixture of SCFAs. The relative role of systemic butyrate in initiating this response is unknown. Similarly, it is unknown where along the crypt villus axis changes in early response gene expression and protein abundance are occurring. *In situ* hybridization studies would enable investigators to determine if the proliferative effects associated with butyrate are specific to the crypt region, as suggested by Velasquez (1996).

2.6) HUMORAL REGULATION OF INTESTINAL ADAPTATION

The mechanism by which SCFAs exert their stimulatory effects is not fully established. Direct effects on proliferation are seen in organ culture studies, in the absence of neural or hormonal factors (Schepach et al., 1992). In contrast, many *in vivo* studies show SCFAs to have distant effects on gastrointestinal tissue. For example, SCFAs infused into the colon stimulate mitosis in the jejunum, suggesting a role for systemic factors (Sakata et al., 1984).

Proglucagon is a 160 amino acid peptide encoded by the glucagon gene, which is present in intestinal L cells and α cells of the islets of Langerhans (Bell et al., 1983; Holst, 1984). In mammals, a single copy gene gives rise to an mRNA transcribed from an identical promoter in the pancreas, intestine and brain (Novak et al., 1987; Heinrich et al., 1984; Lee et al., 1990).

Proglucagon is post-translationally cleaved into several peptide products. The peptides produced differ depending on whether cleavage occurs in the intestinal L cells or the pancreatic α cells. The major product produced in the pancreas is glucagon, while the intestine produces GLP-1 (7-36 amide), GLP-1 (7-37 glycine extended), glicentin, oxyntomodulin, and GLP-2 (Orskov et al., 1987). The products of the intestinal cleavage of proglucagon are referred to collectively as "enteroglucagon". L cells increase along the length of the intestine, with the highest number found in the distal ileum and colon (Holst,1984). Prohormone convertases, PC1 and PC2, appear to play important roles in

the tissue-specific post-translational processing of proglucagon (Tucker et al., 1996). Hill et al. (1999) found that Pax6, an important activator of proglucagon gene expression in islet cells, is also essential for the development and functioning of glucagon-producing intestinal cells. It has recently been suggested that there may be differences between species in the regulation of proglucagon. Nian et al.(1999) analyzed the human proglucagon promoter expression in transgenic mice, and found divergence in the mechanisms utilized for tissue specific regulation of the human and rodent proglucagon genes.

The ontogeny of proglucagon mRNA and encoded precursor was studied in the rat intestine from day 11 of fetal gestation to maturity. A similar magnitude of increases in L cell density and proglucagon mRNA abundance was seen, possibly reflecting an increase in L cells, rather than changes in transcription or mRNA stability (Jin et al., 1990). Increased expression of proglucagon and secretion of proglucagon derived peptides has been observed in various disease states including celiac disease (Besterman et al., 1978), STZ diabetes (Brubaker et al., 1989) and following massive small bowel resection (Gornacz et al., 1984; Taylor et al., 1990).

GLP-1 is an incretin hormone with glucose-dependent insulinotrophic actions. In addition to stimulating the release of insulin, GLP-1 has been shown to stimulate proinsulin gene expression and biosynthesis (Fehmann and Habener, 1992). This action, along with the observation that GLP-1 maintains insulinotrophic activity in non-insulin dependent diabetes (NIDDM), makes GLP-1 an attractive potential therapeutic agent in

the treatment of NIDDM. More recently, GLP-1 has also been shown to play a role in the regulation of food intake (Gunn et al., 1996) and body temperature (O'Shea et al., 1996).

GLP-2 has been found to be an important intestinotrophic factor (Tsai et al., 1997a; Tsai et al. 1997b). Gleeson et al. (1971) found a patient with an enteroglucagon producing renal tumor exhibited small bowel hyperplasia, while Dowling (1982) found that an intraperitoneal injection of the tumor extract resulted in intestinal enlargement in rats. A study on non-luminal influences on bowel mucosa demonstrated a correlation between enteroglucagon and cell proliferation following small bowel resection (Gornacz et al., 1984). Similarly, Drucker et al. (1996) showed that nude mice bearing subcutaneous proglucagon producing tumors exhibited proliferation of the small intestinal epithelium

GLP-2 was found to be the agent that produced the most marked stimulation of intestinal proliferation, increasing bowel weight, villous height and stimulating crypt cell proliferation. In contrast to other growth factors, GLP-2 appears to exert a "tissue specific effect" on the gut, as no evidence of increases in proliferation were found in other tissues including the spleen, heart, brain or liver. The authors speculate that this may be due to the specific expression of a GLP-2 receptor. Monroe et al. (1999) have recently cloned and characterized the GLP-2 receptor as a G protein coupled receptor super family member, which is expressed in the gut and is closely related to the glucagon and GLP-1 receptor.

Although it appears to be normal histologically, the functional capacity of the GLP-2 treated intestine has not been studied. Brubaker et al. (1997) found increased BBM dissacharidase and peptidase activity, parallel to intestinal growth, leading the researchers to conclude that normal to increased digestive capacity was seen following subcutaneous treatment. These animals also exhibited normal glycemic profiles, suggesting that the enteroinsular axis is unaffected. Curiously, decreases in jejunal glucose transporter expression (SGLT1 and GLUT2) were seen with GLP-2 treatment.

The effect of GLP-2 on both BLM and BBM glucose uptake has been characterized. Cheesman and Tsang (1996) showed that 2 hour vascular perfusions of GLP-2 (400 and 800 pM) increased the BLM D-glucose maximal transport rate. A subsequent study by Cheeseman and O'Neill (1998) demonstrated that vascular infusions of GLP-2 increased phloridizin insensitive D-glucose uptake, with no change in the BLM abundance of GLUT2.

Similar work characterizing the effect of GLP-2 on the BBM, showed that brush border glucose uptake, as well as SGLT1 protein, increased following GLP-2 infusion (Cheeseman, 1997). The insertion of SGLT1 into the BBM may be responsible for this increase in uptake, as luminal brefeldin A, an inhibitor of protein trafficking, blocked the changes in transport that were observed with GLP-2 treatment.

GLP-2 has been shown to have potential benefits in various models of disease. The role of GLP-2 in experimental diabetes was examined by Fischer et al. (1997). In a model of untreated streptozotocin induced diabetes, a correlation between enhanced bowel weight and increases in ileal and plasma GLP-2 and proglucagon mRNA levels were seen. Insulin therapy was able to prevent these effects, which were shown to be independent of nutrient consumption. Thulesen et al. (1999) found plasma GLP-2 levels to parallel diabetic intestinal growth, while plasma enteroglucagon increased regardless of the extent of mucosal injury. Drucker et al. (1999) used a model of dextran sulfate induced colitis to show that GLP-2 reverses weight loss, and increases colon length, crypt depth and both the mucosal area and integrity of the colon.

Results from both animal and human studies suggest a potential role for GLP-2 in the management of gastrointestinal diseases. Boushey et al. (1999) showed GLP-2 to decrease the mortality and reduce the severity of indomethacin–induced murine enteritis. Jeppeson and colleagues (1999) demonstrated an impaired meal stimulated GLP-2 response in patients suffering from short bowel syndrome. Patients with inflammatory bowel disease undergo an adaptive response to intestinal injury by increasing circulating levels of bioactive GLP-2, facilitating the repair of intestinal mucosal epithilium (Xiao et al., 2000). Preliminary work by Prasad et al. (2000) demonstrates that patients with intestinal ischemia syndromes may benefit from treatment with GLP-2α, a GLP-2 analog. The authors also speculate that GLP-2α may prove to be a useful adjunct in improving graft survival in intestinal transplantation.

In addition to the well established intestinotrophic effects, GLP-2 has recently been found to have other physiological effects. Intravenous infusions of GLP-2 reduce gastric acid secretion in healthy volunteers (Wojdemann et al., 1999). The same group of investigators demonstrated an "ileal brake effect" in a study of antral motility in intravenously infused pigs (Wojdemann et al., 1998).

The mechanism by which GLP-2 exerts its effects is unknown. A study by Yusta et al. (2000) examined GLP-2 signaling in baby hamster cells expressing a transfected rat GLP-2 receptor. GLP-2 increased cAMP levels and activated both cAMP-response element and AP-1-dependent transcriptional activity in a dose dependent manner. The induction of early response genes and the stimulation of cell proliferation were noted at GLP-2 concentrations of 100nM.

Many studies have looked at the nutritional regulation of proglucagon expression and peptide synthesis. Enteral nutrition is known to play a role, as demonstrated by Hoyt (1996) who found that fasting rats decreased proglucagon mRNA, with increases occurring with refeeding. Similarly, Goodlad (1989) found decreases in plasma enteroglucagon in rats infused with total parenteral nutrition.

Intraluminal glucose stimulates the release of GLP-1 (Orskov et al, 1986). Shima et al. (1990) found that while both metabolizable and non-metabolizeable hexoses stimulated secretion, certain structural features were required. Ordinary mixed meals were found to stimulate the secretion of proglucagon derived peptides (Holst and Orskov,

1994). Xiao et al., (1999) fed healthy volunteers test meals and concluded that the secretion of GLP-2 from the intestine is differentially regulated in a nutrient dependent manner. Rocca and Brubaker (1999) used an *in situ* model of the rat GI system to show that the secretion of GLP-1 and glucagon-like immunoreactivity (gGLI) in response to luminal fat is regulated by a complex neuroendocrine loop. This loop involves the enteric nervous system, the afferent and efferent vagus nerves, as well as the duodenal hormone GIP.

The effect of dietary fiber on proglucagon expression and peptide secretion is well established. Gee et al. (1996) found increases in plasma enteroglucagon levels following the ingestion of fermentable carbohydrates. Reimer et al. (1996) fed diets supplemented with fiber to rats and found increased ileal proglucagon mRNA expression and increased plasma GLP-1 levels following an oral glucose load. A subsequent study using physiological levels of fiber in the diet confirmed these findings. Highly fermentable rhubarb fiber was found to increase ileal proglucagon mRNA levels above that of levels found in animals fed a non-fermentable cellulose fiber source (Reimer et al., 1997).

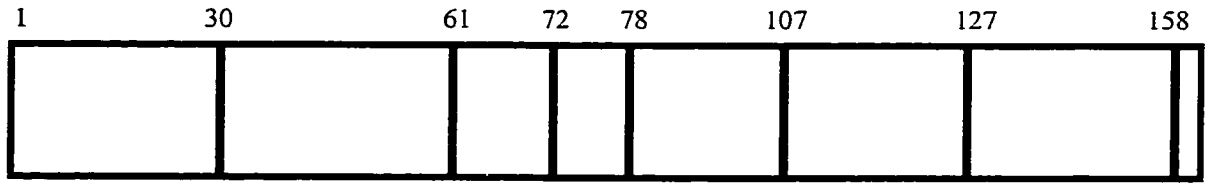
The role of systemic SCFAs in stimulating proglucagon expression and peptide synthesis has been investigated. Tappenden et al. (1996) showed increases in ileal proglucagon mRNA with SCFA supplemented TPN following 80% small bowel resection. A subsequent study showed that 12 hour infusions of TPN supplemented with

SCFAs increased ileal proglucagon mRNA and plasma GLP-2 levels in the non-resected rat (Tappenden et al.,1998).

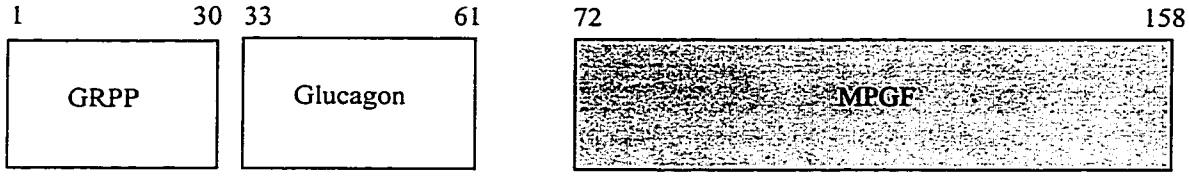
The effect of butyrate on proglucagon expression and peptide synthesis is not well established. Brubaker (1988) found that sodium butyrate did not produce any changes in glucagon-derived peptides in fetal rat intestinal cultures. Work has been done using cultures of a neoplastic pancreatic A cell tumor derived from a patient with glucagonoma syndrome. Incubation with sodium butyrate had no effect on glucagon like immunoreactivity secretion *in vitro,* leading the investigators to conclude that neoplastic A cells may have abnormalities in the biosynthesis and secretion of glucagon (Drucker et al., 1990). In contrast, Philippe et al. (1987) found that insulinoma cells treated with sodium butyrate increased glucagon mRNA expression and induced a pattern of events leading to cell differentiation.

To my knowledge, no *in vivo* studies have looked at the effect of butyrate of proglucagon expression and peptide synthesis. In addition, the effect of systemic butyrate administration has not been investigated.

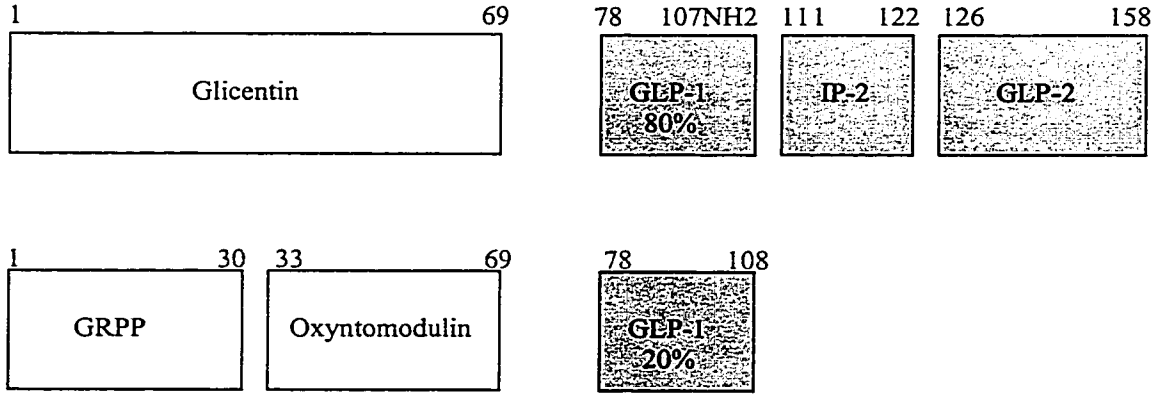
Figure 2. Post-translational processing of proglucagon in the pancreas and the intestinal L-cells. The numbers represent the amino acid at which enzymatic cleavage occurs.



PANCREATIC α CELL



INTESTINAL L-CELL



3) HYPOTHESIS

I have reviewed the links between TPN, short chain fatty acids, and intestinal adaptation. Based on this information and the results of previous studies in our lab, I propose the following hypothesis:

- TPN will decrease the expression of the brush border glucose transporter (SGLT1), Na^+K^+-ATPase, proglucagon and the early response genes *(c-myc, c-fos)* and will increase the expression of the basolateral glucose transporter, GLUT2, as compared to the oral control group.

- SCFA supplemented TPN will increase the expression of the basolateral glucose transporter (GLUT2), proglucagon, and the early response genes (*c-myc, c-fos*) compared to the TPN group. The expression of Na^+K^+-ATPase and the expression and abundance of SGLT1 will be unaffected by SCFA supplemented TPN as compared to the TPN group.

- Butyrate (BUT) supplemented TPN will increase the expression of the basolateral glucose transporter (GLUT2), proglucagon, and the early response genes (*c-myc, c-fos*) compared to the TPN group. The expression of Na^+K^+-ATPase and the expression and abundance of SGLT1 will be unaffected by BUT supplemented TPN.

4) MATERIAL AND METHODS

4.1) ANIMAL CHARACTERISTICS

Thirty two adult male Sprague Dawley rats (193±1.2g) obtained from Health Sciences Laboratory Animal Service (University of Alberta, Edmonton, Canada) were acclimatized and housed in individual metabolic cages in a temperature and humidity controlled facility with 12 hour light/dark exposure. Three days prior to surgery animals were given a nutritionally complete elemental diet (Rodent Laboratory Diet PMI# 5001, see Table 1) in order to decrease residual fiber fermentation and short chain fatty acid production prior to the experimental period. All animals had free access to drinking water. All procedures received ethical approval from the University of Alberta Animal Policy and Care Committee and are consistent with the guidelines of the Canadian Council on Animal Care.

4.2) EXPERIMENTAL MODEL

Prior to surgery, the animals underwent a 12 hour fast, were weighed and anesthetized with halothane. Jugular catheterizations and swivel placements were performed, as described by Popp and Brennan (1981). Postoperatively, animals were randomly assigned to one of three groups. Animals were infused with standard TPN for 66 hours followed by 6 hours of either (a) standard TPN, (b) SCFA supplemented TPN (36mmol/L acetate, 15 mmol/L propionate, 9 mmol/L butyrate) or (c) butyrate

supplemented TPN (9mmol/L butyrate) (see Figure 1). The TPN solutions (see Table 2) were prepared daily under a laminar flow hood in sterile conditions and were filter sterilized before infusion (0.22 μm millipore filter, Millipore Corporation, Bedford MA). The solutions were infused at a rate of 1.75 ml/hour using a Harvard pump (Harvard Apparatus, Wellesley, MA). A final group, which did not undergo jugular catheterization, acted as an oral control. They were fed the elemental diet, similar in macronutrient content to the TPN solutions, such that all animals received the same amount of energy daily (46 kcal/day). All animals had free access to drinking water throughout the study.

4.3) TISSUE PREPARATION

Following the 72 hour experimental period, animals were weighed and anesthetized with halothane. The intestine was rapidly excised from the ligament of Treitz to the ileocecal valve and rinsed in ice cold saline. The intestine was divided into three segments, with the proximal one third segment representing the jejunum and the distal one third segment representing the ileum. One cm segments were removed from the proximal end of the jejunum and frozen in liquid nitrogen for RNA analysis. One cm segments from the distal end of the ileum were also removed and processed in the same manner. The remaining segments of jejunum and ileum were weighed, measured, and cut open along the longitudinal axis in order to obtain mucosal scrapings for subsequent Western Blot analysis.

4.4) RNA ISOLATION

Total cellular RNA was isolated using the Trizol reagant (GIBCO BRL, Burlington, ON). RNA was quantified by measuring absorbance at 260 nm and 280 nm (GeneQuant RNA/DNA Calculator). RNA was equally loaded (15 μg/lane) and electrophoresed on a denaturing 1% agarose/0.66 M formaldehyde gel. The integrity and relative amounts of RNA were evaluated using ultraviolet visualization of ethidium bromide stained RNA. Capillary diffusion was used to transfer the RNA to MSI nitrocellulose membrane (MSI Laboratories, Westboro, MA) for cDNA probes or Zetaprobe GT Genomic nylon membrane (Biorad Laboratories, Hercules, CA) for riboprobes. RNA was fixed to the membranes by baking at 80° *in vacuo* for 2 hours.

4.5) NORTHERN BLOT ANALYSIS

The 440 bp proglucagon cDNA probe was a generous gift from Peter J. Fuller of Prince Henry's Institute of Medical Research in Melbourne, Australia. The probe was labeled with [α^{32}P] -dATP (800 Ci/mmol, Amersham Canada, Oakville, ON) by nick translation (Random Primers DNA Labeling System, Life Technologies, Burlington, ON). Membranes were prehybridized for 1 hour at 65 °C in a medium containing 6xSSPE (0.18 M NaCl, 0.01 M sodium phospate, 1 mM EDTA, ph 7.4), 0.1% sodium dodecyl sulfate (w/v), 5x Denharts solution (0.5 g Ficoll 400, 0.5 g PVP, 0.5 g bovine serum albumin). The membranes were incubated overnight (16-20 hours) at 65°C with

hybridization solution containing the ^{32} P labeled probe. Following hybridization, membranes were washed 3 times with 2xSSPE, 0.1% SDS for 20 minutes at room temperature, followed by a final wash of 0.1xSSC, 0.1% SDS for 20 minutes at 60 °C. Membranes were then exposed to KODAK XAR5 film (Eastman KODAK, Rochester, NY) using an intensifying screen at -70°C. Following adequate exposure, the film was developed using the Kodak M35A X-OMAT processor.

The GLUT2 plasmid was kindly donated by Dr. G.I. Bell, of the Howard Hughes Medical Institute, University of Chicago. The [α^{32}P]-CTP (800 Ci/mmol, Amersham Canada, Oakville, ON) labeled GLUT2 antisense riboprobe was generated from Xba1 linearized plasmid DNA (PGEM4Z-HTL-3) and T7 RNA polymerase. Membranes were prehybridized for 1 hour at 50 °C in a medium containing 60% (v/v) deionized formamide, 1xSSPE (.18M NaCl, 0.01 M sodium phosphate at ph 7.4, 1mM EDTA), 0.5% blotto, 10% (w/v) dextran sulfate, 1% (w/v) sodium dodecyl sulfate and 500 µg/ml salmon testes DNA. The membranes were then incubated at 50 °C overnight (16-20 hours) with the hybridization solution containing the ^{32} P labeled probe. Following hybridization, membranes were washed with 2xSSC (5min @ room temp), 2xSSC/0.1% SDS (10 min @room temp), .2xSSC/1% SDS (5-30 min @70°C) and .2xSSC (2min @ room temp) . Membranes were then exposed to KODAK XAR5 film (Eastman KODAK, Rochester, NY) using an intensifying screen at -70°C. Following adequate exposure, the film was developed using the Kodak M35A X-OMAT processor.

The SGLT-1 cDNA probe was obtained as a generous gift from Dr. N.O. Davidson of the University of Chicago. The cDNA probes encoding the α_1 and β_1 Na^+K^+-ATPase isoforms were obtained from Dr. J. Lingrel of the University of Cincinnati. Hybridization with the non-radioactively labelled β1 and α1 NaK ATPase and SGLT1 cDNA probes was carried out according to the standard protocol for digoxigenin labeled cDNA probes (Roche Molecular Biochemicals, 1999). Briefly, DNA probes were labelled with DIG-11-UTP using random primer labelling. To quantitate the labelled cDNA, serial dilutions of the labelled control DNA and the labelled experimental DNA were dot blotted onto nylon membranes and immunologically detected according to the manufacturers protocol (Roche). The intensity of the probe was compared to the control and the probe concentration estimated.

Membranes were prehybridized for 30 minutes at 42 °C in DIG Easy Hyb solution (Roche Molecular Biochemicals). All probes were heat denatured for 10 minutes at 95°C before being added to prewarmed DIG Easy Hyb (~50 ng/ml solution). Hybridization at 42°C proceeded overnight. Following hybridization, membranes were washed for 2x5 minutes in 2XSSC, 0.1%SDS at room temperature, and 2x15 minutes in 0.1XSSC, 0.1%SDS at room temperature. Following these stringency washes, membranes were briefly rinsed in 1X washing buffer (Roche Molecular Biochemicals) and then blocked in 1x blocking reagant (Roche Molecular Biochemicals) for 30 minutes to reduce non specific binding. Membranes were incubated with anti-digoxigenin-alkaline phosphatase at a dilution of 1:10 000 for 30 minutes. To remove unbound antibody, membranes were washed 2x 15 minutes in 1x washing buffer (Roche

Molecular Biochemicals). Membranes were equilibrated in 1x detection buffer for 5 minutes. The chemiluminescent substrate CDP-Star (Roche Molecular Biochemicals) was applied to the membrane and a reaction with the bound antibody conjugate produced a signal that was detected by exposing the membranes to Kodak X-OMAT AR-5 film for 10 to 30 minutes.

The abundance of mRNA was determined using laser densitometry (Model GS-670 Imaging Densitometer, Biorad Laboratories Ltd, Mississauga, ON). Quantification of the 28S ribosomal units from the membranes was used to account for loading discrepancies.

4.6) REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION (RT-PCR)

200 ng of isolated total RNA was reverse transcribed using Expand RT (GIBCO BRL, Life Technologies) and an oligo dT_{15} primer. Briefly, the RNA and primer were incubated for 10 minutes at 65°C. Following the addition of the dNTP's (2 mM each), 5X buffer (100 mM Tris-HCl pH 8.3, 150 mM KCl, 6 mM MgCl), 20 mM DTT and 50 U of Expand RT, the mixture was incubated for 60 minutes at 42°C.

Coding sequences for c-myc and c-fos were obtained from the Genbank database (NCBI). *c-myc* and *c-fos* primers were designed using GeneJockey II from Biosoft (Ferguson, MO) and Amplify 1.2. An 10 µl aliquot of the RT reaction was amplified by PCR using 1.25 U Taq DNA polymerase (Gibco BRL) in a 50 µl reaction containing 2.25 mM MgCl, 200 µM dNTPs, 20 mM Tris HCl (ph 8.0), 50 mM KCl and 300 nM of each

primer. Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH) was used as an internal control in all PCR reactions as it is constitutively expressed in the adult small intestine (Burant et al, 1994). For *c-myc*, a GAPDH mixture (including 1.5 μM primers, 20 mM Tris HCl (ph 8.0), 50 mM KCl, 2.25 mM MgCl and 0.2 mM dNTP's) was added after 12 cycles of the *c-myc* PCR amplification. For *c-fos*, the GAPDH mixture was added along with the *c-fos* mixture at the start of the PCR reaction. Reaction mixtures were amplified using a DNA thermal cycler (Perkin Elmer Gene Amp PCR System 2400, version 2.11). The conditions included an initial denaturation step for 2 minutes at 94°C, followed by 35 cycles with denaturation for 2 minutes at 94°C, annealing for 30 seconds at 57°C (*c-myc*) or 60 °C (*c-fos*), and elongation at for 1 minute at 72 °C. After the completion of 35 cycles, the mixture was incubated for 7 minutes at 72 °C. PCR products were separated on a 1% (w/v) agarose gel. The gels were stained with .1 mg/ml ethidium bromide to visualize the DNA. The abundance of DNA was determined using laser densitometry (Model GS-670 Imaging Densitometer, Biorad Laboratories Ltd, Mississauga ,ON).

4.7) BRUSH BORDER MEMBRANE (BBM) AND BASOLATERAL MEMBRANE (BLM) PREPARATION

BBM and BLM were isolated from rat intestinal mucosal scrapings using homogenization, differential centrifugation, and Ca^{2+} precipitation. (Maenz and Cheeseman, 1986; Orsenigo et al., 1987; Orsenigo et al., 1985). The Bio-Rad Protein Assay was used to determine the protein concentration of the samples. Aliquots were stored at –70°C for Western immunoblotting.

4.8) WESTERN BLOTTING

BBM and BLM aliquots, containing approximately 20 μg of protein, were solubilized in Sample Buffer Dye (0.125 M Tris-HCl, pH 6.8, 20% glycerol, 4% SDS, 10% β-mercaptoethanol, 0.025% Bromophenol Blue). These samples were incubated at 100°C for 5 minutes to denature the proteins. The samples were stored at –70 °C.

BLM and BBM proteins were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) using a modification of the method developed by Laemmli (1970). Four gels were prepared in a multicaster chamber (Hoefer scientific instruments, San Francisco, California) and stored at 4°C overnight. Gels were composed of two parts: Resolving gel (7.5% gel, 0.375 M Tris, pH 8.8) and Stacking gel (4% gel, 0.123 M Tris, pH 6.8).

The samples along with four Kalaidoscope Prestained Standards (Bio-Rad laboratories, Hercules, Canada), were loaded onto the gel. Electrophoresis was carried out in a Hoefer electrophoresis tank (Hoefer scientific instruments, San Francisco, California). Gels were oriented vertically and submerged in a tank containing electrophoresis buffer (0.025 M Tris, pH 8.3, 0.192 M glycine, 0.1% SDS). Proteins were electrophoresed at room temperature at a constant voltage of 100 Volts through the stacking gel for 30 minutes and 200 Volts through the resolving gel for 2.5 hours.

After migration, proteins were immobilized on a solid support by electroblotting to a nitrocellulose membrane (Towbin et al., 1979). The gel was put into contact with a nitrocellulose membrane and closed tightly in a transfer cassette (Hoefer scientific instruments, San Francisco, California) between filter papers and sponges. Cassettes were placed in a Hoefer transfer tank (Hoefer scientific instruments, San Francisco, California) between two electrode panels and totally submerged in freshly prepared Transfer Buffer (25mM Tris, 192mM glycine, 20% methanol). Electrotransfer was carried out for 160 minutes at a current of 1 Amp.

In order to determine the completeness of protein transfer, the membrane were removed from the cassette and stained with Ponceau S. Membranes were destained with deionized water until no further trace of Ponceau S was visible. Gels were stained with Coomassie Blue Stain (Coomassie Blue R250, methanol, desionised water and glacial acetic acid) to ensure complete protein transfer. The membranes were blocked by incubation overnight in BLOTTO (Bovine Lacto Transfer Technique Optimizer) containing 5% w/v dry milk in Tween Tris Buffered Saline (TTBS: 0.5% Tween 20, 30 mM Tris, 150 mM NaCl.

Membranes were washed three times with TTBS for 10 minutes on a shaking incubator. Then, membranes were probed with specific rabbit anti-rat antibodies. The incubation was carried out at overnight at room temperature. The antibody was diluted in 2% dry milk in TTBS at 1:500.

The polyclonal antibody against SGLT1 was obtained from Biogenesis, Poole, England. It detects the SGLT-1 protein of 75-85 kDa. Following this primary incubation, membranes were washed three times with TTBS for 10 minutes to remove the residual unbound primary antibody. Membranes were then, incubated for one hour with goat anti - rabbit antibody conjugated with horseradish peroxidase (HRP) (Pierce, Rockfort, Illinois, USA). This secondary antibody was diluted at 1:20000 in 2% dry milk in TTBS.

After three 10 minute washes in TTBS to remove residual secondary antibody, membranes were incubated 5 minutes with SuperSignal® Chemiluminescent-HRP Substrate (Pierce, Rockfort, Illinois, USA) composed of 50% Stable Peroxide Solution and 50% of Luminol/Enhancer Solution. The membranes were then exposed to X-OMAT AR films for 5 minutes. The film was developed and fixed using Kodak GBX developer and fixer.

The relative band densities were determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life science group, Cleveland, Ohio, USA).

4.9) STATISTICS

Data was analyzed using a randomized block ANOVA. Sources of variation were block (gel) and treatment. Gel was included as a block in order to account for gel-to-gel variability. The general linear model (GLM) procedure in SAS (Version 6.04, SAS Institute, Cary, NC) was used. When a significant difference was identified, student's t-test was used to make comparisons between means. Significance was defined as $p<0.05$.

5) RESULTS

5.1) Animal characteristics

The initial weights of the animals in the 4 groups did not differ. The change in the weight of the animals over the 72 hour experimental period was not significantly affected by the treatments (Table 3). The mean weight of the intestine (mg per cm length) was significantly higher in both the jejunum and ileum of the orally fed animals as compared to the intravenously fed animals (Table 4).

5.2) α1 and β1 Na^+K^+ATPase mRNA expression

The administration of TPN, SCFA and BUT had no effect on the jejunal expression of basolateral α1 and β1 Na^+K^+ATPase mRNA as compared to the oral control group (Figures 4 and 5).

The administration of TPN, SCFA and BUT had no effect on the ileal expression of basolateral α1 and β1 Na^+K^+ATPase mRNA as compared to the oral control group (Figures 6 and 7).

5.3) GLUT2 mRNA expression

The jejunal expression of GLUT2 mRNA was unaffected by administration of TPN, SCFA and BUT as compared to the oral control group (Figure 8).

The ileal expression of GLUT2 mRNA was increased by the administration of SCFA as compared to the oral control group (Figure 9). Neither TPN or BUT significantly affectd ileal GLUT2 expression as compared to the oral control group.

5.4) SGLT1 mRNA expression

The jejunal expression of brush border SGLT1 mRNA was significantly reduced ($p<0.05$) in animals exposed to BUT as compared to the oral control or TPN group (Figure 10). SCFAs had no effect on jejunal SGLT1 mRNA as compared to the oral control or TPN group.

5.5) SGLT1 protein abundance

The abundance of brush border SGLT1 protein in the ileum was significantly reduced ($p<0.05$) by the administration of TPN and BUT as compared to the oral control group (Figure 11).). SCFAs had no effect on ileal SGLT1 protein as compared to the oral control or TPN group.

5.6) c-myc and c-fos mRNA expression

The ileal expression of c-myc and c-fos mRNA was unaffected by the administration of TPN, SCFA and BUT as compared to the oral control group (Figures 12 and 13).

5.7) Proglucagon mRNA expression

The administration of SCFA significantly increased ($p<0.05$) ileal proglucagon mRNA expression as compared to the TPN group (Figure 14).

6) DISCUSSION

The effect of SCFA or BUT supplemented TPN appears to be site specific. Different responses were seen in the proximal and distal small intestine. The more pronounced ileal response agrees with previous findings (Tappenden et al., 1997;Tappenden et al., 1998a,b) and may reflect increased ileal sensitivity to SCFAs or to GLP2, which is produced from neighboring L cells (Bell et al, 1983).

The effect of SCFAs on ileal GLUT2 expression agrees with previous studies (Tappenden et al, 1997). The responsiveness of GLUT2 expression to SCFAs appears to be specific, as the basolateral membrane $Na^{+}K^{+}$-ATPase was not changed with this treatment. Although not statistically significant ($p= 0.146$), increases in GLUT2 expression were also seen in TPN fed animals as compared to oral controls. This increase was expected as a result of delivering all nutrients systemically. Similarly, work by Cheeseman and Maenz (1989) showed increases in GLUT2 expression following 30 minute systemic glucose infusions. Taken together, these studies demonstrate a rapid component to changes in the gene expression of GLUT2 in response to systemic nutrients, and SCFAs in particular. The more modest increase seen in this study as compared to previous work may be because we did not use an intestinal resection model. Also, diurnal variations in GLUT2 expression have been documented by Corpe et al. (1996). Differences in the levels of expression seen in this study as compared to previous work may, therefore, be due to the differences in the time of day that animals were sacrificed. It is not known whether SCFAs exert their effects by increasing the rate of

transcription or by affecting mRNA stability or turnover. Nuclear run on assays or ribonuclease protection assays could be done in the future to address this question. Although protein was not measured in this study, GLUT2 is transcriptionally regulated (Rencurel et al, 1996). Also, earlier work by Tappenden et al (1998a), demonstrated increases in GLUT2 protein in response to SCFA administration. Thus, while GLUT2 does respond to the systemic delivery of nutrients, the increase seen with SCFA or BUT supplemented TPN was not significantly higher than the TPN group.

Total parenteral nutrition decreases intestinal brush border glucose uptake (Inoue et al, 1993). As expected, the systemic infusion of nutrients, and resulting lack of luminal stimulation, decreased ileal SGLT1 protein abundance. The short-term administration of SCFAs or BUT in this non-resected model was not sufficient to stimulate increases in SGLT1 abundance. Although transport was not measured in this study, earlier work found no changes in D-glucose transport following 24 or 72 hour SCFA administration as compared to standard TPN (Tappenden et al, 1998a). Taken together, these results suggest that in the non-resected intestine, the brush border glucose transporter, SGLT1, is relatively unresponsive to systemic SCFAs. BUT administration decreased jejunal SGLT1 mRNA expression when compared to TPN. Jejunal protein abundance, however, was not measured in this study. The significance of this change in mRNA expression is questionable, as SGLT1 is known to be post-transcriptionally regulated (Shirazi-Beechey et al, 1991). Future studies investigating the roles of phosphorylation and intracellular protein trafficking in the regulation of SGLT-1 activity are warranted.

Although alterations in SGLT1 mRNA expression and protein abundance were found, concominant changes in Na^+K^+-ATPase expression were not seen in this study. The Na^+K^+-ATPase pumps sodium out of the enterocyte, maintaining the gradient necessary for the uptake of glucose from the lumen (Horisberger et al., 1991). It is not known what magnitude of change is necessary in SGLT1 expression or abundance to produce changes in Na^+K^+-ATPase expression In conclusion, the systemic delivery of nutrients decreases ileal SGLT1 abundance, and neither SCFA or BUT supplemented TPN were able to prevent this effect.

The expression of ileal *c-fos* and *c-myc* were not changed by TPN, SCFA or BUT administration. This is in contrast to previous reports of increases in expression following 6 hour SCFA treatment (Tappenden et al, 1998b). Although Northern blotting was used in this previous study, our study utilized RT-PCR as a measure of mRNA expression. Despite the use of this more sensitive technique, we were unable to demonstrate changes in expression. Early response genes demonstrate rapid and transient inductions (1-3 hours) in response to trophic stimuli (Sacks et al, 1 995). It is possible that we missed a transient increase in expression, and therefore measured mRNA expression at a steady state level. A subsequent time course study using shorter infusion times, may identify earlier increases in ERG expression.

Both fermentable fiber and systemic SCFAs increase ileal proglucagon mRNA expression (Tappenden et al, 1998a,b; Reimer et al, 1997; Massimino et al, 1997). Similarly, in our study SCFAs increased proglucagon mRNA compared to the TPN

group. Butyrate (9 mM), however, produced an intermediate response relative to SCFAs (60mM), suggesting that the SCFA effect is not butyrate specific. This is the first *in vivo* study, examining the effect of a single SCFA on proglucagon expression. The mechanism by which SCFAs affect proglucagon expression is unknown, but may involve a cAMP dependent pathway (Rocca and Brubaker, 1995).

Although the administration of SCFAs increased GLUT2 and proglucagon expression, butyrate was not found to be responsible for these effects, as was postulated. Indeed the response to 9 mM butyrate as compared to the 60 mM SCFA mixture, was found to be intermediate in magnitude. It is possible that the amount of butyrate (9 mM) as compared to the SCFA mixture (60 mM), was not sufficient to modulate gene expression. Although similar levels have been used in studies using intraluminal administration of butyrate, (Velasquez et al, 1996; Kripke et al, 1989) systemic administration may result in a dilution in the blood to a level that may not alter gene expression. Future studies comparing 60 mM infusions of butyrate with 60 mM infusions of a SCFA mixture would answer this question.

The lack of effect of butyrate may also be a result of the infusion time used in this study. Unpublished work by Tappenden (2000), showed acute effects on intestinal permeability within only 15 minutes of butyrate administration. Because butyrate is a preferred fuel of colonocytes (Roediger et al, 1982), it may provide a more acute stimulus, as compared to the SCFA mixture. Future studies looking at different infusion times may demonstrate the ability of butyrate to produce changes in gene expression.

As hypothesized, the systemic delivery of TPN increased ileal GLUT2 expression, although the change was not statistically significant ($p=.146$). Similarly, the lack of luminal stimulation in the TPN fed group reduced SGLT1 protein abundance compared to the oral control group ($p<0.05$). We also predicted that parenteral feeding would cause sufficient intestinal atrophy to reduce the ileal expression of proglucagon and the early response genes. No changes were seen, suggesting that the route of nutrient delivery does not affect proglucagon and early response gene expression in this experimental model.

The results of this study partially support our first hypothesis. We expected TPN to cause sufficient atrophy such that decreases in SGLT1, proglucagon, and the early response genes would occur. While our results show that decreases in SGLT1 protein abundance were seen, no changes in proglucagon or early response genes were found. Due to the systemic delivery of nutrients, an increase in the basolateral expression of GLUT2 was anticipated. Our results show only a modest, non-significant increase in GLUT2 expression with TPN as compared to the orally fed group.

Based on the results of previous studies, the second hypothesis predicted increases in GLUT2, proglucagon and early response gene expression in the SCFA supplemented group, as compared to the TPN group. While the results of this study show significant increases in proglucagon expression, SCFA supplemented TPN did not affect the expression of the early response genes or GLUT2.

The third hypothesis predicted that the SCFA effect was butyrate specific. Consequently we hypothesized that the effects seen with SCFAs would also be observed with the administration of butyrate. This hypothesis is not supported by the results of this study.

Table 1. Composition of the elemental diet

INGREDIENT	G/KG DIET
Amino acid mix[1]	200.0
Fat[2]	100.0
Glucose	427.2
Sucrose	213.6
Fiber	---
Mineral Mix[3]	44.1
Vitamin Mix[4]	12.6
Choline Chloride	2.5
Digestible Energy[5] MJ/kg	17.9
Amino Acid Content, g/MJ	11.2
Nitrogen Content, g/MJ	1.72

[1] Supplied (g/kg): L-arginine-HCl, 67.5; L-histidine, 22.5; L-isoleucine, 41; L-leucine, 67.5; L-lysine-HCl, 90; L-methionine, 41; L-phenylalanine, 58; L-threonine, 41; L-tryptophan, 13; L-valine, 47.5; L-alanine,17.5; L-aspartic acid, 17.5; L-glutamic acid, 239.5; glycine, 116.5; L-proline, 27.5; -cystine, 22.5; L-serine, 17.5; L-tyrosine, 22.5; L-asparagine, 30.

[2] Contains (g/kg) hydrogenated beef tallow, 392; linseed oil, 20; and safflower oil 588. Polyunsaturated to saturated ratio was 1.0.

[3] Supplied (g/kg) calcium phosphate dibasic, 500; potassium citrate monohydrate, 220; sodium chloride, 74; potassium sulfate, 52; magnesium oxide, 24; ferric citrate pentahydrate, 6; manganous carbonate, 3.5; zinc carbonate, 1.6; potassium iodate, 0.01; cupric carbonate, 0.3; sodium selenite, 0.01; chromium potassium sulfate, 0.55; sucrose, 118.

[4] Supplied (g/kg) thiamin hydrochloride, 600mg; riboflavin, 600mg; pyridoxine hydrochloride, 700mg; nicotinic acid, 3.0g; D-calcium pantothenate, 1.6g; folic acid, 200mg; D-biotin, 20mg; cyanocobalamin, 1.0mg; retinyl palmitate (250 000IU/g), 1.6g; allrac-αtocopherol acetate (250IU/g), 20g; cholecalciferol (400 000IU/g), 250 mg; menaquinone, 5.0g; sucrose, 972.9g.

[5] Digestible energy was calculated from gross energy measurements of dietary components.

Table 2. Composition of TPN Solutions

INGREDIENT	TPN	TPN+SCFA	TPN+BUTYRATE
Dextrose (g/L)	204	199	201
Nitrogen[1] (g/L)	8.0	8.0	8.0
Lipid[2] (g/L)	26	26	26
NaCl (mmol/L)	60	---	51
Sodium acetate (mmol/L)	---	36	---
Sodium propionate (mmol/L)	---	15	---
Sodium butyrate (mmol/L)	---	9.0	9.0
KCl (mmol/L)	20	20	20
KPO_4 (mmol/L)	15	15	15
$C_6H_{11}O_7 \bullet 1/2Ca$ (mmol/L)	8.0	8.0	8.0
MgSO4 (mmol/L)	3.0	3.0	3.0
Multivitamins[3] (ml/L)	10	10	10

[1] 10% Travasol (Baxter, Toronto, Ontario).

[2] 20% Intralipid (Kabi Pharmacia, Baie D'Urfe, Quebec).

[3] Multi-1000 (Sabex Inc., Boucherville, Quebec).

Table 3. Initial body weights of rats and net body weight change following the 72 hour experimental period

TREATMENT	INITIAL WEIGHT (grams)	NET WEIGHT CHANGE (grams)
BUT	185 ± 3^{a}	3.4 ± 3.6^{x}
SCFA	190 ± 3^{a}	3.1 ± 3.4^{x}
TPN	184 ± 3^{a}	-2.9 ± 3.6^{x}
ORAL	189 ± 3^{a}	2.3 ± 3.2^{x}

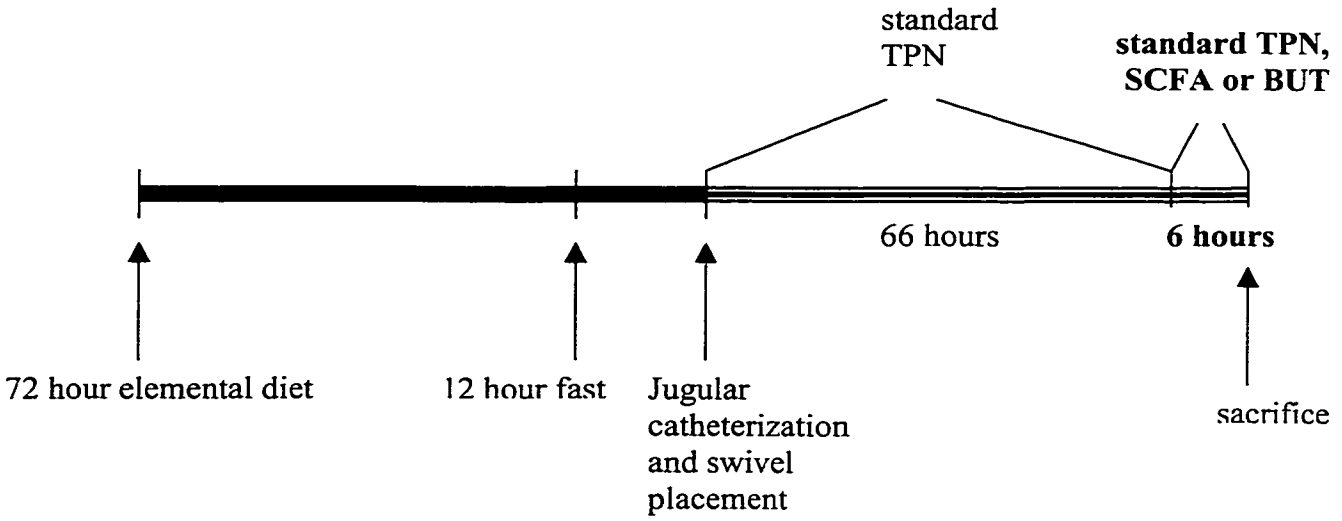
Values are means ± SEM. Values with different letters are significantly different ($p < 0.05$).

Table 4. Intestinal weights following 72 hour experimental period

	WEIGHT PER LENGTH (mg/cm)	
TREATMENT	**JEJUNUM**	**ILEUM**
BUT	37.1 ± 1.3^{b}	33.3 ± 1.6^{b}
SCFA	36.2 ± 1.8^{b}	35.3 ± 2.3^{b}
TPN	36.1 ± 1.8^{b}	34.3 ± 3.3^{b}
ORAL	51.4 ± 1.1^{a}	42.1 ± 0.8^{a}

Values are means ± SEM. Values with different letters are significantly different ($p < 0.05$).

Figure 3. Experimental design



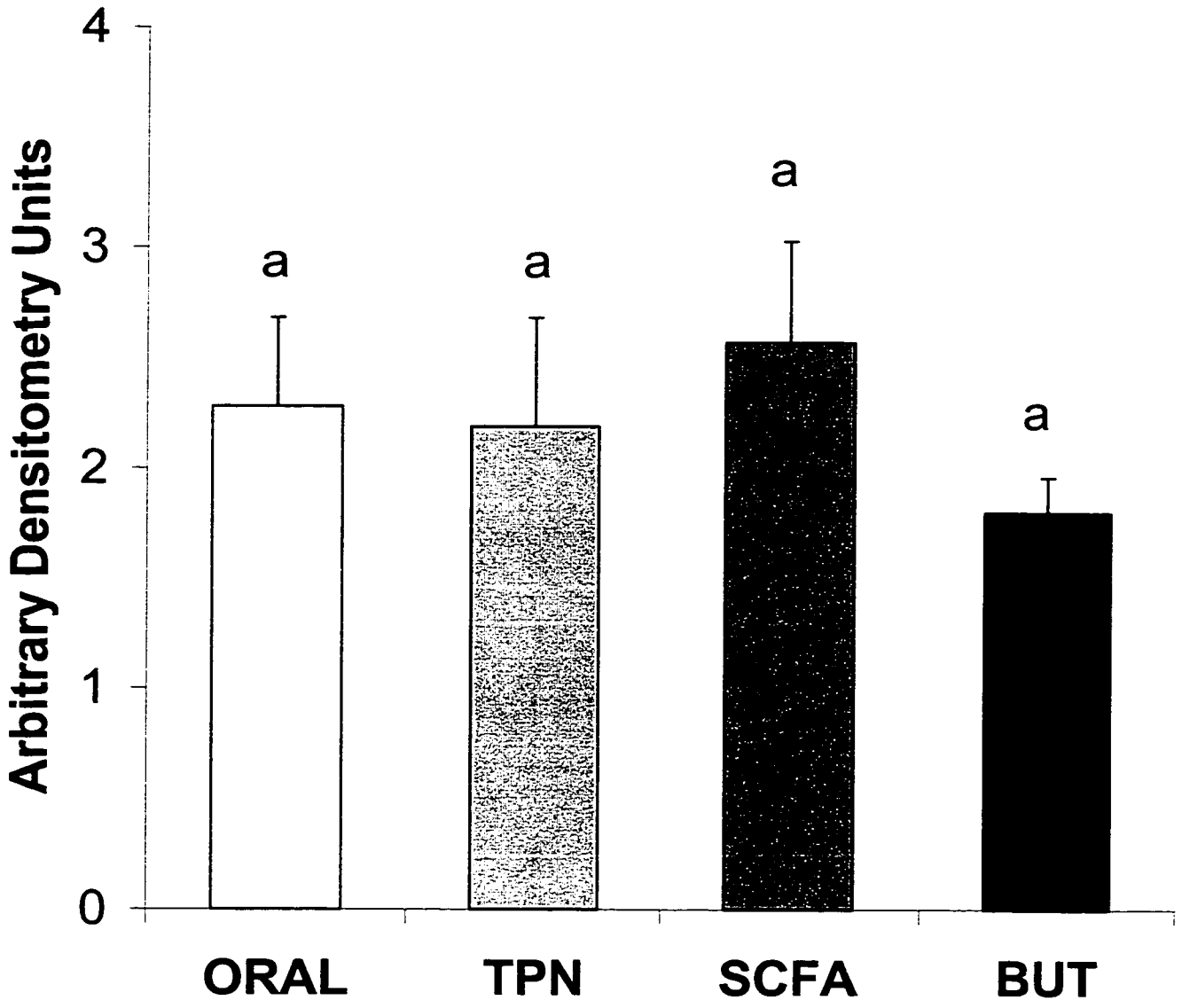


Figure 4. The Effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on jejunal α1 Na^+K^+-ATPase mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

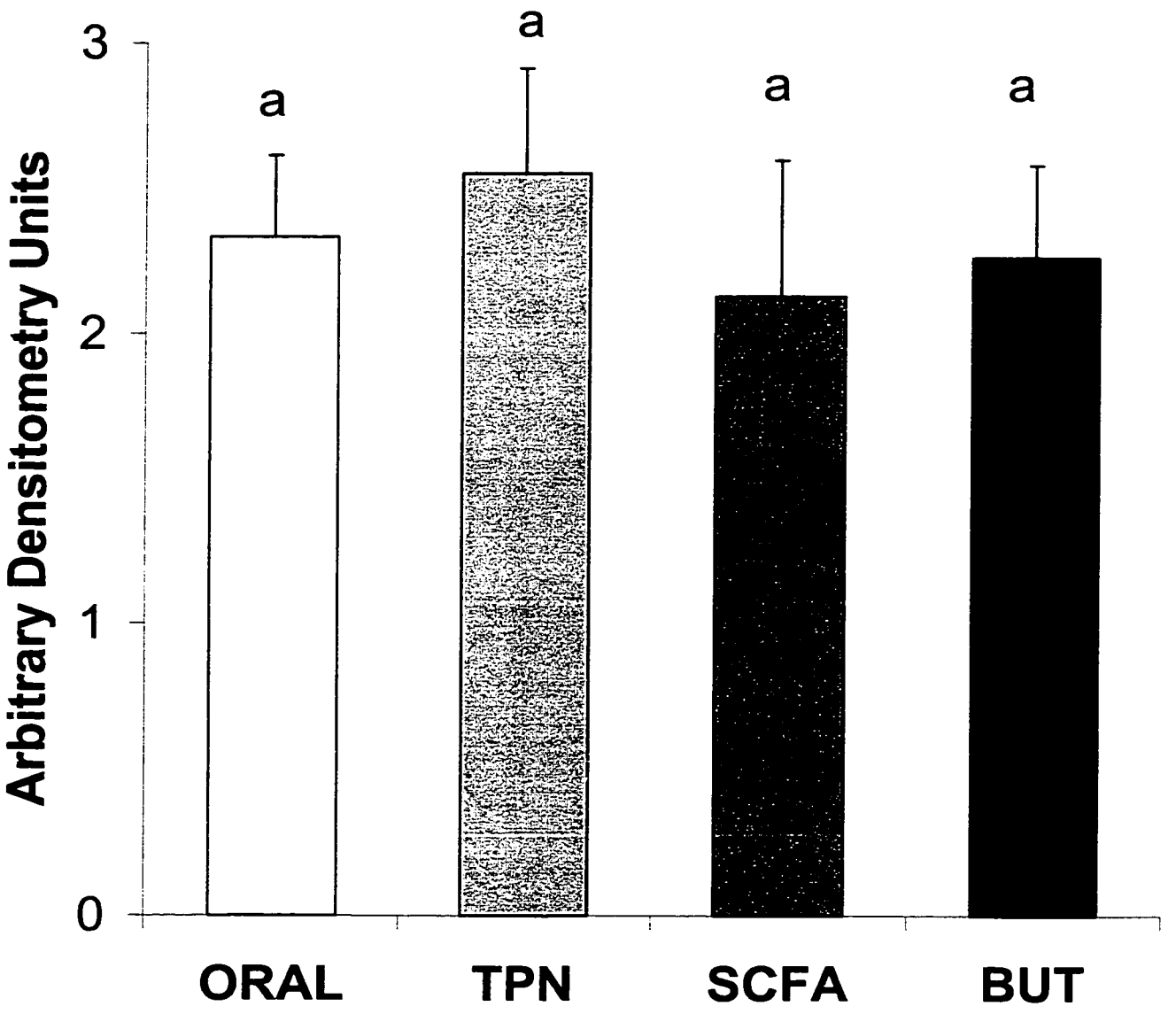


Figure 5. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on jejunal β1 Na^+K^+-ATPase mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

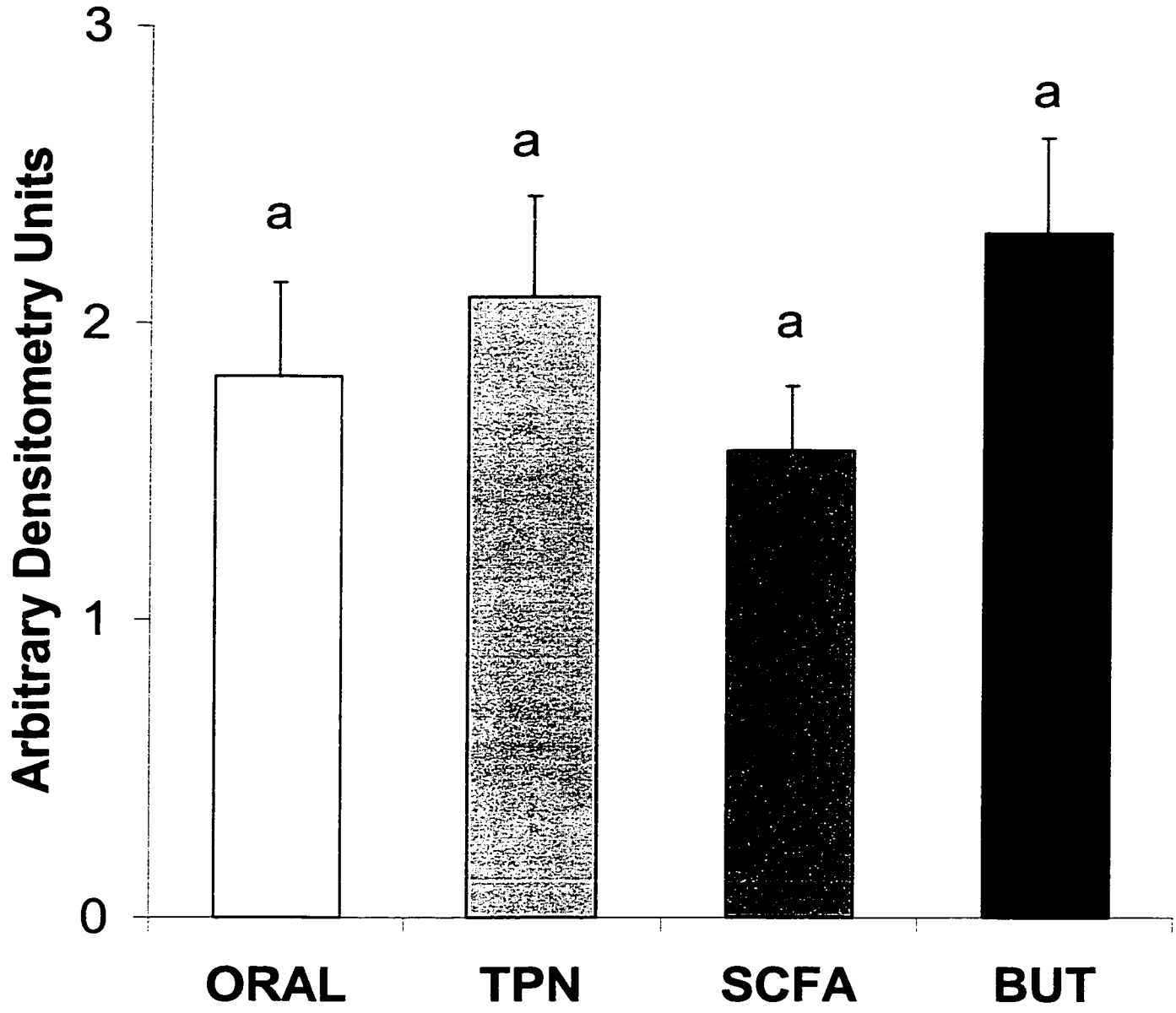


Figure 6. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on ileal α1 Na^+K^+-ATPase mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

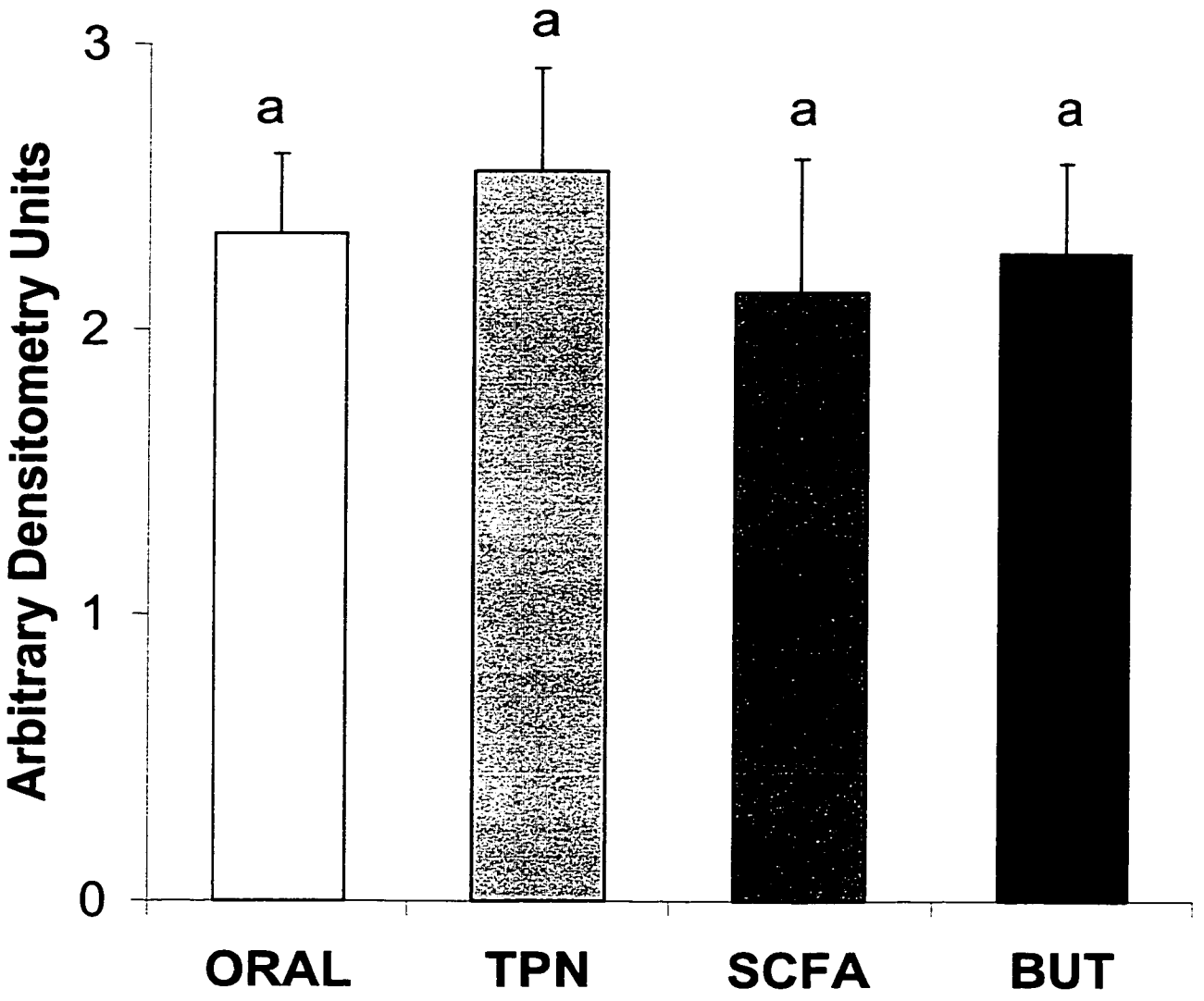


Figure 7. The Effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on ileal β1 Na^+K^+-ATPase mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by a randomized block ANOVA and student's t-test.

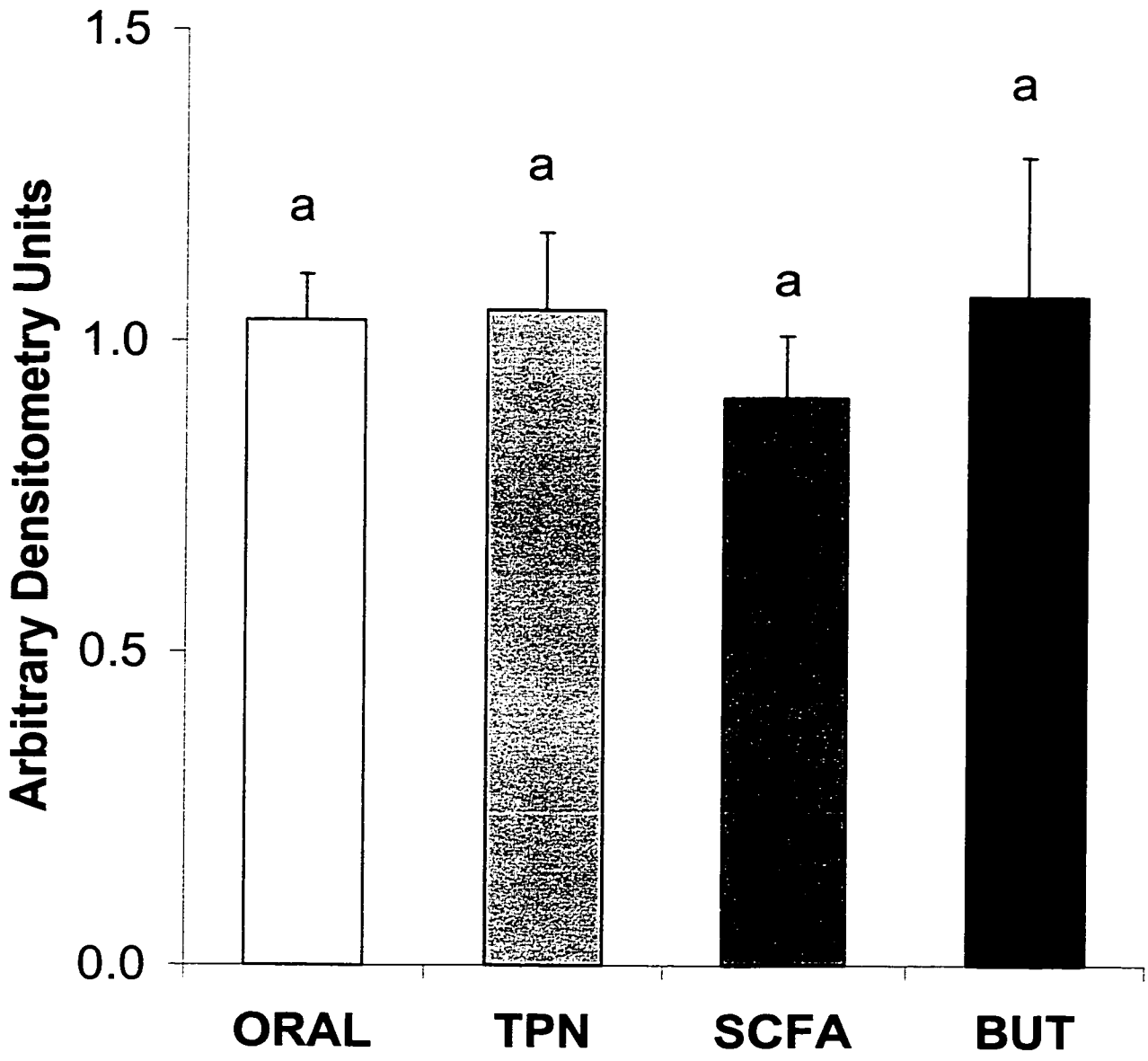


Figure 8. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on jejunal GLUT2 mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

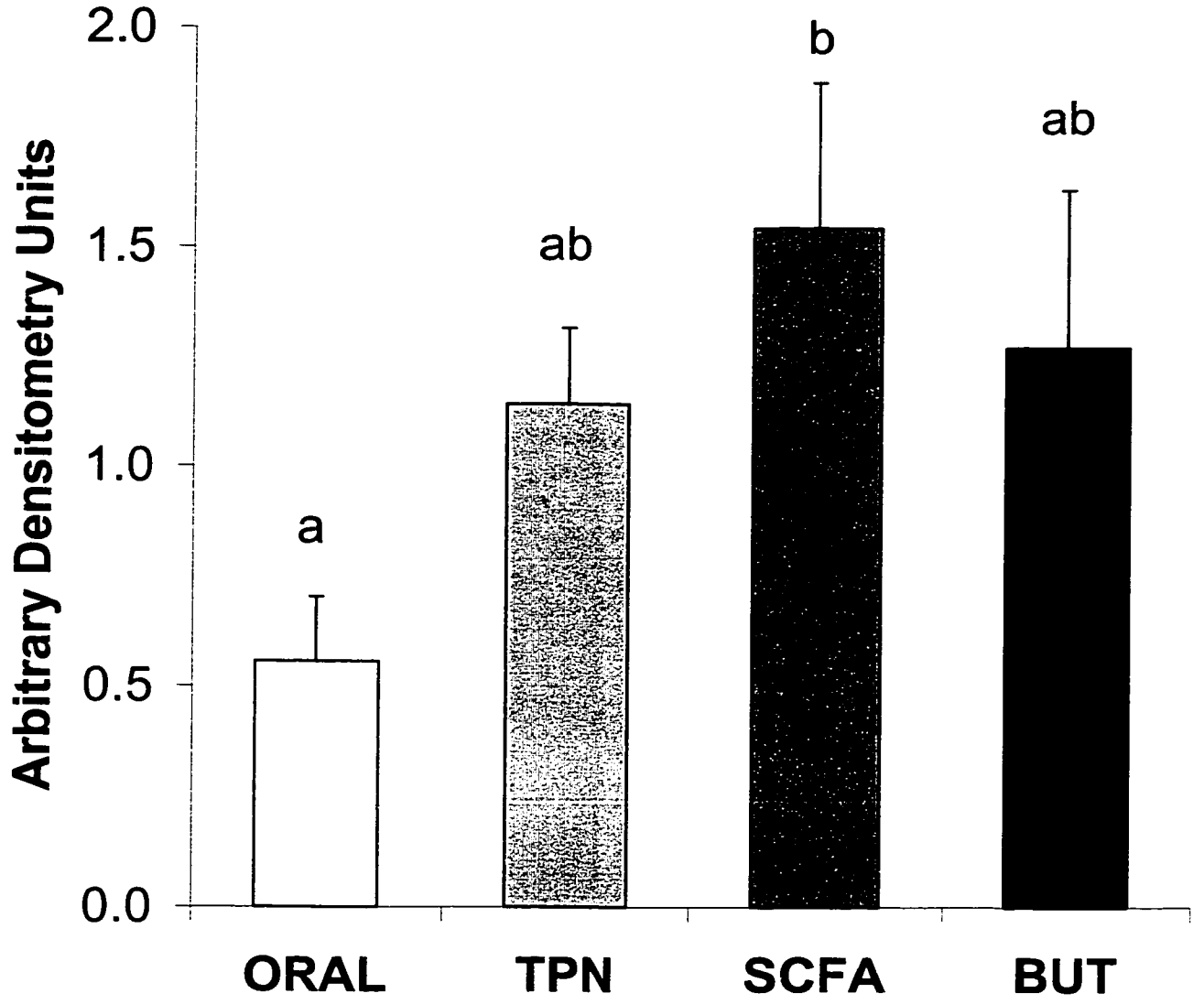


Figure 9. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on ileal GLUT2 mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized ANOVA and student's t-test.

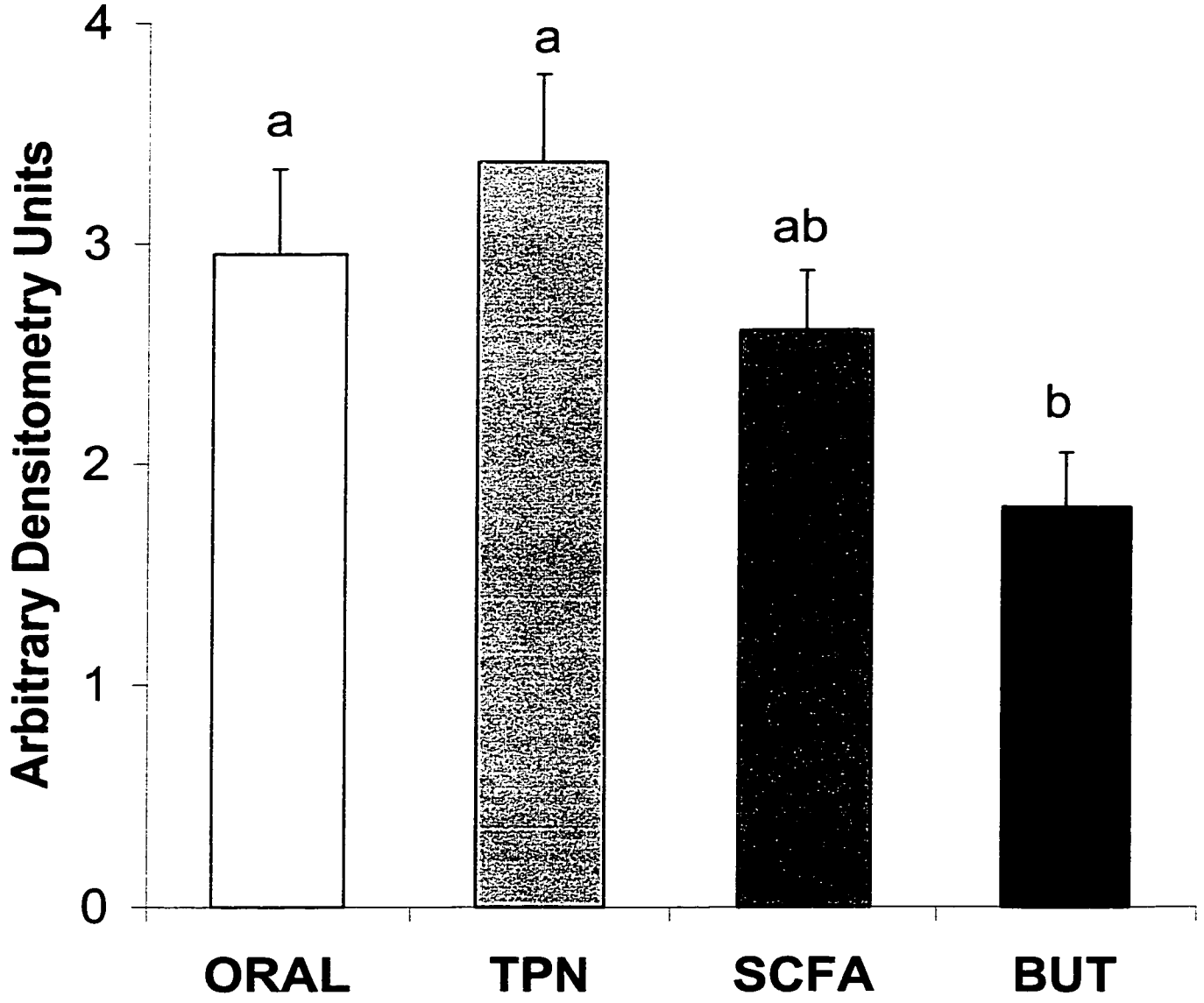


Figure 10. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on jejunal SGLT1 mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

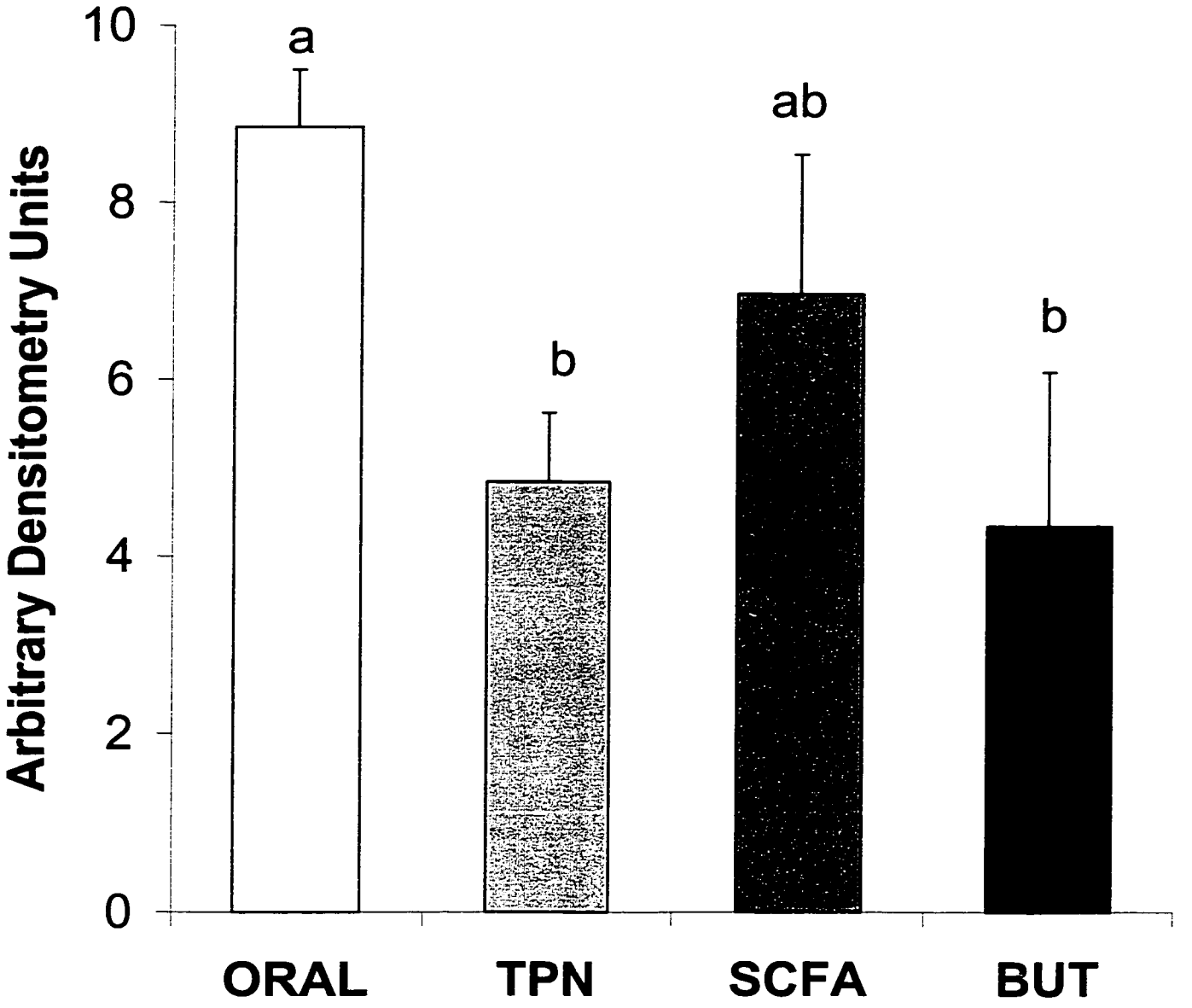


Figure 11. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on ileal SGLT1 protein abundance. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

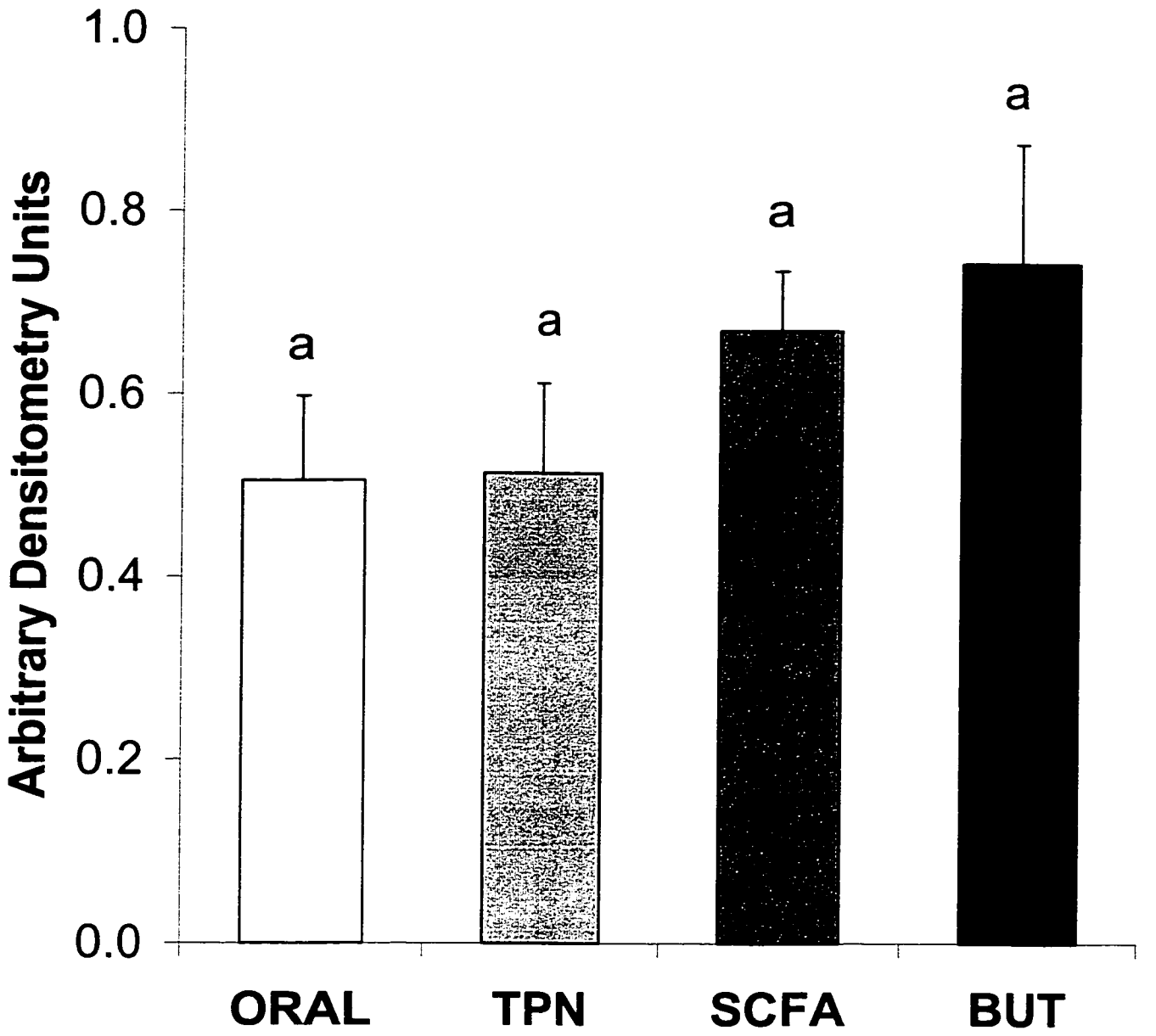


Figure 12. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on ileal c-myc mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

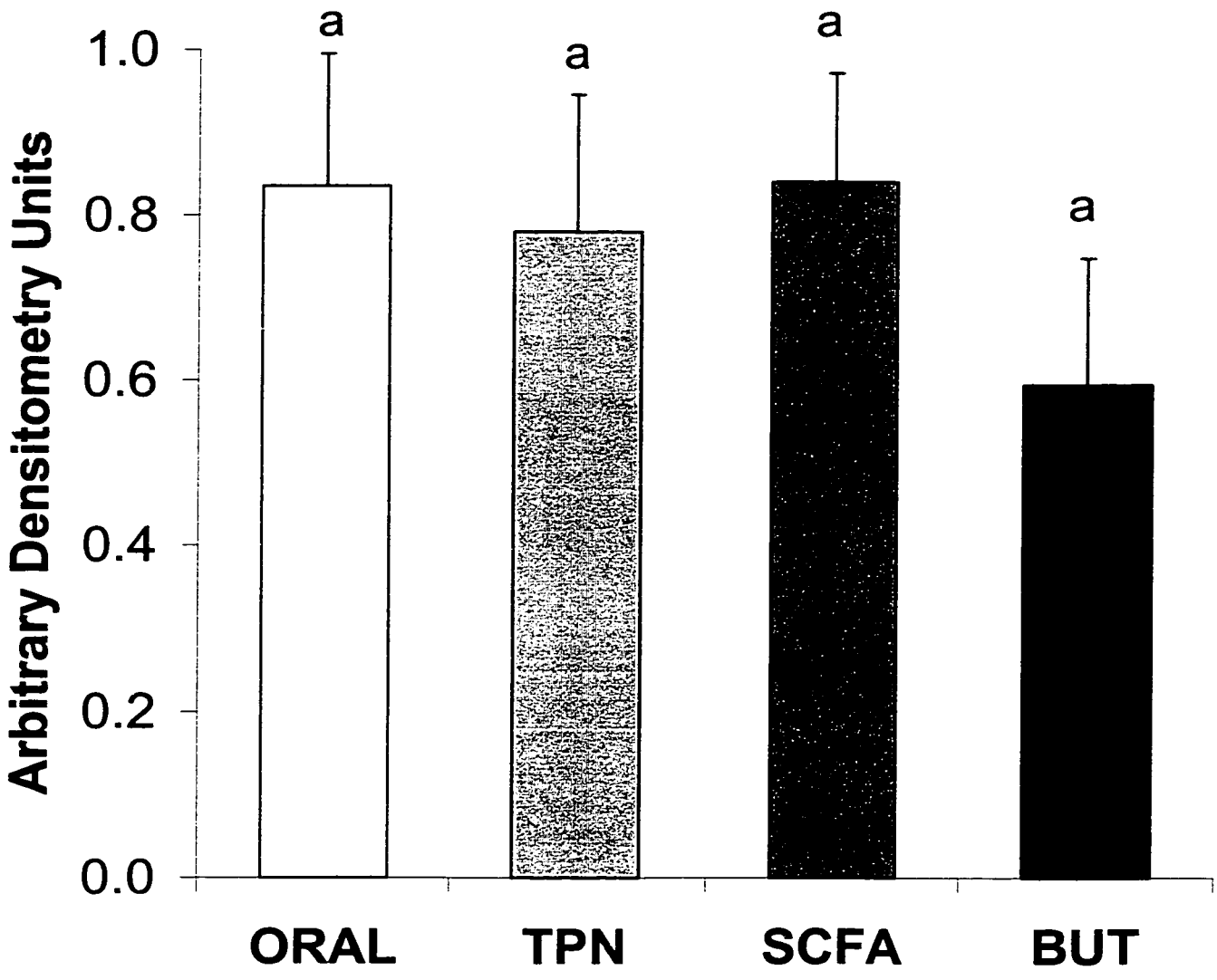


Figure 13. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on ileal c-fos mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

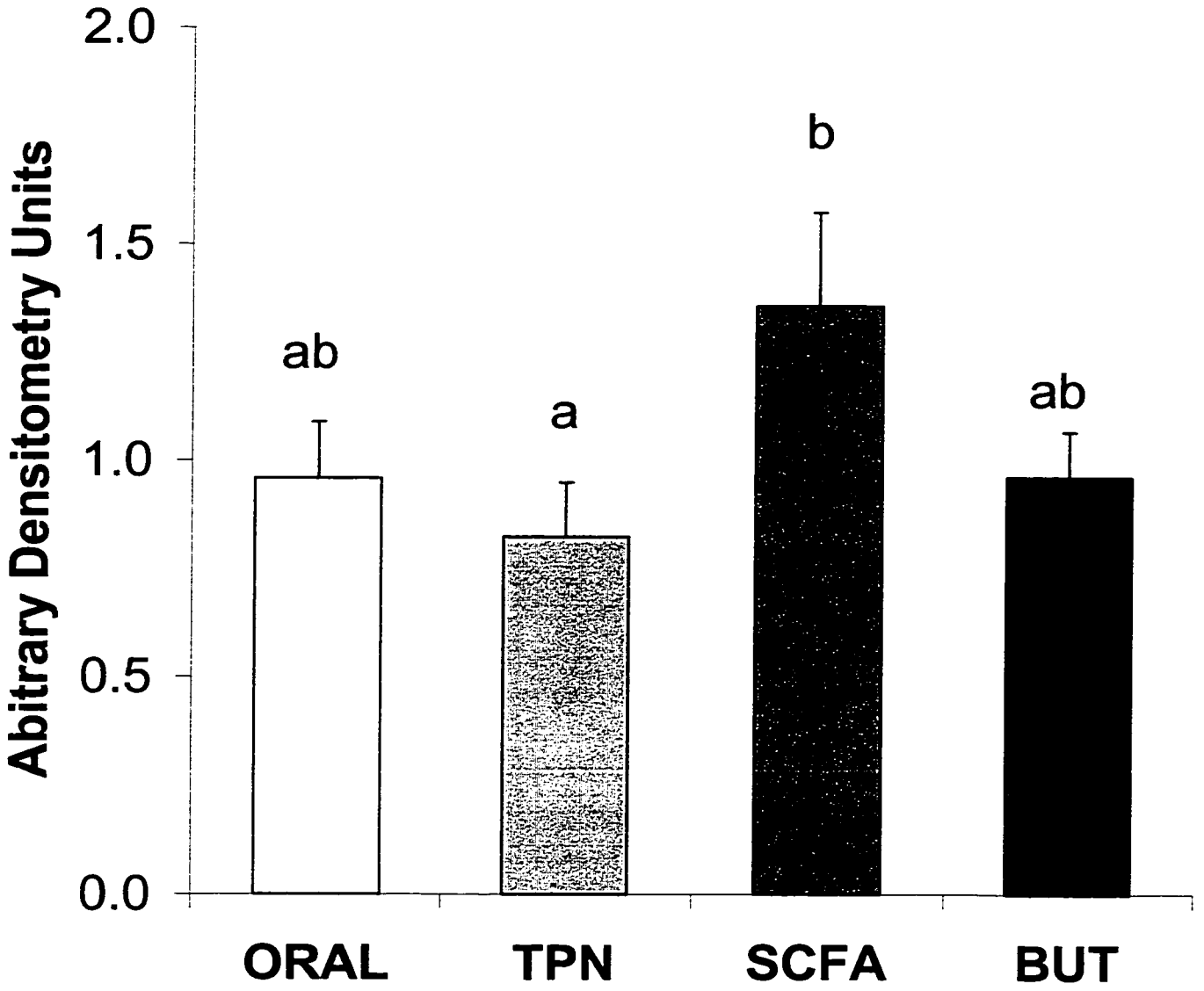


Figure 14. The effect of standard TPN, short chain fatty acid supplemented TPN, and butyrate supplemented TPN on ileal proglucagon mRNA expression. Values are means ± SEM. Bars with different letters are significantly different as identified by randomized block ANOVA and student's t-test.

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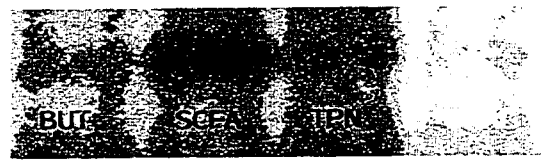
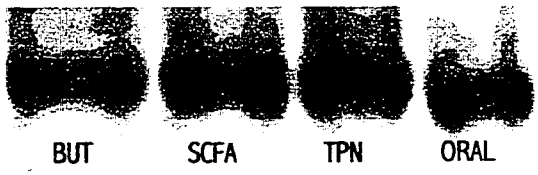
Appendix 1. Representative Northern and Western blot scanned images.

Proglucagon 1.2 Kb transcript

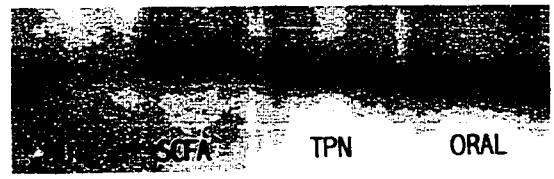
GLUT2 3.8 Kb transcript



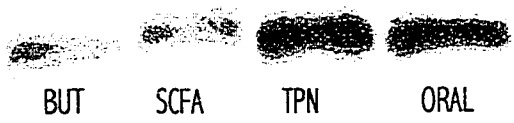
α1 Na^+K^+-ATPase 2.7 Kb transcript



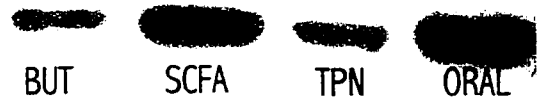
SGLT-1 4.8 Kb transcript



β1 Na^+K^+-ATPase 3.7 Kb transcript



SGLT1 ~75 KDa protein



Appendix 2. Representative RT-PCR scanned images