Absorption Kinetics of Amino Acids, Peptides, and Intact Proteins

Gabriella A.M. Ten Have, Marielle P. K.J. Engelen, Yvette C. Luiking, and Nicolaas E.P. Deutz

The small intestine acts as interface and regulator between the gut lumen and the rest of the body and controls the degree and rate of transport of amino acids coming from dietary protein via the portal vein to the liver and the systemic circulation. To measure protein absorption, kinetics multicatheter animal (pig) models in combination with amino acid tracer technology are available. Dietary factors influence the absorption rates from the lumen to the gut, metabolism of dietary component in the gut, and the release of amino acids to the portal circulation from digested protein. In a balanced-protein meal, the gut dietary amino acid utilization (30–50%) for gut protein synthesis will result in a labile protein pool in the gut that can be beneficial during the postabsorptive state. To enhance gut retention, amount and quality of protein and the presence of carbohydrate are major factors. Besides this the use of a slowly digestible protein or the presence of fiber in the meal can increase retention further. During the absorption of low-quality protein meals, fewer amino acids are utilized by the gut, resulting in higher amounts of amino acid release to the portal circulation. Malnutrition or starvation, protein depletion, deficiencies of specific nutrients, or illness such as sepsis all inhibit the growth and change protein turnover of the intestinal mucosa and therefore affect absorption kinetics. Therefore, the kind of protein meal that has the most optimal absorption kinetics (the most beneficial) for gut and for the rest of the body depends on these (patho)physiological circumstances. Despite the absence of different absorption kinetics between protein, peptides, and amino acids, they could be beneficial in specific circumstances.

Key Words: gut, nutrition, intestine, metabolism

The gastrointestinal tract is the primary organ controlling food digestion, absorption of food components, and release of food components to the rest of the body. Furthermore, the gut has a barrier function that protects the body against invasion (translocation) of endogenous luminary microorganisms and toxins (64).

To cover this variety of functions, the structure of the small intestine has a large luminal surface area (mucosa) made possible through mucosal folding with the presence of villi and microvilli on top and crypts at the bottom. The intestinal mucosal layer is one of the most rapidly replicating tissues in the body, with a

The authors are with the Donald W. Reynolds Institute on Aging, University of Arkansas for Medical Sciences, Little Rock, AR 72205.
continuous process of differentiation of stem cells into specialized cells for absorption (enterocytes or epithelial cells), excretion (enteroendocrine cells), and mucus (water and glycoproteins) secretion (goblet cells) (30).

Digestion of dietary protein begins in the stomach but occurs mainly in the small intestine. Although approximately 95% of dietary protein is absorbed in the small intestine (17), the remaining amino acids, undigested proteins, and unabsorbed peptides, of either dietary or endogenous origin, can enter the large intestine. In the large intestine these components are subject to digestion and metabolism by the intestinal microflora. (63).

The small intestine itself is the absorption organ for amino acids coming from dietary protein and is the first pass for amino acids to the circulation. Therefore, the small intestine acts as interface and regulator between the gut lumen and the rest of the body and controls the degree and rate of transport of amino acids coming from dietary protein via the portal vein to the liver and systemic circulation. However, not all amino acids that pass the gut enter the circulation, because some of the amino acids are used for local metabolism (e.g., oxidation, protein synthesis). The current review focuses on the influence of several dietary factors on gut amino acid absorption, metabolism, and release to the portal system.

The Gut as a Metabolic Active Organ

Besides being the organ that has direct contact with ingested food, the gut is also a very metabolically active organ (61). Supply of amino acids to the gut cells does not rely solely on luminal supply during feeding. There is also a substantial use from arterial supply, and as such the gut is “competing” with other tissues for amino acid utilization (Figure 1). The high level of metabolic activity in the gut is also demonstrated by the fact that rates of protein synthesis in the intestinal mucosa are among the highest in the body (39).

During Feeding

Luminal contact with nutrients is very important in the regulation of intestinal protein metabolism (19, 25, 47). Feeding is known to rapidly stimulate protein synthesis in the gut. Approximately 50% of dietary amino acid intake is used by the portal-drained viscera, but this percentage varies between different amino acids (15, 53). It is known that mucosal protein turnover is high and that amino acids with a different origin, for example, lumen and circulation, contribute to protein synthesis at a different level during feeding (53).

Mucosal protein synthesis rate is not affected by feeding and is less sensitive to short-term variations in nutritional status (4). In neonatal pigs, less than 20% of intestinal amino acid utilization is for constitutive gut growth by the intestinal mucosa (49). It appears that an enhanced secretory (glyco)protein synthesis rate in these neonatal pigs contributes to the increased gut protein synthesis during feeding (53). Approximately 26% of the dietary protein intake reappeared in the portal vein by way of recycling of digested (glyco)proteins. Therefore, intestinal recycling of amino acids apparently contributes to their overall systemic availability. The protein-synthesizing capacity, however, depends on the dietary protein source used, as will be discussed later.
Absorption Kinetics

During Fasting

Malnutrition or starvation, protein depletion, and deficiencies of specific nutrients all inhibit growth and turnover of the intestinal mucosa (14, 33, 64). During fasting, gut protein is considered a (labile) pool (48). After a meal there is a net accumulation of protein in the gut, whereas in the postabsorptive state a net loss of protein takes place. The hypothesis is that net retention of amino acids as protein in the gut serves to “buffer” a bolus meal containing protein. When a protein would be rapidly digested, absorbed, and directly released to the portal system, the large flux of highly concentrated amino acids in the portal vein would give rise to a high rate of urea production, gluconeogenesis, and amino acid oxidation (16). A more gradual release of amino acids from the gut would ensure a more prolonged supply of amino acids in the portal vein, resulting in lower plasma concentrations in the portal vein and a lower urea production and potentially more muscle anabolism (48). During prolonged fasting, the intestine is taking up amino acids from the arterial side. In particular, glutamine coming from the muscle compartment is utilized as a major energy substrate by intestinal mucosal and immune cells (6, 14).

**Figure 1** — Model that explains net amino acid uptake and release into the circulation after a protein–peptide–amino acid meal. AA indicates amino acid.
Measurement of Gut Amino Acid Absorption Kinetics in Vivo

The in- and outflow (flux) of amino acids, protein, and nitrogen in the gut exhibit a complicated pattern (26). The digestion of many dietary proteins by the gut is incomplete. Besides dietary supply, there is also a continuous (but variable) entry into the intestinal lumen of endogenous protein and amino acid nitrogen that is subject to digestion. To gain insight into the absorption of a meal, methods are used that examine the digestion characteristics of a protein meal in the gut lumen, and other techniques are based on amino acid oxidation and the use of stable-isotope techniques. In order to measure absorption and fluxes of amino acids across the gut, a multicatheter technique is needed (50). Combination of catheterization of multiple organs and stable-isotope techniques enables measurement of in vivo gut metabolism and the interaction with other organs (15).

In Humans

Multiorgan catheter techniques are often not applicable in humans. In humans, measurements across the portal-drained viscera (the gastrointestinal tract, pancreas, spleen, and associated adipose tissue) are only possible during surgery (22, 42, 52). Therefore, in studies in healthy conscious subjects, most often splanchnic extraction of alimentary amino acids, which represents the sum of portal-drained viscera and liver amino acid uptake and utilization, is measured (2, 5, 11, 20, 34, 35, 51, 57). Introduction of this doubly labeled stable-isotope technique has enabled a more detailed study of splanchnic conversions and amino acid extraction (29, 38) in which 2 tracers of the same amino acid but with a different label are used. Simultaneously, one tracer is infused intragastrically or orally, and the other intravenously. A multicompartmental computing simulation model has been developed to describe dietary nitrogen postprandial distribution and metabolism in humans. The model uses experimental data on dietary N kinetics in certain accessible pools of the intestine, blood, and urine in healthy adults fed a [15N]-labeled protein meal (24, 31). In this model, however, specialized information on the absorption or utilization of dietary amino acids in the gut is limited.

Large and Small Animal Models

Small animals like rodents are considered suitable for investigating the mechanisms of absorption and bioavailability (14), whereas larger animals are generally used to assess absorption kinetics (26, 50). Pigs are often used in metabolic research because they are very similar to humans with respect to renal, cardiovascular, and digestive anatomy and physiology (13, 32, 40, 46). Furthermore, larger species like the pig are also useful when multiple blood samples have to be taken over time.

Multicompartmental Modeling

Studying amino acid absorption and utilization by the gut is difficult. The complicating factor is that the intestinal mucosa receives nutrients from 2 sources, the diet (brush border membrane) and the systemic circulation (basolateral membrane). Flux of amino acids from the lumen to the portal system is mainly studied in animal
models using multicatheter techniques. This enables sampling of blood entering (afferent blood vessel) and leaving the organ (efferent blood vessel) for measurement of concentration differences across the organ with simultaneous measurement of organ blood flow (50) (see Figure 1). Net balance studies of amino acids across an organ, however, only provide information on the net uptake (net anabolism) or release (net catabolism), and dynamic processes (e.g., disposal and production) cannot be quantified.

Extension of substrate (tracee) studies by the use of isotopes (tracers) enables measurements of amino acid disposal and production across the organ. The principle is based on the dilution principle of Fick. During primed continuous intravenous tracer infusion any change in the enrichment over time occurs as a consequence of dilution from the unlabeled tracee. The rate of appearance of an amino acid is determined by measuring the change of the tracer:tracee ratio in relation to the isotope infusion rate (60).

In the gut, phenylalanine disposal and production (turnover) are related to protein synthesis and protein breakdown and are measured by calculating tracer disappearance rate in that organ and the net balance of tracee. To assess phenylalanine measured protein turnover, tracer:tracee ratio measurements on the arterial and portal sides are needed (Figure 2). This is a so-called 2-compartment model, with plasma and interstitial fluid being the two compartments. A “3-compartment” model can also be applied when tissues or biopsies are available (the third compartment

Figure 2 — Model to measure amino acid (AA) uptake, metabolism, and release into the circulation after a protein–peptide–amino acid meal by using isotopes of amino acids (a, aa) that are present in protein.
being the free tissue pool). Combination of several tracers makes it possible to get quantitative information of multiple metabolic pathways in the gut.

Flux of a single amino acid across the gut coming from a protein meal represents absorption, utilization, and release to the portal system. To gain more insight into the absorption rate in time of a single amino acid, 2 tracers of this amino acid are used (Figure 2). One is done intravenously via a primed continuous infusion protocol, and the other is given orally, added to a protein meal.

**Dietary Factors That Affect Gut Amino Acid Absorption Rate, Metabolism, and Release**

The amount of protein, the protein source, and the presence of other macronutrients in the meal influence the absorption rate and metabolism of amino acids in the gut and affect the release rate of amino acids to the portal system. All these factors determine the anabolic capacity of a meal.

**Protein Amount**

The amount of protein in a diet has a major effect on the magnitude of change of protein metabolism in the gut and in the rest of the body. With a balanced meal approximately 90% of the dietary amino acids are absorbed by the gut. About 30–50% will be used by the intestine itself, and the remainder is released to the portal system. An excessive amount of protein intake potentially could lead to limitation of gut absorption and thus to a reduction of the percentage protein absorption. It is more likely, however, that the maximum that the gut cells can utilize for own metabolism is reached more quickly and that, consequently, higher protein intake will reduce the percentage of protein extracted. The amino acids that are not absorbed and undigested proteins will flow into the colon. During low protein intake, the percentage protein extracted by the gut will be higher, although the intestine can adapt to reduced protein intake by reducing its amino acid oxidation (54) (see Table 1).

**Protein Quality**

The quality (nutritional value) of a dietary protein is related to both the bioavailability of ingested nitrogen and amino acids and the efficiency of their metabolic utilization to meet nitrogen and amino acid requirements for growth and renewal of body proteins (28, 41, 43, 62). It is postulated that a high-quality dietary protein source stimulates amino acid utilization in the gut and is therefore of benefit for the gut and for the rest of the body (48, 53). In a pig study, we compared soy (low-quality) and casein (high-quality) protein and observed that liver urea production and net release of essential amino acids by the gut was higher with soy (15). This was confirmed in a study in healthy subjects, demonstrating a lower net protein synthesis and higher ureagenesis after a soy-containing meal (35). This suggests that amino acid retention across the portal-drained viscera is higher after a casein protein meal. Possible explanations are the fact that soy is deficient in the essential amino acids methionine and lysine, it contains fewer branched-chain amino acids, and the difference in the digestion rate between both proteins (3). In addition,
the high biological value of casein protein potentially is related to the release of peptides that have a local trophic effect on the gut, and that can increase mucin production (10).

Metabolic utilization of amino acids in the gut thus depends on the composition of the meal with respect to the presence or absence of (in)dispensable essential amino acids. Complete lack of amino acids in a protein meal makes the protein of inferior quality. Previous studies in pigs showed that ingestion of an isoleucine-lacking blood protein meal resulted in elevated urea production. Concomitant intravenous isoleucine infusion lowered the increase of urea and promoted amino acid retention in the gut (16) (see Figure 3). In conclusion, high-quality proteins stimulate amino acid utilization in the gut and therefore induce more gut amino acid retention (see Table 1).

Table 1  Summary of the Effects of Dietary Factors on Percentage Gut Retention and Portal Appearance After Bolus Feedings of Proteins, Peptides, and Amino Acids Under Healthy Conditions

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Effect on percentage gut retention</th>
<th>Effect on percentage portal appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing amount of protein</td>
<td>Decrease</td>
<td>Increase</td>
</tr>
<tr>
<td>Excessive amount of protein</td>
<td>Decrease</td>
<td>Decrease</td>
</tr>
<tr>
<td>Lowering amount of protein</td>
<td>Minor increase</td>
<td>Minor decrease</td>
</tr>
<tr>
<td>Improving quality of protein</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Slowing rate of protein digestion</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Adding carbohydrates to the meal</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Adding fats to the meal</td>
<td>Minor increase</td>
<td>Minor decrease</td>
</tr>
<tr>
<td>Adding soluble fiber to the meal (prolonged)</td>
<td>Increase</td>
<td>Decrease</td>
</tr>
<tr>
<td>Partially hydrolyzing protein (mainly</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>represents peptides)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fully hydrolyzing protein (mainly represents free amino acids)</td>
<td>No change</td>
<td>No change</td>
</tr>
</tbody>
</table>

Figure 3 — Pattern of portal-drained viscera amino acid efflux during bolus feeding with proteins. AA indicates amino acid.
Slow Versus Fast Proteins

A difference in the speed of protein digestion and absorption in the gut, the so-called slow- versus fast-protein concept, also affects the quality of a protein (12). Postprandial plasma amino acid profile shows a rapid high increase with whey protein and a prolonged plateau of moderate hyperaminoacidemia with casein protein (1), resulting in a more prolonged positive net protein balance after casein than after whey intake in young healthy males (1, 12) (Figure 3). The discussion of whether a slowly digestible protein (e.g., casein) is better than a quickly digestible protein (e.g., whey) depends on the (patho)physiological situation. The “labile” gut protein pool hypothesis suggests that prolonged amino acid release from the gut is better for the body in the long term. Recent evidence indicates that the optimal dietary protein source and feeding pattern changes with age. Stimulation of muscle protein synthesis in the elderly necessary to counteract sarcopenia is higher with fast proteins like whey, with protein hydrolysates, or with a protein-feeding pulse pattern (58). Therefore, factors such as amino acid profile, digestibility, and absorption rate of a protein can all influence gut retention of that protein.

Role of Adding Carbohydrates to the Meal

In a multicatheterized-pig study, pigs were given a bolus meal consisting of high-quality proteins with and without carbohydrates. Addition of carbohydrates to a protein meal resulted in increased intestinal amino acid retention (Figure 3), lower urea production, and increased gut glutamine uptake and alanine release, indicating stimulated gut metabolism (17). This suggests that adding carbohydrate to a protein meal improves the anabolic quality of the meal in the gut. Recent studies showed that adding high-quality protein to a carbohydrate meal has an insulinotropic effect (56). Intake of free leucine, phenylalanine, and arginine further augmented the insulin response. More recent studies show that coingestion of a protein hydrolysate with additional free leucine maximizes the insulinotropic response (in Type 2 diabetes patients, as well as in normoglycemic controls) (36, 37). Simultaneous ingestion of these amino acids and carbohydrates resulted in a 100% higher insulin response than with carbohydrate only (56).

Role of Adding Fat to the Meal

Until now, the effect of fat intake on amino acid retention in the gut has not been studied directly. Studying splanchnic nitrogen retention after adding fat versus carbohydrate to a protein meal using a simulation model revealed that fat enhances splanchnic dietary N anabolism only transiently, without significantly affecting the global kinetics of splanchnic retention and peripheral uptake (23). This suggests that after fat cointake the absorption rate can be reduced in the lumen side but that no major gut metabolic changes are expected.

Role of Adding Fiber to the Meal

Fiber is a well-known dietary ingredient that stimulates long-term mucosal growth, especially in the colon. The magnitude of growth depends on the type of fiber ingested (30). It is expected that if the structural changes only take place in the colon
they will not influence amino acid absorption in the small intestine. However, extra food retention resulting from the higher viscosity of a fiber-containing meal can lead to more protein digestion retention in the intestinal lumen. Recent studies showed that prolonged ingestion of soluble dietary fiber such as pectin leads to structural changes in the small intestine (44). The total protein amount of the intestine was enhanced, which resulted in higher protein turnover and, consequently, higher energy and amino acid requirements for the digestive tract. It is expected that the enhanced mucosa amount will result in increased absorption capacity and rate.

**Absorption Kinetics of Peptides**

Small peptides can be actively absorbed by the enterocytes via specific transporters. The peptides in the enterocyte are converted to single amino acids, which are released to the portal system or utilized by the gut itself. Another possibility is that the peptides are directly released to the portal system. In a study with healthy young pigs, the gut absorption kinetics of intact proteins, hydrolysates, and free amino acid mixes were found to be comparable (18). In that study the selected protein was a fast protein, with no digestion limitation in the gut lumen itself. This suggests that amino acid absorption coming from hydrolysates can only be higher than that of intact protein, if these hydrolysates come from a slow protein or are in a mixed meal that slows digestion. This hypothesis was confirmed in a study with young man in whom hydrolysates of a fast and slow protein were compared with the intact protein (9).

Modification of the structure of dietary proteins by hydrolysis has been reported to improve the nutritional value of a meal in several studies under specific (patho)physiological circumstances, for instance, in a postexercise situation in which hydrolysate works insulinotropically (55). In another study it was suggested that the fact that milk peptide hydrolysate solutions elicit greater release of glucose-dependent insulinotropic polypeptide than the complete protein from which they are derived, which might be beneficial in clinical situations involving insulin resistance and glucose intolerance (9). In addition, in conditions of reduced digestibility as in critically ill patients, a meal with hydrolyzed protein or peptides might be beneficial (27).

It has been suggested that some proteins also contain bioactive sequences that may affect gut absorption and/or metabolism. Oligopeptides derived from casein, for example, stimulated mucin release from the goblet cells in an isolated vascularly perfused rate jejunum (10).

**Absorption Kinetics of Amino Acids**

Although the gut absorption rates of free amino acids and intact proteins coming from the same native protein are identical (18), this does not mean that the flux rates of all amino acids are identical. The flux rate depends on whether or not the amino acids can be used by the gut itself (protein synthesis, oxidation) or are converted in the gut to other amino acids that can be beneficial to the rest of the body (Figure 1). The quantitative and qualitative utilization of amino acids by the gut depends on the amino acid profile of the dietary protein and the added macronutrients and the interaction with other organs.
How Absorption Is Influenced by Gut Amino Acid Metabolism

Involvement of several amino acids in metabolic pathways in the gut is reflected by their large intestinal extraction during enteral feeding: glutamate (96%), glutamine (64%), threonine (57%), arginine (65%), cysteine (44%), and branched-chain amino acids (50–60%) (8, 15, 21, 45, 53, 59). Recently it was observed that the gut also uses threonine, sulfur amino acids (cysteine, methionine), and branched-chain amino acids (leucine, isoleucine, and valine) to a higher extent than anticipated from the requirements for protein synthesis in the gut. Apparently gut extracted amino acids are involved in different kind of pathways. Limiting these amino acids in a protein meal (low-quality protein meal) will lead to a less than optimal amino acid profile for the gut. Because of this, amino acid flux can be changed into more release to the portal system (16). A single amino acid deficient in a protein meal can be compensated for by extra intravenous infusion of this amino acid, which normalizes the gut metabolism (16). Threonine is important for the structural protein mucus layer, which is probably the reason that in neonatal piglets, more than two-thirds of the enteral threonine intake was used by the portal-drained viscera. In this way, threonine metabolism can influence the absorption rate (53).

How Absorption Is Influenced by Illness

In recent studies, pigs were made septic by 24-h infusion of endotoxins (6, 7). When these pigs were fed a meal with high-quality proteins, the appearance of amino acids in the portal circulation was increased (Figure 4), indicating that amino acid utilization of the gut was reduced in the septic pigs. Tracer studies revealed that protein synthesis was not changed but protein breakdown was enhanced in the septic pigs.

Figure 4 — Effect of sepsis on the appearance of amino acids in pigs. The amount of protein received by continuous infusion was comparable between groups. FedpostS = after 30 h of starvation; FedpostS+LPS = after 30 h of starvation and 24 h of endotoxin infusion. No change in protein synthesis was observed, whereas protein breakdown increased. Percentage absorption was not different. PDV indicates portal-drained viscera; LPS, lipopolysaccharide.
Gut metabolism was clearly affected in the septic pigs because gut glutamine consumption was greatly reduced. Therefore, an altered gut metabolism caused by illness can change the amount of gut amino acid absorption and release to the circulation, as well as the profile of amino acid released to the gut.

**Conclusion**

In this review, we have discussed the factors that lead to enhanced, reduced, or modified absorption kinetics. To study these factors, a sophisticated model in multicatheterized pigs along with the use of isotopes that can calculate absorption and gut metabolism is necessary.

**References**


