Protein delivery and clinical outcomes in the critically ill: a systematic review and meta-analysis

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ABSTRACT

Objectives: Protein is a fundamental component of critical care nutrition, but there has been uncertainty about the optimal amount. We undertook this systematic review and meta-analysis to examine the relationship between delivered protein and mortality in randomised controlled trials (RCTs) of nutritional interventions involving critically ill adults. Secondary outcomes included the effect of protein dose on lengths of stay, mechanical ventilation and incidence of infections.

Methods: We reviewed the relevant English-language literature published between 1966 and 2015 and identified RCTs comparing different strategies of nutritional support lasting at least 48 hours in critically ill adults. Articles were included if mortality was reported and the difference in delivered protein between interventions was significant ($P < 0.05$). We calculated summary estimates for mortality as odds ratios (ORs) with 95% confidence intervals (CIs) using a random-effects estimator, and we used meta-regression to assess the effect of delivered protein on mortality.

Results: From 3016 assessed records, 357 full-text articles were reviewed and 14 studies, investigating various interventions and routes of nutrition and comprising 3238 patients, were included. The mean protein delivered was 42.95 g/day (SD, 20.45 g/day) or 0.67 g//kg/day (SD, 0.38 g/kg/day) in patients receiving less protein, and 67.15 g/day (SD, 28.47 g/day) or 1.02 g/kg/day (SD, 0.42 g/kg/day) in the higher protein group. Provision of less protein did not influence mortality risk (pooled OR, 0.935; 95% CI, 0.716 –1.219; $P = 0.618$; $I^2 = 48.2\%$). Meta-regression analysis did not show a relationship between mean daily protein delivered and mortality ($P = 0.433$; $I^2 = 50.18\%$). There were no differences between groups in any secondary outcomes.

Conclusions: Delivery of varying amounts of nutritional protein was not associated with any effect on mortality.

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Critical illness causes significant alterations in metabolism, including catabolism of muscle protein and total body protein loss.[1,2] Provision of protein (or amino acids, hereafter also referred to as protein) is thought to be imperative in reducing catabolism[3] and preventing negative sequelae, such as immune dysfunction, skeletal muscle loss and mortality.[4,5] It is generally accepted that critically ill patients have elevated protein needs, but the ideal protein target in those with critical illness is unclear[6,7] and the influence of differing protein delivery on clinical outcomes is uncertain.

Observational studies have shown an association between increasing delivery of protein and reduced risk of mortality. A prospective cohort study of 113 intensive care unit patients showed a decrease in ICU mortality with each increasing tertile of protein provision (protein delivered at 0.79 g/kg/day associated with 27% mortality; protein at 1.06 g/kg/day associated with 24% mortality; and protein at 1.46 g/kg/day associated with 16% mortality).[8] Another prospective cohort study involving 886 patients showed a positive association between protein delivery (≥ 1.2 g/kg/day) and survival when energy targets were also met (hazard ratio, 0.47; 95% CI, 0.31–0.73).[9] Finally, a large, international observational study, in 167 ICUs involving 2772 patients who were ventilated, reported a reduction in 60-day mortality with each additional 30 g/day of delivered protein (odds ratio [OR], 0.83; 95% CI, 0.75–0.92).[10]

Nutrition guidelines recommend the delivery of 1.2–2 g/kg/day of protein or amino acids during critical illness,[5,11] but some authors recommend higher doses. A systematic review suggested that 2–2.5 g/kg/day may be optimal.[4] Most of the clinical evidence supporting these recommendations comes from studies of nitrogen balance[4] and there are, to our knowledge, no randomised controlled trials (RCTs) that were adequately powered to assess the impact of protein provision on mortality.

Although few RCTs have specifically addressed optimal protein provision in critically ill patients, many have examined nutritional interventions that result in differing "doses" of protein delivered to each group. We undertook this systematic review and meta-analysis of RCTs of nutritional interventions in critically ill patients with the primary aim of examining the relationship between delivered protein and mortality. Our secondary aims were to assess any relationship between delivered protein and other clinically relevant outcomes, including duration of mechanical ventilation, ICU and hospital lengths of stay and incident infections.

Methods

We undertook study selection and analysis and report our results in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[12] We did not publish a formal review protocol before commencing the study.

Inclusion and exclusion

We included RCTs comparing different strategies of nutritional support delivered for at least 48 hours to adult critically ill patients. Included studies were limited to those in which there was a significant difference in delivered protein between two intervention arms ($P < 0.05$) and mortality was a reported outcome. We only selected studies that reported protein delivery (as g/day or g/kg/day of total protein or nitrogen) or studies with adequate published data (including supplementary files) from which protein delivery could be calculated. Trials of critically ill patients were defined as those in which at least 50% of the trial population received mechanical ventilation. Studies were excluded if they investigated a supplement that did not include protein or amino acid(s), if one group did not receive protein-containing nutrition for the study period, or if nutrition support was instituted at differing times or was delivered orally. Trials were also excluded if they only included elective or cardiac surgical populations.

Search strategy

We performed an online search for original research articles and review articles published in English, using MEDLINE (via PubMed and Ovid) and Embase (Ovid) databases and the Cochrane Database of Systematic Reviews, for the period 1 January 1966 to 31 December 2015. We used the following search terms: *randomized controlled trial, controlled clinical trial, critical care, critical illness, intensive care, mechanical/artificial ventilation, ventilator, enteral nutrition, parenteral nutrition, nutritional support, protein, nitrogen balance, amino acid, caloric intake, mortality, skeletal muscle, muscle strength, fatigue, endurance, infection* and *sepsis.* A full sample search is included in the Appendix (online at cicm.org.au/Resources/Publications/Journal). Our initial search targeted studies with clinical, as well as functional, outcomes, but once the included studies were examined, there were insufficient common functional outcomes to pursue further analysis. We also reviewed the references of recent reviews and nutrition guidelines,[5,11,13,14] as well as any relevant reviews or articles we had identified in the original search.

Study selection, data extraction and assessment for risk of bias

Two reviewers (M D and L C) independently reviewed all studies selected for a full-text review, and applied the inclusion and exclusion criteria. Any discrepancies in study selection were resolved through discussion and consensus with the other investigators.

Data extraction was completed independently and in duplicate by both reviewers. Data extracted from the published reports included: study characteristics (author, year published, calendar period of study and duration of intervention); participant characteristics (number of participants, diagnostic group [eg, medical or surgical], age, sex, body mass index [BMI], bodyweight and Acute Physiology and Chronic Health Evaluation [APACHE] II score); nutritional interventions, including delivery of calories and protein; and clinical outcomes (mortality, ICU and hospital lengths of stay, duration of mechanical ventilation and incident pneumonia or bacteraemia). Any differences in data extraction were resolved through re-review of the published article to reach a consensus.

Last, each included study was assessed independently and in duplicate by both reviewers, using the Cochrane Collaboration's tool,[15] for risk of bias in random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment (divided into objective and subjective outcomes), incomplete outcome data (short-term and long-term outcomes), selective reporting and any other sources of bias. Disagreements were resolved through discussion until consensus was reached.

Data handling and statistical analysis

For continuous variables, we extracted the mean, median, interquartile range (IQR), standard deviation (SD), standard error of the mean (SEM) and 95% confidence interval (95% CI). SDs were calculated from the SEM and 95% CI,[15] and means and SDs were estimated from medians and IQRs.[16] For articles that reported means for individual study days, we calculated an overall mean. Reported nitrogen delivery was converted to an estimated protein dose by multiplying by 6.25.[17] Protein dose estimates in g/day or g/kg/day were interconverted using the group mean weight, if available. When the calorie or protein delivery was reported in graphical form, daily values were estimated from a magnified version of the graph and the mean calculated. If studies reported mortality at more than one time point, we used the primary outcome of the original study for the analytical mortality, or (if mortality was not the primary outcome) we selected the most commonly reported and clinically relevant outcome.

We analysed summary estimates for mortality and other binary outcomes using the DerSimonian–Laird random-effects estimator (as implemented in the user-written Stata metan module[18]) and report them as ORs with 95% CIs and show them as forest plots. We analysed statistical heterogeneity across trials using the I^2 statistic, with values of 35%–50% indicating moderate evidence and > 50% indicating substantial evidence of heterogeneity. Continuous outcomes are reported as weighted mean difference (WMD) in days, with 95% CI.

Potential for small-study bias (which may be a function of publication bias or of heterogeneity itself[19]) was undertaken

using the Harbord modification of the Egger test,[20] and with visual assessment of funnel plot asymmetry using a mixed-effects meta-analytical model.[21] We subsequently adjusted the summary OR to account for any potential bias using:

- methods to assess and adjust for meta-analytical study selection bias:[22]
  - the Copas model (which assumes that [study] selection probability is an increasing function of the observed study effect), and
  - the trim-and-fill routine (a non-parametric method to restore funnel plot symmetry by adding studies); and
- a regression-based method of "limit" meta-analysis (the effect estimate is adjusted for bias in the meta-analysis, in which the underlying model, an extended random-effects model, takes account of possible small-study effects by allowing the treatment effect to depend on the standard error; at the limit, a trial of infinite size produces a standard error of 0),[19,23] as implemented in the metasens *R* package.[24]

We performed sensitivity analysis by exploring the effects of different random-effect distributions (normal, *t* and mixed) on the summary OR. The probability of outlier status for each study was also assessed using the metaplus *R* package.[25] Similarly, we performed statistical outlier and influential case ("leave-one-out") diagnostics on standardised residuals, which were also assessed for normality. We also assessed the consistency of observed outcomes having different precisions, using the Galbraith plot and the metafor[21] *R* package.

Trial sequential analysis was performed using cumulative random-effects meta-analysis with O'Brien–Fleming bounds,[26] first for all included studies, subsequently excluding studies deemed to be at high risk of bias, and finally including this subset of studies with adjustment for heterogeneity. Power was set at 80% with an alpha level of 0.05, assuming a relative risk reduction (RRR) of 19% (based on an average mortality of 26% in the current meta-analysis and an absolute risk reduction [ARR] of 5%). We also conducted sensitivity analyses using an ARR of 1% (RRR, 3.8%) and 10% (RRR, 38.5%).

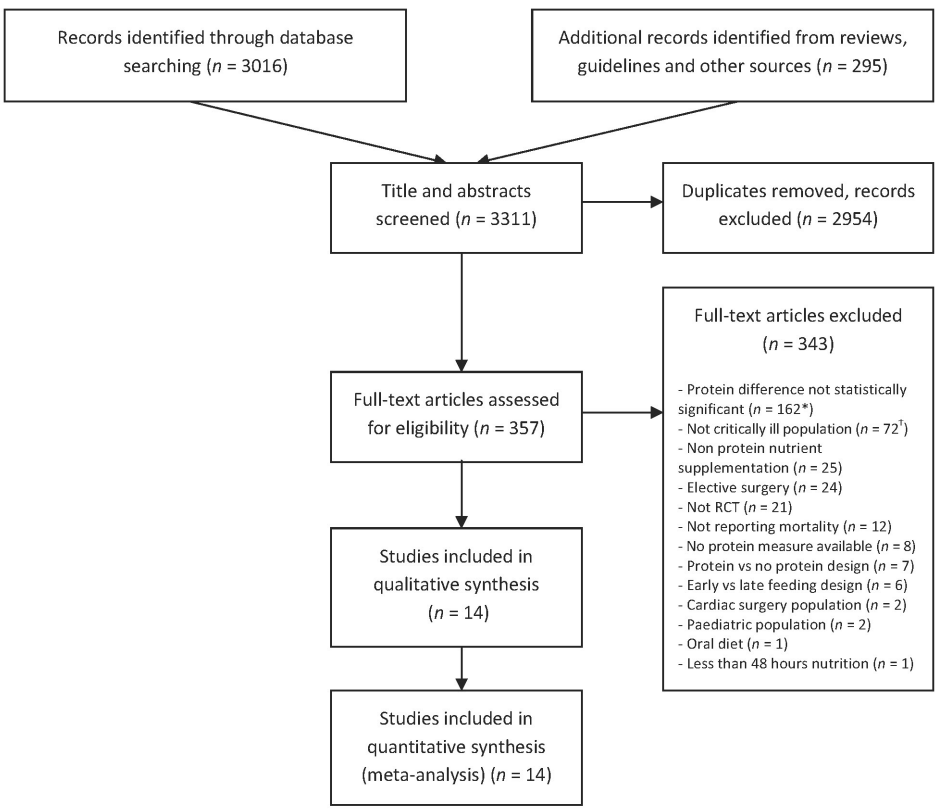
Results

Our initial search identified 3016 records from database searches and a further 295 records from references of articles, reviews, guidelines and other sources (Figure 1). The titles and abstracts were reviewed to identify potentially relevant studies, with 357 full-text articles retrieved for review. Of these, 343 did not meet our inclusion criteria (exclusions are listed in Figure 1), leaving 14 RCTs for inclusion.

Study and patient characteristics

Detailed characteristics of the included studies are shown in the Appendix. The study interventions included enteral nutrition (EN) in differing rates or volumes,[27-30] gastric versus post-pyloric feeding,[31-33] glutamine supplementation,[34-36] parenteral amino acids,[37,38] differing EN formulae[39] and caloric targets in refeeding syndrome.[40] Studies also varied in

Figure 1. Study selection



RCT = randomised controlled trial. * *P* not < 0.05. † Defined as trials in which > 50% of the trial population received mechanical ventilation.

the route of artificial nutrition: in six, EN was the predominant route;[28,29,31-33,39] in four, EN was the primary route but supplemental parenteral nutrition was allowable;[27,30,35,36] two studies used parenteral nutrition (PN) alone;[34,37] and in two, the route of nutrition was not specified.[38,40]

A total of 3238 patients were included across 14 studies: 1608 patients in the group that received less protein (lower protein group) and 1630 receiving more protein (higher protein group). Table 1 summarises the patient characteristics from each group in the 14 included studies. Each study is arranged with the groups divided into lower and higher protein provision. Baseline characteristics were comparable between the two groups in all studies. Patients appeared to be adequately nourished on study entry, with the group mean BMI ranging between 22.8 kg/m^2 and 30.1 kg/m^2. The group mean APACHE II score ranged between 18 and 27.7.

Nutrition delivery

Table 2 summarises measures of protein and energy delivery, when available, from each of the included studies. The duration of measured protein intake ranged from 5 days to 20 days (mean, 9.6 days). The mean daily protein delivery was available from 12 studies and ranged from 5.3 g/day[28] to 69.4 g/day[36] in the group receiving less protein (mean, 42.95 g/day [SD, 20.45 g/day]), and from 18.7 g/day[28] to 127.5 g/day[35] in the higher protein group (mean, 67.15 g/day [SD, 28.47 g/day]). Protein delivery per kilogram of

Table 1. Patient characteristics in included studies*

Study lead author	Year published	Diagnostic group	Protein delivery group	*n*	Mean age, years (SD)	Men, *n* (%)	Mean BMI, kg/m^2 (SD)	Mean APACHE II score (SD)
Braunschweig[27]	2014	ALI/ARDS	Lower	38	58.6 (16.2)	21 (55.3%)	30.1 (8.9)	27.7 (7.9)
			Higher	40	52.5 (17.1)	19 (47.5%)	29.8 (9.3)	23.4 (9.3)
Doig[40]	2015	Mixed	Lower	166	59 (16)	93 (56.0%)	28 (7.3)	18 (6)
			Higher	165	61 (16)	104 (63.0%)	28 (6.7)	18 (6)
Doig[38]	2015	Mixed	Lower	235	62.7 (16.6)	147 (62.6%)	29.5 (6.9)	20.2 (6.8)
			Higher	239	63.3 (15.4)	158 (66.1%)	28.9 (7)	21.7 (7.6)
Ferrie[37]	2015	Mixed	Lower	60	61.3 (15.7)	36 (60.0%)	27.4	23.7 (8.1)
			Higher	59	65.6 (14.3)	38 (64.4%)	25.7	25.5 (9.4)
Goeters[34†]	2002	Surgical	Lower	35	53.6 (18)	23 (65.7%)	25 (4)	–
			Higher	33	48.9 (16.3)	20 (60.6%)	26.3 (4)	–
Heyland[35‡]	2013	Mixed	Lower	607	63.2	355 (58.5%)	29.5	25.9
			Higher	611	63.4	371 (60.7%)	30.0	26.7
Hsu[31]	2009	Medical	Lower	62	67.9 (15.3)	43 (69.4%)	23.1 (4.1)	20.3 (6.9)
			Higher	59	70 (13.1)	42 (71.2%)	23.5 (5.8)	20.5 (6.4)
Huang[32]	2012	Medical	Lower	51	68.3 (6.2)	35 (68.6%)	23.4 (4.1)	19.6 (6.2)
			Higher	50	70.9 (13.2)	37 (74.0%)	24 (6.1)	21 (6.8)
Ibrahim[28]	2002	Medical	Lower	75	59.1 (19)	35 (46.7%)	–	25.6 (8.3)
			Higher	75	56.5 (15.6)	28 (37.3%)	–	24.7 (8.4)
Kearns[33]	2000	Medical	Lower	23	49 (19.1)	16 (69.6%)	–	20 (4.8)
			Higher	21	54 (13.7)	14 (66.7%)	–	22 (9.2)
Ozgultekin[36§]	2008	Neurotrauma	Lower	20	50.9 (18)	14 (70.0%)	–	18.9 (2.3)
			Higher	40	48.0	25 (62.5%)	–	19.35
Qiu[39]	2015	Mixed	Lower	73	63.8 (18.5)	56 (76.7%)	22.8 (2.5)	19.6 (5.8)
			Higher	71	66.6 (17.9)	54 (76.1%)	22.3 (3.7)	19.1 (4.9)
Rice[29]	2011	Medical	Lower	98	53 (19)	39 (39.8%)	29.2 (10.2)	26.9 (8.1)
			Higher	102	54 (17)	47 (46.1%)	28.2 (9.4)	26.9 (6.6)
Singer[30¶]	2011	Mixed	Lower	65	62 (17)	41 (63.1%)	27.4 (7.3)	22.4 (6.8)
			Higher	65	59 (18)	35 (53.8%)	27.8 (6.3)	22.1 (7.4)

BMI = body mass index. APACHE = Acute Physiology and Chronic Health Evaluation. ALI = acute lung injury. ARDS = acute respiratory distress syndrome. * Missing data were not available from published reports. † Only "modified" APACHE II score reported, not included. ‡ Data are weighted averages of two subgroups making up glutamine and no glutamine groups. § Data from two higher protein groups (Group II and Group III) combined into "higher" protein group. ¶ Baseline data from intention-to-treat population.

Table 2. Nutritional delivery data in included studies*

Study lead author	Year published	Duration (days)†	Protein delivery group	Mean protein, g/day (SD)	Mean protein, g/kg/day (SD)	Mean calories, kcal/day (SD)	Mean calories, kcal/kg/day (SD)	Calorie:nitrogen ratio
Braunschweig[27]	2014	20	Lower	60.4 (24)	0.68	1221 (423)	16.6 (5.6)	126.3
			Higher	82 (23)	0.95	1798 (509)	25.4 (6.6)	137
Doig[40‡]	2015	7	Lower	32.2	–	847	–	164.3
			Higher	54.8	–	1366	–	155.8
Doig[38]	2015	7	Lower	–	0.66	968	–	–
			Higher	–	1.63	1216	–	–
Ferrie[37]	2015	7	Lower	60 (21)	0.9 (0.21)	1720 (516)	24.9 (4.2)	179.2
			Higher	76 (26)	1.09 (0.22)	1610 (468)	23.1 (3.9)	132.4
Goeters[34§]	2002	9	Lower	–	1.26	1517	–	–
			Higher	–	1.46	1439	–	–
Heyland[35¶]	2013	7.9	Lower	43.1	–	894 (540)	–	129.6
			Higher	127.5	–	910 (555)	–	44.6
Hsu[31]	2009	10.9	Lower	58.8 (4.9)	0.97 (0.39)	1426 (110)	23.5 (8.8)	151.6
			Higher	67.9 (4.9)	1.11 (0.31)	1658 (118)	27.1 (7.6)	152.6
Huang[32]	2012	17	Lower	56.6	–	1343	–	148.4
			Higher	64.5	–	1575	–	152.6
Ibrahim[28]	2002	5	Lower	5.3 (5.3)	0.06	126 (115)	1.5	148.6
			Higher	18.7 (15.4)	0.23	474 (400)	5.8	158.4
Kearns[33]	2000	8.5	Lower	31 (24)	0.4 (0.48)	812 (585)	12 (9.6)	163.7
			Higher	44 (18.3)	0.7 (0.46)	1157 (394)	18 (4.6)	164.3
Ozgultekin[36**]	2008	10	Lower	69.4	0.97 (0.11)	–	–	–
			Higher	99.0	1.39	–	–	–
Qiu[39]	2015	5	Lower	34.7	–	984	–	176.9
			Higher	41.1	–	1071	–	162.9
Rice[29]	2011	6	Lower	10.9 (6.8)	0.13	300 (149)	3.6	172
			Higher	54.5 (33.2)	0.67	1418 (686)	17.3	162.6
Singer[30††]	2011	14	Lower	53 (16)	0.68	1480 (356)	19.0	174.5
			Higher	76 (16)	0.95	2086 (460)	26.1	171.5

* Missing data were not available from published reports. † Experimental period over which protein intake was measured. ‡ Protein (g/day) reported for Days 1–7 in appendix with study.[40] $P < 0.05$ for only first 5 days. § Nutritional and clinical outcomes only reported for the per-protocol group (received treatment for 9 days). ¶ Protein calculated from total nitrogen received, including glutamine supplement. ** Data from two higher protein groups (Group II and Group III) combined into "higher" protein group. †† Nutritional and clinical outcomes only reported for per-protocol group (n = 112).

body weight was available from only 10 studies. In the lower protein group, mean protein delivery was 0.67 g/kg/day (SD, 0.38 g/kg/day) and in the higher protein group, the mean was 1.02 g/kg/day (SD, 0.42 g/kg/day). The within-trial protein difference ranged from 9.1 g/day[31] to 84.4 g/day.[35] The lower protein group also had fewer calories delivered, with an average of 1049 kcal/day in this group compared with 1367 kcal/day in the higher protein group. Most trials had comparable calorie:nitrogen ratios (see Table 2) between the two groups, with two exceptions.[10,37]

Assessment for risk of bias

A summary of adjudicated risk of bias in each domain for included studies is shown in Table 3. All but two studies were at unclear or high risk of bias due to lack of blinding of participants and personnel. Excluding blinding, three studies were regarded as being at high risk of bias in one other domain. Two studies were regarded as being at high risk of bias in two other domains and these were excluded from the trial sequential analysis.

Table 3. Risk of bias assessment*

Study lead author	Year	Random sequence generation	Allocation concealment	Participant and personnel blinding	Outcome assessment blinding (objective outcomes)	Outcome assessment blinding (infections)	Incomplete outcome data (short-term/ in-hospital)	Incomplete outcome data (> 30 days/post-discharge)	Selective reporting
Braunschweig[27]	2014	L	L	H	L	U	L	U	U
Doig[40]	2015	L	L	H	L	L	L	L	U
Doig[38]	2015	L	L	H	L	U	L	L	L
Ferrie[37]	2015	L	L	L	L	U	L	L	U
Goeters[34]	2002	U	U	U	L	U	H	U	U
Heyland[35]	2013	L	L	L	L	L	L	U	L
Hsu[31]	2009	L	U	H	L	L	L	U	U
Huang[32]	2012	L	U	U	L	U	L	U	U
Ibrahim[28]	2002	H	H	H	L	L	L	U	U
Kearns[33]	2000	L	U	U	L	L	U	U	U
Ozgultekin[36]	2008	U	U	H	L	U	U	U	U
Qiu[39]	2015	L	U	H	L	U	L	U	U
Rice[29]	2011	L	L	H	L	H	L	U	H
Singer[30]	2011	L	U	H	L	U	H	U	U

L = low risk. H = high risk. U = unclear risk or where that domain is not applicable to the study. * Adjudicated risk of bias for included studies. Included are assessments for each of the domains recommended by the *Cochrane handbook for systematic reviews of interventions*, version 5.1.0.[15] The domains of "outcome assessment blinding" and "incomplete outcome data" have been split into two separate columns.

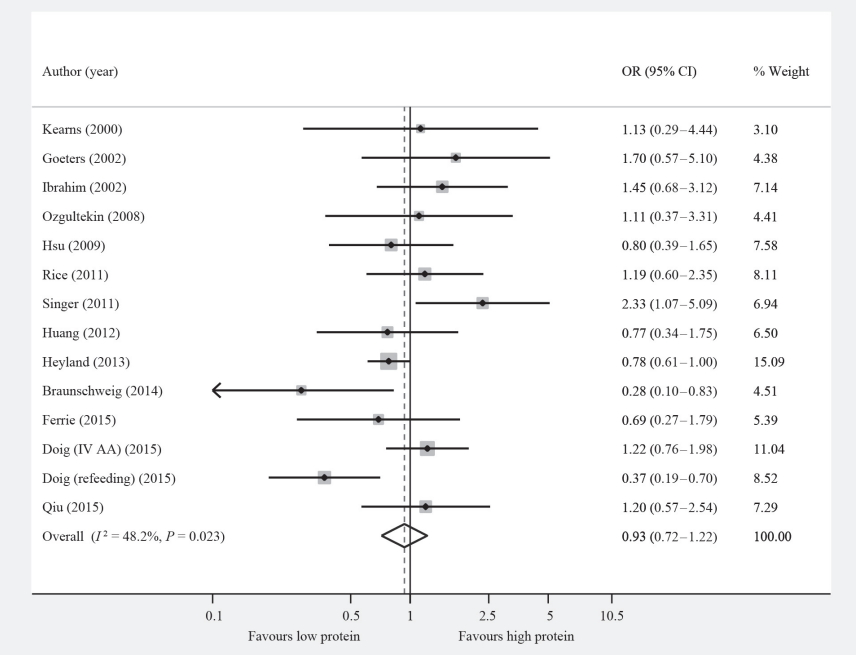
Clinical outcomes

Mortality

Mortality was the primary outcome in three trials: mortality at 28 days[35] and at 60 days,[40] and hospital mortality.[30] Of the remaining studies, the analytical mortality was hospital mortality in six,[28,29,32,37-39] 30-day mortality in two,[34,36] and an unspecified study period in three studies.[27,31,33] Provision of less protein did not increase or decrease mortality risk (pooled OR, 0.935; 95% CI, 0.716–1.219; $P = 0.618$) (Figure 2). However, there was evidence of moderate heterogeneity ($I^2 = 48.2\%$; $P = 0.023$). Mortality was examined in subgroups according to the time point at which it was measured (Appendix Figure S1) and study interventions (Appendix Figure S2) without any consistent subgroup effect across studies. Clinical outcomes for each trial are included in Appendix Table S2.

Summary estimates were robust to differences in random-effect

Figure 2. Effect of protein delivery on mortality*



OR = odds ratio. IV AA = intravenous amino acid. * Individual (square) points denote OR of each study; lines either side of square points denote 95% CIs; size of square is proportional to study size; vertical line denotes null effect; dashed line denotes meta-analytical point estimate. Weights are from random-effects analysis.

distributions (Appendix Figure S3), and the posterior probability of any individual study being an outlier was non-significant (P = 1.0) (Appendix Figure S4). Similarly, there was no evidence of significant outlier or influential studies based on residuals, which also showed normality. All studies were contained within the 95% CI of the Galbraith plot (Appendix Figure S5). Despite the result of the Harbord test being non-significant (P = 0.47), there was evidence of funnel plot asymmetry (Figure 3). Adjustments to the random-effects estimate of the primary mortality OR (OR, 0.934; 95% CI, 0.7164–1.219) using the Copas model (OR, 0.934; 95% CI, 0.719–1.214) or the trim-and-fill routine (OR, 0.844; 95% CI, 0.629–1.129), and using "limit" meta-analysis (OR, 0.782; 95% CI, 0.559–1.094; P = 0.15) produced similar and non-significant estimates. Inference on these estimates was judged to be consistent.

Meta-regression

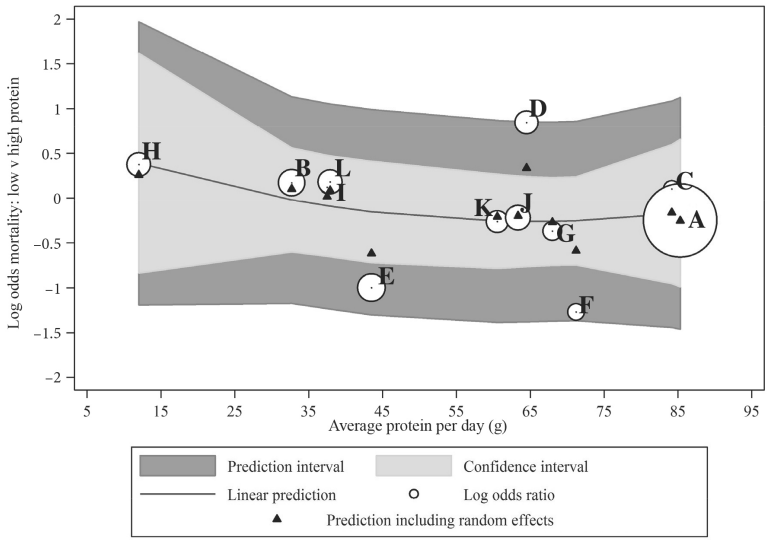
Using meta-regression analysis, we found no effect of protein delivery on mortality according to number of nutrition days (P = 0.38), APACHE II score (P = 0.80), age (P = 0.58) or sex (P = 0.75). There was also no difference in effect for studies investigating supplementation with glutamine (P = 0.34), or for any differing measure of protein provision (g/day or g/kg/day of protein or nitrogen). Finally, there was no significant effect of the provision of calories (P = 0.72) or the interaction of calories and nitrogen (P = 0.66).

Random-effects meta-regression analysis of the effect of increasing average protein provision on mortality did not show an association between mean grams per day of protein and outcome, but there was significant heterogeneity (P = 0.433; I^2 = 50.18%, Figure 4).[41] There was no relationship between the within-trial delivered protein difference (g/day) and mortality (Appendix Figure S6).

Secondary outcomes

When we compared the low-protein and high-protein groups, we found no significant differences in ICU length of stay (11 studies; WMD, 0.039 days; 95% CI, –0.746 to 0.825; P = 0.922), hospital length of stay (10 studies; WMD, –0.963 days; 95% CI, –3.932 to 2.006; P = 0.525) or duration of mechanical ventilation, measured either as absolute days (six studies; WMD, –0.073 days; 95% CI, –0.821 to 0.676; P = 0.849) or ventilator-free days in a 28-day period (two studies; WMD, –0.119 days; 95% CI, –2.067 to 1.829; P = 0.905). There were

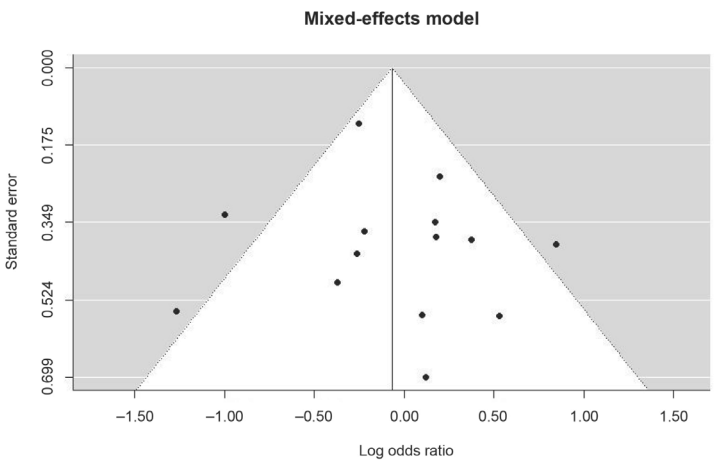
Figure 4. Meta-regression analysis of effect of average protein delivery on mortality*



A = Heyland.[35] B = Rice.[29] C = Ozgultekin.[36] D = Singer.[30] E = Doig.[40] F = Braunschweig.[27] G = Ferrie.[37] H = Ibrahim.[28] I = Kearns.[33] J = Hsu.[31] K = Huang.[32] L = Qiu.[39]

* Random-effects meta-regression analysis using log odds scale with linear prediction effect line, 95% CIs and point estimates with circles that reflect study size. Triangles represent best linear unbiased predictions, inclusive of random effects, assuming the fitted model is correct. These estimates are shrunk towards the population average effect, consistent with random-effects estimation. A prediction interval is shown in dark grey and may be interpreted as the region within which one may realistically hope to find the next large study.[41] A quadratic effect was modelled for average daily protein as being more clinically plausible and supported by reduction in τ^2.

Figure 3. Funnel plot representation for small-study bias*



* Black dots represent individual studies. Log odds ratio is for mortality. In the absence of publication or small-study bias, it is expected that the plot should approximate a symmetrical "funnel" (shown in white).

no differences in the incidence of new-onset pneumonia (six studies; OR, 1.224; 95% CI, 0.814–1.841; $P = 0.332$) or new-onset bacteraemia (four studies; OR, 1.068; 95% CI, 0.463–2.460; $P = 0.878$).

Trial sequential analysis

Trial sequential analysis of included studies (assuming an ARR of 5%), excluding those deemed to be at significant risk of bias[28,29] and adjusting for heterogeneity, showed that with increasing information size (number of patients) the estimate of effect (*Z* statistic) did not approach significance boundaries (12 studies; see Figure 5). This was consistent, regardless of the adjustment for heterogeneity or inclusion or exclusion of studies deemed at high risk of bias, and for different ARRs (1% and 10%).

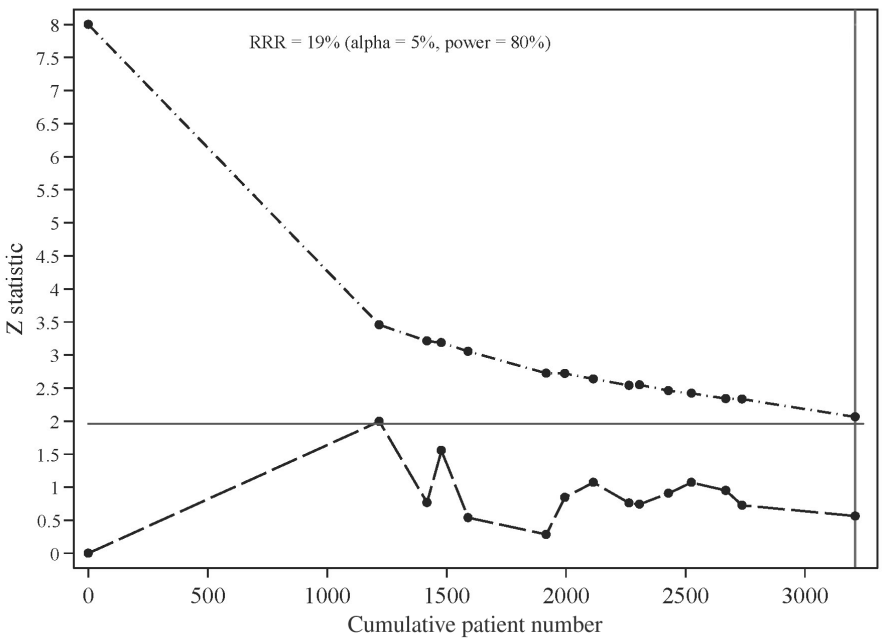
Discussion

In our meta-analysis of 14 RCTs of artificial nutritional support, involving 3238 critically ill adults, we found no effect of lower versus higher protein provision on mortality and no effect of the amount of delivered protein on mortality, when analysed with meta-regression.

This is, to our knowledge, the only meta-analysis examining the effect of protein delivery on mortality in critically ill adults. Two systematic reviews (without meta-analysis) have previously addressed the question of appropriate protein delivery in this population. In 2012, Hoffer and Bistrian[4] examined clinical trials providing different levels of protein to critically ill adults and assessing metabolic or clinical outcomes. Of 13 studies ($n = 291$ patients) that met their inclusion criteria, only four were randomised and only two ($n = 64$) included any assessment of clinical outcome. In their analysis, increasing protein provision consistently improved nitrogen balance, protein turnover and the few measured clinical outcomes. Therefore, they cautiously recommended a protein target of up to 2.5 g/kg/day (the highest studied dose), despite the significant limitations of the included studies. In a 2013 systematic review of RCTs,[42] Ferrie and colleagues assessed protein requirements for hospitalised patients, including the critically ill. The authors noted that most studies used nitrogen balance as the outcome of interest and concluded that high-level evidence was lacking

Two RCTs cited in previous systematic reviews were not included in our analysis. Scheinkestel and colleagues[43] included critically ill, mechanically ventilated patients receiving continuous renal replacement therapy. Patients received EN and/or PN and were randomised to a gradually escalating protein dose ($n = 40$) (2 days each of 1.5 g/kg/day, 2.0 g/kg/day and 2.5 g/kg/day of protein) or a fixed protein dose ($n = 10$) of 2 g/kg/day for 6 days. The authors concluded that increasing protein provision led to a positive nitrogen balance and that this was associated with an increased probability of survival. However, clinical outcomes were not assessed between the two randomised groups. Furthermore, multivariate analysis with adjustments for potential confounding factors failed to show a link between protein intake and outcome. Mesejo and colleagues[44] randomised 50 hyperglycaemic, critically ill patients to two enteral formulae with differing nitrogen content. There was a significant difference in received nitrogen between the two groups (12.8 g/day v 14 g/day nitrogen; $P = 0.04$) despite them having received equivalent energy (1599 kcal/day v 1664 kcal/day; $P = 0.28$). We excluded this trial from our analysis because the proportion of patients receiving mechanical ventilation was not reported. Even so, in this study, there were no significant

Figure 5. Trial sequential analysis*



RRR = relative risk reduction. * Cumulative random-effects meta-analysis of 12 studies (excluding those deemed at high risk of bias) with O'Brien–Fleming bounds and adjusted for heterogeneity; cumulative patient number equates with information size; long-dash line denotes estimate of effect (*Z* statistic); dash–dot line denotes significance boundary; horizontal solid line denotes nominal significance ($Z = 1.96$).

differences in ICU length of stay, days of mechanical ventilation or mortality.

A recently published RCT (included in our analysis) specifically investigated the delivery of differing protein doses using PN in ICU patients (n = 119) and found no impact on mortality (protein delivered, 0.90 g/kg/day [hospital mortality, 15%]; protein delivered, 1.09 g/kg/day [hospital mortality, 20%]; P = 0.47).[37] Our meta-analysis, although at odds with previous observational data,[8-10] is in keeping with this RCT and supports the conclusion that either the amount of protein delivered, within the range provided in our included studies, does not influence survival from critical illness, or that the effect size is quite small. On the basis of the prediction intervals of our meta-analysis (Figure 4)[41] and the trial sequential analysis, a subsequent large RCT may not find a significant result or, if such a difference does exist, it would likely be small. Our analysis also failed to find any significant effect on other relevant clinical variables, including duration of mechanical ventilation, ICU or hospital lengths of stay and the acquisition of pneumonia or bacteraemia.

Strengths and limitations

The primary strength of our analysis over previously published RCTs is its statistical power, with the inclusion of 844 mortality events from 3238 critically ill patients,[45] which, in a conventional mortality trial, would have 92% power to detect a 5% ARR. The inclusion of only randomised studies allows stronger inferences regarding causality, compared with observational data. Our search strategy was broad and included studies of nutritional interventions in critically ill adults delivered by any route. Given that provision of EN and PN appear to be clinically equivalent,[46] our study potentially informs the provision of protein by either route. Finally, we undertook a detailed assessment of the risk of bias within included studies using an accepted tool[15] and undertook methods to adjust for potential publication bias.

Our review was potentially limited by restricting our search to articles published in English, introducing the potential for language bias, and by the absence of a pre-published protocol. Our definition of studies including critically ill patients (at least 50% of patients mechanically ventilated) was chosen pragmatically but may not have captured the entire spectrum of critical illness.

The applicability of these results to practice is further limited by several issues. First, only one of the 14 included studies was designed to deliver different protein doses;[37] therefore, the differences in protein provision in the other studies were achieved either by chance or as a secondary consequence of various trial designs. This clinical heterogeneity is likely responsible for the observed statistical heterogeneity and warrants caution in drawing conclusions from summary statistics in this meta-analysis. Further, many of the studies were small and most were at potential risk of bias in at least one domain. Only two studies adequately blinded participants and treating personnel,[35,37] and four other studies were regarded as being at high risk of bias in at least one other domain.[28-30,34]

Our conclusions are also limited by the low levels of protein provision, relative to guideline recommendations, in the lower protein and higher protein delivery groups (mean, 0.67 g/kg/day v 1.02 g/kg/day, respectively, from 10 studies). These protein doses are too low to assess the impact of meeting or exceeding the 1.2–2 g/kg/day recommended by current guidelines[5,11] and, although this likely reflects the reality that ICU patients are often underfed relative to prescriptions,[10,47] it may yet be shown that there are clinical benefits to meeting these goals. In our analysis, using meta-regression, we saw no effect of up to 127.5 g of mean daily protein,[35] although in this trial, the additional protein in the intervention arm was provided as supplementary glutamine, rather than as mixed amino acids or complete protein. The duration of differing protein provision (mean, 9.6 days) may also be too short for potential benefits to be realised.

We performed our analysis without regard to baseline nutritional status, but no study specifically included malnourished patients and the mean BMIs (range, 22.8–30.1 kg/m^2) suggested that most of the patients were not nutritionally at risk on ICU admission.[47] Hence, caution is required in the application of these results to malnourished patients, who potentially have more to gain from adequate nutrition.[48] Although one trial examined patients with the refeeding syndrome,[40] the syndrome was defined based on incident hypophosphataemia rather than measures of pre-morbid nutrition.

Finally, adding to the uncertainty regarding optimal protein delivery is the potential for confounding by calorie provision. Although ideal caloric delivery in critical illness is also unclear,[49] it has been suggested that there is an important interaction between delivered calories and protein with optimal or supra-threshold amounts of both being required to improve outcomes.[9] However, we found no demonstrable interaction between calorie and protein delivery (P = 0.66), which was in keeping with a recent meta-analysis of caloric delivery in the critically ill.[50] Most included studies delivered different amounts of protein concomitant with differing amounts of energy because the design resulted in different delivery of total nutrition. In 12 of the 14 studies, the calorie:nitrogen ratio was comparable between the groups receiving differing amounts of protein.

With due consideration of these limitations, our meta-analysis suggests that differences in protein provision do not have a significant impact on the analysed outcomes. More robust research examining the relationship between protein dose and clinically important outcomes following critical illness is required.

Competing interests

None declared.

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References

1 Streat SJ, Beddoe AH, Hill GL. Aggressive nutritional support does not prevent protein loss despite fat gain in septic intensive care patients. *J Trauma* 1987; 27: 262-6.

2 Monk DN, Plank LD, Franch-Arcas G, et al. Sequential changes in the metabolic response in critically injured patients during the first 25 days after blunt trauma. *Ann Surg* 1996; 223: 395-405.

3 Ishibashi N, Plank LD, Sando K, Hill GL. Optimal protein requirements during the first 2 weeks after the onset of critical illness. *Crit Care Med* 1998; 26: 1529-35.

4 Hoffer LJ, Bistrian BR. Appropriate protein provision in critical illness: a systematic and narrative review. *Am J Clin Nutr* 2012; 96: 591-600.

5 McClave SA, Taylor BE, Martindale RG, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (ASPEN). *JPEN J Parenter Enteral Nutr* 2016; 40: 159-211.

6 Stroud M. Protein and the critically ill; do we know what to give? *Proc Nutr Soc* 2007; 66: 378-83.

7 Weijs PJ. Fundamental determinants of protein requirements in the ICU. *Curr Opin Clin Nutr Metab Care* 2014; 17: 183-9.

8 Allingstrup MJ, Esmailzadeh N, Wilkens Knudsen A, et al. Provision of protein and energy in relation to measured requirements in intensive care patients. *Clin Nutr* 2012; 31: 462-8.

9 Weijs PJ, Stapel SN, de Groot SD, et al. Optimal protein and energy nutrition decreases mortality in mechanically ventilated, critically ill patients: a prospective observational cohort study. *JPEN J Parenter Enteral Nutr* 2012; 36: 60-8.

10 Alberda C, Gramlich L, Jones N, et al. The relationship between nutritional intake and clinical outcomes in critically ill patients: results of an international multicenter observational study. *Intensive Care Med* 2009; 35: 1728-37.

11 Singer P, Berger MM, Van den Berghe G, et al. ESPEN Guidelines on Parenteral Nutrition: intensive care. *Clin Nutr* 2009; 28: 387-400.

12 Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *BMJ* 2009; 339: b2535.

13 Kreymann KG, Berger MM, Deutz NE, et al. ESPEN Guidelines on Enteral Nutrition: intensive care. *Clin Nutr* 2006; 25: 210-23.

14 Dhaliwal R, Cahill N, Lemieux M, Heyland DK. The Canadian critical care nutrition guidelines in 2013: an update on current recommendations and implementation strategies. *Nutr Clin Pract* 2014; 29: 29-43.

15 Higgins J, Green S, editors. Cochrane handbook for systematic reviews of interventions, version 5.1.0. Cochrane Training, 2011. http://handbook.cochrane.org/ (accessed Jan 2017).

16 Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol* 2014; 14: 135.

17 Mariotti F, Tome D, Mirand PP. Converting nitrogen into protein — beyond 6.25 and Jones' factors. *Crit Rev Food Sci Nutr* 2008; 48: 177-84.

18 Harris R, Bradburn M, Deeks J, et al. Metan: fixed- and random-effects meta-analysis. *Stata J* 2008; 8: 3-28.

19 Rucker G, Carpenter JR, Schwarzer G. Detecting and adjusting for small-study effects in meta-analysis. *Biom J* 2011; 53: 351-68.

20 Harbord RM, Harris RJ, Sterne JAC. Updated tests for small-study effects in meta-analyses. *Stata J* 2009; 9: 197-210.

21 Viechtbauer W. Conducting meta-analyses in R with the metafor package. *J Stat Softw* 2010; 36: 1-48.

22 Schwarzer G, Carpenter J, Rucker G. Empirical evaluation suggests Copas selection model preferable to trim-and-fill method for selection bias in meta-analysis. *J Clin Epidemiol* 2010; 63: 282-8.

23 Dechartres A, Altman DG, Trinquart L, et al. Association between analytic strategy and estimates of treatment outcomes in meta-analyses. *JAMA* 2014; 312: 623-30.

24 Schwarzer G, Carpenter J, Rücker G. Metasens: advanced statistical methods to model and adjust for bias in meta-analysis. R Packages, 2016. https://rdrr.io/cran/metasens (accessed Jan 2017).

25 Beath KJ. A finite mixture method for outlier detection and robustness in meta-analysis. *Res Synth Methods* 2014; 5: 285-93.

26 Miladinovic B, Hozo I, Djulbegovic B. Trial sequential boundaries for cumulative meta-analyses. *Stata J* 2013; 13: 77-91.

27 Braunschweig CA, Sheean PM, Peterson SJ, et al. Intensive nutrition in acute lung injury: a clinical trial (INTACT). *JPEN J Parenter Enteral Nutr* 2015; 39: 13-20.

28 Ibrahim EH, Mehringer L, Prentice D, et al. Early versus late enteral feeding of mechanically ventilated patients: results of a clinical trial. *JPEN J Parenter Enteral Nutr* 2002; 26: 174-81.

29 Rice TW, Mogan S, Hays MA, et al. Randomized trial of initial trophic versus full-energy enteral nutrition in mechanically ventilated patients with acute respiratory failure. *Crit Care Med* 2011; 39: 967-74.

30 Singer P, Anbar R, Cohen J, et al. The tight calorie control study (TICACOS): a prospective, randomized, controlled pilot study of nutritional support in critically ill patients. *Intensive Care Med* 2011; 37: 601-9.

31 Hsu CW, Sun SF, Lin SL, et al. Duodenal versus gastric feeding in medical intensive care unit patients: a prospective, randomized, clinical study. *Crit Care Med* 2009; 37: 1866-72.

32 Huang HH, Chang SJ, Hsu CW, et al. Severity of illness influences the efficacy of enteral feeding route on clinical outcomes in patients with critical illness. *J Acad Nutr Diet* 2012; 112: 1138-46.

33 Kearns PJ, Chin D, Mueller L, et al. The incidence of ventilator-associated pneumonia and success in nutrient delivery with gastric versus small intestinal feeding: a randomized clinical trial. *Crit Care Med* 2000; 28: 1742-6.

34 Goeters C, Wenn A, Mertes N, et al. Parenteral L-alanyl-L-glutamine improves 6-month outcome in critically ill patients. *Crit Care Med* 2002; 30: 2032-7.

35 Heyland D, Muscedere J, Wischmeyer PE, et al. A randomized trial of glutamine and antioxidants in critically ill patients. *N Engl J Med* 2013; 368: 1489-97.

36 Ozgultekin A, Turan G, Durmus Y, et al. Comparison of the efficacy of parenteral glutamine and branched-chain amino acid solutions given as extra supplements in parallel to the enteral nutrition in head trauma. *E Spen Eur E J Clin Nutr Metab* 2008; 3: e211-6.

37 Ferrie S, Allman-Farinelli M, Daley M, Smith K. Protein requirements in the critically ill: a randomized controlled trial using parenteral nutrition. *JPEN J Parenter Enteral Nutr* 2016; 40: 795-805.

38 Doig GS, Simpson F, Bellomo R, et al. Intravenous amino acid therapy for kidney function in critically ill patients: a randomized controlled trial. *Intensive Care Med* 2015; 41: 1197-208.

39 Qiu C, Chen C, Zhang W, et al. A fat-modified enteral formula improves feeding tolerance in critically ill patients: a multicenter, single-blind, randomized controlled trial. *JPEN J Parenter Enteral Nutr* 2015; Sep 8. pii: 0148607115601858. [Epub ahead of print.] http://journals.sagepub.com/doi/abs/10.1177/0148607115601858 (accessed Jan 2017).

40 Doig GS, Simpson F, Heighes PT, et al. Restricted versus continued standard caloric intake during the management of refeeding syndrome in critically ill adults: a randomised, parallel-group, multicentre, single-blind controlled trial. *Lancet Respir Med* 2015; 3: 943-52.

41 Graham PL, Moran JL. Robust meta-analytic conclusions mandate the provision of prediction intervals in meta-analysis summaries. *J Clin Epidemiol* 2012; 65: 503-10.

42 Ferrie S, Rand S, Palmer S. Back to basics: estimating protein requirements for adult hospital patients. A systematic review of randomised controlled trials. *Food Nutr Sci* 2013; 4: 201-14.

43 Scheinkestel CD, Kar L, Marshall K, et al. Prospective randomized trial to assess caloric and protein needs of critically Ill, anuric, ventilated patients requiring continuous renal replacement therapy. *Nutrition* 2003; 19: 909-16.

44 Mesejo A, Acosta JA, Ortega C, et al. Comparison of a high-protein disease-specific enteral formula with a high-protein enteral formula in hyperglycemic critically ill patients. *Clin Nutr* 2003; 22: 295-305.

45 Flather MD, Farkouh ME, Pogue JM, Yusuf S. Strengths and limitations of meta-analysis: larger studies may be more reliable. *Control Clin Trials* 1997; 18: 568-79.

46 Harvey SE, Parrott F, Harrison DA, et al. Trial of the route of early nutritional support in critically ill adults. *N Engl J Med* 2014; 371: 1673-1684.

47 Heyland DK, Dhaliwal R, Wang M, Day AG. The prevalence of iatrogenic underfeeding in the nutritionally "at-risk" critically ill patient: results of an international, multicenter, prospective study. *Clin Nutr* 2015; 34: 659-66.

48 McClave SA, Martindale RG, Rice TW, Heyland DK. Feeding the critically ill patient. *Crit Care Med* 2014; 42: 2600-10.

49 Weijs PJ, Wischmeyer PE. Optimizing energy and protein balance in the ICU. *Curr Opin Clin Nutr Metab Care* 2013; 16: 194-201.

50 Parikh HG, Miller A, Chapman M, et al. Calorie delivery and clinical outcomes in the critically ill: a systematic review and meta-analysis. *Crit Care Resusc* 2016; 18: 17-24. ❑