REVIEW

CURRENT OPINION

Understanding the complex relationship between amino acid absorption kinetics and postprandial muscle protein synthesis rates in healthy adults and critically ill patients

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Purpose of review
Protein digestion and amino acid absorption kinetics are quantifiable metrics commonly utilized to determine the quality of a protein source. This review critically evaluates recent evidence (primarily from studies that provided commonly consumed protein-rich foods) regarding the relationship between *in vivo* protein digestion and amino acid absorption rates with the postprandial stimulation of muscle protein synthesis (MPS), with an emphasis on healthy adults and critically ill patients.

Recent findings
Ingested protein sources that elicit moderate amino acid bioavailability, including leucine, stimulate MPS rates to a comparable extent as protein sources that elicit high amino acid bioavailability in healthy young adults. Amino acid absorption kinetics appear to be modulated in critically ill patients, leading to a marked reduction in postprandial MPS rates. Preliminary studies demonstrate that enteral feeding of high dose free amino acids increase amino acid bioavailability to a greater extent than intact protein, leading to a positive whole-body net protein balance in critically ill patients. However, in practice, the high osmolarity of free amino acids leads to a high prevalence of diarrhoea and thus limits the clinical application of this intervention.

Summary
The enteral provision of free amino acids represents a theoretical, but not practically-relevant, clinical nutrition strategy to mitigate the catabolic response to critical illness. Future studies are warranted to establish targeted protein/amino acid-based interventions to mitigate skeletal muscle atrophy during the metabolic care of critically ill patients.

Keywords
protein digestibility, amino acid kinetics, leucine trigger, muscle protein turnover, critical illness

INTRODUCTION

Dietary protein-derived amino acids provide the primary substrate and signal for muscle protein synthesis (MPS) and thus are fundamental in regulating skeletal muscle mass across the healthspan. Accordingly, the concept of dietary protein quality continues to receive scientific attention with regards to informing protein guidelines across clinical nutrition and metabolic care settings [1]. Protein quality is fundamentally determined by the essential amino acid (EAA) composition and digestibility of a given protein source, and subsequent amino acid absorption from the gastrointestinal lumen in the small intestine [2]. The rate of protein digestion and amino acid absorption determines the postprandial rise in circulating amino acids and is widely recognized to modulate postprandial MPS rates, although this relationship has recently been considered more complex

than is often espoused [3,4]. Contemporary evidence also indicates that amino acid absorption rates may be compromised in critical illness [5], with

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KEY POINTS

- The complex relationship between protein digestion, amino acid absorption and the postprandial stimulation of muscle protein synthesis (MPS) is dependent upon physiological (i.e., healthy and clinical populations, rest and exercise) and nutritional (protein dose, nutrient-nutrient interactions) factors.

- Protein digestion and amino acid absorption rates may be compromised in critically ill patients, leading to an impaired stimulation of MPS.

- Future studies are warranted to establish effective and pragmatic clinical nutrition interventions to mitigate muscle atrophy during the metabolic care of critically ill patients that also have minimal side effects.

implications for personalized protein nutrition recommendations in critically ill patients [6]. This review synthesizes recent evidence regarding the complex relationship of protein digestion and amino acid absorption rates with the postprandial stimulation of MPS, with specific reference to studies conducted in healthy adults and critically ill patients.

TERMINOLOGY

Whilst dietary protein digestion and amino acid absorption kinetics are firmly established as primary factors in determining the quality of a protein source [7], there remains confusion and a lack of precision around relevant terminology [8]. The term 'protein digestion' is defined as the mechanical and chemical breakdown of protein into amino acids within the gastrointestinal (GI) tract, whereas 'amino acid absorption' describes the process of amino acid uptake from the GI lumen [8]. Accordingly, the term 'protein digestibility' refers to the proportion of ingested dietary protein that is broken down into absorbable constituents for the GI tract, and 'amino acid absorbability' describes the proportion of amino acids that are taken up from the GI lumen [8]. In addition, 'protein bioavailability' encapsulates the proportion of ingested protein-derived amino acids (also dipeptides and tripeptides) absorbed into the circulation that renders amino acids available as substrate for the postprandial stimulation of MPS [8]. Finally, the 'postprandial handling of dietary protein' is increasingly recognized as an all-encompassing term used to describe the sequence of metabolic processes pertaining to the digestion, absorption and subsequent stimulation of MPS in response to an ingested protein source [9]. The consistent use of precise terminology

is critical in advancing understanding into the role of protein quality in clinical nutrition.

To achieve a comprehensive assessment of postprandial protein handling *in vivo* in humans, intravenous stable isotope-labelled amino acid infusions can be combined with the ingestion of an intrinsically-labelled protein [9]. This tandem approach enables the simultaneous measurement of protein digestion and amino acid absorption kinetics, alongside informative physiological readouts such as the postprandial stimulation of MPS [10]. Largely attributed to the expensive monetary cost and labour-intensive nature of this oral-intravenous dual tracer method, our understanding of postprandial protein handling for a range of protein sources under a variety of conditions and within different clinical populations continues to emerge, albeit at a steady rate. Nonetheless, based on recent findings, the importance of protein digestion and amino acid absorption kinetics as independent determinants of protein quality may have been over-simplified within a clinical nutrition setting.

PROTEIN DIGESTION AND AMINO ACID ABSORPTION KINETICS IN HEALTHY ADULTS

Current opinion regarding the nutritional profile of a high-quality protein source is rapidly evolving. Traditionally, the rate of protein digestion and amino acid absorption that determines the postprandial rise in circulating amino acids is widely recognized to modulate the postprandial stimulation of MPS [11]. Specifically, it was proposed that a directly proportional positive association exists between the amplitude and rate of postprandial increase in blood leucine concentration, and the magnitude of the MPS response to an ingested protein source. This phenomenon was coined as the 'leucine trigger' hypothesis [12] and is underpinned by robust molecular studies that highlight the potency of leucine (vs. other EAA's) in upregulating the mechanistic target of rapamycin complex 1 (mTORC1) signalling cascade that activates the translation initiation step of MPS [13]. In comparison, the 'leucine threshold' concept quantifies the minimum leucine availability required for the intracellular machinery to stimulate MPS [14■]. Whilst the leucine trigger hypothesis provides valuable mechanistic insight regarding the postprandial regulation of *in vivo* MPS rates in humans, from an applied perspective, we [3] and others [4] have recently highlighted the complex nature of applying the leucine trigger hypothesis across all physiological scenarios and populations. This more cautious approach is primarily based on emerging evidence that, under certain conditions, a

dissociation exists between ingested leucine or plasma leucine concentrations and the magnitude of postprandial MPS rates in response to ingested protein [14▪].

A consistent [15–20], albeit not universal [21], finding across a series of recent experimental studies conducted in healthy young and older adults concerns the apparent disconnect between blood leucine concentration profiles and postprandial MPS rates in response to a variety of ingested protein sources, that is, crystalline free amino acids, plant and animal proteins, and alternative (fungal, algal, insect) proteins (Table 1). For instance, the ingestion of crystalline free amino acids compared with an equivalent 30 g dose of intact milk protein resulted in a more rapid amino acid absorption and greater postprandial plasma amino acid availability, albeit without affecting corresponding MPS rates in healthy young adults [15]. This disassociation between plasma leucine concentrations and postprandial MPS rates has also consistently been reported across a recent programme of studies conducted at Maastricht University that compared various isolated intact plant proteins, namely wheat hydrolysate [18], pea concentrate [17], potato concentrate [22], corn isolate [23] or a blend of wheat hydrolysate, corn isolate and pea concentrate protein [24], with a dose-matched milk concentrate control condition in healthy young adults. Indeed, while the increase in plasma leucine concentration was less pronounced after ingesting 30 g of pea protein vs. 30 g of milk protein, postprandial MPS rates were similar between conditions [17]. Furthermore, and also aligned with the concerted global effort to advance sustainable human nutrition [25,26], recent studies have reported no clear association between the amplitude or rate of postprandial increase in blood leucine concentration and the magnitude of MPS response to the ingestion of alternative proteins in healthy young adults, including fungal-derived mycoprotein (*Fusarium venenatum*) [20,27,28], microalgae [19] or meal worm [29] derived insect protein. Taken together, these data highlight that ingested proteins that elicit a moderate postprandial leucine profile also exhibit the capacity to stimulate a robust increase in MPS, which may be explained (at least partially) via a leucine-independent mechanism(s).

Beyond individual constituents, a concerted effort has recently focussed on advancing understanding regarding the protein digestion, amino acid absorption and postprandial response of MPS to the ingestion of protein-rich whole foods [16,30] and mixed macronutrient meals [31], which is commonly termed a food-first approach. For instance, and highlighting the apparent disassociation between amino acid absorption kinetics and the postprandial stimulation of MPS, an elegant study by Paulussen *et al.*

demonstrated comparable MPS rates with the post-exercise ingestion of a 99 g salmon fillet *vs.* the same nutrients ingested as an isolated mixture of crystalline free amino acids and fish oil in resistance-trained young adults, despite a markedly earlier peak in EAA concentrations in the isolated constituent nutrients group. These data [16] are consistent with an earlier report whereby the consumption of skimmed milk stimulated a greater postprandial MPS response than the ingestion of minced beef, despite the observation that leucine availability was greater in the beef *vs.* milk condition [32]. Given that conditions were matched for protein content between studies, these data suggest that other components of the protein-rich food source beyond protein digestion and amino acid (leucine) absorption kinetics modulated the postprandial response of MPS. Hence, it could be argued that the role of postprandial leucine kinetics in regulating MPS may be less evident within the context of protein-rich whole food ingestion [14▪].

Alternative nutritional components of food (i.e., nutrient-nutrient interactions of protein with lipids, vitamins and minerals) or non-nutrient components of food related to form, preparation and processing has also been proposed to modulate the postprandial response of MPS, via distinct leucine-independent molecular mechanisms [33▪]. Aligned with the concept of a food matrix effect [2], the interaction of nutrients (e.g., protein, carbohydrates, fats, vitamins, minerals) contained in whole foods or nutrient-dense mixed meals are purported to be regulatory for the stimulation of MPS via multiple pathways. For instance, the carbohydrate content of a food source is primarily responsible for insulin secretion that facilitates the delivery of amino acids to skeletal muscle and subsequent activation of mTORC1 translocation and cell signalling [34]. Moreover, vitamins, minerals and other bioactive components of whole/mixed foodstuff also activate nutrient sensing mechanisms within the muscle cell that indirectly modulates the stimulation of MPS [33▪]. Nonnutrient components of food, specifically related to form, preparation and processing, are also implicated in modulating the postprandial MPS response via leucine-independent mechanisms [2]. For example, the ingestion of milk with higher glycation levels (induced via heat treatment) was shown to evoke an attenuated rise in plasma EAA concentrations (compared to lower glycation milk) which, in theory, would be rate-limiting for the postprandial stimulation of MPS [33▪]. Thus, while the anabolic action of leucine appears to be robust when provided via isolated protein sources, a leucine-independent effect on the regulation of MPS appears to be relevant when applied to more complex nutritional scenarios such as whole foods or mixed meals.

Table 1. Summary of recent (2021 onwards) findings from studies that simultaneously measured blood leucine concentration profiles and postprandial rates of muscle protein synthesis in healthy young and older adults

Reference	Participants	Study design/intervention	Muscle fraction for postprandial MPS measurement	Evidence supporting "leucine trigger" hypothesis	Blood leucine concentration profile	Postprandial rates of MPS
Hermans *et al.* (2021)	24 healthy young (25±4 y) males	Double-blind, parallel, RCT with 30g intrinsically labeled mealworm-derived protein (WORM) vs. milk-derived protein (MILK) Unilateral bout of resistance exercise	Mixed MPS 0–5 h	No	Peak plasma leucine concentration: MILK > WORM by 27% ($P<0.001$)	MILK ($0.056\pm0.012\%\cdot h^{-1}$) = WORM ($0.045\pm0.017\%\cdot h^{-1}$, $P>0.05$) at rest MILK ($0.073\pm0.020\%\cdot h^{-1}$) = WORM ($0.059\pm0.015\%\cdot h^{-1}$, $P>0.05$) postexercise
Hermans *et al.* (2022)	20 healthy young (25±4 y) males	Parallel, RCT with 30g cheese protein vs. milk protein concentrate Unilateral bout of resistance exercise	Mixed MPS 0–4 h	No	Peak plasma leucine concentration: Milk >cheese by 38% ($P<0.001$)	Milk ($0.056\pm0.010\%\cdot h^{-1}$) = cheese ($0.055\pm0.018\%\cdot h^{-1}$, $P>0.05$) at rest Milk ($0.063\pm0.010\%\cdot h^{-1}$) = cheese ($0.067\pm0.013\%\cdot h^{-1}$, $P>0.05$) postexercise
Paulussen *et al.* (2023)	Healthy, physically active young (24±4 y) males (n = 5) and females (n = 5)	Crossover, RCT with salmon (SAL) (20.5g protein and 7.5g fat) vs. constituent nutrients of salmon ingested in the form of crystalline amino acids, coconut oil, and fish oil (ISO)	MyoPS 0–5 h	No	AUC plasma leucine: SAL > ISO ($P=0.050$)	SAL ($0.056\pm0.022\%\cdot h^{-1}$) = ISO ($0.046\pm0.025\%\cdot h^{-1}$, $P=0.308$)
Pinckaers *et al.* (2024a)	24 healthy young (24±3 y) males	Double-blind, parallel, RCT with 30g pea protein (PEA) vs. 30g milk protein (MILK)	MyoPS 0–5 h		Peak plasma leucine concentration: MILK ($353\pm45\,\mu mol/L$) > PEA ($282\pm30\,\mu mol/L^{-1}$, $P<0.001$) by ~25%	MILK ($0.053\pm0.013\%\cdot h^{-1}$) = PEA ($0.053\pm0.017\%\cdot h^{-1}$; $P=0.96$)
Pinckaers *et al.* (2024b)	Healthy older (72±4 y) males (n=8) and female (n=8) adults	Crossover, RCT with whole-food omnivorous meal containing beef as the primary source of protein (0.45g protein/ kg body mass; MEAT) vs. Isonitrogenous and isocaloric whole-food vegan meal (PLANT)	Mixed MPS 0–6 h		Peak plasma leucine concentration: MEAT ($198\pm23\,\mu mol/L^{-1}$) > PLANT ($158\pm21\,\mu mol/L^{-1}$, $P<0.001$) by ~25%	MEAT ($0.052\pm0.023\%\cdot h^{-1}$) >. PLANT ($0.035\pm0.021\%\cdot h^{-1}$, $P=0.037$) by ~47%

Table 1 (*Continued*)

Reference	Participants	Study design/intervention	Muscle fraction for postprandial MPS measurement	Evidence supporting "leucine trigger" hypothesis	Blood leucine concentration profile	Postprandial rates of MPS
Pinckaers *et al.* (2024c)	36 healthy young (26±4 y) males	Double-blind, parallel, RCT with 30 g corn protein (CORN) vs. 30 g milk protein (MILK) vs. 30 g protein blend with 15 g corn plus 15 g milk protein (CORN+MILK)	MyoPS 0–5h		Peak plasma leucine concentration: CORN (390±66 µmol/L)=MILK (353±45 µmol/L)=CORN+MILK (395±62 µmol/L, $P>0.05$) Time to peak leucine concentration: MILK (46±43 min) < CORN (130±35 min) and CORN+MILK (133±45 min, all $P<0.001$)	CORN (0.053±0.013%·h^{-1})=MILK (0.053±0.013%·h^{-1})=CORN + MILK (0.052±0.024, $P>0.05$)
Pinckaers *et al.* (2023)	24 healthy young (24±4 y) males	Double-blind, parallel, RCT with 30 g milk protein (MILK) vs. 30 g plant blend combining 15 g wheat, 7.5 g corn, and 7.5 g pea protein (PLANT-BLEND)	MyoPS 0–5h		Peak plasma leucine concentrations: MILK (353±45 µmol/L) > PLANT-BLEND (283±22 µmol/L, $P<0.001$) by ~25% Time to peak leucine concentration: MILK (46±43 min) < PLANT-BLEND (113±46 min, $P=0.001$)	MILK (0.053±0.013%·h^{-1})=PLANT-BLEND (0.064±0.016%·h^{-1}, $P=0.08$)
Pinckaers *et al.* (2022)	24 healthy young (24±4 y) males	Double-blind, parallel, RCT with 30 g potato-derived protein (POTATO) vs. 30 g milk protein (MILK) Unilateral bout of resistance exercise	Mixed MPS 0–5h		Peak plasma leucine concentrations: MILK (341±65 µmol/L) > POTATO (252±23 µmol/L, $P<0.001$) by 26% Postprandial plasma leucine availability: MILK (iAUC: 35±8 µmol/L × 5h) > POTATO (27±4 µmol/L×5h, $P<0.05$) by 23% Time to peak leucine concentration: MILK (48±27 min) < (153±50 min, $P<0.001$)	MILK (0.050±0.012%·h^{-1}), respectively; = POTATO (0.053±0.017%·h^{-1}, $P=0.540$) at rest MILK (0.064±0.015%·h^{-1})=POTATO (0.069±0.019%·h^{-1}, $P=0.520$) postexercise

Table 1 (*Continued*)

Reference	Participants	Study design/intervention	Muscle fraction for postprandial MPS measurement	Evidence supporting "leucine trigger" hypothesis	Blood leucine concentration profile	Postprandial rates of MPS
Pinckaers *et al.* (2021)	36 healthy (24±3 y) males	Double-blind, parallel, RCT 30g milk protein (MILK) vs. 30g wheat protein (WHEAT) vs. 30g blend combining 15g wheat plus 15g milk protein (WHEAT+MILK)	MyoPS 0–5 h		Plasma leucine increase was greater for MILK vs. WHEAT (time × treatment: $P<0.001$) but did not differ between MILK and WHEAT +MILK (time × treatment: $P=0.09$). Peak plasma leucine concentrations: MILK ($353\pm45\,\mu$mol/L) > WHEAT ($280\pm37\,\mu$mol/L, $P<0.001$) and WHEAT+MILK ($301\pm44\,\mu$mol/L l, $P=0.01$) Time to peak leucine concentration: MILK (46 ± 43 min) = WHEAT (58 ± 19 min, $P = 0.42$) = WHEAT+MILK: 64 ± 51 min, $P=0.31$)	MILK ($0.053\pm0.013\%\cdot$h^{-1}) = WHEAT ($0.056\pm0.012\%\cdot$h^{-1}, $P=0.56$) = WHEAT+MILK ($0.059\pm0.025\%\cdot$h^{-1}, $P=0.46$)
van der Heijden *et al.* (2023)	Healthy young (22±3 y males (n =18) and females (n=18)	Double-blind, parallel, RCT 25g protein from fungal-derived from mycoprotein (MYCO) vs. Spirulina (SPIR) vs. Chlorella (CHLO) Unilateral bout of resistance exercise	MyoPS 0–4 h	No	Peak plasma leucine concentration: SPIR ($316\pm66\,\mu$mol/L) > MYCO ($226\pm30\,\mu$mol/L, $P<0.001$) and CHLO ($187\pm30\,\mu$mol/L, $P<0.001$)	MYCO ($0.060\pm0.015\%\cdot$h^{-1}) = SPIR ($0.066\pm0.022\%\cdot$h^{-1}) = CHLO ($0.055\pm0.019\%\cdot$h^{-1}, $P>0.05$) at rest MYCO ($0.092\pm0.024\%\cdot$h^{-1}) = SPIR ($0.086\pm0.028\%\cdot$h^{-1}) = CHLO ($0.090\pm0.024\%\cdot$h^{-1}, $P>0.05$) postexercise
Weijzen *et al.* (2022)	Healthy young (22±3 y) males (n=12) and females (n=12)	Double-blind, parallel, RCT with 30 g intrinsically L-[1–^{13}C]-phenylalanine–labelled milk protein (PRO) vs. 30g of free amino acids (FAA) labelled with L-[1–^{13}C]-phenylalanine	Mixed MPS 0–6 h	No	Peak plasma leucine concentration: FAA ($501\pm42\,\mu$mol/L) > PRO ($326\pm59\,\mu$mol l/L, $P<0.001$)	FAA ($0.053\pm0.014\%\cdot$h^{-1}) = PRO ($0.051\pm0.010\%\cdot$h^{-1}, $P=0.629$)
West *et al.* (2023)	24 healthy young (21±1 y) males	Double-blind, parallel, RCT with 70g mycoprotein (MYC; 31.4g protein, 2.5g leucine) vs. 38.2g of a protein concentrate obtained from mycoprotein (PCM; 28.0g protein, 2.5g leucine) Unilateral bout of resistance exercise	MyoPS 0–1.5 h	No	Peak plasma leucine concentration: PCM > MYC ($P<0.0001$)	MYC = PCM at rest ($P > 0.05$) MYC = PCM postexercise ($P>0.05$)

Table 1 (Continued)

Reference	Participants	Study design/intervention	Muscle fraction for postprandial MPS measurement	Evidence supporting "leucine trigger" hypothesis	Blood leucine concentration profile	Postprandial rates of MPS
West *et al.* (2023a)	Healthy young (21 ± 1 y) males (*n* = 24) and females (*n* = 9)	Double-blind, parallel, RCT with 25 g of protein from mycoprotein (MYC) vs. pea protein (PEA) vs. blend (39% MYC, 61% PEA) of the two (BLEND) Whole-body bout of resistance exercise	MyoPS 0–4 h	No	Time to peak leucine concentration: PEA and BLEND > MYC ($P < 0.0001$)	MYC ($0.076 \pm 0.004\% \cdot h^{-1}$) = PEA $0.087 \pm 0.01\% \cdot h^{-1}$) = BLEND ($0.085 \pm 0.01\% \cdot h^{-1}$; all $P > 0.05$)

AUC, area under curve; g, gram; h, hour; MPC, milk protein concentrate; MPS, muscle protein synthesis; Myo, myofibrillar; RCT, randomized controlled trial; y, years.
Values are presented as means ± SE.

Interestingly, to our knowledge, the only recent study to demonstrate a positive association between blood leucine concentration profiles and the postprandial response of MPS to an ingested protein source was conducted in healthy older adults [21]. In this study, peak plasma leucine concentrations were 25% greater after ingesting a protein-matched (36 g) beef-based omnivorous meal *vs.* an isocaloric vegan meal that constituted plant-based whole foods, and was accompanied by a corresponding ~47% greater postprandial MPS response over the subsequent 6-h postprandial period [21]. These data are consistent with findings from two recent systematic reviews [3,4] that concluded the utility of the leucine trigger hypothesis in regulating the postprandial response of MPS appears to be age dependent in healthy adults. In this regard, a strong relationship ($r^2 = 0.64$) was observed between the dose of ingested leucine and the postprandial response of MPS in healthy older adults, but this association was absent ($r^2 = 0.01$) in healthy young adults [4]. The precise explanation for this apparent age-effect on the utility of the leucine trigger hypothesis is difficult to reconcile, but is possibly related to impairments in the absorption, transport and delivery of leucine to senescent skeletal muscle, coupled with a reduced sensitivity of intramuscular cell signalling proteins to leucine that is evident in healthy older adults [7]. Hence, the higher leucine threshold that is required to stimulate a postprandial response of MPS in older adults dictates that the rate of leucine appearance into the circulation features more prominently as both a limiting factor in activating the muscle protein synthetic machinery and in regulating the magnitude of postprandial MPS rates in 'anabolic-resistant' healthy older adults than 'anabolic-sensitive' healthy young adults. At the other end of the health spectrum, given that recent evidence indicates that amino acid absorption rates may be compromised in critical illness [5], intuitively the leucine trigger hypothesis may play a prominent role in devising amino acid/protein recommendations in this more compromised, albeit under-studied, clinical population. Accordingly, the remainder of this review summarizes the limited evidence base pertaining to protein digestion and amino acid absorption kinetics within an intensive care setting with a view to advancing clinical nutrition guidelines in critically ill patients.

PROTEIN DIGESTION AND AMINO ACID ABSORPTION KINETICS IN CRITICALLY ILL PATIENTS

Intensive care poses an extreme challenge to critically ill patients whereby a rapid decline in skeletal muscle mass and function is associated with poor

Table 2. Summary of recent (2022-) studies utilizing protein-based interventions in critically ill patients with functional and clinical endpoints

Reference	Participants	Study design	Intervention	Outcomes
Heuts et al. (2025)	n = 4164 patients aged ≥ 18 years	Systematic review and meta-analysis	Higher vs. lower protein groups (mean protein intake: 1.5 ± 0.6 vs. 0.9 ± 0.4 g/kg/day)	Risk ratio (RR) (1.01 (95% CI: 0.84–1.16) for mortality
Lee et al. (2024)	n = 3,303 aged ≥ 18 years	Systematic review and meta-analysis	Higher vs. lower protein groups (mean protein intake: 1.49 ± 0.48 g/kg/day vs. 0.92 ± 0.30 g/kg/day)	Risk ratio (0.99 (95% CI: 0.88–1.11) for mortality Self-reported quality-of-life physical function measurements at day-90 (Standardized mean difference → 0.40, 95% CI: –0.04–0.84) In patients with acute kidney injury: Mortality ↑ (RR 1.42; 95% CI: 1.11–1.82)
Qin et al. (2024)	n = 2,965 aged ≥ 18 years	Systematic review and meta-analysis	Higher (≥1.2 g/kg/day) vs. lower (<1.2 g/kg/day) doses of protein supplementation	RR (1.03; 95%CI: 0.92–1.15) for mortality Length of intensive care unit stay (0.19; 95% CI: -0.67–1.04) Length of hospital stay (0.73; 95%CI: -1.59–3.04) Duration of mechanical ventilation (-0.14; 95%CI: -0.83–0.54) RR (1.11; 95%CI: 0.87–1.41) for incidence of acute kidney injury
Araujo et al. (2024)	High group: n = 206; 60.3 ± 15.7 years Medium group: n = 325; 58.8 ± 14.3 years	Retrospective cohort study	Protein intake (g/kg/day): low (<0.8), medium (0.8–1.19), high (1.2–1.5), and very high (>1.5)	Protein dose 1.2–1.5 g/kg/day was associated with superior functional capacity at ICU discharge compared with other doses
van Gassel et al. (2024)	Protein group: n = 7; 55 (45, 60) years Amino acid group: n = 7; 71 (54, 73) years	Randomized controlled trial	20 g intrinsically L-[1–13C]-phenylalanine-labelled milk protein vs. 20 g intrinsically L-[1–13C]- phenylalanine amino acids for 6 h	Whole-body protein net balance became positive after nutrient administration (P-time < 0.001) and tended to be more positive after free amino acid in provision (P-time treatment = 0.07)
van Gassel et al. (2022)	n = 21; 47 (40, 60) years vs. 9 healthy controls 37 (19, 73) years	Non-randomized controlled trial	100 mL of a formula feed (Ensure) and 2 g of 3-O-Methyld-glucose (3-OMG) via postpyloric feeding tube for 60 to 240 min	Postprandial rise in essential amino acids was not apparent in critically ill patients compared with healthy controls (iAUC 60 min, −4858 [−6859 to 2886] vs 5406 [3099–16,853] μmol/L; P=0.039). No significant differences were observed from 0–240 min
Chapple et al. (2022)	n = 15; 50 ± 17 years vs. 10 healthy controls 54 ± 23 years	Non-randomized controlled trial	Primed intravenous L-[ring-2H5]-phenylalanine, L-[3,5–2H2]-tyrosine, and L-[1–13C]-leucine infusion over 9.5 h and a duodenal bolus of intrinsically-labelled (L-[1–13C]-phenylalanine and L-[1–13C]-leucine) intact milk protein (20 g protein) over 60 min	Plasma amino acid availability did not differ between groups (ICU patients, 54.2 ± 9.1%, vs. healthy control subjects, 61.8 ± 13.1%; P=0.12) Myofibrillar protein synthesis rates increased in both groups (0.028 ± 0.010%/h vs. 0.043 ± 0.018%/h with main time effect (P=0.046), with lower rates in ICU patients vs. healthy control subjects (main group effect P=0.001) Incorporation of protein-derived phenylalanine into myofibrillar protein was 60% lower in ICU patients (0.007 ± 0.007 mol percentage excess vs. 0.017 ± 0.009 mol percentage excess; P=0.007)

long-term functional recovery and increased mortality [6]. Indeed, critically ill patients are estimated to lose ~2% of their muscle mass per day following admission to intensive care which exacerbates risk of morbidity and mortality in this patient group [35,36]. The physiological mechanisms that underpin muscle atrophy in critical illness are not fully elucidated, but are clearly multifactorial with contributing features including elevated levels of under-nutrition, inflammation, immobilization, hormonal alterations, and a muscle catabolic state underpinned by anabolic resistance. Gastrointestinal dysfunction is also common during critical illness [37] and some recent evidence indicates that postprandial amino acid absorption rates may be compromised among critically ill patients [5], although this observation is not universal. For instance, intravenous and enteral stable isotopic tracer studies reported a reduced systemic availability of phenylalanine in critically ill patients *vs.* healthy controls, although this observation may be ascribed to an impaired digestion rate or a higher splanchnic extraction of amino acids among critically ill patients [38]. Interestingly, another study reported an attenuated postprandial increase in EAA concentrations in critically ill patients which was not associated with small intestinal function [5]. Conversely, despite the enteral administration of a 20 g intrinsically-labelled milk protein eliciting comparable amino acid absorption kinetics between critically ill patients and healthy controls, Chapple *et al.* reported an ~60% reduction in the postprandial response of MPS in critically ill patients [39], thus reflecting a state of anabolic resistance to enteral protein provision. Hence, it seems intuitive that dietary-derived amino acid availability and the subsequent postprandial stimulation of MPS may be impaired in this more compromised, but under-studied, clinical population, although it remains unclear whether impaired digestion is a causal factor. Nonetheless, moving forward there is a need for experimental studies to establish optimal and pragmatic protein feeding strategies within a clinical setting of critical illness.

Accordingly, a recent proof-of-concept study investigated the acute (2-h) postprandial response of amino acid kinetics and whole-body net protein balance to manipulation of enteral amino acid intake in critical illness [40**]. Specifically, van Gassel *et al.* compared the efficacy of enteral feeding via a nasogastric tube with 20 g of intrinsically-labelled intact milk protein *vs.* a custom made free amino acid mixture that contained an identical amino acid profile. The amino acid mixture condition elicited a more rapid and marked increase in systemic phenylalanine and leucine concentrations, and whole-body protein net balance tended to be more positive

compared to the intact protein condition over a 2-h period in critically ill patients [40**]. Whilst these preliminary data indicate that enteral administration of free amino acids may mitigate the catabolic response in critically ill patients, the high molarity of these products anecdotally leads to a high prevalence of diarrhoea which nullifies the clinical utility of free amino acids into clinical practice. Conversely, negligible side effects have been reported with semi-elemental products such as peptide-based diets. Moving forward, future studies that include direct tissue-specific, rather than whole-body, measurements of MPS are warranted to establish targeted and pragmatic protein or amino acid based interventions to mitigate skeletal muscle atrophy during critical illness (Table 2).

CONCLUSION

The relationship of protein digestion and amino acid absorption kinetics with the postprandial stimulation of MPS is complex. While the leucine trigger hypothesis provides invaluable mechanistic insights into the postprandial regulation of MPS, recent *in vivo* studies report a disassociation between blood leucine availability and postprandial MPS rates that is contingent upon physiological (i.e., healthy and clinical populations, rest and exercise) and nutritional (protein dose, isolated intact protein sources and protein-rich foods) factors. Preliminary evidence suggests that amino acid absorption kinetics are compromised in critically ill patients. Accordingly, future studies are warranted to determine best practice protein/amino acid-based guidelines to mitigate muscle atrophy during critical illness.

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