Amino Acids for Optimal Intestinal Mucin Synthesis and Gut Protection in Health and Disease

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Abstract

Amino acid requirements are defined in healthy conditions. In pathological situations, including intestinal inflammation, the body defense is associated with anabolic reactions involving the splanchnic area and especially the gut. Intestinal defense and repair processes dramatically increase the synthesis rate of proteins implicated in the gut barrier function, such as mucins. It augments the host's need of specific amino acids, particularly those enriched in mucins. A "healthy" diet is therefore not adapted. Increasing the dietary supply of threonine, serine, proline and cysteine is required to promote mucin synthesis and strengthen the non-immune intestinal barrier function.

Glossary of Abbreviations
ASR: Absolute Synthesis Rate
IBD: Inflammatory Bowel Disease
FSR: Fractional Synthesis Rate
MUC2: Mucin 2 Gene
Muc2: Mucin 2 Protein

Introduction

The gastrointestinal tract is one of the most metabolically active organs of the body, which reflects its important and numerous biological functions. Whereas the gastrointestinal tract contributes 3% to 6% of the mammalian body weight, it accounts for more than 20% of the whole-body protein turnover.[1] This is mainly due to a high protein synthesis rate and to a continuous and significant secretory activity. This translates into a high demand in certain amino acids required for the protein synthesis process. Such a high requirement has been ascribed to support the non-immune gut barrier, in particular the synthesis of intestinal mucins. Inflammatory situations further increase the intestinal protein synthesis and consequently the utilization of certain amino acids by the intestine. In this context, adequate nutritional management is required to maintain or repair the intestinal barrier integrity and function.

The Non-Immune Intestinal Barrier

The intestinal protection of the host is ensured by both the intestinal immune system and a physical, non-immune intestinal barrier. The intestinal barrier ensures protection of the host from the external environment (luminal pathogens, noxious agents, etc.) while allowing absorption of nutrients for adequate supply of the whole body. Its optimal function relies on the close interplay of several intestinal compartments. The major key players are: the commensal intestinal microbiota presence and equilibrium, which antagonizes the adhesion of potentially pathogenic bacteria[2]; the intestinal mucus layer, which covers and protects the delicate epithelial cells[3]; the intestinal epithelium itself, ensuring the separation between the luminal contents and the underlying tissue compartments;[4] the Paneth cells, producing antimicrobial peptides;[5] the tight junctions between epithelial cells, contributing to the modulation of paracellular pathways[6]; and the enteric nervous system, recently recognized as a key regulator of the epithelial barrier integrity.[7]

Complex regulatory mechanisms are taking place to ensure the subtle equilibrium among these different components of the non-immune intestinal barrier. Optimal nutritional support is crucial to maintain this intestinal homeostasis, favoring a global healthy status of the body and preventing gut-related diseases.

Composition and Role of the Intestinal Mucus Layer

The gastrointestinal epithelium is covered by a viscoelastic mucus gel layer composed of: a complex mixture of glycoproteins named mucins; peptides, including trefoil peptides and antimicrobial peptides; water; macromolecules, such as secretory immunoglobulin A; electrolytes; microorganisms; and sloughed cells.[3,8] The mucus gel constitutes the front line of innate host defense; one of its main documented functions is to protect delicate epithelial surfaces against mechanical stresses and constant attacks from digestive fluids, microorganisms and toxins.[3,9] Its protective effect is directly related to its thickness and composition. The unique protection capacity of the mucus gel is conferred, in part, by its high content in mucin glycoproteins, which are continuously synthesized and secreted by intestinal goblet cells and mucosal epithelial cells throughout the entire gastrointestinal tract.[3]

The mucus thickness, composition and protective effect vary along the gastrointestinal tract[10] as a result of the differential expression of various distinct mucins and the dynamic balance between opposing anabolic (expression, synthesis and secretion from goblet cells) and catabolic (physical and proteolytic degradation) processes. The mucus layer is thickest in the stomach and

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large intestine in order to provide strong protection from acidic conditions (stomach) and microbiota (colon). It is thinnest in the small intestine likely to avoid interference with the absorption of nutrients.[10] An inner, firmly adherent mucus layer consisting of membrane-bound mucins adheres to the apical side of epithelial cells and contributes to the formation of glycocalyx, a polysaccharide matrix coating the surface of intestinal epithelial cells. A soluble, loosely adherent mucus outer layer, consisting of secreted gel-forming mucins, covers the inner mucus layer. This soluble layer favors the establishment and maintenance of a balanced commensal microbiota that antagonizes potentially pathogenic bacteria.[11,12]

Characteristics of Intestinal Mucins

To date, 21 mucin genes have been identified, of which 15 have been shown to be expressed in the human gastrointestinal tract.[13] Intestinal mucins share particular compositional features. They are usually large polypeptides (10%-20% of the mucin mass) that are heavily glycosylated (up to 80%-90% of the mucin mass). The oligosaccharide side chains are mainly composed of N-acetylgalactosamine, N-acetylglucosamine, galactose and fucose primarily linked to serine and threonine residues of the mucin polypeptide core via O-glycosidic bonds. Post-translational modifications, including sialylations and sulfations, complete the macromolecule.[3]

The mucin polypeptide size usually ranges from 200 kDa up to 900 kDa, with the exception of the salivary form MUC7 (39 kDa).[14] As compared to other mammalian proteins, mucins are particularly enriched in the amino acids threonine, serine and proline, which account for up to 28%, 14% and 13%, respectively, of the total amino acid composition of mucins.[3] For comparison, the average threonine content of body proteins ranges from 3% to 7% of total amino acids. The threonine, serine and proline residues are concentrated into central tandem repeat PTS (proline, threonine, serine) regions made of conserved sequences repeated about 100-fold. Cysteine-rich domains also are present on the mucin polypeptides.[14] They allow mucins to assemble into homo-oligomers via intermolecular disulphide bonds formed between the cysteine-rich domains, which confer the viscoelastic and protective property of the mucus gel.[13]

Among the 15 mucins expressed in the human gastrointestinal tract, MUC2, MUC5AC, MUC5B, MUC6, MUC7, and MUC19 are secreted mainly by specialized goblet cells.[14] In the small and large intestines, MUC2 is the predominant gel-forming mucin. Its critical role to protect the colonic epithelium from colitis has been clearly demonstrated in a Muc2-deficient mice model.[15] MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, and MUC17 are membrane-associated mucins expressed by mucosal epithelial cells of the human gastrointestinal tract.[13] In the small and large intestines, MUC3, MUC4, MUC13, and MUC17 are the predominant membrane-associated forms that have been identified.[13] They extend above the cell surface and form the glycocalyx. Specific roles in anti-adhesive and signaling mechanisms,[16] intestinal cell restitution[17] and protection of intestinal epithelial cells from infection[18] have been proposed for membrane-associated mucins.

Complex regulatory mechanisms are taking place to ensure adequate mucin expression and secretion for optimal intestinal protection. These mechanisms have been shown to involve neuronal, hormonal and paracrine pathways.[19-21] The nutritional status that allows the supply of adequate amounts of amino acids required for mucin synthesis[22-26] and the microbiota[11,27] also are key regulators of intestinal protection.

Metabolic Disorders in Intestinal Diseases Impair Mucin Production and Gut Protection

Many intestinal diseases involving chronic inflammation, such as inflammatory bowel disease (IBD), are associated with intestinal barrier dysfunctions. The two major types of IBD, ulcerative colitis and Crohn's disease, are accompanied by an increase in small and large intestinal permeability.[28,29] Among modifications observed at the gut barrier level, an altered gut microbiota composition[30,31] and qualitative and quantitative impairment of the mucus layer and mucin production have been reported.[13,32] In particular, the synthesis of a mature, glycosylated form of Muc2, the primary mucin secreted in the colon, is decreased in ulcerative colitis patients, which reduces the mucus barrier.

Abnormal expression of gastric-secreted mucins in ileum and colon also has been reported, which may reflect an adaptive response to strengthen the defense reaction.[13] The expression of membrane-bound mucins MUC3, MUC4 and MUC17 has been observed to be decreased, further corroborating the reduction of epithelial protection. However, and interestingly, the expression of MUC13, recently documented to inhibit toxin-induced apoptosis of the colonic epithelium,[33] has been shown to be increased in inflamed colonic mucosa biopsies, reflecting a defensive mechanism that remains nevertheless insufficient to maintain or restore the intestinal barrier function.

Metabolic disorders associated with acute systemic inflammatory reactions, as observed in sepsis, for instance, also impact the intestinal barrier function. Acute inflammation stimulates the synthesis of acute-phase proteins in the liver[34] and mucosal proteins and mucins in the intestines.[33] These anabolic reactions are important adaptations aiming at ensuring the body's defense against primary and secondary aggressions. A key factor in the initiation and maintenance of such body defenses is therefore the ability of the host to sustain such stimulation of protein synthesis. In this context, there is a strong increase in amino acid requirements,[36] especially in those present at high levels in mucins. In a disease state, food intake is often decreased, and the dietary amino acid supply is too low to meet the metabolic demand. Amino acids are thus obtained through increased muscle catabolism.[37]

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Amino Acid Requirements for Optimal Mucin Synthesis and Gut Protection

The gastrointestinal tract contributes only 3% to 6% of the mammalian body weight, whereas it accounts for more than 20% of the whole-body protein turnover.[1] This is, in part, due to its high proliferative and secretory activities that support the non-immune gut barrier function, particularly the rapid renewal of intestinal epithelial cells and the continuous synthesis of intestinal mucins. The amino acid composition of synthesized and secreted proteins largely affects the amino acid requirements of the gut, which has to be met by dietary nutrition and endogenous synthesis (for nonessential amino acids).

Under Healthy Conditions

Threonine is an essential amino acid, which means it cannot be synthesized by the organism and must therefore be supplied in the diet. Under healthy conditions, threonine is key for the maintenance of the gut. Indeed, compared with other essential amino acids, a large proportion of dietary threonine (up to 60%) is retained by the healthy pig[38] or human[39] intestine. Since the core protein of intestinal mucins contains high amounts of threonine (up to 30% of their amino acid composition[3]), their continuous synthesis explains the high rate of threonine utilization by the gastrointestinal tract. Along this line, a lack of Muc2 in knock-out mice indeed inducies the metabolic oxidation of unused threonine,[40] which reflects an excessive supply of threonine occurring in the absence of Muc2 synthesis.

In contrast, when dietary threonine supply is below the requirements, threonine can become a limiting amino acid for the synthesis of intestinal mucins, as shown in rats[23] and in pigs and piglets.[24-26] Indeed, the mucin fractional synthesis rate, defined as the percentage of mucins synthesized per day, has been shown to decrease by half in the upper small intestine of rats fed a diet covering 30% of their threonine requirements for growth (Figure 1). Nevertheless, it has no major limiting effect on total mucosal protein synthesis[23] (Figure 1), with these proteins containing about seven times less threonine than Muc2.

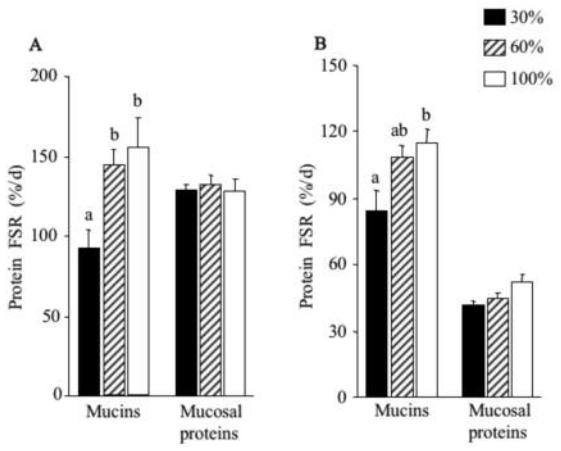

Figure 1. Fractional synthesis rate (FSR), expressed in %/day, of mucins and total mucosal proteins in the upper small intestine (A) and colon (B) of rats fed semisynthetic diets meeting 30%, 60% or 100% of their threonine requirements for growth. Diets were isonitrogenous (adjusted with alanine) and administered to the rats for 14 days. All groups of rats were pair-fed to the mean intake of rats from the group 30%. The *in vivo* protein synthesis was measured using the flooding dose method following injection of L-(1-13C)-valine. Values are means ± SEM, n=8. For each intestinal compartment (mucins or mucosal proteins), means without a common letter differ, $p<0.05$.

Because mucins are particularly resistant to digestive enzyme activities, the threonine recycling from mucins secreted in the upper gastrointestinal tract is very low[41] and the threonine loss is very high in respect to the whole body threonine requirement.[42] In summary, under healthy conditions, it is crucial that the dietary threonine supply accurately meets the body's threonine requirement in order to maintain optimal mucin synthesis and intestinal protection, to favor a global healthy status of the body, and to prevent gut-related diseases.

In Inflammatory Diseases

As shown in animal models and humans, inflammatory situations, such as those observed in IBD (chronic inflammation) and sepsis (acute inflammation), are associated with an overall increased anabolic reaction occurring mainly in the intestines and the liver, respectively.[43-46] This anabolic response increases the utilization of amino acids and, in particular, those present at high levels in intestinal and hepatic proteins. Therefore, the requirements for threonine and for other amino acids, such as serine and cysteine, is strongly increased.[47]

The availability of those amino acids for the synthesis of intestinal mucins for which they are primary (threonine) or likely secondarily (serine, cysteine) limiting[23-26,47] is probably too low because of a limited nutritional quality (insufficient levels of these amino acids) and quantity (poor appetite) of the dietary intake. As an example, two days after infection, the utilization of threonine for the synthesis of rat intestinal mucins has been shown to be 70% greater than in pair-fed rats.[35] Overall, the daily absolute threonine utilization for the synthesis of intestinal proteins (gut wall) plus the plasma proteins (minus albumin) increased by 23%, which represented 2.6 times the dietary intake of rats.[35] Similarly, proline, which is highly represented in the composition of intestinal mucins (13%[3,48] as compared to 4%–7% in body proteins, except collagen), also may be a secondary limiting amino acid for mucin synthesis.

In inflammatory situations, adequate and well-balanced nutritional support is therefore required to promote the defensive response, the repairing mechanisms and consequently the maintenance or restoration of an effective intestinal barrier function. The definition of "adequate and well-balanced nutritional support" will depend on the metabolic condition associated with the disease and therefore can't refer to that defined for the healthy condition.

As previously observed in IBD animal models, the intestinal

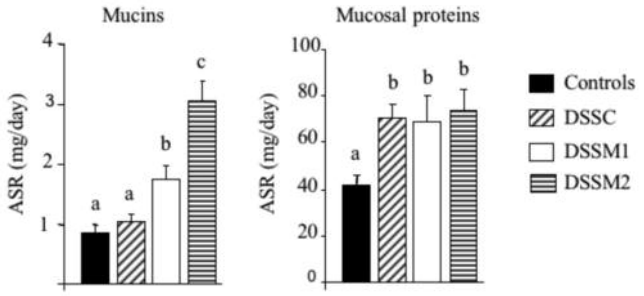

Figure 2. Absolute synthesis rates (ASR), expressed in mg/day, of mucins and mucosal proteins in the colons of dextran sodium sulfate (DSS) treated rats. The rats were fed for 28 days with isonitrogenous (adjusted with alanine) semisynthetic powder diets providing the following supplementation levels as compared to rat's requirements: DSSM1; twofold increases in threonine, proline, serine and cysteine; DSSM2; fourfold increases in threonine and proline; and threefold increases in serine and cysteine. Values are means ± SEM (n=8). For each intestinal compartment (mucins or mucosal proteins), means without a common letter differ, p<0.05.

mucin production is not stimulated with a healthy, balanced diet.[45,46,49-52] However, increasing the threonine, serine, proline and cysteine dietary supply in a rat model for colitis has been shown effective in promoting the colonic mucin synthesis in a dose-dependent manner, while having no effect on total mucosal proteins[52] (Figure 2). The higher dose of amino acids increased the presence of Muc2-containing goblet cells in the surface epithelium of the ulcerated area.[52] It also promoted the growth of all commensal bacterial populations tested, including *Lactobacillus*.[52]

Conclusion

The amino acids threonine, serine, proline and cysteine are relatively high in the composition of intestinal mucins, which explains, in part, their high utilization by the gut. Adapted nutritional support, in particular with accurate levels of these four amino acids, is therefore crucial to maintain an effective intestinal barrier function. Pathological situations, including intestinal inflammation, intestinal defense and tissue repair processes, further increase the host's need of such amino acids. In such situations, an increased dietary supply of threonine, serine, proline and cysteine is advised to promote the mucin synthesis and the growth and equilibrium of the commensal microbiota and consequently to strengthen the non-immune intestinal barrier function.

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