24 Gastrointestinal protein and amino acid metabolism in growing animals

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The tissues of the gastrointestinal tract play a major role in the metabolism of protein and amino acids in growing animals. Intestinal tissue has a high rate of protein metabolism, which is directly linked to the high rates of proliferation, protein secretion, cell death and desquamation of various epithelial and lymphoid cells within the mucosa. Because the small intestine is the first tissue exposed to the diet, it has a key regulatory role in the digestion, absorption, metabolism and availability of dietary protein and amino acids for growth. The major oxidative fuels for the intestine are glutamate, glutamine, aspartate and glucose; however, some essential amino acids are also oxidized, such as lysine, leucine and phenylalanine. Among the essential amino acids, threonine utilization is particularly high, and may be critical for normal intestinal function. Intestinal protein and amino acid metabolism is primarily regulated by the modality (enteral versus parenteral) and quantity of nutrient intake, and to a lesser extent by the composition of nutrients.

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Intestinal metabolism has a significant influence on the quantity and pattern of amino acids required for growth, particularly arginine and threonine.

1. INTRODUCTION

During the last 20 years, research studies established that the tissues of the gastrointestinal tract have a substantial impact on whole-body protein and amino acid metabolism in growing animals. The portal-drained visceral (PDV) tissues, comprised largely of gastrointestinal tissues, contribute between 3 and 6% of body weight, but they account for 20 to 35% of whole-body protein turnover and energy expenditure (Burrin et al., 1989; Yen et al., 1989; Nieto and Lobley, 1999; Stoll et al., 1999a). The disproportionate impact of gastrointestinal tissues on whole-body metabolism is a function of their relatively high fractional rates of protein synthesis and oxygen consumption. Studies with domestic animals have demonstrated that the fractional synthesis rate of protein in the intestinal tissues is several-fold higher than that in peripheral tissues, such as muscle (Lobley et al., 1980, 1992; Attaix et al., 1986; Burrin et al., 1992; Davis et al., 1996). Although we know much about the heterogeneous phenotypes and characteristic functions of the cells that comprise the intestinal mucosa, the quantitative influence of these different cell types on mucosal protein metabolism as a whole has yet to be fully delineated. Moreover, we have a limited knowledge of how the spatial localization of cells within the mucosa determines the source of substrate, its supply, and rate of metabolism.

Two critically important physiological functions of the intestine that are necessary for maximal growth of the organism are: (1) to digest and absorb dietary nutrients, and (2) to serve as a biological barrier in order to prevent or minimize effects of environmental pathogens, toxins and other antigenic molecules. Studies during the past decade have suggested that ensuring an adequate nutrient supply to the intestine, mainly via the oral or enteral route, is essential to maintain these functions. Currently, efforts are increasing to quantify the extent to which the intestine uses nutrients for its own purposes, and modifies the rate and pattern of amino acid supply for peripheral tissue growth (Reeds et al., 1993; Reeds et al., 1999; Burrin et al., 2001). Given the significance of the intestinal tissues to whole-body metabolism, the investigation of those factors that substantially alter intestinal mass has become increasingly relevant to the study of whole-animal growth.

The intent of this review is to characterize gastrointestinal protein and amino acid metabolism by examining (1) the underlying influence of anatomy and cellular function, (2) the major metabolic fates of amino acids,
2. ANATOMICAL AND MORPHOLOGICAL BASIS OF GUT METABOLISM

2.1. Regional metabolism within the gastrointestinal tract

There are notable differences in the rates of metabolism within the tissues of the gastrointestinal tract. Studies in developing animals indicate that the rates of protein synthesis are higher in the small intestine than in the stomach and large intestine (Attaix and Arnal, 1987; Attaix et al., 1992; Burrin et al., 1999a). Within the small intestine, the protein synthesis rate is highest in the duodenum, and declines longitudinally (fig. 1) (Attaix et al., 1992; Stoll et al., 2000a). There are numerous estimates of non-essential amino acid metabolism (mainly glutamine, arginine, citrulline and ornithine) in primary isolated enterocytes (Darcy-Vrillon et al., 1994; Quan et al., 1998; Wu, 1998), colonocytes (Mouille et al., 1999), and colon carcinoma cell-lines (Selamnia et al., 1998). One study comparing isolated cells from the jejunum, caecum and colon found no difference in the glutamine metabolism.

![Fractional rates of protein synthesis](image)

**Fig. 1.** Fractional rates of protein synthesis is small and large intestinal segments of milk-fed and weaned lambs. (Adapted from Attaix et al., 1992).
oxidation rate, despite regional differences in glucose and short-chain fatty acid oxidation (Fleming et al., 1991).

2.2. **Cellular basis of mucosal metabolism**

The high rates of protein metabolism in the gut are directly linked to the high rates of proliferation, protein secretion, cell death and desquamation of various epithelial and lymphoid cells within the mucosa. *In vivo* labeling studies in young pigs show that the epithelial cells have a life span of about 3 to 10 days, depending on the intestinal region and stage of development (Smith and Jarvis, 1978; Fan et al., 2001). Within the small intestinal epithelium, the proliferative crypt compartment contains pluripotent stem cells, which undergo mitosis and differentiation into four cell lineages: absorptive enterocytes, mucin-producing goblet cells, antibacterial peptide-producing Paneth cells, and enteroendocrine cells. The differentiated phenotype of these cells defines their main functions, which are to digest and absorb dietary nutrients and provide an innate mucosal barrier to the luminal environment. Lymphoid cells are the other major class of cells located mainly in the lamina propria region beneath the epithelial monolayer. These cells include T- and B-lymphocytes, macrophages, neutrophils, mast cells, dendritic cells and fibroblasts that are diffusely organized around a highly vascular and neuronal network. Studies in vitro show that these epithelial (Higashiguchi et al., 1993; Wu 1998) and lymphoid (Szondy and Newsholme, 1990; Dugan et al., 1994) cell types exhibit high rates of protein synthesis and glutamine metabolism. The protein secretory capacity of goblet cells is also substantial, and mucin protein represents a significant component of endogenous secretions that pass undigested to the terminal ileum (Lien et al., 1997; Montagne et al., 2000).

2.3. **Luminal vs arterial substrate input**

Another important anatomical consideration with respect to the metabolism of epithelial cells is that they derive nutrients from two separate sources: the intestinal lumen, via the apical/brush-border membrane, and the blood, via the basolateral membrane. Studies in piglets with isotopically labeled substrates have shown that there is significant utilization of both luminal and arterial amino acids. It is of interest to note that, in pigs fed a milk-based formula, the input of amino acids from the luminal route (i.e. diet) is far greater than that from the arterial circulation (fig. 2). With respect to the uptake of amino acids, particularly glutamate and glutamine, there is markedly greater uptake from the luminal (67–96%) than the arterial (11–21%)
Interestingly, the uptake of luminal glucose, both on a relative and absolute basis, is considerably less than that of amino acids (~6% of input). It is possible that this combined phenomenon is a function of the inherent amino acid and glucose transport capacities of the apical (luminal) and basolateral (arterial) surfaces of intestinal enterocytes. However, in absolute molar terms, the uptake of some amino acids from the arterial and luminal routes is nearly equal, because the rate of arterial amino acid input is more than five times greater than that from the diet. Thus, some amino acids used by the gut are derived equally from the diet and arterial supply. In contrast, intestinal glucose is supplied largely by the arterial circulation (85% total), with only a small contribution from the diet (15%). The relatively high rates of first-pass metabolism of dietary amino acids may be a phenomenon that is largely confined to the small intestine rather than the stomach or large intestine. That is because when intact protein is consumed, the level of free amino acids is normally low in the stomach as well as the large intestine due to bacterial fermentation. If the digestive environment in the stomach and large intestine limits the luminal amino acid availability, then the arterial circulation must meet the amino acid needs of these tissues. This idea is supported by the evidence that during total parenteral nutrition, atrophy occurs primarily in the proximal region.
of the small intestine, while the mass of the distal small intestine, stomach and large intestine is preserved (Goldstein et al., 1985; Morgan et al., 1987; Ganessunker et al., 1999).

2.4. Crypt vs villus cell metabolism

The extent to which epithelial cells derive their nutrients from the luminal or vascular input is further influenced by their stage of differentiation and physical location along the crypt-villus axis. Early studies (Alpers, 1972) showed that crypt cells are more highly labeled with isotopic tracers derived from the blood, whereas villus cells are more highly labeled with tracers given luminally (fig. 3). Although these findings seem logical, in a general sense, we still know very little about how the stage of differentiation and amino acid transporter expression on the apical and basolateral surface of enterocytes affect the rate and pattern of nutrient utilization. Studies with cultured enterocytes indicate that the mitogenic effect of glutamine appears to be dependent on its metabolism, but interestingly, it cannot be reproduced with glutamate. This apparent specificity of glutamine as a crypt cell mitogen may be due to the fact that the crypt cell lines used in these studies had a limited capacity to transport glutamate across the cell membrane. Yet, this contradicts the findings of extensive intestinal glutamate metabolism documented *in vivo* (Windmueller and Spaeth, 1975; Reeds et al., 1996). On the

![Fig. 3. Relative incorporation of luminally (●-³H-leucine) and intravenously (○-¹⁴C-leucine) administered leucine into enterocytes isolated from the crypt to villus axis of the intestinal mucosa (adapted from Alpers, 1972).](image)
other hand, it may be that crypt cells are capable of basolateral transport of blood-borne glutamine (but not glutamate) for proliferative processes, but as the cells differentiate and mature, they acquire the ability to transport luminal glutamate for use as a metabolic fuel and as a biosynthetic precursor (fig. 4). This idea is supported by evidence that shows that glutamine transport is high in undifferentiated Caco-2 cells, but in confluent differentiated cells glutamine transport is decreased, whereas glutamate transport is substantially increased (Mordrelle et al., 2000).

3. METABOLIC FATE OF AMINO ACIDS

Once taken up by the intestinal tissues, amino acids can be utilized for three major metabolic purposes: (1) incorporation into protein; (2) conversion via

Fig. 4. Model of mucosal glutamate, glutamine, and aspartate metabolism derived from dietary intake and the arterial circulation.
transamination into other amino acids, metabolic substrates and biosynthetic intermediates; and (3) complete oxidation to CO$_2$. In the first two pathways, amino acids can be deposited and recycled by the body for purposes of growth or other biological functions. In the case of some amino acids, namely threonine and cysteine, incorporation into endogenous secretions that are fermented in the large intestine represents a net nutritional loss. Likewise, if the amino acids are completely oxidized to CO$_2$ by the mucosal cells, this is also a nutritional loss, especially in the case of essential amino acids.

3.1. Protein synthesis and degradation

The major metabolic fate of amino acids in the gut is considered to be their incorporation into cellular proteins. Although this is true of essential amino acids, there is extensive metabolism of several non-essential amino acids. Numerous studies have measured the rates of protein synthesis in various tissues of the gastrointestinal tract. In general, these studies show that the fractional rates of protein synthesis are relatively high compared to other major tissues such as muscle, due to the high rates of cell turnover. Moreover, gut protein synthesis rates are modulated by several nutritional and hormonal factors, the details of which are described below. However, there is a lack of fundamental knowledge about the cellular and molecular mechanisms that regulate protein synthesis in gut tissues in comparison with our knowledge of other tissues, such as liver and muscle. Recent evidence has shown that the stimulation of muscle protein synthesis by food intake, insulin and amino acids is mediated by activation of intracellular signal pathways which include IRS-1, PI-3 kinase, MAP-kinase, mTOR and p70-S6-kinase (Kimball et al., 1998; Davis et al., 2000; Kimball et al., 2000; Anthony et al., 2001). These signaling pathways play a critical role in the proliferation and differentiation of intestinal epithelial cells (Taupin and Podolsky, 1999; Wang et al., 2001) and can be activated by amino acids (Rhoads et al., 2000); yet, their role in the regulation of protein synthesis is largely unknown. We know even less about the regulation of protein degradation in gut tissues, whether at the molecular and cellular level, or with respect to nutritional and hormonal regulation (Baracos et al., 2000).

3.2. Nonessential amino acid metabolism

Of the amino acids ingested in the diet, the intestinal metabolism of glutamine and glutamate has been studied most extensively (fig. 5). It has been known for more than 25 years that there is substantial utilization of
glutamine and glutamate by the intestine, and much of the glutamine is derived from the arterial circulation (Windmueller and Spaeth, 1975; 1980). More important, however, is the fact that in situ studies (Windmueller and Spaeth, 1980) have shown that at least 50% of the dietary glutamate and glutamine taken up by the gut is completely oxidized to CO₂. These results were recently confirmed by \textit{in vivo} studies of 13C-labeled glutamate and glutamine metabolism by the PDV tissues in piglets indicating that as much as 95% of enteral glutamate is metabolized by the gut, largely to CO₂ (Stoll et al., 1999a) (fig. 6). Consistent with this finding, studies in premature infants and adult humans, based on estimates of whole-body kinetics of enterally fed 13C- and 15N-labeled glutamate and glutamine, indicate that approximately 50 to 95% of the dietary glutamine and glutamate is extracted by splanchnic tissues, and most of this is oxidized to CO₂ (Battezzati et al., 1995; Darmaun et al., 1997; Haisch et al., 2000).

In addition to the role of glutamate as metabolic fuel, studies in rats (Horvath et al., 1996) have shown that adding 4% glutamate to an elemental diet deficient in both glutamine and glutamate significantly increases intestinal growth, crypt cell mitosis, and lactase activity, without affecting body weight gain. These results serve to challenge the long-standing notion that glutamine is the primary oxidative fuel for the gut, and suggest that glutamate and perhaps aspartate ingested in the diet are equally important intestinal fuels. Moreover, studies with short-term cultures of isolated enterocytes have shown that, along with glutamine, glucose is a major intestinal oxidative fuel (Darcy-Vrillon et al., 1994; Kight and Fleming, 1995; Wu et al., 1995). Although our results indicate that significant quantities of glucose
are indeed oxidized by the gut, they demonstrate a preferential utilization of arterial rather than dietary glucose. It is important to note that, although glucose represents a major oxidative fuel (29%) in terms of total PDV CO$_2$ production, the proportion of glucose oxidized completely to CO$_2$ is substantially less than that of either glutamate or glutamine. This finding is entirely consistent with results from cultured enterocytes showing that glutamine can potently suppress glucose oxidation, whereas glucose has little impact on glutamine oxidation (Kight and Fleming, 1995; Wu et al., 1995). The implication is that glutamine and glutamate are preferentially channeled towards mitochondrial oxidation, while most of the glucose is utilized for other metabolic or biosynthetic purposes. A small percentage of the portal glucose utilization is metabolized to alanine (7.5%) and lactate (5.9%), which leaves approximately 60% unaccounted for. The biosynthetic products potentially derived from gut glucose utilization are numerous; however, incorporation into mucin glycoproteins, fatty acids and lipids presents a variety of intriguing possibilities.

The importance of glutamine and glutamate to gut metabolism goes beyond their roles as oxidative fuel for rapidly proliferating cells, such as enterocytes and lymphocytes; they also function as important precursors

Fig. 6. Relative rates of arterial and dietary substrate uptake and oxidation by the portal-drained visceral tissues in young pigs fed cow’s milk formula (GLN, glutamine; GLU, glutamate; GLUC, glucose; ASP, aspartate; Adapted from stoll et al., 1999a).
of nucleic acids, nucleotides, amino sugars, amino acids, and glutathione (Smith, 1990; Souba et al., 1990; Rouse et al., 1995; Klimberg, 1996; Gate et al., 1999). A number of studies with adult rats, neonatal pigs and porcine enterocytes have demonstrated that glutamine/glutamate are precursors for the synthesis of several non-essential amino acids, including alanine, aspartate, proline, citrulline and arginine (Windmueller and Spaeth, 1980; Blaicher et al., 1993; Wu et al., 1995; Wu and Knabe, 1995; Stoll et al., 1999a). In addition, Reeds et al. (1997) reported that luminal glutamate, rather than glutamine-derived glutamate, is the preferential source of glutathione synthesis in the intestinal mucosa of the infant pig, suggesting a highly compartmentalized catabolism of intestinal glutamate and glutamine. It is also noteworthy that the enzymes necessary for de novo synthesis of both glutamate and glutamine are present in gut tissues, and their expression is up-regulated during the suckling-to-weanling transition (Shenoy et al., 1996; Madej et al., 1999).

Studies with cultured intestinal epithelial cells have demonstrated that glutamine has pluripotent actions, including stimulation of cell proliferation, differentiation, ornithine decarboxylase (ODC) and immediate early gene (c-jun) expression, and polyamine synthesis (Kandil et al., 1995; Wang et al., 1996). Glutamine also suppresses apoptosis, which suggests that it may be an important survival factor (Papaconstantinou et al., 1998). In a recent review, Rhoads (1999) postulated that glutamine stimulates cell proliferation by a signaling mechanism which involves the activation of two related, but distinct, classes of mitogen-activated protein kinases. The biochemical mechanism whereby glutamine affects intestinal function may be related to its conversion to glucosamine, which reduces the cellular NADPH and suppresses nitric oxide synthesis (Wu et al., 2001).

3.3. Essential amino acid metabolism

A number of essential amino acids play a key role in gut function and thus their metabolism by the gut has a critical influence on essential amino acid requirements. Dietary threonine and cysteine are utilized by the intestinal mucosa for mucin and glutathione synthesis. From a nutritional perspective, although threonine is considered essential, cysteine is not. However, cysteine can be synthesized from the essential amino acid, methionine. Therefore, increased metabolism of methionine to meet cysteine needs may become limiting for growth, and nutritionally significant. The secretory mucins play a key role in the innate immune defense of the mucosa, and the core protein of the major intestinal mucins (van Klinken et al., 1997) contains a large amount of threonine and cysteine. Likewise, cysteine is a
component of the tripeptide antioxidant glutathione, which is critical for the maintenance of the structural integrity and barrier function of the intestinal mucosa (Martensson et al., 1991). Given that the rates of mucosal synthesis and secretion of glutathione and mucins are likely significant, it follows that their production by the gut could have a quantitatively significant impact on the animal’s dietary requirement for threonine, cysteine, and perhaps methionine. For threonine, but not cysteine, there is evidence to support this hypothesis. First, based on net portal utilization, as much as 60 to 85% of dietary threonine is utilized by the gut (Stoll et al., 1998; Van Goudoever et al., 2000). More conclusive, however, was a recent study (Bertolo et al., 1998) demonstrating that the threonine requirement of piglets maintained by parenteral nutrition was only 40% of that observed in piglets receiving enteral feedings. Moreover, a subsequent report found that feeding threonine-deficient diets to piglets significantly reduces intestinal mass and goblet cell numbers, and this suppression of intestinal growth cannot be fully restored by providing threonine parenterally (Ball et al., 1999). Another study demonstrated that the methionine requirement of piglets maintained by parenteral nutrition was 35% lower than that of enterally fed piglets (Shoveller et al., 2000; 2001). This value is lower than the value of 46% based on our measurements of net portal utilization of dietary methionine. Nevertheless, both findings suggest that the gut utilizes substantial amounts of dietary methionine, and raise the question of whether methionine is being metabolized to cysteine and used for intestinal mucin synthesis.

Dietary arginine is essential for young piglets. Studies have shown that the small intestine is an important site of arginine and proline synthesis (fig. 7) (Murphy et al., 1996; Wu, 1998; Stoll et al., 1999a). Similarly, in adult rats and weanling pigs (Windmueller and Spaeth, 1980; Dugan et al., 1995), intestinal citrulline synthesis from glutamine, glutamate and proline is the main source for circulating citrulline, which plays a critical role in arginine homeostasis. In many adult animals and humans, arginine is synthesized by the kidney from intestinally derived citrulline at rates that are inadequate to support growth in neonatal animals. As the milk of humans, pigs, rats and many other mammals is deficient in arginine (Davis et al., 1994), it is considered an essential amino acid for growing animals. In the healthy suckling pig, the intestinal synthesis of arginine provides only about half of the animal’s needs for growth; thus, arginine is required in the diet. Moreover, the net intestinal synthesis of arginine declines substantially during the late suckling period (Wu and Morris, 1998). These observations raise the possibility that both the endogenous (via gut synthesis) and dietary arginine supply may be limiting for maximal growth of suckling piglets. Studies with enterocytes from newborn pigs (Blaicher et al.,
1993; Wu and Knabe, 1995) and developing mouse and rat small intestine (Hurwitz and Kretchmer, 1986; DeJonge et al., 1998) have shown developmental changes and metabolic zonation along the crypt-villus axis of arginine-metabolizing enzymes. At birth, the enterocytes of the upper villus (DeJonge et al., 1998) are the major site of arginine synthesis, but gradually become the major site of net citrulline production as intestinal arginase expression increases via a glucocorticoid-dependent mechanism. This transition is compensated by the gradually increasing capacity of the kidney to use citrulline for arginine synthesis. Thus, following the transition from suckling to weanling, the intestine appears to become a site of arginine degradation rather than synthesis (see review, Wu and Morris, 1998).

The limited arginine degradation by enterocytes from newborn pigs ensures a maximum output of arginine (synthesized from glutamine or derived from milk) into the portal circulation for utilization by extraintestinal tissues. Type II arginase, a mitochondrial enzyme that is expressed at lower levels in kidney, brain, small intestine, mammary gland, and macrophages but not in liver, is distinct from type I arginase, a cytosolic enzyme that is highly expressed in liver as a component of the urea cycle. The induction of type II arginase in enterocytes after weaning possibly regulates the availability of arginine for the synthesis of nitric oxide (NO), ornithine and thus, polyamines, proline, and glutamate, as well as ureagenesis in the small intestinal mucosa (fig. 7). Although considerably less than in the liver,
gut ureagenesis may be a first-line defense in detoxification of ammonia derived from tissue metabolism and luminal microorganisms (Wu, 1995). However, the major end-products of arginine metabolism by intestinal enterocytes from weaned pigs are proline and ornithine. Proline, required for collagen synthesis, is one of the most abundant amino acids in human milk (Davis et al., 1994). Arginine-derived proline is not detectable in enterocytes of newborn or suckling pigs. However, providing proline in the diet can ameliorate the hyperammonemia associated with dietary arginine deficiency in neonatal pigs (Brunton et al., 1999). Thus, while proline is not considered an absolute dietary essential nutrient, it may be conditionally essential for maintaining arginine synthesis in neonates. It is apparent from a number of studies that a normally functioning gut is important for maintenance of whole-body arginine and proline status, especially in neonates. Furthermore, these amino acids become conditionally essential under conditions that markedly reduce gut mass or compromise function, such as massive small bowel resection (Wakabayashi et al., 1995).

Recent studies in cultured intestinal cells (Blaicher et al., 1995) have shown that ornithine derived from arginine metabolism is converted to polyamines (fig. 8). Polyamines (putrescine, spermidine, spermine, cadaverine)
are ubiquitous cationic amines involved in cell proliferation and differentiation in many tissues, including the gastrointestinal tract. Ornithine decarboxylase (ODC) and S-adenosyl-methionine decarboxylase (SAMDC), converting ornithine to putrescine and putrescine to spermine, respectively, are the rate-limiting enzymes in polyamine synthesis. The synthesis of polyamines from arginine is negligible in enterocytes of newborn and suckling animals (Blaicher et al., 1991; Blaicher et al., 1992), but increases in enterocytes of postweaning animals, concurrent with the induction of both arginase and ODC (Wu and Morris, 1998). Luminal administration of polyamines increases intestinal growth in adult rats (Seidel et al., 1985) and has been shown to enhance intestinal maturation (Grant et al., 1990) and cell proliferation in developing rats (Dufour et al., 1988). Polyamines are present in human milk in micromolar concentrations for up to 4 months of lactation (Pollack et al., 1992; Buts et al., 1995). Results of a recent study in cultured intestinal cells suggested that both human and rat milk, but neither bovine milk nor infant formula, contain sufficient amounts of polyamines to sustain cell growth during inhibition of polyamine synthesis with difluoromethylornithine (DFMO), a specific and irreversible inhibitor of ODC (Capano et al., 1998). Thus, when the ingestion of milkborne polyamines by the neonate ceases after weaning, the induction of intestinal polyamine synthesis from ornithine, arginine and proline may become physiologically significant for the maintenance of normal intestinal growth and function (Wu et al., 2000a; 2000b). Furthermore, the induction of intestinal polyamine synthesis is dependent on the weaning-induced cortisol surge.

### 3.4. Essential amino acid oxidation

The catabolism of essential amino acids by the gut has received little recent attention, in part because early studies failed to demonstrate the presence of the catabolic enzymes in the small intestinal mucosa (Wu, 1998). However, studies based on isotopic tracer kinetics in PDV tissues suggest that dietary essential amino acids may indeed be oxidized by the intestinal mucosa. Studies in sheep (Pell et al., 1986; Cappelli et al., 1997; Yu et al., 2000), pigs (Burrin et al., 1999b) and dogs (Yu et al., 1992) have found that approximately 5–10% of whole-body leucine flux is oxidized by the portal-drained viscera. Recent studies in piglets enterally fed [U-13C] threonine, leucine, lysine and phenylalanine suggested significant first-pass metabolism by the intestinal mucosa (Stoll et al., 1998; Stoll et al., 1999; Van Goudoever et al., 2000; Van der Schoor et al., 2001). In growing pigs, lysine is considered to be the first limiting dietary amino acid. Studies in young piglets showed that intestinal oxidation of dietary lysine accounted for about
one-third of whole-body lysine oxidation. Interestingly, although about 10% of the arterial flux of lysine was taken up by the portal-drained viscera, none of this was oxidized, suggesting a preferential oxidation of dietary lysine. Studies in young pigs suggest that approximately 15 to 30% of the whole-body leucine and phenylalanine flux is oxidized by the PDV tissues (Van der Schoor et al., 2001; Bush et al., 2001). The oxidation of phenylalanine implies that phenylalanine hydroxylation occurs in the gut and is consistent with previous observations suggesting de novo gut tyrosine production. However, threonine seems to be unique among the essential amino acids in that there is negligible $^{13}$C-threonine oxidation to CO$_2$ when given either enterally or systemically to young piglets. This may be due to the fact that it is preferentially used for synthesis of mucins.

4. ROLE OF ONTOGENY

The fractional rates of whole-body protein metabolism are highest during foetal life, and decline progressively with advancing post-conceptual age, commensurate with fractional growth rates (Waterlow et al., 1978; Goldspink and Kelly, 1984). In rats, the intestinal protein mass increases approximately 750-fold (2.5 to 1893 mg between 18 days gestational age and 105 weeks postpartum age (Goldspink et al., 1984). During this life span, however, the fractional protein synthesis rate (FSR) in the small and large intestine declines by 43%. After weaning, the decline in intestinal FSR with age is largely due to a decreased synthesis in the muscularis and serosal layers, whereas the mucosa remains constant (Merry et al., 1992). Despite the overall age-related decline, studies in neonatal rats and mice indicate that the FSRs of the stomach, small intestine and pancreas increase significantly after weaning (Burrin et al., 1991; Burrin et al., 1999a). In domestic animals, there are few, if any, estimates of gastrointestinal FSRs before birth and beyond pubertal ages, yet the changes between birth and weaning in pigs and sheep tend to parallel those in rodents (Seve et al., 1986; Attaix et al., 1992; Davis et al., 1996). In milk-fed animals, the intestinal FSR declines during the neonatal period, but increases markedly (40–50%) after weaning. Even more striking is the relative contribution of the small and large intestine to whole-body FSR, which was found to be approximately 10% in milk-fed lambs and 20% in weaned lambs. The explanation for this sharp increase in gut protein synthesis after weaning is likely the substantial change in the composition diet and resultant stimulation of mucosal cellularity and proliferation (Attaix and Meslin, 1991; Pluske, 1997; Jiang et al., 2000). The extent to which these changes are related to alterations in the gut microflora is also a probable factor.
With respect to the ontogenic changes in the metabolism of specific amino acids, most studies have focused on glutamine, arginine, citrulline, ornithine and proline. The rates of glutamine and glucose oxidation in isolated enterocytes decrease by roughly 90% in the first 3 weeks of life (Darcy-Vrillon et al., 1994; Wu et al., 1995). The percentage of glutamine and glucose oxidized to CO₂ at birth is 36 and 21%, respectively, and declines at 3 weeks of age to 4 and 2%, respectively. After weaning, in intraepithelial lymphocytes, glutamine is mainly metabolized to glutamate and ammonia (92%), with minimal oxidation (4%). The decline in glutamine oxidation with age is paralleled by increased activities of glutamine synthetase, glutaminase, and glutamate dehydrogenase (Hahn et al., 1988; Shenoy et al., 1996; Madej et al., 1999). Other studies in isolated enterocytes show that the synthesis of citrulline and ornithine increases with age, particularly after weaning, and proline is a major precursor for their synthesis (Wu, 1997). Recent studies indicate that this shift in ornithine production is associated with increased ODC activity and is dependent on increased circulating cortisol (Wu et al., 2000a; Wu et al., 2000b).

5. ROLE OF NUTRITION

5.1. Enteral vs parenteral

Oral feeding is a potent stimulus of intestinal protein and amino acid metabolism in growing animals (McNurlan et al., 1979; Patureau Mirand et al., 1990; Burrin et al., 1991; Burrin et al., 1995). Prolonged fasting leads to markedly reduced protein mass in gut tissues via suppressed protein synthesis and increased protein degradation, especially in the small intestine (Burrin et al., 1991; Samuels et al., 1996). Recent studies have shown that the stimulatory effect of nutrient intake on gut protein metabolism is highly dependent on the administration of nutrients via the enteral route (Dudley et al., 1998; Burrin et al., 2000; Stoll et al., 2000a). These piglet studies showed that net loss of intestinal protein occurs during 7 days of TPN, and that intestinal protein accretion is highly correlated with the level of enteral intake; intestinal protein balance occurred at 20% enteral intake, and maximal intestinal protein accretion at 60% enteral intake (fig. 9).

Numerous studies have shown positive effects of glutamine-supplemented TPN in preventing atrophy, stimulating protein anabolism and maintaining intestinal permeability (Tamada et al., 1992; Platell et al., 1993; Inoue et al., 1993; Haque et al., 1996; Naka et al., 1997; Khan et al., 1999). In contrast, other studies have shown no effect of glutamine on gut growth or protein metabolism in animals receiving glutamine supplementation.
parenterally (Spaeth et al., 1993; Burrin et al., 1994; Marchini et al., 1999; Humbert et al., 2001). Furthermore, parenteral infusion of lipid has been shown to stimulate intestinal protein synthesis more than either glucose or glutamine (Stein et al., 1994). Thus, the intestinal trophic effects of glutamine-supplemented TPN are evident under conditions of compromised gut function, such as sepsis, inflammation, and small-bowel resection, while in healthy animals, glutamine provides limited benefit.

Although the provision of nutrition via the enteral route is critical for intestinal growth, the effects of first-pass metabolism can significantly increase whole-body requirements for arginine, threonine, and methionine (Brunton et al., 2000). Moreover, studies in piglets have shown that when first-pass intestinal metabolism is bypassed during TPN, arginine and proline become essential amino acids because their biosynthesis requires the enteral input of precursors (Bartolo et al., 1999a). In contrast, the consequences of reduced gut mass following TPN may enhance the absorption of nutrients when enteral feeding is resumed. Our preliminary studies indicated that the 52% reduction in intestinal mass observed after 7 days of TPN was associated with a 30% increase in the net portal absorption of enterally administered leucine (Burrin et al., 1999b). Moreover, the increased absorption was largely due to reduced portal utilization of arterial leucine,
secondary to the reduced intestinal mass. This finding indicates that a 50% reduction in gut mass actually increased the net systemic amino acid availability for growth. Interestingly, these results provide a metabolic explanation for the phenomenon of compensatory growth, which occurs when animals are fed *ad libitum* following periods of restricted food intake. Previous studies in ruminants have shown that the reduction in the mass and energy expenditure of the visceral organs during nutrient restriction results in increased dietary energy availability for compensatory growth (Ferrell, 1988). It would appear equally plausible that the increased systemic amino acid availability resulting from a reduction in intestinal mass and amino acid metabolism also could lead to enhanced efficiency of dietary protein use and growth rate, typical of compensatory growth.

### 5.2. Nutrient Composition

In addition to the quantity of dietary intake, the composition of dietary nutrients can also substantially affect gut protein and amino acid metabolism. There is an extensive literature describing the impact of dietary composition on gut growth and function (Spector et al., 1977; Weser et al., 1986; Buts et al., 1990; Bragg et al., 1991; Jenkins and Thompson, 1994). However, considerably less is known about how the composition of the diet alters gut protein metabolism. Some studies have shown that restriction of dietary protein has limited effects on intestinal protein synthesis and growth (Seve et al., 1986; Ebner et al., 1994), whereas others have reported a decrease in protein synthesis (McNurlan and Garlick, 1981; Wykes et al., 1996). The study with neonatal pigs demonstrated that, although protein malnutrition significantly reduced whole-body growth and amino acid absorption, this was associated with reduced carcass growth, since gut tissue growth was maintained (Ebner et al., 1994). A recent study based on metabolism of enterally fed $^{13}$C-lysine showed that in pigs fed low-protein diets, the PDV tissues utilized more than 75% of the lysine intake, compared to 45% in high-protein-fed pigs (Van Goudoever et al., 2000). Another important finding of this study was that on the high-protein diet, all of the PDV lysine utilization was derived from the arterial circulation, whereas on the low-protein diet, the gut used lysine equally from the enteral and arterial input. This study highlights the preferential use of dietary amino acids by the gut and how this limits the systemic availability of lysine for lean tissue growth during protein malnutrition.

Studies focusing on the impact of dietary macronutrients found that enteral amino acids, but not carbohydrate or lipid, stimulated intestinal protein synthesis, whereas each of these stimulated gut protein accretion, suggesting
that carbohydrate and lipid suppressed gut proteolysis (Stoll et al., 2000). In contrast, in other studies, enteral amino acids rapidly decreased intestinal protein synthesis and proteolysis in young pigs (Adegoke et al., 1999). Feeding a high-fat versus high-carbohydrate diet to young pigs stimulated intestinal protein synthesis (Ponter et al., 1994). Numerous studies have examined the effect of dietary supplementation with specific amino acids on intestinal growth and metabolism. Supplementing glutamine, glutamate, and arginine has been shown to be stimulatory to the gut in some cases, but not in others (Michail et al., 1995; Wu et al., 1996; Horvath et al., 1996; Vanderhoof et al., 1997; Hasebe et al., 1999; Bertolo et al., 1999b; Ewtushik et al., 2000). Dietary factors that stimulate mucosal proliferation and cell turnover also tend to increase protein synthesis. Feeding fibre and lectins have been shown to stimulate intestinal protein synthesis in some cases (Southern et al., 1985; Palmer et al., 1987), but not in others (Nyachoti et al., 2000). Studies show that neonatal pigs fed elemental diets have higher rates of protein synthesis and cell proliferation than those fed cow’s milk formula, even though intestinal mucosal growth and villus morphology are similar (Stoll et al., 2000c).

6. INFECTION AND INFLAMMATION

Infestation with pathogenic microbial and viral organisms, or exposure to the toxins they produce, is known to adversely affect both intestinal structure and function. Studies with growing animals indicate that exposure to pathogenic and non-pathogenic organisms impart a protein metabolic cost to the animal associated with stimulation and maintenance of the pro-inflammatory, acute-phase response (Von Allmen et al., 1992; MacRae, 1993; Higashiguchi et al., 1994; Johnson, 1997; Breuille et al., 1998; Wang et al., 1998; Breuille et al., 1999; Mack et al., 1999). The studies demonstrate that treatment with pro-inflammatory stimuli such as bacteria, enteric parasites, endotoxin and cytokines significantly increases protein synthesis in visceral tissues, especially the liver, gut and spleen, while increasing catabolism and net loss of muscle protein body weight. Recent work in sheep demonstrated that parasitic infection increases the rate of leucine utilization and oxidation by PDV tissues, thereby reducing the systemic availability of dietary amino acids by 20–30% (Yu et al., 2000). In addition, most of the increased PDV leucine utilization is either oxidized or lost as endogenous protein secretion; together, these losses account for most of the reduced nitrogen retention associated with infection (Yu et al., 2000).

These results illustrate a mechanism whereby the gastrointestinal microflora activate the immune system and suppress the growth rate in domestic
animals. Antimicrobial compounds are fed to domestic animals in order to suppress the activity of the gut microflora and enhance growth; however, the exact mechanism for this effect is unknown. It has been shown that by suppressing microbial activity, antimicrobials reduce the luminal concentration and associated toxic insult of ammonia, and thereby diminish the thickness and mass of the intestinal mucosa and associated lymphoid tissue (Visek, 1978). Studies in pigs and chickens show that feeding antimicrobial compounds significantly reduces small intestinal mass, cell proliferation and intestinal ammonia absorption (Yen et al., 1987; Yen and Pond, 1990; Krinke and Jamroz, 1996). Additional evidence indicates that much of the luminal ammonia originates from bacterial hydrolysis of urea and deamination of dietary amino acids. Thus, given the recent evidence that the gut may be an important site of dietary amino acid catabolism, the question arises as to whether this activity is associated with the luminal microbes or the cell populations of the mucosa. Although it is likely that both possibilities exist, the end result is the catabolism and loss of dietary amino acids, especially those that are essential, that otherwise would be used for growth.

7. FUTURE PERSPECTIVES

It is evident from past studies that the tissues of the gastrointestinal tract play an integral role in the whole-body protein and amino acid metabolism of growing animals. It has also become increasingly evident that the gut not only consumes a substantial fraction of the dietary intake, but is critical for the metabolism of key amino acids, such as arginine and threonine, that are essential for the growth of developing animals. There has been considerable progress in our understanding of intestinal amino acid metabolism, especially nonessential amino acids such as glutamate, glutamine, citrulline, ornithine, and proline. However, with the exception of arginine and, to some extent, threonine, there is only a very limited understanding of the cellular basis for how other essential amino acids are metabolized within the gut tissues. With the expanding range of analytical tools, from the molecular to the whole-organ level, there is a clear rationale and opportunity to explore these issues in growing animals. For example, many of the biochemical pathways responsible for oxidation of essential amino acids have either not been well characterized or even shown to exist in intestinal tissues. Although it is well established that glutamine and glutamate are major oxidative fuels for the gut, the metabolic fate of nitrogen from these substrates is only vaguely understood. There is also a compelling need to identify the cellular signaling mechanisms whereby nutrients and other extracellular molecules affect the rates of protein and amino acid metabolism of intestinal cells. Many of
the pathways and cellular targets that have been shown to be important in
other cell types are obvious candidates (e.g., MAP kinases, PI3 kinase,
mTOR, proteasome, NF-kB). As we begin to establish the underlying cellu-
lar basis of protein metabolism, it should become increasingly evident as to
which specific nutrients are important for intestinal growth and function;
this will then lead to more rational diet formulation. In addition, continued
efforts should be aimed at establishing how regulatory elements, such as
nutrients, hormones and environmental factors, modulate the growth and
metabolism of the gut tissues, and then quantifying their impact on nutrient
availability and growth of the animal. Further studies that couple the use of
trans-organ substrate balance and isotopic kinetic measurements will be par-
ticularly useful in attempting to answer these questions. An especially cru-
cial area will be the impact of the gut microflora, and the extent to which it
modifies gut amino acid utilization and the nitrogen economy of the animal.

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