Developmental Regulation of the Expression of Nutrient Transporter and BrushBorder Membrane Hydrolase Genes in the Small Intestine of Piglets

By

Xunjun Xiao

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Dr. K. E. Webb, Jr., Chairman
Dr. E. A. Wong
Dr. H. Jiang
Dr. A. F. Harper
Dr. A. P. McElroy

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(ABSTRACT)

The objective of this study was to evaluate developmental regulation of the expression of nutrient transporter and brushborder hydrolase genes in the small intestine of piglets. Seventy piglets from seven sows were killed at birth (d 0), during suckling (d 1, 3, 7, 14, 21) and postweaning (d 22, 24, 28, 35), and intestinal segments (duodenum, jejunum and ileum) were collected. The mRNA abundance was determined by Northern blot using specific cDNA probes for three disaccharidases (lactase-phlorizin hydrolase, LPH, sucrase-isomaltase, SI, and maltase-glucoamylase, MGA), three peptide hydrolases (aminopeptidase A, APA, aminopeptidase N, APN, and dipeptidyl peptidase IV, DPP IV), two sugar transporters (Na^+-dependent glucose transporter 1, SGLT1, and facilitated glucose transporter 5, GLUT5), a peptide transporter (H^+-dependent peptide transporter 1, PepT1), four amino acid transporters (excitatory amino acid carrier 1, EAAC1, Na^+-dependent neutral amino acid transporter, ATB^0, the light chain of a heterodimeric transport system $b^{0,+}$ involved in the heteroexchange of cationic and neutral amino acids, $b^{0,+}AT$, and Na^+-independent large branched and aromatic neutral amino acid transporter 2, LAT2), and two iron transporters (divalent metal ion transporter 1, DMT1, and iron-regulated transporter 1, IREG1). Protein expression was quantified by Western blot using specific antibodies for LPH, SI, SGLT1, and PepT1. During suckling, the abundance of LPH, APA, APN, DPP IV, $b^{0,+}AT$ mRNA increased quadratically ($P < 0.001$) with age from birth to d 7 or 14 then remained unchanged or slightly declined with age to d 21. The mRNA abundance of SI increased and LAT2 decreased linearly ($P < 0.001$) with age, and the abundance of MGA and GLUT5 mRNA remained unchanged with age. There was an age x intestinal segment interaction ($P < 0.001$) for the abundance of EAAC1 and ATB^0 mRNA. The abundance of EAAC1 mRNA increased from d 0 through 14 and remained stable to d 21 in the ileum, and it was low and slightly increased with age through d 21 in the duodenum and jejunum. The abundance of ATB^0 mRNA generally increased from d 0 to 21 in the duodenum and ileum, and increased from d 0 to 7 and then decreased to d 21 in the jejunum. The abundance of SGLT1 and PepT1 mRNA was substantial at birth and transiently declined to d 1. The abundance of SGLT1 mRNA generally increased from d 1 to 21, and PepT1 mRNA abundance increased to d 3 and then plateaued through d 21. Postweaning, the mRNA abundance of all of these carbohydrate and protein assimilation related genes increased during the first day (3 d for ATB^0) after weaning then declined to the levels at weaning in the jejunum and ileum, followed by a subsequent change pattern that varied among genes. During suckling, the mRNA abundance of LPH, SGLT1, and APA was greater in the duodenum and jejunum than the ileum ($P < 0.001$). The PepT1 and APN mRNA was evenly distributed among intestinal segments, and the expression of MGA, DPP IV, EAAC1, $b^{0,+}AT$, ATB^0, and LAT2 mRNA was generally greater in the jejunum and ileum than the duodenum or greatest in the ileum. Postweaning, the mRNA abundance of all of these carbohydrate and protein assimilation related genes examined was generally greater in the jejunum and ileum than the duodenum or highest in the ileum. From d 0 through 35, DMT1 and IREG1 mRNA was predominantly ($P < 0.05$) distributed in the duodenum, where the abundance

of DMT1 and IREG1 mRNA increased with age during suckling, and then rapidly decreased after weaning. The protein expression of LPH and SI exhibited a similar developmental pattern as that for the mRNA abundance. Unlike the developmental regulation of their respective mRNA abundance, the protein expression of SGLT1 exhibited a general decline from suckling to postweaning. The protein expression of PepT1 gradually decreased with age from birth to d 35 in the duodenum, and initially declined from birth to the lowest value then slightly increased with age through d 21, followed by an increase to d 35 in the jejunum and ileum. In conclusion, the gene expression of these brushborder hydrolases and nutrient transporters was not only differentially regulated by age but also differentially distributed along the small intestine of piglets at early stages of life. These differences in ontogenetic regulation and the distribution may be related to the luminal substrate concentration as well as the nutrient categories, and the developmental regulation of these genes may occur not only at the transcriptional level but also at the posttranscriptional level.

Key words: transporter, hydrolase, developmental regulation, small intestine, pig

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Chapter I. Introduction

The growth, development and general health of mammals rely heavily on dietary nutrient intake. The small intestine is the primary organ responsible for the final digestion and absorption of dietary nutrients, and these functions are largely dependent on the growth and development of the intestine. In mammalian species, the growth, development and maturation of the small intestine is particularly rapid during the early stages of life, which is largely stimulated by the transition from mainly parenteral nutrition before birth (via the placenta) to exclusively enteral nutrition after birth, and later by the transition from mother's milk during suckling to solid diets after weaning. Hence, enteral intake of nutrients in newborns and subsequent weaning to an adult diet elicit structural and functional gastrointestinal changes, although the responses vary among species, sources of nutrients and specific gastrointestinal tract functions. It is well understood that intestinal hydrolases and nutrient transporters are essential for the efficient digestion and absorption of dietary nutrients including proteins and carbohydrates. Therefore, recent research efforts have been focused on nutritional, physiological, and regulatory aspects of these intestinal hydrolases and nutrient transporters. Reviewed in this dissertation are the reports from recent studies on the ontogenetic regulation of intestinal growth and development, intestinal digestion and absorption of dietary nutrients, and the basic characterization of intestinal hydrolases and transporters, and their nutritional and physiological functions and regulatory mechanisms.

Although specific activities of intestinal hydrolases and nutrient transporters change with age in mammalian species, little is known about the ontogeny of specific brushborder membrane hydrolases and nutrient transporters at the molecular level in pigs. The studies in this dissertation were conducted to investigate the ontogeny regulation of gene expression of intestinal hydrolases and nutrient transporters in the small intestine of piglets at early stages of life, as well as their

distribution along the small intestine. The hydrolases are those involved in the final digestion of carbohydrates and proteins, and the nutrient transporters are those responsible for the absorption of peptides, amino acids, monosaccharides, and iron. Because the measurements for a variety of hydrolases and nutrient transporters were made at three sites (duodenum, jejunum and ileum) along the entire length of the small intestine at ten ages between birth and d 35, 14 d after weaning, our results provide better resolution of spatial and temporal changes and broader pictures than previous studies that evaluated either fewer or more distant time points, and in most cases only the jejunum. Results from these studies provide information on developmental regulation of intestinal hydrolase and transporter gene expression in piglets at early stages of life. They may also provide indirect information regarding the gastrointestinal tract development in newborns and infants. In terms of development, structure, and function, the pig small intestine exhibits more similarity to the human small intestine than other laboratory animal models, such as mouse, rat, rabbit, and guinea pig. Together, knowledge gained from these studies will aid nutritionists in formulating appropriate diets. These specialized diets may not only stimulate the intestinal digestive and absorptive function for optimal growth performance and health status of pigs at both these critical early stages and at later stages, but also provide neonates under critical conditions with more appropriate diet components.

Chapter II. Literature Review

Introduction

The ontogenetic development of the mammalian intestine is a topologically and temporally highly organized process, which results in the formation of specialized intestinal epithelia and mucosal layers (Pachá, 2000). The small intestinal mucosal layer fulfills a variety of important physiological roles, including digestive and absorptive functions, maintenance of a physical barrier, and secretion of water and electrolytes (Pachá, 2000). The early ontogenetic development can be divided into five phases: 1) morphogenesis, 2) cytodifferentiation and fetal development including preparation of the epithelium for colostrum and milk, 3) birth, 4) suckling, and 5) weaning. The first two phases occur during gestation and prepare the intestine for the postnatal life at which time the intestine assumes complete responsibility for nutritional intake (Puchal and Buddington, 1992). During the suckling period, the immature gastrointestinal tract is offered a monotonous diet, the mother's milk. At weaning, the dietary transition from milk to solid diets triggers profound modifications in digestive and absorptive functions when neonatal properties are lost and mature ones are acquired (Pachá, 2000).

After birth, the gastrointestinal tract must possess efficient digestion and transport systems to obtain adequate nutrients. In the small intestine, the final digestion and absorption of dietary nutrients including monosaccharides, peptides, amino acids, fatty acids, vitamins and minerals, occurs through specific digestive enzymes and transporters located on the brushborder membrane of enterocytes. The transporters located on the basolateral membrane then mediate the movement of the absorbed nutrients within the enterocytes into bloodstream to meet metabolic needs of the extra-intestinal tissues. To date, research on intestinal digestive and absorptive functions has been primarily focused on several areas. The first is the study of the ontogenetic

regulation of intestinal growth and morphogenesis, particularly around birth and weaning. The development of the intestine has a direct impact on the rate and capacity of digestion and absorption of dietary nutrients. Understanding how intestinal digestive and absorptive functions change with age will enhance our ability to formulate appropriate diets. The specialized diets may reduce morbidity and enhance growth performance through the improvement of the digestive and absorptive functions of intestine. The second area is the basic study of specific intestinal hydrolase(s) and nutrient transporter(s) in terms of molecular characterization, preferred substrates, and nutritional and physiological functions. The third research area is the study of how intestinal digestive and absorptive function is regulated not only by intrinsic properties of enterocytes, but also by external factors. The knowledge from the second and third research areas provides fundamental information about major hydrolases and nutrient transporters, including their nutritional and physiological roles, and regulatory mechanisms. This allows researchers to exploit this knowledge to manipulate the intestinal digestive and absorptive functions under various conditions. To match with or even stimulate intestinal digestive and absorptive capability through formulating appropriate diets, the underlying mechanisms that control the expression of certain genes and the activities of respective proteins must be explored.

This chapter will begin with a brief description of the functional unit in the intestine, and the patterns of intestinal growth and development. Dietary nutrient digestion and absorption in the gastrointestinal tract will also be briefly addressed. The major intestinal hydrolases and transporters involved in the terminal digestion and absorption of dietary nutrients will then be discussed, with particular focus on tissue and cellular distribution, nutritional and physiological roles, and regulatory mechanisms.

The Crypt-Villus Functional Unit in the Intestine

The crypt-villus axis represents the functional unit in the intestine (van Dongen et al., 1976). It can be defined by typical morphological and functional properties displayed by the mature villus enterocytes that differ from crypt cells. The villi are mainly lined by absorptive, goblet and enteroendocrine cells, while the crypts contain stem cells, proliferative and undifferentiated cells, and a subset of differentiated secretory cells, namely Paneth, goblet and enteroendocrine cells (van Dongen et al., 1976). The differentiation and maturation of each cell type takes place as the cells move either upwards towards the villus (absorptive, mucus and endocrine cells) or downwards to the bottom of the crypt (Paneth cells; Uni et al., 1998). As for enterocytes, they arise from stem cells in the crypts, then undergo differentiation as they migrate towards the villus tip, where they are eventually exfoliated 2 to 5 d after emerging from the crypt, and the life span of these cells depends mainly on species, intestinal region, and life stages of animals (Ferraris, 2001). Many hydrolases and nutrient transporters are found only on the brushborder membrane of mature enterocytes along the villus, and not in cells lining the crypt. It is noteworthy that in all species studied, the crypt-villus junction represents a physical limit from which enterocytes acquire their final functional characteristics (Ferraris, 2001).

Growth and Development of the Intestine

Maturation and morphogenesis of the gastrointestinal tract in some mammalian species, such as the pig, continues from as early as the middle of gestation until 6 to 8 wk after birth, and is characterized by increases in intestinal weight and length, villus and crypt height, cell migration rate, RNA and DNA contents, and the adaptation of intestinal enzymatic activity (Rome et al., 2002). There are three distinct phases in which critical development occurs for adaptation to nutritional resources, namely gestation (particularly late gestation), at birth, and at weaning.

Prenatally, the morphogenesis and cytodifferentiation in utero prepare the intestinal epithelium for digestion and absorption of colostrum and milk components (Pachá, 2000). In pigs, during the last 20 % of gestation, the relative weight of small intestine to body weight increased 79 %, with a 37 % increase in the relative percentage of mucosa, and no change in the relative growth in length. As a result, the relative intestinal mucosa weight to body weight rose by 146 % (Boudry et al., 2004). Similar results for pig intestinal growth were reported during the last 10 % of gestation (Buddington et al., 2000). The stimulation of intestinal growth might be due to endocrine regulation, including fetal glucocorticoid secretion, and the swallowing of amniotic fluid, which contains dilute concentrations of proteins, free amino acids, and a variety of growth factors. Swallowed fluids seemed to be the major factor inducing intestinal growth and thereby enhancing digestive and absorptive capacity due to the increased gut mass (Boudry et al., 2004). The intestinal growth, uptake capacity of certain nutrients, and the activity of selected intestinal enzymes was enhanced by the provision of artificial enteral diets, such as elemental nutrients, milk or growth factors. The potential mechanisms for the changes of digestive and absorptive functions involve changes in enterocyte turnover rate and/or the biosynthesis of specific proteins, and increases in the intestinal mass (Boudry et al., 2004).

After birth, the most rapid increases in intestinal dimensions occur during the first few days, particularly during the first 6 h of suckling milk (Zhang et al., 1997; Buddington et al., 2000). The growth and morphological changes of the small intestine of piglets were examined during the first 3 d after birth (Xu et al., 1992). There was a 72 % increase in small intestinal weight, virtually all of which occurred during the first day and was primarily a 115 % increase in mucosal weight. The dramatic increase in mucosal weight was associated with a 40 % increase in intestinal thickness and a 100 % increase in absorptive surface during the first 6 h after birth.

There was also a 24 % increase in small intestinal length, a 35 % increase in small intestinal diameter, a 61 % increase in villus height, and a 28 % increase in villus diameter during the first day. These results were supported by later studies (Zhang et al., 1997; Buddington et al., 2000). Xu et al. (1992) also demonstrated that the cellular population in the small intestinal mucosa, as indicated by DNA content, increased progressively with age, with a 120 % increase during the first 3 d. The increase in cell population is primarily due to the stimulation of the crypt cell proliferation, and to a lesser extent enterocyte proliferation, by suckling (Zhang et al., 1997). The rapid intestinal growth, morphological maturation, and formation of enterocyte ultrastructure during the first few days after birth was largely attributed to the dramatic shift from parenteral to enteral nutrition, and the changes in systemic or local luminal factor(s) around birth may also be an important contributor (Simmen et al., 1990; Burrin et al., 1994). Of critical importance to developing animals is whether the postnatal growth and morphological maturation have any affect on digestive and absorptive capacities of the entire small intestine.

Lactase specific activity (Burrin et al., 1994; Zhang et al., 1997) and transport rates of most nutrients per milligram of tissue decline during the first day after birth, whereas total intestinal lactase activities and transport capacities for most nutrients slightly increased (Buddington 1992; Zhang et al., 1997). The increase in total intestinal lactase activity and transport rates of most nutrients may be caused by the induction of gene expression and the synthesis and processing of gene products by birth and onset of suckling (Burrin et al., 1994; Zhang et al., 1997). What is less clear is whether the changes in gene expression reflect reprogramming of existing enterocytes or the production of enterocytes with adult characteristics that eventually replace the fetal ones. The postnatal decline in specific activities of hydrolases and transport rate of nutrients per milligram of tissue may be attributable to several factors. First,

the mature enterocyte proliferation rate is slower than that of the crypt cell proliferation, which results in dilution of the enterocytes in the entire intestinal cell population. Second, functional protein synthesis and processing takes time during the translocation and insertion into the brushborder membrane. Third, the respective gene products redistribute along the entire crypt-villus axis to the villi at birth (Freeman et al., 1993). Fourth, the intestinal hypertrophy and hyperplasia, villus swelling (Strocchi and Levitt 1993), and changes in the physical and chemical characteristics of the brushborder membrane (Alessandri et al., 1990) that occur after birth may also impact the uptake rate of nutrients and even the hydrolase activities.

At weaning, piglets under commercial conditions are subjected to nutritional and environmental/psychological stresses. Abrupt weaning is typically accompanied by low feed intake, which seems to be the main reason for the growth stasis after weaning (Boudry et al., 2004). The change from maternal milk and dependency on the sow, to a physically and chemically different diet as well as different feeding regimens and environmental stress causes profound changes in the gastrointestinal tract of piglets (Kelly et al., 1991). A recent study examined the major changes in the small intestinal morphology and activities of intestinal peptidases in piglets during the first 9 d after weaning (Hedemann et al., 2003). Villus height decreased to a minimum during the first 3 d after weaning and then recovered to the pre-weaning value by 5 to 9 d after weaning, and this is in accordance with other studies showing that villus height is minimal 2 to 5 d after weaning (Hampson, 1986; Kelly et al., 1991; van Beers-Schreurs et al., 1998). Crypt depth increased with age after weaning, which has also been observed in several other studies (Hampson, 1986; Kelly et al., 1991; van Beers-Schreurs et al., 1998). In contrast, in unweaned pigs, a gradual increase in crypt depth occurred with age, while villus height did not change (Hampson, 1986). The greatest loss of villus height after weaning occurred

in the proximal part of the small intestine, and the crypt elongated most in the distal part (Hampson, 1986; Hedemann et al., 2003). It is well recognized that increased crypt depth is an indication of increased cell production in the crypts, which is confirmed by a good correlation between the crypt depth and the mitotic counts (Hedemann et al., 2003), and decreased villus height is associated with cell loss. The presence of food in the gastrointestinal tract is necessary for structural and functional maintenance of the intestinal mucosa, and food deprivation(e.g. postweaning anorexia) results in villus atrophy and a decrease in crypt cell production rate (Pluske, 2000).

Coincidentally, with the shortened villi and elongated crypt depths, a decreased activity of brusborder membrane lactase and peptidases has been observed (Hampson and Kidder, 1986). The Na^+-dependent glucose uptake was also decreased in the jejunum and ileum of piglets after weaning, except for a transient increase in the jejunum during the first 2 d postweaning (Boudry et al., 2004). The postweaning decline is most likely connected to the villus atrophy with the loss of mature enterocytes and their replacement with immature enterocytes (Hampson and Kidder, 1986; Pluske, 2000). The possible interpretation of the transient increase in glucose uptake is the upregulation by short-term starvation, in anticipation of upcoming nutrients (Boudry et al., 2004).

Dietary Nutrient Digestion and Absorption in the Gastrointestinal Tract

Digestion and absorption of luminal nutrients in the gastrointestinal tract is a complex and well-integrated process. Feed initially enters the mouth where chewing reduces particle size to expose more surface area for enzyme action, and salivation wets the feed particles and provides salivary amylase to initiate starch digestion. Feed then moves further to the stomach where the wetting continues and HCl is introduced. The resulting acidic environment serves to

unravel proteins for further digestion and activate pepsinogen into pepsin, and also serves as a barrier to the passage of bacteria into the small intestine. The unraveling of proteins exposes the primary peptide bonds to subsequent enzymatic hydrolysis in the small intestine. The principle enzymes involved in the digestion of proteins, fats and carbohydrates are present in the small intestine. Some are secreted by the pancreas, and others are produced by intestinal enterocytes. Specific digestive enzymes attack various chemical bonds of large food molecules to break them down to absorbable molecules, which are then primarily absorbed across the brushborder membrane of intestinal enterocytes via specific transporters.

Carbohydrates. Carbohydrate assimilation in the gastrointestinal tract is fundamental for energy supply in humans and animals. Dietary carbohydrates can be divided into the digestible and undigestible fractions. Among digestible carbohydrates, polysaccharides and oligosaccharide must be digested into their constituent monosaccharides before being absorbed. The processes of carbohydrate digestion and absorption are accomplished through the action of specific digestive enzymes and transporter proteins (Goda et al., 1999). The primary digestible carbohydrates in the diets of mammals are starch, lactose, and sucrose. The digestion of starch begins with salivary amylase in the mouth, but this activity is much less effective than that of pancreatic α-amylase in the small intestine (Semenza et al., 2001). Pancreatic α-amylase hydrolyzes starch, with the primary end products being maltose, maltotriose, α-destrins, and a limited amount of glucose. The intermediate products of α-amylase digestion are hydrolyzed into their component monosaccharide glucose via disaccharidases expressed on the brushborder membrane of small intestinal enterocytes (Semenza et al., 2001). Lactose and sucrose can only be digested by brushborder membrane lactase and sucrase into galactose and glucose, and glucose and fructose, respectively (Nichols et al., 2003). In the small intestine, glucose and galactose are transported

across the brushborder membrane of enterocytes via a Na^+-dependent glucose transporter 1 (SGLT1; Wright et al., 1994), and fructose is absorbed across the brushborder membrane through a Na^+-independent glucose facilitated transporter 5 (GLUT5; Ferraris, 2001). The intracellular monosaccharides are then transported across the basolateral membrane via a Na+-independent glucose facilitated transporter 2 (GLUT2) into the bloodstream (Bird et al., 1996).

Proteins. The luminal proteins in the gastrointestinal tract are derived from both dietary sources and endogenous sources (i.e., secretion and sloughed cells). Proteins are initially denatured by stomach acid, in conjunction with limited proteolysis by pepsin (Britton and Koldovsky, 1989). In young mammals, gastric rennin partially hydrolyzes and precipitates milk casein and increases gastric retention time (Britton and Koldovsky, 1989). Protein digestion is largely completed in the small intestine at a slightly alkaline pH (Daniel, 2004). The denatured proteins are broken down through highly efficient endo- and C-terminal cleavage by pancreatic proteases (trypsin, chymotrypsin and elastase) and carboxypeptidases. The resultant end products, mostly large peptides, undergo further hydrolysis by a variety of peptidases (e.g. aminopeptidases, and dipeptidyl peptidases) present on the brushborder membrane of intestinal epithelium. Analysis of luminal contents after albumin administration has shown that amino acids are present in the lumen primarily in peptide form rather than in free amino acid form (Adibi and Mercer, 1973). The peptides present in the lumen consist mostly of two to six amino acids. The concentration of these peptide mixtures was 120 to 145 m*M*, while a total concentration of all amino acids was just 30 to 60 m*M*. The di- and tripeptides and free amino acids are efficiently absorbed via a H^+-dependent peptide transporter 1 (PepT1) and multiple amino acid transport systems across the brushborder membrane of enterocytes, respectively (Ganapathy et al., 1994 and 2001). The extent to which di- or tripetides released during digestion

are finally broken down to free amino acids on the brushborder membrane of intestinal enterocytes or are taken up across the brushborder membrane into enterocytes is still unknown. It depends on both the affinity and the substrate concentration of peptides, which compete simultaneously for the binding sites of digestive enzymes or peptide transporters (Daniel, 2004).

In human adults, protein assimilation is generally not considered to be limited. Adult human volunteers ate 320 to 480 g of protein (equivalent to 1.5 to 2.8 kg of lean meat) within 8 h and did not exhibit a limitation in protein assimilation (Matthews, 1991). However, there is no similar result reported in domestic animals, which require high dietary protein intake for rapid growth. Although most proteins and oligopeptides are rapidly degraded in the small intestine, some structures are fairly resistant to hydrolysis (Daniel, 2004). Thus, the extent and velocity to which a dietary protein is finally hydrolyzed to its constituent free amino acid and small peptides depends on its composition (amino acid sequence) and posttranslational modifications, such as glycosylation and thermal effects of food processing (Kuwata et al., 2001). The low activity of brushborder membrane-bound peptidases, as rate-limiting enzymes in the small intestine, determines the digestive breakdown of these peptides (Kuwata et al., 2001).

Fats. In recent years, research on fat digestion and absorption has been focused on improving fat utilization in infants (especially preterm infants) and reducing fat digestion and absorption in adults to prevent obesity (Lowe et al., 1998). Triglycerides, the predominant form of dietary fats, play an important role in nutrition, such as a major energy source, precursors for cellular membranes, and for prostaglandins, thromboxanes, and leukotrienes, and vehicles for fat-soluble vitamins (Carey and Hernell, 1992). Dietary fats are particularly important for newborns whose major energy consumed is from the mother's milk fats, with dietary fats contributing up to 90 % of the energy retained in new tissues during the first several weeks

(Fomon et al., 1970). Consequently, the development of newborns depends on the efficient digestion of dietary triglycerides, which must be cleaved into free fatty acids and monoacylglycerol before absorption (Hajri and Abumrad, 2002). Adult mammals require pancreatic triglyceride lipase and colipase along with bile salts for efficient digestion of dietary fat (Lowe et al., 1998). Large lipid droplets are first broken down into smaller droplets by emulsification. The resulting lipid substrates are then digested to a monoacylglycerol and two fatty acids, in the presence of pancreatic lipase, colipase, and bile salts in the small intestine (Hajri and Abumrad, 2002). Digested products and bile salts form amphipathic micelles. These micelles keep the insoluble products in soluble aggregates from which small amounts are released and absorbed by epithelial cells via diffusion. The uptake of unesterified long chain fatty acids also occurs through saturable, carrier-mediated modes. The most prominent and best characterized of these carriers are fatty acid transporter /CD36 (Hajri and Abumrad, 2002) and fatty acid transport protein 4 (FATP4; Stahl et al., 1999). Free fatty acids and monoglycerides then recombine into triacylglycerol within enterocytes then enter the lymphatic fluid as droplets called chylomicrons, which are then taken up by the lacteals in the intestine. In newborns, the situation is a little different. Firstly, the digestion of fats in infants begins in the mouth with the function of several digestive enzymes originated from the mouth or milk. Secondly, pancreatic triglyceride lipase does not contribute to dietary fat digestion in newborns due to its absence before the suckling-weaning transition (D'Agostino et al., 2002). In newborns, colipase appeared to interact with a homologue of pancreatic triglyceride lipase, pancreatic lipase-related protein 2, to participate in dietary fat digestion (Lowe et al., 1998).

Minerals. Minerals are essentially required for health, development, and growth of animals and humans. Most minerals are toxic when present at higher than normal required

concentrations. The majority of mineral absorption occurs in the small intestine, and in many cases, intestinal absorption is the key regulatory step in mineral homeostasis. At present, the best studied and characterized mechanisms of mineral absorption are for calcium and iron. For the purpose of this dissertation, this literature review will focus on iron.

Iron homeostasis is regulated at the level of intestinal absorption to ensure adequate but not excessive quantities of iron absorbed from the diet. Inadequate absorption can lead to iron-deficiency disorders such as anemia, while excessive iron is toxic because mammals do not have a physiologic pathway for its elimination (Leong et al., 2003). Dietary nonheme iron is generally found in the ferric (Fe^{3+}) oxidation state. It must be reduced to ferrous (Fe^{2+}) ion to be transported by a H^{+}-dependent divalent metal ion transporter 1 (DMT1), the predominant transmembrane iron transporter on the brushborder membrane of intestinal enterocytes (Gunshin et al., 2005). The reduction of dietary iron is thought to be mediated by an enzymatic ferric reductase on the intestinal brushborder membrane (Riedel et al., 1995). Iron is primarily absorbed via enterocytes in the proximal duodenum, and the efficient absorption requires an acidic environment. Once inside enterocytes, iron is trapped by incorporation into ferritin or exported out of enterocytes via a basolateral iron-regulated transporter 1 (IREG1) or ferroportin 1 (FPN1). It then binds to the iron carrier transferrin for transport throughout the body. The heme iron, from ingestion of hemoglobin or myoglobin, is also readily absorbed (Gunshin et al., 2005). It appears that intact heme is taken up by small intestinal enterocytes by endocytosis. Once inside the enterocyte, iron is liberated and essentially follows the same pathway for export as nonheme iron. Some heme may be transported intact into the circulation.

Distribution, Physiological Functions, and Regulation of Expression and Activity of Protein Digestion and Absorption Related Genes

Under normal physiological conditions, dietary protein undergoes a series of degradative steps carried out by the digestive enzymes originating from the stomach, pancreas, and small intestine, resulting in a final product mixture of free amino acids and small peptides, which are efficiently absorbed by enterocytes in the small intestine (Erickson et al., 1995). During this integrated process, brushborder membrane-bound hydrolases, including three major peptidases, aminopeptidase A (APA), aminopeptidase N (APN), and dipeptidyl peptidase IV (DPP IV), play a pivotal role in the final digestion of short- and medium chain peptides into di-, and tripeptides, and free amino acids. The cellular transport of small peptides (di-, and tripeptides) from the intestinal lumen into enterocytes is primarily mediated by PepT1, which is located on the brushborder membrane of intestinal enterocytes (Daniel, 2004). The intracellular transported small peptides are then rapidly hydrolyzed to free amino acids by intracellular peptidases. The absorption of free amino acids from the intestinal lumen involves various amino acid transport systems expressed on the brushborder membrane with distinct functional characteristics, such as substrate specificity, affinity, and driving force (Ganapathy et al., 1994). The intracellular free amino acids are then transported via amino acid transport systems across the basolateral membrane into the bloodstream. A limited amount of intact peptides are transported across the basolateral membrane via a peptide transporter located herein (Ganapathy et al., 1994).

Carrier-mediated transport of amino acids across the intestinal epithelium supplies amino acids (peptides and free amino acids) from intestinal lumen to not only the circulating bloodstream but also the mucosal cells. Mucosal cells need amino acids for energy, synthesis of proteins, nucleosides, and polymides, as well as for the maintenance of the intestinal defense system, including glutathione and mucin production (Reeds et al., 2000). In humans and pigs, the absorbed dietary amino acids utilized by intestinal mucosa accounted for 30 to 40 % for some

essential amino acids (Stoll et al., 1998), and most of glutamine, glutamate, and aspartate (Battezzati et al., 1995; Stoll et al., 1998). Under a meal condition, oxidation of arterial glutamine, luminal glutamine plus glutamate plus aspartate, and luminal glucose accounted for 38 %, 39 %, and 6 %, respectively, of the CO_2 produced by rat small intestine (Windmueller et al., 1980). This indicates that amino acids, rather than glucose, are the major fuel for the small intestine mucosa. Radioactive tracer studies have shown that amino acids from both luminal and arterial sources by uptake across brushborder and basolateral membranes are used in mucosal protein synthesis and energy generation; and, even in the fed state, there is uptake from both brushborder and basolateral membranes (Reeds et al., 2000). Over the past decade, a number of research groups have been actively involved in molecular cloning and functional characterization of amino acid and/or peptide transporter(s) in various animal species. A variety of transporters involved in the absorption of amino acids were found to be expressed in the small intestine, including peptide transport system, PepT1, and amino acid transport systems, such as L, y^+, y^+L, $b^{0,+}$, A, ASC, B^0, $B^{0,+}$, and X^-_{AG}. In our lab, we have concentrated on molecular cloning and functional characterization, examination of tissue distribution, and dietary and developmental regulation of intestinal peptide and/or amino acid transporter(s) in domestic animals, including sheep (Pan et al., 2001), chicken (Chen, 2001), turkey (Van, 2005), and pig (Klang, 2005), as well as wild animal species, such as black bear (Gilbert, 2005).

Before addressing the regulatory mechanisms of intestinal hydrolases and transporters involved in the intestinal protein digestion and absorption, it is necessary to give a brief description about their molecular structure, tissue and cellular distribution, nutritional and physiological functions. For the purpose of this dissertation, this literature review will focus on three major brushborder membrane-bound peptide hydrolases, APA, APN, and DPP IV, a

peptide transporter, PepT1, and five amino acid transporters, excitatory amino acid carrier 1 (EAAC1), Na^+-dependent neutral amino acid transporter (ATB^0), the light and heavy chain of a heterodimeric transport system $b^{0,+}$ involved in the heteroexchange of cationic and neutral amino acids ($b^{0,+}AT$, and NBAT), and Na^+-independent large branched and aromatic neutral amino acid transporter 2 (LAT2).

Aminopeptidase A. Aminopeptidase A (APA, EC 3.4.11.7) is a homodimeric membrane-bound zinc metallopeptidase. It is an extensively glycosylated protein composed of two 140 to160 kDa disulfide-linked subunits (Troyanovskaya et al., 2000). This enzyme specifically catalyzes the removal of the N-terminal glutamyl or aspartyl residue from oligopeptide substrates, and as such has been implicated in the *in vivo* metabolism of angiotensin II to angiotensin III, and cholecystokinin-8 (Wilk and Healy, 1993). The APA cDNA has been cloned and functionally characterized in human, rat, and mouse (Jiang et al., 2000), and APA is widely distributed, but is found primarily in the renal and intestinal brushborder of epithelial cells, and in the vascular endothelium of many organs, where APA might be involved in metabolism of circulating or locally formed bioactive peptides (Li et al., 1993; Troyanovskaya et al., 2000).

In the small intestine of rats, APA mRNA and protein expression was highest in the ileum, where APA protein was restricted to the brushborder membrane of enterocytes lining the intestinal microvilli, and APA mRNA levels were higher in the proximal portion of the villi than in the distal tips (Troyanovskaya et al., 2000). On the brushborder membrane of the small intestine, APA is a type II integral membrane protein of 945 amino acids, with a short 17 amino acid residual N-terminal cytoplasmic tail, a 22 amino acid residual transmembrane domain, and an extracellular domain containing the active site, which is located at the interface of the N- and C-terminal subdomains (Troyanovskaya et al., 2000),

Aminopeptidase N. Aminopeptidase N (APN, EC 3.4.11.2) is a 150 kDa membrane-bound ectoenzyme belonging to the zinc metallopeptidases family, which includes APA. The APN preferentially catalyzes the removal of neutral and basic amino acids from the N terminus of small peptide substrates (Riemann et al., 1999). The APN gene has been cloned and functionally characterized in human, rat and pig, and APN mRNA and protein is highly expressed in the liver, placenta, and renal and intestinal brushborder of epithelial cells, and to a lesser extent, in the brain, lung, blood vessels, and primary cultures of fibroblasts (Riemann et al., 1999). Given its wide distribution and broad substrate specificity, APN has been implicated in a variety of tissue-specific functions (Terenius et al., 2000). In peripheral organs, it participates in the enzymatic cascade of the renin–angiotensin system. In the brain, it cleaves angiotensin III. Moreover, APN is identical to a marker CD13 and serves as a retrovirus receptor (Look et al., 1989).

The mRNA abundance of APN gradually increases from the proximal to distal part of small intestine in rabbits and pigs (Freeman, 1995), and APN protein is expressed mainly in the enterocytes located in the villi and upper crypt in the jejunum of pig (Hansen et al., 1994). On the brushborder membrane of small intestine, APN is a transmembrane ectoenzyme that catalyzes the removal of neutral or basic amino acids from the N termini of a number of small peptides (Shipp and Look, 1993; Riemann et al., 1999). Depending on species, APN protein is composed of 963 to 967 amino acids, with a 9 to10 amino acid N-terminal tail in the cytoplasm, a 23 to 24 amino acid transmembrane segment, and a large extracellular ectodomain containing the active site (Luciani et al., 1998).

Dipeptidyl Peptidase IV. Dipeptidyl peptidase IV (DPP IV; CD26; EC 3.4.14.5) is a 110 kDa integral type II membrane glycoprotein and is present on the plasma membrane as a

homodimer (Morimoto and Schlossman 1998). The DPP IV has at least two functions, a signal transduction function and a proteolytic function (Morimoto and Schlossman 1998). In terms of a proteolytic function, DPP IV is a glycosylated ectoenzyme that preferentially cleaves dipeptides from the N-terminus of peptides containing a proline or alanine as the second residue, and to a lesser extent, when that residue is replaced by serine, glycine, valine, and leucine (Bongers et al., 1992). It has been shown to cleave a variety of biologically active peptides (Frohman et al., 1989; Mentlain et al., 1993; Medeiros and Turner, 1996). The DPP IV cDNA has been cloned and characterized in rat, mouse, and human, and DPP IV is expressed primarily in the renal and intestinal brushborder of epithelial cells (Morimoto and Schlossman, 1998).

The DPP IV is expressed at relatively high levels along the entire small intestine and at low levels in the stomach and large intestine in rats (Hong et al., 1989; McCaughan et al., 1990). The DPP IV enzyme activity is lower in crypt cells than in villus cells, which is associated with an increasing gradient of DPP IV mRNA and protein levels along the crypt-villus axis. The expression of DPP IV is regulated largely at the mRNA level (Hong et al., 1989).

Peptide Transporter. A number of mammalian peptide transporters have been cloned and functionally characterized. One intestinal peptide transporter, PepT1, is a H^+-coupled transmembrane protein capable of transporting a broad array of neutral, acidic, and basic di-and tri-peptides, as well as peptidomimetics (Daniel, 2004). The PepT1 cDNA from a variety of species have been cloned and characterized in cell lines and/or *Xenopus* oocytes, and the expression of PepT1 mRNA is predominantly detected in the small intestine, or omasal and ruminal epithelium of sheep, and dairy cows, and less expressed in the liver and kidney of some species (in rabbit, Fei et al., 1994; in human, Liang et al., 1995; in rat, Saito et al., 1995; in mouse, Fei et al., 2000; in sheep, Pan et al., 2001; in chicken, Chen et al., 2002; in bear, Gilbert,

2005; in pig, Klang et al., 2005; in turkey, Van et al., 2005). The expression of PepT1 exhibited a differential spatial pattern along the small intestine longitudinal and crypt-villus axis in rabbits (Freeman et al., 1995). The PepT1 mRNA was most abundant in duodenum and jejunum. In all sections of small intestine, PepT1 mRNA abundance increased from undetectable level in the crypt base to a maximum level at 50 to 70 % of the height of the villus. The PepT1 was highly expressed in the absorptive epithelial cells of the villi in the rat small intestine (Ogihara et al., 1999). The distribution of PepT1 protein was exclusively on the brushborder membrane of enterocytes from both prenatal and mature rats (Hussain et al., 2002). However, immediately after birth, PepT1 protein extended to the subapical cytoplasm and to the basolateral membrane of enterocytes.

In pigs, two major transcripts of approximately of 2.9 and 3.5 kb were observed throughout the entire small intestine (Klang et al., 2005). Analysis of pig PepT1 protein predicts that it is composed of 708 amino acids and has 13 putative transmembrane domains, with the large hydrophilic loop located between transmembrane domains 10 and 11, and the amino terminus extracellular and the carboxy terminus intracellular. The PepT1 protein has a number of potential N-glycosylation and protein kinase recognition sites, which suggests that the transporter may be regulated by reversible phosphorylation to alter the V_{max} and K_m (Brandsch et al., 1994). The functional characterization of PepT1 by transiently transfecting CHO cells demonstrated that, like all other PepT1 clones, pig PepT1 can transport a wide range of di- and tripeptides but not tetrapeptides in a substrate saturable manner, with an optimal pH of 6.0 to 6.5, and peptide transport activity is driven by an inwardly directed H^+ gradient and is independent of Na^+ and K^+ (Klang et al., 2005).

Amino Acid Transporters. Free amino acid transport is very complex because of the existence of multiple amino acid carriers with overlapping substrate specificity, and this complexity is further confounded by factors such as species, developmental differences, regional variations along the intestine, incompleteness of available information, and the differences in the investigational techniques applied. Below is the partial summary of classified amino acid transport systems occurring in the small intestine.

System X^-_{AG}. System X^-_{AG} is defined as a high affinity transport system that is specific for acidic amino acids aspartate and glutamate. Glutamate transport across plasma membranes of neurons, glial cells and epithelial cells of the small intestine and kidney is mediated by high- and low-affinity transport systems (Kanai and Hediger, 1992). High-affinity transport systems have been described to be coupled to the inwardly directed electrochemical potential gradients of Na^+ and H^+, and to the outwardly directed gradient of K^+, with the preferred substrates of L-glutamate and D- and L-aspartate. To date, five high affinity glutamate transporters have been identified. Among them, a cDNA encoding a high affinity glutamate transporter, excitatory amino acid carrier -1 (EAAC1) was isolated from rabbit small intestinal cDNA library, and EAAC1 transcripts were detected in specific neuronal structures in the central nervous system, small intestine, kidney, liver, and heart (Kanai and Hediger, 1992). In the small intestine of neonatal piglets, glutamate tracer uptake data revealed that system X^-_{AG} was the major pathway responsible for transporting luminal L-glutamate across the brushborder membrane of enterocytes (Fan et al., 2004). It was further shown that EAAC1 was the predominant isoform of system X^-AG and the primary Na^+-dependent glutamate transporter expressed in these epithelial cells.

The expression of EAAC1 has been detected in the small intestine of various species, including rat (Erickson et al., 1995; Rome et al., 2002; Howard et al., 2004), cattle (Howell et al., 2001), lamb (Howell et al., 2003), and pig (Fan et al., 2004). In rats, EAAC1 mRNA abundance displayed a pronounced gradient along the proximal-distal intestinal axis, with the greatest abundance in the ileum (Erickson et al., 1995; Rome et al., 2002; Howard et al., 2004**),** suggesting the distal part of the small intestine is an important area for acidic amino acid absorption. In pigs, two predominant immunoreactive bands of approximately 55 and 70 kDa, representing the nonglycosylated and glycosylated forms of EAAC1, respectively, were detected in the small intestine (Fan et al., 2004). The EAAC1 protein was observed in the microvilli of enterocytes confined to the lower third of the villi and the crypts in piglets, which is consistent with the results reported in rats (Rome et al., 2002). They further demonstrated that the V_{max} of L-glutamate transport activity across the brushborder membrane into enterocytes was high in proliferating and differentiating midvillus epithelial cells and low in the differentiated upper villus cells. The glutamate transport affinity of system X_{AG}^- was significantly lower in crypt and middle villus than in upper villus cells. It is well established that transporters with a low transporter affinity are usually associated with a large V_{max} value. Thus, these results collectively suggest that the principal function of EAAC1 may be to meet the anabolic requirements of rapidly proliferating epithelia rather than to provide the extra-intestinal tissues with dietary glutamate.

System ASC. System ASC is a ubiquitous system which mediates Na^+-dependent transport of small zwitterionic amino acids, with a high affinity for alanine, serine, threonine, and cysteine (Kanai and Hediger, 2004). At present, two isoforms designated as ASCT1 and ASCT2, which share 57 % identity, have been isolated and functionally characterized. In addition to the

common substrates of ASC transport systems, ASCT2 also accepts glutamine and asparagine with high affinity, and methionine, leucine and glycine with low affinity (Kanai and Hediger, 2004). Furthermore, ASCT2 transports glutamate with low affinity, and the transport activity is enhanced at low pH (Pinilla et al., 2001). Like ASCT1, ASCT2 mediates Na^+-dependent obligatory exchange of substrate amino acids. Results from recent studies indicate that ASCT2 is identical to amino acid transporter B^0 (ATB^0) in the rabbit small intestine (Avissar et al., 2001a). The ASCT2/ATB^0 has the similar tissue and intracellular distributions, transport properties to that of system B^0 (Utsunomiya-Tate et al., 1996; Kekuda et al, 1996 and 1997), which is a Na^+-dependent, broad-spectrum neutral amino acid transport system, and the major brushborder membrane glutamine transporter (Fan et al., 1998; Costa et al., 2000; Ray et al., 2003). Avissor et al. (2004) demonstrated that changes in ASCT2/ATB^0 mRNA and protein paralleled changes in the transport activity of system B^0. These data indicate that ASCT2/ATB^0 protein might be responsible for system B^0 activity. However, the transport characteristics of ASCT2/ATB^0 may not be identical to those of classical system B^0 because it has not yet been determined whether ATB^0/ASCT2 participates in non-obligatory exchange (Torreszamorano et al., 1998). The ATB^0/ASCT2 is primarily expressed on the brushborder membrane of epithelial cells in the proximal kidney tubule and small intestine in rabbits. In rabbit intestine, ATB^0/ASCT2 expression exhibited an increasing gradient along the proximal-distal intestinal axis, with the lowest expression in the duodenum and highest in the colon (Avissar et al., 2001a and b).

System L. System L is responsible for the Na^+-independent transport of large branched and aromatic neutral amino acids (Rajan et al., 2000). Members of this family form heterodimers with a heavy chain protein, 4F2hC. This review section will only focus on one of its family members, LAT2. The LAT2-4F2hC heterodimeric complex is present on the basolateral

membrane, where it serves as a low affinity exchanger to equilibrate the concentration of neutral amino acids across the membrane with broad substrate specificity. The preferred substrates include small neutral amino acids (alanine, cysteine, glycine, and serine), glutamine, and large neutral amino acids (Rajan et al., 2000). It has a lower affinity for intracellular amino acids than for extracellular ones. It also serves as an exporter of cystine (Rajan et al., 2000).

The LAT2 cDNA was first isolated from a rabbit intestinal cDNA library, and consists of 535 amino acids, 12 putative transmembrane domains, with the intracellular amino and carboxy termini (Rajan et al., 2000). The putative structure contains phosphorylation sites for protein kinase A and C and tyrosine kinase. The LAT2 expression is widespread, with the primary expression in the basolateral membrane of epithelial cells in the proximal kidney tubule and small intestine (Wagner et al., 2001). In MDCK cells, the cotranslocation of LAT2 and 4F2hC to the basolateral membrane was observed by immunofluorescence microscopy (Bauch et al., 2003)

The 4F2hC protein is a type II glycoprotein. The rat 4F2hC protein consists of 527 amino acids, with a molecular weight of approximately 60 kDa (Deves and Boyd, 1998). It consists of a single transmembrane domain, with an intracellular amino terminus and a glycosylated extracellular carboxyl terminus. The 4F2hC protein is believed to control the signaling that translocates the LAT2-4F2hc complex to the basolateral membrane.

System $b^{0,+}$. System $b^{0,+}$ facilitates the Na^+-independent transport of neutral or basic amino acids into the cells in exchange for intracellular neutral amino acids. This heterodimeric amino acid transporter consists of a light chain ($b^{0,+}AT$) and a heavy chain (NBAT) through a disulfide linkage, with the carboxyl and amino terminus of $b^{0,+}AT$ intracellular (Verrey et al., 2003). Coexpression of $b^{0,+}AT$ and NBAT induces a Na^+-independent, high affinity transport for cystine and cationic amino acids, and a lower affinity for neutral amino acids, occurring via an

exchange mechanism (Palacin and Kanai, 2004). The functional expression of system $b^{0,+}$ in HeLa cells showed a high affinity for arginine and leucine from the outside of cells, and a low affinity of leucine from inside. A unidirectional transporter on the apical membrane maintains a high intracellular concentration of neutral amino acids, resulting in a transmembrane concentration gradient that drives obligatory exchange (Bauch et al., 2003). Coimmunoprecipitation analysis showed that NBAT and $b^{0,+}$AT displayed an opposite expression pattern in the brushborder membrane of the kidney proximal tubule, with highest expression of $b^{0,+}$AT in the proximal region of the tubule and lowest expression in the distal region, and highest expression of NBAT in the distal region of the tubule and lowest in the proximal region, suggesting that they may bind to other subunit proteins to for their full transport function (Bauch and Verrey, 2002; Fernandez et al., 2002). In transfected HeLa and MDCK cells, $b^{0,+}$AT protein assumed full transport activity in the absence of NBAT, but in enterocytes, coexpression with NBAT is required for the translocation of $b^{0,+}$AT to the brushborder membrane (Reig et al., 2002).

The $b^{0,+}$AT cDNA was first isolated from a mouse intestinal cDNA library and then functionally characterized in COS-7 cells (Chairoungdua et al., 1999). The mouse and human $b^{0,+}$ AT protein consists of 487 amino acids with a molecular weight of approximately 50 kDa, with 12 putative transmembrane domains, and a conserved cysteine residue in the putative extracellular loop between domains three and four (Chairoungdua et al., 1999; Wagner et al., 2001). The $b^{0,+}$AT protein has putative phosphorylation sites for tyrosine kinase, protein kinase C, and cAMP (Wagner et al., 2001). The mutation in $b^{0,+}$AT led to the loss of system $b^{0,+}$ transport activity. The stability of NBAT protein is increased by heterodimerization with $b^{0,+}$AT, but the stability of $b^{0,+}$AT protein is independent of NBAT. These results suggest that $b^{0,+}$AT is

the catalytic subunit (Wagner et al., 2001). The $b^{0,+}$AT gene is widely expressed, with the primary expression on the brushborder membrane of epithelial cells in small intestine and kidney (Verrey et al., 2000; Wagner et al., 2001; Palacin and Kanai, 2004).

The NBAT cDNA has been isolated and functionally characterized by different research groups (Tate et al., 1992; Wells and Hediger, 1992; Bertran et al., 1993). The human NBAT protein consists of 685 amino acids of approximately 85 kDa and a single transmembrane domain, with an extracellular carboxyl terminus, an intracellular N-terminus, and a large extracellular domain (Palacin and Kanai, 2004). The mouse NBAT protein has four putative N-glycosylation sites (Segawa et al., 1997). The primary function of NBAT is to signal the trafficking of $b^{0,+}$AT to the brushborder membrane. It has been suggested that the cysteine residues on the carboxyl terminus of NBAT play an important role in the transport function of system $b^{0,+}$. However, the breakage of disulfide bonds did not completely eliminate the expression of the heterodimer at the brushborder membrane. These results demonstrated the presence of non-covalent interactions between NBAT and $b^{0,+}$AT protein (Palacin and Kanai, 2004). Like $b^{0,+}$AT, the NBAT gene is widely distributed, with the primary expression on the brushborder membrane of epithelial cells in small intestine and kidney (Verrey et al., 2000; Wagner et al., 2001; Palacin and Kanai, 2004). Expression of NBAT protein is restricted to the brushborder membrane of the intestinal epithelia and kidney proximal tubule, and to a lesser extent, in the cytoplasm, and along the crypt-villus axis, where it exhibits an increasing gradient, with no protein detected in the crypts (Palacin and Kanai, 2004).

Regulation of Intestinal Protein Digestion and Absorption

The ability of the small intestine to efficiently digest luminal protein and then absorb amino acids and small peptides varies in response to a variety of factors. These variations are

seen during development, pregnancy, and lactation, and also in response to disease, the quality and quantity of dietary proteins, diurnal cycle, local or systemic hormone levels, and so on. The underlying mechanisms for this process may be specific or nonspecific. The specific mechanisms are involved in the specific changes in the digestion and absorption of individual or a specific group of nutrients. The nonspecific mechanisms are involved in the generalized alterations in the digestive and absorptive ability of all nutrients in the small intestine.

Dietary Substrate Regulation. The dietary regulation of intestinal brushborder membrane hydrolases has been extensively researched. An early study was conducted to investigate the effects of dietary protein levels on small intestinal brushborder and cytosolic peptide hydrolase activities in rats (Nicholson et al., 1973). The small intestinal brushborder membrane peptide hydrolase activity (L-leucyl-P-naphthylamide as substrate) was significantly greater in rats fed a high protein diet (55 % casein) for 7 d compared to in those fed a low protein diet (10 % casein). In contrast, the activities of cytosolic peptide hydrolase (L-prolyl-L-leucine as substrate) and brushborder membrane non-proteolytic enzymes (sucrase, maltase, and alkaline phosphatase) did not vary significantly with diets. Raul et al. (1987) demonstrated that, compared to those re-fed a 10 % protein diet, adult rats fed a 70 % protein diet for 15 h after overnight food deprivation exhibited increased intestinal APN activities, with a slight increase in the jejunum and a substantial (51 %) increase in the ileum. This result supported the previous study in which intestinal brushborder membrane peptide hydrolase activity was significantly higher in rats fed high-protein diets than in those fed a low protein diet, particularly in the ileum (McCarthy et al., 1980). The increase in the ileal APN activities was more prominent in the mature cells of the upper villi. They also showed that increased APN activity associated with high protein intake paralleled an increased amount of immunoreactive APN protein. A later study investigated the

effects of dietary protein levels on the mRNA abundance of brushborder hydrolases angiotensin-converting enzyme (ACE) and DPP IV (Erickson et al., 1995). The hydrolase ACE exhibited a pronounced gradient of mRNA abundance along the intestinal axis with highest amounts in the proximal and middle regions. The DPP IV mRNA was evenly distributed along the intestinal axis with elevated levels detected distally. Switching from a 4 % protein diet to a 50 % protein diet for 14 d resulted in elevated ACE and DPP IV mRNA abundance throughout the small intestine, particularly in the proximal region for ACE (three- to fivefold increase), and in the distal region for DPP IV (1.3 to 1.5 fold increase).

Several studies have been conduced to explore the mechanisms by which dietary substrate regulates brushborder membrane peptide hydrolases. Reisenauer and Gray (1985) demonstrated that the biosynthetic rate of APN protein can be rapidly increased by perfusing specific peptide substrates in the small intestine of rats. A later study (Suzuki et al., 1993) was conducted to investigate the effect of the amount and types of dietary proteins on the specific activities and gene expression of brushborder membrane peptidase in the small intestine of rats. Two hydrolases, DPP IV and ACE, play a critical role in the terminal digestion of prolyl peptides, which are generally resistant to hydrolysis by peptide-hydrolyzing enzymes from stomach and pancreas. It was shown that diets high in proteins and proline (gelatin) led to five- to sixfold increases in intestinal activities of DPP IV and ACE after 7 d of administration, but a diet high in proline was particularly effective in stimulating intestinal activities of these two enzymes. In addition, these changes were accompanied by a 1.5- to 3.5-fold increase in the gene transcription of DPP IV and ACE and a parallel increase in mRNA levels (Suzuki et al., 1995). To understand the mechanism by which ACE in the proximal part of the small intestine was much more responsive to dietary protein than that in the distal part in rats, Erickson et al. (2001)

examined the changes in enzyme activity, mRNA level, and protein biosynthetic rate simultaneously in the proximal and distal intestinal parts during the dietary induction of ACE. It was shown that there was a five- to sixfold increase in the biosynthetic rate of ACE protein and a 1.6-fold increase in mRNA level in the proximal intestine within 24 h after switching to a high-protein diet. No change in ACE protein biosynthesis was observed in the distal intestine despite a 1.3- and 2.4-fold increase in mRNA level by 2 d and 14 d, respectively. These results indicate that intestinal ACE is differentially regulated in the proximal and distal parts of small intestine, and the regulation primarily occurs at both transcriptional and translational levels.

A possible regulator of function and/or gene expression of any nutrient transporter is its own substrates. Such is the case for peptide and amino acid transporters. Two *in vitro* studies (Thamotharan et al., 1998; Walker et al., 1998) provided solid evidence for substrate regulation of PepT1 by excluding the involvement of other systemic factors, such as hormones. In these two studies, an intestinal cell line, Caco-2, was employed. The Caco-2 cells in culture differentiate into polarized cell monolayers with microvilli on the apical membrane expressing various intestinal hydrolases and nutrient transporters, such as PepT1. It was shown that previous exposure of Caco-2 cells to a peptide-rich medium (10 m*M* Gly-Sar, a synthesized dipeptide resistant to hydrolysis) for 24 h led to a twofold increase in the V_{max} of Gly-Sar uptake without any notable change in the K_m. Western and Northern blot analysis revealed that there was a more than twofold increase in the abundance of PepT1 protein and mRNA, respectively, after previous exposure of Caco-2 cells to the peptide-rich medium. Substituting Gly-Sar with a corresponding mixture of glycine and sarcosine did not stimulate peptide transport, which indicated that the enhancement in peptide uptake is stimulated by dipeptides rather than free amino acids. In a different study, a natural dipeptide Gly-Gln was used to evaluate the possible regulation of

PepT1 expression (Walker et al., 1998). It was observed that previous incubation of Caco-2 cells in 4 m*M* Gly-Gln medium for 3 d resulted in a twofold increase in the V_{max} of Gly-Sar uptake with no change in the K_m. A twofold increase in cellular PepT1 mRNA level and apical membrane PepT1 protein abundance was also observed. Therefore, these results indicate selected dipeptides can upregulate the transport activity of Caco-2 cells, and this induction is due to the enhanced PepT1 gene expression at mRNA levels (Walker et al., 1998).

Several studies were conducted to investigate dietary substrate regulation of PepT1 in vivo. Erickson et al. (1995) demonstrated that rats fed a high protein diet (50 % casein) for 14 d exhibited a 1.5- to twofold higher PepT1 mRNA level in the middle and distal parts of small intestine, compared to those fed a low protein diet (4 %). This finding was consistent with results reported elsewhere (Shiraga et al., 1999). They demonstrated that, in comparison with a protein free diet, feeding rats a high protein diet (50 % casein) for 3 d enhanced the transport of Gly-Sar in ileal brushborder membrane vesicles (BBMV; a 1.2-fold increase in the V_{max} but no change in the K_m), mRNA abundance (100 %), and protein levels of PepT1 (90 %). Additionally, this study demonstrated that the increase in peptide transport activity and gene expression of PepT1 could be induced by feeding rats a protein-free diet supplemented with a single dipeptide (20 % Gly-Phe) or a free amino acid (10 % Phe). So far, the only reported study regarding the dietary regulation of PepT1 gene expression in domestic animals was conducted in our laboratory. In chickens, PepT1 mRNA abundance increased with age from d 0 through 35 in those fed 18 % and 24 % CP (restricted food intake) diets, whereas decreased with age in those fed a 12 % CP diet (Chen et al., 2005). In those fed a 24 % CP diet *ad libitum*, PepT1 mRNA abundance declined with age until d 14 and then increased from d 14 through 35 to an intermediate level, which was lower than that in intake restricted chickens. The results indicated

that expression of chicken intestinal PepT1 mRNA was regulated by dietary protein intake. Dietary protein intake alters the concentration of free amino acids and small peptides in the gut and may also cause a wide range of other metabolic alterations. Thus, these studies provided only circumstantial evidence for small peptide regulation of PepT1.

To further understand the underlying molecular mechanisms of transcriptional activation of intestinal PepT1 gene by dietary protein (protein, free amino acids and dipeptides), the promoter region of rat PepT1 gene was isolated and characterized by transient transfection (Shiraga et al., 1999). Within the PepT1 promoter region, there are certain elements which respond to selected dipeptides and amino acids with enhanced activity. The effects of Phe or Gly-Phe were most pronounced, and several other amino acid and selected dipeptides stimulated lesser activity, which are consistent with their dietary stimulatory effects. Among the elements within the PepT1 promotor region, it was suggested that the AP-1 binding site (TGACTCAG, nt-295), the AARE-like element-binding site (CATGGTG, nt -277), and an octamer-binding protein site for Oct1/Oct2 might be involved in responding to dietary proteins. The AP-1 is a transcription factor associated with the regulation of gene expression under amino acid deprivation conditions (Pohjanpelto and Holtta, 1990), and a similar AARE-like element was shown to control asparagine synthetase gene expression under essential amino acid deprivation (Guerrini et al., 1993).

Transport of amino acids across the brushborder membrane of intestinal enterocytes also can be regulated by dietary substrate levels. In general, high levels of dietary proteins or amino acids increase intestinal transport of amino acids, but the pattern of this upregulation varies (Ferraris and Diamond, 1989; Ganapathy et al., 1994). Karasov et al. (1987) demonstrated that feeding a high protein diet resulted in a varied (32 to 81 %) increase in the uptake of amino acids

tested in mouse jejunal BBMV, whereas feeding a protein deficient diet led to a decrease in the uptake for aspartate and proline, no change for lysine, and a slight increase for leucine and alanine. In goats and sheep, Schröder et al. (2003) demonstrated long-term low dietary protein intake (10 % vs 19 % as controls) for 25 d resulted in a 25 to 30 % decrease in alanine transport, but a greater than 50 % increase in leucine transport. The alterations in amino acid transport paralleled the changes in the V_{max} without any notable change in the K_m. These results are generally consistent with those reported elsewhere (Levine, 1986; Stein et al., 1987; Bierhoff et al., 1988). They demonstrated that intestinal perfusion of free amino acid solution resulted in a variable and nonspecific effect on the *in vitro* uptake of amino acids tested, and that individual amino acid did not necessarily induce their own transport but increased the transport of unrelated amino acids. Collectively, these data suggest that the dietary regulation of amino acid transport may be not only dependent on dietary protein or amino acid levels, but also the individual amino acid characteristics, such as metabolic roles and toxicity.

Salloum et al. (1990) demonstrated that a glutamine enriched diet induced a 75 % and 250 % increase in glutamine uptake in BBMV from the rat jejunum, compared to those of a glycine or glutamate enriched diet, respectively. In a more recent study conducted with chicken intestine, it was shown that 0.3 % dietary L-lysine supplementation also resulted in upregulation of L-lysine transport (Torras-Llort et al., 1998). However, an early report indicated that dietary L-phenylalanine and L-tyrosine supplementation in rats reduced the intestinal transport of L-phenylalanine and L-tyrosine, respectively (Wapnir et al., 1972). Since L-phenylalanine and L-tyrosine show higher toxicity than L-lysine (Harper et al., 1970) and all of these three amino acids are essential amino acids, these results indicate that the toxicity of substrates may also determine the pattern of regulation. Soriano-García et al. (1999) reported results from a study, in

which they investigated the effect of 0.4 % dietary L-methionine supplementation on L-methionine and L-lysine uptake in the chicken small intestine. The L-methionine is an essential amino acid in poultry nutrition, and possesses the highest toxicity (Harper et al., 1970). The kinetic analysis of L-methionine uptake in BBMV showed that methionine supplementation overall resulted in a 30 % decrease in the V_{max} and a 10 % decrease in the K_m. Methionine supplementation also reduced the L-lysine uptake by a 15 % reduction in the V_{max} without any change in the K_m. The downregulation of amino acid uptake induced by L-methionine, L-phenylalanine, and L-tyrosine supplementation may be an adaptive response to reduce the risk of intoxication by dietary excess of these amino acids. These results support the notion that the toxicity of supplemented substrate can be an important factor in the regulation of amino acid transport. Together, these regulatory patterns can be seen as a compromise among conflicting constraints imposed by the diverse roles of proteins and by the toxicity of essential amino acids at concentrations higher than the normal requirement.

Compared with the studies of dietary regulation of peptide transport, little information is available for dietary regulation of amino acid transport at the molecular level. Erickson et al. (1995) reported that, switching from a low (4 %) to a high (50 % casein) protein diet, rat EAAC1 mRNA abundance increased 1.5- to threefold in the small middle intestine, with little change in other parts of the small intestine, whereas NBAT mRNA abundance did not change throughout the small intestine. In a different study (Ogihara et al., 1999), the intestinal NBAT mRNA abundance did not differ in mice fed a protein free or a 20 % casein diet for 4 d after consuming a protein free diet for 14 d. Compared with a 20 % casein and protein free diet, of the amino acids tested (a protein free diet plus 5 % lysine, arginine, alanine, glycine, aspartate or glutamate), only aspartate led to a 2.8-fold increase in the mRNA abundance of NBAT (subunit

of system $b^{0,+}$) in the ileum, and a significant increase in cystine uptake via system $b^{0,+}$, with an increase in the V_{max} but no change in the K_m. The rat NBAT gene promoter region contains putative binding sites for various transcription factors, such as CCAAT-enhancer-binding protein, hepatocyte nuclear factor 1-b, and hepatocyte nuclear factor 4. The induced expression of the NBAT gene by aspartate may be through the action of aspartate itself or an intermediate messenger on the binding sites within the promoter region.

From the literature cited above, it is clear that the small intestine selectively up- or downregulates specific substrate uptake ability in response to the loads of specific amino acids. However, studies are limited regarding the cellular mechanism by which amino acids in the intestinal lumen modulates specific substrate absorption. Pan et al. (2002) addressed this issue by investigating specific substrate-regulated amino acid transport activity in Caco-2 cells. The amino acid substrate used was alanine, a neutral amino acid, which is primarily transported across the intestinal brushborder membrane by a Na^+-dependent system B^0 (90 %). The functional activity of system B^0 on apical membranes is dependent on the expression product of the ATB^0 gene. They found that depleting alanine from the medium attenuated the uptake activities of system B^0 within 30 min, reaching the lowest levels within 3 h. Extracellular alanine added to depleted Caco-2 cells increased alanine transport activities within 5 min. Kinetic analysis showed that acute alanine exposure increased both the K_m and V_{max} of transport system B^0, an indicative of a *trans*stimulation effect. This induction was associated with no substantial change in ATB^0 mRNA abundance and protein synthesis. Increasing intracellular alanine levels by using the cytosolic alanine aminotransferase inhibitor, increased alanine uptake activity. Acute exposure to other substrates of system B^0 also increased the uptake of alanine, whereas nonsubstrates did not affect alanine uptake. These results suggest that increasing alanine

availability to intestinal cells, by either exogenous substrate exposure or inhibition of intracellular catabolism, acutely and reversibly increases brushborder membrane alanine transport activity via a posttranslational *trans*stimulation mechanism.

In summary, dietary proteins tend to increase the activities of intestinal brushborder membrane peptide hydrolases as well as peptide and amino acid transport activity, primarily through gene expression upregulation. The patterns of dietary regulation of amino acid transport are complex, which appears to be the compromise of the metabolic needs, dietary protein or free amino acid levels, and the toxicity of the amino acid(s) to be transported.

Food Deprivation. Food deprivation is a common malnutritional condition in patients who become acutely ill and in domestic animals that are under various stresses, such as weaning and transportation. Food deprivation causes atrophic changes in intestinal mucosal architecture by reducing cell turnover and crypt cell production rate (Ihara et al., 2000). Food deprivation also has been shown to cause decreased concentration of nutrients including amino acid and small peptides in the gut lumen, and subsequently to cause alterations in the brushborder membrane peptide hydrolases, and in the intestinal peptide and amino acid transporters.

Kim et al. (1973) demonstrated that a short period (24 h) of food deprivation of adult rats markedly decreased the specific activity of brushborder membrane peptide hydrolases, but significantly increased the cytosolic peptide hydrolase activity in the small intestine. Buts et al. (1990) reported that brushborder membrane-bound aminopeptidase specific activity was enhanced after 4 d of food deprivation in adult rats. The conflicting results regarding the effects of food deprivationof brushborder membrane peptidase activities may be attributable to differences in the substrates used for enzyme assay, or the methodological problems. However,

none of these studies investigated changes in the specific peptide hydrolase at the molecular level.

Ihara et al. (2000) reported a study in which they examined changes in the activity of several brushborder hydrolases under food deprivation conditions, and investigated the molecular background of the expression of these hydrolases. They used total parenteral nutrition (TPN) as a food deprivation model, which could distinguish the influence of whole-body malnourishment from direct effects due to the absence of luminal nutrition. In this study, rats were starved or given TPN for 5 d. Rats allowed free access to food were used as controls. Changes in the activity and expression of jejunal brushborder membrane hydrolases were compared among three groups. In the food deprived group, APN and DPP IV activities were significantly elevated by 77 % and 66 %, respectively, compared to those in control group. Upregulation of peptidase activity was not observed in the TPN group. Western and Northern blot analysis revealed that changes in APN and DPP IV activity were attributable to increases in their corresponding protein and mRNA levels. Western blot analysis for DPP IV also demonstrated that the protein molecular size was slightly smaller in the food deprived group than in the control group. This indicated that the alteration in DPP IV activity may be also due to some modification in the posttranslational processing. Taken together, the activity and expression of brushborder membrane APN and DPP IV in rat jejunum is upregulated during food deprivation, and these changes are considered to be an effect of whole-body malnourishment, rather than an absence of luminal nutrition. Although little is known about the factors regulating the activity of these peptidases, the hormones as systemic regulatory factors seem to be a potential contributor. It also can not be ruled out that local effects were associated with atrophic changes in the intestinal mucosa.

Recently, several studied have been conducted to elucidate the effect of food deprivation on intestinal peptide transport. Food deprivation for 24 h greatly increased the rate of Gly-Gln uptake by the BBMV from the rat jejunum, which is associated with a twofold increase in the V_{max} and no notable alteration in the K_m (Thamotharan et al., 1999). Ogihara et al. (1999) demonstrated that 4 d food deprivation markedly increased the amount of PepT1 protein present in rat jejunum, whereas dietary administration of free amino acids reduced the amount of PepT1 protein. A study was conducted to clarify regulatory mechanisms of PepT1 expression by starvation, in which rats were food deprived for 4 d, semi-food deprived (50 % amount of control) for 10 d, or given TPN for 10 d, and rats with free access to diets were used as control (Ihara et al., 2000). This study demonstrated that both food deprivation and TPN treatment caused a significant decrease in mucosal weight by 41 % and 50 %, respectively. The intestinal PepT1 mRNA levels increased by 79 %, 61 %, and 64 % in the food deprived, TPN, and semi-food deprived groups, respectively, when compared to those in the control group. The upregulation of PepT1 mRNA levels by TPN in the middle and distal parts of small intestine in rats was also reported by Howard et al. (2004). These results suggest that the enhanced PepT1 gene expression and transport activity under the malnourished condition is most likely induced by luminal signals such as the presence of amino acids and small peptides but not the whole-body nutrition status.

Food deprivation is also known to alter the intestinal ability to transport free amino acids. Muniz et al. (1993) demonstrated that 4 d food deprivation stimulated L-alanine transport by the Na^+-dependent system A and the Na^+-independent system L in the isolated jejunal enterocytes from guinea pigs, without any changes in either the Na^+-dependent systems ASC or passive uptake. Food deprivation resulted in a twofold increase in the V_{max} of system A and L without

any change in the K_m, which suggests that food deprivation stimulates amino acid transport across the brushborder membrane of enterocytes by inducing the number of specific transporters. This is generally consistent with the results from an earlier study, in which they demonstrated that food deprivation not only enhanced the density of valine transporting sites (as measured by autoradiography) in the villus tip, but also resulted in their appearance in the lower villus regions of rat intestine (Thompson and Debnam, 1986). However, food deprivation did not alter the rate of basolateral transport of free amino acids. This implies that certain amino acids whose brushborder but not basolateral membrane transport increases may remain within enterocytes and be metabolized (Ferraris and Carey, 2000). They also demonstrated that the protein synthesis inhibitor cycloheximide abolished food deprivation-induced increase in alanine uptake, which may be due to cycloheximide inhibitory effect on the synthesis of amino acid transporters or other proteins regulating amino acid transporter synthesis or function (Muniz et al., 1993). Thompson and Debnam (1986) demonstrated that an increase in the ratio of enterocytes to nonenterocytes may also be responsible for food deprivation induced increases in valine absorption.

Although food deprivation generally increased intestinal amino acid transport, the effects of TPN on amino acid transport are less clear. In vivo aspartic acid, valine, and lysine uptake (per milligram intestine) was much higher in the intestine from 24-h food deprived and parenterally fed rats, compared with that in enterally fed rats (Bierhoff and Levine, 1988). Given 50 % decreases in intestinal mass per centimeter, amino acid absorption per centimeter was lower in parenterally fed rats compared to those in enterally fed rats (Miura et al., 1992). Howard et al. (2004) reported a study in which they investigated changes in amino acid transporter mRNA expression in TPN treated rats. Compared to orally fed rats, 7 d of TPN administration

led to an increase in the expression of ASCT1, SAT2, and GLYT1 mRNA in the duodenum, ASCT2, EAAC1, and NBAT mRNA in the ileum, and no alteration in the abundance of CAT1, PAT1, and SN2 mRNA. This may reflect differing roles for substrates of transporters located on the brushborder and basolateral membranes, and along the proximal-distal axis of the intestine.

In summary, food deprivation generally increases the functional activity of brushborder membrane peptide hydrolases and intestinal peptide and amino acid transporters primarily through the increased gene expression of these hydrolases and transporters. These food deprivation-induced increases are most likely related to luminal signals for peptide and amino acid transport, and to systemic factors for peptide hydrolases. The protein digestion and absorption capacity of the entire small intestine depends on the magnitude of the decrease in mucosal mass and the increases in the activity per unit, with the physiological significance to maximize intestinal protein assimilation through counterbalancing the loss of mucosal mass during food deprivation.

Developmental Regulation. Protein assimilation in the gastrointestinal tract is a complex process in which most aspects have a developmental pattern (Austic, 1985). Gastric pH, and intestinal peptide and amino acid transport, and the activities of pepsinogen, trypsin, chymotrypsin, enterokinase and intestinal peptide hydrolases vary during development. This section will focus on a relatively integrated developmental view of protein assimilation in the small intestine.

It is well established that there exists a prenatal development of brushborder membrane peptide hydrolases in the small intestine of mammals. In the proximal and middle intestine of pigs, during the last 10 to 20 d of gestation, APN activity increased with fetal age, while APA and DPP IV activities were not significantly affected by fetal age (Sangild et al., 1995 and 2002).

The total intestinal hydrolytic capacity increased (at least 1.5-fold) with fetal ages for all three peptide hydrolases partly due to the increase in total intestinal weight. The prepartum rise in endogenous cortisol secretion also stimulated the prenatal expression of certain brushborder membrane hydrolases in the small intestine at this critical time (Sangild et al., 1995). The fetal fluid swallowing in late gestation seemed to be the main factor inducing intestinal growth in pig fetuses, which in turn nonspecifically affects the brushborder membrane peptide hydrolysis. Auricchio et al. (1981) demonstrated that the activities of brushborder peptidases (APN, APA, and DPP IV) were present in rat fetuses (17 to 19 d of fetal life). The activities of these peptidases increased then at a different rate, reaching their maximal values in the second and third week after birth, which then decreased to adult values during the first month of postnatal life. Only APN activity increased steadily after birth, reaching maximal activity at the end of the first month. In piglets, the intestinal brushborder membrane peptidase activity increased and then declined with age during suckling (Sangild et al., 1991; Tarvid et al., 1994), and the intestinal brushborder membrane APN and DPP IV declined during the first 3 d postweaning and then increased to d 9 postweaning (Hedemann et al., 2003). The increase in the peptidase activity during the first few days after birth may be attributable to the stimulation of gene expression and protein synthesis by suckling colostrum and milk, which are rich in nutrients and growth factors. The age-dependent decline during late suckling and postweaning may be because of a genetic inherent reduction of activity. The postweaning decline in peptidase activity is most likely connected to the villus atrophy with the loss of mature enterocytes (Hedemann et al., 2003).

In a study to determine whether there exist postnatal changes in the V_{max} and K_m of brushborder membrane APN in the jejunum of pigs, Fan et al. (2002) observed that the V_{max} of APN in the BBMV was highest at postweaning, intermediate during adulthood, and lowest

during suckling. This is consistent with previous work in rats (Reisenauer et al., 1992), in which weaning stimulated the translocation of soluble cytosolic APN onto the brushborder membrane. However, in another study, pig jejunal APN activities (expressed as per unit of total mucosal protein) were found to decrease steadily from suckling to adulthood (Tarvid et al., 1994). This discrepancy in APN activities is most likely related to the different denominators used to express enzyme activities. The K_m of APN was highest at weaning and postweaning, intermediate during adulthood, and lowest during suckling. The postnatal variation in the glycosylation of the intestinal brushborder membrane hydrolases and the changes in microvillus lipid composition and membrane fluidity might be responsible for developmental changes in the K_m values.

More than two decades ago, it was noted that there existed age-dependent changes in the absorption of dipeptides. Himukai et al. (1980) demonstrated that the uptake of Gly-Gly or Gly-Leu in the jejunum and ileum of guinea pigs was significantly greater in sucklings (3 to 4 d) than in weanlings (10 to14 d), which, in turn, were greater than in adults. Kinetically, this developmental change in dipeptide uptake was characteristic with the altered V_{max} and the constant K_m. In a different study (Guandalini and Rubino, 1982), Gly-Pro uptake in the jejunum and ileum of rabbits increased gradually from 25 d of gestation to a striking perinatal peak. After the first 6 d of postnatal life, the uptake of Gly-Pro declined continually, reaching a minimal value at adulhood. In contrast, glycine uptake did not display a well-defined developmental pattern in the jejunal and ileal tissues. Furthermore, the uptake of Gly-Gly was substantially higher than that of glycine in the jejunal and ileal tissues of rabbits from gestation through adulthood. Together, results from these two studies indicated that there existed a developmental change in the activity of intestinal dipeptide transport and a preferential uptake of small peptides over their constituent free amino acids.

After the intestinal PepT1 was cloned, many laboratories began to study the aspects of molecular expression of PepT1 during development. Miyamoto et al. (1996) first showed the level of jejunal PepT1 mRNA in rats was highest on d 4, and then decreased to reach the adult level by d 28 after birth. Shen et al. (2001) investigated intestinal PepT1 mRNA and protein levels in rats at regular intervals from 17 d of gestation to 75 d after birth. The expression of PepT1 mRNA was present as early as 20 d of fetal life. The intestinal PepT1 mRNA level increased rapidly to birth and reached the highest levels by d 3 to 5 after birth, which then declined rapidly to 11 to 13 % of the highest level by d 14, followed by an increase to 23 to 58 % of the highest levels by d 24, 3 d after weaning. A similar pattern of expression was observed for PepT1 protein, but PepT1 protein levels at d 24 and at d 75 were approximately 59 to 88 % and 70 % of maximal level, respectively. The quantifiable detection of a transient PepT1 mRNA and protein expression was also observed in the colon during the first days after birth. The ontogenetic regulation of intestinal PepT1 gene expression might be induced postpartum by suckling and later weaning, in an adaptive response to changes in the diet, from high-protein milk to an adult diet containing more carbohydrate than protein. It was reported that the serum concentration of thyroid hormone rose from d 5 to 15 (Henning, 1981) as the expression levels of PepT1 decline. Thyroid hormone was reported to downregulate the expression of PepT1 in Caco-2 cells (Ashida et al., 2002). Therefore, it seems likely that thyroid hormone regulates the expression of PepT1 during development. The trend for PepT1 mRNA and protein levels to alter with age provided the molecular basis for the age-dependent change in dipeptide transport activity previously reported in guinea pigs (Himukai et al., 1980) and rabbit (Guandalini and Rubino, 1982). Hussain et al. (2002) reported a study in which the protein expression of PepT1 was determined in the duodenum of rats by immunostaining at 18 d of gestation, birth, weaning

and adulthood. The distribution of PepT1 protein was exclusively on the brushborder membrane of enterocytes from both prenatal and mature rats. However, immediately after birth, PepT1 protein extended to the subapical cytoplasm and to the basolateral membrane of enterocytes. Rome et al. (2002) also demonstrated that PepT1 exhibited the same pattern along the small intestine in rats from postnatal d 4 to 50, and PepT1 protein was detected exclusively on the brushborder membrane of enterocytes.

Two recent studies have been conducted in our laboratory to investigate the developmental expression of PepT1 mRNA in the small intestine of domestic animals. In chickens, the abundance of PepT1 mRNA rapidly increased over 14-fold from 18 d of embryogenesis to just before hatch, and the postnatal change patterns were dependent on dietary protein levels (Chen et al. 2005). The PepT1 mRNA abundance increased with age from d 0 through 35 in chickens fed 18 % and 24 % CP (restricted food intake) diets, whereas they decreased with age in those fed a 12 % CP diet. In chickens fed a 24 % CP diet *ad libitum*, PepT1 mRNA abundance declined with age until d 14 and then increased from d 14 through 35 to an intermediate level. A similar prenatal pattern was observed in the turkey (Van et al., 2005). The PepT1 mRNA was barely detectable in turkey small intestine at d 23 of embryogenesis, but it increased 3.2-fold from embryo d 23 to just before hatch.

Although amino acids are recognized as essential substrates for intestinal anabolic and catabolic processes, particularly for early postnatal growth, little is known about ontogenetic development of free amino acid transport in the small intestine. In pigs, Buddington et al. (1996) showed that the BBMV of small intestine already possessed the active uptake ability for L-leucine in fetuses at ~ 7 wk of gestation, and the uptake rate of L-leucine remained stable through 12 wk of gestation, and then increased to birth when a distal to proximal gradient was

established. The prenatal appearance of transporters including amino acid transporters provides a mechanism for absorption of dilute nutrients present in swallowed amniotic fluid, which are critical for normal growth and maturation of intestine and fetus (Pitkin et al., 1975). Buddington et al. (2001) examined the developmental patterns of intestinal absorption of five amino acids (aspartate, lysine, leucine, methionine, and proline) from 14 to 15 wk of gestation through 42 d after birth (measured on 12 d before birth, 3 h after birth, d 1, 3, 7, 28, and 42; weaned on d 30) in pigs. The rates of absorption were highest at birth (except for proline) and declined by an average of 30 % during the first 24 h of suckling. The absorption rates increased from d 1 to 7 and then either decreased gradually for leucine, methionine, and proline or remained unchanged for aspartate and lysine. The V_{max} of saturable absorption by the mid-intestine increased during the last 2 wk of gestation, was highest at birth, and then declined. The K_m did not change between 14 to 15 wk of gestation and birth and was lower for weaned pigs compared with those for sucklings. Regional differences for the rates of absorption were not detected until after birth, and only for aspartate and proline. The increases in the rates of carrier-mediated amino acid absorption and the lack of changes in the K_m between14 to 15 wk of gestation and at birth suggest the highest V_{max} at birth are caused by increases in the densities of the same transporter(s), and not the appearance of other transporter types. The decline in the V_{max} or the rate of carrier-mediated amino acid absorption during the first 24 h of suckling is not caused by a loss of transporters but instead by the rapid increase in tissue mass that effectively dilutes the transporters. The decline in carrier-mediated amino acid transport also coincides with the postnatal replacement of fetal enterocytes, leading to a redistribution of transport functions along the crypt- villus axis. The continuing decline in rates of carrier-mediated amino acid absorption, particularly after weaning is consistent with results from other species. In adult rats, there were

modest decreases with age in rates of tyrosine, phenylalanine, tryptophan and histidine uptake per gram intestinal protein in BBMV from the jejunum (Syme and Smith, 1982; Teillet et al., 1995). In mice, Ferraris et al. (1993) found that brushborder membrane uptake of five amino acids tested decreased with age. However, unlike the other amino acids tested, the rate of carrier-mediated absorption of lysine slightly increased postweaning. The different ontogenetic patterns present suggest that there may be specific regulation of various transport systems to match changes in dietary inputs and requirements for different amino acids.

In summary, the intestinal brushborder membrane peptide hydrolases as well as peptide and amino acid transporters are regulated by developmental stages. Generally, peptide hydrolases are abundant and active during late gestation and at birth, and with a slight increase during the early suckling and a decrease during the late sucking and at weaning. Active amino acid transport is present early in middle gestation and does not exhibit a well-defined developmental pattern toward birth, at which time amino acid transport activity is high, followed by a general decline with age. Peptide transport is present in late gestation and dramatically increases to peak around birth, followed by a general decline to the end of weaning and then an increase to an intermediate level at adulthood.

Circadian Influence. In animals, most physiological, biochemical, and behavioral processes vary in a periodic manner with respect to time of day. Intestinal digestion and absorption of dietary protein is no exception. Stevenson et al. (1975) demonstrated that rats fed both *ad libitum* and restricted exhibited the highest peptide hydrolase activity (leucylnaphthylamide as substrate) around feeding time in terms of time, although restricted feeding decreased leucylnaphthylamide hydrolyzing activity. The circadian rhythm of intestinal amino acid absorption was also reported. Furuya and Yugari (1975) demonstrated that in pigs fed

once daily at 0830 h, the absorption of L-histidine in the jejunum decreased with time after feeding.

A recent study was conducted to investigate the diurnal rhythms of intestinal absorption of small peptides and its molecular mechanism (Pan et al., 2002). The rats were allowed free access to water and standard laboratory chow and were maintained in a 12-h photoperiod (0800-2000 h). Intestinal transport of Gly-Sar by in situ intestinal loops and everted intestine, intestinal PepT1 mRNA and protein levels were measured at 0400 h with a 4 h interval. The results indicated that the transport of Gly-Sar was greater in the dark than in the light phase. The PepT1 mRNA and protein levels varied markedly with time, with a maximum at 2000 h and a minimum at 0800 h. In contrast, renal PepT1 showed little diurnal rhythmicity in protein and mRNA expression. These findings indicate that intestinal PepT1 undergoes diurnal regulation in activity and expression, and this could affect the intestinal absorption of dietary proteins. In a later study, Pan et al. (2004) further examined the effects of various feeding conditions on the diurnal rhythm of intestinal PepT1. In rats deprived of feed for 2 to 4 d, PepT1 protein level did not differ between 0800 and 2000 h, whereas PepT1 mRNA level was still significantly higher at 2000 h than at 0800 h in rats deprived of feed for 4 d. Refeeding for 2 d after 4 d of feed deprivation returned the diurnal variation in PepT1 protein levels to normal. Consuming feed during the daytime (0900–1500 h) only shifted the peaks of PepT1 mRNA and protein levels from the dark phase to the light phase. These findings suggest that feed intake, rather than the light cycle, greatly affects the diurnal rhythm of PepT1 expression.

Hormonal Regulation. In recent years, a number of studies have demonstrated that intestinal protein digestion and absorption can be regulated by a variety of hormones. In this

literature review, the hormones discussed will be limited to insulin, epidermal growth factor, leptin, thyroid hormone, insulin-like growth factors, and glucagon-like peptide 2.

Although the intestinal mucosa is not a classic target for insulin, a number of studies have shown that insulin has important physiological effects on intestinal growth, cell maturation, and enzyme expression in several mammalian species (Marandi et al., 2001). Thamotharan et al. (1999) demonstrated the possible regulatory effect of insulin on PepT1 activity in Caco-2 cells. The uptake of Gly-Gln into Caco-2 cells was enhanced twofold after preincubation with 5 n*M* insulin for 60 min, which was associated with an increase in the V_{max} and no alteration in the K_m. The increased V_{max} indicated that insulin increased the number of peptide transporters in the apical membrane of Caco-2 cells. This was confirmed by Western blot analysis that apical membrane PepT1 protein level increased in insulin-treated cells. However, Northern blot analysis showed no difference in PepT1 mRNA abundance between control and insulin-treated cells. The colchicine-mediated disruption of microtubular structures, but not inhibition of protein synthesis, prevented the incorporation of PepT1 protein into the apical membrane in the presence of insulin. This indicates that insulin appears to increase the PepT1 protein density in the apical membrane by recruitment of intracellular preformed transporters. They also found that the binding of insulin to its receptor was required since a tyrosine kinase inhibitor, which alone did not affect the uptake of Gly-Gln into Caco-2 cells, completely blocked the stimulatory effect of insulin. It was later found that previous exposure of the basolateral but not apical membrane of Caco-2 cells to insulin significantly increased the uptake of Gly-Sar in a dose-dependent manner (Nielsen et al., 2003). These results collectively suggest that the acute stimulation of peptide transport into Caco-2 cells by insulin most likely results from the increased recruitment of

preformed PepT1 protein into the apical membrane triggered by the binding of insulin to its receptors on the basolateral membrane.

Epidermal growth factor (EGF) is secreted by the gastrointestinal glands into the gut lumen and has stimulatory effects on the proliferation of epidermal cells (Fisher and Lakshmanan, 1990). Nielsen et al. (2001) demonstrated that the incubation of Caco-2 cells with 5 ng/ml EGF for 5 d inhibited dipeptide uptake and the maximal inhibitory effect was achieved after 15 d of incubation. The EGF treatment caused a 50 % and 80 % decrease in transepithelial transport and apical uptake of Gly-Sar in Caco-2 cells, respectively, but no substantial reduction was observed in basolateral uptake of Gly-Sar. There was no difference in the K_m between control and EGF-treated cells. The RT-PCR analysis showed that in EGF-treated cells, the level of PepT1 mRNA was reduced by 40 % of that in control cells. Western blot analysis also showed a 35 % decrease in PepT1 protein in EGF-treated cells compared to control cells. Results from a more recent study demonstrated that cells exposed to EGF on the basolateral but not apical side exhibited significantly lower Gly-Sar uptake than in control cells (Nielsen et al., 2003). However, a short term (5 min) exposure of the basolateral membrane of Caco-2 cells to EGF induced a dose-dependent increase in the apical uptake of Gly-Sar (Nielsen et al., 2003). The kinetic analysis showed an increase in the V_{max} of PepT1-mediated transport of Gly-Sar without any change in the K_m. The PepT1 mRNA level of Caco-2 cells was not affected by the short term EGF treatment. This rapid stimulatory effect may result from the increased recruitment of PepT1 protein from an internal preformed pool into the apical membrane. Besides peptide transport, the uptake of amino acid also has been shown to be regulated by EGF (Salloum et al., 1993). In adult rats, subcutaneous administration of EGF at a dosage of 10 μg/100 g BW every 8 h for three doses resulted in a 120 % increase in the uptake of glutamine and alanine by jejunal BBMV,

which was accompanied with a 70 % increase in the V_{max} and no change in the K_m. Together, the long-term treatment of EGF inhibits PepT1 transport activity into Caco-2 cells by a reduction in PepT1 gene expression, primarily at the mRNA level, whereas the short-term treatment of EGF stimulates the uptake of dipeptide and amino acid probably by the increased recruitment of transporter protein from an internal preformed pool into the apical membrane. These EGF-induced effects are triggered by binding of EGF with its receptors on the basolateral membrane of enterocytes.

Leptin is produced by adipose cells as well as nonadipose tissues, including the stomach. Leptin originating from adipose cells is released into the circulation, and the stomach-derived leptin secreted in the gastric juice is not fully degraded by proteolysis and reaches the intestine in an active form to manipulate intestine functions (Sobhani et al., 2000). In Caco-2 cells and rat jejunal enterocytes, 2 n*M* leptin treatment markedly increased the uptake of Gly-Sar and the peptidomimetic drug cephalexin (CFX) within 30 to 60 min, with a 51 % increase in the V_{max} and no alteration in the K_m (Buyse et al., 2001). In leptin treated cells, PepT1 protein content increased by 60 % in the apical membrane and decreased by 50 % in the intracellular compartment, whereas PepT1 mRNA level did not change. The increase in the uptake of Gly-Sar was observed only when leptin was added to the apical but not basolateral side of these cells. Furthermore, leptin-induced stimulation of CFX and Gly-Sar uptake was completely suppressed by colchicines but not by brefeldin pretreatment. Thus, the short-term stimulatory effect of leptin on the dipeptide uptake into cells involves the increased recruitment of PepT1 protein from an intracellular preformed pool into the apical membrane mainly through the action of leptin on the apical sides of cells.

Thyroid hormone (T3) is secreted from the thyroid to maintain growth, development, body temperature, and energy levels, and it also has effects on gastrointestinal development, structure, and functions (Levin, 1969). Most of its effects appear to be mediated by activation of nuclear receptors to regulate the formation of mRNA and then protein synthesis (Ribeiro et al., 1995). A recent study (Ashida et al., 2002) demonstrated that pretreatment of Caco-2 cells with 100 n*M* T_3 for 4 d substantially inhibited the uptake of Gly-Sar. Kinetic analysis showed that T3 treatment resulted in a twofold decrease in the V_{max} and no change in the K_m. A 30 % and 75 % decrease in apical membrane PepT1 protein content and cellular PepT1 mRNA level was observed, respectively. The formation of ligand-bound T3 receptor complexes interacting with T3-responsive elements within the regulatory regions of target genes is presumably a first step for regulation of target genes (Ribeiro et al., 1995). Together, it is suggested that T_3-induced inhibition of dipeptide transport at least partly results from decreased transcription and/or stability of PepT1 mRNA, probably by acting on T3-reponsive elements within PepT1 gene to suppress its expression, though the precise mechanism remains to be determined.

A series of studies have been conducted to evaluate the effects of insulin-like growth factor-I (IGF-I) on intestinal digestive and absorptive function in neonatal piglets and to explore the underlying mechanisms (Alexander and Carey, 1999, 2001, 2002). Human recombinant IGF-I (3.5 mg $\cdot$ kg^{-1}BW $\cdot$ d^{-1}) or control vehicle was given orogastrically to colostrum-deprived piglets for 4 d (d 1 to 5). Carrier-mediated uptake of alanine and glutamine per milligram jejunal tissue was increased by IGF-I treatment, which was accompanied by the increased V_{max} and constant K_m. Generally, the rates of Na^+-dependent nutrient absorption were enhanced in piglets treated with oral IGF-I, and this effect was independent of changes in mucosal mass or surface area. The changes in alanine and glutamine uptake were abolished by preincubation of tissues

with a PI 3-kinase inhibitor. Thus, these results suggest that the stimulatory effect of IGF-I on jejunal alanine and glutamine transport involves the activation of PI 3-kinase.

Glucagon-like peptide 2 (GLP-2) is produced as a 33-amino acid peptide derived from post-translational processing of the proglucagon polypeptide in enteroendocrine L cells, which are located predominantly in the distal small intestine and colon (Petersen et al., 2002). The GLP-2 has been shown to affect intestinal digestive enzyme and nutrient transport activity and is a potent intestinotropic factor in neonatal and adult animals (Petersen et al., 2002). Compared with orally feeding, TPN administration to the rats for 7 d increased the expression of ASCT1, SAT2, and GLYT1 mRNA in the duodenum and ileum, and of ASCT2, EAAC1, NBAT, and PepT1 mRNA in the ileum only (Howard et al., 2004). The GLP-2 infusion in TPN-fed rats for 7 d led to a decrease in the gene expression of these specific transporters along the small intestinal length. Thus, decreased plasma GLP-2 concentration during TPN may contribute to the observed increases in the gene expression of these amino acid and peptide transporters. In a recent study, Guan et al. (2003) demonstrated that the uptake of some amino acids by the portal drained viscera was increased by 4 h GLP-2 infusion in neonatal piglets after 7 d of TPN. Preservation of cellular protein after chronic GLP-2 treatment may reduce the requirement for amino acid uptake, thus leading to the decrease in amino acid and peptide transporter mRNA expression, whereas the short-term GLP-2 infusion in TPN treated neonatal piglets may increase amino acid uptake to prevent intestinal atrophy. In one study to investigate the role of GLP-2 in regulating amino acid and peptide transport under normal conditions, the results showed that exogenous GLP-2 given to orally fed rats increased glycine uptake in the jejunum (Guan et al., 2003). However, more studies are needed to elucidate the actual roles of GLP-2 in intestinal protein assimilation and the potential mechanism(s).

In summary, intestinal protein digestion and absorption as well as related brushborder peptide hydrolase and transporters can be regulated by a wide range of hormones, and the majority of studies have been concentrated on PepT1 expression and transport activity in epithelial cells. The acute regulation of PepT1 transport activity by insulin, EGF and leptin results from the alteration in the recruitment of PepT1 protein from an internal preformed pool into the apical membrane, and the chronic regulation of PepT1 transport activity by EGF, T3, IGF-1 and GLP-2 is primarily due to the alteration in PepT1 gene expression. Both acute and chronic regulations of PepT1 transport activity by hormones are triggered by the binding of the hormone with its receptor located on either the apical or basolateral membrane.

Effect of PepT1-Mediated Uptake on Amino Acid Transport. It is well established that amino acid transport across the brushborder membrane of enterocytes is mediated via multiple distinct amino acid transport systems (Malandro and Kilberg, 1996; Palacin et al., 1998) and a peptide transport system, PepT1 (Daniel, 1996; Adibi, 1997). Thus, it is important to know whether there exists the interaction between the uptake of amino acids and small peptides.

It has been shown that free amino acids are not substrates for PepT1 (Brandsch et al., 1994; Liang et al., 1995). Previous studies have demonstrated that free amino acids induced not only the activity and gene expression of amino acid transporters but also PepT1 in the small intestine (Shiraga et al., 1999). However, little is known about the effects of peptide uptake on amino acid transport activities of epithelial cells within the small intestine. The only such study was reported by Wenzel et al. (2001) by using Caco-2 cells. System $b^{0,+}$ mediated 85 % of L-arginine uptake, and transport of L-alanine into Caco-2 cells was mediated mainly by systems other than systems $b^{0,+}$. They further demonstrated that, except for glycine in free or dipeptide form, preincubation of Caco-2 cells with either neutral, mono- or dibasic dipeptides or the

constituent amino acids tested all led to an increase in the rate of L-arginine uptake compared to the cells preincubated with buffer only. The stimulation was higher when preincubations were done with dipeptides than with free amino acid, and was highest (4.6-fold) when a combination of dipeptides and free amino acids was used. In contrast, L-alanine uptake was not significantly induced by any preincubation treatment. Therefore, the stimulation from the preincubation appears to be specific to system $b^{0,+}$. However, preincubation with hydrolysis-resistant dipeptide D-Phe-L-Ala or with intracellular aminopeptidase inhibitor amastatin eliminated the stimulatory effect of dipeptides on L-arginine uptake, whereas amastatin had an effect on the stimulation caused by free amino acid. These results indicated that intracellular hydrolysis of dipeptides was a prerequisite for the trans-stimulation of L-arginine uptake. The concentrations of Gly-Arg and Lys-Lys needed to achieve the half maximal stimulation of L-Arginine uptake are very similar to their corresponding affinities of these peptides for transport by PepT1. Therefore, it appears that the uptake of dipeptides into the cells causes the stimulation of amino acid uptake via system $b^{0,+}$ and is the rate-limiting step for this stimulation.

This study demonstrated that a functional interaction exists between uptake of free amino acids and peptides at the cellular level, and provided evidence for PepT1 as a modifier of amino acid transporter. Given its high capacity, PepT1 can supply intestinal enterocytes with a large amount of peptides that are then hydrolyzed to release free amino acids within the cells. These amino acids in turn are able to transstimulate the uptake of rare or essential amino acids through the induced amino acid transporter. Therefore, PepT1 could maximize the absorption of limiting or essential amino acids by transporting peptides containing these amino acids and also by transstimulating the uptake of these amino acids through certain amino acid transporters. Since most amino acid transporters cloned thus far operate as an exchanger model, loading of cells

with amino acids via PepT1 might be especially relevant with regard to the net movement of amino acids across the epithelium (Chen, 2001).

Distribution, Physiological Functions, and Regulation of Expression and Activity of Carbohydrate Digestion and Absorption Related Genes

Dietary carbohydrate is an important dietary energy source for animals and humans. In weaned pigs, plant starches provide the largest percentage of energy in the diet. Starches are a mixture of two structurally different polysaccharides, amylose, a linear (4-O-α-D-glucopyranosyl-D-glucose)$_n$ polymer, and amylopectin, with additional 6-O- D-glucopyranosyl-D-glucose links (about 4 % of total), which results in a branched configuration (Semenza et al., 2001). Dietary starches are a mixture of approximately 25 % amylose in amylopectin (Semenza et al., 2001). The α-amylase derived from the salivary and pancreatic secretion hydrolyzes starches into linear maltose oligosaccharides by attacking the α-1-4 linkage, and branched isomaltose oligosaccharides by bypassing the α-1-6 linkage of amylopectin (Nichols et al., 2003). The starch-derived oligosaccharides are hydrolyzed to the final monosaccharide glucose by mucosal brushborder membrane disaccharidases including but not limited to sucrase-isomaltase (SI) and maltase-glucoamylase (MGA) in the small intestine (Semenza et al., 2001). Lactose and sucrose are another two common carbohydrate sources in the milk and/or diets. Lactase phlorhizin hydrolase (LPH) and SI are responsible for the hydrolysis of lactose to glucose and galactose and of sucrose to fructose and glucose, respectively. The glucose and galactose are primarily transported across the brushborder membrane into intestinal enterocytes via a Na^+-dependent glucose transporter 1 (SGLT1; Ferraris, 2001). Although new evidence suggests that some other glucose transport systems including SGLT2 and SGLT3 may also play a role in intestinal glucose and galactose uptake, their function needs to be elucidated. A

facilitated glucose transporter 2 (GLUT2) has been implicated in the conditional uptake of glucose (Wright et al., 2004). Fructose is transported across the brushborder membrane via a facilitated glucose transporter 5 (GLUT5; Ferraris, 2001). The intracellular monosaccharides are then mainly translocated across the basolateral membrane to the bloodstream through GLUT2 (Bird et al., 1996). For the purpose of this dissertation, this review section will only focus on three disaccharidases, LPH, SI, and MGA, and two monosaccharide transporters, SGLT1 and GLUT5.

Lactase-Phlorizin Hydrolase. Lactase-phlorizin hydrolase (LPH, EC 3.2.1.23, 3.2.1.62) is present in the small intestine of most mammals and is responsible for the hydrolysis of lactose into glucose and galactose (Hollox et al., 2001). The LPH is synthesized only in the villus enterocytes. Enzyme synthesis is a complex process controlled by a series of transcriptional and post-transcriptional events that culminate in insertion of the mature protein into the brushborder membrane. The transcription of the LPH gene starts in the cells at the base of the villi (Dudley et al. 1992). The LPH protein is synthesized as a glycosylated polypeptide with a molecular weight of approximately 245 kDa. During translocation or immediately following insertion of the enzyme into the brushborder membrane, precursor LPH is proteolytically cleaved to form the mature brushborder protein with an apparent molecular weight of 150 kDa (Dudley et al. 1996). Mature LPH dimerizes on the brushborder membrane of intestinal enterocytes to form the active enzyme, with a C-terminal membrane-spanning domain.

The LPH mRNA and lactase activities are already present during the fetal period and increase markedly during final fetal stages. Lactase activity is highest at birth and then declines during suckling and especially after weaning. The LPH mRNA and lactase activities are higher in the proximal part than the distal part of the small intestine (Goda et al., 1999). The lactase

activities were very low in the crypts, and they were gradually elevated from the base of the villi to the mid-villus region, with maximal activity at 75 % height of the villus-crypt axis, followed by a decrease toward the top of the villi. The distribution of LPH protein and mRNA abundance along the villus-crypt axis paralleled that of lactase activity (Goda et al., 1999).

Sucrase-Isomaltase. In vivo, sucrase-isomaltase (SI, EC 3.2.148 and 3.2.1.10) accounts for 80 % of maltase (1, 4-O-α-D-glucanohydrolase) activity, all sucrase activity and almost all isomaltase (1,6-O-α-D-glucanohydrolase) activity. It is synthesized as a single polypeptide chain, which is cleaved by protease to form the two catalytic subunits with different substrate specificities during the translocation and insertion into the brushborder membrane (Semenza et al., 2000). The polypeptide is attached to the brushborder membrane via its N-terminal end located on one subunit. A SI transcript of approximately 6 kb was primarily detected in the small intestine of adult rats, and SI mRNA abundance and sucrase activity was greatest in the jejunum (Leeper and Henning, 1990). In adult rats, SI activities were very low in the crypt, and they were gradually elevated from the base of the villi to the mid-villus region, with maximal activity at around 55 to 65 % of the heights of the villus-crypt axis, and then decreased toward the top of the villus (Goda et al., 1999). The distribution of SI protein and mRNA abundance along the villus-crypt axis paralleled that of sucrase activity.

Maltase-Glucoamylase. Maltase-Glucoamylase (MGA, EC 3.2.1.20 and 3.2.1.3) is responsible for the final digestion of linear starch to glucose in the small intestine. In vivo, MGA accounts for all glucoamylase (1, 4-O-α-D-glucanohydrolase) activity, 20 % of maltase activity, and 1 % of isomaltase activity. Thus, MGA and SI complement one another in the digestion of starch. The MGA has two catalytic sites, which are identical to those of SI. The MGA and SI are members of the glycosyl hydrolase family 31, but the proteins show only 59 % amino acid

sequence identity. Like SI, MGA is synthesized as a single glycosylated polypeptide chain, which is cleaved by protease to form the two subunits with different substrate specificities during the translocation and insertion into the brushborder membrane (Semenza et al., 2000). Results from studies of MGA in humans, pigs, and chickens have shown that the enzyme consists of a single polypeptide chain with a molecular weight of 335, 240, and 240 kDa, respectively (Noren et al., 1986; Hu et al., 1987; Nichols et al., 1998). This protein probably forms homodimers, and the complex is anchored to the brushborder membrane by the N terminus of the polypeptides.

SGLT1. The SGLT1 is a high affinity/low capacity Na^+/glucose cotransporter primarily expressed on the brushborder membrane of epithelial cells in the small intestine and kidney where it is responsible for the absorption of glucose and galactose (Ferraris, 2001). The uphill transport of sugar is coupled to Na^+ transport down its electrochemical potential gradient across the membrane (Wright et al., 1994). The SGLT1 cDNA have been cloned and functionally characterized from rabbit, mouse, human, rat, dairy cow, and pig (Wright and Turk et al., 2004). The rabbit SGLT1 protein consists of 662 amino acids, with a molecular weight of approximately 73 kDa. More recent analysis by freeze-fracture electron microscopy suggests that the SGLT1 putative protein structure contains 14 transmembrane domains, with the amino and carboxyl termini facing extracellularly, one N-linked glycosylation site, and a number of potential protein kinase A (PKA) and protein kinase C (PKC) phosphorylation sites (Wright et al., 2003; Wright and Turk, 2004).

In situ hybridization analysis indicated that SGLT1 mRNA expression was limited to the villi, with undetectable expression in the crypts in the jejunum of rats (Ramsanahie et al., 2004). In the villi, SGLT1 mRNA is mainly distributed in the mid and upper thirds of the villi, with very little expression in the tips (Ramsanahie et al., 2004). Immunofluorescence staining further

revealed the expression of SGLT1 protein is largely restricted to the brushborder membrane of enterocytes in jejunum and to a lesser extent in the cytoplasm of villus enterocytes (Ramsanahie et al., 2004). Shirazi-Beechey et al. (1994) suggested the presence of a 58 kDa non-glycosylated SGLT1 precursor protein in enterocytes at the crypt region. As enterocytes migrate out of the crypt, the expression of this protein declines, while the expression of the 75 kDa mature glycosylated SGLT1 appears and begins to increase. The authors suggest the presence of sugar sensor(s) in the crypt cells responsible for stimulating the synthesis of the precursor protein in response to the presence of luminal sugars.

GLUT5. The GLUT5 is a high affinity fructose facilitative transporter, and its cDNA clone has been isolated from human (Kayano et al., 1990), rat (Inukai et al., 1993), rabbit (Miyamoto et al., 1994) and mouse (Corpe et al., 2002) cDNA libraries. The composite sequence of the clones obtained from the mouse kidney cDNA libraries was 2,069 bp in length, and its encoded protein consisted of 501 amino acids, with a molecular weight of approximately 55 kDa. The predicted protein structure contains 12 putative transmembrane domains, with the amino and carboxyl terminal coding sequence the most divergent regions among the species. The GLUT5 possessed potential asparagine-glycosylation sites, protein kinase, casein kinase II, and tyrosine kinase phosphorylation sites. Northern blot analysis revealed mouse GLUT5 mRNA expression to be confined to the small intestine, kidney, and testis with transcript sizes of approximately 2.1, 2.4, and 2.8 kb, respectively. In situ hybridization studies demonstrated that mouse GLUT5 expression is confined to the mature villus of the small intestine (Shu et al., 1997).

Regulation of Intestinal Carbohydrate Digestion and Absorption

Like intestinal protein digestion and absorption, the ability of the small intestine to efficiently assimilate luminal carbohydrates is regulated by a variety of factors, including dietary

carbohydrates, food deprivationor feed restriction, developmental stage, disease, diurnal cycle, and local or systemic hormone levels.

Dietary Substrate Regulation. It has been known for many years that the activities of disaccharidase and monosaccharide transport in the small intestine vary in response to dietary carbohydrates, though the molecular mechanism is still not completely clear. Feeding a high starch diet to rats caused a parallel increase in sucrase and lactase activities (Yamada et al., 1981), and a low starch diet resulted in a decrease in both sucrase and lactase activities (Goda et al., 1983). Furthermore, sucrose was able to induce not only sucrase activity but also maltase and lactase activities (Samulitis-dos et al., 1992), and inversely, lactose (a substrate for lactase) was able to induce sucrase activity as well (Goda et al., 1985). It appeared that this diet-induced change in the activities of these disaccharidases was not evoked by the substrate itself. Therefore, there may be a common mechanism for the regulation of these disaccharidases by dietary carbohydrates.

Kishi et al. (1999) demonstrated that force-feeding a fructose and sucrose diet (40 % of energy as fructose or sucrose) for 6 or 12 h led to parallel increases in the mRNA levels of SI and SGLT1, GLUT5, and GLUT2 in the jejunum of adult rats within 12 h. However, feeding a diet containing glucose or α-methylglucoside (a transportable but non-metabolizable sugar) generally did not increase the mRNA levels of SI or the monosaccharide transporters. The specific lactase activities and LPH mRNA levels in the jejunum of adult rats were coordinately increased by consumption of various carbohydrates, including sucrose, fructose, galactose, and glycerol, whereas feeding glucose and α-methyglucoside caused a slight or no increase in lactase activities and LPH mRNA levels, respectively (Tanaka et al., 1998). Among the monosaccharides examined, fructose induced the most prominent increases in mRNA levels of SI and LPH, which

were accompanied by a coordinate rise in mRNA levels of SGLT1 (Kishi et al., 1999) or GLUT5 (Tanaka et al., 1998). Nuclear run-on assays revealed that fructose as well as sucrose was able to elicit an increase in transcription of LPH and SI genes, and that their transcription was affected only slightly, if at all, by glucose intake. Therefore, the sucrose induced increases in the transcription of LPH and SI genes are most likely attributable to fructose produced by the hydrolysis of sucrose. These results suggest that fructose is capable of increasing the mRNA levels of LPH, SI and monosaccharide transporters in the small intestine, and that the transcriptional regulation might play a pivotal role in the carbohydrate-induced coordinate enhancement of the expression of these genes. The direct signal for this induction appeared to be luminal fructose rather than some systemic factors secondary to luminal fructose (Kishi et al., 1999b). In this study, a solution containing either fructose or glucose was simultaneously perfused into two consecutively cannulated and irrigated loops of the rat jejunum sharing the blood circulation system, which ensured these two loops were subjected to the same systemic environment. The loops with fructose perfusion exhibited significantly higher levels of sucrase activity as well as SI and GLUT5 mRNA than those in the loops perfused with glucose.

To explore the underlying molecular mechanism whereby dietary carbohydrates modulate the expression of intestinal digestion and absorption related genes, 5' flanking regions of SI and LPH have been cloned and analyzed (Goda, 2000). The DNase I footprinting analysis of the rat LPH gene showed that the protected region conserved the same sequence as the *cis*-element (CE-LPH1) reported in the pig LPH gene and the *cis*-regulatory element (SIF1) in the mouse SI gene (Traber et al., 1992). Electrophoretic mobility shift assays using CE-LPH1 and SIF1 revealed that nuclear extracts from the jejunum of rats fed a high-starch or high–fructose diet displayed greater density of retarded bands than those from rats fed a low-starch diet. Force-

feeding a fructose diet led to greater binding of the dimeric nuclear protein (Cdx-2) to both CE-LPH1 and SIF1 than feeding a low-starch diet. These results suggest that the *cis*-elements CE-LPH1 and SIF1 may be involved in the carbohydrate-induced increases of the transcription of LPH and SI, presumably through changes in the expression and/or binding activity of Cdx-2.

It is well known that dietary carbohydrate regulates intestinal sugar transporter activity. Early studies have demonstrated that feeding a high-carbohydrate diet for 1 to 3 d increased intestinal glucose transport (in rats, Diamond et al., 1984; in mice, Ferraris and Diamond, 1992; in sheep, Dyer et al., 1994). The increase in intestinal glucose transport was accompanied by increases in glucose transporter density (Ferraris and Diamond, 1992 and 1993) and in the levels of SGLT1 mRNA (Miyamoto et al., 1993). Although dietary starch or glucose did not induce the activities and mRNA levels of intestinal disaccharidases and monosaccharide transporters within 6 to 12 h (Tanaka et al., 1998; Kishi et al., 1999), the adult rats fed a high-glucose or high-starch diet for 5 to 7 d exhibited elevated mRNA levels of SGLT1, GLUT2, and SI in the jejunum when compared to those fed a low-carbohydrate diet (Miyamoto et al., 1993; Yasutake et al., 1995). Thus, there may exist at least two different mechanisms for the short- or long-term carbohydrate-induced gene expression of SI and intestinal monosaccharide transporters.

The mechanisms of dietary carbohydrate regulation of SGLT1 were elucidated in adult sheep (Lescale-Matys et al., 1993). The small intestine of adult sheep normally does not express SGLT1 protein or mRNA due to the absence of glucose, which is degraded by microorganism fermentation in the rumen. Small intestinal infusion of D-glucose resulted in a 60 to 90-fold increase in the activity of glucose uptake and in the number of glucose transporters, but only a twofold increase in SGLT1 mRNA level. Dyer et al. (1997) demonstrated that the amount of SGLT1 protein but not the SGLT1 mRNA abundance correlated well with the measured glucose

transport activity, which indicates that the modulation of glucose transport activity by luminal glucose is primarily due to changes in SGLT1 protein levels. This is consistent with the results reported in adult mice (Ferraris et al., 1993). The intestinal glucose uptake was enhanced by twofold in mice fed a high-carbohydrate diet for 7 d compared to those fed a low-carbohydrate diet, and this induction in glucose uptake was tightly correlated with an increase in the number of intestinal SGLT1 molecules. Together, these results suggest that the principal level of SGLT1 regulation by luminal glucose is translational or posttranslational. Regulation of glucose transport by dietary carbohydrates may involve increased transcription of SGLT1 in crypt cells. As cells migrate to the villi, SGLT1 mRNA is degraded, and transporter proteins are then inserted into the membrane, leading to increases in glucose transport within 1 to 3 d (Ferraris, 2001).

Unlike SGLT1 regulation, the regulation of GLUT5 expression in the small intestine of mammals is rapid, and changes in fructose transport are typically paralleled by similar changes in GLUT5 mRNA and protein abundance. In adult rats, GLUT5 mRNA abundance doubled within 3 h after intestinal perfusion with fructose solutions (Kishi et al., 1999). Consuming a high-fructose diet for 3 d gave rise to an increase in the V_{max} of intestinal fructose transport in rats, and a parallel increase in GLUT5 mRNA and protein abundance (Crouzoulon and Korieh, 1991). A more recent study demonstrated that intestinal GLUT5 mRNA abundance and fructose uptake rate in adult rats appeared to increase with the levels of dietary fructose, although a content of 30 % dietary fructose was required for significant increases in the expression of GLUT5 mRNA (Shu et al., 1997). This fructose-induced increase in GLUT5 mRNA abundance is most likely through enhancing transcriptional rate (Kishi et al., 1999). Unlike SGLT1, GLUT5 regulation by dietary fructose seems to involve *de novo* synthesis of GLUT5 mRNA and protein

in cells lining the villi, leading to increases in fructose transport a few hours after consumption of diets containing fructose (Ferraris, 2000). Thus, fructose transport activity via GLUT5 can be reprogrammed in mature enterocytes lining the villi.

Results from a number of studies indicate that glucose transport activity and SGLT1 gene expression can be induced by a wide range of substrates, including glucose, galactose, fructose, xylose, mannose or sucrose, glycerol, and transportable, but non-metabolizable substrates of SGLT1, such as 3-*O*-methyl glucose, and a non-transportable analogue, 2-deoxy-D-glucose. The possible explanation for this phenomenon is that there are a variety of receptors for these signals inducing SGLT1, or there is a single receptor with a wide range of specificity for a variety of signals (Ferraris, 2001). In contrast, fructose uptake activity and GLUT5 expression is regulated only by diets containing its substrate, fructose (Burant and Saxena, 1994; Shu et al., 1997). Dietary regulation of SGLT1 and GLUT5 may require the interaction between dietary signals and the small intestinal mucosa. Lescale-Matys et al. (1993) demonstrated that luminal but not intravenous infusion of glucose induced a marked increase in glucose uptake activity and SGLT1 protein abundance. The fructose-enriched diet did not increase GLUT5 mRNA or protein level in a segment of small intestine that was isolated from the rest of the small intestine, but continued to have mesenteric blood supply (Burant and Saxena, 1994; Kishi et al., 1999b).

In summary, dietary carbohydrates generally upregulate intestinal carbohydrate digestion and absorption by increasing the gene expression of intestinal disaccharidases and monosaccharide transporters. The dietary regulation of the gene expression of these intestinal disaccharidases and monosaccharide transporters primarily occurs at the transcriptional level, except for SGLT1, which is regulated largely at the translational or posttranslational levels. The gene expression of intestinal disaccharidase and monosacharide transporter can be regulated by a

wide range of carbohydrates, except for GLUT5, which is only specifically regulated by its substrate, fructose.

Food Deprivation. Since the luminal carbohydrate concentrations change during food deprivation, it is reasonable to speculate that intestinal brushborder membrane disaccharidase and monosaccharide transport is downregulated under this condition. However, a number of studies reported increases in brushborder membrane disaccharidase and glucose transport (expressed as per milligram of intestine or per milligram of protein), independent of decreases in mucosal mass, during food deprivation (Ferraris and Carey, 2000).

Sucrase activities (expressed per tissue protein or DNA as well as per intestinal segment) showed a progressive decrease during food deprivation in the proximal and middle segments but not the distal segment of the small intestine in rats food deprived for 1 to 3 d, whereas lactase activities in all segments increased significantly (Yamada et al., 1983). The changes in enzyme activities during food deprivation occurred in the upper and middle villus for lactase and in the lower and middle villus for sucrase. To understand the mechanism by which food deprivation influences the activities of sucrase and lactase, SI and LPH protein expression in the jejunum of rats was measured along with enzyme activities (Holt and Yeh, 1992). Sucrase activity and SI protein amount declined after 2 to 3 d of food deprivation. In contrast, lactase activity and LPH protein content increased after food deprivation. In vivo incorporation analysis demonstrated that protein synthesis increased for LPH and decreased for SI during food deprivation. Therefore, the regulation of jejunal lactase and sucrase activities by food deprivation may be through modulating LPH and SI protein biosysthesis. Ihara et al. (2000) demonstrated that, compared with those fed a normal diet, food deprivation or TPN for 5 d in adult rats led to a 40 % and 20 % decrease in jejunal sucrase and maltase activities, respectively, and a 10 % decrease in SI and

MGA mRNA abundance. However, the ileal and jejunal segments appeared to respond differently to food deprivation. In the ileal segment, sucrase and maltase activities increased during food deprivation by 60 % and 40 %, whereas no effect was observed in the TPN group. The regional response of SI activities to food deprivationwas generally in agreement with results reported elsewhere (Yamada et al., 1983). Miura et al. (1992) also demonstrated that a substantial decrease in the activities of brushborder membrane sucrase and maltase was observed in the proximal and middle segments of the intestine of TPN rats. This complements previous studies demonstrating that the regulation of sucrase or maltase activity might depend on luminal nutrition, rather than on the whole-body nutritional state.

The effect of food deprivation on intestinal monosaccharide uptake has been well reviewed (Ferraris and Carey, 2000). In general, the intestinal glucose uptake (per milligram tissue, per milligram intestinal or brushborder membrane protein) was increased during food deprivation compared with that of well-fed animals. The 2 to 3 d food deprivation of the mice previously fed a carbohydrate-free diet still led to a 50 to 100 % increase in glucose uptake per milligram intestine (Diamond et al., 1984). This indicates that food deprivation-induced increase in intestinal glucose uptake is not likely related with changes in luminal carbohydrate concentration. In mildly malnourished rats that had no substantial change in intestinal mass, glucose uptake per milligram intestine also increased (Chadha et al., 1992). Therefore, these results indicated that some systemic or local factor rather than changes of luminal carbohydrate substrate concentration were responsible for food deprivation-induced increases in glucose uptake. Potential candidates are increases in the Na^+ electrochemical gradient, increases in microvillus surface area and membrane fluidity, increase in the ratio of absorptive to nonabsorptive cells, or changes of some systemic hormonal factors. Little is known about the

effect of food deprivation on basolateral monosaccharide transport mediated by GLUT2. In rats whose brushborder uptake and transepithelial transport of glucose were enhanced with semi-food deprivation, basolateral transport was not affected (Ferraris and Carey, 2000).

In summary, food deprivation generally decreases SI and sucrase activity and increases LPH and lactase activity in the proximal and middle part of the small intestine by changes in their protein biosynthesis, whereas the effect may differ in the distal part of the small intestine. This effect appears to be related to the changes in luminal factors rather than systemic factors. Glucose uptake in intestine is generally upregulated during food deprivation, which is most likely due to changes in systemic factors, while the basolateral glucose transport is less affected during food deprivation.

Developmental Regulation. In mammals, intestinal lactase specific activity was first observed during early gestation and dramatically increased during the last 10 to 20 % of gestation (Sangild et al., 1995). Lactase activity is high during the first few days after birth and slightly declines during late suckling, followed by a substantial decrease during weaning, and then subsequently maintains a relatively low level throughout adulthood (Krasinski et al., 1994). In contrast, intestinal specific sucrase and maltase activities are undetectable or very low during gestation and at birth (Sangild et al., 1995), and increase slightly during suckling, followed by a rapid increase after weaning (Krasinski et al., 1994). Krasinski et al. (1994) demonstrated that lactase and sucrase specific activities correlated well with their respective protein and mRNA levels in the rat small intestine. The mRNA abundance of LPH was abundant from birth through suckling, decreased two- to fourfold during weaning, whereas SI mRNA was first detected 14 d after birth and increased rapidly to abundant levels by d 28, 7 d after weaning. The levels of LPH and SI premature mRNA paralleled those of their respective mRNAs. Transcriptional rate,

determined by RNase protection assay and nuclear run-on assays, declined for LPH and increased for SI during weaning. In adult rats, LPH mRNA was restricted to the jejunum and proximal ileum, whereas SI mRNA was detected throughout the small intestine, a pattern regulated by the transcriptional rate. These results suggest that gene transcription plays a major role in the developmental and horizontal regulation of LPH and SI biosynthesis, and that these two genes are regulated differently in the rat small intestine.

During ontogenetic development, specific changes in intestinal monosaccharide transport occur in parallel to the nutrient requirements. The fetal small intestine of many mammals is known to actively transport glucose (Buddington and Diamond, 1989; Ferraris and Diamond, 1997) or to express SGLT1 mRNA at substantial levels (in rats; Matsumoto et al., 1993; in human, Wang et al., 1994). In rabbits, active uptake of glucose, fructose, and galactose (per milligram intestine) increased by 3.3-, 6.2-, and 2.1-fold, respectively, during the final 7 d of gestation (Phillip et al., 1990). Transport rate of glucose and galactose is typically highest directly after birth, but then decreases gradually afterward (in chicken, Moreno et al., 1996; in mink, Buddington et al., 2000; in rat, Khan et al., 2000). Transport rate of fructose is generally modest right after birth, and then decreases gradually in rats and rabbits (Buddington et al., 1990; Toloza and Diamond, 1992). Later in development, after the completion of weaning, fructose transport in the small intestine of rats increases dramatically by three- to sixfold (Castello et al., 1995; Shu et al., 1997). Thus, during rat development, the expression of GLUT5 is considerably delayed compared to that of SGLT1 (Rand et al., 1993). In pigs, intestinal transport rates of glucose, galactose, and fructose (per milligram intestine) were highest at birth, with a steep decline after the onset of suckling (Puchal and Buddington, 1992). This transient decline is most probably connected to the dilution of mature enterocytes in the small intestine due to the rapid

cell proliferation in the crypt triggered by suckling colostrum or milk. It was also observed that the highest rates of glucose and fructose transport shifted from the proximal to middle part of small intestine after weaning, whereas galactose transport remained highest in the proximal intestine. This phenomenon may be related to the changes in the velocity and extent of monosaccharides released from carbohydrate digestion during suckling and after weaning. Changes in fructose to glucose and galactose to glucose transport ratios before weaning suggest transporter development is partly genetically hard-wired, apparently to prepare pigs for weaning. Furthermore, the ratios of fructose to glucose transport in pigs were lower than those seen in rats and rabbits (Puchal and Buddington, 1992).

Although glucose and fructose transport in adult rats can be regulated by diets, intestinal fructose and glucose transport did not change in neonatal (particularly early suckling) rats fed high-carbohydrate pellets or intestinally perfused with high-glucose or fructose solutions (David et al., 1995; Shu et al., 1997; Jiang and Ferraris, 2001). This unresponsiveness may be due to slow intestinal cell turnover rate in suckling rats (> 7 d transit time from crypt to villus) (Yeh, 1977). Also, it is possible that monosaccharide transporters in cells present in the small intestine at birth cannot be regulated, and dietary regulation begins only when these cells are eventually replaced.

To analyze whether the developmental regulation of SGLT1 was due to the changes in diet compositions or caused by other factors, Barfull et al. (2003) evaluated the ontogenesis of intestinal glucose transport by feeding chickens a standard diet (37.5 % carbohydrate). The results showed that jejunal glucose transport (per milligram brushborder membrane protein) was 40 % lower in adults (5 wk old) than in newly hatched chickens (2 d old). This was matched by a parallel decline in SGLT1 protein abundance. However, SGLT1 mRNA abundance did not differ

between age groups. The immunohistochemical staining showed that SGLT1 protein was exclusively located on the brushborder membrane of enterocytes along the entire villus and was absent in the crypts in both age groups. These results indicated that the age-related decline in jejunal glucose transport was due to a reduction in SGLT1 protein expression and was regulated at the posttranscriptional level.

In summary, the developmental regulation of intestinal carbohydrate digestion and absorption is highly specific for each disaccharidase or monosaccharide transporter. Lactase activity is high at birth and then declines during late suckling and weaning, whereas the activity of sucrase or maltase is low at birth and increases during the middle and late suckling and is then dramatically upregulated after weaning. The developmental regulation of disaccharidases is accompanied by a similar pattern of gene expression, which is likely regulated by the developmental change in the transcription rate. The SGLT1 protein and transport activity is high at birth and declines with age later on, whereas GLUT5 and fructose transport activity is not abundant until late suckling and is rapidly upregulated after weaning.

Circadian Influence. Samulitis-dos et al. (1992) demonstrated that activities of disaccharidases (sucrase, lactase, and maltase) in 7-wk old rats exhibited typical diurnal variation by displaying a peak around 2200 h and a lowest point at approximately 1000 h, regardless of diet type (low or high starch diets). These results are consistent with those from an early study (Stevenson et al., 1975), in which they also demonstrated that the change of feeding regime from *ad libitum* to restricted feeding (food available between 1400 and 1800 h) shifted the diurnal rhythm with the altered daily levels of enzyme activities.

It has been well recognized for many years that intestinal sugar transport is subject to a strong diurnal influence. In adult rats, intestinal glucose and fructose absorption increased by two

to threefold from 0900 to 2000 h (no feed consumed during this period), and then gradually decreased at night when food consumption began, followed by an increase to early morning (Ferraris et al., 1990; Castello et al., 1995; Corpe et al., 1996; Tavakkolizadeh et al., 2001). The diurnal alteration in intestinal glucose and fructose transport was accompanied by the changes in mRNA and protein abundance of SGLT1 and GLUT5, respectively (Castello et al., 1995; Corpe et al., 1996; Rhoads et al., 1998; Pan et al., 2002). In a recent study, Pan et al. (2004) examined the effects of various feeding regimes on diurnal rhythm of intestinal SGLT1 in adult rats. It was shown that food deprivation for 2 to 4 d eliminated the diurnal alteration in SGLT1 protein levels but not mRNA levels, and refeeding for 2 d after 4 d of feed deprivation returned the diurnal variation in SGLT1 protein levels to normal. Consuming feed during the daytime (0900 to 1500 h) only shifted the peaks of SGLT1 mRNA and protein levels from the dark phase to the light phase. These findings suggest that feed intake, rather than the light cycle or an inherent diurnal signal, greatly affects the diurnal rhythm of SGLT1 expression. However, the specific mechanisms linking feed intake to gene expression remain obscure.

To delineate the mechanisms diurnally regulating SGLT1, SGLT1 expression was examined in rats maintained in a 12-h photoperiod with free access to chow (Rhoads et al., 1998). The SGLT1 mRNA levels varied markedly, with a maximal level occurring near the onset of dark and a minimal level near the onset of light. They further demonstrated that the transcription rate of SGLT1 was sevenfold higher in the morning (1000-1100 h) than in the afternoon (1600-1700 h). The change in SGLT1 transcription rate was accompanied by the change in hepatocyte nuclear factor 1 (HNF-1) isoform complement at the HNF-1 site within the SGLT1 promoter. Serological tests indicated that HNF-1α was present in complexes throughout the day, while HNF-1β binding exhibited diurnal periodicity. Thus, it is possible that SGLT1

transcription is in part regulated by the exchange of HNF-1 dimerization partners during transcriptional initiation. These data suggest that diurnal regulation of SGLT1 gene expression may be due to change in transcription rate, although the contribution of mRNA stability can not be ruled out. The regulation of circadian rhythm of glucose transport seems to occur independent of dietary regulation, hence the effects of diet and circadian rhythm may be additive. There are two distinct mechanisms proposed to be responsible for regulating monosaccharide transporter gene expression and function in intestinal epithelial cells. One pathway involves induction of long-term changes in transport rates by intestinal luminal signals such as those arising from the change in dietary carbohydrate levels (Ferraris, 2001). The other pathway involves a daily anticipatory mechanism preparing the intestine for an expected alteration in luminal contents (Tavakkolizadeh et al., 2001).

In summary, the diurnal rhythm of intestinal carbohydrate digestion and absorption activity to prepare for the expected alteration in luminal contents is established by feed intake rather than inherent signals or light cycles. The potential mechanism at least in part involves a diurnal change in the pattern of transcriptional rate and subsequently the accumulation of respective mRNA and protein, through change in the binding activity of transcriptional factor(s) with the element(s) within the promoter region of the target gene.

Hormonal Regulation. Like intestinal protein digestion and absorption, a number of studies have been conducted to evaluate the effect of hormones on intestinal carbohydrate digestion and absorption and elucidate the underlying mechanism(s).

The intraperitoneal administration of insulin for 3 d (d 11 to 14) caused a precocious induction of SI and SI mRNA and stimulated maltase activities without effect on LPH activity in the small intestine of suckling rats in a dose-dependent manner (Buts et al., 1998). The insulin-

induced stimulatory effect was prevented by pretreatment with insulin receptor monoclonal antibody through inhibiting SI and MGA expression. Thus, these data suggest that the premature induction of SI and MGA by insulin is mediated by binding of insulin to its intestinal receptors, which in turn indirectly triggers the transcription of SI and MGA. The mechanisms by which insulin enhances intestinal SI and MGA in suckling rats have been further explored (Marandi et al., 2001). In response to insulin, a number of proteins were rapidly phosphorylated on tyrosine residues, including MAP kinase and PI 3-kinase. Administration of an antireceptor antibody or a MAP kinase-2 inhibitor but not a PI 3-kinase inhibitor to sucklings inhibited insulin stimulatory effects. Compared to controls, insulin enhanced the intestinal activity of MAP kinase-2 by twofold. Thus, the insulin-induced stimulation of SI and MGA appears to be caused at least in part by the cascade of MAP kinase-2.

The Na^+-dependent uptake of D-glucose via SGLT1 in the small intestine of rats was increased by diabetes mellitus, which was restored by insulin treatment (Kurokawa et al., 1995). They further investigated the mechanism by which diabetes mellitus and insulin regulated the transport activity of small intestinal SGLT1. The induced diabetes at 2 wk after the injection of streptozotocin increased the intestinal BBMV SGLT1 protein without changing the SGLT1 mRNA level. The increased content of BBMV SGLT1 protein was restored by the subcutaneous injection of insulin. In contrast, there was no change in the mRNA levels of SGLT1 in diabetic and insulin-treated diabetic rats. These results suggest that downregulation of intestinal SGLT1 transport activity by insulin involves translational or posttranslational mechanisms.

In Caco-2 cells, 20 to 200 ng/ml EGF treatment for 12 d resulted in a marked reduction in sucrase activity, whereas other brushborder enzymes (APN, DPP IV, and alkaline phosphatase) were only marginally affected (Cross and Quaroni, 1991). The EGF regulated SI expression at

two different levels. At a 20 ng/ml level, EGF affected primarily SI mRNA processing in the endoplasmic reticulum and/or increased its degradation. At a 200 ng /ml level, a marked reduction in SI mRNA levels and biosynthesis was observed. These results suggest that the long-term treatment of EGF downregulates sucrase activities by decreasing SI gene transcription and/or mRNA stability. The regulation of glucose transport and SGLT1 expression by EGF was investigated in rabbit jejunal loops (Chung et al., 1999). Luminal exposure of tissue to 60 ng/ml EGF for 1 h resulted in a 53 % increase in the V_{max} of glucose uptake, and no change in the K_m, which was accompanied by a ~ 50 % increase in BBMV SGLT1 protein content. These stimulatory effects were abolished by the concurrent treatment of cytochalasin D, an inhibitor of actin polymerization, which alone had no effect on glucose uptake. Thus, these findings suggest that EGF-induced upregulation of jejunal glucose uptake results from the recruitment of SGLT1 protein into the brushborder membrane through an actin polymerization-related mechanism.

In fetal and suckling rats, the subcutaneous administration of T3 for 4 d increased the sucrase activity along the entire length of the small intestine in a dose-related manner (Vaucher et al., 1982). However, in adult pigs (Tivey et al., 1993) and rats (Hodin et al., 1992), T3 treatment had no effect on sucrase activities. In differentiating but not differentiated Caco-2 cells, addition of 50 n*M* T3 for 3 d enhanced sucrase activity by 65 % compared to untreated cells, which was associated with a twofold increase in the V_{max} and no change in the K_m, and a twofold increase in SI protein content and a notable increase in SI mRNA level (Jumarie et al., 1996). These results suggest that T3 induced an increase in sucrase activity primarily through upregulating SI gene expression. The regulation of SGLT1 expression by T3 was investigated in Caco-2 cells (Matosin-Matekalo et al., 1998). In differentiated cells, the addition of 100 n*M* T3 induced a tenfold increase in glucose uptake and a sixfold increase in the V_{max}, which was

accompanied by a T3 dose-dependent increase in SGLT1 mRNA abundance. In a later study (Matosin-Matekalo et al., 1998), they demonstrated that T3 treatment also upregulated the mRNA abundance of GLUT5 in a dose-dependent manner, in fully differentiated Caco-2 cells. The analysis of the GLUT5 promoter region revealed that the -308/-290 region of the GLUT5 promoter specifically binds T3 receptor/retinoid X receptor heterodimers, and that T3 load dissociates the cosuppressor protein complex to enhance GLUT5 gene expression. Thus, upregulation of SI, SGLT1 and GLUT5 by T3 treatment is probably accomplished by enhancing gene expression.

Recent studies have shown that intestinal galactose and glucose uptake can be downregulated by leptin treatment. Lostao et al. (1998) demonstrated the uptake of D-galactose by rat small intestinal rings was inhibited by 33 % after 5 min of incubation with 78 n*M* leptin, and by 56 % after 30 min of incubation. This inhibitory effect was accompanied by a 75 % decrease in the V_{max} and a 60 % decrease in the K_m. Ducroc et al. (2005) further demonstrated that luminal addition of leptin decreased glucose uptake in rat jejunum mucosa in a dose-dependent manner and the maximal inhibition (> 90 %) was achieved after 5 min. Western blot analysis showed that the rapid inhibition of glucose uptake by leptin was associated with a parallel decrease in the abundance of SGLT1 protein in brushborder membranes. Biochemical assays revealed that the inhibitory effect of leptin was accompanied by a twofold increase in PKA and PKC activities, and the inhibition was prevented by inhibitors of protein PKC isoforms but was not blocked by a PKA inhibitor (Barrenetxe et al., 2004). Together, these data suggest that gut leptin induces the rapid inhibition of glucose uptake by decreasing the recruitment of SGLT1 protein from the preformed intracellular pool into the brushborder membrane at least

partly through the activation of PKC. However, this does not exclude that leptin may also affect the affinity of the transporters.

Daily administration of 2.5 ug GLP-2 for 10 d increased the activities of maltase, sucrase, and lactase by ~ twofold in the duodenum of mice (Brubaker et al., 1997). The GLP-2 infusion in the neonatal piglet for 6 d (d 1 to 7) during chronic TPN increased lactase activity and intestinal uptake of glucose, which was associated with an increase in SGLT1 BBMV protein (Cottrell et al., 2005). Infusion of GLP-2 rapidly (within 1 h) increased glucose transport activity in BBMV isolated from the rat jejunum (Cheeseman et al., 1997). Kinetic analysis showed this stimulation resulted from a threefold increase in the V_{max} and no consistent change in the K_m. Consistent with this, the acute GLP-2-mediated increase in brushborder membrane glucose transport was associated with a similar increase in SGLT1 protein abundance. The effect of GLP-2 could be blocked by luminal brefeldin A or wortmannin. These results indicate that the acute upregulation of intestinal glucose uptake by GLP-2 treatment is associated with the increased trafficking of SGLT1 protein from an intracellular pool into the brushborder membrane.

In summary, intestinal carbohydrate digestion and absorption is regulated by a variety of hormones. How the activity of individual disaccharidase or monosaccharide transporters is regulated by hormones depends on hormone type, treatment duration and dosage, and developmental stages of the small intestines. The general mechanism involves the alteration in the recruitment of protein from the intracellular preformed pool into the apical membrane for acute regulation, or changes in gene expression and probably in the K_m for chronic regulation. These hormonal effects are initiated by binding of the hormone with its receptor located on either the apical or basolateral membrane.

Intestinal Iron Absorption and Its Regulation

Rapid growth and expansion of hemoglobin mass make neonates or infants particularly susceptible to iron deficiency. Iron deficiency not only results in anemia, but also delayed cognitive and psychomotor development. Unlike adults, for neonates, the dietary iron (mother's milk or iron supplementation) is mainly nonheme iron. Iron homeostasis is maintained primarily by regulating iron absorption via intestinal enterocytes. In other words, the efficiency of iron absorption is regulated by iron status. Many genes involved in the transport of nonheme iron across enterocytes have been identified and functionally characterized, including a brushborder membrane ferric iron reductase (DcytB), a brushborder membrane divalent metal transporter 1 (DMT1), and a basolateral membrane ferroportin 1 (IREG1, FPN1). The DcytB reduces dietary ferric iron to ferrous iron (McKie et al., 2001), and ferrous iron is then actively transported into enterocytes via DMT1 (Fleming et al., 1997; Gunshin et al., 1997). The intracelluar iron traverses within enterocytes and is exported across the basolateral membrane via IREG1 (Abboud and Haile, 2000; Donovan et al., 2000; McKie et al., 2000).

The DMT1 is a H^+-dependent divalent metal ion transporter with broad substrate range including but not limited to Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, and Cu^{2+} (Gunshin et al., 1997). It is widely expressed, but is most abundant in intestine and kidney. In the small intestine of adult rats and mice, DMT1 is expressed predominantly in the proximal duodenum, where its protein expression is limited to the villi and is absent in the crypts (Gunshin et al., 1997; Canonne-Hergaux etal., 1999). In the villi, DMT protein is limited to the columnar absorptive epithelium of mucosa, with the strongest expression on the brushborder membrane of enterocytes in two thirds of the villi. The IREG1 is expressed in tissues involved in body iron homeostasis including small intestine, and pregnant uterus, as well as muscle and central nervous system cells in the

embryo (Abboud and Haile**,** 2000). In the small intestine, IREG1 is primarily localized to the basolateral membrane (Abboud and Haile, 2000).

A number of recent studies have shown that the gene expression of intestinal iron transporter can be regulated by dietary iron levels in adults. The duodenal DMT1 mRNA (Gunshin et al., 1997) and protein expression (Canonne-Hergaux et al., 1999) was dramatically (50- to 100-fold) upregulated by dietary iron deficiency, and was downregulated by dietary iron overload (Trinder et al., 2000). Like DMT1, dietary iron deficiency induced IREG1 mRNA and protein expression in the duodenum in adult rats, and overload reduces its expression (Abboud and Haile**,** 2000, and Zoller et al. 2001). The mechanisms that regulate DMT1 and FPN1 expression by dietary iron intake are not well known. The regulation of DMT1 and IREG1 expression may be controlled by the interaction of iron responsive element (IRE) binding protein(s) and IRE in the 3' UTR on the DMT1 and FPN1 mRNA or alternatively may be caused by transcriptional activation of the gene (Canonne-Hergaux et al., 1999). Many mRNAs encoding proteins involved in metabolism of iron contain IREs in their untranslated regions that mediate changes in protein levels in response to IRE binding protein availability based on iron status through posttranscriptional mechanisms.

Domellöf et al. (2001 and 2002) found that daily iron supplementation increased hemoglobin in infants at age of 6 mo, regardless of initial iron status, whereas at age of 9 mo, a significant increase in hemoglobin by iron supplementation was only observed in infants with low hemoglobin concentrations at 6 mo of age. Domellöf et al. (2002) further demonstrated that daily iron supplementation did not influence the fractional iron absorption from human milks in infants at 6 mo of age, whereas it did decrease the fractional iron absorption from human milk in infants at 9 mo of age. Similar results were reported in neonatal rats (Leong et al., 2001), in

which they demonstrated that the efficiency of dietary iron absorption in the duodenum did respond to dietary iron intake in rats at d 20 only but not at d 10.

Leong et al. (2003) further investigated this phenomenon at molecular levels. They demonstrated that duodenal DMT1 mRNA and protein expression at d 10 did not differ among groups with different dietary iron intakes, whereas it was significantly (four- to fivefold) greater in the unsupplemented low-Fe group compared to that in the control and supplemented high-Fe groups at d 20. Duodenal IREG1 mRNA level was significantly lower in the unsupplemented low-Fe group at d 10 but was eightfold higher than that in the control group at d 20, and IREG1 protein level at d 10 did not differ among groups but was 3.3-fold higher by d 20 in the unsupplemented low-Fe group compared with controls. Both duodenal DMT1 and IREG1 mRNA level increased significantly by iron deficiency at d 20, which corresponded to changes in protein levels. This is in agreement with previous findings that DMT1 and IREG1 gene expression increased in humans with iron deficiency (Zoller et al., 2001) and in iron-deficient rats (Canonne-Hergaux et al., 1999). However, iron deficiency had no effect on duodenal DMT1 mRNA level and appeared to decrease IREG1 mRNA level at d 10, and DMT1 and IREG1 protein expression was unaffected by iron deficiency. This provides the molecular basis for the unresponsiveness of intestinal iron absorption to dietary iron intake reported in infants at age of 6 mo of life (Domellöf et al., 2001) or rats at d 10 (Domellöf et al., 2002). Thus, these results collectively suggest that these intestinal iron transporters are not responsive to iron status at the early life stages. The mechanism is not yet clear, but it is possible that gut development may be immature during the early life stages. Additionally, the sensory communication between body iron stores and the intestinal enterocyte may not be well developed. The iron intake of mammalian neonates is supplied solely from milk during the early life stages, hence, it is

possible that intestinal regulation of iron absorption during this period may not be as crucial as later on when they depend on dietary iron sources other than milk.

Leong et al. (2003) demonstrated the gene expression of DMT1 and IREG1 at both mRNA and protein levels is developmentally regulated in rats. On d 1 after birth, DMT1 mRNA was at a low level. It increased by seven- to ninefold to d 10 and d 20 and slightly declined to d 30, followed by a 110-fold increase by d 40, and then a decline to d 50. The similar but more modest changes were seen for IREG1 mRNA during development. The underlying mechanism for this developmental change is not clear. The increase of iron transporter gene expression with age during suckling may be to maximize the iron intake from milk to improve or maintain the iron homeostasis through genetic programming. Expression of both genes increased dramatically on d 40 after birth, which it is not likely to be caused by low iron status because iron status is improved by d 40. This pronounced increase in the expression of iron transporters on d 40 may be regulated by mechanisms that have been observed in other developmentally regulated intestinal genes such as SI.

In summary, two important iron transporters, DMT1 and IREG1, participate in intestinal iron absorption. In adults, intestinal iron absorption is up- or downregulated by dietary iron deficiency or overload, respectively, through the corresponding changes in DMT1 and IREG1 gene expression. However, for animals or infants who are too young, intestinal iron absorption as well as DMT1 and IREG1 gene expression can not be regulated in response to dietary iron intake. Besides the developmental changes of iron absorption in response to dietary iron status, intestinal iron absorption is also regulated by the development stages alone.

Summary

The small intestine is the primary organ in which the assimilation of most of the dietary nutrients occurs. The intestinal digestion and absorption of dietary nutrients is a complex and well-integrated process, and a wide range of brushborder membrane hydrolases and nutrient transporters expressed in the epithelial cells play an essential role in this process.

Many studies have been conducted to investigate the dietary, developmental, diurnal, and hormonal regulation of dietary nutrient digestion and absorption in the small intestine at the tissue, cellular, and molecular levels. The activity of intestinal digestion and absorption can be regulated through specific and/or nonspecific mechanism(s). The potential specific mechanisms involve either the changes in gene expression of specific hydrolases or nutrient transporters or the posttranslational alterations (e.g. the trafficking of the respective protein from an intracellular preformed pool into the brushborder membrane), and the nonspecific mechanism involves the changes in intestinal mucosal mass and surface area. The knowledge gained from these studies has enhanced our ability to formulate the appropriate diets paralleling the small intestinal digestive and absorptive ability of animals or humans, and provide better medical treatment or nutritional supplementation to those under critical conditions.

The small intestine of neonates experiences dramatic changes in the mass, morphological structure, and function in response to the onset of suckling colostrum or milk right after birth and then weaning onto a solid adult diet. However, during the early life stages, the small intestine or enterocyte exhibits unresponsiveness to the load of certain dietary nutrients, which is most likely related to the genetic rigidity of the small intestine. To better understand the postnatal developmental changes in intestinal digestive and absorptive function of neonates, it is necessary

to investigate a variety of hydrolases and nutrient transporters along the entire length of the small intestine at a closely spaced time intervals for better resolution of spatial and temporal changes.

Although numerous studies were conducted to investigate the interrelationship between gastrointestinal development and nutrient assimilation in young animals, most were restricted to measuring specific activities of intestinal nutrient transporters and digestive enzymes. In recent years, there have been an increasing number of studies related to the ontogenetic changes in intestinal nutrient digestion and absorption at the molecular level in rodent animals, however, few such studies have been reported in pigs. The present study was designed and conducted to increase our knowledge regarding the developmental regulation of intestinal digestion and absorption activity at the molecular level and therefore to explore the underlying regulatory mechanisms.

Chapter III.

Developmental Regulation of the Expression of Nutrient Transporter and Brushborder Membrane Hydrolase Genes in the Small Intestine of Piglets

ABSTRACT

Seventy piglets from seven sows were killed at birth (d 0), during suckling (d 1, 3, 7, 14, 21) and postweaning (d 22, 24, 28, 35), and intestinal segments (duodenum, jejunum and ileum) were collected. The mRNA abundance was determined by Northern blot using specific cDNA probes for three disaccharidases (lactase-phlorizin hydrolase, LPH, sucrase-isomaltase, SI, and maltase-glucoamylase, MGA), three peptide hydrolases (aminopeptidase A, APA, aminopeptidase N, APN, and dipeptidyl peptidase IV, DPP IV), two sugar transporters (Na^+-dependent glucose transporter 1, SGLT1, and facilitated glucose transporter 5, GLUT5), a peptide transporter (H^+-dependent peptide transporter 1, PepT1), four amino acid transporters (excitatory amino acid carrier 1, EAAC1, Na^+-dependent neutral amino acid transporter, ATB^0, the light chain of a heterodimeric transport system $b^{0,+}$ involved in the heteroexchange of cationic and neutral amino acids, $b^{0,+}AT$, and Na^+-independent large branched and aromatic neutral amino acid transporter 2, LAT2), and two iron transporters (divalent metal ion transporter 1, DMT1, and iron-regulated transporter 1, IREG1). Protein expression was quantified by Western blot using specific antibodies for LPH, SI, SGLT1, and PepT1. During suckling, the abundance of LPH, APA, APN, DPP IV, $b^{0,+}AT$ mRNA increased quadratically ($P < 0.001$) with age from birth to d 7 or 14 then remained unchanged or slightly declined with age to d 21. The mRNA abundance of SI increased and LAT2 decreased linearly ($P < 0.001$) with age, and the abundance of MGA and GLUT5 mRNA remained unchanged with age. There was an age x intestinal segment interaction ($P < 0.001$) for the abundance of EAAC1 and ATB^0 mRNA. The abundance of EAAC1 mRNA

increased from d 0 through 14 and remained stable to d 21 in the ileum, and it was low and slightly increased with age through d 21 in the duodenum and jejunum. The abundance of ATB^0 mRNA generally increased from d 0 to 21 in the duodenum and ileum, and increased from d 0 to 7 and then decreased to d 21 in the jejunum. The abundance of SGLT1 and PepT1 mRNA was substantial at birth and transiently declined to d 1. The abundance of SGLT1 mRNA generally increased from d 1 to 21, and PepT1 mRNA abundance increased to d 3 and then plateaued through d 21. Postweaning, the mRNA abundance of all of these carbohydrate and protein assimilation related genes increased during the first day (3 d for ATB^0) after weaning then declined to the levels at weaning in the jejunum and ileum, followed by a subsequent change pattern that varied among genes in a gene-dependent manner. During suckling, the mRNA abundance of LPH, SGLT1, and APA was greater in the duodenum and jejunum than the ileum ($P < 0.001$). The PepT1 and APN mRNA was evenly distributed among intestinal segments, and the expression of MGA, DPP IV, EAAC1, $b^{0,+}AT$, ATB^0, and LAT2 mRNA was generally greater in the jejunum and ileum than the duodenum or greatest in the ileum. Postweaning, the mRNA abundance of all of these carbohydrate and protein assimilation related genes examined was generally greater in the jejunum and ileum than the duodenum or highest in the ileum. From d 0 through 35, DMT1 and IREG1 mRNA was predominantly ($P < 0.05$) distributed in the duodenum, where the abundance of DMT1 and IREG1 mRNA increased with age during suckling, and then rapidly decreased after weaning. The protein expression of LPH and SI exhibited a similar developmental pattern as that for the mRNA abundance. Unlike the developmental regulation of their respective mRNA abundance, the protein expression of SGLT1 exhibited a general decline from suckling to postweaning. The protein expression of PepT1 gradually decreased with age from birth to d 35 in the duodenum, and initially declined from

birth to the lowest value then slightly increased with age through d 21, followed by an increase to d 35 in the jejunum and ileum. In conclusion, the gene expression of these brushborder hydrolases and nutrient transporters was not only differentially regulated by age but also differentially distributed along the small intestine of piglets at early stages of life. These differences in ontogenetic regulation and the distribution may be related to the luminal substrate concentration as well as the nutrient categories, and the developmental regulation of these genes may occur not only at the transcriptional level but also at the posttranscriptional level.

Introduction

In mammals, the growth, development, and maturation of the gastrointestinal (GI) tract initiates early from the middle of gestation and continues several weeks after birth (Rome et al., 2002). These developmental changes are rapid and dramatic during early postnatal life, especially at birth when the GI tract assumes the complete responsibility for the provision of nutrients and shifts from processing the dilute amniotic fluid swallowed to the nutrient-enriched colostrum or milk ingested, and around weaning when the dietary transition occurs from milk to a physically and chemically different solid diet (Zhang et al., 1997; Hedemann et al., 2003).

The pig is one of the major domestic animals raised for meat production. With the modern commercial practice of early weaning, the gastrointestinal system of neonatal pigs tends to become challenged to its limit, resulting in numerous problems, such as malnutrition and the postweaning growth check (Xu and Cranwell, 2003). In the last two decades, many studies have been conducted to investigate the interrelationship between gastrointestinal development and nutrient assimilation in piglets at early stages by measuring specific activities of intestinal nutrient transporters and digestive enzymes (Xu and Cranwell, 2003). However, the examination and comparison of the ontogeny of the small intestinal digestive and absorptive function is

complicated and even confused by the choice of denominator (tissue weight, tissue protein, BBMV protein, or DNA) used to normalize the specific activity and the methods and substrates used as well (Fan et al., 2004). In recent years, there have been an increasing number of studies on the ontogenetic changes in intestinal nutrient digestion and absorption at the molecular level in rodents aimed at providing us with a better understanding of this process, however, few such studies have been reported in pigs.

Carbohydrates and proteins are two important nutrient categories that impact the growth performance, development, and general health of piglets, and the assimilation of carbohydrates and proteins occurs primarily in the small intestine (Adibi, 2003). In the small intestine, dietary carbohydrates and proteins undergo a series of degradative steps carried out by a wide array of digestive enzymes derived from the stomach, pancreas, and small intestine, resulting in a final mixture of free amino acids and small peptides from proteins and constituent monosaccharides from carbohydrates, respectively (Daniel, 2004). During this process, intestinal brushborder membrane disaccharidases and peptide hydrolases play an essential role in the final digestion of proteins and carbohydrates into absorbable molecules, which are efficiently absorbed by a spectrum of specific nutrient transporters expressed in the enterocytes. During the early stages of life, iron is a very important and essential nutrient for young animals and humans. Iron deficiency is a common problem in neonatal piglets under commercial conditions and in infants during lactation, and iron supplementation is often needed (Egeli and Framstad, 1999). It is well understood that the absorption of dietary nonheme iron (the primary iron source during lactation) is mediated through a brushborder membrane divalent metal ion transporter 1 (DMT1), and subsequently a basolateral iron-regulated transporter 1 (IREG1; Leong et al., 2003). Hence, the objectives of this study were to investigate the ontogenetic regulation of the gene expression of

selected brushborder membrane hydrolases and nutrient transporters in the small intestine of piglets, as well as their distribution along the small intestine. The hydrolases include three disaccharidases (lactase-phlorizin hydrolase, LPH, sucrase-isomaltase, SI, and maltase-glucoamylase, MGA), and three peptide hydrolases (aminopeptidase A, APA, aminopeptidase N, APN, and dipeptidyl peptidase IV, DPP IV). The nutrient transporters include a peptide transporter (H^+-dependent peptide transporter 1, PepT1), two monosaccharide transporters (Na^+-dependent glucose transporter 1, SGLT1, and Na^+-independent facilitated glucose transporter 5, GLUT5), five amino acid transporters (excitatory amino acid carrier 1, EAAC1, Na^+-dependent neutral amino acid transporter, ATB^0, the heavy chain and light chain of a heterodimeric transport system $b^{0,+}$ involved in the heteroexchange of cationic and neutral amino acids, NBAT and $b^{0,+}AT$, and Na^+-independent large branched and aromatic neutral amino acid transporter 2, LAT2), and two iron transporters (DMT1 and IREG1). The knowledge from the present study will provide useful information to guide the formulation of diets. These specified diets may best meet the biological needs of the piglets for optimal growth performance and health status during the early stages of life. It has been widely accepted that intestinal ontogenesis in the pig resembles human more closely than other animal species. Thus, this knowledge may also guide feeding of newborn infants with diets that are optimized to match and even stimulate normal intestinal organ function and maturation.

Materials and Methods

Chemicals and Reagents. All chemicals and reagents were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma -Aldrich (St. Louis, MO) unless otherwise stated. Tri-Reagent was purchased from Molecular Research Center (Cincinnati, OH). The 2X PCR Master kit and the pGEM-T easy vector system were purchased from Promega (Madison, WI). The

Quick-Gel Extraction kit, Qiaprep Spin Miniprep kit, and High Speed Midi kit were purchased from Qiagen (Valencia, CA). Restriction enzymes were purchased from New England Biolabs (Beverly, MA). Nylon membranes were purchased from Osmonics, Inc. (Westborough, MA). Polyvinylidene difluoride (PVDF) membranes were purchased from Bio-Rad (Hercules, CA). The [^{32}P]dNTP was purchased from Perkin-Elmer Life Sciences (Boston, MA). The nick translation labeling system was purchased from Invitrogen Life Technologies (Carlsbad, CA). Sephadex G-50 quick-spin columns were purchased from Roche (Indianapolis, IN).

Animals and Sample Collections. All animal procedures were reviewed and approved by Virginia Tech's Animal Care Committee. Seven sows with 10 or more piglets at birth were selected for this study at the Virginia Tech Swine Center in Blacksburg, Virginia. The sows were moved into individual cages in a farrowing room 3 d before the due date and were kept there with piglets until the end of the 21-d lactation phase. Two Heat lamps were placed in each cage to provide extra warmth for neonatal piglets. The piglets were routinely immunized and muscle injected with 1 mL of iron-dextran (200 mg) on d 3. On d 21, piglets from each sow were assigned to double-ducked, stainless steel weaner cages in the nursery room and weaned onto a nursery diet (NRC, 1998), with the dietary ingredients and nutrient composition listed in Table 1. Each weaner cage was equipped with a removable stainless steel feeder, a nipple drinker and with perforated flooring. Both the farrowing and nursery rooms were maintained with continuous lighting and an ambient temperature of 27 to 30 ºC.

In this study, one piglet from each of seven sows was removed and killed by intravenous injection of a lethal dose of pentobarbital sodium, on d 0 (right after birth without suckling), d 1 (24 h after birth), 3, 7, 14, 21, 22, 24, 28, and 35. The small intestine from the pylorus to the ileocecal valve was quickly excised and then divided into three segments, duodenum, jejunum

and ileum. Each intestinal segment was opened longitudinally, and rinsed three times with fresh ice-cold phosphate buffered saline (PBS; 1.47 mM NaH_2PO_4, 8.09 mM Na_2HPO_4, 0.145 M NaCl) to rinse out digesta contents. The mucosa was gently scraped off with glass slides, mixed thoroughly, and then weighed out (0.25 to 0.3 g for total RNA isolation per package; 1 to 2 g for brushborder membrane isolation per package). The tissue was then wrapped in aluminum foil and dropped into liquid N. The packaged intestinal samples were then stored at –80 °C for later use.

Total RNA Isolation. Total RNA was isolated from intestinal samples by using Tri-Reagent following the manufacturer's protocol with slight modifications. The final RNA pellet was dissolved in 0.1 % diethylpyrocarbonate (DEPC) water containing 0.1 % SDS. The quality of isolated RNA was tested by measuring absorbance at 260 nm and 280 nm by spectrophotometry (Model U-2000, Hitachi Instrument Inc, Tokyo, Japan), and the integrity of isolated RNA samples was further checked by size fractionation on a denaturing agarose gel. Total RNA samples were stored at –80 °C until use.

Preparation of Pig cDNA Probes. The pig PepT1 probe was a full length cDNA clone isolated in our lab (Klang et al., 2005). The other pig specific cDNA probes were generated by reverse transcription polymerase chain reaction (RT-PCR) and subcloning. Two different RT-PCR methods were used in this study. Two-step RT-PCR was performed for the following genes: Glyseraldehyde-3-phosphate dehydrogenase (GAPDH), $b^{0,+}AT$, ATB^0, NBAT, LAT2, EAAC1, SGLT1, GLUT5, DMT1, and IREG1. Briefly, for the reverse transcription of RNA into cDNA, total RNA from the pooled samples (duodenum, jejunum and ileum) was treated with DNase and then denatured at 80 °C for 15 min. The denatured RNA was incubated at 42 °C for 2 h with a reverse transcription system kit (Promega, Madison, WI), and the enzyme was

denatured by heating the mixture at 95 °C for 5 min. After cDNA synthesis, PCR was carried out in a PTC-200 Peltier DNA Engine (MJ Research, Reno, NV) using corresponding sets of primers as follows: the two primers, 2 x 1 μL 20 pmol/L primers, 3 μL of the above reaction mixture containing synthesized cDNA as the template, 25 μL of 2 X PCR Master mix, and 20 μL of dd H_2O. After preheating at 94 °C for 4 min, 35 amplification cycles were performed at 92 °C for 1 min, 55 °C for 1 min, and 72 °C for 1.5 min. The final extension was carried out at 72 °C for 15 min. One-step RT-PCR was performed by using a One-step RT-PCR kit (Qiagen, Valencia, CA) according to the manufacturer's manual for the following genes: APA, APN, DPP IV, LPH, SI, and MGA. Briefly, 1 μg of total RNA was added to a set of gene specific primers and the reaction mix and amplified in a PTC-200 Peltier DNA Engine with the conditions as follow: reverse transcription at 50 °C for 30 min, initial PCR activation at 95 °C for 15 min, 35 amplification cycles (94 °C for 1 min, 55 °C for 1 min, 72 °C for 1 min), and final extension at 72 °C for 10 min. The sequences of primers for these specific genes are listed in Table 2.

After PCR, the reaction mixtures were run on a 1 % agarose gel, along with a 100 to 1000 bp DNA marker (Promega, Madison, WI). The band was visualized under UV-light by ethidium bromide staining, and those bands with the desired size were digested and extracted from the agarose gel using the Qiagen Quick-Gel Extraction kit. The purified PCR products were ligated with a pGEM-T Easy vector system and introduced into competent *E. coli* cells by electroporation using a BTX-Harvard Apparatus ECM Electro Cell Manipulation System (Holliston, MA). The cells were then plated out at 37 °C for 24 h with 100 μg/mL ampicillin, 0.5 m*M* IPTG, and 80μg/mL X-GAL for positive clone screening. Five positive clones (white) were picked and cultured in 2 to 3 mL LB medium containing 100 μg/mL ampicillin and incubated at 37 °C for 12 to16 h.

The plasmid was isolated from cultured cells by using the Qiagen Qiaprep Spin Miniprep kit, and then digested by specific restriction enzymes to confirm the presence of the correct inserts. The identity of the fragments was further confirmed by sequencing at the Virginia Bioinformatics Institute (Blacksburg, Va). The sequences were compared using BLAST (NCBI) and aligned with the corresponding gene sequences to confirm their molecular identities. The correct clone was then cultured in 200 mL LB medium plus 100 μg/mL ampicillin, and the High Speed Midi Kit was used to harvest high yields of purified plasmid following the manufacturer's protocol. After digestion of purified plasmid with appropriate restriction enzymes, the insert fragment was purified for later use as a cDNA probe.

Northern Blot Analysis. For Northern blot analysis, 20 μg of total RNA from each sample were sized-fractionated at 35 V for 12 to 18 h on a denaturing agarose gel containing 2.2 mol/L formaldehyde. Ethidium bromide staining was used to visualize RNA bands under UV-light, and a 0.24 to 9.5 kb RNA ladder (Invitrogen, Carlsbad, CA) was run along with RNA samples to determine RNA size. The gel was rinsed with purified H_2O and subsequently immersed for 20 min in transfer buffer, a 20 X SSC solution (3 *M* NaCl, 0.3 *M* sodium citrate, pH 7.0). The Nylon membrane was immersed in purified H_2O and then placed in transfer buffer for 5 min before being added to the transfer system. A downward capillary membrane transfer system was set overnight in transfer buffer. After the completion of transfer, the membrane was rinsed twice in purified H_2O and then in a 2 X SSC solution for 15 min. After the membrane was completely air-dried, RNA was cross-linked onto the membrane by UV-light at 2 X 0.30 J/cm^2 using a BIOS Bioslink UV crosslinker (New Haven, CT).

The membranes were prehybridizated for 4 h in a solution containing 50 % formamide, 5X Denhardt's solution, 6X SSPE (1X SSPE = 0.15 mmol/L NaCl, 10 mmol/L NaH_2PO_4, and 1

mmol/L EDTA), 0.5 % SDS and 10 mg/L yeast tRNA at 42 °C. The membranes were then hybridized for 16 h under identical conditions with the addition of pig specific cDNA probes. The probe was labeled with [α-^{32}P]-dNTP by nick translation and purified by Sephadex G-50 spin column chromatography. Posthybridization washing was done under high-stringency conditions as follows: twice in 5X SSPE, 0.5 % SDS at room temperature for 15 min, twice in 1X SSPE, 0.5 % SDS at 42 °C for 15 min, and twice in 0.1X SSPE, 1 % SDS at 65 °C for 15 min. The washing was either terminated earlier or extended in 0.1X SSPE, 1 % SDS at 65 °C for optimal hybridization signals for individual genes. The GAPDH signals were used as an internal standard to correct for differences in RNA loading onto gels or in RNA transfer to membranes, by performing the hybridization simultaneously with the gene of interest.

The membranes were then wrapped in saran wrap and exposed to Kodak phosphor imaging screens for 20 min to 4 h in the dark. After exposure to the membranes, phosphor image screens were scanned with the Molecular Imager FX using PDQUEST software (Bio-Rad, Hercules, CA). The images were analyzed using the volume tools of Quantity One Quantification Software (Bio-Rad, Hercules, CA) to quantify the density of the hybridization bands. The mRNA abundance of the gene of interest was expressed as a ratio of the density of that gene to the density of GAPDH.

Brushborder Membrane Preparation. The brushborder membrane from frozen mucosa scrapings was prepared by using a slightly modified method described by Kessler et al. (1978) and Coleto et al. (1998). All procedures below were performed either on ice or at 4 °C. The frozen mucosa scrapings (1 to 2 g wet weight) were quickly dropped into 10 ml ice-cold buffer (100 m*M* mannitol, 2 m*M* HEPES/Tris, pH 7.4, 0.1 m*M* PMSF). Once thawed, the mucosa scrapings were homogenized with a Janke and Kunkel Ultra-Turrax T25 homogenizer at full

speed for four 30 sec periods, and more ice-cold buffer was added to achieve a 5 % homogenate. About 2.0 mL of the homogenate was removed and saved for subsequent protein and marker enzyme assays. The remaining homogenate was centrifuged at 500 × *g* for 12 min and the pellets were discarded. A 1*M* $MgCl_2$ solution was added to the remaining supernatant to a final concentration of 10 m*M*, followed by occasional gentle mixing for 20 min. The suspension was centrifuged at 3,000 × *g* for 15 min, and the supernatant was removed and centrifuged again at 3,000 × *g* for 15 min. The supernatant was gently poured into another centrifuge tube and centrifuged at 30,000 × *g* for 30 min. The resultant pellet was resuspended in 20 mL buffer (100 m*M* mannitol, 2 m*M* HEPES/Tris, pH 7.4, 1 m*M* $MgSO_4$, and 0.1 m*M* PMSF). The suspension was homogenized for 1 min with the same homogenizer used before and centrifuged at 30,000 × *g* for 30 min. The final pellet containing purified brushborder membrane vesicles was resuspended in buffer (300 m*M* mannitol, 20 m*M* HEPES/Tris, pH 7.4, and 0.1 m*M* $MgSO_4$). The suspension was homogenized by passing it through a 25-gauge needle 15 times. The resulting suspension was aliquoted and stored at -80 ^{o}C for later use.

Marker Enzyme and Protein Determination. Sucrase (EC 3.2.1.48) has been used as a reliable marker for the brushborder membrane (Schmitz et al., 1973). The brushborder membrane preparations were routinely evaluated by the enrichment of sucrase activities. The enrichment factor was calculated as a ratio of the sucrase activity per mg protein in the brushborder membrane fraction divided by the sucrase activity per mg protein in the whole intestinal homogenates. Sucrase activity was assayed according to the method described by Dahlquist (1964). Protein content was determined by using the protein 2-D Quant kit (Amersham Biosciences, Piscataway, NJ) following the manufacturer's protocol with bovine serum albumin serving as a standard.

Western Blot Analysis. Four primary antibodies and two secondary antibodies were used successfully for Western blots in this study. The rabbit anti-pig PepT1 polyclonal antibody (serum) was custom generated against a synthetic peptide corresponding to amino acid 694 to 708 (DSLYPKLDTDVQTQM) in the C-terminus of the pig intestinal PepT1 (Sigma Genosys, Woodlands, TX). The mouse anti-rabbit SGLT1 polyclonal antibody was generously provided as a gift by Dr. Kasahara (Teikyo University, Japan). This polyclonal antibody was raised in a mouse against the synthetic peptide corresponding to amino acid 564 to 575 (RNSKEERIDLDA) of the deduced amino acid sequence of rabbit intestinal SGLT-1, and this antibody has been used successfully to detect a single SGLT1 protein band in rats (Takata et al., 1991; Yoshida et al., 1995) and chickens (Barfull et al., 2002). The mouse anti-pig SI and LPH monoclonal antibodies (Heath et al., 1996) were generously given as a gift by Dr. Nichols at the Baylor College of Medicine in Houston, Texas.

The brushborder membrane samples were solubilized in sample loading buffer (1 % SDS, 50 m*M* Tris-HCl, pH 7.0, 20 % glycerol, 0.2 *M* DTT and 0.01 mg/mL bromphenol blue) and boiled for 5 min. Ten micrograms of protein per sample were subjected to 7.5 % polyacrylamide gel electrophoresis containing 0.1 % SDS (SDS-PAGE) and resolved proteins were then electrotransferred onto the PVDF membrane. The prestained precision plus protein kaleidoscope standard (Bio-Rad, Hercules, CA) was used to confirm the quantitative estimation of transfer efficiency and serve as a molecular weight marker for the protein bands on the membrane.

The blots were washed twice in a TBS-T solution (20 m*M* Tris-HCl, pH 7.5, 150 m*M* NaCl, 0.1 % Tween 20) for 5 min and then incubated in a blocking solution (1 % BSA in TBS-T solution) for 2 h. The blots were then incubated for 1.5 h in the blocking solution containing a specific primary antibody with the optimal dilution condition (1/800 for rabbit anti-pig PepT1

polyclonal antibody; 1/1500 for mouse anti-rabbit SGLT1 polyclonal antibody; 1/10,000 for mouse anti-pig SI monoclonal antibody; 1/100, 000 for mouse anti-pig LPH monoclonal antibody). The solution containing the primary antibody was poured off. The blots were washed 5X 10 min in the TBS-T solution, and then incubated for 1 h in a blocking solution plus a secondary antibody. For PepT1, goat anti-rabbit IgG (Pierce Biotechnology, Inc., Rockford, IL) was used as a secondary antibody with a 1/5,000 dilution, and for SGLT1, SI, and LPH, goat anti-mouse IgG (Bethyl Laboratories) was used as a secondary antibody at a 1/5,000 dilution. After incubation, the solution was poured off and the blots were washed 5X 10 min in TBS-T. The blots were visualized using the ECL detection kit (Amersham Biosciences, Piscataway, NJ) following the manufacturer's protocol, and then exposed for varying times to Kodak X-ray film to determine the optimal exposure time. The autoradiographs were scanned with an Epson Expression scanner (Epson America, Inc., Long Beach, CA), and the mean densities of each band of interest were calculated and exported.

Statistical Analysis. The data were analyzed by using the Proc Mixed procedure (SAS Institute, Cary, NC) and the following statistical model: $y_{ijkn} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha\beta_{ij} + \alpha\gamma_{ik} + \beta_{\gamma jk} + \varepsilon_{ijkn}$, in which α, β, and γ represent age, sex and intestinal segment, respectively. As seen above, for mRNA and protein abundance, the model included the main effect of age, intestinal segment, and sex, and all two-way interactions but not the three-way interaction. The main effects of age were further tested for linear and quadratic responses using orthogonal contrasts in the General Linear Model procedure.

Results

Feed Intake and Growth Performance. The piglet growth performance and feed intake during the experimental period is shown in Table 3. During the 21-d suckling period, piglet average daily gain (ADG) was 278 g. Milk consumption was not recorded and no creep feed was offered. Postweaning, average daily feed intake (ADFI) was low during the first 7 d. In the gastrointestinal tract, few feed particles were observed in all of the piglets killed during the first 3 d after weaning and even in two of seven piglets killed on d 28, 7 d after weaning (data not shown). Accordingly, piglet ADG was negative or near zero during the first 7 d after weaning. During 7 to 14 d after weaning, piglet ADFI was approximately 500 g and ADG was approximately 250 g.

Quality and Purity of the Brushborder Membrane Preparations. Protein expression of intestinal hydrolases and nutrient transporters was quantified by Western blot using brushborder membrane protein samples. The quality and purity of brushborder membrane preparation was checked by the enrichment factor of the specific activity of sucrase, a reliable brushborder marker enzyme. The average enrichment factor calculated from the samples collected from d 7 through 35 was 18.7 ± 4.6, which is in the range (10 to 25) reported from previous studies (Zhang et al., 1997). The sucrase activities from the samples collected on d 0, 1, and 3 was very low, which made it difficult to accurately calculate the enrichment factors of sucrase activities on these time points.

Developmental Regulation of Expression of Intestinal Disaccharidases and Monosaccharide Transporters. The mRNA abundance of LPH changed quadratically ($P < 0.05$) with age during suckling (Figure 3.1). The mRNA abundance of LPH rapidly increased with age during the first few days after birth then remained relatively constant or slightly declined with

age through d 21. The mRNA abundance of LPH changed quadratically ($P < 0.05$) with age postweaning. The LPH mRNA abundance rapidly declined then increased slightly or remained unchanged to d 35. The mRNA abundance of LPH was greater ($P < 0.05$) in the duodenum and jejunum than the ileum during suckling and was greatest ($P < 0.05$) in the jejunum postweaning.

The expression of LPH protein changed quadratically ($P < 0.01$) with age during suckling (Figure 3.2). The expression of LPH protein increased from birth to d 7 then remained stable or slightly declined with age to the end of suckling. The expression of LPH protein changed quadratically ($P < 0.01$) with age postweaning. The expression of LPH protein was relatively constant during the first day after weaning and then rapidly decreased to a low level to d 35. During suckling, the expression of LPH protein was higher ($P < 0.01$) in the duodenum and jejunum than the ileum. Postweaning, the expression of LPH protein was also higher ($P < 0.01$) in the duodenum and jejunum than the ileum.

The mRNA abundance of SI increased linearly ($P < 0.001$) with age during suckling (Figure 3.3). Postweaning, there was an age x intestinal segment interaction for the mRNA abundance of SI. In the duodenum, the mRNA abundance of SI decreased with age, with an 80 % decrease from d 21 to 35. In the jejunum and ileum, SI mRNA abundance first peaked on d 22 (1 d after weaning) then rapidly declined to d 24, followed by a subsequent increase to d 35. On d 35, the mRNA abundance of SI in the jejunum was close to the value at weaning, whereas the mRNA abundance in the ileum was threefold greater than that at weaning. The mRNA abundance of SI was greater ($P < 0.05$) in the duodenum and jejunum than in the ileum during suckling and greater ($P < 0.05$) in the jejunum and ileum than in the duodenum postweaning.

The expression of SI protein increased linearly ($P < 0.001$) with age during suckling, and there was an age x intestinal segment interaction in the expression of SI protein postweaning

(Figure 3.4). Postweaning, the expression of SI protein generally declined with age in the duodenum. In the jejunum and ileum, the expression of SI protein was relatively constant during the first 7 d after weaning, followed by a slight increase to d 35. The protein expression of SI was greatest ($P < 0.001$) in the jejunum and lowest ($P < 0.001$) in the duodenum during suckling and was greater ($P < 0.001$) in the jejunum and ileum than the duodenum postweaning. The developmental regulation of intestinal sucrase activity is shown in Figure 3.5. Like the expression of SI protein, the sucrase activity (expressed as per mg BBMV protein) increased linearly ($P < 0.001$) with age during suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.01$) in sucrase activity. The sucrase activity remained relatively stable with age in the duodenum and jejunum from d 21 to 35. In the ileum, sucrase activity stayed unchanged with age during first 7 d after weaning then increased to d 35. The sucrase activity was higher ($P < 0.01$) in the jejunum than the duodenum and ileum during suckling and was higher ($P < 0.001$) in the jejunum and ileum than the duodenum postweaning.

The mRNA abundance of MGA was low and relatively constant with age during suckling, and increased quadratically ($P < 0.05$) with age postweaning, with a slight increase during the first 7 d after weaning and a rapid increase afterward (Figure 3.6). The abundance of MGA mRNA was greatest ($P < 0.05$) in the ileum during suckling and was greater ($P < 0.05$) in the jejunum and ileum than the duodenum postweaning.

The developmental regulation of intestinal SGLT1 mRNA abundance is shown in Figure 3.7. In the duodenum and jejunum, the mRNA abundance of SGLT1 declined by > 50 % from birth to d 1 then gradually increased to d 14, followed by a slight decline to d 21. In the ileum, SGLT1 mRNA abundance declined by 20 % from birth to d 1 then slightly increased through d 21. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) in the mRNA

abundance of SGLT1. In the duodenum, the mRNA abundance of SGLT1 decreased during the first 3 d after weaning then plateaued through d 35. In the jejunum and ileum, it rapidly (150 % in the jejunum and 70 % in the ileum) increased during the first day after weaning then declined by d 24, followed by an increase to d 35. The overall developmental effect on the mRNA abundance of SGLT1 from birth to d 35 was a 10 % and 80 % increase in the jejunum and ileum, respectively, and a 50 % decrease in the duodenum. The SGLT1 mRNA abundance was greater ($P < 0.05$) in the duodenum and jejunum than the ileum during suckling and was greater ($P < 0.05$) in the jejunum and ileum than the duodenum postweaning.

The developmental regulation of intestinal SGLT1 protein expression is shown in Figure 3.8. There was an age x intestinal segment interaction ($P < 0.05$) in the expression of SGLT1 protein during suckling. During suckling, after initially increasing from birth to d 1 (in the duodenum and ileum) or 3 (in the jejunum), the expression of SGLT1 protein generally declined with age through d 21 in the duodenum and jejunum. In the ileum, the protein expression of SGLT1 slightly declined from d 1 to 14 then increased to the end of suckling. There was an age x intestinal segment interaction ($P < 0.05$) in the expression of SGLT1 protein postweaning. In the duodenum, the expression of SGLT1 protein remained stable during the first 3 d after weaning and then declined through d 35. In the jejunum and ileum, the protein expression of SGLT1 decreased with age during the first 3 d after weaning then remained stable (in the jejunum) or slightly declined (in the ileum) with age through d 35. From birth through d 35, the expression of SGLT1 protein generally declined, and the expression of SGLT1 protein was higher ($P < 0.01$) in the duodenum and jejunum than the ileum during suckling.

There was an interaction ($P < 0.01$) between age and intestinal segment in GLUT5 mRNA abundance during suckling (Figure 3.9). Generally, the mRNA abundance of GLUT5

decreased from birth to d 3 and then increased to d 21 in the duodenum, and increased from birth to d 7 and subsequently declined to d 21 in the jejunum and ileum. Postweaning, the mRNA abundance of GLUT5 peaked at d 22 then rapidly declined to d 24, followed by a slight increase to d 35. The mRNA abundance of GLUT5 tended to be greater in the duodenum and jejunum than the ileum during suckling ($P = 0.18$) and postweaning ($P = 0.14$).

Developmental Regulation of Expression of Intestinal Peptide Hydrolases and Peptide and Amino Acid Transporters. The mRNA abundance of APA increased quadratically ($P < 0.001$) with age during suckling (Figure 3.10). The APA mRNA abundance increased by twofold from birth to d 7 then slightly declined through the end of suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) for the mRNA abundance of APA. In the duodenum, APA mRNA abundance decreased with age. In the jejunum and ileum, APA mRNA abundance increased by 50 to 60 % during the first day after weaning and then decreased to close to the level at weaning by d 24, followed by a decline in the jejunum and an increase in the ileum to d 35, respectively. From d 0 to 35, the mRNA abundance of APA increased by 2.4-fold in the ileum but only increased slightly in the duodenum and jejunum. A higher ($P < 0.05$) expression of APA mRNA was observed in the jejunum and ileum than the duodenum both during suckling and postweaning.

The mRNA abundance of APN increased quadratically ($P < 0.001$) with age during suckling (Figure 3.11). The mRNA abundance of APN increased by over sixfold from birth to d 3 (in the jejunum and ileum) or d 7 (in the duodenum) then plateaued through the end of suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) in the mRNA abundance of APN. In the duodenum, APN mRNA abundance slightly decreased with age during the first 3 d after weaning then remained unchanged with age through d 35. In the

jejunum and ileum, APN mRNA abundance transiently increased (up to 150 % in the jejunum and 50 % in the ileum) then declined to the previous level on d 21 to d 24, accompanied by a subsequent increase to d 28 then a slight decline to d 35. The mRNA abundance of APN did not differ among intestinal segments during suckling and was greater ($P < 0.05$) in the jejunum and ileum than the duodenum postweaning.

The mRNA abundance of DPP IV increased quadratically ($P < 0.001$) with age during suckling (Figure 3.12). The mRNA abundance of DPP IV increased by over fivefold from birth to d 7 then changed slightly with age through the end of suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) in the abundance of DPP IV mRNA. In the duodenum, DPP IV mRNA abundance slightly decreased with age. In the jejunum and ileum, DPP IV mRNA abundance increased (110 % in the jejunum and 30 % in the ileum) during the first day after weaning and declined to a value greater than that at weaning, and then remained relatively stable through d 35. The abundance of DPP IV mRNA was greater ($P < 0.05$) in the jejunum and ileum than the duodenum both during suckling and postweaning.

The effect of developmental stages on the mRNA abundance of intestinal PepT1 is shown in Figure 3.13. The intestinal PepT1 mRNA abundance declined from birth to d 1. The abundance of PepT1 mRNA then increased to the values at birth by d 3 to 7 and remained relatively constant through the end of suckling. Postweaning, in the duodenum, the mRNA abundance of PepT1 was stable during the first day after weaning and declined to d 24, followed by a slight increase to d 35. In the jejunum and ileum, the mRNA abundance of PepT1 increased during the first day after weaning, and declined to a value less than that on d 21, followed by a subsequent slight increase to d 35. Generally, PepT1 mRNA was evenly distributed among the

three intestinal segments during suckling and tended to be expressed higher ($P = 0.11$) in the jejunum than the duodenum and ileum postweaning.

There was an age x intestinal segment interaction ($P < 0.05$) in PepT1 protein expression during suckling and postweaning (Figure 3.14). At birth, the expression of PepT1 protein was at the highest level and did not differ among intestinal segments. The expression of PepT1 protein gradually decreased from birth to d 1 then remained relatively unchanged to the end of suckling in the duodenum, whereas PepT1 protein level rapidly (> 70 %) decreased from birth to d 3 and then slightly increased through the end of suckling in the jejunum and ileum. Postweaning, the expression of PepT1 protein generally declined to d 35 in the duodenum. In the jejunum and ileum, the expression of PepT1 protein increased throughout the period. The PepT1 protein level was higher ($P < 0.05$) in the duodenum than the jejunum and ileum during suckling.

There was an age x intestinal segment interaction ($P < 0.05$) in the abundance of EAAC1 mRNA both during suckling and postweaning (Figure 3.15). In the duodenum and jejunum, EAAC1 mRNA abundance was low and remained unchanged or slightly increased with age from birth through d 35. In the ileum, the abundance of EAAC1 mRNA increased by 1.6-fold from birth to d 14 then slightly increased to d 21. Postweaning, the abundance of EAAC1 mRNA in the ileum increased to d 22 then declined to the value at weaning by d 28, followed by a 60 % increase to d 35. The EAAC1 mRNA abundance exhibited an increased gradient along the proximal-distal intestinal axis, with the greatest abundance in the ileum ($P < 0.05$) both during suckling and postweaning.

There was an interaction ($P < 0.001$) between age and intestinal segment in the abundance of ATB^0 mRNA both during suckling and postweaning (Figure 3.16). The abundance of ATB^0 mRNA generally increased by 30 to 60 % from birth to d 14 then remained unchanged

with age to d 21 in the duodenum and ileum. In the jejunum, ATB^0 mRNA abundance increased by 50 to 60 % from birth to d 7 then decreased to close to the value at birth by d 21. Postweaning, in the duodenum, the abundance of ATB^0 mRNA generally increased from d 21 to 35. In the jejunum and ileum, ATB^0 mRNA abundance initially increased (60 % in the ileum and twofold in the jejunum) from d 21 to 24 (3 d after weaning) and declined to a value greater than that at weaning by d 28, and then changed slightly with age to d 35. The abundance of ATB^0 mRNA was generally greater in the ileum than the duodenum and jejunum during suckling and greater in the duodenum and ileum than the jejunum postweaning.

The abundance of $b^{0,+}AT$ mRNA increased quadratically ($P < 0.001$) with age during suckling (Figure 3.17). The abundance of $b^{0,+}AT$ mRNA increased from birth to d 3 (in the ileum) or d 7 (in the duodenum and jejunum) then slightly declined to d 21, at which time, the abundance was a little greater than that at birth. Postweaning, the abundance of $b^{0,+}AT$ mRNA increased (twofold in the jejunum, 30 % and 60 % in the duodenum and ileum, respectively) during the first day after weaning then rapidly declined to the level at weaning, followed by either a slight increase in the jejunum and ileum or a slight decrease in the duodenum to d 35. The abundance of $b^{0,+}AT$ mRNA was greater ($P < 0.05$) in the jejunum and ileum than the duodenum during suckling and was greatest ($P < 0.05$) in the jejunum postweaning.

The abundance of LAT2 mRNA declined linearly ($P < 0.001$) with age during suckling, with an overall 60 to 85 % decrease from d 0 to 21(Figure 3.18). Postweaning, the abundance of LAT2 mRNA increased (one-, two-, and fivefold in the duodenum, jejunum and ileum, respectively) from d 21 to 22, and rapidly declined to a barely detectable level on d 24 and then remained relatively stable with age through d 35. The LAT2 mRNA was predominantly ($P <$

0.05) distributed in the ileum during suckling and tended to be higher ($P = 0.09$) expressed in the jejunum and ileum than the duodenum postweaning.

Developmental Regulation of the mRNA Expression of Intestinal Iron Transporters. There was an age x intestinal segment interaction ($P < 0.05$) in the abundance of DMT1 mRNA both during suckling and postweaning (Figure 3.19). During suckling, DMT1 mRNA abundance increased with age in the duodenum, with an overall sevenfold increase from birth to d 21. In the jejunum and ileum, DMT1 mRNA abundance slightly increased from birth to d 3 and then plateaued through d 21. Postweaning, the abundance of DMT1 mRNA initially declined then generally remained unchanged through d 35, with a more rapid decline in the duodenum than in the jejunum and ileum during the first day after weaning. The DMT1 mRNA was predominantly ($P < 0.01$) distributed in the duodenal tissue both during suckling and postweaning.

There was also an age x intestinal segment interaction ($P < 0.05$) in the abundance of IREG1 mRNA both during suckling and postweaning (Figure 3. 20). During suckling, the abundance of IREG1 mRNA increased from birth to d 3 and remained relatively constant until d 14, and then slightly declined to d 21 in the duodenum. In the jejunum and ileum, the abundance of IREG1 mRNA generally increased from birth through d 14 and then slightly declined to d 21. Postweaning, the abundance of IREG1 mRNA declined from d 21 to 24 then slightly increased through d 35 in all intestinal segments. The IREG1 mRNA abundance was greater ($P < 0.01$) in the duodenum than the jejunum and ileum both during suckling and postweaning.

Discussion

It is well known that the growth, development, and maturation of the small intestine in piglets is rapid during the early stages of life, especially around birth and weaning, which may dramatically impact the intestinal digestive and absorptive function (Pachá, 2000). Many studies

have been conducted to investigate the interrelationship between gastrointestinal development and nutrient assimilation in piglets at early stages of life by measuring specific activity of intestinal nutrient transporters and digestive enzymes (Xu and Cranwell, 2003), however, little information is available for such development specific changes at the molecular level.

Thus, this study was conducted to investigate the developmental regulation of important nutrient transporters and brushborder membrane hydrolases in the small intestine of piglets during early stages of life at the molecular level. The aim was to better understand this process and the corresponding regulatory mechanisms and to eventually apply this knowledge to swine production and even clinical treatments.

During the 21-d suckling period, the piglet ADG was 15 to 20 % higher than reported from sows with 10 or more piglets (Aherne, 2001). The higher growth performance during suckling is probably attributable to the reduction in the number of littermates with age by the removal of piglets as the experiment progressed. From the middle to the end of the suckling period, the growth performance of piglets is apparently limited by the milk yield of sows, especially for those with a large number of littermates (Eissen et al., 2003). The decrease in the number of littermates during suckling could increase milk supply to individual piglets and therefore improve growth performance. After weaning, adequate feed intake by piglets was delayed up to 7 d, which is much longer than 1 to 3 d normally occurring during weaning (Bruininx et al., 2002). This phenomenon may be caused by two possible factors. One is that nutrient supply from the sow's milk was sufficient for piglet rapid growth, even during late suckling. Under this situation, the piglets are more willing to solely rely on milk for nutrients than those under larger litter conditions of semifood deprivationdue to milk shortage. Another factor may be that no creep feed was offered to piglets during the middle and late suckling period

in the present study. In commercial or other experimental conditions, the consumption of a limited amount of creep feed by piglets during suckling improved the transition from milk to physically and chemically different adult diets (Bruininx et al., 2002). The negative ADG during the first 7 d after weaning indicated that nutrient intake was not sufficient to maintain or promote the growth of the weaned piglets. Seven days after weaning, piglet ADG gradually improved as ADFI increased. The normal weaning transition usually causes a transient decrease in villus height and net absorption in the small intestine and the decrease is recovered by 7 to 9 d after weaning as feed intake resumes (Hedemann et al., 2003). However, in this study, the prolonged food deprivation may have had different and more deleterious effects on gut health and function.

The small intestinal brushborder membrane disaccharidases LPH, SI, and MGA are crucial in the final digestion of carbohydrates. In the present study, the abundance of intestinal LPH mRNA rapidly increased with age from birth to d 7 and remained relatively constant through the end of suckling. Postweaning, the abundance of LPH mRNA rapidly declined. Inversely, the abundance of SI and MGA mRNA slightly increased or remained unchanged with age during early suckling, and the abundance of SI mRNA then dramatically increased with age during middle and late suckling, and the abundance of MGA mRNA was stable during middle and late suckling and increased after weaning onto a solid diet. The SI and LPH protein expression generally exhibited similar developmental change patterns with their respective mRNA abundance. These data are consistent with the previous studies reported in rats. Intestinal sucrase and maltase activities were undetectable or very low during gestation and at birth (Sangild et al., 1995), and slightly increased during suckling, followed by a rapid increase after weaning (Krasinski et al., 1994). In contrast, lactase activity was high during the first few days after birth and slightly declined during late suckling, followed by a substantial decrease during

weaning, and then subsequently maintained a relatively low level throughout adulthood (Krasinski et al., 1994). Lactase and sucrase specific activities also correlated well with their respective protein and mRNA levels in rat small intestine (Krasinski et al., 1994). The authors speculated that the developmental regulation of these disaccharidases is largely due to the alterations in the transcriptional rate mediated by the interaction of transcriptional factor(s) with the regulatory element(s) within the promoter regions of these genes (Krasinski et al., 1994; Motohashi et al., 1997).

Functional analysis of the promoter region of the rat lactase gene revealed that a *cis*-element, CE-LPH1 interacted specifically with a nuclear protein, NF-LPH1 to regulate lactase transcriptional rate (Boukamel et al., 1994). The amount of NF-LPH1 was in direct proportion to lactase expression and activity during the suckling-weaning transition, thus NF-LPH1 was crucial in defining the developmental decline of lactase expression at weaning, primarily at the transcriptional level. Within a short, evolutionarily conserved promoter region of the mouse SI gene, a hepatocyte nuclear factor-1 (HNF-1) regulatory element (SIF3) was shown to be able to regulate SI gene expression (Tung et al., 1997; Boudreau et al., 2001). Nuclear proteins isolated from enterocytes showed increased binding of the HNF-1α complex with a concomitant decrease in the HNF-1β-containing complex to the SIF3 element during the suckling-weaning developmental transition. These changes coincided with a strong induction of SI gene transcription. In transfection experiments, HNF-1α activated the SI promoter via the SIF3 element, and coexpression of HNF-1β impaired this transcriptional activation. These findings demonstrate the essential role of HNF-1 regulatory element (SIF3) to regulate SI gene transcription and suggests that the ratio of HNF-1α to HNF-1β plays a role in the transcriptional activity of SI gene during intestinal development. However, further studies are needed to explore

what causes the alterations in the expression of these specific nuclear transcriptional factors and the binding activity to the regulatory element(s) within the promoter region during the suckling-weaning developmental transition.

The SGLT1, a Na^+-dependent glucose transporter, located on the brushborder membrane of enterocytes, is the primary transporter responsible for the uptake of luminal glucose and galactose in the small intestine (Wright et al., 2003). In the present study, SGLT1 mRNA and protein levels were high at birth. The developmental patterns in SGLT1 mRNA and protein expression differed afterward. The brushborder membrane SGLT1 protein expression generally remained unchanged or slightly declined with age during suckling and then substantially decreased to d 35. However, intestinal SGLT1 mRNA abundance generally increased with age during early and middle suckling. Postweaning, SGLT1 mRNA abundance also generally increased with age in the jejunum and ileum, except for a spike right after weaning, whereas SGLT1 mRNA abundance decreased with age in the duodenum. Results from previous studies have shown that Na^+/glucose cotransport is already detectable in the intestine during late embryogenesis in various animal species, such as chickens (Barfull et al., 2002), rats (Buddington and Diamond, 1989), and lambs (Lescale-Matys et al., 1993). The intestinal maximal capacity per tissue weight to transport glucose is reached the day after hatch or birth and declines afterward until attainment of adult levels, which is directly correlated with the alteration in the levels of intestinal SGLT1 protein but not mRNA (Lescale-Matys et al., 1993; Barfull et al., 2002). These findings collectively suggest that changes in the Na^+/glucose transport capacity and SGLT1 protein expression are due to a posttranscriptional regulatory mechanism. Barfull et al. (2002) demonstrated that intestinal SGLT1 protein level was 40 % lower in chickens at an age of 5 wk than at an age of 2 d, when chickens were fed a single standard diet from hatch until 5

wk of age. Conversely, chickens at 5 wk of age had twice the glucose concentration in the luminal contents compared with 2 d old chickens. In the present study, intestinal SGLT1 protein expression was higher during suckling than postweaning, although luminal glucose concentration is supporsedly to be higher in piglets after the completion of the transition from high protein milk to a high carbohydrate diet than in those during suckling. Therefore, these results suggest that, during the ontogenetic development of intestinal SGLT1, some signal(s) other than substrate concentration is responsible for the control of SGLT1 expression. Further research is needed to fully elucidate the precise molecular pathways that regulate SGLT1 expression by developmental stages.

Intestinal GLUT5 is a facilitative transporter specifically responsible for luminal fructose uptake, and the gene expression and the transport activity of GLUT5 is only regulated by its specific substrate, fructose but not other dietary carbohydrates (Shu et al., 1997). In the present study, intestinal GLUT5 mRNA abundance did not exhibit a well-defined developmental pattern of change in piglets during the early stages of life. In general, intestinal GLUT5 mRNA abundance was relatively constant during the time period studied. In a preliminary study, intestinal GLUT5 mRNA was undetectable by Northern blot using the standard radioactively labeled cDNA probe, but was later detected using the labeled cDNA probe with sevenfold higher specific activity (present study). Together, these results may indicate that intestinal GLUT5 mRNA abundance in piglets is low from birth through the end of weaning. In rat and rabbit small intestine, GLUT5 mRNA is normally undetectable by Northern blot at birth and during suckling, but is stimulated to substantial levels after the completion of weaning (Castello et al., 1995; Shu et al., 1997). Furthermore, results from studies have shown that this developmentally regulated time course of GLUT5 expression can be reprogrammed by precocious consumption of high

fructose diets, which results in marked increases in GLUT5 activity and mRNA abundance several days ahead of the natural schedule (David et al., 1995; Shu et al., 1997). The low GLUT5 mRNA abundance in the small intestine of piglets in this study, even after weaning, is probably related to the absence or low levels of fructose in the normal diets of piglets, and other dietary carbohydrates lack the ability to stimulate GLUT5 gene expression and transport activity. However, more information at the mRNA level by using a much more sensitive technique, such as real time RT-PCR and at the protein level is needed to fully and precisely understand developmental regulation of GLUT5 gene expression in the small intestine of piglets.

In pigs (Sangild et al., 1995) and rats (Krasinski et al. 1994), the LPH activity and gene expression increases during late gestation to high levels at birth (Sangild et al., 1995), preparing the intestine for the digestion of lactose from colostrum and milk after birth. This prenatal increase in LPH activity and gene expression may be related to genetic programming or the action of some hormone(s). In this study, an increase in LPH gene expression in the small intestine of piglets was observed during the early and middle suckling periods. This increase is probably stimulated by lactose intake from milk. The physiological significance of this stimulation is to increase the intestinal capability to digest lactose. It was reported that dietary lactose was able to upregulate intestinal SI and MGA activity and gene expression in adult rats (Goda et al., 1985). In the present study, intestinal SI and MGA gene expression was not substantially stimulated by lactose intake from milk during the first few days. This may be related to the genetic nature of intestinal enterocytes in neonates. However, SI mRNA and protein expression and hydrolytic activity did increase with age during the middle and late suckling period. It is unclear if the induction of SI gene expression and hydrolytic activity results from genetic programming which prepares the small intestine for high starch diets and/or the

cumulative action of milk lactose stimulation or other mechanism(s). The distribution pattern of the gene expression of disaccharidases along the small intestine in piglets is primarily related to their luminal substrate concentrations. Lactose and starch are the primary carbohydrate sources in milk and adult diets, respectively. Lactose is a direct substrate for LPH, whereas starch takes a long digestion journey along the small intestine to provide the substrate for SI and MGA. Thus, it is reasonable that LPH gene expression was greater in the duodenum and jejunum than the ileum during suckling, and the gene expression of SI and MGA was greater in the jejunum and ileum than the duodenum postweaning in the present study. Together, these resulted in more glucose and galactose released into the proximal and middle parts of the small intestine during suckling, and more monosaccharides released in the middle and distal parts of the small intestine postweaning. The resulting gradient of luminal monosaccharides can explain why SGLT1 mRNA and protein expression was greater in the duodenum and jejunum than the ileum during suckling and then greater in the jejunum and ileum than the duodenum postweaning.

Aminopeptidase A, APN, and DPP IV are three major brushborder membrane peptide hydrolases involved in the final digestion of luminal proteins into small peptides and free amino acids, which are efficiently absorbed in the small intestine via PepT1 and a variety of amino acid transporters expressed on the brushborder membrane of enterocytes, respectively (Daniel, 2004). We observed that, in the small intestine of piglets, the mRNA abundance of these three peptide hydrolases increased with age during the first few days after birth then remained constant or slightly decreased to the end of suckling. Results from previous studies have shown that piglet intestinal brushborder membrane peptidase activity increased then declined with age during suckling (Sangild et al., 1991; Tarvid et al., 1994). Thus, the results from the present study provided the molecular basis for the ontogenetic changes in intestinal brushborder membrane

peptidase activities during suckling. The increase in mRNA abundance and potentially in the protein abundance of these brushborder peptide hydrolases may be attributable to the stimulation of gene expression and protein synthesis by suckling colostrum and milk, which are rich in proteins and growth factors. The physiological function of this stimulation is to increase the intestinal peptide hydrolytic activities to match the increased protein intake. The decrease in mRNA expression of these brushborder hydrolases during late suckling may be related to the relatively low luminal protein content compared to the rapidly increased intestinal dimension and mucosal mass. Later on, the mRNA abundance of these peptidases was transiently upregulated after weaning then recovered to the previous level during suckling. However, Hedemann et al. (2003) demonstrated that the intestinal hydrolytic activities of APN and DPP IV declined during the first 3 d after weaning then increased to d 9 after weaning. This discrepancy between the mRNA abundance and specific activities of peptide hydrolases can be explained by two potential mechanisms. One mechanism involves the time lag between the synthesis of mRNA and the final insertion of mature peptide hydrolase protein into the brushborder membrane of enterocytes. Another mechanism involves the villus atrophy with loss of mature enterocytes due to weaning, which greatly decreases the specific activities of the brushborder peptide hydrolases but reduces the mRNA abundance to a much lesser extent (Hedemann et al., 2003).

In the present study, the mRNA abundance of intestinal PepT1 was generally constant with age, and PepT1 mRNA was evenly distributed among intestinal segments. The PepT1 protein expression was high at birth. It gradually decreased from birth through d 35 in the duodenum. In the jejunum and ileum, PepT1 protein level rapidly decreased from birth to d 3 then slightly increased to the end of suckling, followed by an increase with age to d 35. In rats, a similar developmental pattern of PepT1 mRNA and protein expression was observed. After

increasing to achieve the perinatal peak value around d 3 to 5, the abundance of intestinal PepT1 mRNA and protein declined rapidly to lowest levels by d 14, followed by an increase to an intermediate level by d 24, 3 d after weaning, and at adulthood (Miyamoto et al., 1996; Shen et al., 2001). The ontogenetic regulation of intestinal PepT1 gene expression might be induced postpartum by suckling and later weaning, in an adaptive response to changes in the diet, from high-protein milk to an adult diet containing more carbohydrate than protein (Shen et al., 2001). It was reported that the serum concentration of thyroid hormone rose from d 5 to 15 in rats (Henning, 1981) as the expression levels of PepT1 declined. Thyroid hormone was reported to downregulate the expression of PepT1 in Caco-2 cells (Ashida et al., 2002). Therefore, thyroid hormone may be involved in the developmental regulation of PepT1 expression.

The EAAC1 is a high affinity glutamate transporter. In neonatal piglets, EAAC1 is the primary glutamate transporter in the small intestine (Fan et al., 2004). It is well recognized that glutamate is the major energy fuel in the small intestine of animals (Windmueller et al., 1980). In neonatal piglets, EAAC1 expression was restricted to the lower villus and the crypt of the small intestine, and the V_{max} of glutamate transport via EAAC1 was higher in the differentiating and proliferating cells than the differentiated cells (Fan et al., 2004). Therefore, the major physiological role of EAAC1 in the small intestine may be to meet the catabolism of enterocytes themselves rather than to provide the nutritional supply to the extra-intestinal tissues. In the present study, EAAC1 mRNA abundance was predominantly distributed in the distal part of the small intestine in piglets, which is consistent with the results reported in rats (Erickson et al., 1995; Rome et al., 2002; Howard et al., 2004) and pigs (Zhao, 2005). These results may suggest that the distal part of the small intestine is the major glutamate absorption site. In the ileum, EAAC1 mRNA abundance was low at birth and generally increased with age during the

experimental period studied, which may suggest that EAAC1 provides more glutamate as energy fuel to mucosal cells as the intestine matures. The increase in EAAC1 mRNA abundance with age in the ileum may possibly be related to the nutritional transition as well as the increases in the activities of brushborder membrane peptide hydrolases to break the peptides into free amino acids.

The ATB^0 is a Na^+-dependent, broad-spectrum neutral amino acid transporter and is the major brushborder membrane glutamine transporter in the small intestine (Avissar et al., 2004). The $b^{0,+}AT$ is the light chain of amino acid transport system $b^{0,+}$, which facilitates the Na^+-independent transport of neutral or basic amino acids into enterocytes in exchange for intracellular neutral amino acids (Verrey et al., 2003). The heavy chain of this heterodimeric amino acid transporter is NBAT (Verrey et al., 2003). For system $b^{0,+}$, $b^{0,+}AT$ protein assumes full transport activity in the absence of NBAT, but in enterocytes, coexpression with NBAT is required for the translocation of $b^{0,+}AT$ to the brushborder membrane (Reig et al., 2002). In the present study, we observed that the mRNA abundance of ATB^0 and $b^{0,+}AT$ increased with age during early and middle suckling and then slightly declined or remained unchanged to the end of suckling. The increase in the mRNA abundance of ATB^0 and $b^{0,+}AT$ with age during early suckling is most likely induced by the high concentration of luminal amino acid substrates after suckling colostrum or milk. Interestingly, the mRNA abundance of ATB^0 was upregulated during the first 3 d after weaning in the jejunum and ileum, unlike other genes examined, where the transient spike of their mRNA abundance was observed only during the first day after weaning. The important physiological function of this prolonged upregulation of ATB^0 gene expression may be to scavenge the luminal glutamine into the mucosal cells. The absorbed glutamine may serve as potent fuel to prevent intestinal atrophy caused by weaning. The general

increase in ATB^0 mRNA abundance from birth to d 35 may be related to the changes in amino acid composition and levels of milk and adult diets. The lowest ATB^0 mRNA abundance in the jejunum was observed during late suckling and postweaning. This distribution pattern is different from the results reported in rabbits, in which ATB^0 mRNA and protein expression exhibited an increasing gradient along the proximal-distal intestinal axis, with the greatest expression in the ileum (Avissar et al., 2001a and b).

Unlike other amino acid transporters examined in the present study, LAT2 mRNA abundance displayed a reverse developmental pattern. The abundance of LAT2 mRNA generally decreased with age from birth through d 35, except for a transient spike on d 22, 1 d after weaning. In the small intestine of mice (Rossier et al., 1997; Dave et al., 2004), LAT2 mRNA and protein was detected primarily on the basolateral membrane of enterocytes, where it colocalized with 4F2hc, a heavy chain subunit of system L. The physiological role of LAT2 is to release smaller intracellular neutral amino acids in exchange for larger extracellular neutral amino acids and thereby equilibrate their relative concentrations in intestinal enterocytes (Dave et al., 2004). Reeds et al. (2000) demonstrated that amino acids from arterial sources taken across basolateral membranes are used in mucosal protein synthesis and energy generation, and even in the fed state, there is uptake from basolateral membranes. Thus, the high level of LAT2 mRNA in the small intestine of piglets at birth may indicate that LAT2 plays a critical role in absorbing certain neutral amino acids from the bloodstream to nourish mucosal cells during late gestation. During that period, luminal nutrients are mainly derived from the swallowed amniotic fluid, which contains a dilute concentration of nutrients, including glucose, and free amino acids (Boudry et al., 2000). Therefore, luminal nutrient supply is limited for intestinal enterocytes, especially for those located in the distal part of the small intestine. However, this necessity of

amino acid uptake from bloodstream to nourish the mucosal cells gradually decreased as the nutrient transporters, including amino acid transporters expressed on the brushborder membrane of intestinal enterocytes, take over the responsibility of the uptake of luminal nutrients from colostrum or milk after birth.

During early suckling, intestinal PepT1 mRNA abundance was generally constant, and PepT1 protein expression decreased with age, whereas the mRNA abundance of amino acid transporters and peptide hydrolases expressed on the brushborder membrane of enterocytes increased with age. These results suggest that the increased amount of free amino acids generated in the small intestinal lumen is due to the increase in the peptide hydrolase activities, which have been shown to be the rate-limiting enzyme(s) in the final digestion of medium and short chain peptides into free amino acids and small peptides (Daniel, 2004). The increase in the activities of brushborder peptide hydrolases may be induced by high protein intake through the upregulation of their gene expression. The mRNA abundance of these peptide hydrolases was generally higher in the middle and distal parts of the small intestine than in the proximal part, which is consistent with the previous reports in rabbit (Freeman, 1995), and rats (Hong et al., 1989; Troyanovskaya et al., 2000). This indicated that the middle and distal parts of the small intestine are the major sites for the terminal digestion of luminal protein to generate free amino acids, which lead to the concomitant greater mRNA abundance of amino acid transporters in the middle and distal parts of the small intestine than in the proximal part. In the jejunum and ileum, the rapid decrease in PepT1 protein expression during the early and middle suckling period is accompanied by a rapid increase in the gene expression and functinal activities of the brushborder membrane peptide hydrolases and free amino acid transporters. The decreased PepT1 protein along with the increased specific activities of these peptidases and free amino acid

transporters may be due to the competition of the hydrolytic enzymes over PepT1 for di-and tripeptide substrates to break them down into free amino acids.

It is well recognized that iron transporters DMT1 and IREG1 play a critical role in intestinal iron absorption and body iron homeostasis, and intestinal iron absorption is tightly correlated with intestinal DMT1 and IREG1 gene expression (Leong et al., 2003). In the present study, intestinal DMT1 and IREG1 mRNA was predominantly distributed in the duodenum of piglets. In the duodenum, during suckling, DMT1 and IREG1 mRNA abundance increased with age then slightly declined to the end of suckling. The DMT1 and IREG1 mRNA abundance dramatically decreased after weaning then stayed at low values through d 35. The similar distribution pattern of DMT1 and IREG1 mRNA and protein was also observed in mice and rats. In the small intestine of mice and rats, DMT1 and IREG1 mRNA and protein was predominantly expressed in the duodenum and exhibited a decreased gradient along the intestinal proximal-distal axis (Gunshin et al., 1997; Canonne-Hergaux et al., 1999; Abboud and Haile**,** 2000). The DMT1 and IREG1 gene expression was regulated by dietary iron intake in the duodenum but not in the rest of the small intestine (Gunshin et al., 1997; Zoller et al., 2001). Thus, the duodenum appears to be the primary site for intestinal iron absorption and its regulation. The increase in DMT1 and IREG1 gene expression with age during early suckling was also observed in rats (Leong et al., 2003). In the present study, the piglets were supplemented with 200 mg iron by muscle injection on d 3, which should eliminate the possibility of iron deficiency afterward. Interestingly, although piglets declined to eat feed during the first 3 to 7 d after weaning, DMT1 and IREG1 mRNA abundance was substantially downregulated with age during this period. The increases in DMT1 and IREG1 gene expression with age during suckling to increase iron absorption regardless of body iron status may be related to the genetic rigidity of intestinal

enterocytes and/or the lack of communication between the body iron status and iron absorption in immature intestine (Leong et al., 2003). Its physiological role is to maximize the iron absorption from milk, which is usually low in iron compared to the iron requirement of neonates during early stages of life (Domellöf et al., 2001). The rapid decrease of DMT1 and IREG1 mRNA abundance after weaning may also be due to the inherent genetic programming, which signals the small intestine to decrease iron absorption from high iron diets by downregulation of gene expression of intestinal iron transporters.

Among the hydrolases and nutrient transporters tested in this study, the mRNA abundance of several genes (SGLT1, GLUT5, PepT1, LPH, LAT2, and DMT1) exhibited a transient decline during the first day after birth. This transient decline most likely results from the rapid cell proliferation in the crypts triggered by the onset of suckling colostrum or milk (Zhang et al., 1997). The rapid cell proliferation in the crypt generally decreases the relative content of mature enterocytes in the whole intestinal cell population. The cells derived from the crypt region migrate toward the tip of the villus as they mature and this maturation process takes 3 to 7 d, and only mature enterocytes display maximal gene expression and functional activity (Ferraris, 2001). This notion was further supported by the results of gene expression at protein levels, in which PepT1, SGLT1, and LPH protein content in the BBMV did not differ between d 0 and 1, as the intestinal BBMV are solely derived from enterocytes. The magnitude of the regulation of gene expression and cell proliferation will determine how the intestinal gene expression changes at the mRNA level. Generally, gene expression of most nutrient transporters and hydrolases is upregulated by the onset of suckling colostrum or milk, which are rich in nutrients and growth factors, thus, the hydrolyzing and transport activity of the entire small intestine increases due to the dramatic increase in the intestinal mucosal mass (Zhang et al.,

1997). A transient increase during the first day after weaning was observed in the jejunum and ileum for many of the genes examined. This phenomenon could be interpreted by the transient food deprivationrelated to weaning and/or the changes in systemic or local hormones. More studies are needed to elucidate the precise underlying mechanisms, since most of the previous studies missed this sampling time point. The later decline in gene expression during the first 3 to 7 d after weaning is largely related to the loss of mature enterocytes due to villus atrophy and the elongation of the crypts, which together results in decreasing the percentage of mature enterocytes in the entire intestinal cell population and consequently the decrease in the relative mRNA abundance (Hedemann et al., 2003).

In this study, we investigated the developmental regulation of gene expression of various important hydrolases and nutrient transporters, as well as their distribution along the small intestine. This is the first reported study with an attempt to simultaneously investigate the developmental regulation of the important genes involved in the intestinal assimilation of dietary nutrients. The results from the present study have broadened our knowledge on the ontogenetic regulation of intestinal digestive and absorptive functions at the molecular level in piglets during the early stages of life. With the new knowledge gained from the present study at hand, swine nutritionists may be able to formulate more appropriate diets to achieve the genetic potential of pig lines at the critical early stages of life. To fully understand this developmental regulation process, further studies are needed in several fields. These research fields include but are not limited to: 1) provide detailed information about the intestinal mass, mucosal weight, and architectural structure along developmental regulation of nutrient transporters and hydrolases at the molecular level, 2) provide detailed information about developmental regulation of these genes at the protein and functional activity levels, as well as their distribution along the crypt-

villus axis, 3) elucidate the mechanisms by which the expression of these genes are developmentally regulated and differentially distributed, and 4) quantify the nutritional role of PepT1 in overall amino acid absorption in the small intestine of young animals.

Table 1. Ingredient and nutrient composition of the postweaning diet

Ingredients,	%
Corn	38.97
Dried whey, 12 % CP	20.75
Soybean meal, 46.5 % CP	20.00
Fish meal, 60 % CP	6.00
Lactose	5.00
Spray-dried animal plasma	4.00
Corn oil	2.50
Dicalcium phosphate	0.69
Salt	0.40
Limestone	0.35
Vitamin premix [a]	0.25
Trace mineral premix [b]	0.15
L-lysine·HCl, 78.8 %	0.11
DL-Methionine, 99 %	0.10
Analyzed nutrient composition	
CP, %	23.59
Lysine, %	1.42
Calculated nutrient composition	
Lysine, %	1.60
ME, kcal/lb	1,554
Crude fat, %	5.59
NDF, %	6.44
Ca, %	0.90
P, %	0.79
P, avail. %	0.55

[a]Supplied per kg of diet: Vitamin A (as retinyl acetate), 6,600 IU; Vitamin D (as cholecalciferol), 659 IU; Vitamin E (as DL-tocopherol acetate), 66.15 IU; Vitamin K (as menadione sodium bisulfite), 4.35 mg; riboflavin, 11.0 mg; d-pantothenic acid, 22.0 mg; niacin, 33.0 mg; vitamin B12, 22 ug; folate,1.65 mg; biotin, 0.22 mg.

[b]Supplied per kg of diet: Zn (as ZnO), 126.5 mg; Fe (as $FeSO_4 \cdot H_2O$), 126.5 mg; Mn (as MnO), 30.0 mg; Cu (as $CuSO_4 \cdot 5\ H_2O$), 10.45 mg; I (as $Ca(IO_3) \cdot H_2O$, 0.29 mg; Se (as N_a2SeO_3), 0.30 mg.

Table 2. Primer sequences for synthesis of cDNA probes [a]

Gene Names [b]	Sense primer (5' to 3')	Anti-sense primer (5' to 3')
GAPDH	ATGCCTCCTGTACCACCAAC	CACAACCTGGTGCTCAGTGT
LPH [c]	CTCAGGTGTACAAGTTCT	AGCATGAAGTGCAAGAAG
SI [c]	TGGCCATCCAGTCATGCC	CCACCACTCTGCTGTGGA
MGA [c]	CAGAAGCTGCCAAGACTGTG	AGAACTGTTGGTGCACATCC
APA [c]	GTCTCTACCACCTGACGAT	CTCTGTAAGTGATGAGTCC
APN [c]	ACATCACTCTCATCCACCCT	GCAATCACAGTGACAACTCG
DPP IV[c]	CCTCCGGCGTCTGTGTTA	TGGATTCAGCTCACAGCT
SGLT1	AGTGGGCAGCTCTTCGATTA	TGAATGTCCTCCTCCTCTGC
GLUT5	CACGTCCAGACCCAAGATG	GGGACAACATTGGAGGAGAG
ATB^0	ATCACTATCCTGGTCACCGC	AGTCAAGAACGGGGGAGATT
EAAC1[d]	GGGAAGATCAT(ACT)GA(AG)GTC	AACCGGTCCAGCA(AG)CCA(AG)TC
$b^{0,+}AT$ [d]	AGACTTGTTTATGTGGCGGG	GGAGGCTGAGCTTGTTACTC
LAT2	CGGAGACTGGTTCTGGAGAG	AGTTGACCCATGTGAGGAGC
DMT1	AGGATCTAGGGCATGTGGTG	TGTGTGGCCATCATAGGGTA
IREG1	TCCCTGAGATGAGCCCTAAA	GGACACCCAGCCATTTATTG

[a] All the primers were synthesized by MWG-Biotech (High Point, NC).

[b] GAPDH: glyceraldehyde-3-phosphate dehydrogenase; LPH: lactase-phlorizin hydrolase; MGA: maltase-glucoamylase; SI: sucrase-isomaltase; APA: aminopeptidase A; APN: aminopeptidase N; DPP IV: dipeptidyl peptidase IV; SGLT1: Na^+-dependent glucose transporter 1; GLUT5: Na^+-independent glucose facilitated transporter 5; EAAC1: excitatory amino acid carrier 1;

ATB^0: Na^+-dependent neutral amino acid transporter; $b^{0,+}AT$: the light chain of a heterodimeric transport system $b^{0,+}$ involved in the heteroexchange of cationic and neutral amino acids; LAT2: Na^+-independent large branched and aromatic neutral amino acid transporter 2; DMT1: divalent metal ion transporter 1; IREG1: iron-regulated transporter 1.

[c] The primers of LPH, SI, MGA APA, APN, and DPP IV were adopted from Petersen et al. (2002).

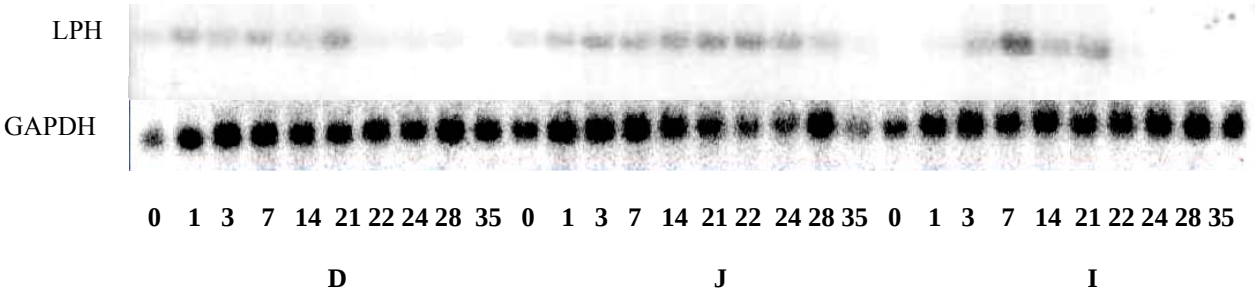
[d] The primers were designed as degenerate primers based on the conserved sequence region among human, mouse, rat, and rabbit.

Table 3. Growth performance and feed intake of piglets during the experimental period

Item	No. of Piglets	ADFI [a], g	ADG [a], g
Suckling (d 0 to 21)	7	Not recorded	278
Postweaning (d 21 to 35)			
d 21 to 22	7	52	-5
d 22 to 24	7	126	-102
d 24 to 28	7	189	6
d 28 to 35	7	494	251

[a] average daily feed intake (ADFI) and average daily gain (ADG).

A.



B.

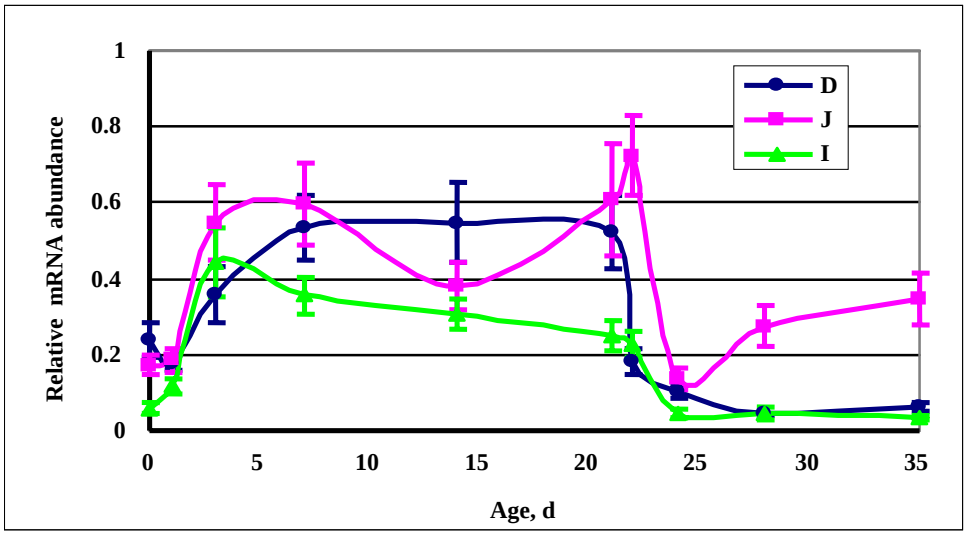
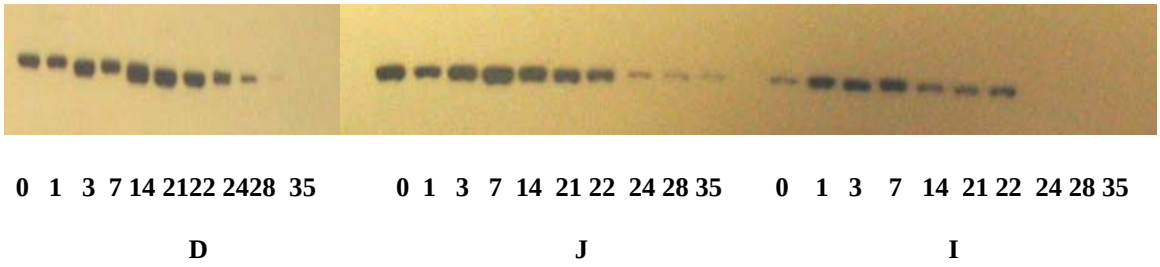


Figure 3.1. Northern blot analysis of lactase-phlorizin hydrolase (LPH) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent LPH mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of LPH to GAPDH. Each point represents average ratio of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). During suckling, there was a quadratic ($P < 0.05$) effect with age and LPH mRNA abundance was greater ($P < 0.05$) in the duodenum and jejunum than the ileum. Postweaning, there was a quadratic ($P < 0.05$) effect with age and LPH mRNA abundance was greatest ($P < 0.05$) in the jejunum.

A.



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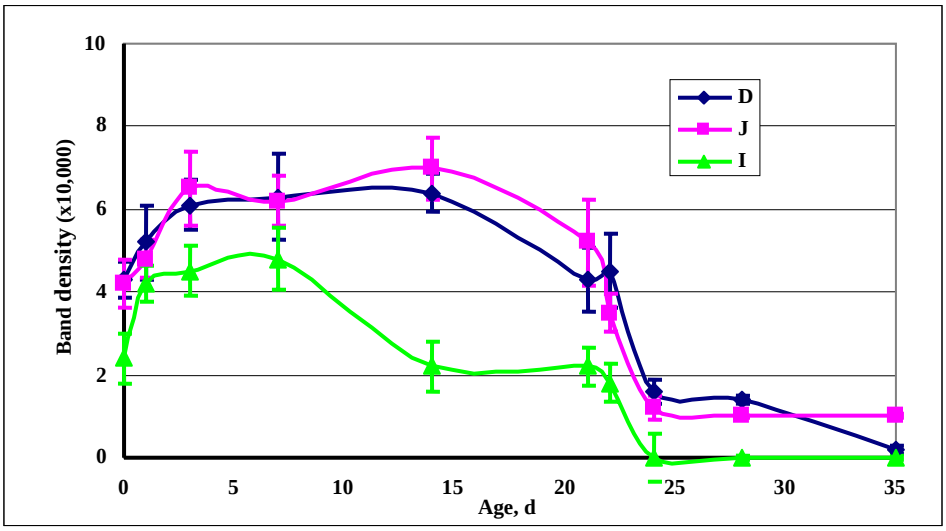
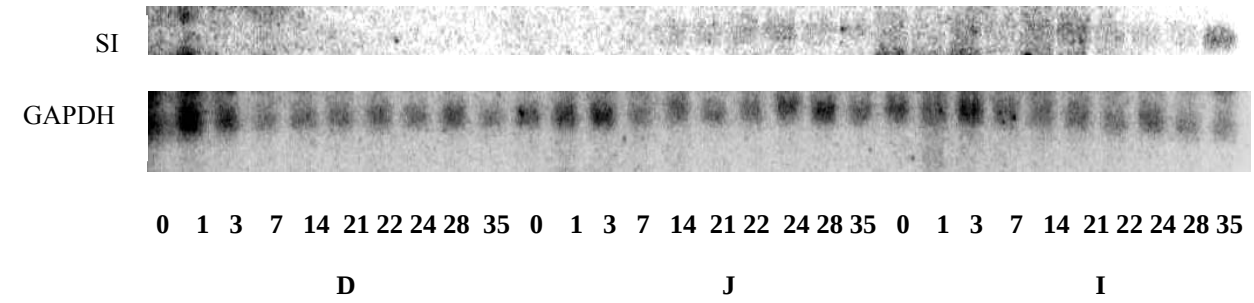


Figure 3.2. Western blot analysis of lactase-phlorizin hydrolase (LPH) protein expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Brushborder membrane fraction was isolated from individual intestinal tissues. Ten microgram of brushborder membrane protein from each sample was run on 7.5 % SDS-PAGE then transferred on PVDF membranes. The blot represents one of the seven replicate membranes (**A**). Each membrane comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Bright bands represent LPH protein. The densitometric data from the Western blots are presented in **B.** Data are presented as the absolute densitometric readings of LPH. Each point represents average reading from seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The expression of LPH protein changed quadratically ($P < 0.01$) with age both during suckling and postweaning. The expression of LPH protein was higher ($P < 0.01$) in the duodenum and jejunum than the ileum both during suckling and postweaning.

A.



B.

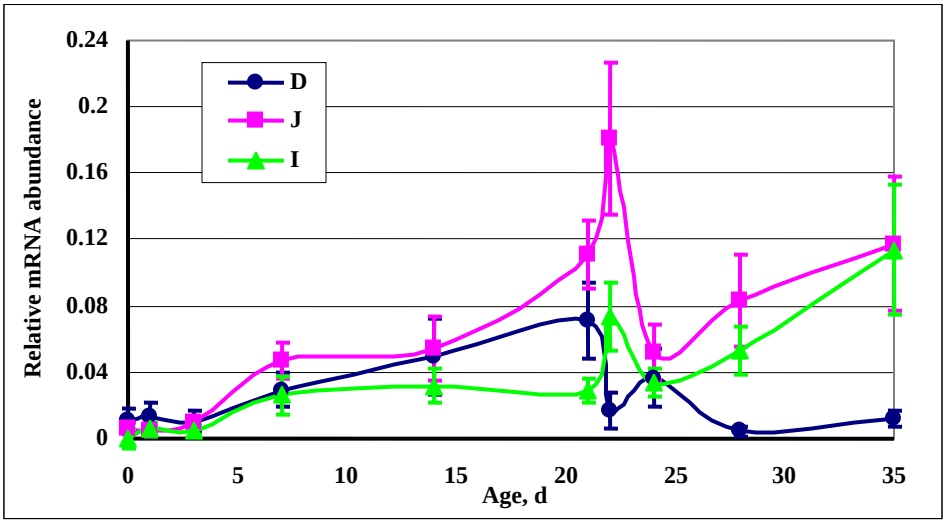
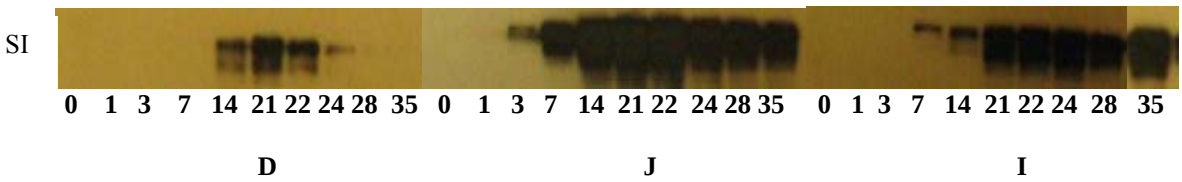


Figure 3.3. Northern blot analysis of sucrase-isomaltase (SI) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent SI mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of SI to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The mRNA abundance of SI increased linearly ($P < 0.001$) with age during suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.01$) for the mRNA abundance of SI. The mRNA abundance of SI was greater ($P < 0.05$) in the duodenum and jejunum than in the ileum during suckling and was greater ($P < 0.05$) in the jejunum and ileum than in the duodenum postweaning.

A.



B.

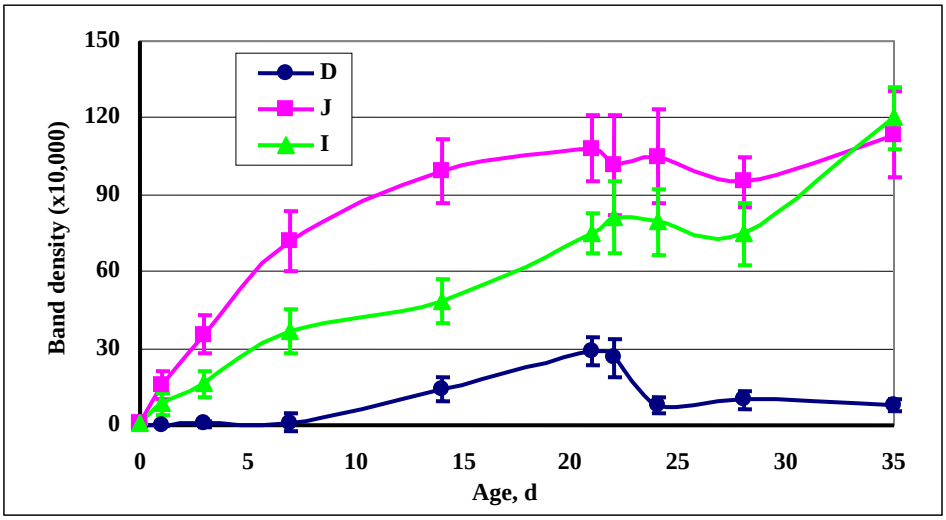


Figure 3.4. Western blot analysis of sucrase-isomaltase (SI) protein expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 per age group). Brushborder membrane fraction was isolated from individual intestinal tissues. Ten microgram of brushborder membrane protein from each sample was run on 7.5 % SDS-PAGE then transferred on PVDF membranes. The blot represents one of the seven replicate membranes (**A**). Each membrane comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Bright bands represent SI protein. The densitometric data from the Western blots are presented in **B.** Data are presented as the absolute densitometric readings of SI. Each point represents average reading from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The expression of SI protein increased linearly ($P < 0.001$) with age during suckling, and there was an age x intestinal segment interaction ($P < 0.01$) in the expression of SI protein postweaning. The protein expression of SI was greatest ($P < 0.001$) in the jejunum and lowest ($P < 0.001$) in the duodenum during suckling and was greater ($P < 0.001$) in the jejunum and ileum than the duodenum postweaning.

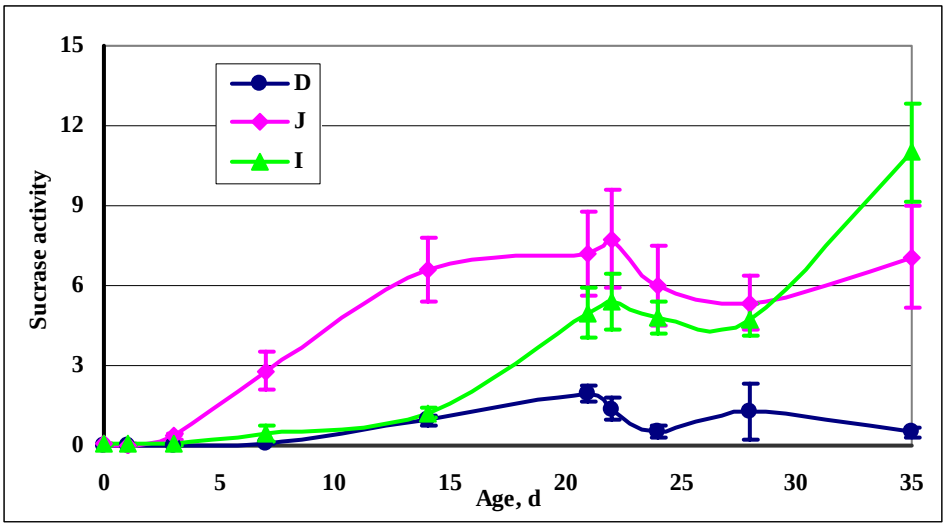
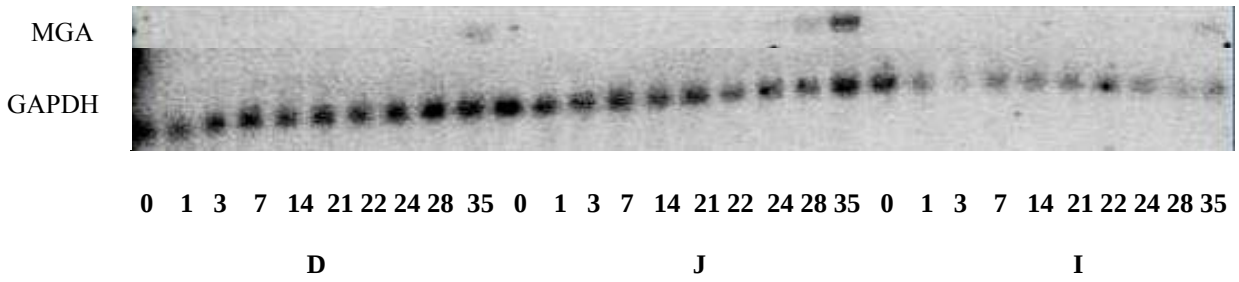


Figure 3.5. Developmental regulation of sucrase activity in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Brushborder membrane fraction was isolated from individual intestinal tissues. Sucrase activities were measured by using brushborder membrane fraction samples. Each point represents average specific activity from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM) and was expressed as μg of glucose released per mg brushborder membrane protein when incubated at 37 ^{o}C for 30 min. For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The intestinal sucrase activity increased linearly ($P < 0.001$) with age during suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.01$) in sucrase activity. The sucrase activity was higher ($P < 0.01$) in the jejunum than the duodenum and ileum during suckling and was higher ($P < 0.001$) in the jejunum and ileum than the duodenum postweaning.

A.



B.

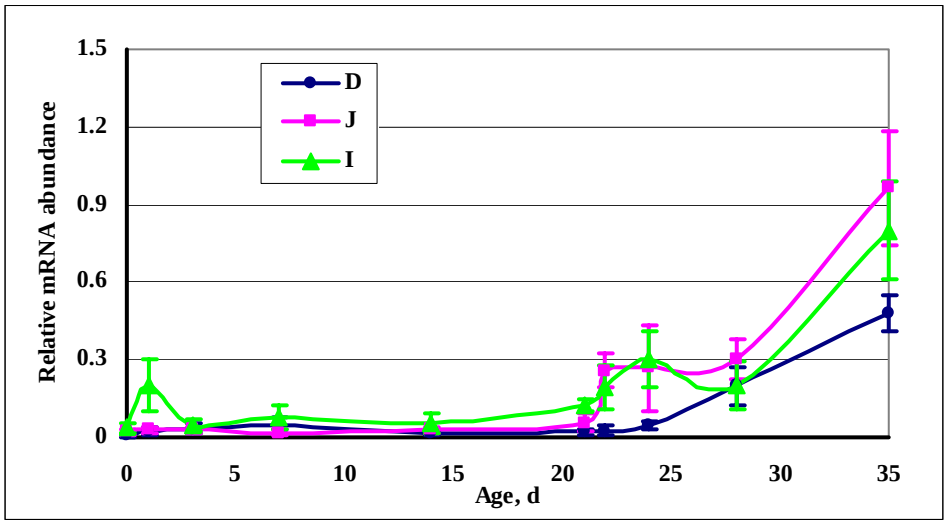
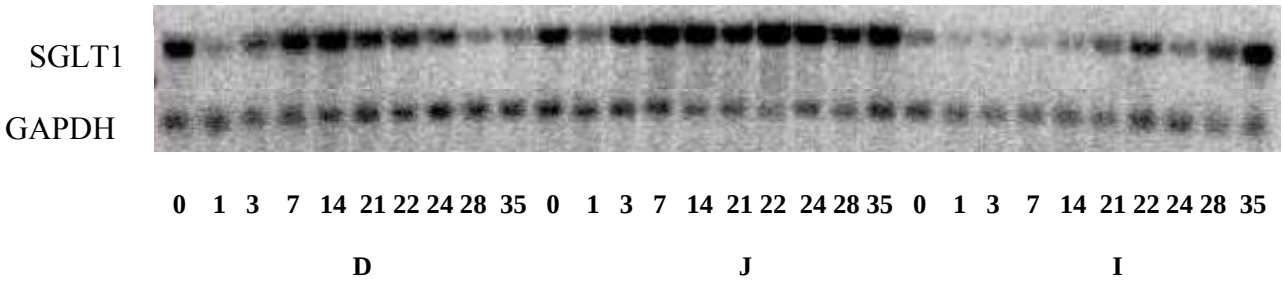


Figure 3.6. Northern blot analysis of (maltase-glucoamylase) MGA mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent MGA mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of MGA to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The mRNA abundance of MGA was low and relatively constant with age during suckling, and increased quadratically ($P < 0.05$) with age postweaning. The abundance of MGA mRNA was greatest ($P < 0.05$) in the ileum during suckling and was greater ($P < 0.05$) in the jejunum and ileum than the duodenum postweaning.

A.



B.

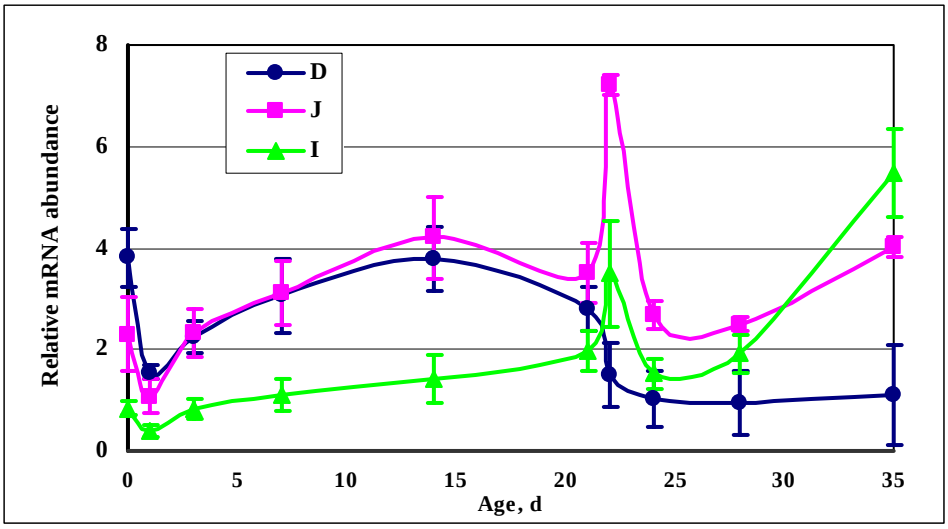
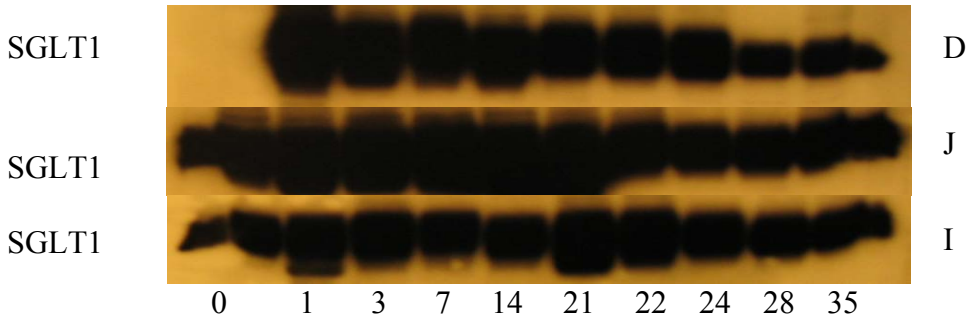


Figure 3.7. Northern blot analysis of Na^+-dependent glucose transporter 1 (SGLT1) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent SGLT1 mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of SGLT1 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). During suckling, the mRNA abundance of SGLT1 declined from birth to d 1 then generally increased to d 21.Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) in the mRNA abundance of SGLT1. The SGLT1 mRNA abundance was greater ($P < 0.05$) in the duodenum and jejunum than the ileum during suckling and was greater ($P < 0.05$) in the jejunum and ileum than the duodenum postweaning.

A.



B.

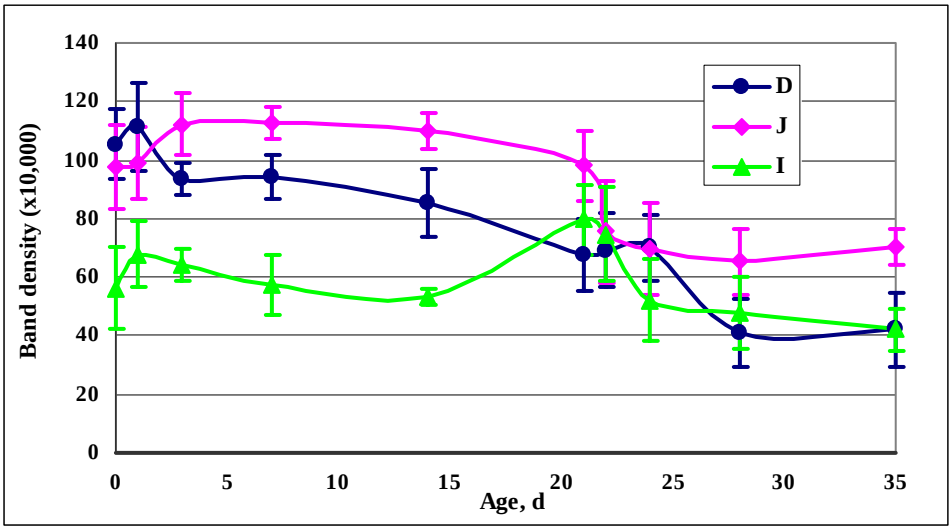
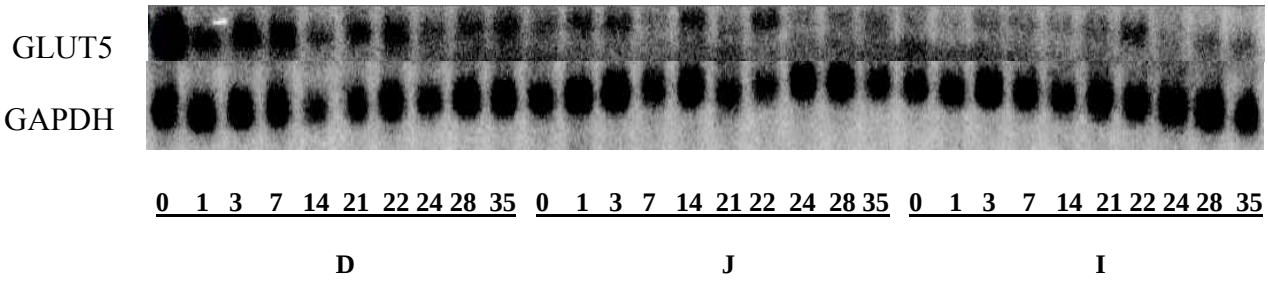


Figure 3.8. Western blot analysis of Na^+-dependent glucose transporter 1 (SGLT1) protein expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Brushborder membrane fraction was isolated from individual intestinal tissues. Ten microgram of brushborder membrane protein from each sample were run on 7.5 % SDS-PAGE then transferred on PVDF membranes. The blot represents one of the seven replicate membranes (**A**). Each membrane comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Bright bands represent SGLT1 protein. The densitometric data from the Western blots are presented in **B.** Data are presented as the absolute densitometric readings of SGLT1. Each point represents average reading from seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). There was an age x intestinal segment interaction ($P < 0.05$) in the expression of SGLT1 protein both during suckling and postweaning. The expression of SGLT1 protein was higher ($P < 0.01$) in the duodenum and jejunum than the ileum during suckling.

A.



B.

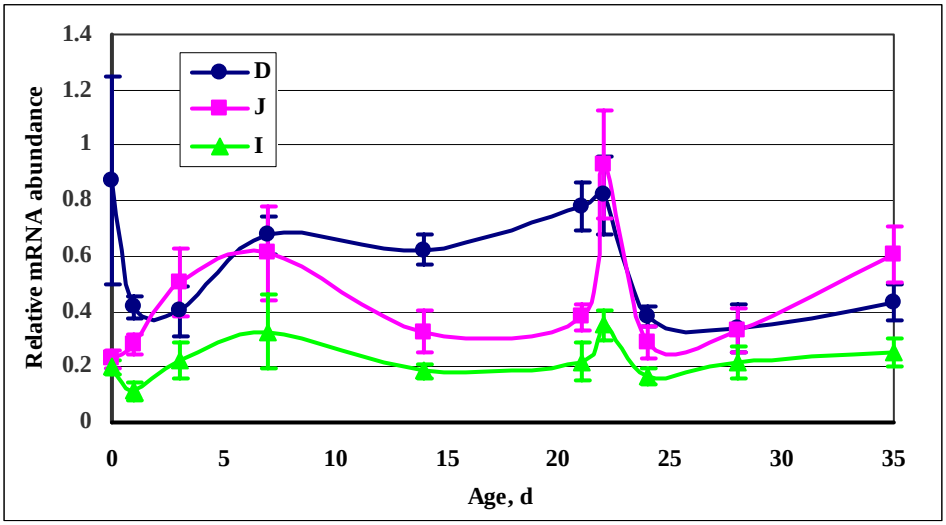
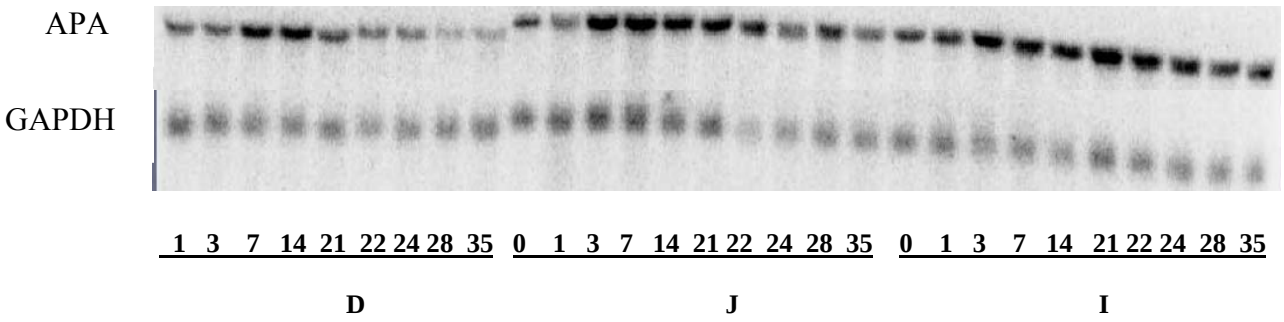


Figure 3.9. Northern blot analysis of GLUT5 mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent GLUT5 mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of GLUT5 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). There was an interaction ($P < 0.01$) between age and intestinal segment in GLUT5 mRNA abundance during suckling. Postweaning, the mRNA abundance of GLUT5 peaked at d 22 then rapidly declined to d 24, followed by a slight increase to d 35. The mRNA abundance of GLUT5 tended to be greater in the duodenum and jejunum than the ileum during suckling ($P = 0.18$) and postweaning ($P = 0.14$).

A.



B.

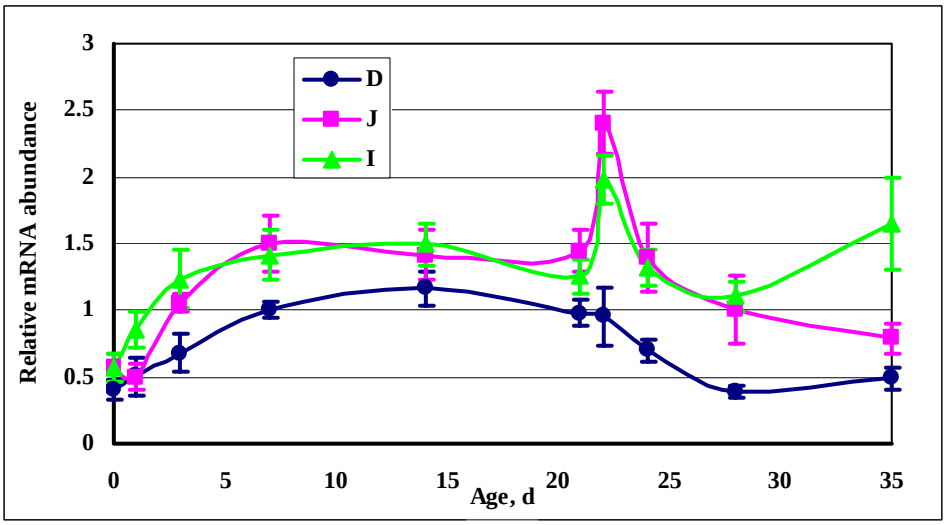
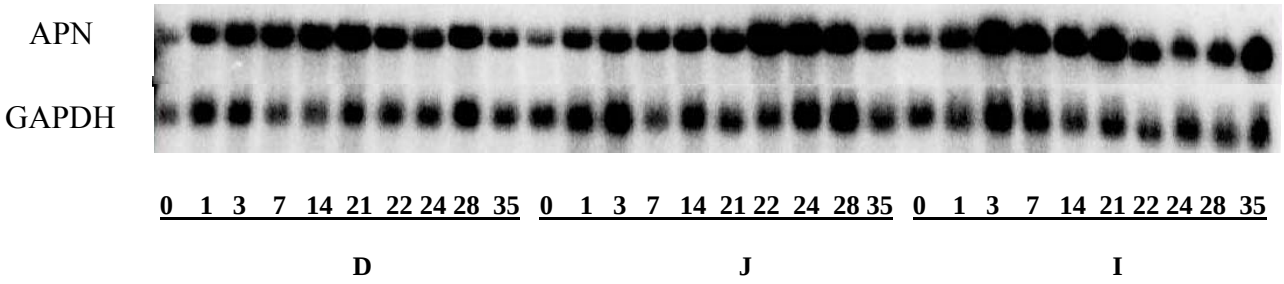


Figure 3.10. Northern blot analysis of aminopeptidase A (APA) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent APA mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of APA to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The mRNA abundance of APA increased quadratically ($P < 0.001$) with age during suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) for the mRNA abundance of APA. The expression of APA mRNA was higher ($P < 0.05$) in the jejunum and ileum than the duodenum both during suckling and postweaning.

A.



B.

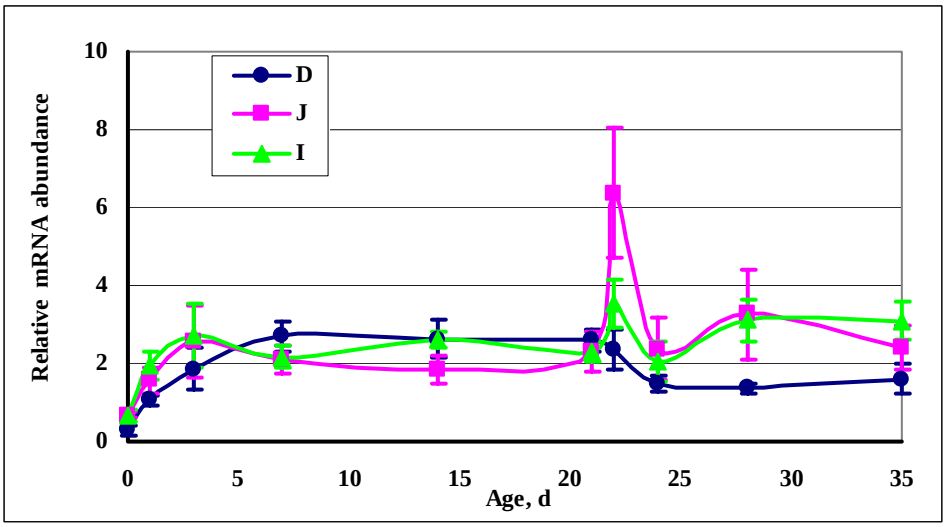
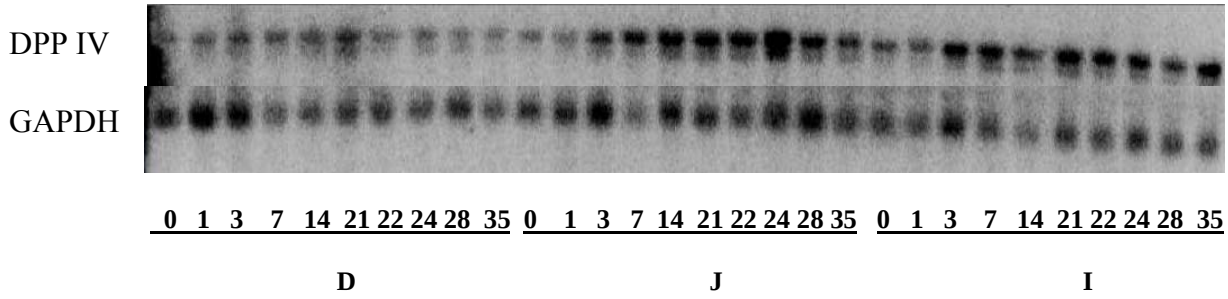


Figure 3.11. Northern blot analysis of aminopeptidase N (APN) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent APN mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of APN to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The mRNA abundance of APN increased quadratically ($P < 0.001$) with age during suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) in the mRNA abundance of APN. The mRNA abundance of APN did not differ among intestinal segments during suckling and was greater ($P < 0.05$) in the jejunum and ileum than the duodenum postweaning.

A.



B.

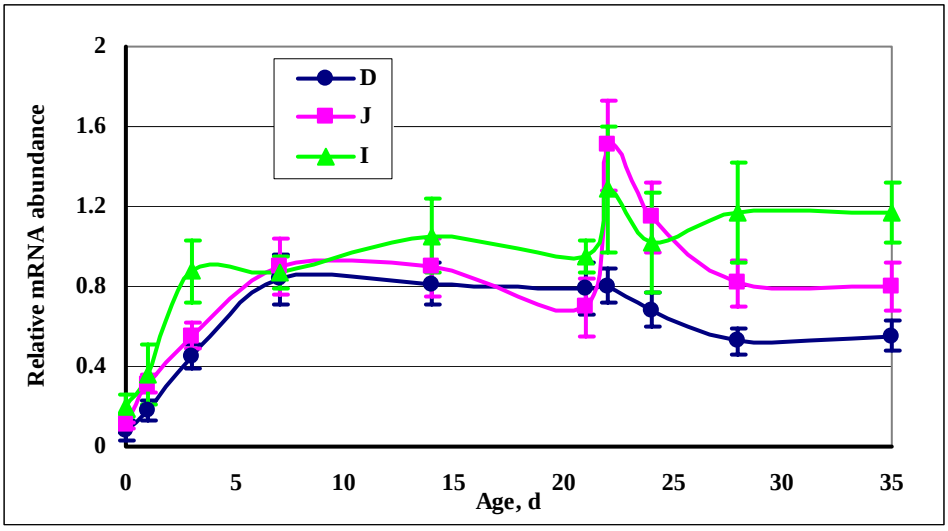
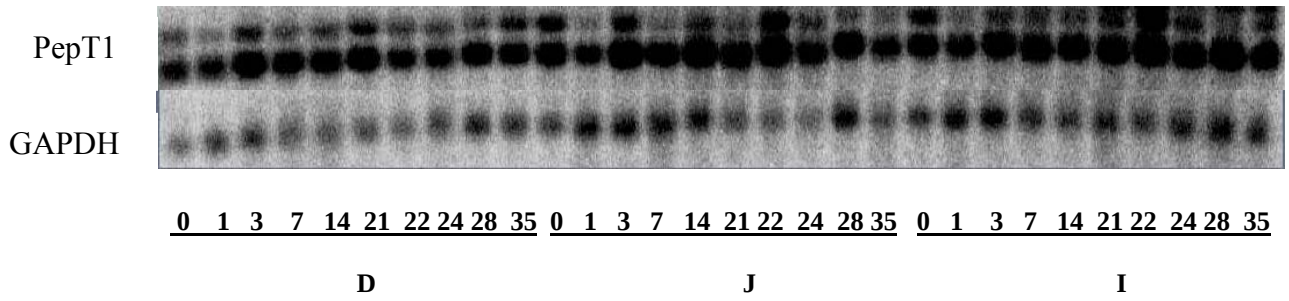


Figure 3.12. Northern blot analysis of dipeptidyl peptidase IV (DPP IV) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent DPP IV mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of DPP IV to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The mRNA abundance of DPP IV increased quadratically ($P < 0.001$) with age during suckling. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) in the abundance of DPP IV mRNA. The abundance of DPP IV mRNA was greater ($P < 0.05$) in the jejunum and ileum than the duodenum both during suckling and postweaning.

A.



B.

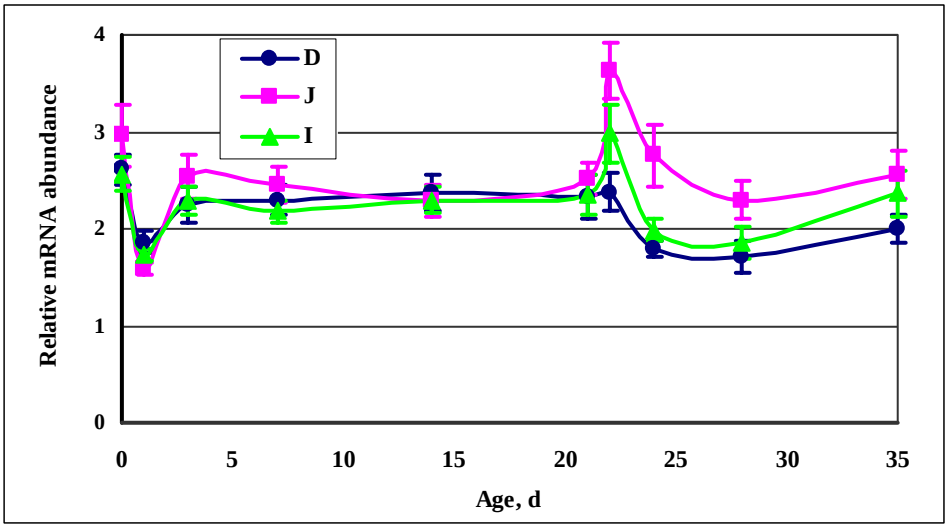
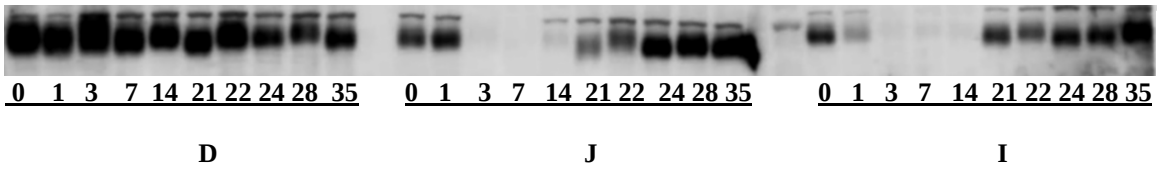


Figure 3.13. Northern blot analysis of peptide transporter 1 (PepT1) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent two mRNA variants of PepT1 and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of PepT1 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The intestinal PepT1 mRNA expression was generally constant during suckling and postweaning, except for a decline on d 1 and a peak on d 22. Generally, PepT1 mRNA was evenly distributed among the three intestinal segments during suckling and tended to be expressed higher (P = 0.11) in the jejunum than the duodenum and ileum postweaning.

A.



B.

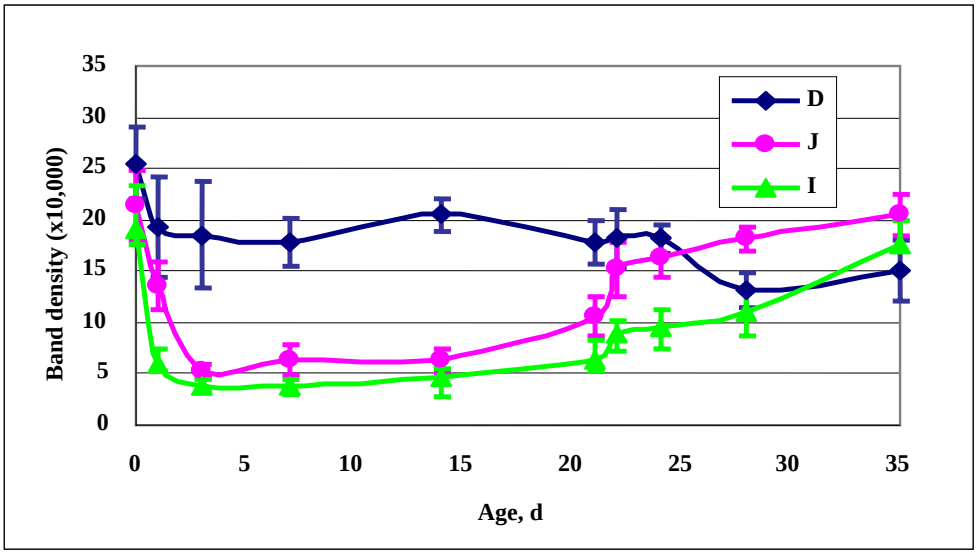
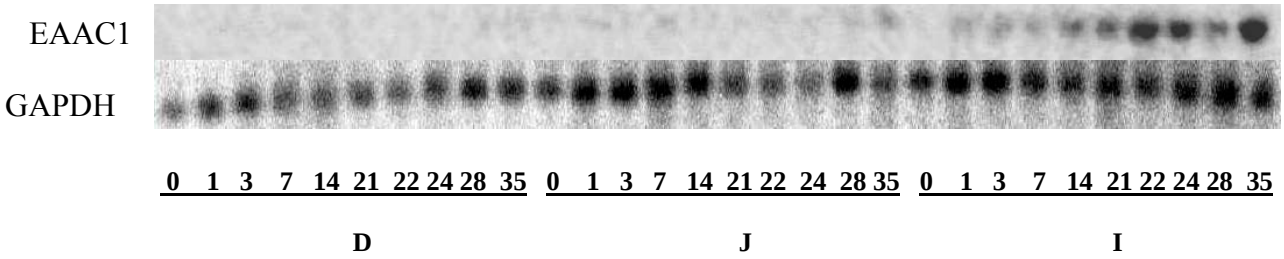


Figure 3.14. Western blot analysis of PepT1 transporter 1 (PepT1) protein expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Brushborder membrane fraction was isolated from individual intestinal tissues. Ten micrograms of brushborder membrane protein from each sample were run on 7.5 % SDS-PAGE then transferred on PVDF membranes. The blot represents one of the seven replicate membranes (**A**). Each membrane comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Bright bands represent PepT1 protein. The densitometric data from the Western blots are presented in **B.** Data are presented as the absolute densitometric readings of PepT1. Each point represents average reading from seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). During suckling, there was an age x intestinal segment interaction ($P < 0.05$) in PepT1 protein abundance and PepT1 protein abundance was greatest ($P < 0.05$) in the duodenum. Postweaning, there was an age x intestinal segment interaction ($P < 0.05$) in PepT1 protein abundance and PepT1 protein abundance did not differ ($P = 0.24$) among intestinal segments.

A.



B.

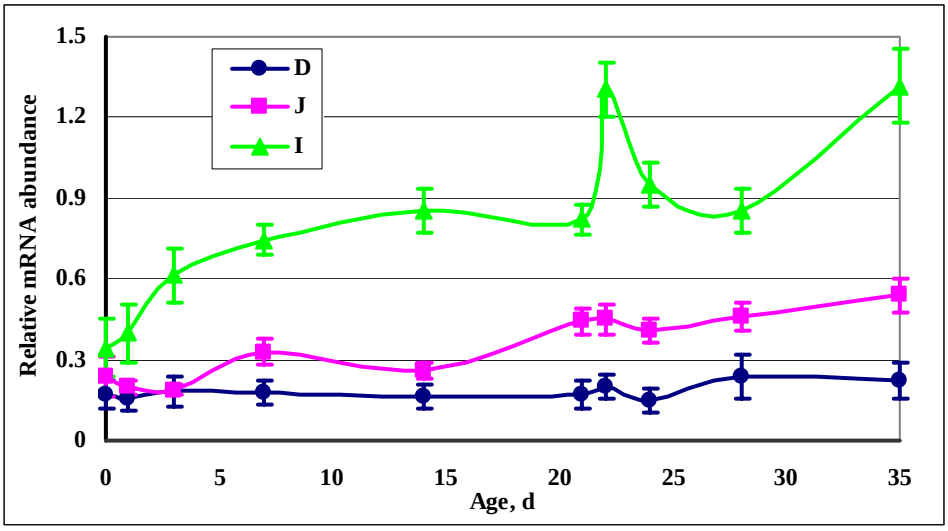
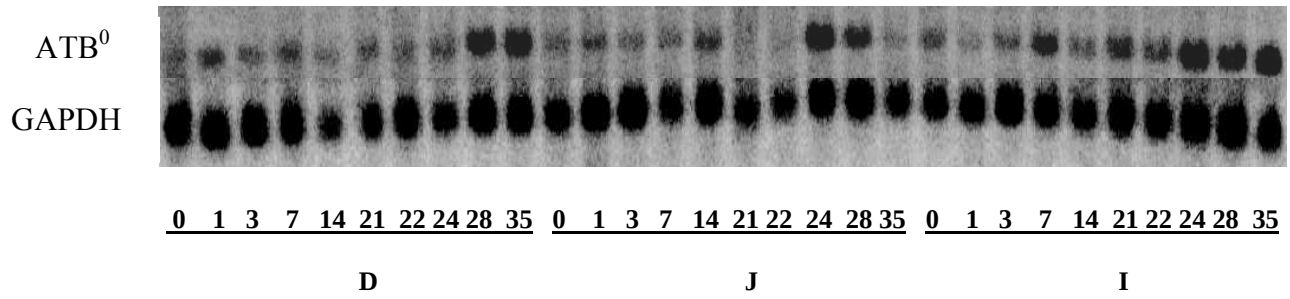


Figure 3.15. Northern blot analysis of excitatory amino acid carrier 1 (EAAC1) mRNA expression in the small intestine of piglets. Small intestinal segments duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent EAAC1 mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of EAAC1 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). There was an age x intestinal segment interaction ($P < 0.05$) in the abundance of EAAC1 mRNA both during suckling and postweaning. The EAAC1 mRNA abundance exhibited an increased gradient along the proximal-distal intestinal axis, with the greatest ($P < 0.05$) abundance in the ileum both during suckling and postweaning.

A.



B.

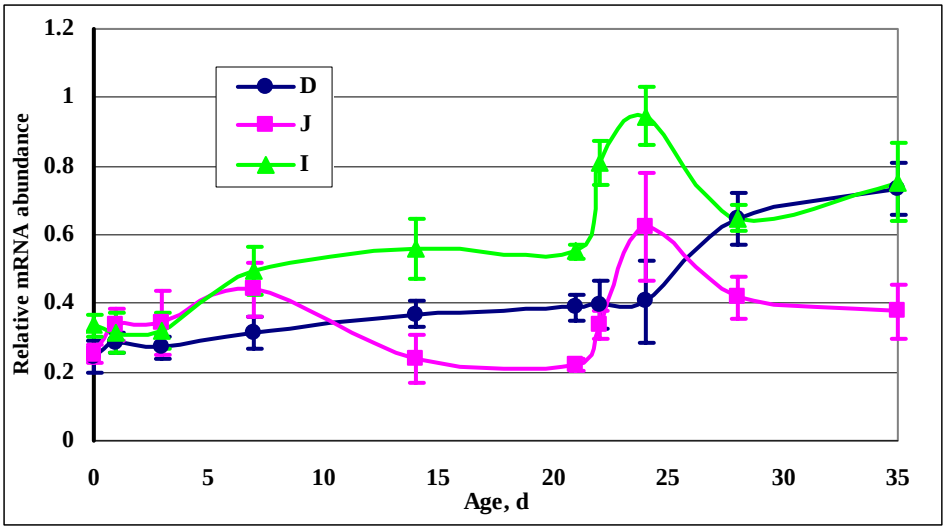
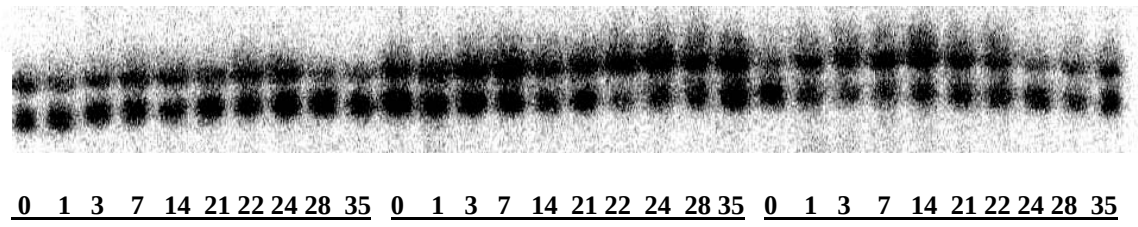


Figure 3.16. Northern blot analysis of neutral amino acid transporter B^0 (ATB^0) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent ATB^0 mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of ATB^0 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). There was an interaction ($P < 0.001$) between age and intestinal segment in the abundance of ATB^0 mRNA both during suckling and postweaning. The abundance of ATB^0 mRNA was generally greater in the ileum than the duodenum and jejunum during suckling and greater in the duodenum and ileum than the jejunum postweaning.

A.



B.

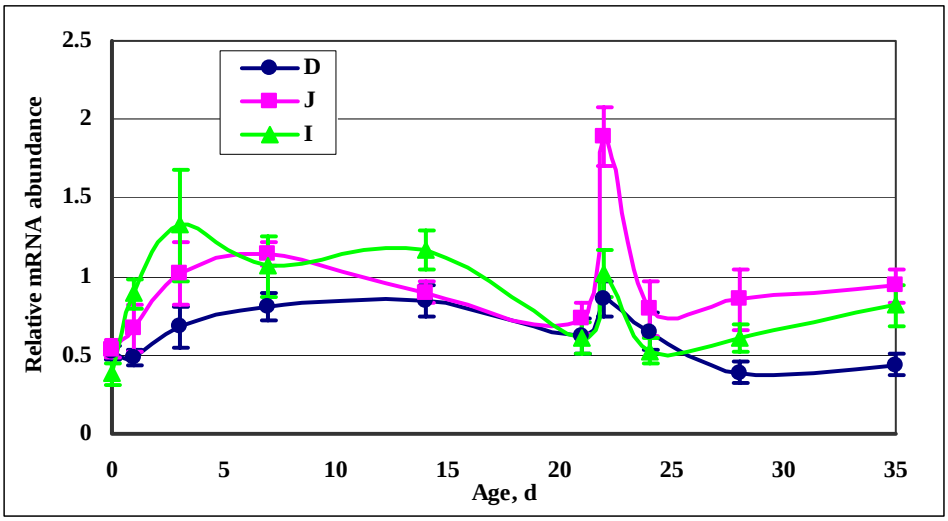
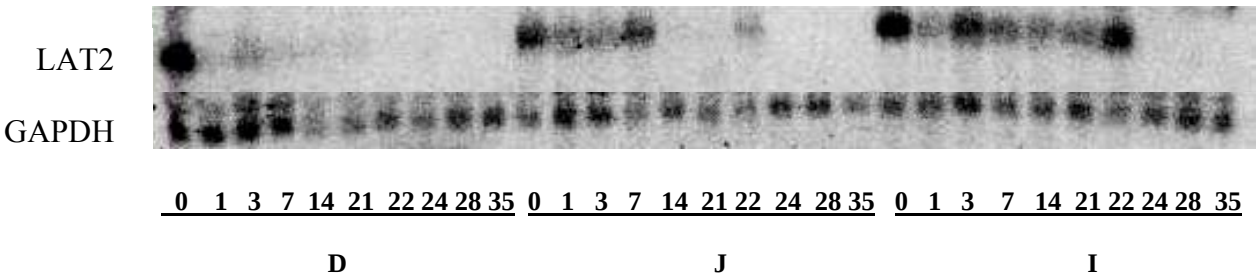


Figure 3.17. Northern blot analysis of a light chain of amino acid transporter $b^{0,+}$ ($b^{0,+}$AT) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent $b^{0,+}$AT mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of $b^{0,+}$AT to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The abundance of $b^{0,+}$AT mRNA increased quadratically ($P < 0.001$) with age during suckling. Postweaning, the abundance of $b^{0,+}$AT mRNA rapidly increased then declined to the level at weaning, followed by either a slight increase in the jejunum and ileum or a slight decrease in the duodenum to d 35. The abundance of $b^{0,+}$AT mRNA was greater ($P < 0.05$) in the jejunum and ileum than the duodenum during suckling and was greatest ($P < 0.05$) in the jejunum postweaning.

A.



B.

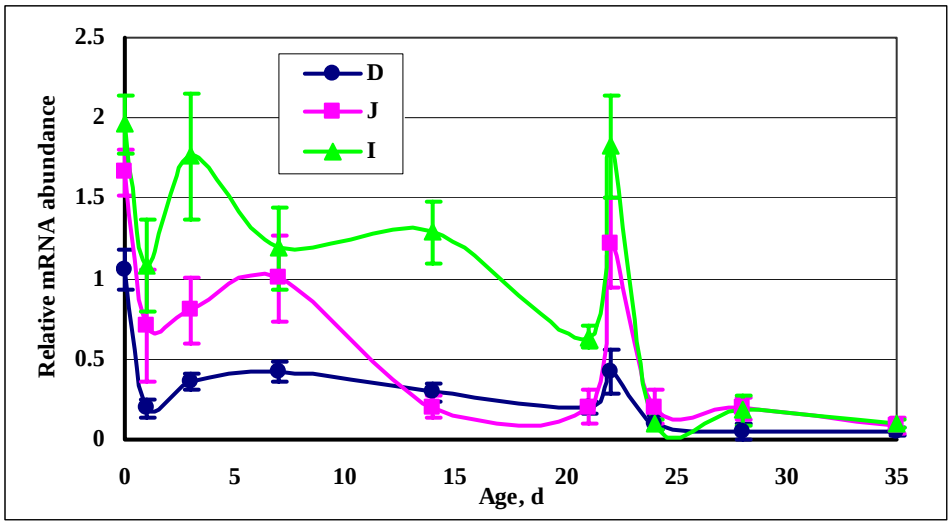
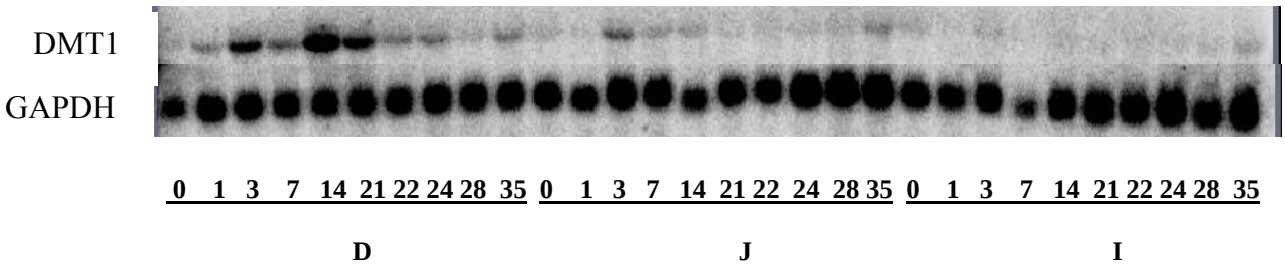


Figure 3.18. Northern blot analysis of a large branched and aromatic neutral amino acid transporter (LAT2) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent LAT2 mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of LAT2 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). The abundance of LAT2 mRNA declined linearly ($P < 0.001$) with age during suckling. Postweaning, the abundance of LAT2 mRNA increased from d 21 to 22, and rapidly declined to a barely detectable level on d 24 then remained relatively stable with age through d 35. The LAT2 mRNA was predominantly ($P < 0.05$) distributed in the ileum during suckling and tended to be higher ($P = 0.09$) expressed in the jejunum and ileum than the duodenum postweaning.

A.



B.

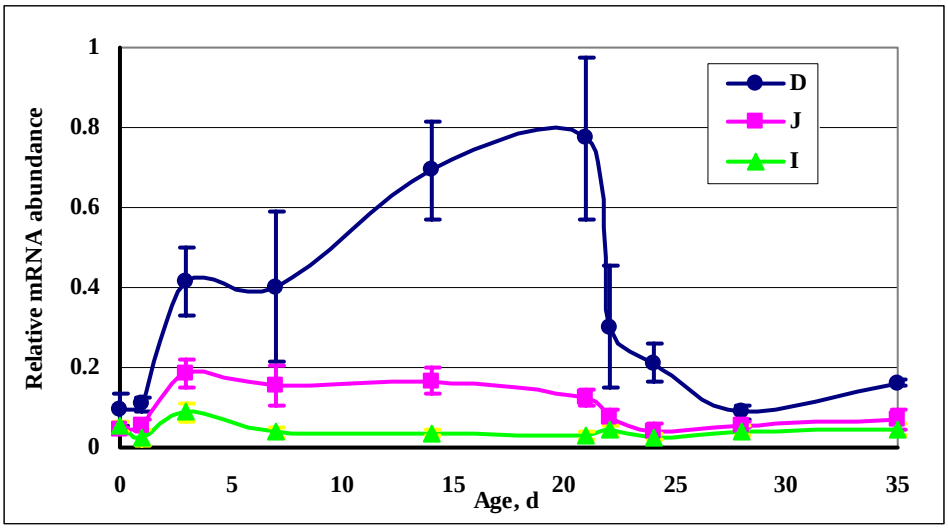
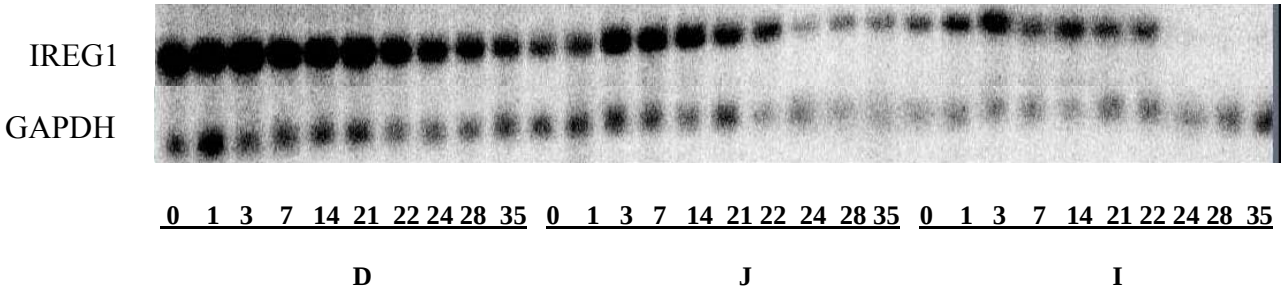


Figure 3.19. Northern blot analysis of divalent metal ion transporter 1 (DMT1) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent DMT1 mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of DMT1 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). There was an age x intestinal segment interaction ($P < 0.05$) in the abundance of DMT1 mRNA both during suckling and postweaning. The DMT1 mRNA was predominantly ($P < 0.01$) distributed in the duodenal tissue both during suckling and postweaning.

A.



B.

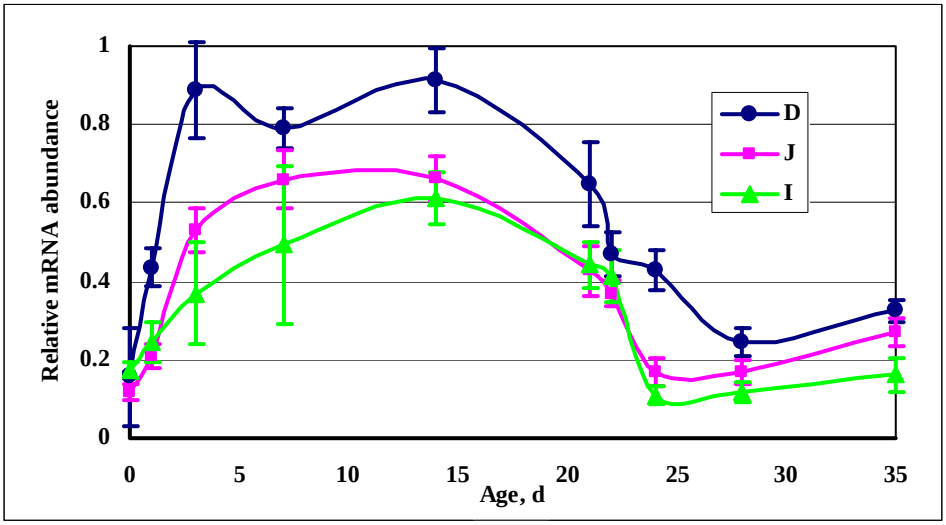


Figure 3.20. Northern blot analysis of iron-regulated transporter 1 (IREG1) mRNA expression in the small intestine of piglets. Small intestinal segments including the duodenum (D), jejunum (J), and ileum (I) were collected from piglets at birth (d 0), during suckling (d 1, 3, 5, 7, 10, 14, 21; weaning occurred at d 21), and postweaning (d 22, 24, 28, 35) from each of seven sows (n = 7 piglets per age group). Total RNA was extracted from individual intestinal tissues. The blot represents one of the seven replicate gels (**A**). Each gel comprised one replicate including each of the three small intestinal segments from one of the 10 piglets of each sow. Upper bands represent IREG1 mRNA and lower bands represent the internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), GAPDH, respectively. The densitometric data from the Northern blots are presented in **B.** Data are presented as ratio of densitometric readings of IREG1 to GAPDH. Each point represents average ratio from one of seven piglets sampled at the indicated time point (n = 7, mean ± SEM). For data analysis, the whole experimental period was divided into two sections, during suckling (from d 0 through d 21) and postweaning (from d 21 through d 35). There was also an age x intestinal segment interaction ($P < 0.05$) in the abundance of IREG1 mRNA both during suckling and postweaning. The IREG1 mRNA abundance was greater ($P < 0.01$) in the duodenum than the jejunum and ileum both during suckling and postweaning.

Chapter IV. Epilogue

The present study was designed to evaluate the ontogenetic regulation of the gene expression of nutrient transporters and brushborder membrane hydrolases in the small intestine of piglets at the early stages of life. The expression of these genes was examined at the mRNA level, and only four of these genes were further evaluated at the protein level due to the unavailability of specific antibodies. The data from the present and previous studies demonstrated that the developmental regulation of intestinal nutrient transporters or hydrolases at the mRNA level was not always accompanied by the similar patterns at the protein and functional activity levels, since the membrane-bound nutrient transporters and hydrolases can be regulated at multiple levels, i.e., the transcription, protein synthesis, processing, and final insertion into the membrane (Dudley et al., 1996; Goda et al., 1999; Fan et al., 2002). The functional activity of intestinal nutrient transporters and hydrolases can also be regulated by their distribution along the crypt-villus axis as the cells mature from the crypts toward the tip of villi. Thus, it is of importance to further investigate the developmental regulation of these nutrient transporters and hydrolases at the protein and functional activity levels, and their cellular distribution by using *in situ* hybridization and immunohistochemistry. The dramatic increase in intestinal mass, mucosal weight, and the alterations in intestinal structure and cell population during the early stages of life, should be taken into account before the overall conclusion is drawn.

A number of studies have shown that intestinal assimilation capacity of certain nutrients is limited in young animals and humans. So far, the best characterized nutrient is fructose. The malabsorption of fructose commonly occurs in children under age of three when they consume a

large quantity of fructose containing juice (Ferraris, 2001). The fructose malabsorption is primarily due to the scarcity of the intestinal fructose transporter, GLUT5. It is generally believed that the intestinal assimilation capacity of dietary proteins and carbohydrates (starch) is also limited in young animals such as piglets (Xu and Cranwell, 2003). The dilute concentration of free amino acids and glucose in the amniotic fluid swallowed are the main enteral nutrients for the fetuses during gestation. Results from previous studies demonstrated that glucose and peptide transport reached a maximal level at birth or a few days after birth then generally declined to an intermediate level at weaning and adulthood. In the present study, PepT1 protein level gradually decreased with age from birth through the end of suckling in the duodenum. In the jejunum and ileum, PepT1 protein level decreased with age during the first few days after birth then slightly increased with age to the end of suckling. Concomitantly, the mRNA abundance of the amino acid transporters and peptide hydrolases expressed on the brushborder membrane of intestinal enterocytes increased with age during early suckling then remained constant through the end of suckling. It is reasonable to speculate that peptide transport activity was high at birth then declined with age during suckling in the small intestine of piglets, whereas amino acid transport activity was low at birth then increased with age during early suckling then remained constant through the end of suckling. The inverse developmental patterns in peptide and free amino acid transport activity may be due to alterations in the composition of the final digestion products (free amino acids and small peptides), which is largely controlled by the changes in the specific activity of peptide brushborder hydrolases. Thus, the data from the present study may suggest that, from a nutritional view point, artificial nutritional supplementation to neonates may be more appropriate in a small peptide form rather than in a intact protein or free amino acid form, based on the limited peptide hydrolytic activity and amino acid transport activity.

It is well recognized that intestinal amino acid absorption is more efficient and rapid in a peptide form than a free amino acid form in animals and humans (Webb et al., 1992; Li et al., 1999; Daenzer et al., 2001). In a clinical setting, synthetic glutamine dipeptides are widely used as suitable constituents of nutritional supplementation due to the obvious advantages (high stability, solubility and bioavailability) over crystalline free amino acids. Although demonstrated as essential to life in patients suffering from inherited diseases of amino acid transporters (Adibi, 1997), the quantitative significance of PepT1 in overall amino acid absorption is not fully understood. In our lab, real time RT-PCR analysis revealed that the absolute mRNA abundance of PepT1 was several fold higher than that of amino acid transporters examined in the intestine of black bears (Gilbert, 2005). It is important to compare the absolute mRNA and even protein abundance of PepT1 and amino acid transporters in other species such as pigs in order to determine whether there are more peptide transporter molecules present in the small intestine than amino acid transporters. However, studies have shown that only one single primary peptide transporter (PepT1) responsible for small peptide absorption is expressed in the small intestine, whereas there are multiple amino acid transporters coordinately involved in amino acid absorption. Thus, this may give us some confounding and misleading information in terms of the single paired comparison of the abundance of individual amino acid transporters and PepT1, and no solid conclusion may be drawn. It would be of interest to generate a unique animal model, which is deficient in the gene encoding intestinal PepT1 to elucidate the quantitative nutritional and metabolic significance of peptide absorption.

Previous studies have shown that diets including concentrates of protein hydrolysates (random peptide mixture) improved the growth performance of young animals (Zimmerman et al., 1997; Lindemann et al., 1998; Wang et al., 2003). The authors explained that the enhanced

growth performance of young animals by feeding peptide based diets was primarily due to the more efficient assimilation of small peptides than intact proteins in the small intestine. However, these studies neglected to include the same source of proteins in both its intact and hydrolyzed forms. Therefore, the enhanced growth performance of young animals may solely result from the higher quality of peptide hydrolysates as protein sources in term of protein composition, compared to the control protein used. The free amino acid based diets have been shown to be inferior for animal growth and health compared to intact protein and peptide based diets (Daenzer et al., 2001; Dabrowski et al., 2003). Since the crystalline free amino acid based diets are much more expensive and physically disadvantageous (stability, solubility and bioavailability) than the peptide hydrolysate and intact protein based diets, it is not practical to apply this kind of diet in animal production. Thus, it is extremely interesting to investigate whether the peptide hydrolysate based diets improve animal growth performance compared to intact protein diets by using the same source of proteins, and then to explore the mechanisms by which the peptide hydrolysate diets do so if this is true. To me, it seems that the brushborder membrane peptide hydrolases, which are key rate-limiting enzymes in the terminal digestion of luminal proteins into small peptides and free amino acids, may play a much more important role in this enhancement than amino acid absorption, even though peptide transport is more efficient and rapid than free amino acid absorption. Thus, peptide based diets may require optimization of the ideal proportions of intact proteins, peptides, and free amino acids to support animal maximal growth by making the most use of peptide and free amino acid transport activity and enzymatic hydrolytic activity and by stimulating and maintaining the optimal gut functions. The quantitative evaluation of different peptide proteins as carriers of essential amino acids to improve bioavailability is also required. The new knowledge from this series of research may

modify the current strategy for estimating the protein requirements of domestic animals, and thus lead to better understanding of the process of protein accretion and animal growth.

The data from the present study indicated that the mRNA abundance of LAT2, an amino acid transporter expressed on the basolateral membrane of intestinal enterocytes was high at birth then declined rapidly with age to a relatively low level at weaning. Since LAT2 functions as an exchange for amino acids, during late gestation and at birth, the putative role of LAT2 may be to obtain amino acids from the bloodstream into intestinal enterocytes for nourishment, especially for those enterocytes located in the distal part of the small intestine and have limited luminal amino acid supply from amniotic fluid swallowed. Thus, it is of importance to 1) elucidate the developmental pattern of LAT2 during late gestation and the expression distribution along the intestine and to 2) explore the precise physiological function of LAT2 by using the LAT2 deficient animal model in terms of the intestinal morphology, structure and mass during late gestation and early birth. The knowledge from this study will provide potential guidance for the clinical treatment of intestinal atrophy in preterm and newborn infants.

In the present study, sucrase gene expression and the specific activities of enzyme were upregulated linearly with age during suckling, while MGA, which is a very critical enzyme involved in the final digestion of starch into monosaccharides, was low and remained unchanged with age during the suckling period. This may indicate that sucrose, which is the substrate for sucrase, is a better carbohydrate source than starch for weanling piglets in terms of digestibility. Previous studies have shown that the gene expression of sucrase was dramatically upregulated to a substantial level at the completion of weaning in many mammalian species. The authors speculated that the increase in the gene expression and specific activity of sucrase was regulated

by genetic programming, in anticipation of the intake of sucrose. However, the data for gene expression and specific activity of sucrase from the present study indicated that this upregulation initiated in the early suckling period, and previous study also demonstrated that lactose intake can upregulate the gene and specific activity of sucrase in adult animals. Thus, it is of importance to verify that the upregulation of sucrase gene expression and specific activity is attributable to the intake of lactose from milk or genetic programming.

In summary, future studies should focus on the further evaluation of developmental regulation of the intestinal digestive and absorptive process at both the protein and functional activity levels and the cellular distribution of these nutrient transporters and hydrolases as well. The underlying mechanisms by which developmental stages regulate the expression of these nutrient transporter and hydrolase genes also need to be elucidated. The quantitative significance of PepT1 and brushborder peptide hydrolases in the overall protein assimilation is yet to be determined. These will provide insight into better diet formulation to enhance the health status and growth performance of pigs.

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Vita

Xunjun Xiao, the son of Jiahua Xiao and Jiazhi Zhang, grew up in Huabei Province, a central part of China with his younger sister. He earned his Bachelor's degree in Animal Nutrition and Feed Science in 1998, and Master's degree in Animal Nutrition in 2001, from the College of Animal Sciences and Technologies at China Agricultural University in Beijing, China. He came to the United States to pursue his Ph. D. degree in Dairy Sciences in fall of 2001 with Dr. Josheph Herbein in the Department of Dairy Sciences and transferred to work under the direction of Dr. Kenneth E. Webb in the Department of Animal and Poultry Sciences in spring of 2002. He received financial support from the John Lee Pratt Animal Nutrition foundation from 2001 to 2004, with an extension to 2005. He is a member of the American Society of Animal Science, and the American Society of Nutritional Sciences.

He married Xiaomei Min on June 4, 2001 and their twin-daughters Annie and Angela were born on August 31, 2003 in Roanoke, Virginia.