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Increasing protein dose does not further augment muscle protein synthesis in critical illness: a randomized, controlled clinical trial

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Abstract

Rationale: **Critical illness impairs the muscle protein synthetic response to protein administration. Whether increased protein dose can overcome this anabolic resistance is unknown.**

Objectives: **To assess the impact of a single intraduodenal bolus of 40 g protein compared to 20 g of protein in mechanically ventilated critically ill patients on the primary outcome of postprandial muscle protein synthesis rates.**

Methods: **Mechanically ventilated patients were randomized to 40 or 20 g of whey protein isolate delivered intraduodenally over 1 h. Primed continuous intravenous L-[ring-$^{13}C_6$]-phenylalanine and L-[3,5-$^{2}H_2$]-tyrosine infusions were applied with repeated arterial blood and skeletal muscle tissue sampling over 2 h fasting and 6 h postprandial periods to assess plasma amino acid responses and rates of fasting and postprandial muscle protein synthesis (primary outcome). Data are mean ± SD and area under the curve (AUC), analyzed with ANCOVA adjusted for fasting rate and paired *t*-tests ($P < .05$).**

Measurements and main results: **Twenty patients ($n = 10$/group: 40 g: 90% male, 49 ± 21 y and 20 g: 80% male, 51 ± 13 y) were studied. Postprandial muscle protein synthesis rates (primary outcome) did not differ between groups (40 g vs 20 g: 0.030 ± 0.012 vs 0.025 ± 0.010%·h^{-1}; adjusted mean difference 0.007 (95% CI, −0.003 to 0.016) %·h^{-1}; $P = .152$). Postprandial plasma leucine and tyrosine availability (AUC) were higher following 40 g vs 20 g protein (leucine: 263 ± 87 vs 194 ± 54 µmol·L^{-1}, $P = .005$; tyrosine: 92 ± 24 vs 63 ± 17 µmol·L^{-1}, $P = .006$). Fasting muscle protein synthesis rates did not differ between groups (40 g vs 20 g: 0.020 ± 0.012 vs 0.025 ± 0.023%·h^{-1}; $P = .558$). The post hoc uncontrolled analysis of muscle protein synthesis rates from fasting to postprandial periods increased in the 40 g group only ($P = .005$).**

Conclusion: **Higher enteral protein does not further augment postprandial muscle protein synthesis rates to overcome anabolic resistance during critical illness, despite increased plasma amino acid availability.**

Trial Registration Number: **Australia New Zealand Clinical Trials Registry Identifier: ACTRN12620000776909.**

Keywords protein, critical illness, anabolic resistance, muscle protein synthesis, enteral nutrition

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At a Glance Commentary

Scientific Knowledge on the Subject: Critical illness is accompanied by impairments in the muscle protein synthesis response to dietary protein administration. Using contemporary stable isotope amino acid methodology, muscle protein synthesis was quantified in patients receiving 40 g compared to 20 g of whey protein. Providing a higher dose of enteral protein (40 g) further increased plasma amino acid availability but did not augment postprandial muscle protein synthesis during critical illness.

What This Study Adds to the Field: This study demonstrates that providing greater amounts of protein (40 g vs 20 g) does not overcome anabolic resistance to dietary protein in critically ill patients.

Introduction

Skeletal muscle wasting occurs early and rapidly during critical illness.[1] Muscle atrophy is associated with increased intensive care unit (ICU) and hospital length of stay,[2] and poor functional recovery after hospital discharge.[3] To aid improved outcomes in survivors of critical illness, strategies to reduce muscle wasting are required.[4]

Muscle mass maintenance is regulated by rates of muscle protein synthesis and breakdown. In health, following intake of dietary protein, plasma amino acid availability increases, which in turn stimulates muscle protein synthesis.[5] As such, increasing protein dose administered during critical illness has the potential to attenuate muscle loss, with international critical care guidelines recommending provision of 1.2-2.0 g protein/kg body weight/day,[6,7] higher than that recommended in health (0.7-0.8 g protein/kg body weight/day).[8] Our group has previously shown that dietary protein digestion and amino acid absorption are not impaired in critical illness when compared to healthy participants following duodenal administration of 20 g protein. However, incorporation of amino acids into skeletal muscle was 60% lower in critically ill patients, demonstrating anabolic resistance to enteral protein provision[9] as a key contributor to the muscle loss observed during critical illness.

It has been recognized that increasing protein doses can maximize anabolic potential, improving rates of muscle protein synthesis in other patient groups.[10,11] Whether there is a dose-response of enteral protein administration on rates of muscle protein synthesis in the critically ill has not been investigated. We hypothesized that greater protein provision will result in higher postprandial muscle protein synthesis rates in critical illness. In this study, we assessed the impact of enteral provision of 20 g vs 40 g protein on muscle protein synthesis rates in critically ill patients. Preliminary results of this study have been previously reported in abstract form.[12]

Methods

Trial design

This prospective, single-center, proof-of-concept, parallel-group randomized trial was approved by the Central Adelaide Local Health Network Human Research Ethics Committee (reference number: 12960), with cross-institutional approval obtained from the University of Adelaide Human Research Ethics Committee (application ID: 37643) and prospectively registered on the Australia New Zealand Clinical Trials Registry (ACTRN12620000776909). This study follows the Consolidated Standards of Reporting Trials (CONSORT) guideline for parallel-group randomized trials.[13]

Participants

Patients admitted to the Royal Adelaide Hospital ICU were eligible if they were: (1) aged ≥ 18 years; (2) mechanically ventilated and expected to remain ventilated until the end of the calendar day after recruitment; and (3) suitable to receive enteral nutrition. Full inclusion/exclusion criteria are provided in Table S1. Prior written informed consent was obtained from the patient's surrogate decision maker.

Randomization

Following consent, participants were randomized to receive either 40 g (higher protein) or 20 g (lower protein) of enteral protein. The randomization schedule was computer-generated in a 1:1 ratio for treatment allocation and placed in sealed, consecutively numbered, opaque envelopes. The randomization schedule was created by a research staff member not involved in the study conduct. Envelopes were opened by research staff following consent to enable the enteral protein preparation. The randomization schedule was designed so that participants who withdrew were replaced until the target number of participants with complete data for the primary outcome was achieved.

Experimental protocol

The experimental protocol is detailed in Figure 1 and Supplementary Methods. Prior to the study day, a nasoduodenal feeding tube was placed using the Cortrak device (Avanos Medical, New South Wales, Australia) and postpyloric position confirmed via X-ray. At 0600 h on the study day, following a 4 h fast, plasma and intracellular pools of phenylalanine and tyrosine were primed with a single intravenous dose of labeled L-[ring-$^{13}C_6$]-phenylalanine (2.25 μmol·kg^{-1}) and L-[3,5-$^{2}H_2$]-tyrosine (0.867 μmol·kg^{-1}) (Cambridge Isotope Laboratory, Massachusetts, United States) delivered via an intravenous pump, to rapidly increase the plasma amino acid enrichment. Immediately following (t=−240 min), a continuous intravenous infusion of L-[ring-$^{13}C_6$]-phenylalanine (0.050 μmol·kg^{-1}·min^{-1}) and L-[3,5-$^{2}H_2$]-tyrosine (0.019 μmol·kg^{-1}·min^{-1}) was administered for 10 h, to maintain a stable plasma amino acid enrichment throughout the entire experimental study period. At t = 0 min, 40 or 20 g of 100% whey enteral protein isolate Beneprotein (Nestle, New South Wales, Australia; amino acid profile in Table S2), dissolved in water to

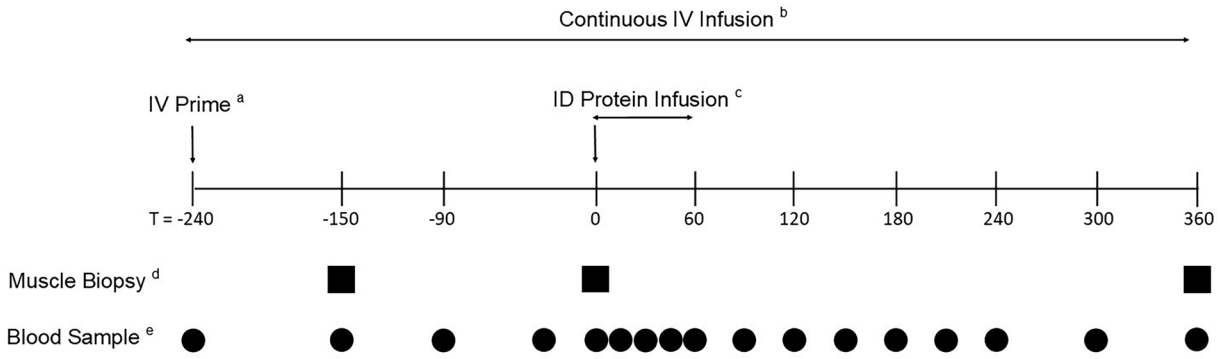

Figure 1 Study design. [a]Intravenous priming dose of L-[ring-$^{13}C_6$]-phenylalanine and L-[3,5-$^{2}H_2$]-tyrosine. [b]Intravenous continuous infusion of L-[ring-$^{13}C_6$]-phenylalanine and L-[3,5-$^{2}H_2$]-tyrosine. [c]Intraduodenal administration of whey protein isolate (Beneprotein, Nestle) to provide 40 vs 20 g of protein made with water to 240 mL volume, enriched with 4% of L-[ring-$^{13}C_6$]-phenylalanine delivered over 60 min via an enteral feeding pump. [d]Skeletal muscle biopsy sample using the Bergstrom needle technique to determine fasting (t = −150 min to t = 0 min) and postprandial (t = 0 to t = 360 min) muscle protein synthesis rates. [e]Arterial blood samples were collected at pre-specified timed intervals. ID, intraduodenal; IV, intravenous; T, time (min).

240 mL, was delivered over 60 min via an enteral feeding pump (Flocare, Nutricia, New South Wales, Australia). The enteral protein was enriched with 4% free, crystalline L-[ring-$^{13}C_6$]-phenylalanine (Cambridge Isotope Laboratory, Massachusetts, United States) to match the expected plasma L-[ring-$^{13}C_6$]-phenylalanine enrichment and minimize dilution of the steady-state precursor pool implemented by constant infusion.

Plasma and muscle tissue collection and analysis

Arterial blood samples were collected at timed intervals in relation to intraduodenal enteral protein commencement (t = 0 min) as outlined in Figure 1 and Supplementary Methods. Skeletal muscle tissue biopsies were collected under aseptic conditions at t = −150, 0, and 360 min from the middle region of the *M. vastus lateralis* of a randomly assigned initial leg, proceeding in alternate order using separate incisions. Muscle tissue samples were collected using a Bergstrom needle biopsy technique[14] following local anesthetic administration (Xylocaine 2% with 1:200 000 adrenaline). Plasma and muscle sample analyses were conducted in line with previous work,[9] with details presented in Supplementary Methods.

Tracer calculations

The study involved the primed, continuous intravenous infusion of L-[ring-$^{13}C_6$]-phenylalanine and L-[3,5-$^{2}H_2$]-tyrosine combined with arterial blood sampling to determine the rate of muscle protein synthesis in fasting and postprandial states. Muscle protein fractional synthesis rates (%·h^{-1}) of mixed muscle protein were calculated by dividing the increment in enrichment of muscle protein-bound L-[ring-$^{13}C_6$]-phenylalanine (eg, the product: labeled amino acids bound into skeletal muscle protein), by the enrichment of the precursor, eg, plasma L-[ring-$^{13}C_6$]-phenylalanine enrichment, as per previous methodologies[15] (Supplementary Methods). Total area under the curve (AUC) was calculated using the trapezoidal rule for plasma time curves and was divided by total minutes (eg, AUC/360) to report results in the original units of measurement.

Statistical analyses

Based on the observed variability from previous studies,[9,16-18] a sample of ten patients per group with complete data for the primary outcome were required to detect a difference in postprandial (0-360 min) muscle protein fractional synthesis rates between treatment groups (primary outcome) of 0.0164%·h^{-1}, with 80% power, 5% significance level, and SD of 0.0124%·h^{-1} (effect size = 1.3). This difference following ingestion of a higher protein dose would reflect a 50% increase in postprandial muscle protein synthesis rate, which would translate to expected changes in muscle mass attenuation that are of clinical relevance.[19]

Demographic data and clinical characteristics at baseline (Supplementary Methods) were presented as mean ± SD, median (IQR), or n (%) by randomized group. The difference in postprandial (0-360 min; primary outcome) muscle protein fractional synthesis rates between groups was analyzed using analysis of covariance (ANCOVA) adjusted for fasting (t = −150 to 0 min—prior to enteral protein) muscle protein fractional synthesis rate. For secondary outcomes, differences in plasma amino acid concentrations—peak values, time to peak, and AUC calculated for plasma time curves—were analyzed using ANCOVAs adjusted for fasting timepoints or periods of the outcome. Two-factor repeated measures ANOVAs were used to analyze differences over time between intervention groups during the fasting period (t = −150 to 0 min) and postprandial period (0-360 min) of plasma amino acid enrichments separately, with time as a within-subjects variable and group as between-subjects variable. In case of significant time × group interactions, pairwise comparisons between intervention groups at each timepoint and between timepoints for each group were performed, with Bonferroni adjustments for multiple comparisons. Fasting (t = −150 to 0 min) muscle protein fractional synthesis rates were compared between groups using an independent samples t-test. Change in muscle protein fractional synthesis rates between fasting and postprandial periods in each group was analyzed using paired t-tests; these were performed as a post hoc uncontrolled analysis; however, was added to the statistical analysis plan at trial completion, prior to data analysis. While glucose, insulin, gastrointestinal regulatory hormone profile (cholecystokinin, peptide YY, and ghrelin) and whole-body protein balance and breakdown

were registered as secondary outcomes, based on the results of our previous study which demonstrated that protein digestion and amino acid absorption in critically ill patients is normal compared to health,[9] the inclusion of these outcomes was considered to be less relevant to the research question and therefore not analyzed.

All data are expressed as mean ± SD, estimated mean differences, and 95% CI. Data were analyzed for participants with complete data for the primary outcome only. No imputation of missing outcome data was conducted. Significance was set at $P < .05$ with no adjustment for multiple testing. All calculations were performed using IBM SPSS Statistics (IBM Statistics, version 29, IBM Corp).

Results

Patients admitted to the Royal Adelaide Hospital ICU from 7 September 2020 to 27 October 2022 were screened for eligibility. Fifty patients met all inclusion and no exclusion criteria and had their surrogate decision maker approached for written informed consent, with consent obtained from 31 patients. Twenty-nine patients underwent randomization (40 g: $n = 14$; 20 g: $n = 15$), and 10 patients in the 40 g group and 11 patients in the 20 g group completed the study. One patient in the 20 g group had a protocol violation (a suspected interruption to the intravenous stable isotope amino acid infusion) and was excluded, such that 10 patients in each group were included in the final analysis (CONSORT, Figure 2). Participant characteristics are presented in Table 1.

Postprandial muscle protein synthesis rates (primary outcome)

Postprandial muscle protein fractional synthesis rates did not differ between groups (40 g vs 20 g: 0.030 ± 0.012 vs 0.025 ± 0.010%·h^{-1}, adjusted mean difference 0.007 (95% CI, −0.003 to 0.016) %·h^{-1}; $P = .152$; Figure 3).

Plasma amino acid concentrations

Fasting plasma phenylalanine, leucine, and tyrosine concentrations ($t = 0$ min) did not differ between groups (Figure 4A-C). No differences in postprandial plasma phenylalanine concentrations ($AUC_{0\text{-}360}$) were observed between groups (40 g vs 20 g: 75 ± 12 vs 76 ± 14 μmol·L^{-1}; $P = .641$; Figure 4A). Postprandial plasma leucine and tyrosine concentrations ($AUC_{0\text{-}360}$) were higher following administration of 40 g compared to 20 g protein (leucine—40 g vs 20 g: 263 ± 87 vs 194 ± 54 μmol·L^{-1}; adjusted mean difference 70.6 (95% CI, 25-116) μmol·L^{-1}; $P = .005$; Figure 4B and tyrosine—40 g vs 20 g: 92 ± 24 vs 63 ± 17 μmol·L^{-1}; adjusted mean difference 17 (95% CI, 6-29) μmol·L^{-1}; $P = .006$; Figure 4C). Peaks in plasma leucine concentrations were higher in the 40 g group compared to the 20 g (40 g vs 20 g: 472 ± 231 vs 314 ± 116 μmol·L^{-1}; $P = .0496$); however, peak concentrations were not different between groups for tyrosine ($P = .079$) and phenylalanine ($P = .272$). Time to peak in phenylalanine, leucine, and tyrosine concentrations did not differ between groups ($P = .114$, $P = .485$, and $P = .494$, respectively).

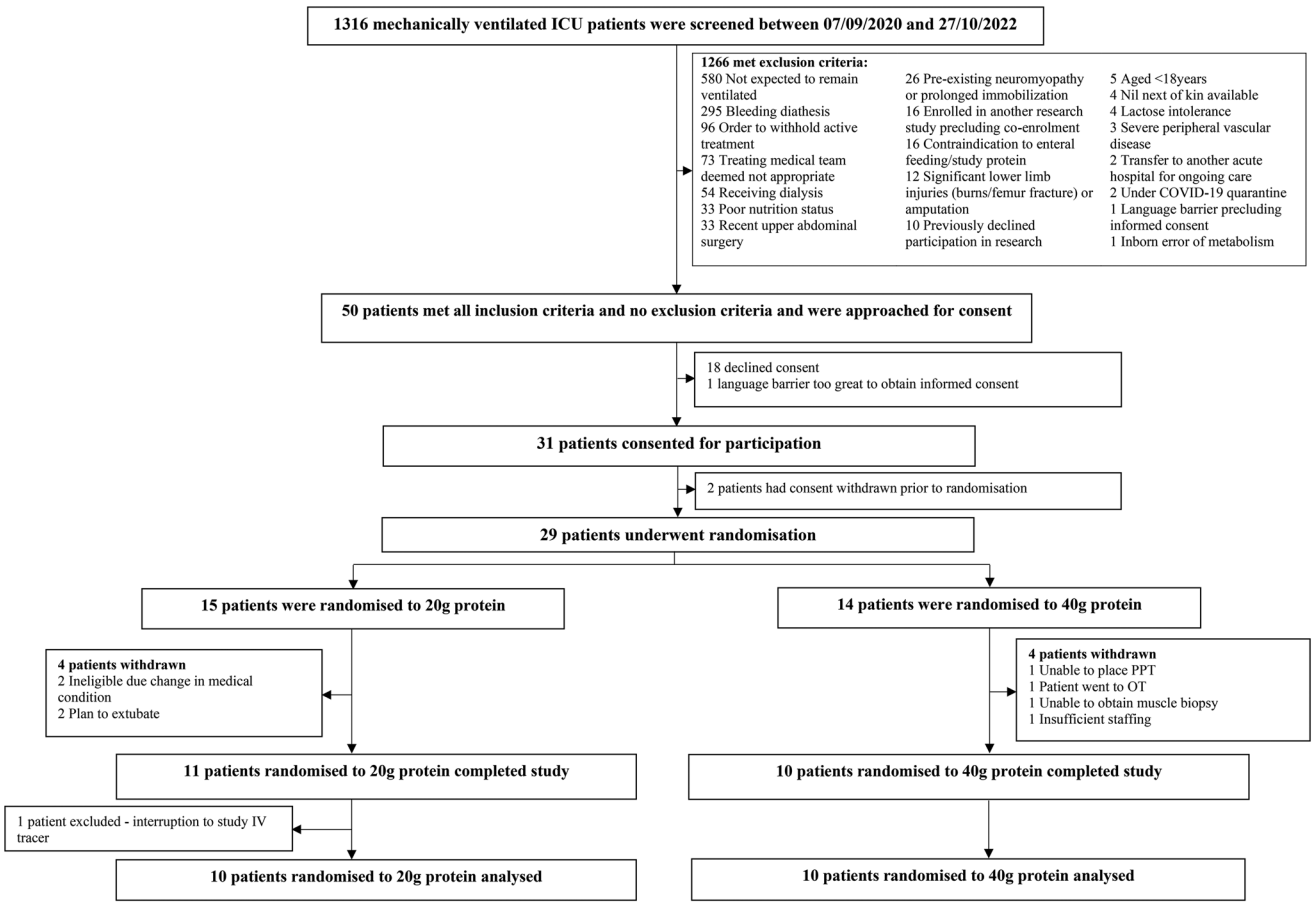

Figure 2 Flow of participants through study. ICU, intensive care unit; IV, intravenous; OT, operating theatre; PPT, postpyloric tube.

Table 1 Demographic and clinical characteristics of the study participants.

	40 g group *n* = 10	20 g group *n* = 10
Age, years	49 ± 21	51 ± 13
Male, *n* (%)	9 (90)	8 (80)
Weight, kg	85.3 ± 15.1	81.1 ± 17.8
Body mass index, kg m^{-2}	27.2 ± 5.1	26.5 ± 3.9
APACHE II score		
On ICU admission	18 ± 6	17 ± 8
On study day	17 ± 5	17 ± 5
Days in ICU prior to study	3 (2, 7)	4 (2, 5)
Length of ICU stay, days	12.3 (11.2, 21.7)	13.7 (9.4, 16.7)
Duration of mechanical ventilation, days	9.2 (4.0, 15.4)	9.7 (6.7, 12.3)
Diagnostic category, *n (%)*		
Neurological	5 (50)	4 (40)
Trauma	3 (30)	3 (30)
Respiratory	1 (10)	2 (20)
Cardiovascular	0 (0)	1 (10)
Sepsis	1 (10)	0 (0)
Average energy intake in 72 h pre-study		
kcal·day^{-1}	657 ± 447	895 ± 579
kcal·kg^{-1}·day^{-1}	8.2 ± 6.5	11.8 ± 9.2
Average protein intake in 72 h pre-study		
g·day^{-1}	37.3 ± 35.2	46.0 ± 26.8
g·kg^{-1}·day^{-1}	0.48 ± 0.52	0.61 ± 0.44
RIFLE criteria on study day, *n (%)*		
No AKI	8 (80)	10 (100)
Injury	1 (10)	0 (0)
Failure	1 (10)	0 (0)
Creatinine on study day, μmol·L^{-1}	72 (60, 92)	55 (53, 69)
Opiates, μg·mL^{-1}	*n* = 7	*n* = 4
Peak dose administered during study	100 (40, 120)	200 (160, 200)
Propofol	*n* = 10	*n* = 8
During study day (mg·24 h^{-1})	2940 (1178, 6145)	4370 (2645, 5063)
Total 72 h prior to study day (mg·72 h^{-1})	7855 (1278, 13 208)	5750 (4093, 7758)
Insulin units in 72 h prior to study fasting period, *n* (%)		
0 units	9 (90)	8 (80)
0-20 units	0 (0)	1 (10)
20-60 units	1 (0)	0 (0)
>60 units	0 (0)	1 (10)

Data are presented as mean±SD or median (IQR). Data were analyzed using an independent samples *t*-test, $P < .05$.
AKI, acute kidney injury; APACHE, acute physiology and chronic health evaluation; h, hours; ICU, intensive care unit; IU, International Unit, RIFLE, risk injury failure loss end-stage renal disease score.

Plasma amino acid enrichments

Prior to ingestion of the meal, fasting plasma L-[ring-$^{13}C_6$]-phenylalanine weighted mean enrichments did not differ between groups (40 g vs 20 g: 7.1 ± 0.9 vs 6.8 ± 1.7 MPE; $P = .663$). In the fasting period ($t = -150$ to 0 min), no differences in plasma L-[ring-$^{13}C_6$]-phenylalanine enrichments between groups were observed (P-time×group interaction = 0.840, time main effect $P < .001$; group main effect $P = .652$; Figure S1A). Postprandial plasma L-[ring-$^{13}C_6$]-phenylalanine weighted mean enrichments, used for the calculation of muscle protein fractional synthesis rates, were comparable between groups (40 g vs 20 g: 7.6 ± 0.8vs 7.4 ± 1.7 MPE; $P = .766$). Following intraduodenal protein commencement, no differences in L-[ring-$^{13}C_6$]-phenylalanine between groups were observed (group main effect $P = .671$); however, enrichments were increased from baseline in the 40 g group at 15 min ($P = .005$), 30 min ($P = .011$), and 300 min ($P = .044$). Fasting and postprandial L-[ring-$^{13}C_6$]-tyrosine and L-[3,5-$^{2}H_2$]-tyrosine enrichments also did not differ between groups (Figure S1B and C).

Fasting muscle protein synthesis rates

Muscle protein fractional synthesis rates in the fasting period did not differ between groups (40 vs 20 g: 0.020 ± 0.012 vs 0.023 ± 0.023%·h^{-1}; mean difference −0.005 (95% CI, −0.022 to 0.012) %·h^{-1}; $P = .558$; Figure 3).

Change from fasting to postprandial muscle protein synthesis rates

Muscle protein fractional synthesis rates significantly increased from the fasting to the postprandial period in the 40 g group (mean difference: 0.010 (95% CI, 0.004-0.016) %·h^{-1}; $P = .005$), but not in the 20 g group (mean difference: 0.00003 (95% CI, −0.0146 to 0.0146) %·h^{-1}; $P = .997$; Figure 3).

Discussion

Our study compared plasma amino acid availability and postprandial muscle protein synthesis rates following the administration of 40 g compared with 20 g of whey protein isolate in critically ill patients. Postprandial plasma amino availability was greater following administration of 40 g compared to 20 g of protein; yet, both fasting and postprandial muscle protein synthesis rates did not differ between groups.

We have previously shown that protein digestion and amino acid absorption following a 20 g protein dose delivered intraduodenally is not impaired in critically ill patients when compared to healthy participants. Our data now demonstrate that plasma amino acid availability is greater following provision of 40 g compared to 20 g of protein in critically ill adults. This aligns with studies in other cohorts that show a dose-response in amino acid availability following increased protein dose. A study in 33 healthy older men randomly assigned to ingest 10 g, 20 g, or 35 g of intrinsically labeled whey protein showed that 35 g protein resulted in a greater postprandial rise in plasma amino acid availability when compared with the provision of 10 and 20 g protein.[11] Similarly, in

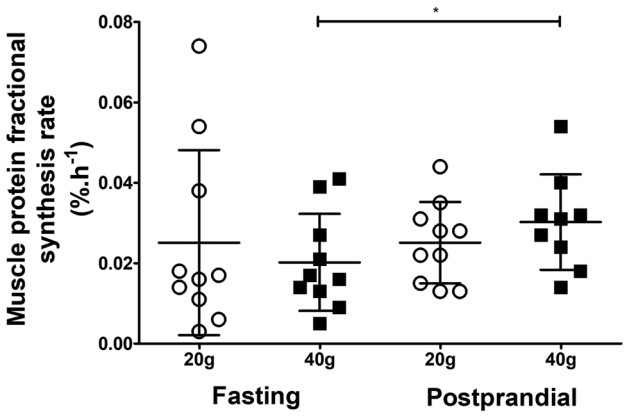

Figure 3 Fasting and postprandial muscle protein fractional synthesis rates (FSR, %·h^{-1}) during the fasting (−150 to 0 min) and postprandial (0-360 min) periods in the 40g and 20 g protein groups. Mean ± SD. * difference between fasting and postprandial periods, $P < .05$.

25 patients with cancer randomly assigned to medical food containing 40 or 24 g protein, plasma leucine availability was greater in the 40 g group.[10] Our data extend on previous findings, demonstrating a clear dose-response relationship in postprandial plasma amino acid availability following protein administration during critical illness.

We did not observe any differences in postprandial muscle protein synthesis rates between groups. This is despite the greater rise in postprandial plasma amino acid availability following provision of 40 g vs 20 g protein, and within-group comparisons showing evidence of an absolute increase in rates of muscle protein synthesis from fasting to postprandial in the group receiving 40 g of protein, while 20 g of protein did not elicit a measurable anabolic response, albeit these were post hoc uncontrolled analyses. These data suggest that the anabolic resistance to dietary protein observed in critical illness previously[9] could not be overcome by increasing the delivered protein dose up to 40 g. This is in contrast to data from other cohorts with anabolic resistance to dietary protein (eg, elderly, patients with cancer), that have demonstrated a dose-dependent stimulation of muscle protein synthesis following protein administration.[10,11] The reason for this anabolic resistance in critical illness is unknown, but is likely to be multi-factorial, driven by systemic inflammation, insulin resistance, and immobility, resulting in impaired signaling and cellular stress involved in the protein synthesis pathway.[20]

These data have implications for the interpretation of three recent large randomized controlled trials evaluating augmented protein doses in critical illness, all which have failed to show benefits on patient outcomes.[21-23] The EFFORT Protein trial randomized 1301 critically ill patients to two protein doses (mean 1.6 vs 0.9 g/kg body weight/day) and reported no between-group difference in the primary outcome (time-to-discharge alive from hospital up to 60 days after ICU admission). However, harm in the higher protein arm was observed in two of the 11 sub-groups: patients with acute kidney injury and higher organ failure scores at baseline.[21,24] The PRECISe trial enrolled 935 critically ill patients and reported that higher enteral protein delivery (mean 1.48 vs 0.95 g/kg body weight/day) resulted in significantly worse health-related quality of life during 180 days after randomization.[22] The TARGET Protein cluster-randomized trial compared a very high protein enteral formula (100 g/L) with a standard protein formula (63 g/L) in 3397 critically ill patients and found no between-group difference in the primary outcome (number of days free of the index hospital and alive at Day 90).[23] Our data provide plausible mechanistic evidence of the inability for increased plasma amino acid supply to be utilized for muscle protein synthesis in order to elicit benefit on muscle health and functional

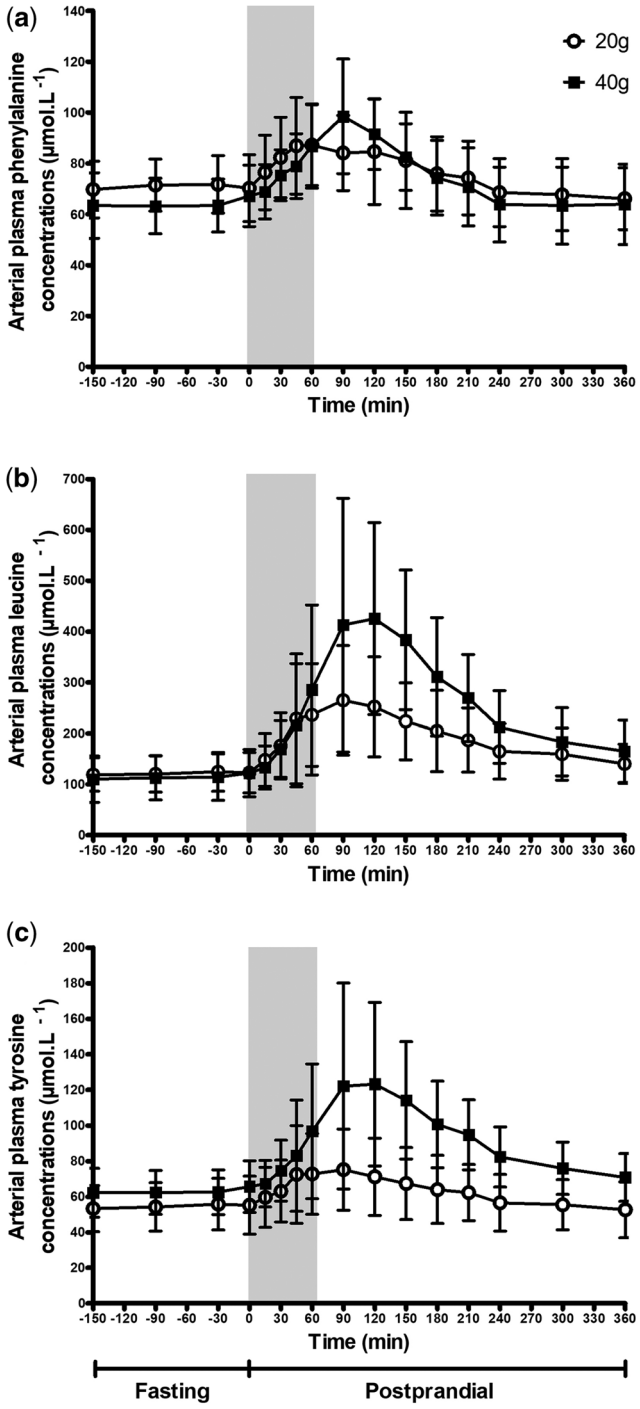

Figure 4 Total arterial plasma (a) phenylalanine, (b) leucine, and (c) tyrosine concentrations (µmol·L^{-1}) during fasting (−150 to 0 min) and postprandial (0-360 min) periods following intraduodenal protein administration. Mean ± SD. The gray section represents the period of intraduodenal protein administration.

outcome and, as such, improve important clinical or patient-centered outcomes. With greater amounts of protein administered and not being directed toward increased muscle tissue protein synthesis, one hypothesis is that excess protein supply may act as a substrate for other tissue protein synthesis and/or energy provision, which may lead to oxidation of excess amino acids into toxic byproducts, such as ammonia and urea, driving harm[25]; however, these were not measured in the present study.

Strengths and limitations

Our study has several strengths: firstly, the study used an established stable isotope amino acid methodology to measure fasting and postprandial muscle protein synthesis rates to provide world-first data on the inability of greater protein doses to overcome the observed anabolic resistance to dietary protein in critical illness. Secondly, administration of enteral protein directly into the small intestine bypasses the effect of gastric emptying, which is known to be delayed in ∼50% of critically ill patients.[26] Thirdly, study group characteristics were well-matched at baseline including for age and body mass index, which is of significance given the influence of increased age and obesity on rate of muscle protein synthesis.[27,28] Finally, whey-based protein, as used in this study, is a high-quality protein and effective in the stimulation of muscle protein synthesis in other patient cohorts displaying anabolic resistance.[29] Limitations of our study include that there was no pre-published statistical analysis plan; however, the analyses were pre-specified before data evaluation. In addition, the timing of intervention/measurements, wherein patients were studied in the acute phase of illness while mechanically ventilated, on an average of three to four days after ICU admission. As such, the observed anabolic resistance to the higher protein dose may not persist across the full duration of ICU stay and may vary over time.[30] Finally, the provision of protein as a bolus in this study does not reflect continuous enteral nutrition administration routinely used in clinical care,[6,7] but enabled the direct measurement of the muscle protein synthetic response to the administration of a single protein dose.

Conclusion

Enteral provision of a greater protein dose (40 g vs 20 g) increases plasma amino acid availability but does not further augment postprandial muscle protein synthesis rates to overcome anabolic resistance in critically ill patients.

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Author contributions

M.J.S., I.W.K.K., A.M.D., L.J.C.v.L., M.J.C., and L.S.C. made substantial contributions to the conception or design of the work. M.J.S., I.W.K.K., I.E.A., R.L., N.A., T.A.M., M.P.P., A.M.D., E.R., K.M.L., S.C.-K., N.Y., Y.C., L.J.C.v.L., M.J.C., and L.S.C. were responsible for the acquisition, analysis, or interpretation of data for the work. M.J.S., I.W.K.K., L.J.C.v.L., M.J.C., and L.S.C. drafted the work, and all other authors revised it critically for important intellectual content. All authors provided final approval of the version submitted for publication and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Supplementary material

Supplementary material is available at *American Journal of Respiratory and Critical Care Medicine* online.

Conflicts of interest

Please see the ICMJE disclosure forms, which have been provided as Supplementary material. The authors report no conflicts of interest related to this trial.

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Data availability

Nonidentifiable data that underlie the results reported in this trial will be made available 3 years after publication and ending 5 years after publication of the manuscript. Availability will only be made to independent researchers who provide a written proposal for data evaluation that is judged to be methodologically sound by an independent committee approved by the sponsor. Proposals should be directed to lee-anne.chapple@adelaide.edu.au. If the proposal is approved, applicants will be required to sign a data access agreement and will remain responsible for all costs incurred.

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