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Original Article

Gut amino acid absorption in humans: Concepts and relevance for postprandial metabolism

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SUMMARY

Dietary amino acid absorption kinetics are an important determinant of protein quality. The term “amino acid digestibility” is commonly used to refer to the amount of ingested amino acids that become available following absorption. However, one should differentiate between the subsequent processes of converting protein into smaller constituents (protein digestion) and luminal amino acid uptake (amino acid absorption). Amino acid “absorbability” or “bioavailability” is assessed by quantifying the disappearance of amino acids across (part of) the gastrointestinal tract. The assessment of fecal, apparent ileal (AID), standardized ileal (SID), and true ileal disappearance (TID), reflect amino acid absorbability with increasing accuracy, due to correction for microbial metabolism in the large intestine, basal gut endogenous amino acid losses, and total gut endogenous amino acids losses, respectively. A substantial amount of absorbed amino acids undergo first-pass splanchnic extraction, but the majority is immediately released in the circulation and becomes available for peripheral tissues. The assessment of amino acid “bioavailability” or “absorbability” is used in protein quality ranking systems such as the Digestible Indispensable Amino Acid Score (DIAAS). However, such scores neglect that the rate of absorption is also an important determinant of postprandial metabolism. In addition, amino acid absorption and/or its rate are highly dependent on factors such as the duration of the postprandial assessment period. Therefore, amino acid absorption kinetics should be assessed under the relevant experimental conditions. To this end, an oral-

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intravenous dual tracer approach can be applied to assess dietary protein derived amino acid release into the circulation and allows the assessment of the subsequent impact on postprandial whole-body protein metabolism.

1. Introduction

Proteins in all living biological tissues are in a constant turnover as a result of the balance between protein synthesis and protein breakdown rates [1,2]. Dietary protein ingestion provides amino acids that stimulate muscle protein synthesis by both functioning as substrate and as signaling molecules that up-regulate anabolic pathways [3–5]. Various factors modulate the protein synthetic response to protein ingestion, including the amount and quality of the ingested protein [6–8]. The capacity of dietary protein to stimulate protein synthesis appears to largely depend on both its amino acid profile and its digestion and amino acid absorption kinetics [9,10]. The latter two are, therefore, the main components of dietary protein quality ranking systems such as the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and Digestible Indispensable Amino Acid Score (DIAAS) [11]. The DIAAS has been recommended to replace the PDCAAS, primarily due to its use of a more accurate assay of amino acid absorption. Clearly, accurate assessment of protein digestion and amino acid absorption is essential to understand postprandial protein metabolism and to define dietary protein intake recommendations.

Protein digestion and amino acids absorption describe the sequential processes by which ingested protein provide amino acids made available to the organism to support anabolic and catabolic pathways (Fig. 1). In brief, protein digestion starts with chewing to mechanically increase the surface area [12]. After swallowing, contractions of the stomach facilitate gastric mixing and chemical breakdown by gastric acid and pepsin [13]. Gastric emptying determines the rate at which the ingested protein is delivered in the duodenum [14], where segmentation contractions further facilitate hydrolysis by pancreatic enzymes, such as trypsin, chymotrypsin, carboxypeptidase A and intestinal brush-border enzymes [15]. Subsequently, amino acids, dipeptides, and tripeptides, are released and taken up across the intestinal mucosa, after which they are considered to be absorbed [16]. Following absorption, a substantial part of the ingested amino acids undergoes first-pass splanchnic extraction, i.e. amino acid uptake in intestinal and hepatic tissues [17]. However, the majority of the absorbed amino acids are released into the systemic circulation where they are transported and taken up by peripheral tissues [18]. The fraction of ingested protein that is not digested and absorbed in the small intestine reaches the large intestine where amino acids are not quantitatively absorbed but deaminated and metabolized by microbiota [19,20].

Much work has been performed to quantify the various aspects of protein digestion and amino acid absorption which are often expressed as an absolute amount, absolute rate, or as a proportion of the ingested amount. The purpose of this review is to describe the processes by which dietary protein-derived amino acids are made available to the organism, their impact on postprandial protein metabolism, and to critically assess how these processes are described.

1.1. Protein digestion and intestinal amino acid absorption

The basic concepts of protein digestion and amino acid absorption are easily understood and widely known. However, their exact definitions and assessment may be more challenging [21] (Table 1). The protein digestion and amino acid absorption terminology is not always evident and can be confusing. The term amino acid “digestibility” is commonly used to represent the amount or proportion of dietary protein derived amino acids that are made available to the organism after digestion and absorption.

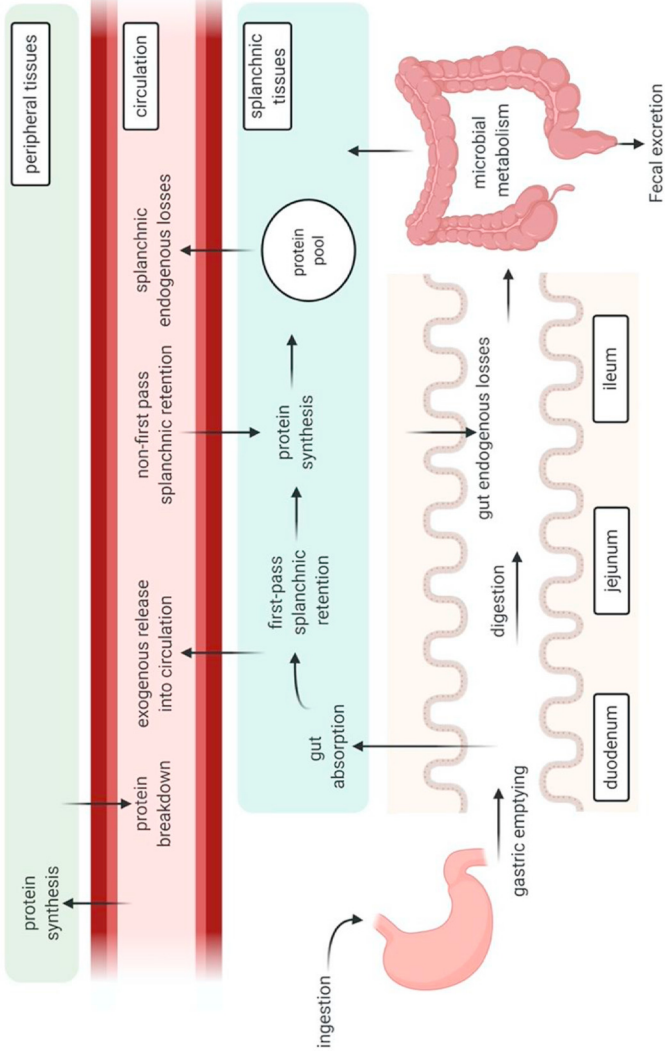

Fig. 1. Schematic representation of various processes and compartments involved in gut amino acid absorption and amino acid release in the circulation.

Table 1
Overview of definitions in protein digestion and amino acid absorption.

• Protein digestion	• Mechanical and chemical breakdown of protein into smaller constituents in the GI tract
• Protein digestibility	• Proportion of ingested protein that is broken down into absorbable constituents for the GI tract
• AA absorption	• Uptake of AA, di- and tripeptides from the gastrointestinal lumen by enterocytes
• AA absorbability	• Proportion of ingested AA, di- and tripeptides, that are taken up from the gastrointestinal lumen by enterocytes
• AA fecal disappearance	• Proxy of AA absorbability: proportion of ingested AA, di- and tripeptides, that disappear between oral intake and fecal excretion
• Apparent Ileal Disappearance (AID)	• Proxy of AA absorbability: proportion of ingested AA, di- and tripeptides, that disappear between oral intake and the terminal ileum
• Standardized Ileal Disappearance (SID)	• Proxy of AA absorbability: proportion of ingested AA, di- and tripeptides, that disappear between oral intake and the terminal ileum, corrected for basal gut endogenous losses
• True Ileal Disappearance (TID)	• Proxy of AA absorbability: proportion of ingested AA, di- and tripeptides, that disappear between oral intake and the terminal ileum, corrected for basal and specific gut endogenous losses
• Protein bioavailability	• Proportion of ingested AA, di- and tripeptides, that are absorbed in a chemical form that renders them potentially suitable for protein synthesis
• Splanchnic AA extraction (SPE)	• Uptake of ingested AA, di- and tri-peptides into splanchnic tissues
• Exogenous AA appearance into the circulation (EXO_{plasma})	• Release of ingested AA, di- and tri-peptides, into the circulation

However, digestion and absorption are distinct and subsequent processes. Protein digestion is the process of cleavage of protein into absorbable smaller fragments constituted by amino acids, dipeptides, and tripeptides. Amino acid absorption is the process of amino acid, dipeptide, and tripeptide uptake from the gastrointestinal lumen. As the capacity of the small intestinal mucosa to absorb amino acids, dipeptides, and tripeptides, is generally far greater than the amounts in which they are released in the lumen, protein digestion typically represents the limiting step in amino acid absorption. However, it is important to note that while protein digestion and amino acid absorption are distinct physiological processes, their scoring systems are dependent on downstream processes, *i.e.* the amino acid absorbability of ingested protein is modulated by the digestibility of that protein. Therefore, the protein digestibility and amino acid absorbability score of an ingested protein will be quantitatively similar under most conditions. Nevertheless, the term amino acid absorbability as a proxy of bioavailability would be more appropriate when referring to the amount of amino acids made bioavailable following protein digestion and absorption.

As a proxy for measuring dietary amino acid absorption, the disappearance of a dietary amino acid in (part of) the gastrointestinal tract is assessed [22]. Amino acid absorbability is assessed with various assays that differ in their complexity and validity. The oldest and most basic approach to determine amino acid absorption is to assess the disappearance of ingested amino acids between oral intake and fecal excretion (oro-fecal amino acid disappearance).

$$Oro-fecal\ dissapearance\ (\%) = \frac{\left(AA_{intake} - AA_{fecal}\right)}{AA_{intake}} \cdot 100\% \quad [1]$$

AA_{intake} represents the amino acid content of ingested amino acids and/or protein. AA_{fecal} represents the amino acid content of fecal material. A major limitation of this oro-fecal approach is that the amino acid composition of fecal material is impacted by substantial microbial metabolism in the large intestine [23]. It is generally believed that net microbial amino acid degradation occurs in the large intestine [22]. Therefore, the disappearance of amino acids in the large intestine does not necessarily reflect amino acid absorption by the host organism.

To avoid interference from microbial metabolism in the large intestine, the disappearance of ingested amino acids can be determined at the terminal ileum, i.e. at the end of the small intestine (oro-ileal disappearance). While there is some suggestion that amino acids can be absorbed in the large

intestine, there is no evidence that this occurs in relevant amounts [20]. Therefore, intestinal amino acid absorption is practically complete at the end of the small intestine. For this reason, oro-ileal disappearance is considered to more accurately represent gut amino acid absorption when compared to fecal disappearance.

A limitation for both fecal and ileal disappearance approaches is that digesta in the feces or terminal ileum contain amino acids from both exogenous (*i.e.* undigested and/or unabsorbed dietary protein) and endogenous (*e.g.* gut amino acid losses from digestive enzymes and other proteins secreted into the intestinal lumen, and desquamated epithelial cells) origin [24]. Gut endogenous amino acid losses can be distinguished into basal and specific losses. Basal losses represent the minimal amount of loss and are not impacted by dietary composition, such as digestive enzyme secretion and epithelial cell turnover. Specific losses are amino acid losses above basal losses that result from dietary composition. For example, dietary fiber and anti-nutritional factors have been shown to enhance digestive enzyme secretion and epithelial cell turnover and consequently increase specific losses [25,26]. When oro-fecal or oro-ileal disappearance is not corrected for endogenous losses either in the feces or at the terminal ileum, the terms apparent fecal or ileal "digestibility" are used [21]. When oro-fecal or oro-ileal disappearance are corrected for basal fecal or ileal endogenous amino acid losses, the terms standardized fecal or ileal "digestibility" are used. When fecal or ileal digestibility is corrected for total fecal or ileal endogenous losses (*i.e.* both basal and specific losses), the term true fecal or ileal "digestibility" is used. The True Ileal "Digestibility" (TID) assay is the best currently available approach to assess amino acid absorption [27].

For ileal "digestibility" values that are considered as more accurate than fecal values:

$$\textit{Apparent ileal disappearance}\ (AID,\%) = \frac{(AA_{intake} - AA_{ileum})}{AA_{intake}} \cdot 100\% \quad [2]$$

$$\textit{Standardized ileal disappearance}\ (SID,\%) = \frac{(AA_{intake} - AA_{ileum} - AA_{endo,basal})}{AA_{intake}} \cdot 100\% \quad [3]$$

$$\textit{True ileal disappearance}(TID,\%) = \frac{\left(AA_{intake} - AA_{ileum} - AA_{endo,basal} - AA_{endo,spec}\right)}{AA_{intake}} \cdot 100\% \quad [4]$$

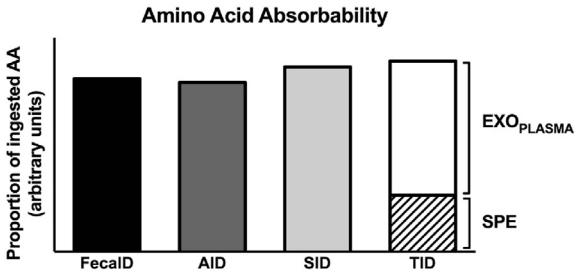

Fig. 2. Conceptual framework of amino acid absorbability assessed with various assays. Arbitrary units are used because amino acid absorbability is dependent on contextual factors such as the amount and type of protein ingested. In general, fecal disappearance is higher when compared to apparent ileal disappearance (AID), due to the net amino acid loss in the large intestine due to microbial metabolism. AID underestimates true ileal disappearance (TID), as it does not account for basal and (possible) specific gut endogenous protein losses. Standardized ileal disappearance (SID) may underestimate TID as it does not account for (possible) specific gut endogenous protein losses. A substantial amount of absorbed amino acids undergo first-pass splanchnic extraction (SPE), but the majority is released in the circulation and becomes available for peripheral tissues (EXO_{plasma}).

AA_{intake} represents the amino acid content of ingested amino acids and/or protein. AA_{ileum} represents the amino acid content of digesta at the terminal ileum. $AA_{endo,basal}$ represent basal gut endogenous amino acid losses. $AA_{endo,spec}$ represents specific gut endogenous amino acid losses (see also Fig. 2). As determination of amino acid absorbability is currently performed by measuring the disappearance of amino acids from the gut, the term "disappearance" would be more appropriate than "digestibility" when referring to these assays. Furthermore, there is also some inconsistency in the terminology used to describe these various formulas. More particularly, the terms "apparent", "standardized", "true" and "real" ileal amino acid disappearance are not always used consistently and equivalently [28–32]. There is an urgent need to homogenize and more precisely define the terminology. Given what the word "true" implies, we believe it should include all possible corrections (i.e. basal + specific endogenous ileal amino acid losses). Likewise, standardized ileal disappearance seems to be the appropriate term for ileal digestion corrected for basal ileal endogenous amino acid losses. Therefore, the terminology described by Stein et al. [21] to distinguish between these formulas, *i.e.* apparent ileal disappearance (no correction for gut endogenous losses), standardized ileal disappearance (correction for basal losses), and true ileal disappearance (correction for total gut endogenous losses) seems to be the most precise.

1.2. Splanchnic extraction and exogenous amino acid release in the circulation

Once ingested amino acids are taken up over the gastrointestinal barrier, they are considered as absorbed and bioavailable for the host organism. Both subsequent splanchnic extraction and/or release in the circulation are also important elements of post-prandial protein-derived amino acid handling. A substantial part of the absorbed amino acids are taken up and metabolized in splanchnic tissues, termed first-pass splanchnic extraction [17]. Splanchnic extraction does not impact overall protein bioavailability but directs absorbed amino acids towards retention in splanchnic tissues at the expense of amino acids available for the peripheral tissues further downstream. This first-pass splanchnic extraction can be assessed by an oral-intravenous dual tracer technique in which a labeled amino acid (*e.g.* $^{2}H_5$-phenylalanine) is continuously infused into the circulation, and the same amino acid with a different label (*e.g.* 1-^{13}C-phenylalanine) is ingested in regular boluses to obtain a steady state. The ingested free amino acid isotope does not need to be digested and is assumed to have a 100% absorbability. Therefore, its metabolic fate include metabolism in the splanchnic area and/or the release in the blood. By assessing the plasma enrichments of both labeled amino acids and normalizing for their delivery rate, splanchnic extraction can be calculated.

$$\textit{First pass splanchnic extraction} = TID - \frac{E_{p,oral} \Big/ F_{oral}}{Ep,\, iv / F_{iv}} \quad [5]$$

$E_{p,oral}$ and $E_{p,iv}$ are the plasma enrichments of the oral and intravenous tracer, respectively. F_{oral} and F_{iv} are the oral ingestion rate and the intravenous infusion rate, respectively. TID represents absorbability of ingested amino acids as assessed by true ileal disappearance, which is assumed to be 100% for free amino acids. Theoretically, this approach can also be used to estimate splanchnic extraction of dietary protein-derived amino acids. However, this requires the use of intrinsically labeled protein in which the oral amino acid isotope is incorporated within the dietary protein [33]. Moreover, it requires the TID value of the dietary protein which is often not feasible to assess during the experimental conditions. While it can be theoretically estimated from literature values, such an approach is unlikely to be accurate as discussed in later sections. It is even more complex to assess splanchnic extraction during non-steady state conditions. Substantially higher splanchnic extraction rates have been observed following the ingestion of a protein bolus when compared to values obtained during a free amino acid sip feeding protocol [4,34,35]. This apparent discrepancy can be explained by a limited duration of the postprandial assessment period. Sometimes, more time is needed to allow the ingested protein to become fully digested and the protein derived amino acids to become absorbed and released in the circulation. Without full protein digestion and amino acid absorption during the assessment period the estimated splanchnic extraction rates should be regarded overestimates.

Absorbed amino acids that escape first-pass splanchnic extraction are released into the peripheral circulation, from where they are transported and taken up in the various peripheral tissues [18]. Due to the ease of sampling, plasma amino acid concentrations are often assessed as a proxy of protein digestion and amino acid absorption [36,37]. However, plasma amino acids levels are strongly influenced by endogenous amino acid release into the circulation as a result of tissue protein breakdown. As protein ingestion inhibits protein breakdown rates, changes in plasma amino acid concentrations following protein ingestion are a consequence of both exogenous amino acids appearing in the circulation and a reduction of endogenous amino acids being released in the circulation [4]. Therefore, plasma amino acid concentrations can only provide an overall picture and should not be used to quantify protein digestion and amino acid absorption kinetics or quantify the amount of exogenous amino acids absorbed and appearing in the circulation.

The release of dietary protein derived amino acids into the circulation can be assessed by an oral-intravenous dual tracer technique in which a stable amino isotope (*e.g.* $^{2}H_{5}$-phenylalanine) is continuously infused into the circulation, and a different stable isotope of the same amino acid (*e.g.* 1-^{13}C-phenylalanine) is ingested [38–40]. By assessing the dilution of the ingested amino acid enrichment in plasma and relating it to the total plasma amino acid rate of appearance, the exogenous amino acid rate of appearance can be calculated.

$$EXO\ R_a = Total\ R_a\ x\ \frac{E_{p,oral}}{E_{oral}} \quad [6]$$

EXO R_a represents the exogenous amino acid rate of appearance. Total R_a represents the total amino acid rate of appearance. $E_{p,oral}$ represents the plasma enrichments of the oral amino acid isotope, E_{oral} represents the enrichment of the exogenous amino acids. The exogenous amino acid rate of appearance can be assessed for dietary protein when the amino acid tracer is incorporated into the dietary protein. In addition, the exogenous amino acid rate of appearance can be assessed for the ingestion of a single bolus by using Steele's correction for the non-steady state [40]. By calculating the incremental area under the curve of exogenous amino acid rate of appearance, the total amount of exogenous amino acid appearing in the circulation during a time period can be calculated (EXO_{plasma}) and expressed as a percentage of the amino acid content of the ingested protein.

$$EXO_{plasma}\ (\%) = \frac{iAUC\ EXO\ R_a}{AA_{intake}}\ x\ 100\% \quad [7]$$

AA represents amino acids. iAUC EXO R_a represents the incremental area under the curve of exogenous amino acid rate of appearance. AA_{intake} represents the amount of the ingested amino acid.

The use of the oral-intravenous dual tracer technique to quantify the exogenous amount of amino acids appearing in the circulation has recently been questioned [41]. Following the ingestion of labeled amino acids, some of these labeled amino acids get incorporated into splanchnic tissues such as the intestine and the liver. In addition, unlabeled amino acids can be released by these tissues because of their own turnover. Consequently, the ratio of tracer (labeled amino acids) to tracee (unlabeled amino acids) decreases as the ingested amino acids are transferred through the various splanchnic regions (Fig. 3). This dilution of the tracer to tracee ratio (TTR) would then result in an underestimation of the amount of exogenous amino acids appearing into the circulation as determined by the oral-intravenous dual tracer method. However, this is based on an incorrect interpretation of the oral-intravenous dual tracer technique. The purpose of the oral tracer is to quantify the appearance rate of ingested amino acids into the circulation. In simple terms, for each labelled amino acid appearing in the circulation, a known amount of unlabelled ingested (exogenous) amino acids has appeared. This requires a constant ratio between the labelled and unlabelled exogenous amino acids that were present in the protein that was consumed (TTR_{exo}), not between labelled amino acids and all (exogenous + endogenous) unlabelled amino acids (TTR). In contrast to TTR, the TTR_{exo} remains constant throughout the splanchnic region (Fig. 3). The TTR_{exo} of intrinsically labelled protein is an inherent property of the ingested protein and is not affected by its subsequent metabolism. Therefore, TTR_{exo} in Eq. (6) is the enrichment

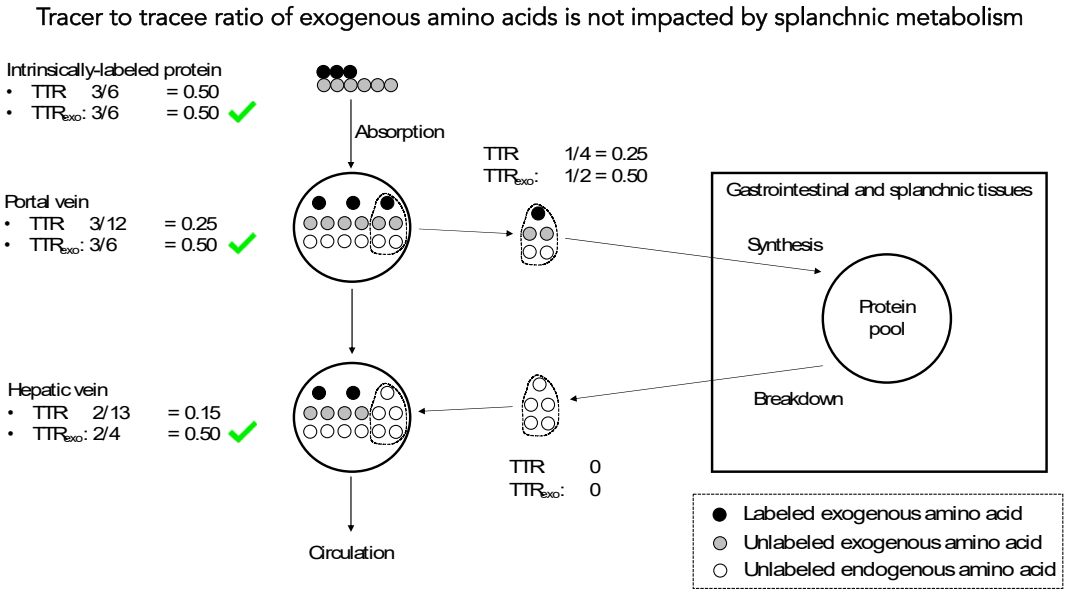

Fig. 3. Schematic representation of the tracer to tracee ratio of both total and exogenous (intrinsically labeled) protein derived amino acids, in the portal vein, and the hepatic vein, when gastrointestinal and splanchnic tissue net protein balance is zero. Using unlabeled amino acids as the tracee (TTR), the tracer to tracee ratio decreases as a result of splanchnic metabolism. However, the tracer to tracee ratio of the intrinsically labeled protein derived amino acids (TTR_{exo}) is not affected by splanchnic metabolism. Therefore, the tracer to tracee ratio of exogenous protein can be used to accurately assess the metabolic fate of the ingested protein derived amino acids.

level of the ingested protein (E_{oral}) and is used to calculate the rate at which the ingested protein derived phenylalanine is released in the circulation. Fig. 3 provides a mathematical and visual representation on how the oral-intravenous dual tracer technique accurately determines the exogenous rate of (protein) derived amino acid appearance [42].

The oral-intravenous dual tracer method is expensive and labor intensive, especially when intrinsically labeled proteins are used. Therefore, the exogenous amount of protein derived amino acids appearing in the circulation is sometimes estimated based upon values taken from the literature, termed the protein bioavailability estimation approach [41,43]. For example, the exogenous amount of protein derived amino acids appearing in the circulation has been estimated by correcting amino acid intake for established splanchnic extraction values. However, the "bioavailability" of ingested protein derived amino acids is highly context dependent and may not be extrapolated to different conditions. For example, a splanchnic extraction of 27% has been observed when 40 g free amino acids where ingested as a frequent sip protocol to establish steady state conditions under resting conditions [17]. This splanchnic extraction value has been extrapolated to the bolus ingestion of 70 g meat protein in a large mixed meal consumed following exercise [43]. Notably, plasma essential amino acid concentrations continued to increase throughout the entire postprandial assessment period, implying that protein digestion and amino acid absorption was still ongoing at the end of the relatively short assessment period. Therefore, even if the 27% splanchnic extraction value could be extrapolated to this specific feeding protocol, it would require a postprandial assessment period which allows plasma amino acid levels to return to baseline concentrations. Consequently, the protein bioavailability approach based upon literature values can result in substantial over- or under-estimation of the amount of exogenous protein derived amino acids appearing in the circulation.

The protein bioavailability approach to estimate the amount of exogenous protein derived amino acids appearing in the circulation has undergone several iterations. The most recent version first corrects amino acid intake with literature values of true ileal disappearance. Subsequently, a second correction involves estimating splanchnic clearance based on the postprandial increase in protein hydroxylation [41]. However, it is questionable if protein hydroxylation above the basal value represents a good proxy for splanchnic extraction. For example, it requires the assumption that splanchnic tissue net protein balance is exactly zero during the postprandial period. Moreover, this iteration does not solve the major issue that protein digestion and absorption still need to be extrapolated based on a static literature value. The duration of the postprandial assessment period, subjects' age, nutrient co-ingestion, food matrix and processing, amount of protein, physical activity prior to food ingestion, the presence or absence of disease, etc., all impact dietary protein digestion and amino acid absorption kinetics [44–48]. Any deviation from the experimental conditions under which literature values for protein absorbability were previously established will introduce some error in the estimation of dietary protein derived amino acid appearance in the circulation. Therefore, the protein bioavailability estimation approach should only be applied when literature values obtained by the oral-intravenous dual tracer technique are available from studies applying nearly identical experimental conditions.

1.3. The relevance of protein digestion and absorption kinetics for protein quality

Amino acids need to be absorbed to become available for the organism and amino acid absorbability is therefore an important factor in the assessment of protein quality. The Food and Agriculture Organization of the United Nations (FAO) has recently recommended the Digestible Indispensable Amino Acid Score (DIAAS) to quantify dietary protein quality [11]. The DIAAS is based on the amino acid profile and their individual absorbability (assessed as true ileal disappearance) of the protein. The DIAAS approach highlights the importance of amino acid absorbability but does not fully appreciate the impact of the kinetics of protein digestion and amino acid absorption for assessing protein quality. This is despite different results suggesting that the rate of amino acid absorption is an important modulator of protein quality. Specifically, the ingestion of proteins that allow a more rapid release of amino acids into the circulation appear to be more potent to stimulate muscle protein synthesis rates when compared to more slowly digestible proteins [8], even when corrected for amino acid contents [9,49]. Consequently, both the total amount of amino acids available as well as the rate of protein derived

amino acid absorption and subsequent systemic release should be considered when defining protein quality.

The quality of dietary protein could also be defined based on its capacity to increase (whole-body) net protein balance. The assessment of whole-body protein metabolism is often based on the assessment of plasma amino acid kinetics [4,44,45,50–52]. A triple tracer technique can be used to extend on the oral-intravenous dual tracer method. In the fasted state, protein breakdown is the only source of amino acid release into the circulation. Therefore, the total plasma amino acid rate of appearance equals the endogenous plasma amino acid rate of appearance and represents protein breakdown rates. However, in the fed state, total plasma amino acids rate of appearance consists of both the endogenous and the exogenous plasma amino acid rate of appearance as a result of tissue protein breakdown and protein ingestion, respectively. Therefore, postprandial protein breakdown rates can be assessed by subtracting the exogenous plasma amino acid rate of appearance from the total plasma amino acid rate of appearance.

$$PB = ENDO\ R_a = Total\ R_a - EXO\ R_a \quad [8]$$

PB represent whole-body protein breakdown rate. ENDO R_a represents endogenous amino acid rate of appearance into the circulation. Total R_a represent total amino acid rate of appearance into the circulation. EXO R_a represents the exogenous amino acid rate of appearance into the circulation. Eq. (8) shows that the assessment of protein breakdown rates directly depends on the accurate assessment of EXO R_a. An overestimation in EXO R_a directly results in an underestimation of protein breakdown rates. As discussed earlier, the protein bioavailability estimation approach requires extrapolation and typically overestimates EXO R_a. Indeed, substantial reductions in postprandial protein breakdown rates have been reported when the protein bioavailability estimation approach has been used [43,53,54]. These observations are likely to represent artefacts that reflect the inaccuracy in the EXO R_a estimation. Therefore, to accurately assess the exogenous amino acid rate of appearance and its impact on postprandial protein metabolism, it is preferred to apply the oral-intravenous triple tracer approach. The triple tracer approach allows the assessment of dietary protein quality based on the consideration of all aspects of protein digestion and amino acid absorption kinetics and its impact on (whole-body) protein metabolism.

2. Conclusions

There is a need for more consistency and accuracy in the terminology of protein digestion and amino acid absorption. Traditionally, the term “amino acid digestibility” has been used to describe the amount of amino acids made available to the organism after digestion and absorption (as a proxy of their “bioavailability”). However, protein digestion is the process of cleaving protein into smaller fragments while amino acid absorption is the process of luminal uptake of protein constituents. Therefore, the absorbability of amino acids then describes and quantifies the overall process of protein digestion and amino acid absorption. This amino acid absorbability can be assessed by various assays that differ in sampling location and/or correction for endogenous amino acid losses. However, there is some inconsistency for describing these assays. It can be considered (oro)-fecal disappearance when sampling feces, apparent (oro-)ileal disappearance (AID) when sampling in the terminal ileum, standardized (oro)-ileal disappearance (SID) when ileal disappearance is corrected for basal gut endogenous amino acid losses, and true (oro-) ileal disappearance (TID) when ileal disappearance is corrected for total gut endogenous amino acid losses. Following amino acid absorption, a substantial amount of amino acids undergoes first-pass splanchnic extraction. However, the majority is released in the circulation and becomes available for peripheral tissues in the post-prandial phase. Both amino acid absorbability and the rate of post-prandial amino acid absorption are important determinants of protein quality. However, the digestion and absorption kinetics of dietary protein should not be considered as static properties of protein, as they are highly dependent on experimental and individual conditions such as the ingested amount of protein, the duration of the postprandial assessment period, age, and the presence or absence of disease. An oral-intravenous dual tracer method can be applied to

accurately assess the total amount and rate of ingested amino acids appearing in the circulation under specific conditions and on an individual level. This dual tracer approach is a crucial element of the oral-intravenous triple tracer approach to quantify postprandial plasma amino acids kinetics and assess whole-body protein metabolism.

Statement of authorship

JT, DT and LvL wrote the manuscript. All authors edited and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Declaration of interests

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare no conflict of interest for the current work. LvL and JT have received research grants, consulting fees, speaking honoraria, or a combination of these for work on postprandial protein metabolism; full overview is provided here: https://www.maastrichtuniversity.nl/l.vanloon.

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