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Original article

Timing of PROTein INtake and clinical outcomes of adult critically ill patients on prolonged mechanical VENTilation: The PROTINVENT retrospective study

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SUMMARY

Background & aims: Optimal protein intake during critical illness is unknown. Conflicting results on nutritional support during the first week of ICU stay have been published. We addressed timing of protein intake and outcomes in ICU patients requiring prolonged mechanical ventilation.

Methods: We retrospectively collected nutritional and clinical data on the first 7 days of ICU admission of adult critically ill patients, who were mechanically ventilated in our ICU for at least 7 days and admitted between January 1st 2011 and December 31st 2015. Based on recent literature, patients were divided into 3 protein intake categories, <0.8 g/kg/day, 0.8–1.2 g/kg/day and >1.2 g/kg/day. Our primary aim was to identify the optimum protein dose and timing related to the lowest 6 month mortality. Secondary endpoints were ventilation duration, need for renal replacement therapy (RRT), ICU length of stay (LOS) and mortality and hospital LOS and mortality.

Results: In total 455 patients met the inclusion criteria. We found a time-dependent association of protein intake and mortality; low protein intake (<0.8 g/kg/day) before day 3 and high protein intake (>0.8 g/kg/day) after day 3 was associated with lower 6-month mortality, adjusted HR 0.609; 95% CI 0.480–0.772, $p < 0.001$) compared to patients with overall high protein intake. Lowest 6-month mortality was found when increasing protein intake from <0.8 g/kg/day on day 1–2 to 0.8–1.2 g/kg/day on day 3–5 and >1.2 g/kg/day after day 5. Moreover, overall low protein intake was associated with the highest ICU, in-hospital and 6-month mortality. No differences in ICU LOS, need for RRT or ventilation duration were found.

Conclusions: Our data suggest that although overall low protein intake is associated with the highest mortality risk, high protein intake during the first 3–5 days of ICU stay is also associated with increased long-term mortality. Therefore, timing of high protein intake may be relevant for optimizing ICU, in-hospital and long-term mortality outcomes.

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1. Introduction

Nutritional support during critical illness is heavily debated [1]. Many studies have evaluated effects of nutritional support on clinical outcomes in ICU. Most studies have focused on energy provision [2–4], however there is growing evidence that protein intake may be more important than caloric intake [5–7]. As fixed protein to energy ratios in most feeding regimens are used, it is complex to separate effects of protein intake from those of energy intake. Furthermore, in several studies both energy and protein intake were similarly associated with clinical outcomes in univariate analyses [8]. Other studies showed that high protein intake was associated with reduced mortality risk [9], whereas energy overfeeding was associated with increased mortality risk [10].Lower mortality and more ventilator free days were reported in patients with sepsis or severe pneumonia reaching higher protein and

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caloric intake in the early phase of ICU stay [11]. This might even be more relevant for patients with Body Mass Index (BMI)<25 or >35 kg*m^{-2} [12]. Recently, a retrospective analysis of energy provision during the first week of ICU stay in 475 patients with prolonged mechanical ventilation showed beneficial effects from early full energy feeding on mortality and quality of life 3 months post ICU discharge [13]. In this study protein intake was not studied separately. From the non-nutritional calories (*e.g.* dextrose, citrate and propofol infusions) only propofol infusions were taken into account, although non-nutritional calories may contribute for up to 20% of total caloric intake in individual patients [14]. Moreover, in that study cumulative caloric intake over one week was studied and daily effects of intake were not assessed.

Casaer and co-workers, based on a post hoc analysis of the EPANIC randomized trial, suggested a time-dependent association of protein intake and clinical outcome, with possible harmful effects of protein intake during the first 3 days of ICU admission [15].

In order to achieve a personalized nutritional approach several questions need to be answered [16,17]. Therefore, we addressed how protein intake during the first week of ICU admission influences clinical outcomes among prolonged mechanically ventilated critically ill patients.

Our primary aim was to determine the best timing and dose of protein intake to support the lowest 6-month mortality. Secondary outcome measures were the effect of timing and dose of protein intake on ICU and hospital length of stay (LOS), ICU and hospital mortality, ventilation duration and need for renal replacement therapy (RRT).

2. Materials and methods

For this single center cohort study we retrospectively collected data from patients fulfilling inclusion criteria, who were admitted to our ICU between January 1st 2011 and December 31st 2015. Inclusion criteria were: adult critically ill patients (≥18 years), requiring invasive mechanical ventilation for a minimum duration of 7 days. Patients were excluded if the time from admission to start of mechanical ventilation exceeded 48 h, if data on nutritional needs were incomplete, in case of contraindications to full nutrition, if their condition influenced their nutritional needs in a way that we were unable to estimate or compare results with other patients, such as pregnancy, preexistent neuromuscular diseases, known protein malabsorption or metabolic abnormalities. In patients with multiple ICU admissions during the study period, to avert bias we excluded data from ICU readmissions. An ICU admission was considered a readmission when the patient was admitted within 6 months of the primary ICU admission.

2.1. Ethical approval

The institutional review board of Gelderse Vallei Hospital approved the study and waived informed consent for reasons of the retrospective design and anonymization of patient identifiers before analysis.

2.2. Data collection

Data extraction was performed using SAS Enterprise Guide queries (version 7.12HF1), from our MetaVision (Patient Data Management System, iMDsoft, Tel Aviv, Israel) database and other hospital electronic patient records. Baseline characteristics were listed; age, gender, primary admission diagnosis, baseline APACHE-II and SOFA-scores, several baseline blood tests, admission type (medical, elective and non-elective surgery), comorbidities, modified Nutrition Risk in Critically ill (mNUTRIC) score [18] and administered non-nutritional calories (dextrose infusion, propofol and trisodium citrate) [14].

Data to calculate the Charlson Comorbidity Index (CCI) [19] were obtained from the quality management system for hospital mortality registration. All deaths in the Netherlands are registered in the municipal personal records database of the Dutch government. As our electronic patient management system is directly connected to this database date of death could be extracted. When date of death was not registered the patient was presumed alive. Days were defined as calendar days.

2.3. Nutritional parameters

We collected data on nutritional intake for the first 7 days of ICU admission, including protein and energy targets, actual given doses of proteins (g) and calories (kcal) from enteral (EN) and parenteral nutrition (PN). Additionally non-nutritional calories from trisodium citrate, glucose and propofol infusions were calculated and added to calculate total caloric intake [14]. We divided total caloric intake into adequacy categories based on recent literature [6,10] (3 groups: hypocaloric: <80% of energy target, normocaloric: 80–110% of energy target and hypercaloric: more than 110% of energy target).

2.4. Calculation of nutritional goals

In all patients body weight and height were measured on ICU admission. The World Health Organization/Food and Agricultural Organization of the United Nations (WHO/FAO) formulas were used to calculate caloric and protein targets by our computerized feeding protocol [14]. According to BMI, the actual, corrected (weight on BMI 27) or ideal body weight (women weight on BMI 21, men weight on BMI 22.5) was used. An addition to resting energy expenditure (REE) of 20% was used to correct for disease activity.

Our target protein intake was 1.5 g per kilogram bodyweight per day (g*kg^{-1}*day^{-1}) for patients with BMIs up to 27 kg*m^{-2}. In case of BMI 27–30 kg*m^{-2}, weight was corrected to BMI 27 kg*m^{-2}. In case of BMI >30 kg*m^{-2} we used ideal body weight and protein administration was set to 2.0 g*kg^{-1}*day^{-1}, whereas patients with a BMI >40 kg*m^{-2} prescription was 2.5 g per kg ideal weight per day according to international guidelines [20].

2.5. Protein categories

We used protein targets in grams per kilogram uncorrected body weight on ICU admission to divide patients into categories according to their mean protein intake during the first week of ICU admission. The chosen cut-off values are based on recent literature [10]; protein intake less than 0.8 g*kg^{-1}*day^{-1}, 0.8 g*kg^{-1}*day^{-1} to 1.2 g*kg^{-1}*day^{-1} and more than 1.2 g*kg^{-1}*day^{-1}.

2.6. Study end points

Our primary endpoint was the association of 7-days protein intake and 6-months survival. We considered this to be the most appropriate time window, because the effects of protein provision may not be expected within a short timeframe and previous studies on critical care nutrition feeding interventions show effects on long-term but not early mortality endpoints [21]. Moreover, long-term outcomes are clinically very important for patient prognosis and recovery. Secondary endpoints included ICU and in hospital mortality, ICU and hospital LOS, ventilation duration, need for and duration of RRT and all cause hospital readmission within 6 months from ICU admission.

2.7. Data analysis

Descriptive data are reported as means and standard deviation (SD) or median and interquartile range (IQR) in case of skewed distributions, or as frequencies and percentages or ranges (minimum–maximum).

2.8. Statistical analysis

Baseline characteristic differences and secondary endpoints were assessed with Chi square tests or Fisher's exact tests and ANOVA or Kruskal–Wallis tests where appropriate. Six-month survival was assessed by Kaplan Meier survival estimate curves and Cox Proportional Hazards Models. A P-value <0.05 was considered statistically significant. For univariate analysis all variables considered to be relevant based on literature were included. For the primary outcome measure, when univariate analysis revealed $p < 0.10$ multivariate analysis was performed. Multicollinearity of variables included into multivariate analyses was assessed by calculation of the variance inflation factor (VIF), we considered a VIF above 2 as an indicator of relevant collinearity. IBM SPSS Statistics for Windows, version 24.0 (IBM Corporation, released 2014, Armonk, New York, USA) was used to perform analyses.

3. Results

3.1. Patients

During the study period 2237 patients were admitted to our ICU, of which 546 were considered eligible for inclusion. We excluded 91 patients; reasons were delayed intubation (N = 59), ICU admission within the six months previous to the selected admission (N = 25) and insufficient data on nutritional intake due to participation in a blinded tube feeds study (N = 7, Fig. 1). In total, 455 individual patients were enrolled in our study, of which four were enrolled twice.

Baseline characteristics and feeding parameters are shown in Tables 1 and 2. Significant differences were observed between the 3 protein intake subgroups for BMI, SOFA-score, admission type, hours to start feeding, route of feeding, daily protein target, total protein and caloric intake, adequacy of protein and caloric intake and percentage of non-nutritional calories.

3.2. Primary outcome

The 6-months survival was 65.6%, 68.9% and 55.6% in the low (0.8 $g*kg^{-1}*day^{-1}$), intermediate (0.8–1.2 $g*kg^{-1}*day^{-1}$) and high

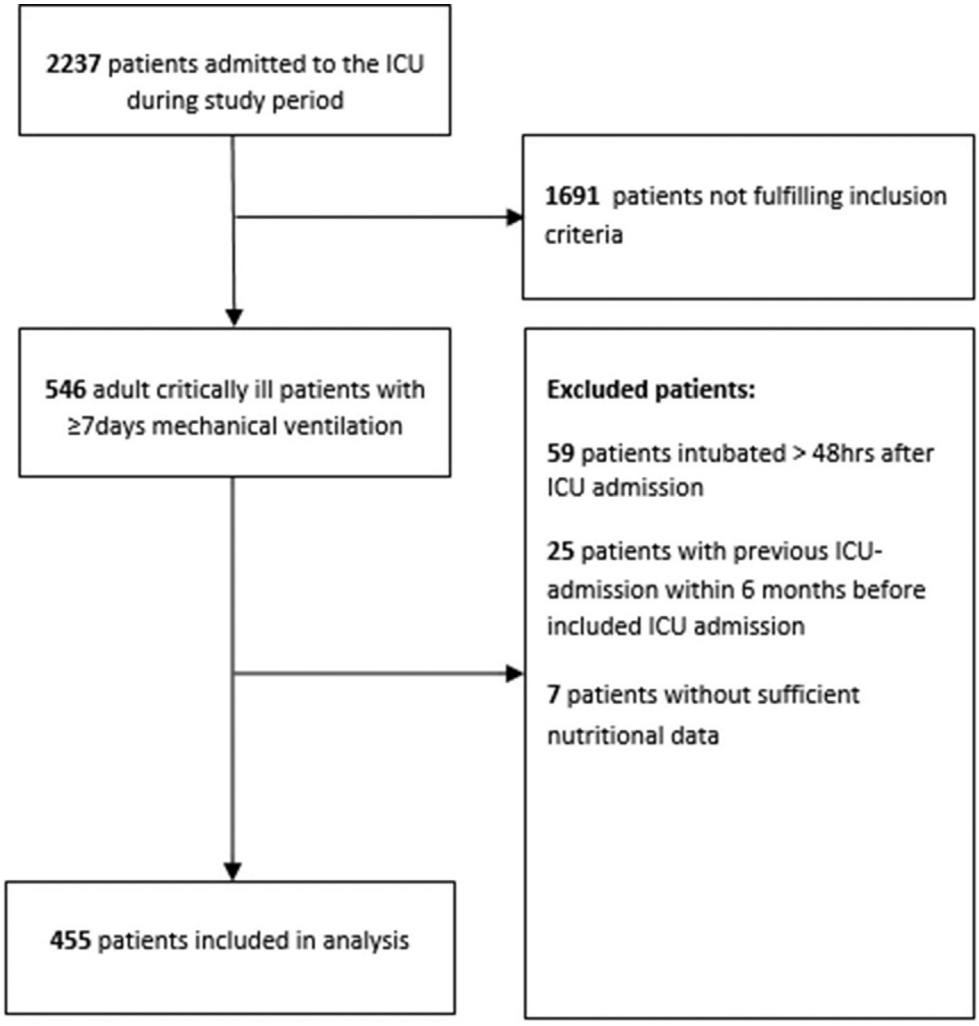

Fig. 1. Flow chart of the study population.

Table 1
Baseline characteristics.

	Total population	Protein intake categories			p-value[a]
Protein intake in g*kg^{-1}*day^{-1}		LOW	INTERMEDIATE	HIGH	
		<0.8	0.8–1.2	>1.2	
N (%)	455 (100)	128 (28.1)	264 (58.0)	63 (13.8)	
Females N (%)	170 (37.4)	47 (36.7)	98 (37.1)	25 (39.7)	0.933
Age, median [IQR]	70 [61–77]	68 [60–77]	70 [61–76]	70 [61–79]	0.633
BMI,kg*m^{-2}, median [IQR]	26.4 [23.5–30.0]	28.4 [24.7–32.9]	26.2 [23.6–29.4]	24.6 [21.4–26.6]	<0.001
BMI categories, N (%)					<0.001
<18.5	16 (3.5)	4 (3.1)	10 (3.8)	2 (3.2)	
18.5–25	157 (34.5)	31 (24.2)	93 (35.2)	33 (52.4)	
25–35	234 (51.4)	68 (53.1)	140 (53.0)	26 (41.3)	
>35	48 (10.5)	25 (19.5)	21 (8.0)	2 (3.2)	
ICU admission year, N (%)					0.818
2011	105 (23.1)	35 (27.3)	59 (22.3)	11 (17.5)	
2012	84 (18.5)	20 (15.6)	51 (19.3)	13 (20.6)	
2013	81 (17.8)	21 (16.4)	48 (18.2)	12 (19.0)	
2014	91 (20.0)	25 (19.5)	50 (18.9)	16 (25.4)	
2015	94 (20.7)	27 (21.1)	56 (21.2)	11 (17.5)	
APACHE II score, median [IQR] n = 433	22 [18–28]	24 [19–29]	22 [18–27.5]	23 [18–28.5]	0.167
SOFA score, median [IQR] N = 435	8.0 [6–10]	8.0 [6–11]	8.0 [6–9]	7.0 [5–9.75]	0.050
CCI, median [IQR]	4.0 [2–5]	4.0 [2–6]	4.0 [3–5]	4.0 [2–6]	0.985
mNUTRIC score, median [IQR]	5 [4–6]	5 [4–6]	5 [4–6]	5 [3–6]	0.648
mNUTRIC risk group					0.524
Low (<5), N (%)	183 (40.2)	46 (35.9)	111 (42.0)	26 (41.3)	
High (5–9), N (%)	272 (59.8)	82 (64.1)	153 (58)	37 (58.7)	
Admission categories, N (%)					
Surgical emergency	90 (19.8)	35 (27.3)	47 (17.8)	8 (12.7)	0.032
Surgical	63 (13.8)	17 (13.3)	41 (15.5)	5 (7.9)	
Medical	302 (66.4)	76 (59.4)	176 (66.7)	50 (79.4)	

N = number of patients, g*kg^{-1}*day^{-1} = gram per kilogram uncorrected bodyweight per day, BMI = body mass index, APACHE II score = Acute Physiologic and Chronic Health Evaluation II score, SOFA score = sequential organ failure assessment score, CCI = Charlson Comorbidity Index, mNUTRIC score = modified Nutrition Risk in Critically Ill score, IQR = interquartile range (1st–3th quartile), percentiles by Tukey's Hinges distributions.
[a] Calculated by Pearson's Chi square or Fishers exact test, Anova or Kruskal Wallis test as appropriate.

(>1.2 g*kg^{-1}*day^{-1}) protein intake groups, respectively. Univariate analysis showed a significant survival benefit of the intermediate protein intake category compared with the high protein intake category (p = 0.043). However, this significance was lost in Cox regression multivariate analysis (p = 0.209).

3.3. Time dependent effect of protein intake

We subsequently analysed the early (days 1–3) and late phase (days 4–7) of ICU admission separately (Table 3). Protein intake was classified for mean daily protein intake during early and late phase. Low protein intake during days 1–3 was associated with a statistically significant reduction in 6-month mortality, whereas higher protein intake during days 4–7 was associated with better outcome by unadjusted Cox proportional hazard regression (Table 3). For days 1–3 a Hazard Ratio (HR) of 1.231 (95% CI: 1.040–1.457; p = 0.016) in the >0.8 g*kg^{-1}*day^{-1} group compared to the <0.8 g*kg^{-1}*day^{-1} group was found. Low protein intake <0.8 g*kg^{-1}*day^{-1} during days 4–7 has a HR of 1.605 (95% CI 1.118–2.186; p = 0.003) compared to the high protein intake group. The lowest HR was found in the group with intermediate protein intake during days 4–7 (HR 0.716 95% CI 0.558–0.917; p = 0.008). Further validation of these results was done by assessing days 1–2, showing similar association of low protein intake and 6-month survival. When considering days 1–4, no difference between the low and high intake group was observed (data not shown).

3.4. Time-dependent protein intake subgroups

We subsequently compared patients with protein intakes less than 0.8 g*kg^{-1}*day^{-1} during the whole week (group 1 (g1)), with patients who initially received less than 0.8 g*kg^{-1}*day^{-1} during day 1–3 but advanced to more than 0.8 g*kg^{-1}*day^{-1} (group 2 (g2)) on day 4 and later and patients who had protein intake of more than 0.8 g*kg^{-1}*day^{-1} during the whole week (group 3 (g3)). A significant difference in 6-month survival was observed between g1 and g2 (p = 0.005) and g2 and g3 (p = 0.004) in univariate analysis (Fig. 2). In multivariate analysis the significance between g1 and g2 was lost. However, the survival benefit was confirmed between g2 and g3, HR 0.609 (95% 0.480–0.772; p < 0.001). Moreover, a significant difference was observed between and g1 and g3 in multivariate analysis, HR 1.495 (95% CI 1.020–2.190; p 0.039).

3.5. Time-dependent optimal protein intake

Furthermore, we analysed the 6-month mortality risk of low, intermediate and high protein intake of each ICU admission day separately for the first week of admission in order to find daily optimum protein intake. On day 1–2 the lowest mortality was found with low protein intake, day 3 and 5 for intermediate protein intake and day 6 and 7 for high protein intake. When comparing this model to the previous mentioned protein intake categories and groups a survival benefit was shown with a 6-month survival of 76.6% for the group advancing from low, to intermediate to high intake.

3.6. Secondary outcomes

Secondary outcome measures were assessed based on time-dependent subgroups. Statistical significant differences between groups were found in 6-month mortality (g1 48.6%, g2 28.7%, g3 42.7%, p = 0.004) ICU mortality (g1 40.0%, g2 13.5%, g3 22.2%, p = 0.001) and hospital mortality (g1 48.6%, g2 20.8%, g3 33.3%,

Table 2
Feeding parameters.

	Total population	Protein intake categories			p-value[a]
Protein intake in g*kg^{-1}*day^{-1}		LOW	INTERMEDIATE	HIGH	
		<0.8	0.8–1.2	>1.2	
Time to start feeding, hours, median [IQR]	5.55 [2.8–14.4]	11.7 [4.4–25.8]	5.1 [2.6–11.8]	3.4 [2.1–6.5]	<0.001
Route of feeding (EN vs PN)					0.035
EN, N (%)	362 (79.7)	92 (71.9)	213 (81.0)	57 (90.5)	
PN, N (%)	59 (13.0)	22 (17.2)	32 (12.2)	5 (7.9)	
EN + PN, N (%)	33 (7.3)	14 (10.9)	18 (6.8)	1 (1.6)	
Protein target in g*day^{-1}, median [IQR]	115 [102–128]	121 [111–135]	114 [102–125]	109 [95–117]	<0.001
7-day protein intake, g, median [IQR]	535 [423–624]	391 [317–471]	554 [471–641]	639 [577–737]	<0.001
Protein adequacy days 1–3, %, median [IQR]	51 [31–69]	27 [9–39]	55 [40–69]	83 [69–90]	<0.001
Protein intake (g*kg^{-1}*day^{-1}) days 1–3					<0.001
<0.5, N (%)	182 (40.0)	105 (82.0)	77 (29.2)	0 (0)	
0.5–0.8, N (%)	156 (34.3)	19 (14.8)	127 (48.1)	10 (15.9)	
0.8–1.0, N (%)	82 (18.0)	3 (2.3)	52 (19.7)	27 (42.9)	
1.0–1.2, N (%)	28 (6.2)	1 (0.8)	7 (2.7)	20 (31.7)	
>1.2, N (%)	7 (1.5)	0 (0)	1 (0.4)	6 (9.5)	
Protein adequacy days 4–7, %, median [IQR]	88 [72–99]	66 [53–75]	92 [82–99]	103 [98–111]	<0.001
Protein intake (g*kg^{-1}*day^{-1}) days 4–7					<0.001
<0.5, N (%)	12 (2.6)	12 (9.4)	0 (0)	0 (0)	
0.5–0.8, N (%)	28 (6.2)	27 (21.1)	1 (0.4)	0 (0)	
0.8–1.0, N (%)	62 (13.6)	44 (34.4)	18 (6.8)	0 (0)	
1.0–1.2, N (%)	102 (22.4)	39 (30.5)	62 (23.5)	1 (1.6)	
>1.2. N (%)	251 (55.2)	6 (4.7)	183 (69.3)	62 (98.4)	
Caloric target, kcal*day^{-1}, median [IQR]	1750 [1492–1957]	1687 [1357–1978]	1768 [1523–1955]	1763 [1618–1886]	0.263
7-day caloric intake, kcal, median [IQR]	10,068 [8179–11,485]	7907 [6229–9903]	10,321 [8723–11,522]	11,617 [10,566–12,5322]	<0.001
Caloric adequacy days 1–3, %, median [IQR]	91.4 [78.3–102.5]	46.4 [25.8–70.1]	70.1 [52.6–88.7]	86.3 [76.4–95.9]	<0.001
<80%, N (%)	297 (65.3)	106 (82.8)	168 (63.6)	23 (36.5)	<0.001
80–110%, N (%)	132 (29.0)	19 (14.8)	76 (28.8)	37 (58.7)	
>110%, N (%)	26 (5.7)	3 (2.3)	20 (7.6)	3 (4.8)	
Caloric adequacy days 4–7, %, median [IQR]	103.9 [93.3–116.2]	94.3 [75.7–116.5]	105.2 [96.6–115.4]	108.8 [101.8–116.7]	<0.001
<80%, N (%)	55 (12.1)	40 (31.3)	14 (5.3)	1 (1.6)	<0.001
80–110%, N (%)	241 (53.0)	49 (38.3)	159 (60.2)	33 (52.4)	
>110%, N (%)	159 (34.9)	39 (30.5)	91 (34.5)	29 (46.0)	
Non nutritional to total caloric intake, %, median [IQR]	5.6 [2.1–11]	9.3 [5.6–22.8]	4.6 [1.8–8.8]	3.3 [1.0–8.0]	<0.001

N = number of patients, g*kg^{-1}*day^{-1} = gram per kilogram uncorrected bodyweight per day, IQR = interquartile range (1st–3th quartile), percentiles by Tukey's Hinges distributions, EN = Enteral nutrition, PN = Parenteral nutrition.
[a] Calculated by Pearson's Chi square or Fishers exact test, Anova or Kruskal Wallis test as appropriate.

Table 3
Cox Proportional Hazard Model Analysis: Average protein intake during day 1–3 and day 4–7 and 6-month mortality comparing protein intake categories.

Average protein intake	N	B	Hazard Ratio	95% CI	p-value
Days 1–3					0.019
<0.8 g*kg^{-1}*day^{-1}	338	Reference			
>0.8 g*kg^{-1}*day^{-1}	117	0.208	1.231	1.040–1.457	0.016
Days 4–7					0.008
<0.8 g*kg^{-1}*day^{-1}	40	0.473	1.605	1.178–2.186	0.003
0.8–1.2 g*kg^{-1}*day^{-1}	164	−0.335	0.716	0.558–0.917	0.008
>1.2 g*kg^{-1}*day^{-1}	251	Reference			

N = number of patients included in analysis, B = parameter estimate, 95% CI = 95% confidence interval, g*kg^{-1}*day^{-1} = gram per kilogram uncorrected bodyweight per day.

$p < 0.001$). In addition, 6-months a significant difference was found in 6-months all cause hospital readmission (g1 14.3%, g2 33.3%, g3 24.8%, $p = 0.025$). No significant differences were observed in ventilation duration, need for RRT, ICU readmission within six months, ICU and hospital LOS and discharge destination (Table 4).

4. Discussion

We found a time-dependent association of protein intake and 6-month mortality, suggesting that increasing protein intake from low on day 1–2 (<0.8 g/kg/day) to intermediate on day 3–5 (0.8–1.2 g/kg/day) to high after day 5 (>1.2 g/kg/day) confers the best long-term outcome. The worst long-term outcome was observed with overall low protein intake (<0.8 g/kg/day).

Previous studies on efficacy of protein intake in adult critically ill patients show divergent results. Weijs reported improved outcomes in adult ICU patients with early high protein intake [10]. High intake was defined as >1.2 g*kg^{-1} protein on day 4 of ICU admission. In our study day 4 is part of the late phase in which high protein intake indeed confers benefits for long-term mortality. Therefore we suggest that our findings are not in contrast with these observations.

Our results are in line with the findings of Casaer, who demonstrated comparing early and late PN to supplement EN, that providing higher amounts of protein might lead to inhibition of autophagy, which in turn leads to persisting cell damage and cell dysfunction [15] and worse clinical outcomes. This group suggested that proteins may lead to an autophagy deficient phenotype associated with lower survival rates. Strikingly, this deleterious effect of higher protein intake reached statistical significance only on day 3, not on day 5 and 7. Although this study was performed largely in short-stay surgical critically ill patients, we now show similar findings in prolonged mechanically ventilated ICU patients suggesting an early negative effect in the first three days after ICU admission.

Arabi [2] reported no significant differences in 180 days mortality between early caloric underfeeding and standard caloric feeding when maintaining a similar protein intake in both study arms. These results are not in contrast as the average weight in the studied patients was 80 kg with an average protein intake of 58 g per day suggesting an average intake of 0.725 g*kg^{-1}, below our cutoff value of 0.8 g*kg^{-1} per day.

Although protein turnover and net balance between muscle protein synthesis and break down was subject of investigation for

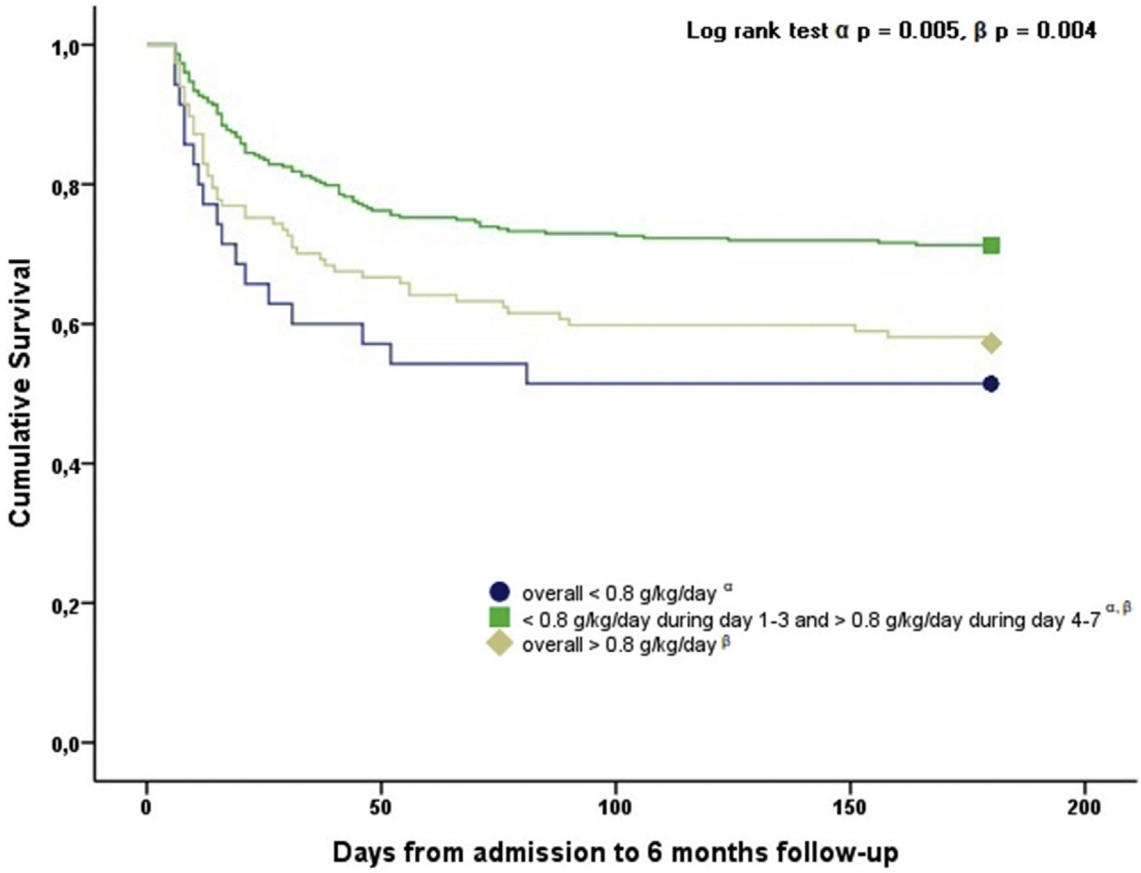

Fig. 2. Six-months survival by Kaplan–Meier estimates for time-dependent protein intake groups.

Table 4
Secondary outcomes for average protein intake during first week comparing time-dependent protein intake groups.

	<0.8 g*kg^{-1}*day^{-1} During first week	<0.8 g*kg^{-1}*day^{-1} During day 1–3 and >0.8 g*kg^{-1}*day^{-1} During day 4–7	>0.8 g*kg^{-1}*day^{-1} During first week	p-value[a]
Patients at risk, N	35	303	117	
6-month mortality, N (%)	17 (48.6)	87 (28.7)	50 (42.7)	0.004
ICU-mortality, N (%)	14 (40.0)	41 (13.5)	26 (22.2)	<0.001
In-hospital mortality, N (%)	17 (48.6)	63 (20.8)	39 (33.3)	<0.001
ICU LOS, days, median [IQR]	16 [11–29]	16 [11–25]	15 [11–24.5]	0.798
Hospital LOS, days, median [IQR]	22 [15–43]	30 [20–44]	26 [17.5–41.5]	0.076
ICU TDA, days, median [IQR] (N = 374)	18 [12.5–30.5]	16 [12–25.25]	16 [12–26]	0.693
Hospital TDA, days, median [IQR] (N = 336)	24.5 [20–45]	32 [22–45]	30.5 [20.75–45.25]	0.741
Ventilation duration, days, median [IQR]	10 [8–21]	11 [8–17]	11 [8–15]	0.583
Need for CVVH, N (%)	11 (31.4)	86 (28.4)	20 (17.1)	0.037
CVVH, days, median [IQR]	9 [6–16]	8 [4–11.25]	6.5 [3.25–10.75]	0.402
6-months all cause hospital readmission, N (%)	5 (14.3)	101 (33.3)	29 (24.8)	0.025

g*kg^{-1}*day^{-1} = gram per kilogram uncorrected bodyweight per day, IQR = interquartile range (1st–3th quartile), percentiles by Tukey's Hinges distributions, N = number of patients, LOS = length of stay, TDA = time to discharge alive = length of stay measure which is not biased by the shorter LOS of in-hospital dead patients, CVVH = continuous venovenous hemofiltration.

[a] Assessed by Fishers' exact test or Kruskal–Wallis test where appropriate.

decades, mechanisms are still poorly understood in critically ill patients. During the initial phase of critical illness, catabolic pathways are activated, causing high protein turnover in order to enhance production of proinflammatory mediators and provide endogenous energy [22,23]. We speculate that in this phase autophagocytic capacity is blunted due to the inflammatory response and this mechanism may be further compromised by external protein administration. In later phases of ICU stay however, proteins and amino acids are strongly needed to provide substrate to synthesize proteins. Moreover during critical illness anabolic thresholds seem to be elevated suggesting that more protein is needed to achieve similar protein synthesis rates.

4.1. Secondary endpoints

We also found an association between time-dependent protein intake and ICU and hospital mortality. Overall low protein intake was associated with the highest ICU and hospital mortality (40.0% and 48.6% respectively). In contrast, no effects on ICU and hospital mortality were found by Casaer [24] who studied early versus late

initiation of parenteral nutrition conferring an early difference in protein and energy intake. It could well be that the benefits of larger late protein intake have been counteracted by the negative effects of early high intake. We found no significant differences related to protein intake and timing with respect to ICU and hospital length of stay. Casaer [24] did find a small beneficial effect of late initiation of PN on ICU and hospital LOS and observed a reduction in ventilation duration and need for RRT, which we could not confirm.

4.2. Strengths and weaknesses

A large number of critically ill patients were included in this study of which an extensive amount of (non)-nutritional variables were available. Only 7 patients were excluded for incomplete data. Due to strict adherence to our feeding protocol, early EN was started shortly after ICU admission (median 5.6 h) and high nutritional adequacy was found, as reported earlier [12]. Therefore evaluation of protein intake in a very early phase was possible (days 1–3). Furthermore, since our patient groups were heterogeneous from the start, we were able to correct for many nutritional (i.e. caloric intake from feeding and non-nutritional calories [14]) and other (i.e. SOFA-score, age, BMI) covariates. The prolonged duration of mechanical ventilation circumvented effects of nutritional intake on outcome in patients with short ICU stay. In patients with early ICU discharge it has been shown that lower intake is associated with better outcome as patients with lower mortality risk are discharged earlier [25,26]. In this study limited protein intake in the first days after ICU is not confounded by early discharge, as all patients were in the ICU for at least 1 week. Another strength is the long follow-up period (6 months).

Limitations of our study are mainly related to its retrospective design potentially introducing bias and residual confounding. Not all information could be collected in retrospect, for instance we lack data on muscle mass and on feeding after day 7 of ICU admission. Also, because of a gradual increase in intake over the first 72 h is specified in our feeding protocol the sample of patients receiving >1.2 g/kg/day of protein was too small for statistical power and could therefore not be analysed separately. Additionally, data are from a single center and inclusion criteria have selected patients with a high severity of illness and prolonged ICU LOS, potentially reducing external validity. Therefore, generalization of study results should be done with caution.

4.3. Implications of the study

Our findings suggest that although overall low protein intake is associated with the worst short- and long-term outcomes it may be beneficial in the first 3 days of ICU admission in adult ICU patients. After day 3 higher protein intake is associated with better outcome. This should change our ideas on aggressive early build-up schedules particularly for protein intake. As early high caloric intake may induce overfeeding, as endogenous production of energy may be marked, we suggest to gradually build-up nutritional support over 5 days to reach 0.8–1.2 g/kg/day of protein on day 3–5 and >1.2 g/kg/day on day 6 and later. Our findings are not contradictory to recent practice guidelines, however suggest that another approach in the early phase would be needed.

4.4. Unanswered questions and future research

Our study shows a time-dependent association of protein intake on outcome. However, prospective research is needed to confirm this. Furthermore, analysis of this effect in specific subgroups may be valuable as differences were shown in earlier studies regarding protein intake (i.e. septic vs non-septic patients) [10,27]. As time-dependence of protein intake may be caused by autophagy interacting with critical illness and the immune response, outcomes may be different when studying less severely ill patients. New research should focus on underlying (patho)physiological mechanisms causing time-dependent effects of protein intake.

5. Conclusions

A time-dependent effect of protein intake in critically ill patients is observed. A gradual increase from low protein intake during the first 2 days of ICU stay to intermediate on day 3–5 and high protein intake from day 6 is associated with lower 6-month mortality. In addition, overall low protein intake is associated with the highest 6-month, ICU and hospital mortality and should be avoided.

Statement of authorship

Dr Van Zanten had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and designing: Van Setten, Van Zanten.

Acquisition of data: Van Setten, Olthof, Kars, Van Zanten.

Statistical analysis and interpretation of data: Van Setten, Van Zanten.

Drafting the manuscript: Van Setten, Koekkoek, Van Zanten.

Critical revision of the manuscript for important intellectual content: Van Setten, Koekkoek, Van Zanten.

Administrative, technical or material support: Van Setten, Olthof, Kars.

Study supervision: Van Setten, Van Zanten.

Final approval of the version submitted: Van Setten, Koekkoek, Olthof, Kars, van Zanten.

Conflict of interest

Arthur van Zanten reported that he has received honoraria for advisory board meetings, lectures, and travel expenses from Abbott, Baxter, BBraun, Danone-Nutricia, Fresenius Kabi, Lyric and Nestle -Novartis. Inclusion fees for patients in nutrition trials were paid to the local ICU research foundation. The remaining authors have disclosed that they do not have any conflicts of interest.

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