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Medical nutrition therapy in long-term adult intensive care patients: effects of protein quantity on muscle mass, biochemical markers, and clinical outcome

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Publications

Scientific manuscripts

Dresen E, Weißbrich C, Fimmers R, Putensen C, Stehle P. Medical high-protein nutrition and loss of muscle mass in adult ICU patients: a randomized controlled trial. Clin Nutr. 2021; 40 (4): 1562–70. doi: 10.1016/j.clnu.2021.02.021.[1]

Dresen E, Weißbrich C, Bühlmeier J, Weisheit C, Egert S, Putensen C, Stehle P. Retrospektive Evaluation der Umsetzung einer klinikinternen Handlungsanweisung zur Ernährungstherapie von Intensivpatienten. Aktuel Ernahrungsmed. 2019; 44 (04): 237-47. doi: 10.1055/a-0915-4994.

Oral presentations

Dresen E, Weißbrich C, Fimmers R, Putensen C, Stehle P. Quantitative Proteinzufuhr und Muskelschwund bei kritisch Kranken: Ergebnisse einer randomisierten kontrollierten Interventionsstudie. Proc. Germ. Nutr. Soc. 2021; 27: 19.[1]

Dresen E, Weißbrich C, Fimmers R, Putensen C, Stehle P. Quantitative Protein Intake and Loss of Muscle Mass in Critically Ill Patients: Results of a Randomized Controlled Trial. Clin Nutr ESPEN. 2021; 40: 422. doi: 10.1016/j.clnesp.2020.09.056.[1]

Posters

Dresen E, Weißbrich C, Fimmers R, Putensen C, Stehle P. Ermittlung des Energieumsatzes von Intensivpatienten: Berechnungsverfahren *vs.* Messungen mittels Indirekter Kalorimetrie. Proc. Germ. Nutr. Soc. 2020; 26: 55.[1]

Dresen E, Bühlmeier J, Weisheit C, Egert S, Weißbrich C. Retrospektive Analyse der Ernährungstherapie chirurgischer Intensivpatienten während des stationären Aufenthalts auf der Intensivstation des Universitätsklinikums Bonn. Proc. Germ. Nutr. Soc. 2017; 23: 65.

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[1] Parts of this dissertation have already been published in these publications.

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List of abbreviations

ALB2	Aluminium diboride
ALT/GPT	Aminotransferase/glutamate pyruvate transaminase
ALTP	Alanine aminotransferase with pyridoxal phosphate activation
Akt	Activated protein kinase B
APACHE	Acute physiology and chronic health evaluation
AR	Acidic tail region
ARA	Arachidonic acid
ARDS	Acute respiratory distress syndrome
ASIS	Anterior superior iliac spine
AST/GOT	Aspartate aminotransferase/glutamate oxaloacetate transaminase
ASTPM	Aspartate aminotransferase with pyridoxal phosphate activation
B-Box	B-box domain
BCAA	Branched-chain amino acid
BIA	Bioelectrical impedance analysis
BILT3	Bilirubin total gen. 3
BMI	Body mass index
BMR	Basal metabolic rate
BMR-oBW	Basal metabolic rate calculated using 'optimal' body weight
BMR-PDMS	Basal metabolic rate documented in the patient data management system
BRAHMS PCT	Immunological test for determination of procalcitonin
BW	Body weight
BW-PDMS	Body weight documented in the patient data management system
C3a	Complement factor 3a
C5a	Complement factor 5a
CA2	Calcium gen. 2
CC	Coiled-coin domain
C/EBP β	CCAAT/enhancer-binding protein-beta
CHF	Chronic heart failure
CI	Confidence interval
CIM	Critical illness myopathy
CIP	Critical illness polyneuropathy
CIPMN	Critical illness polyneuromyopathy

CK   Creatine kinase

CKD   Chronic kidney disease

CLIA   Chemiluminescence immunoassay

CNS   Central nervous system

CO_2 Carbon dioxide

COPD   Chronic obstructive pulmonary disease

CREJ2   Creatine jaffé gen. 2

CRP   C-reactive protein

CRPL3   C-reactive protein gen. 3

CRRT   Continuous renal replacement therapy

CSA   Cross-sectional area

CT   Computed tomography

DEXA   Dual energy X-ray absorptiometry

DGEM   German Society for Nutritional Medicine

DHA   Docosahexaenoic acid

DRKS   German Clinical Trials Register

E %   Energy percent

E1   Ubiquitin-activating enzyme

E2   Ubiquitin-conjugating enzyme

E3   Ubiquitin-protein ligase

EAA   Essential amino acid

ECLIA   Electrochemiluminescence immunoassay

ECMO   Extracorporeal membrane oxygenation

EE   Energy expenditure

eIF2B   Eucaryotic initiation factor 2 subunit B

EPA   Eicosapentaenoic acid

ESPEN   European Society for Clinical Nutrition and Metabolism

f   Female

F-Box   F-box domain

FFA   Free fatty acid

FiO_2 Inspiratory oxygen fraction

FOXO1   Forkhead transcription factor-1

FOXO3a   Forkhead transcription factor-3a

G   Giga

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GDF-15	Growth differentiation factor-15
GFR	Glomerular filtration rate
GRV	Gastric residual volume
GSK-3ß	Glycogen synthase kinase-3-beta
HMB	Beta-hydroxy-beta-methylbutyrate
IC	Indirect calorimetry
ICM	Integrated care manager
ICU	Intensive care unit
ICUAW	Intensive care unit acquired weakness
IFN-γ	Interferon-gamma
IGF-1	Insulin-like growth factor 1
IL-1	Interleukin-1
IL-6	Interleukin-6
IL-10	Interleukin-10
IVNAA	In-vivo neutron activation analysis
KAI	Department of Anesthesiology and Intensive Care Medicine
KLF-15	Krupple-like factor-15
LA	Linoleic acid
LCD	Leucine-charged residue-rich domain
LC-MS/MS	Liquid chromatography combined with mass spectrometry
LOS	Length of stay
LRR	Leucine-rich repeat domain
LZ	Leucine-zipper domain
m	Male
MCT	Medium-chain triglycerides
MAFbx	Muscle atrophy F-box
MFC	Muscle really interesting new gene finger 1 family conserved domain
MOF	Multi-organ failure
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRC	Medical Research Council
MRI	Magnetic resonance imaging
mTORC1	Mechanistic target of rapamycin in complex 1

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MUFA	Monounsatturated fatty acid
MuRF1	Muscle really interesting new gene finger 1
N/A	Not applicable
NaOH	Natriumhydroxide
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B cells
NLS	Nuclear localization domain
oBW	'Optimal' body weight
OR	Odds ratio
PaO_2	Partial pressure of oxygen
PC-APRV	Pressure control-airway pressure release ventilation
PC-BIPAP	Pressure control-biphasic positive airway pressure
PCI	Percutaneous coronary intervention
PCT	Procalcitonin
PDMS	Patient data management system
PDZ	Postsynaptic density protein, drosophila disc large tumor suppressor, zonula occludens-1 protein
PEEP	Positive end expiratory pressure
P_{high}	Upper pressure level
PUFA	Polyunsatturated fatty acid
QMLT	Quadriceps muscle layer thickness
RAG	Recombination activating gene
Rbx1	Really interesting new gene finger protein
RCT	Randomized controlled trial
REE	Resting energy expenditure
REE-IC	Resting energy expenditure measured by indirect calorimetry
REML	Restricted maximum likelihood
RING	Really interesting new gene
RQ	Respiratory quotient
S	Substrate
SAPS	Simplified acute physiology score
SCF	S-phase kinase-associated protein-cullin-F-box protein
SD	Standard deviation
SE	Standard error
SIRS	Systemic inflammatory response syndrome

Skp1	S-phase kinase-associated protein 1
SMA	Skeletal muscle area
Smad 3	Small mothers against decapentaplegic 3 gene
SMOF	Mixture of soybean oil, medium-chain triglycerides, olive oil, and fish oil
SOFA	Sequential organ failure assessment
SOP	Standard operating procedure
SPN	Supplemental parenteral nutrition
SPN-CPAP/PS	Spontaneous-continuous positive airway pressure/pressure support
SPSS	Statistical package for social sciences
TBP	Total body protein
TEE	Total energy expenditure
TEE-oBW	Total energy expenditure calculated using 'optimal' body weight
TEE-PDMS	Total energy expenditure documented in the patient data management system
TGF-ß	Transforming growth factor-beta
TIA	Turbidimetric immunoassay
TISS	Therapeutic intervention scoring system
TNF-α	Tumor necrosis factor-alpha
TP2	Total protein gen. 2
TPN	Total parenteral nutrition
TPUC3	Total protein urine/cerebrospinal fluid gen. 3
TRIGL	Triglycerides
TSC 1/2	Tuberous sclerosis proteins 1 and 2
U	Unit
Ubc	Ubiquitin-carrier protein
UKB	University Hospital of Bonn
UPP	Ubiquitin-proteasome pathway
UREAL	Test for quantitative determination of urea/nitrogen in human serum, plasma, and urine
VCO_2	Carbon dioxide production
VIS	Visible
VO_2	Oxygen consumption

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VT	Tidal volume
WD	Tryptophan-aspartic acid protein domain
y	Years
1.25(OH)$_2$D	1.25-dihydroxyvitamin D3
25-OHD	25-Hydroxyvitamin D3

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1 Introduction

Critical illness is characterized by substantial hormone- and cytokine-mediated changes in protein metabolism in various organs leading to both increased breakdown and decreased synthesis rates (Cuthbertson and Zagreb 1979). Consequently, a considerable and life-threatening loss of muscle mass occurs (Puthucheary et al. 2013; Duan et al. 2020). Acute medical therapeutic measures, such as long-term sedation and mechanical ventilation, as well as the lack of physical activity during an intensive care unit (ICU) stay, can further accelerate this muscle degradation (up to 2 % muscle mass per day). This may lead to the so-called ICU-acquired weakness (ICUAW) (Puthucheary et al. 2013; Friedrich et al. 2018; Arabi et al. 2020), a clinical symptom that is classified as a secondary disorder (Wandrag et al 2019a; Vanhorebeek et al. 2020). If this symptomatology is left untreated, the patient's clinical outcome may be negatively affected in the long term (Brook et al. 2017; Friedrich et al. 2018).

In addition to targeted medication and exercise, research suggests that a quantitatively higher protein intake than recommended for healthy adults (0.8–1.0 g/kg body weight [BW]/d) (Richter et al. 2019) may be useful during critical illness to meet disease-specific increased nitrogen/amino acid requirements and, thus, help mitigate the pronounced loss of muscle and functional proteins (Brook et al. 2017). Based on observational studies (Allingstrup et al. 2012; Elke et al. 2014; Weijs et al. 2014; Nicolo et al. 2016; Compher et al. 2017), the European Society for Clinical Nutrition and Metabolism (ESPEN) (Singer et al. 2018) strongly recommends a daily intake of 1.3 g protein equivalents/kg BW, while the US-American guidelines suggest even higher protein quantities (1.2–2.0 g/kg BW/d) for critically ill patients (McClave et al. 2016). Few randomized controlled trials (RCTs) examined the effects of higher (> 1.2 g/kg BW/d) protein/amino acid administration on patient clinical outcome (morbidity, mortality, length of stay [LOS]) and selected metabolic markers (e.g., nitrogen balance; urine urea, creatinine, and protein; glomerular filtration rate [GFR]), with inconsistent results. The latter might be due to the broad variations in the study designs, e.g., different patient populations, lack of individualized nutrition concepts including adequate energy supply, and different routes of administration (Peake et al. 2014; Doig et al. 2015; Ferrie et al. 2016; Rugeles et al. 2016). In a recent review summarizing the results of RCTs published between 1966 and 2015 comparing different strategies of nutritional therapy with different protein quantity in ICU, no effects on mortality rates could be postulated (Davies et al. 2017). According to the current state of research, no scientifically supported recommendation for the adequate protein supply in critically ill patients can be established at present (Arabi et al. 2020).

2 Scientific rationale

2.1 Metabolic changes during critical illness

Stressors, such as infection, intoxication, burn, state of shock, trauma, and sepsis resulting from injuries and surgical procedures, are triggers of the post-aggression syndrome (Preiser et al. 2014). Its episodes follow a homogenous pattern common in all ICU patients, leading to modifications in substrate utilization, haemodynamic, and immune response, whereby the intensity and duration vary individually (Cuthbertson and Zagreb 1979).

In the early phase of critical illness (shock/ebb phase), a specific reaction of the central nervous system (CNS) characterized by the activation of the sympathetic nervous system and the hypothalamic-pituitary axis is triggered. Subsequently, anti-insulinary hormones are released, e.g., adrenaline, noradrenaline, and cortisol from the adrenal glands, and glucagon from the pancreas. Additionally, the formation of pro-inflammatory cytokines (e.g., interleukin-1 [IL-1], interleukin-6 [IL-6], tumor necrosis factor-alpha [TNF-α]) is triggered by an innate and specific immune response, leading to a so-called systemic inflammatory response syndrome (SIRS) (Cuthbertson and Zagreb 1979; Preiser et al. 2014). These neuroendocrine and immunological pathways influence flow and utilization of nutrients decisively, resulting in a metabolic shift from anabolism to catabolism. Moreover, research suggests that there are distinctive alterations in the secretion of adipokines from the adipose tissue (e.g., leptin, resistin, adiponectin) and specific hormones from the intestinal mucosa (e.g., ghrelin, cholecystokinin, peptid YY) in critically ill patients (Deane et al. 2010; Al-Tarrah et al. 2020).

The neuroendocrine and immunological alterations described above influence the metabolic reaction to disease-related stress, leading to substantial modifications in glucose utilization due to insulin resistance, distinctive peripheral protein catabolism, and increased energy expenditure (Mizock 2001; Wu et al. 2015). This phase of the post-aggression syndrome is called post shock or catabolic flow phase (Cuthbertson and Zagreb 1979). Herein, the overabundance of anti-insulinary hormones (cortisol, glucagon, catecholamines) triggers the upregulation of glycogenolysis, lipolysis, and proteolysis (Barton 1985). Because the glycogen stores are depleted within 24–36 hours, energy is generated via alternative sources, such as amino acids from proteolysis, glycerin and free fatty acids (FFAs) from lipolysis, and ketone bodies from hepatic ketogenesis. Ketone bodies are used by the CNS as alternative substrates to glucose, while FFAs serve as an energy source for peripheral tissues (Patkova et al. 2017). Due to a disturbance of the central insulin sensitivity, there occurs an inadequate suppression

of the glucose production in the liver (Aramendi et al. 2017). Thus, glycerin is transformed to dihydroxyacetone phosphate, thereby maintaining the endogenous formation of glucose via hepatic gluconeogenesis. Additionally, amino acids, especially glucoplastic amino acids, such as glutamine and alanine mainly released by proteolysis of the skeletal muscle proteins, are used as substrates for the gluconeogenesis. Moreover, lactate and pyruvate, generated in hypoxic areas, are re-transformed to glucose via gluconeogenesis in the liver (cori cycle). All the mechanisms described above are generally induced during critical illness and cannot be prevented by exogenous nutrient administration (Cuthbertson and Zagreb 1979; Mizock 2001; Preiser et al. 2014).

Besides upregulation of the endogenous glucose production, a peripheral insulin resistance can be observed. It impairs the insulin-dependent absorption of glucose to the skeletal muscles and adipose tissue (Aramendi et al. 2017). This adaptive mechanism ensures the energy supply to vital organs such as CNS, erythrocytes, and adrenal medulla, which are able to reabsorb glucose without insulin. The interaction of increased endogenous glucose production and peripheral insulin resistance leads to distinctive hyperglycemia (Marik and Bellomo 2013). Increased depletion of endogenous tissue reserves, triggered by adaptive mechanisms, temporarily maintains the supply of substrates such as amino acids, glucose, and fatty acids without exogenous nutrient supply (Cuthbertson and Zagreb 1979). Additionally, due to the increased synthesis of acute phase proteins and specific antibodies combined with the proliferation of immunocompetent cells, the protein catabolism is stimulated as well (Sharma et al. 2019).

After overcoming the post shock/catabolic phase, disease-related hemodynamic, immunological, and metabolic modifications ease, and anabolic mechanisms are induced (Lheureux and Preiser 2017). In the so-called anabolic or recovery phase, the insulin receptors become stimulable again, and the build-up of lean body mass resumes. Thus, energy and substrate resources required during the post-shock/catabolic phase are filled up again. Thereby, reparation mechanisms are enabled supporting the healing and recovery processes (Cuthbertson and Zagreb 1979).

Besides passing the recovery phase, patients often remain in the post shock/catabolic phase of critical illness for an indefinite period. This chronic phase is characterized by persistent organ dysfunction and a catabolic state, resulting in a prolonged need for critical care (Schulman and Mechanick 2012; Elke et al. 2018).

All the described phase-specific changes in substrate utilization, which are generally induced by critical illness, are essential for patient survival and for the healing and recovery processes. While the utilization of endogenous substrates within the disease-mediated metabolic stress response cannot be prevented completely by exogenous substrate administration, the extent of the metabolic changes might be ameliorated by an adequate nutrient supply and, thus, might affect patients' outcome. Therefore, metabolic alterations during critical illness should be treated and monitored carefully within an individual nutrition therapy (Lheureux and Preiser 2017; Sharma et al. 2019).

The metabolic changes described previously during the different phases of critical illness are accompanied by remarkable changes of energy expenditure (EE). The ebb phase is characterized by decreased metabolic actions resulting from anemic and anoxic states, presence and extent of pain, and body heat loss. The extent of temporally reduced EE depends on the severity of illness (Cuthbertson 1942; Sharma et al. 2019). When overcoming the ebb phase and reaching the catabolic flow phase, EE increases and exceeds the normal state. In this post shock phase, hyperemia, exsudative states, emigration of leucocytes, modifications of heart rate, as well as distinct degradation and metabolism of endogenous body reserves occur (Cuthbertson 1942; Cuthbertson and Zagreb 1979; Preiser et al. 2014). Subsequently, EE slowly decreases in the anabolic flow phase. Here, the body becomes stimulable again for exogenous nutrient supply, and mechanisms for recovery from the catabolic state and the loss of body reserves predominate (Cuthbertson 1942; Cuthbertson and Zagreb 1979). These alterations in EE within the different phases of critical illness have to be considered carefully when performing an individual nutrition support therapy in ICU patients (Berger et al. 2019).

2.2 Muscle wasting during critical illness

During critical illness several mechanisms occur that lead to shifts in (muscle) protein balance (increased protein breakdown and concomitant occurrence of decreased protein synthesis), resulting in increased muscle wasting (Puthucheary et al. 2013; Duan et al. 2020). The extent of these impairments in protein metabolism is influenced by several non-communicable (e.g., age, gender, nutritional status prior to ICU admission, severity of illness, occurrence of complications) and communicable factors (e.g., medication, energy and nutrient supply during ICU stay) (Martindale et al. 2017; van Gassel et al. 2020).

Muscle protein degradation

In critically ill patients, protein catabolism is hormonally influenced. Thus, inflammatory cytokines, such as TNF-α, IL-1, IL-6, and interferon-gamma (IFN-γ), induce the activity of proteolytic cascades. In this context, the ubiquitin-proteasome pathway (UPP) is of particular importance (Schwartz and Ciechanover 2009). In this process, the protein ubiquitin binds to other protein structures, marking them for degradation by the proteasome. The binding of ubiquitin to protein structures is mediated by three enzymes: the ubiquitin-activating protein (E1) activates ubiquitin, afterwards ubiquitin is transferred to the target protein structure by the ubiquitin-conjugating protein (E2), and, finally, ubiquitin is conjugated to the target protein structure by an ubiquitin-protein ligase (E3) (Verma and Deshaies 2000; Bloch et al. 2012). Of these latter E3-enzymes, the muscle really interesting new gene (RING) finger 1 (MuRF1) and the muscle atrophy F-box (MAFbx)/atrogin-1 were identified to be upregulated in transcription and expression under catabolic states during critical illness (Gomes et al. 2001; Bodine and Baehr 2014). Several stressors, such as systemic inflammation, cytokine release, oxidative stress, neural inactivity, malnutrition, and administration of glucocorticoids, lead to increased expression of several transcription factors, for example the forkhead transcription factors-1 (FOXO1) and -3a (FOXO3a), the nuclear factor 'kappa-light-chain-enhancer' of activated B cells (NF-κB) transcription factors, the CCAAT/enhancer-binding protein-beta (C/EBP β), and the krupple-like factor-15 (KLF-15). Concomitantly, activation of the glucocorticoid receptor is triggered. Through binding of these transcriptional mediators to the promoter regions of MuRF1 and MAFbx, expression of the E3 enzymes increases, whereby protein degradation by the 26S proteasome is induced. The muscle protein breakdown (MPB) and atrophy are triggered by ubiquitylation and, thereby, targeting proteins for degradation (Bodine and Baehr 2014; Duan et al. 2020). The described mechanisms are shown in **Figure 2-1**.
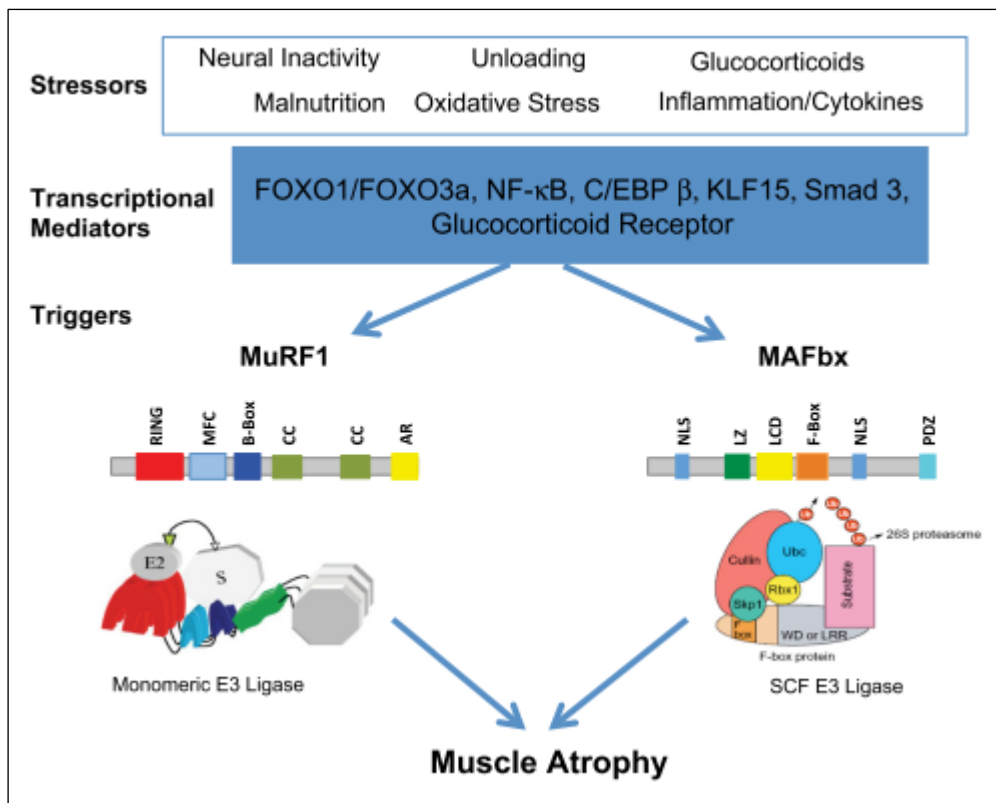
Figure 2-1 Regulation of MuRF1 and MAFbx expression in skeletal muscle (Bodine and Baehr 2014[1]). Skeletal muscle wasting is triggered by different stressors (e.g., neural inactivity, malnutrition, unloading, oxidative stress, inflammatory states, cytokines, and administration of glucocorticoids), which can enhance the expression of specific transcription factors, such as FOXO1/FOXO 3a, NF-κB, C/EBP ß, KLF15, Smad 3, and glucocorticoid receptor. These transcriptional mediators can trigger the expression of MuRF1 and MAFbx by binding to the genes' promotor regions. While MuRF1 comprises the domains RING, MFC, B-Box, CC, and AR, MAFbx includes the domains NLS, LZ, LCD, F-Box, and PDZ. Both MurF1 and MAFbx are E3 ubiquitin ligases (monomeric E3 ligase, SCF ligase), which mediate the binding of ubiquitin and, thus, muscle atrophy is triggered.

Abbreviations: FOXO1/FOXO3a, forkhead transcription factors 1/3a; NF-κB, nuclear factor 'kappa-light-chain-enhancer' of activated B cells; C/EBPβ, CCAAT/enhancer-binding protein-β; KLF-15, krupple-like factor-15; Smad 3, small mothers against decapentaplegic 3 gene; RING, really interesting new gene; MuRF1, muscle really interesting new gene finger 1; MAFbx, muscle atrophy F-box; MFC, muscle really interesting new gene finger 1 family conserved domain; B-Box, B-box domain; CC, coiled-coin domains; AR, acidic tail region; NLS, nuclear localization domain; LZ, leucine-zipper domain; LCD, leucine-charged residue-rich domain; F-Box, F-box domain; PDZ, postsynaptic density protein, drosophila disc large tumor suppressor, zonula occludens-1 protein; SCF, S-phase kinase-associated protein-cullin1-F-box protein; Rbx1, really interesting new gene ring-box 1; Skp1, S-phase kinase-associated protein 1; WD, tryptophan-aspartic acid protein domain; LRR, leucine-rich repeat domain; S, substrate; Ubc, ubiquitin-carrier protein; E2, ubiquitin-conjugating enzyme; E3, ubiquitin-protein ligase.

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[1] This article was published in: American Journal of Physiology-Endocrinology and Metabolism; 2014; 307 (6); Bodine SC, Baehr LM; Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1; p469–484; ©The Author(s) 2014 (reproduced with permission).

Muscle protein synthesis

Research suggests that signaling pathways that initiate the translational process are impaired in ICU patients, leading to notable imbalances in muscle protein metabolism (Martindale et al. 2017). In general, muscle protein synthesis (MPS) is regulated through the protein mechanistic target of rapamycin in complex 1 (mTORC1) and the eukaryotic initiation factor 2 subunit B (eIF2B), triggered by hormonal signals (insulin/insuline-like growth factor 1 [IGF-1]) and amino acids. On the one hand, insulin and IGF-1 activate the protein kinase B (activated protein kinase B [Akt]), after which glycogene synthase kinase-3-beta (GSK-3ß) is phyosphorylated (Song et al. 2005). Thereby, GSK-3ß is inactivated leading to reversed inhibition of eIF2B. In the following, eIF2B mediates the formation of the translation initiation complex and, thus, promotes MPS (Saxton and Sabatini 2017; Bogorad et al. 2018). On the other hand, activation of protein kinase B by insulin and IGF-1 inhibits tuberous sclerosis proteins 1 and 2 (TSC1/2), whereby activity of mTORC1 is triggered indirectly (Bodine et al. 2001; Laplante and Sabatini 2012). Moreover, the activity of mTORC1 is controlled by amino acids via interaction with the recombination activating genes (RAGs). Only in the case of inactive TSC1/2, mTORC1 can be stimulated to promote MPS by amino acids. Like eIF2B, mTORC1 is an important regulator of MPS (Sancak et al. 2010; Martindale et al. 2017). Several conditions, such as sarcopenia, cachexia, immobilization, critical illness, and other diseases (e.g., chronic kidney disease [CKD]), impair the regulation of MPS (Gordon et al. 2013). Generally, these pathophysiological states are associated with inflammation and concomitant development of insulin resistance, inhibiting the activity of Akt (Hu et al. 2009; Slee 2012). In patients with CKD, insulin and IGF-1 signaling is decreased through disease-related release of glucocorticoids. Additionally, pro-inflammatory cytokines stimulate the expression of myostatin, which suppresses the exprimation of IGF-1 itself and the insulin/IGF-1-mediated activation of Akt (Mak and Rotwein 2006). Apart from increasing the expression of myostatin, muscle disuse increases the activity of TSC1/2, leading to inhibition of mTORC1 (Gordon et al. 2013). Moreover, sepsis and sarcopenia cause the inhibition of eIF2B activity (Bertsch et al. 2011). In addition, the described conditions are associated with the development of malnutrition, which is accompanied by decreased plasma amino acid levels. Thereby, stimulation of MPS via RAG/mTORC1 is decreased (Bröer and Bröer 2017). In elderly patients, resistance to the anabolic effects of amino acids occurs. Thus, compared to younger counterparts, higher amino acid concentrations, especially of essential amino acids (EAAs), are needed to stimulate MPS (Rasmussen and Volpi 2012). In summary, all of these conditions reduce MPS by impairing the Akt/mTORC1 signaling pathway and inhibiting the eIF2B

activity (Gordon et al. 2013). The physiological and pathophysiological mechansims that affect the initiation of protein translation decribed above are summarized in **Figure 2-2**.
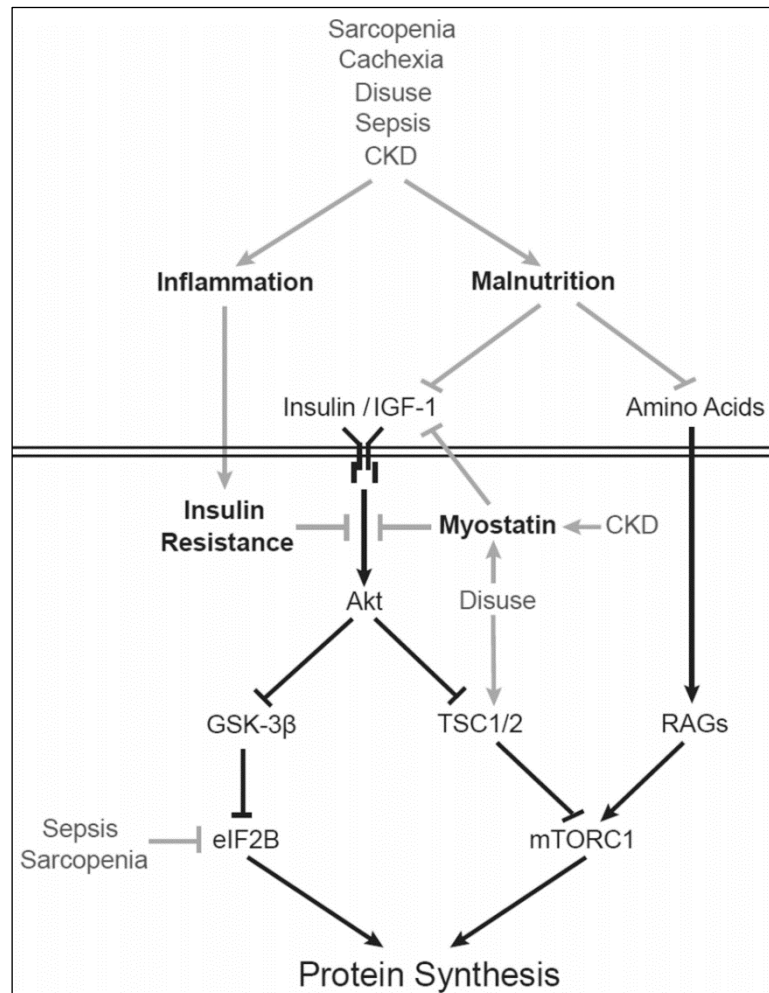
Figure 2-2 Impairment of muscle protein synthesis during several muscle wasting conditions (Gordon et al. 2013[1]). Under physiological states, protein synthesis via the Akt/mTORC1 pathway and induction of eIF2B is triggered by hormones such as insulin and IGF-1. In addition, mTORC1 is activated through RAGs by amino acids (black lines; arrows: stimulation; blunt ends: inhibition). Pathophysiological states, such as sarcopenia, cachexia, disuse, sepsis, and CKD, which lead to inflammation, insulin resistance, and malnutrition, adversely affect these mechanism (grey lines; arrows: stimulation; blunt ends: inhibition) and, thus, protein synthesis is reduced.
Abbreviations: CKD, chronic kidney disease; IGF-1, insulin-like growth factor 1; Akt, activated protein kinase B; GSK-3ß, glycogen synthase kinase-3-beta; TSC 1/2, tuberous sclerosis proteins 1 and 2; RAG, recombination activating gene; eIF2B, eukaryotic initiation factor 2 subunit beta; mTORC1, mechanistic target of rapamycin in complex 1.

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[1] This article was published in: The International Journal of Biochemistry and Cell Biology; 2013; 45 (10); Gordon BS, Kelleher AR, Kimball SR; Regulation of muscle protein synthesis and the effects of catabolic states; p2147–2157; ©Elsevier Ltd. 2013 (reproduced with permission).

Intensive Care Unit Acquired Weakness (ICUAW)

While an increase in MPB with a concomitant decrease in MPS characterizes the disease-related muscle wasting that usually occurs during critical illness, there is an additional risk for the development of what is known as ICUAW. The term describes an onset of muscle weakness acquired during the medical treatment in an ICU that can be diagnosed clinically. This neuromuscular disorder occurs in about 40–50 % of the ICU patients (Lipshutz and Gropper 2013; Yang et al. 2018) and is associated with delayed weaning of mechanical ventilation, prolonged ICU LOS, overall impairment of physical recovery, persisting functional restrictions, as well as higher risk of ICU and in-hospital mortality (Hermans et al. 2014; Peñuelas et al. 2018).

Besides remarkable metabolic and hormonal modifications, several factors of the ICU treatment influence the extent of muscle wasting during critical illness and increase the risk of developing ICUAW (Phillips et al. 2017; Vanhorebeek et al. 2020). **Figure 2-3** illustrates the complexity of interactions between inflammatory response, metabolic and hormonal changes, medical treatment, and disease-related muscle wasting leading to ICUAW following critical illness.
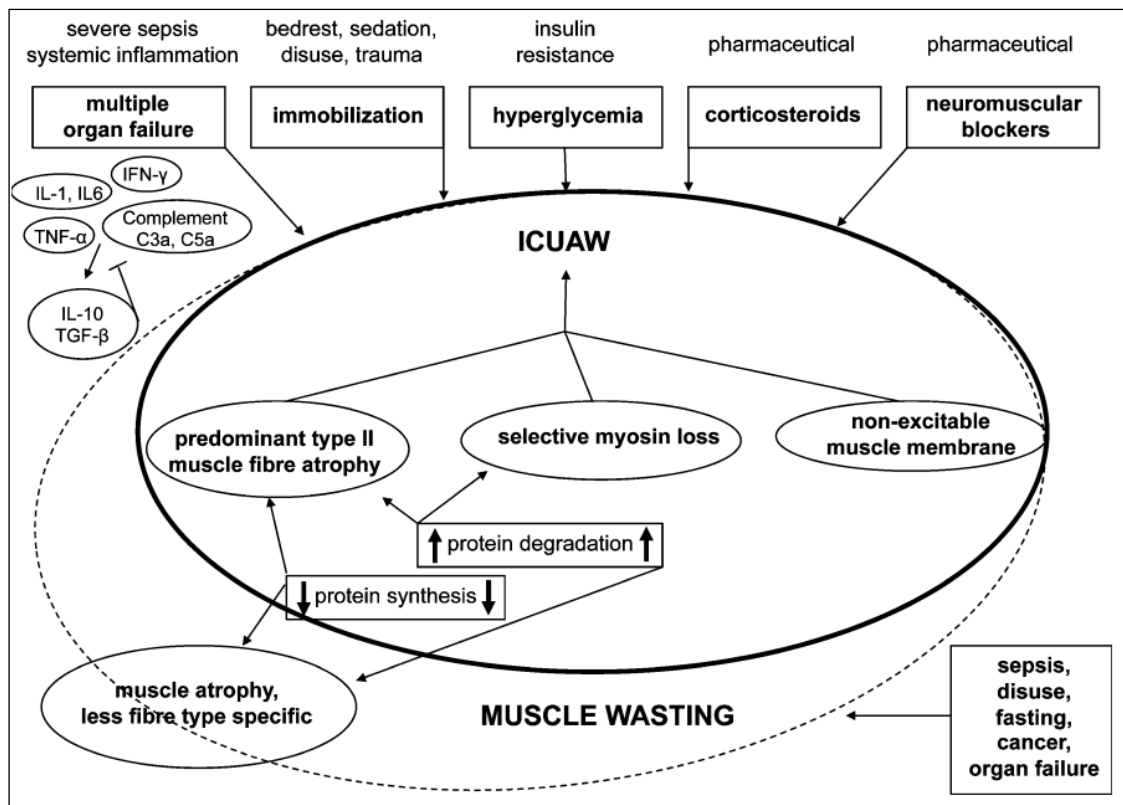
Figure 2-3 Overview of risk factors of disease-related muscle wasting and ICUAW (Schefold et al. 2010[1]). Even though both disease-related muscle wasting and ICUAW are partially overlapping in their risk factors, they describe two different complications resulting from critical illness. While disease-related skeletal muscle wasting always occurs in ICU patients, it does not necessarily need to induce skeletal muscle weakness (ICUAW). In contrast, by diagnosing ICUAW, disease-related skeletal muscle wasting is always preceded.
Abbreviations: IL-1, interleukin-1; IL-6, interleukin-6; IL-10, interleukin-10; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon-gamma; TGF-ß, transforming growth factor-beta; C3a, complement factor 3a; C5a, complement factor 5a; ICUAW, intensive care unit acquired weakness.

On the one hand, disease-related muscle wasting is triggered by sepsis, overall muscle disuse, immobility, fasting states, cancer, and organ failure. As described previously, it is characterized by an increase in MPB and a concomitant decrease in MPS, both leading to generalized muscle atrophy. On the other hand, phases of sepsis and systemic inflammation (e.g., in the case of multi-organ failure [MOF]), overall immobilization (e.g., bedrest, sedation, disuse, trauma), hyperglycemia due to insulin resistance, and medication (e.g., administration of corticosteroids and neuromuscular blocking agents) represent independent risk factors for the development of ICUAW (Schefold et al. 2010). As these factors enhance the imbalance between MPS and

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[1] This article was published in: Journal of cachexia, sarcopenia and muscle; 2010; 1 (2); Schefold JC, Bierbrauer J, Weber-Carstens S; Intensive care unit-acquired weakness (ICUAW) and muscle wasting in critically ill patients with severe sepsis and septic shock; p147–157; ©The Author(s) 2010 (reproduced with permission).

MPB, predominant type II muscle fiber atrophy, selective myosin loss, and non-excitability of muscle membrane are induced. These pathological findings are characteristic signs of ICUAW (Schefold et al. 2010; Hermans and Van den Berghe 2015; Vanhorebeek et al. 2020).

Histological and electrophysiological studies allow classification of ICUAW into critical illness myopathy (CIM), critical illness polyneuropathy (CIP), and critical illness polyneuromyopathy (CIPNM), the latter being a combined clinical picture of the two previously mentioned forms (Shepherd et al. 2017). The term CIM describes an acute and primary muscle disease that results in muscle weakness and paralysis pimarily in the extremity and respiratory muscles. It is characterized by an impaired excitability of muscle tissues (Jolley et al. 2016). The pathophysiological mechanisms of CIM include decreased synthesis and extended usage of myosin, functional impairment of membrane-bound sodium channels and muscular contraction, changes in calcium homeostasis, reduction in membrane potential for chloride, mitochondrial dysfunction, decreased autophagy in muscular cells, aggregation of toxic substances, and impairment in integrity of muscle fibers (Hermans and Van den Berghe 2015; Senger and Erbguth 2017). As a consequence, functional and structural muscle changes occur, leading to overall muscle wasting.

In contrast, CIP is characterized by impairment of motoric and sensory axons due to vascular and cellular events. As a result, there is muscle weakness of the distal extremities, reduction in sensitivity to pain, vibration, and temperature, attenuation of deep tendon reflexes, and flaccid paresis of the upper and lower limbs (Hermans et al. 2008). Due to complex and interacting factors, the pathophysiological mechanisms of CIP have not been fully elucidated. Nevertheless, research suggests that cytokines like IL-1 and TNF-α might lead to increased release of E-selectin in the endothelium. As a marker for cell activation, on the one hand, E-selectin induces the activation of leukocytes in the endoneural space and, on the other hand, it triggers a local production of cytokines. Thus, transfer of neurotoxic substances into the endoneurium is facilitated by the increase in cytokine production and its effects on the vascular permeability. Subsequently, redirection of action potentials is reduced leading to functional impairment of neurons, endoneural edema formation, energy deficiency, hypoxemia, damages of nerve structures, and muscle atrophy (Senger and Erbguth 2017; Shepherd et al. 2017).

In both CIP and CIM, release of proinflammatory cytokines following sepsis further influences muscle wasting by triggering proteolytic cascades of UPP (see above). As the pathogenesis of CIP and CIM is multifactorial and very complex, in ICU patients, both forms often occur in combination as CIPNM (Kress and Hall 2014).

Generally, diagnosis of ICUAW, also differentiated from other neurologic diseases, is of special importance in the clinical setting. It should be performed early after patients' ICU admission to prevent associated adverse outcomes such as prolonged weaning from mechanical ventilation, overall impairment of rehabilitation, as well as increased risk of mortality and morbidity (Siao et al. 2020). In clinical practice, several techniques can be used to examine muscle function and structure to diagnose ICUAW. These include volitional functional testing, electrophysiology, biopsies, and imaging techniques (Vanhorebeek et al. 2020).

Volitional functional testing comprises several methods for bedside testing of muscle strength and to obtain functional capacity, such as the Medical Research Counsil (MRC) sum score, handheld dynamometers to measure handgrip strength, and 6-minute walking distance. Hereby, ICUAW can only be diagnosed in general, while differentiation between CIP and CIM cannot be made (Lipshutz and Gropper 2013; Vanhorebeek et al. 2020).

Electrophysiological testing, such as nerve conduction studies and electromyography, can be applied to diagnose ICUAW in general and to differentiate between CIP and CIM (Weber-Carstens et al. 2009; Jolley et al. 2016). In both CIP and CIM, normal to minimally decreased nerve conduction velocity and reduced compound muscle action potential can be observed. In contrast, CIP is characterized by reduction in sensory-nerve action potential, while in CIM, there is a reduction in muscle excitability on direct muscle stimulation, increased duration of the compound muscle action potential, and normal sensory-nerve action potential (Kress and Hall 2014).

Muscle biopsies can be performed to comprehensively diagnose CIM. However, due to the invasive nature of this technique, implementation into routine clinical practice is difficult (Senger and Erbguth 2017).

In practice, bedside ultrasound measurement of the musculature is becoming increasingly important. This technique can be used in routine clinical practice and allows the observation of atopic muscle changes. However, additional methods (e.g., volitional functional and electrophysiological testing) should be performed as well, to comprehensively identify causes of muscle abnormalities and to make a reliable diagnosis of ICUAW (Witteveen et al. 2017).

In general, the diagnosis of ICUAW by differentiating CIP and CIM is very difficult due to the complexity of causes and contributing factors and the overlap of symptoms and clinical signs. Additionally, medication (e.g., administration of muscle relaxants) and general medical treatment (e.g., bedrest, mechanical ventilation) may interfere with the performance of various

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methods to diagnose ICUAW (Vanhorebeek et al. 2020). The differential diagnosis of ICUAW in general and CIP and CIM from other muscle-damaging diseases is highly relevant in clinical practice. In this context, potential pre-existing diseases such as spinal and brainstem lesions, neuromuscular transmission failures, muscular deficits due to medication, abnormalities in electrolyte concentrations, as well as systemic and other muscle diseases should be excluded before diagnosing ICUAW (Senger and Erbguth 2017).

Besides critical illness itself, research suggests that several factors of medical treatment might contribute to the development of ICUAW. In this context, sepsis and SIRS, MOF, hyperglycemia, application of renal replacement therapy, administration of catecholamines, neuromuscular blocking agents and corticosteroids, and duration of mechanical ventilation are associated with higher risk for the development of ICUAW (Lipshutz and Gropper 2013; Yang et al. 2018; Vanhorebeek et al. 2020).

General predictors of muscle status

In addition to various medical factors and treatment conditions, such as diagnosis, severity of illness, need for mechanical ventilation and duration of its use, as well as administration of catecholamines (Yang et al. 2018), muscle atrophy during ICU stay could be influenced by patient characteristics that generally predict (pre-hospital) muscle status, such as sex, age, height, BW, overall lifestyle, and activity (Curtis et al. 2015).

Since the body composition is influenced by gender-specific hormones, women have a higher percentage of fat, while men have more muscle mass (Abe et al. 2003). In men, testosterone promotes MPS due to its potent anabolic effects. In contrast to men, testosterone is only present in small amounts in women, and this effect has not yet been observed for estrogen, the female sex hormone (Anderson et al. 2017).

The age-related loss of muscle mass, also called sarcopenia, is described extensively in literature. Research suggests that muscle mass decreases physiologically with increasing age due to qualitative and quantitative modifications of its structure and function (Larsson et al. 2019).

Lower quantity and quality of muscle mass at ICU admission itself is associated with increased risk of mortality, regardless of developing muscle wasting due to illness (Weijs et al. 2014; Looijaard et al. 2016). Moreover, low pre-hospital muscle mass is associated with increased risk for longer duration of mechanical ventilation during ICU stay (Moisey et al. 2013).

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As height is predicted by the length of the bones and muscles, taller subjects are generally expected to have more muscle mass than smaller subjects (Gallagher and Heymsfield 1998).

Additionally, it could be assumed that subjects with higher BW contain more muscle mass, which is needed for mobility. In contrast, research findings suggest a non-linear association between BW and muscle mass. Thus, if muscle mass is considered as a percentage of total body mass, increasing BW is associated with a relative decrease in skeletal muscle mass (Forbes 1987; Janssen et al. 2000).

Monitoring of changes in muscle changes

Monitoring of changes in muscle mass during ICU stay can be conducted by several methods, such as physical assessment (e.g., MRC), imaging procedures (e.g., ultrasound, computed tomography [CT], magnetic resonance imaging [MRI], dual energy X-ray absorptiometry [DEXA]), bioelectrical impedance analysis (BIA), and measuring of selected biomarkers (e.g., creatine kinase [CK], growth differentiation factor-15 [GDF-15], serum creatinine to serum cystatin C ratio, urine creatine, 3-methylhistidine) (Nakanishi et al. 2020). Additionally, in-vitro neutron activation analysis (IVNAA) can be performed to determine total body protein (TBP) and nitrogen balance to estimate nitrogen loss (Joskova et al. 2018; Singer 2020).

In contrast to bedside measurement techniques of skeletal muscle mass (e.g., ultrasound), the performance of CT, MRI, and DEXA measurements in clinical practice is associated with higher effort and costs, and is accompanied by increased stress for the ICU patients (e.g., transportation, radiation exposure). Moreover, the influence of hydration status on the obtained values of lean body mass remains unclear in DEXA measurements. Despite the high reliability of CT and MRI, these techniques are not suitable for monitoring of skeletal muscle mass in routine clinical practice. However, if these methods have to be conducted due to other medical reasons, abdominal scans can additionally be analyzed regarding the content of muscle mass (Rooyackers and Wernerman 2014).

In addition to these imaging techniques, as mentioned above, biochemical markers (e.g., CK, GDF-15) can also be analyzed to provide indications about the patients' muscle status and to monitor changes during the ICU stay. Since disease-specific features, such as renal dysfunction, medication, and several organ dysfunctions, may interfere with the results of biochemical analysis, the validity of these biomarkers to estimate muscle mass and monitor its changes might, however, be compromised (Nakanishi et al. 2020).

Moreover, as IVNAA requires patients' exposition to radiation as well, its implementation in routine clinical practice is not indicated. Additionally, performing a 24-h-urine sampling to estimate nitrogen balance and draw conclusions about protein metabolism is very difficult in clinical practice. On the one hand, the results might be influenced by aspects such as age, severity of illness, medical treatment (e.g., application of renal replacement therapy), and nutrition therapy, especially by the administration of protein and amino acids, respectively. On the other hand, organization of samples collection is very difficult to handle in routine clinical practice (Fernández et al. 2019).

In practice, ultrasound is the most commonly used technique to observe muscle changes during the ICU stay. Advantages of this method are that the measurements can be performed bedside and do not place additional stress on the ICU patients. However, ultrasound offers different possibilities for performing muscle mass measurements (Mourtzakis and Wischmeyer 2014; Duan et al. 2020). Thus, various validation and reliability tests show large heterogeneity with respect to aspects such as fixation of measurement points (e.g., thigh *vs.* arm, variation of exact landmark), definition of patient position (e.g., supine [with legs extended] *vs.* standing), and the extent of transducer compression during measurement (maximal *vs.* minimal *vs.* none). Regarding thigh muscle mass, both quadriceps muscle layer thickness (QMLT) and quadriceps cross-sectional area (CSA), can be measured (Sanada et al. 2006; Seymour et al. 2009; Baldwin et al. 2011; Thomaes et al. 2012; Ema et al. 2013; Tillquist et al. 2014). Ultrasound measurement is easy to perform in clinical routine providing precise and reliable information on the muscle mass content and potential changes during ICU stay (Tourel et al. 2020). But, due to the heterogeneity in methodology of measurements and difficulties in dealing with disease-specific aspects (e.g., presence of edema), guidance for standardized proceeding in monitoring muscle mass is required (Mourtzakis and Wischmeyer 2014; Looijaard et al. 2018; Weinel et al. 2019).

2.3 Nutrition therapy in critically ill patients

As described previously, critical illness is accompanied by loss of metabolic functions and trauma-related degradation of muscle mass, and is associated with adverse outcome of ICU patients. In this context, in addition to medical and physical treatment, nutrition therapy is becoming increasingly important during acute illness and in the rehabilitation phase of ICU patients (Kou et al. 2019; Duan et al. 2020).

Adequate energy and nutrient supply, especially of protein and amino acids, respectively, can contribute to influence the course of disease and overall recovery process. Additionally, secondary damages due to illness (e.g., malnutrition, excessive loss of lean body mass) might be prevented (Lambell et al. 2020). As critically ill patients represent a large heterogeneity in diagnosis, medical treatment conditions, demography, and potential pre-existing diseases, the implementation of individual disease- and phase-specific nutrition regimes is indispensable (van Zanten et al. 2019). Moreover, as critically ill patients often require mechanical ventilation and sedation, oral nutrition is not possible and, thus, enteral and/or parenteral nutrition is administered. When selecting the route (enteral and/or parenteral), product, and dosage of nutrition therapy, patients' individual disease- and phase-specific situation, gastrointestinal tolerance, as well as potential pre-existing intolerances and diseases (e.g., lactose intolerance, diabetes mellitus) have to be considered and monitored carefully (Koekkoek and van Zanten 2017).

In general, there is plethora of international guidelines on the nutrition therapy for ICU patients, which attempt to capture the current state of research in this field and are therefore updated regularly. However, clinical trials which investigate diverse aspects of nutrition therapy for ICU patients are very heterogeneous in their findings, which might be a consequence of the great variance in study design (e.g, clinical picture of the study participants, sample size, intervention, measured outcome parameters, overall study conduction). These factors affect the comparability of current studies and make it difficult to derive valid recommendations (Preiser et al. 2015). Additionally, clinical centers differ markedly regarding aspects such as interdisciplinarity in staff, equipment, and selection of nutrition products. Therfore, internal standard operating procedures (SOPs) based on international guidelines and current research results, but according to the local possibilities, are often defined by the clinics itself to give specific step-by-step instructions for the nutrition therapy in their setting (Berger et al. 2019).

Despite conflicting results of current research and heterogeneity in details of some recommendations of international guidelines, there is agreement that determination of energy expenditure, calculation of target energy and protein supply, timing of initiation of nutritional support, route of administration, and monitoring of individual metabolic and gastrointestinal tolerance are of particular importance for the patient outcome (Sharma et al. 2019).

Nutrition therapy: Timing and route

Current research suggests that nutrition therapy should be initiated early (within 24–48 hours after ICU admission), whereby enteral nutrition should be the preferred route, as it represents the physiological way of nutrient absorption and is beneficial for the gut mucosa (van Zanten et al. 2019; Lambell et al. 2020). This aspect is implemented in several international guidelines and already established as standard of care (McClave et al. 2016; Elke et al. 2018; Singer et al. 2018). However, in the case of obstruction, states of uncontrolled shock, bowel ischemia, abdominal compartment syndrome, intra-abdominal hypertension, gastric residual volume (GRV) > 500 ml per 6 hours, and other gastrointestinal contraindications, enteral nutrition should be delayed (Elke et al. 2018; Singer et al. 2018; van Zanten et al. 2019). Moreover, supported by recent research findings, parenteral nutrition should be used if there are any contraindications for enteral nutrition, or if enteral energy and nutrient supply is insufficient. Contrary to previous views, parenteral nutrition is a safe alternative to enteral nutrition. However, since parenteral nutrition is associated with an increased risk of infectious complications compared to enteral nutrition, it should be used cautiously and only as much and for as long as necessary (Elke et al. 2016). Despite numerous studies, the optimal timing of initiation of parenteral nutrition and supplemental parenteral nutrition (SPN) is still debated because of the heterogeneity and the methodical weaknesses of these trials (Heyland et al. 1998; Braunschweig et al. 2001; Elke et al. 2008; Casaer et al. 2011; Kutsogiannis et al. 2011; Elke et al. 2013). Current international guidelines suggest to start parenteral nutrition within 72 hours after ICU admission in the case of contraindications for enteral nutrition. Additionally, according to recent clinical findings (Heidegger et al. 2013), European guidelines generally suggest that SPN is indicated if enteral nutrition cannot cover energy and nutrient needs adequately within the first week after ICU admission (Elke et al. 2018; Singer et al. 2018). Contrarily, American guidelines recommend the use of SPN only in malnourished patients if enteral nutrition is still inadequate to reach pre-defined energy and nutrient targets on days 7–10 after ICU admission (McClave et al. 2016).

Nutrition therapy: Energy supply

As both hypo- and hyperalimentation of energy are associated with increased morbidity and mortality, calculation of target energy supply according to the patients' individual disease- and phase-specific needs is of special importance (Reintam Blaser and Berger 2017; Koekkoek and van Zanten 2018). In clinical practice, determination of patients' individual EE is recommended

being performed by indirect calorimetry (IC) (De Waele et al. 2021). As this technique can be conducted bedside through connection with the mechanical ventilation, the patients' individual disease- and phase-specific EE can be assessed regularly throughout the ICU stay (Delsoglio et al. 2019). If IC is not available, recent European guidelines suggest to use a weight-based estimation of 20–25 kcal/kg actual BW/d for non-obese patients, 11–14 kcal/kg actual BW/d (body mass index [BMI] 30–50 kg/m²), and 22–25 kcal/kg ideal BW/d (BMI > 50 kg/m²) (Elke et al. 2018; Singer et al. 2018). Contrarily, American guidelines recommend using a weight-based estimation of 25–30 kcal/kg BW/d, if IC is not available. Hereby, ideal BW should be used for obese patients (McClave et al. 2016).

Besides determining EE, dosing of energy supply during several phases of critical illness according to the patients' individual state of disease is subject of current research and already implemented in international guidelines. Research suggests that hypocaloric feeding (< 70 % of measured/estimated EE) should be preferred in the early phase of critical illness, while afterwards, isocaloric feeding should be targeted (Weijs et al. 2012; Zusman et al. 2016). Contrarily, in the recovery phase, energy supply should achieve > 100 % of measured/estimated EE, to ensure restoration of substrates and body mass lost in the catabolic phases of illness and cover higher energy needs that occur due to physical rehabilitation (Kristensen 2013; van Zanten et al. 2019). Thus, European guidelines suggest to start with 75 % of measured/estimated EE in the early phase of critical illness and to increase the supply up to > 100 % in the recovery and rehabilitation phase (Elke et al. 2018; Singer et al. 2018). In general, American guidelines recommend an energy supply according to the measured/estimated EE. Especially in patients who are at nutritional risk, > 80 % of EE should be provided within 28–72 hours after ICU admission (McClave et al. 2016).

Nutrition therapy: Protein supply

In the context of a medical nutritional approach in ICU patients, protein supply is of particular importance to stimulate MPS and, thus, minimize MPB and amino acid oxidation that occurs due to changes in substrate utilization and metabolism in critically ill patients (De Waele et al. 2020). Therefore, in addition to targeted medication and exercise, a quantitatively higher protein intake in critically ill patients compared with current recommendations for healthy adults might be usefull to meet the disease-specific increased nitrogen/amino acid requirements and, thus, contribute to overcoming the pronounced loss of functional proteins. While an adequate supply of protein within a medical nutritional approach is already suggested to

minimize MPB, specificities on appropriate dosage, timing, and composition (e.g., protein sources, amino acid pattern) are still under debate (Brook et al. 2017).

In general, research suggests that protein supplementation at levels of 1.2–2.5 g/kg BW/d is safe and may be needed by ICU patients to increase protein/amino acid intake and improve nitrogen balance (Hoffer and Bistrian 2012; Arabi et al. 2020). As proteins/amino acids are indispensable substrates of muscle metabolism, potential effects of high-protein supply on prevention of muscle mass and thickness, and on overall quality of life at ICU discharge, are of particular interest (Ferrie et al. 2016; Fetterplace et al. 2018; Nakamura et al. 2020).

One of the unsolved problems for a reliable definition of protein targets is the optimal timing of uptake to achieve predefined therapeutic goals. While few studies indicate that a protein provision higher than 1.0 g/kg BW/d in the early phase of critical illness seems to be associated with reduced mortality (Weijs et al. 2012; Bendavid et al. 2019), other investigations show that early high-protein administration (> 1.2 g/kg BW/d) might worsen instead of improving the patient outcome, especially during hyperenergetic nutrition therapy (Weijs 2014; Davies et al. 2017; Koekkoek et al. 2018). Generally, it is well known that patients in the acute phase (ebb phase) of the stress response are less capable of utilizing nutrients, thereby implying that early high-dose protein administration might not be beneficial (Cuthbertson and Zagreb 1979). In the later phase (flow phase) of metabolic stress, the insulin sensitivity gradually improves, and the human body's ability to metabolize exogenous substrates increases accordingly (Cuthbertson and Zagreb 1979). Consequently, it could be hypothesized that a high-protein medical diet after the onset of trauma could effectively reduce endogenous proteolysis, which could contribute to muscle mass preservation.

The protein (enteral nutrition) and amino acid (parenteral nutrition) composition, respectively, is of special importance for the muscle metabolism. In this context, EAAs in general and especially branched-chain amino acids (BCAAs; valine, leucine, isoleucine), glutamine, beta-hydroxy-beta-methylbutyrate (HMB) metabolised from leucine, creatine metabolised from arginine, glycine, and methionine, and taurine, a metabolite from cysteine, seem to play a key role (Wandrag et al. 2015; Wolfe 2017). Additionally, in critically ill patients, the conditionally-EAA glutamine is of special importance as it is a crucial factor in the metabolism and homeostasis of energy, protein, and nitrogen and in the immune response. In pathophysiological conditions characterized by substantial hormone-mediated catabolism, endogenous glutamine synthesis and dietary glutamine intake in routine clinical care are insufficient to meet the disease-related greater demand (Stehle and Kuhn 2015). Research suggests that adequate

amounts of glutamine provided within a balanced (parenteral) nutrition therapy contribute to improve outcome parameters such as hospital infections, LOS, and mortality in critically ill patients and, thus, should be an integral part of medical nutrition products (Goeters et al. 2002; Cruzat et al. 2018). As enteral products are made of protein already containing glutamine by nature, especially in the case of parenteral nutrition, supplemental glutamine dipeptides should be provided to optimize protein/nitrogen balance and immune functions and to improve the patient outcome (Stehle et al. 2017).

In routine clinical care, protein supply is performed according to the guidelines for nutrition therapy in critically ill patients defined by international societies. While the ESPEN strongly recommends a daily intake of 1.3 g protein equivalents/kg BW/d in ICU patients (Singer et al. 2018), the US-American guidelines suggest even higher protein quantities of 1.2–2.0 g/kg BW/d (McClave et al. 2016). Moreover, according to the German Society for Nutritional Medicine (DGEM), the protein target is defined as 1.0 g protein/kg BW/d and 1.2 g amino acids/kg BW/d, respectively (Elke et al. 2018). Based on this, protein supply should begin at approximately 75 % of the calculated protein target in the early phase of critical illness and be increased to 100 % by the end of the acute phase. During recovery and rehabilitation, administration of > 100 % of the patients' individual protein target should be achieved (Elke et al. 2018).

Due to the heterogeneity of studies that have investigated the effects of higher amounts of EAAs, conditionally-EAAs, and BCAAs, as well as enrichment with HMB and creatine on outcome parameters such as nitrogen balance, MPS, and overall muscle status in critically ill patients, no recommendations for optimal protein/amino acid composition can be made (Pavlickova Aimova et al. 2014). Therefore, products containing all EAAs, non-EAAs, and conditionally-EAAs should be used in routine clinical practice. Moreover, the use of parenteral glutamine dipeptides in the amount of 0.3–0.5 g/kg BW/d (max. 30 % of nitrogen supply) is recommended, while there is no indication for enteral glutamine pharmacotherapy (Elke et al. 2018).

In general, due to the heterogeneity of the studies (e.g., with respect to target protein supply, measured outcomes, and timing of administration) used as a basis for defining recommendations for routine clinical care, there is still a lack of sufficient evidence for optimal dosing and timing of protein supply in ICU patients. Therefore, there is an urgent need to investigate different higher protein doses (> 1.2 g/kg BW/d) administered at different time points (early *vs.* late phase of critical illness) on outcome parameters such as ICU LOS, duration

of mechanical ventilation, morbidity and mortality rates, loss of body weight, and especially skeletal muscle wasting.

Nutrition therapy: Carbohydrate and fat supply

In addition to energy provided by protein/amino acids, distribution of non-protein energy to carbohydrates/glucose and fat/lipids should be planned carefully as well.

Due to the modifications in glucose utilization and metabolism (e.g., increased glycogenolysis and gluconeogenesis in the liver), and the development of a temporary insulin resistance following critical illness, high-carbohydrate/glucose intake should be avoided. High administration of exogenous carbohydrates/glucose might rather worsen instead of improving patient outcome, by increasing the sympathetic activity, maintaining physical stress, and promoting hyperglycemic states (Patkova et al. 2017). However, carbohydrates/glucose are mandatory essential to provide energy to organs such as the CNS, immunocompetent cells, erythrocytes, epithelial tubules, and cells of proliferating tissues involved in wound healing (Sharma et al. 2019). This makes them elementary components of a medical nutrition concept in ICU patients. The exogenous administration of carbohydrates/glucose aims to preserve endogenous skeletal muscle mass, whose degradation is triggered when using (glucoplastic) amino acids for hepatic gluconeogenesis during the metabolic response following illness (Mundi et al. 2017). Nevertheless, utilization of endogenous substrates cannot be fully prevented by exogenous carbohydrate/glucose administration. Therefore, dosage should be calculated with respect to the patients' individual tolerance (e.g., monitoring of blood glucose level, insulin needs, overall state of disease), whereby normoglycemia should be strived (Gunst et al. 2019).

Regarding fat/lipids, exogenous administration is crucial for providing both energy and essential fatty acids, as well as for preventing depletion of FFAs used as alternative energy substrates during the stress metabolism (Patkova et al. 2017). Metabolic complications, such as hyperglycemia and increased carbon dioxide (CO_2) production following endogenous lipolysis, can be reduced by exogenous fat/lipid administration. Additionally, lipids are essential for the building and function of cell membranes, the profile of inflammatory mediators (e.g., eicosanoid and cytokine activity), and the gene expression (McCarthy and Martindale 2018). Regarding the modulation of inflammatory processes, ω-3 polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), enhance the production of anti-inflammatory mediators and, thus, seem to be beneficial with respect to

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overall patient outcome (Donoghue et al. 2019; Langlois et al. 2019). Contrarily, as ω-6 PUFAs (e.g., linoleic acid [LA], arachidonic acid [ARA]) promote pro-inflammatory mediators, research suggests to reduce their amounts administered within a medical nutrition concept in ICU patients to a minimum (Patkova et al. 2017). Moreover, a mixture of soybean oil (administration of essential amino acids), medium-chain triglycerides (MCTs; administration of energy that is quickly available), olive oil (administration of immuno-neutral monounsaturated fatty acids [MUFAs]), and fish oil (rich in EPA and DHA) might be beneficial to optimize aspects such as triglyceride profile, immune response, inflammatory reaction, and LOS (Dai et al. 2016). However, the fat/lipid dosage and fatty acid composition for nutrition therapy in critically ill patients are still debated, especially depending on the route of administration (enteral *vs.* parenteral), which also affects the current recommendations for routine clinical practice (Calder et al. 2018).

In practice, based on measured/estimated EE, non-protein energy is simplistically distributed between carbohydrates/glucose and fat/lipids in a 60 : 40 energy percent (E %) ratio. However, intravenous administration should not exceed an upper limit of 4.0 g glucose/kg BW/d and 1.5 g lipids/kg BW/d, respectively (Elke et al. 2018; Singer et al. 2018). In terms of fatty acid composition, parenteral lipid solutions should contain higher amounts of MCTs, ω-9 MUFAs, and ω-3 PUFAs by reducing the amount of ω-6 PUFAs. Thus, using a mixture of soybean oil, MCTs, olive oil, and fish oil (SMOF) might be more beneficial than soybean oil alone. Although fish oil-enriched enteral nutrition also seems to be beneficial for patient outcome, the available data of previous studies are very inconclusive and do not allow defining clear recommendations for clinical routine (Calder et al. 2018; Elke et al. 2018; Singer et al. 2018) .

Nutrition therapy: Monitoring

As described previously, in clinical practice, international guidelines are often adapted to internal SOPs of medical centers according to the local conditions and possibilities for action. This should integrate clear instructions for monitoring the patients' individual gastrointestinal and metabolic tolerance, which also influences the efficiency of the particular nutrition regime, in addition to recommendations for the implementation of energy and nutrient intake in general (Berger et al. 2019). On the one hand, parameters such as blood glucose, triglycerides, lactate, electrolytes, phosphate, albumin, C-reactive protein (CRP), and IL-6, markers of liver function (e.g., alanine aminotrans-ferase/glutamate pyruvate transaminase [ALT/GPT], aspartate

aminotransferase/glutamate oxaloacetate transaminase [AST/GOT]), gastrointestinal tolerance (e.g., GRV), and nitrogen balance may give information about the patients' individual metabolic functions and the adequacy of nutrient supply. On the other hand, the implementation of the prescribed individual nutrition regimes (e.g., volume, application time, energy and nutrient target) should be monitored by controlling the applicator and infusion settings (van Zanten et al. 2019; Nienow et al. 2021). Additionally, as described above, energy and nutrient supply should be adapted regularly by determining actual EE and assessing the phase and state of disease if possible (McClave et al. 2016; Elke et al. 2018; Singer et al. 2018; Berger et al. 2019; van Zanten et al. 2019). However, because there are no valid parameters to clearly distinguish between the different phases of stress response following critical illness, implementation of a phase-specific nutrition therapy is difficult and therefore can only be accomplished through non-specific markers (e.g., secondary infections, impaired wound healing, muscle wasting, respiratory complications) and according to the physicians' expertise (Preiser et al. 2015; Lambell et al. 2020).

3 Objectives

The primary objective of this RCT integrated into a standardized critical care therapy (according to internal SOP) was to evaluate, whether a 50 % increase in daily protein supply (combined enteral and parenteral administration) in the later phase of critical illness can significantly contribute to the preservation or even regain of muscle mass during a long-term stay in the ICU. In addition, secondary objectives were defined as the effects of protein quantity on the incidence of clinical symptoms such as pneumonia and wound infections, and on nitrogen balance.

In this context, the study tested the following hypotheses:

1. The amount of protein within a medical nutrition therapy modifies the rates of protein degradation and protein synthesis, thereby influencing muscle mass atrophy and preservation, respectively. Thus, patients receiving a high-protein supply (1.8 g/kg BW/d) show less muscle wasting compared with patients receiving a standard nutritional care (1.2 g protein/kg BW/d).

2. Individual patient and medical characteristics (e.g., sex, age, height, weight, QMLT on admission, severity of illness, administration of catecholamines, duration of mechanical ventilation) influence the rates of protein degradation and synthesis during ICU LOS and, thus, a high-protein supply (1.8 g *vs.* 1.2 g/kg BW/d) contributes to reduction of muscle wasting.

3. A higher protein quantity (1.8 g *vs.* 1.2 g/kg BW/d) within a medical nutrition concept influences the patients' blood biochemical parameters during the study period.

4. A quantitatively higher protein supply (1.8 g *vs.* 1.2 g/kg BW/d) improves the nitrogen balance in critically ill patients.

5. A higher protein quantity (1.8 g *vs.* 1.2 g/kg BW/d) within a medical nutrition therapy reduces the incidence of clinical symptoms such as pneumonia and wound infections.

6. In general, the clinical outcome of critically ill patients (e.g., severity of illness, ICU LOS, duration of mechanical ventilation) is improved by a high-protein supply (1.8 g/kg BW/d) compared with standard nutritional care (1.2 g/kg BW/d).

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4 Subjects and methods

4.1 Study design and setting

This single-center, randomized, controlled, observer-blinded trial was conducted following the Declaration of Helsinki and approved by the Ethics Committee of the Medical Faculty of the Rheinische Friedrich-Wilhelms-University of Bonn, Germany (ethics approval code: 300/15). The study protocol was registered at the German Clinical Trials Register (DRKS; http://www.drks.de; DRKS-ID: DRKS00013594). Written informed consent was obtained from all patients or their legal representatives after the study protocol was explained to them by an investigator.

Screening, recruitment, and intervention took place at five interdisciplinary surgical ICUs of the Department of Anesthesiology and Intensive Care Medicine (KAI) of the University Hospital of Bonn (UKB), Germany, between May 2017 and January 2020.

4.2 Study participants

Sample size and power calculation

Sample size and power calculation were based on a pilot study (unpublished data) that was previously conducted at the KAI (n = 18) to examine the effects of standard nutritional care on muscle mass by ultrasound measurement of QMLT. In this context, a mean decrease in muscle mass thickness of 1.21 mm (9.4 %) with a standard deviation (SD) of 0.8 mm (5.3 %) was observed within 28 days after hospitalization. According to our working hypothesis, a 50 % increase in protein supply should reduce the loss of muscle mass by half (0.56 mm; 4.7 %). Considering a power of 80 % and the use of a two-group t-test at 5 % significance, 21 patients had to be included in both study groups.

Inclusion and exclusion criteria

During the study period, all patients admitted to the ICUs of KAI were screened for eligibility. The basic inclusion criteria for study participation were: (i) age range of > 18–90 years, (ii) necessity of mechanical ventilation, (iii) overcoming the early period of hemodynamic instability (ebb phase) according to the definitions published by ESPEN (Singer et al. 2018) (sustainable decrease in inflammatory response assessed by lowered concentrations of procalcitonin [PCT], CRP, and leukocyte numbers; decreased need for fluid resuscitation; less use of catecholamines), (iv) prediction of a long-term ICU stay (valid statement by the

physicians that the patient will need more than 28 days support of organ function, e.g., respiratory support and renal replacement therapy). Patients were enrolled in the study no later than 20 days after admission to the ICU. Exclusion criteria were: (i) presence of terminal chronic renal failure, (ii) persistent acute respiratory distress syndrome (ARDS: persistent bilateral infiltrates in the chest X-ray or CT scan plus ongoing comprised oxygenation defined by a need for an inspiratory oxygen fraction [FiO_2] higher than 60 %), (iii) ongoing extracorporeal membrane oxygenation (ECMO), (iv) previously diagnosed myopathies, traumatic brain injuries, intracerebral hemorrhages, and cerebral ischemia followed by muscular failures, (v) utilization of inhalation sedation.

All patients were recorded and tracked using an approved patient data management system (PDMS; integrated care manager, ICM; Dräger, Germany), which documents all patient characteristics and medical care orders.

Randomisation and blinding

After their inclusion, participants were randomly assigned using a computer-generated list (1:1 block randomization) to either the intervention (target: 1.8 g protein/kg BW/d) or control group (target: 1.2 g protein/kg BW/d). Nutrition therapy was planned unblinded to ensure exact calculation of protein supply according to group allocation. In contrast, ultrasound measurements of QMLT were performed by a blinded investigator to ensure independent collection of the primary outcome parameter.

4.3 Anthropometric parameters

Anthropometric parameters such as sex, age, and height were collected on admission to the ICU from the patient's identity card, health card, and previous medical records, or by interviewing relatives.

Since no bed-scales to measure actual BW were available at the time of admission, a so-called 'optimal' BW (oBW) was calculated assuming a BMI of 25 kg/m², which allowed a comprehensive and comparable calculation of individual protein intake in both the groups:

$$oBW = 25 \ kg/m^2 \ x \ (height \ [m] \ x \ height \ [m])$$

In general, the (energy and) nutrient supply during the study period was planned using the oBW. However, for comparison, BW recorded in the PDMS (BW-PDMS; predominantly estimated by the physicians) was also documented.

4.4 Nutrition therapy

Quantification of energy expenditure

Quantification of energy supply was based on resting energy expenditure (REE) measured by IC (REE-IC) at the time of enrollment and repeatedly during the study period using the *Quark RMR* (Cosmed Rome, Italy). Generally, in mechanically ventilated patients, IC was conducted in combination with the respirator. By connecting the *Quark RMR* to the respirator, gas exchange was detected through breath-by-breath technique. Additionally, gas was sampled via connection of the sampling line with the filter at the Y-piece of the ventilator tube. Thereby, inspiratory and expiratory gases of the patients' minute volume were measured by the flowmeter located at the turbine connected with the expiratory port of the respirator (Sundström et al. 2013). By detection of oxygen consumption (VO_2) and carbon dioxide production (VCO_2), energy expenditure was calculated automatically by the system via the modified Weir formula (Weir 1949):

$$REE\ (kcal/d) = (3.941\ x\ VO_2\ [ml/min] + 1.106\ x\ VCO_2\ [ml/min])\ x\ 1.44$$

Additionally, the respiratory quotient (RQ) was calculated for providing information about the predominantly oxygenated substrates by the formula (Weir 1949):

$$RQ = VCO_2\ (ml/min) / VO_2\ (ml/min)$$

Due to the integrated battery, the metabolic cart could have been moved between the patients' rooms without shutting down and restarting the system. Thus, one calibration process at the beginning of each day of measurement was sufficient. Before starting a measurement, the bias flow was adjusted to the patients' actual breathing cycle. Afterwards, EE was determined over a period of 30 minutes. To obtain a sequence as stable as possible, the first and last 5 minutes of each measurement were eliminated, and only the steps of the intervening 20 minutes were considered for the calculation of the patients' actual REE. In spontaneously breathing patients, the REE was then adjusted for individual tidal volume (VT) by eliminating outliers in the measurement logs. In this context, all the steps of VT higher than individual dead space volume (generally estimated at about 2 ml/kg BW) were eliminated (Larsen et al. 2012). Within the scope of this study, measured REE served as a solid basis for assessing energy targets.

Additionally, aspects such as the mode of mechanical ventilation (e.g., pressure control-biphasic positive airway pressure [PC-BIPAP], pressure control-airway pressure release ventilation [PC-APRV], spontaneous-continuous positive airway pressure/pressure support [SPN-CPAP/PS]), FiO_2, the pressure of the respiratory tract during the expiration (positive end expiratory pressure [PEEP]) or upper pressure level (P_{high}), and patients' body temperature during IC-measurements were documented for controlling the measurement conditions.

In exceptional cases, when IC was not applicable (patients who were no longer mechanically ventilated or who needed $FiO_2 > 60$ %), EE was calculated according to internal SOP using the Harris-Benedict-equation for determining basal metabolic rate (BMR) (Harris and Benedict 1918), whereby oBW (see above) served as the respective factor:

- Women:

  BMR (kcal/d) = 655.096 + 1.850 x height (cm) + 9.563 x weight (kg) – 4.676 x age (years)

- Men:

  BMR (kcal/d) = 66.473 + 5.003 x height (cm) + 13.752 x weight (kg) – 6.755 x age (years)

Afterwards, following the internal SOP, the calculated BMR was further modified by multiplication with a disease-specific coefficient based on the physicians' assessment resulting in the total energy expenditure (TEE) (UKB 2016):

- Ebbphase: BMR (kcal/d) x 0.5
- Katabaolic phase: BMR (kcal/d) x 1.0
- Anabolic phase:
  - BMR (kcal/d) x 1.1 (bed rest, mild stress)
  - BMR (kcal/d) x 1.4 (large surgical interventions, moderate stress)
  - BMR (kcal/d) x 1.6–1.8 (septic shock, burns, massive stress)

To compare the methods of determining EE for study purpose (IC measurement [REE-IC] or, only in exceptional cases, calculation using the Harris-Benedict-equation, specific coefficients, and oBW [TEE-oBW]) with the routine clinical procedure (calculation using the Harris-Benedict-equation, specific coefficients, and BW documented in the PDMS [TEE-PDMS]), the respective data were also excerpted from the PDMS.

Nutrient supply

A continuous (24-h) medical nutrition therapy covering 100 % of individual actual EE was adjusted at the time of enrollment and (based on actual REE measurements) repeatedly qualified

during the intervention period. Macronutrient supply was planned individually based on the target protein supply that was given through randomization (intervention: 1.8 g/kg BW/d; standard: 1.2 g/kg BW/d) and the previously determined target energy supply (see above). The remaining non-protein energy was given as 60 E % carbohydrates and 40 E % fat (instruction of hospital internal SOP) (UKB 2016; Elke et al. 2018). Enteral nutrition contained all the necessary micronutrients. In the case of parenteral nutrition, micronutrients were additionally supplemented. The administration route (enteral, parenteral, or both) was decided daily upon physicians' assessment of the clinical situation.

Following the internal SOP (UKB 2016), enteral nutrition was the preferred route of nutritional support in study patients. If the target REE and/or target supply of single nutrients could not be completely covered by enteral nutrition, single solutions (amino acids, glucose, lipids) were supplemented parenterally to close the gap. If enteral nutrition was not applicable due to medical and disease-related reasons, energy and nutrients were provided using total parenteral nutrition (TPN). Moreover, in the case of continuous renal replacement therapy (CRRT), a total of 24 g protein or amino acids/d was added to the individual target protein supply to balance treatment-related loss.

Nutrition products

Nutrition therapy was conducted using commercially available enteral and parenteral products (B. Braun Melsungen AG, Melsungen, Germany; Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany). The products were selected following the physicians' instructions according to the product range and availability at the local hospital pharmacy. The respective products as well as their composition and characteristics are presented in **Table 4-1.**

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Table 4-1 Specifications of the enteral nutrition products; modified according to (Fresenius Kabi Limited 2016).

Product	Specification
Fresubin® Original Fiber	• 1 kcal/ml • Fat (rapeseed, sunflower, and fish oils): 3.4 g/100 ml (30 E %) • Carbohydrate (maltodextrin): 13.0 g/100 ml (52 E %) • Protein (milk and soya): 3.8 g/100 ml (15 E %) • Fiber-enriched (inulin, wheat dextrin, and cellulose): 1.5 g/100 ml (3 E %) • Free from gluten and lactose • Daily requirements of vitamins, minerals and trace elements are covered by 1500 ml (≙ 1500 kcal)
Fresubin® Energy Fiber	• Energy: 1.5 kcal/ml • Fat (rapeseed, sunflower, and fish oils): 5.8 g/100 ml (35 E %) • Carbohydrate (maltodextrin): 18.5 g/100 ml (48 E %) • Protein (milk and soya): 5.6 g/100 ml (15 E %) • Fiber-enriched (inulin, wheat dextrine, and cellulose): 1.5 g/100 ml (2 E %) • Free from gluten and lactose • Daily requirements of vitamins, minerals, and trace elements covered by 1500 ml (≙ 2250 kcal)
Fresubin® 2 kcal HP Fiber	• Energy: 2 kcal/ml • Fat (rapeseed, sunflower, and fish oils): 10.0 g/100 ml (45 E %) • Carbohydrate (glucose syrup and maltodextrin): 16.7 g/100 ml (33.5 E %) • Protein (milk): 10.0 g/100 ml (20 E %) • Fiber-enriched (inulin and wheat dextrin): 1.5 g/100 ml (1.5 E %) • Free from gluten, low in lactose ($\leq$ 0.3 g/100 ml) • 26 % MCT in lipid content • Daily requirements of vitamins, minerals, and trace elements covered by $\geq$ 1000 ml (≙ $\geq$ 2000 kcal)

Product	Specification
Survimed® OPD	• Energy: 1 kcal/ml • Fat (MCT; rapeseed, safflower, and fish oils): 2.8 g/100 ml (25 E %) • Carbohydrate (maltodextrin): 14.3 g/100 ml (57 E %) • Protein (whey protein hydrolysate): 4.5 g/100 ml (18 E %) • Low in fiber: 0.08 g/100 ml (0 E %) • Low in lactose: ≤ 0.1 g/100 ml • ω-fatty acids (EPA/DHA) derived from fish oil • 51 % MCT in lipid content • Completely absorbable • Daily requirements of vitamins, minerals, and trace elements covered by 1500 ml (≙ 1500 kcal)
Diben®	• Energy: 1 kcal/ml • Fat (rapeseed, sunflower, and fish oils): 4.6 g/100 ml (41.4 E %) • Carbohydrate (modified starch, fructose, maltodextrin): 9.3 g/100 ml (37 E %) • Protein (milk): 4.7 g/100 ml (18.6 E %) • Fiber-enriched (inulin, cellulose): 1.5 g/100 ml (3 E %) • Free from gluten and lactose • Carbohydrate-modified with modified starch and fructose • ω-fatty acids (EPA/DHA) derived from fish oil • Daily requirements of vitamins, minerals, and trace elements covered 1350 ml (≙ 1350 kcal)
Fresubin® Soya Fiber	• Energy: 1 kcal/ml • Fat (rapeseed, sunflour, and fish oils): 3.6 g/100 ml (32 E %) • Carbohydrate (maltodextrin, fructose): 12.1 g/100 ml (49 E %) • Protein (soya): 3.8 g/100 ml (15 E %) • Fiber-enriched (inulin, cellulose, and wheat dextrin): 2.0 g/100 ml (4 E %) • Free from gluten and lactose • Carbohydrate-modified with fructose

Product	Specification
Fresubin® Soya Fiber (Continuation)	• Daily requirements of vitamins, minerals, and trace elements covered by 1500 ml (≙ 1500 kcal)

Abbreviations: E %, energy percent; MCT, medium-chain triglycerides; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

The characteristic amino acid profiles of the enteral products and the parenteral amino acid solution used for nutrition therapy during the study period are shown in **Tables 4-2 and 4-3, respectively**.

Table 4-2 Amino acid profile of the enteral nutrition products; modified according to (Fresenius Kabi Limited 2016).

Product	Fresubin® Original Fiber	Fresubin® Energy Fiber	Fresubin® 2kcal HP Fiber	Survimed ® OPD	Diben®	Fresubin® Soya Fiber
Essential amino acids (g/100 ml)						
Lysine	0.29	0.44	0.83	0.44	0.37	0.27
Threonine	0.17	0.26	0.46	0.36	0.21	0.17
Methionine	0.08	0.13	0.28	0.10	0.12	0.06
Phenylalanine	0.21	0.32	0.52	0.15	0.23	0.22
Tryptophan	0.05	0.80	0.14	0.08	0.07	0.06
Valine	0.26	0.39	0.72	0.26	0.31	0.22
Leucine	0.37	0.56	0.99	0.50	0.45	0.36
Isoleucine	0.22	0.33	0.56	0.29	0.27	0.21
Conditionally essential amino acids (g/100 ml)						
Tyrosine	0.19	0.28	0.56	0.13	0.24	0.16
Cysteine	0.04	0.05	0.05	0.09	0.03	0.05
Taurine[1]	N/A	N/A	N/A	N/A	N/A	N/A
Histidine	0.11	0.17	0.29	0.07	0.13	0.11

Product	Fresubin® Original Fiber	Fresubin® Energy Fiber	Fresubin® 2kcal HP Fiber	Survimed ® OPD	Diben®	Fresubin® Soya Fiber
Arginine	0.23	0.34	0.37	0.09	0.16	0.32
Glutamine	0.35	0.52	0.91	0.40	0.42	0.33
Non-essential amino acids (g/100 ml)						
Glycine	0.14	0.21	0.19	0.08	0.09	0.21
Alanine	0.16	0.24	0.33	0.24	0.15	0.20
Proline	0.32	0.48	1.01	0.31	0.46	0.23
Serine	0.24	0.36	0.61	0.25	0.27	0.24
Glutamic acid	0.48	0.73	1.28	0.50	0.59	0.49
Aspartic acid and asparagine	0.39	0.58	0.91	0.54	0.35	0.51

[1]Milk protein contains taurine by nature, whereby the exact amounts of the products used during the study period was not given within their specifications.

Abbreviation: N/A, not applicable.

Table 4-3 Specification of the parenteral amino acid solution Aminoplasmal®, 10 %; modified according to (B. Braun Melsungen AG 2017).

Product	Aminoplasmal®, 10 %
Essential amino acids (g/100 ml)	
Lysin hydrochloride (≙ lysine)	0.86 (≙ 0.69)
Threonine	0.42
Methionine	0.44
Phenylalanine	0.47
Tryptophan	0.16
Valine	0.62
Leucine	0.89
Isoleucine	0.50

Product	Aminoplasmal®, 10 %
Conditionally essential amino acids (g/100 ml)	
Tyrosine	0.04
Acetylcysteine	N/A
Taurine	0.00
Histidine	0.30
Arginine	1.15
Non-essential amino acids (g/100 ml)	
Glycine	1.20
Alanine	1.05
Proline	0.55
Serine	0.23
Glutamic acid	0.72
Aspartic acid	0.56

Abbreviation: N/A, not applicable.

During the study period, several glucose solutions and lipid emulsions with varying concentration were used for nutrition therapy **(Table 4-4)**.

Table 4-4 Specifications of the parenteral glucose solutions and lipid emulsion; modified according to (B. Braun Melsungen AG 2017; Fresenius Kabi Deutschland GmbH 2017).

Product	Specification
Glucose 40 %	• 440 g glucose monohydrate/l ≙ 40 g anhydrous glucose
	• Total energy: 1600 kcal/l
	• pH-value: 3.5–5.5
	• Titration acidity: < 1 mmol/l
	• Theoretical osmolarity: 2220 mosm/l
	• Water for injection purposes
	• Concentrate for mixing infusion solutions

Product	Specification
Glucosteril® 20 %	• 220 glucose monohydrate/l ≙ 200 g anhydrous glucose • Total energy: 800 kcal/l • pH-value: 3.0–5.5 • Titration acidity: < 1 mmol NaOH/l • Theoretical osmolarity: 1110 mosm/l • Water for injection purposes, hydrochloric acid 25 % and sodium hydroxide for pH-value adjustation
Glucosteril® 10 %	• 110 g glucose monohydrate/l ≙ 100 g anhydrous glucose • Total energy: 400 kcal/l • pH-value: 3.5–5.5 • Titration acidity: < 1 mmol NaOH/l • Theoretical osmolarity: 555 mosm/l • Water for injection purposes, hydrochloric acid 25 % and sodium hydroxide for pH-value adjustation
Glucosteril® 5 %	• 55 g glucose monohydrate/l ≙ 50 g anhydrous glucose • Total energy: 200 kcal/l • pH-value: 3.5–6.5 • Titration acidity: < 1 mmol NaOH/l • Theoretical osmolarity: 277 mosm/l • Water for injection purposes, hydrochloric acid 25 % and sodium hydroxide for pH-value adjustation
SMOFlipid®	• 200 mg lipids/ml • Total energy: 2000 kcal • Lipid profile (g/l): 60 g refined soybean oil; 60 g medium-chain triglycerides; 50 g refined olive oil; 30 g fish oil • Other ingredients: alpha tocopherol, glycerol, egg lecithin, sodium hydroxide for pH-value adjustation, sodium oleate, water for injection purposes

Abbreviation: NaOH, natriumhydroxide.

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Implementation of nutrition protocols

Individual nutrition plans included all relevant information such as measured/calculated EE (target energy supply), target protein, carbohydrate, and fat supply (g/d; kcal/d), ordered product of enteral formula, if necessary the specific parenteral nutrients (amino acids, glucose, lipids) including the concentration needed, and the respective application rate (ml/h). After calculation and conceptualization of the respective nutrition regime, the individual plans were handed out to the physicians who inserted the order to the PDMS. Additionally, the plans and modifications in nutrition therapy were instructed to the nursing staff who finally put the order into praxis and adjusted the nutrition products and perfusors.

Monitoring of nutrition therapy

Nutrition therapy was monitored daily by strict documentation of the prescribed energy and nutrient supply including the physicians' product choice, and by a retrospective acquisition of the actual energy and nutrient intake considering the application rates used, reflux, and/or GRV.

In addition to recording the nutrition therapy throughout the study phase, energy and nutrient supply was also documented from ICU admission to study inclusion (start of intervention phase; data not shown). Here, the energy and nutrient supply recorded in the PDMS (e.g., sort of enteral and/or parenteral products, rate of application over 24-h) represents the prescribed 'standard' nutrition therapy (according to internal SOP). From this, actual intake during this phase was calculated retrospectively by subtracting energy and nutrients lost via reflux/GRV.

4.5 Measurement of skeletal muscle mass

The skeletal muscle mass was monitored by measuring QMLT via ultrasound (HD15 PureWave Ultrasound system, Philips Healthcare, Bothell, USA) as described by *Tillquist et al. (2014)*. Measurements were performed at the beginning of the study period and two weeks (intermediate) and four weeks (final) thereafter by a 'blind' attending physician who did not know the assignment of the respective patients. Based on the experience from the pilot study (see Chapter 4.2) and considering already published data (Tillquist et al. 2014), two fixed measuring points were defined to standardize the collected data. The first measuring point was positioned between the upper two-thirds and the lower one-third of the anterior superior iliac spine (ASIS) to the patella's upper pole. The second measuring point was set at the midpoint between the ASIS and the patella's upper pole (Tillquist et al. 2014). The position of the respective measuring points is illustrated in **Figure 4-1**.
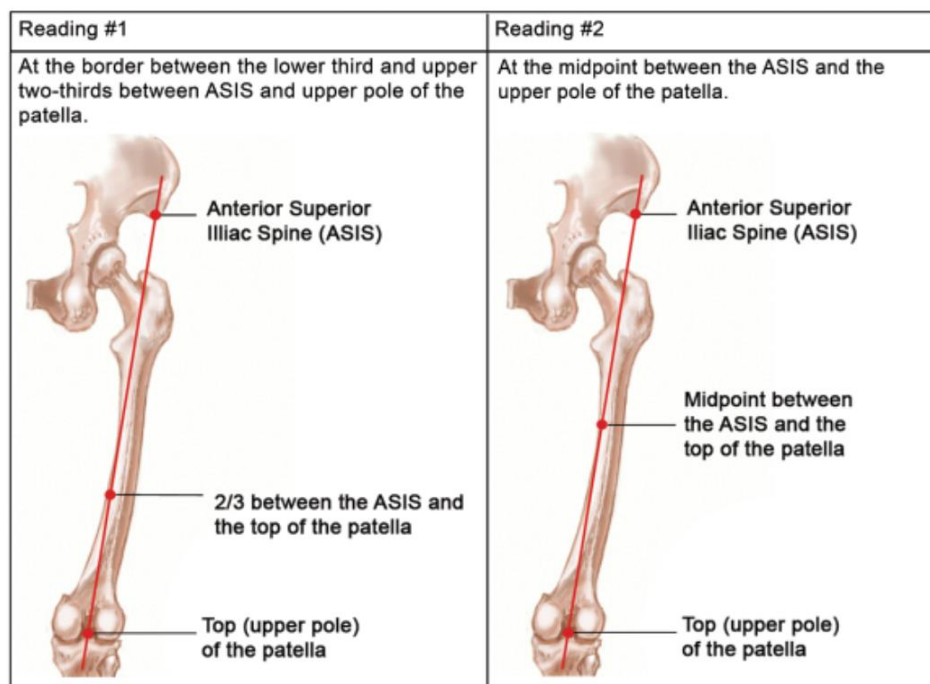
Figure 4-1 Measuring points of QMLT (Tillquist et al. 2014[1]). The measuring points were positioned between the upper two-thirds and the lower one-third of the ASIS to the patella's upper pole (Reading#1) and at the midpoint between the ASIS and the patella's upper pole (Reading#2).
Abbreviations: ASIS, anterior superior iliac spine; QMLT, quadriceps muscle layer thickness.

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[1] This article was published in: Journal of Parenteral and Enteral Nutrition; 2014; 38 (7); Tillquist M, Kutsogiannis DJ, Wischmeyer PE, Kummerlen C, Leung R, Stollery D, Karvellas CJ, Preiser JC, Bird N, Kozar R, Heyland DK; Bedside Ultrasound Is a Practical and Reliable Measurement Tool for Assessing Quadriceps Muscle Layer Thickness; p886–890; ©American Society 2013 (reproduced with permission).

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The measuring points were marked (water-soluble pen) using a tape line and measurements were performed employing the 'Philips ATL Linear 3-12 MHz probe' with the preset 'Abdominal 42 Hz' and using the saggital plane (left-right direction) in a short-axis view of QMLT. After application of ultrasound gel, the transducer was applied perpendicular to the skin surface of the leg. Maximal compression was spent to push away the upper-fat layer and to place the transducer optimally on the underlying muscle layer (monitor control). After fixing one mark at the border between fat and muscle layer and one mark between muscle layer and bone, the distance between these points was calculated automatically (= QMLT). Measurements were conducted twice on both legs; a third measurement (triplicate analysis) was performed when duplicates showed a variation of > 10 %. Mean values at the respective measurement points were calculated for each leg separately. Examplarily, **Figure 4-2** shows an ultrasound sonogram with fixed marks of QMLT within.

Figure 4-2 Example of a sonogram with fixed marks of QMLT (distance between the red circles; own recording; ©Dresen E).
Abbreviation: QMLT, quadriceps muscle layer thickness.

In the case of medical reasons and/or organizational problems the final QMLT measurement was delayed by one or two days and the nutrition therapy was prolonged.

4.6 Biochemical markers

Routine clinical diagnosis

Most of the biochemical markers analyzed within this study were determined in daily clinical routine. For study purpose, creatine kinase, CRP, and IL-6 were ordered additionally. Blood samples were collected standardly by the nursing staff at about 03:00 a.m. and analyzed at the Central Laboratory of the Institute of Clinical Chemistry and Clinical Pharmacology of the UKB. All the parameters, the respective measuring methods, as well as the reagents and instruments used for analyses are presented in **Table 4-5**.

Table 4-5 Parameters of routine clinical diagnosis and their measuring methods; modified according to (UKB 2019).

Parameter	Method	Reagent	Instrument
Albumin, g/l	VIS-photometry	ALB2 (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
Total protein, g/l	VIS- photometry	TP2 (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
Serum urea, mg/dl	VIS- photometry	UREAL (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
Serum creatinine, mg/dl	VIS- photometry	CREJ2 (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
Creatine kinase, U/I	VIS- photometry	CK (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
ALT/GPT, U/l	VIS- photometry	ALTPM (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
AST/GOT, U/l	VIS- photometry	ASTPM (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
Total bilirubin, mg/dl	VIS- photometry	BILT3 (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)

Parameter	Method	Reagent	Instrument
Calcium, mmol/l	VIS- photometry	CA2 (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
Serum triglycerides, mg/dl	VIS- photometry	TRIGL (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
1,25(OH)$_2$D, pg/ml	CLIA	–	IDS isys (Immunodiagnostic Systems)
25-OHD, ng/ml	LC-MS/MS	Mass *Chrom*, 25-OHVitamin D3/D2 in serum/plasma, automated with Hamilton MassSTAR (Chromsystems Instruments and Chemicals GmbH)	KIT* PTQ QQQ 4500 (AB SCIEX Germany GmbH), MassSTAR (Hamilton Germany GmbH)
C-reactive protein, mg/dl	TIA	CRPL3 (Roche Diagnostics)	Cobas c702 (Roche Diagnostics)
Procalcitonin, µg/l	Electrochemiluminescence immunoassay (ECLIA)	Elecsys BRAHMS PCT (Roche Diagnostics)	Cobas e801 (Roche Diagnostics)
Interleukin-6, pg/ml	ECLIA	Elecsys IL-6 (Roche Diagnostics)	Cobas e801 (Roche Diagnostics)
Leukocytes, G/l = x10^9/l	Fluorescence flow cyto-metry, resistance method, photometry	–	XN9000, XN1000 (Sysmex)
Erythrocytes, G/l = x10^9/l	Fluorescence flow cyto-metry, resistance method, photometry	–	XN9000, XN1000 (Sysmex)

Parameter	Method	Reagent	Instrument
Thrombocytes, G/l = x10^9/l	Fluorescence flow cyto-metry, resistance method, photometry	–	XN9000, XN1000 (Sysmex)
Hemoglobin, g/dl	Fluorescence flow cyto-metry, resistance method, photometry	–	XN9000, XN1000 (Sysmex)

Abbreviations: VIS, visible; ALB2, aluminium diboride; TP2, total protein gen. 2; UREAL, test for quantitative determination of urea/nitrogen in human serum, plasma, and urine; CREJ2, creatine jaffé gen. 2; ALT/GPT, alanine aminotrans-ferase/glutamate pyruvate transaminase; U, unit; ALTP, alanine aminotransferase with pyridoxal phosphate activation; AST/GOT, aspartate aminotransferase/glutamate oxaloacetate transaminase; ASTPM, aspartate aminotransferase with pyridoxal phosphate activation; BILT3, bilirubin total gen. 3; CA2, calcium gen. 2; TRIGL, triglycerides; 1.25(OH)$_2$D, 1.25-dihydroxyvitamin D3; CLIA, chemiluminescence immunoassay; 25-OHD, 25-hydroxyvitamin D3; LC-MS/MS, liquid chromatography combined with mass spectrometry; Mass *Chrom*, 25-OHVitamin D3/D2, test kit for specific determination of 25-hydroxyvitamin D; TIA, turbidimetric immunoassay; CRPL3, C-reactive protein gen. 3; Elecsys BRAHMS PCT, immunological test for determination of procalcitonin; IL-6, interleukin-6; ECLIA, electrochemiluminescence immunoassay; G, giga.

Additionally, glycemic control and lactate levels were monitored as part of the usual blood gas analysis (Rapidlab 1265, Siemens Healthcare GmbH, Erlangen, Germany) and the respective data were recorded in the PDMS. The highest and lowest daily values of glucose (mg/dl) and lactate (mmol/l) were also documented for study purpose.

24-h-urine sampling

Evaluation of nitrogen balance by 24-h-urine sampling was performed to monitor nitrogen utilization and to estimate nitrogen and protein/amino acid requirements. Therefore, the nursing staff was instructed to perform the collection frequently, if possible, throughout the study period in each patient. Afterwards, one sample of each collection was analyzed at the laboratory of the Institute of Animal Sciences, Department Animal Nutrition of the Rheinische Friedrich-Wilhelms-University of Bonn, Germany, to determine urine nitrogen (mg/dl). The analysis was conducted by high temperature combustion processes (Rapid Nu cube, Elementar Analysis systems GmbH, Langenselbold, Germany) using the Dumas-method (Dumas 1833). Measurements were performed by double- or, in exceptional cases (very different values), three-fold determination.

To compare the results of the urine nitrogen analysis, nitrogen excretion was calculated using urine urea by the formula (Schwab et al. 2015):

Nitrogen excretion (g/d) = urine urea (mmol/d) x 0.028 + 4 g

Additionally, nitrogen admission was calculated taking into account the nitrogen content of proteins and amino acids supplied via enteral and/or parenteral nutrition:

Nitrogen admission (g/d) = enteral and/or parenteral protein/amino acids supply (g/d) / 6.25

Afterwards, nitrogen balance was determined by calculating the difference between nitrogen admission and nitrogen excretion:

Nitrogen balance (g/d) = nitrogen admission (g/d) – nitrogen excretion (g/d)

Moreover, one sample of each 24-h-urine sampling was analyzed at the Central Laboratory of the Institute of Clinical Chemistry and Clinical Pharmacology of the UKB regarding the following parameters:

- Urine protein, mg/l and mg/d (Turbidimetry; reagenz: total protein urine/cerebrospinal fluid gen. 3 [TPUC3], Roche Diagnostics; instrument: Cobas c702, Roche Diagnostics); modified according to (UKB 2019).
- Urine urea, mg/dl, mg/d, and mmol/d (visible [VIS]-photometry; reagenz: UREAL for quantitative determination of urea/nitrogen in human serum, plasma and urine, Roche Diagnostics; instrument: Cobas c702, Roche Diagnostics); modified according to (UKB 2019).
- Urine creatinine, mg/dl and mg/d (VIS-photometry; reagenz: Creatine jaffé gen. 2 [CREJ2], Roche Diagnostics; instrument: Cobas c702, Roche Diagnostics); modified according to (UKB 2019).

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4.7 Medical characteristics and clinical outcome

Information on selected medical characteristics and clinical outcome parameters were excerpted from the PDMS:

- Diagnosis

- Day of study inclusion (counted from ICU admission)

- Occurrence of septic phases at ICU-admission and during ICU stay

- Daily values of simplified acute physiology score (SAPS) II and therapeutic intervention scoring system (TISS)

- Administration of catecholamines and laxatives (yes/no)

- Last defecation (date)

- Peristalsis (yes/no/sparse)

- Application of CRRT (yes/no)

- Daily insulin provision (Unit [U]/d)

- Length of study period (d)

- ICU LOS (d)

- Length of mechanical ventilation per day (h/d), during the study period (h), and during the ICU stay (h)

- Actual sequential organ failure assessment (SOFA-) score; elevated by taking into account relevant data from the PDMS: respiration (partial pressure of oxygen [PaO_2]/FiO_2), coagulation (thrombocytes), and function of the liver (serum bilirubin), the kidneys (serum creatinine, urinary output), and the cardiovascular system (blood pressure, application of dobutamine/noradrenaline/adrenaline)

- Development of pneumonia and wound infections

- Application of physical treatment throughout the study phase and the ICU stay; recorded qualitatively (yes/no)

_____

4.8 Statistical analysis

Statistical analysis was performed using the statistical package for social sciences (SPSS) software (version 25, IBM Corporation, Somers, USA). Baseline patient characteristics (including anthropometrics and biochemical markers), parameters representing severity of illness, ICU LOS, duration of mechanical ventilation, and data of nutrition therapy are shown descriptively as mean ± SD differentiated according to treatment group. The baseline characteristics of the patients (continuous data) were almost normally distributed, which allowed the application of the t-test. Based on box-plot analyses, a logarithmic transformation was performed for all other continuous data to be qualified for t-test. For discrete variables, chi-squared tests were used to perform group comparison. Additionally, correlation analysis was performed to compare the results of IC measurements to determine the patients' energy expenditure during the study period with the energy expenditure documented routinely at the PDMS, which was estimated by default using the Harris-Benedict-equation.

Linear mixed model procedures were used to test the effects of protein delivery (group), study day (time), and their interaction on daily QMLT changes. For calculation, group (intervention, standard), time, and interaction (group x time) were set as fixed factors and subject identifier as a random factor. As estimation method, restricted maximum likelihood (REML) was set. Random intercepts were used to account for repeated measurements on the individual; repeated measurements at different time points were accounted for. In a second step, data were adjusted for absolute protein intake and fulfillment of target (protein adequacy) and tested for separate and interaction effects (group, time, protein intake/protein adequacy, group x protein intake/protein adequacy, time x protein intake/protein adequacy). Accordingly, carbohydrate intake and adequacy, and fat intake and adequacy were tested as additional confounding factors. In addition, QMLT changes observed during the study period were tested for confounding by individual patient and medical characteristics, such as sex, age, height, oBW, baseline QMLT, diagnosis, severity of illness (SAPS II, TISS, and SOFA-score), number of days with administration of catecholamines, and duration of mechanical ventilation, both individually and in combination of all factors. The QMLT data are presented separately for each measurement point and on average of all measurement points. All the results of the linear mixed model procedures are shown as estimation ± standard error (SE). In all the analyses, significance level was set at $P < 0.05$.

5 Results

5.1 Patient recruitment

A total of 3946 ICU patients admitted within the study period were screened, of whom 211 met the eligibility criteria. Due to several medical events and related organizational problems, 163 patients had to be excluded at short notice (**Figure 5-1**). After obtaining consent from legal representatives, 48 patients were enrolled in the study. Due to unexpected clinical adverse events and organizational reasons, no follow-up QMLT measurement could be performed in six patients (dropouts; intervention: n = 4; standard: n = 2). Finally, 21 patients in each group with at least one follow-up QMLT measurement were included in the analysis (**Figure 5-1**). Patients who met the predefined criteria were included (d 0) on day 13 ± 2 after ICU admission (intervention: day 13 ± 3; standard: day 13 ± 2; P = 0.613).
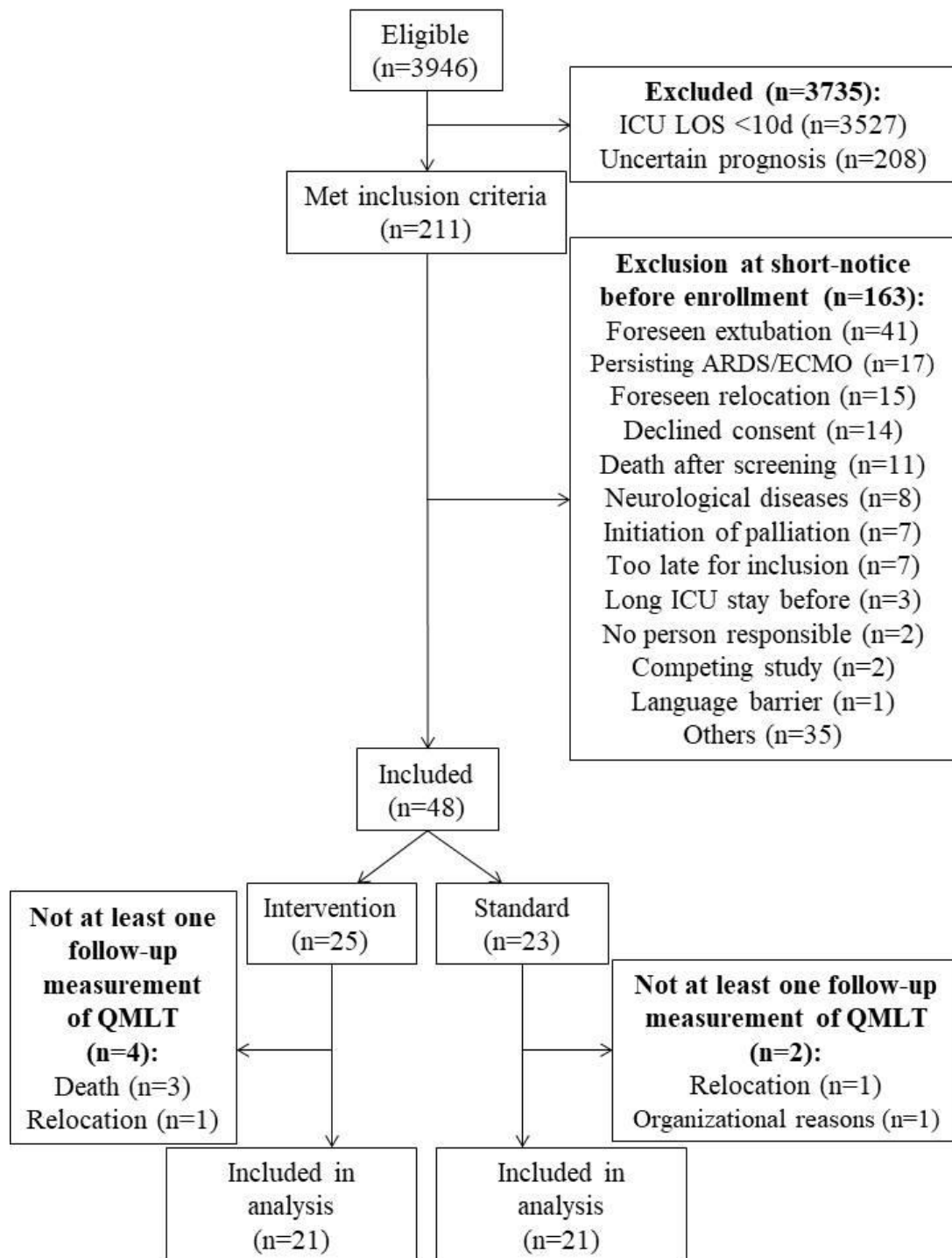
Figure 5-1 Flowchart of patient eligibility.
Abbreviations: ICU, intensive care unit; LOS, length of stay; ARDS, acute respiratory distress syndrome; ECMO, extracorporeal membrane oxygenation; QMLT, quadriceps muscle layer thickness.

5.2 Baseline characteristics

Baseline characteristics of the patients are shown in **Table 5-1**. Gender distribution, age, height, and BW-PDMS did not differ between the groups, whereas diagnosis showed some variations (all data originally documented at ICU admission). Clinical scores and oBW calculated at study entry were comparable between the groups **(Tables 5-1 and 5-2, respectively)**. Within the study period (28 days), 6 patients died (intervention: n = 2; standard: n = 4; P = 0.378).

Table 5-1 Baseline characteristics of study patients.

Parameter	Total (n=42)	Intervention (n=21)	Standard (n=21)	P-values[1]
Sex, n (m/f)	30/12	15/6	15/6	1.0
Age (y)	65 ± 15^2	66 ± 16^2	64 ± 15^2	0.707
Height (cm)	174 ± 10^2	173 ± 9^2	176 ± 11^2	0.260
oBW (kg)	76 ± 9^2	75 ± 8^2	78 ± 10^2	0.249
BW-PDMS (kg)	91 ± 27^2	94 ± 28^2	89 ± 26^2	0.561
SAPS II	45 ± 11^2	46 ± 12^2	45 ± 10^2	0.801
TISS	20 ± 7^2	21 ± 7^2	19 ± 6^2	0.215
SOFA-score	7 ± 3^2	6 ± 3^2	8 ± 3^2	0.102
Sepsis	$36 (86)^3$	$19 (90)^3$	$17 (81)^3$	0.378

[1]P-values for between-group differences; [2]Data shown as mean ± SD; [3]Data shown as n (%). Abbreviations: m, male; f, female; y, years; oBW, 'optimal' body weight; BW-PDMS, body weight documented in the patient data management system; SAPS II, simplified acute physiology score II; TISS, therapeutic intervention scoring system; SOFA, sequential organ failure assessment.

Table 5-2 Distribution of diagnoses on ICU-admission among study groups[1].

Diagnosis	Total (n=42)	Intervention (n=21)	Standard (n=21)	P-values[2]
General surgery	7 (17)	2 (10)	5 (24)	**< 0.001**
Abdominal surgery	10 (24)	5 (24)	5 (24)	1.0
Urogenital diseases	3 (7)	3 (14)	0 (0)	**< 0.001**
ARDS[3]	11 (26)	3 (14)	8 (38)	**< 0.001**
Trauma surgery	4 (9)	3 (14)	1 (4)	**< 0.001**
Cardiovascular surgery	7 (17)	5 (24)	2 (10)	**< 0.001**

[1]Data shown as n (%); [2]P-values for between-group differences; [3]No bilateral infiltrates in the chest X-ray or CT scan and no need of inspiratory oxygen fraction (FiO_2) > 60 % at inclusion.
Abbreviation: ARDS, acute respiratory distress syndrome.

5.3 Determination of energy expenditure

Throughout the study period, daily energy targets were adjusted based on repeated IC measurement (REE-IC) or, in exceptional cases, by modification of disease-specific coefficients to calculate TEE according to the Harris-Benedict-equation (Harris and Benedict 1918), whereby oBW served as the respective factor (TEE-oBW).

In contrast, outside the present study, in routine clinical practice, EE was estimated using the Harris-Benedict-equation and the estimated BW (see Chapter 4.4). For comparison, **Table 5-3** shows the results of the several methods to determine BW (BW-PDMS, estimated by the physicians; oBW, calculated assuming a BMI of 25 kg/m²), BMR (Harris-Benedict-equation using either BW-PDMS [BMR-PDMS] or oBW [BMR-oBW]), TEE (Harris-Benedict-equation modified by disease-specific coefficients using either BW-PDMS [TEE-PDMS] or oBW [TEE-oBW]), and REE-IC (inclusive RQ value) at baseline (d 0).

Table 5-3 Baseline data of energy expenditure[1].

Parameter	Total	Intervention	Standard	P-values[2]
BW-PDMS (kg)	91 ± 27^3	94 ± 28^5	89 ± 26^5	0.561
oBW (kg)	76 ± 9^3	75 ± 8^5	78 ± 10^5	0.249
BMR-PDMS (kcal)	1702 ± 453^3	1719 ± 510^5	1686 ± 399^5	0.815
BMR-oBW (kcal)	1535 ± 216^3	1498 ± 206^5	1572 ± 225^5	0.274
Disease-specific coefficient	1.1 ± 0.2^3	1.1 ± 0.2^5	1.1 ± 0.2^5	0.813
TEE-PDMS (kcal)	1826 ± 464^3	1806 ± 447^5	1846 ± 492^5	0.787
TEE-oBW (kcal)	1673 ± 367^3	1616 ± 358^5	1731 ± 374^5	0.312
REE-IC (kcal)	2524 ± 725^4	2370 ± 705^6	2708 ± 741^7	0.288
RQ	0.70 ± 0.10^4	0.74 ± 0.05^6	0.82 ± 0.14^7	0.099

[1]Data shown as mean ± SD; [2]P-values for between-group differences; [3]n = 42; [4]n = 22; [5]n = 21; [6]n = 12; [7]n = 10.
Abbreviations: BW-PDMS, body weight documented in the patient data management system; oBW, 'optimal' body weight; BMR-PDMS, basal metabolic rate documented in the patient data management system; BMR-oBW, basal metabolic rate calculated using 'optimal' body weight; TEE-PDMS, total energy expenditure documented in the patient data management system; TEE-oBW, total energy expenditure calculated using 'optimal body weight'; REE-IC, resting energy expenditure measured by indirect calorimetry; RQ, respiratory quotient.

To compare the different methods of calculating the patients' target energy supply (TEE-PDMS, TEE-oBW, REE-IC), the results of a correlation analysis are shown in **Table 5-4**. Generally, there was a weak correlation between these methods.

Table 5-4 Correlation between different methods to determine patients' energy expenditure at baseline[1].

Method for determining energy expenditure	Method for determining energy expenditure		
	TEE-PDMS (n = 42)	TEE-oBW (n = 42)	REE-IC (n = 22)
TEE-PDMS			
- Correlation coefficient (Pearson)	1	0.686	0.449
- P-value	–	**< 0.001**	**0.036**
TEE-oBW			
- Correlation coefficient (Pearson)	0.686	1	**0.044**
- P-value	**< 0.001**	–	0.846
REE-IC			
- Correlation coefficient (Pearson)	0.449	**0.044**	1
- P-value	**0.036**	0.846	–

[1]Data shown for the overall study collective (total).

Abbreviations: TEE-PDMS, total energy expenditure documented in the patient data management system; TEE-oBW, total energy expenditure calculated using 'optimal' body weight; REE-IC, resting energy expenditure measured by indirect calorimetry.

## 5.4 Quantitative energy and nutrient intake

Energy intake in the intervention group strongly approaches the energy targets (predominantly determined by IC), whereas only 87 % of the target was achieved in patients with standard nutrition care (**Table 5-5 and Figure 5-2**).

Table 5-5 Energy and nutrient prescription and actual intake during the study period[1].

Parameter	Intervention (n=21)	Standard (n=21)	P-values[2]
Prescribed energy supply			
- kcal/kg BW/d	29.3 ± 7.4	28.7 ± 8.6	0.295
- kcal/d	2182 ± 644	2274 ± 732	**0.032**
Actual energy intake			
- kcal/kg BW/d	27.0 ± 8.9	24.6 ± 9.8	**< 0.001**
- kcal/d	1989 ± 655	1951 ± 828	0.403
- % of target	97 ± 34	87 ± 30	**< 0.001**
Prescribed protein supply			
- g/kg BW/d	1.8	1.2	**< 0.001**
- g/d	132.6 ± 14.9	94.9 ± 11.6	**< 0.001**
Actual protein intake			
- g/kg BW/d	1.5 ± 0.5	1.0 ± 0.4	**< 0.001**
- g/d	112.4 ± 35	81.8 ± 31.9	**< 0.001**
- % of target	86 ± 26	86 ± 12	0.667
Prescribed carbohydrate supply, (60 % non-protein energy)			
- g/kg BW/d	3.2 ± 1.1	3.5 ± 1.3	**< 0.001**
- g/d	239.7 ± 89.8	275.7 ± 104.6	**< 0.001**
Actual carbohydrate intake			
- g/kg BW/d	2.6 ± 0.9	2.8 ± 1.3	**0.025**
- g/d	192.2 ± 72.6	217.8 ± 111.6	**< 0.001**
- % of target	87 ± 35	81 ± 39	**0.007**
Prescribed fat supply (40 % of non-protein energy)			
- g/kg BW/d	0.9 ± 0.3	1.0 ± 0.4	**< 0.001**
- g/d	70.5 ± 26.4	81.0 ± 30.7	**< 0.001**
Actual fat intake			
- g/kg BW/d	1.0 ± 0.5	0.9 ± 0.4	**<0.001**
- g/d	76.0 ± 35.5	75.0 ± 37.3	<0.666
- % of target	119.4 ± 63.0	97 ± 47	**< 0.001**

[1]Data shown as mean ± SD throughout all study days; [2]P-values for between-group differences. Abbreviation: BW, body weight.
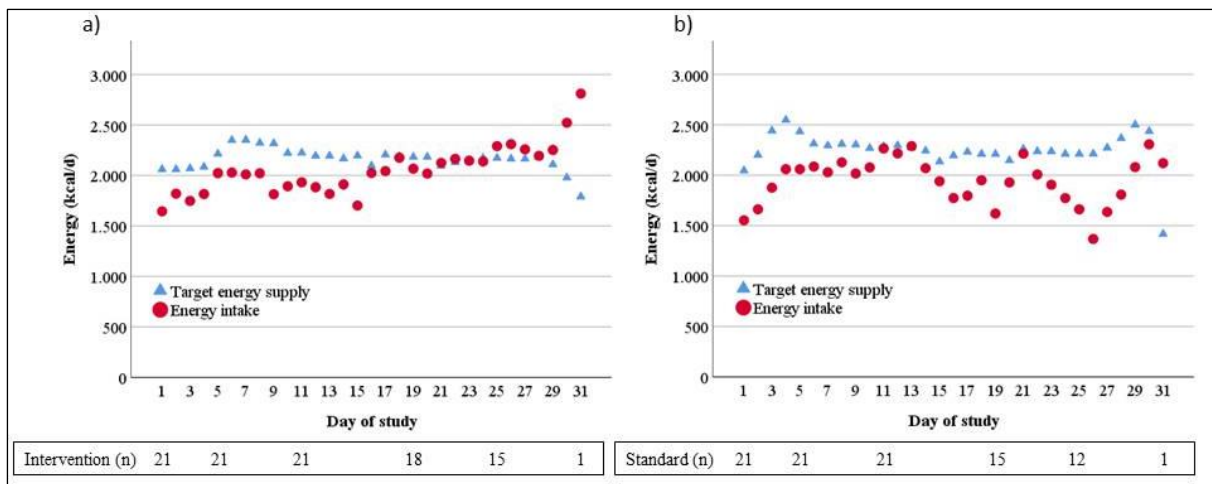
Figure 5-2 Mean target energy supply *vs.* energy intake (kcal/d) in the intervention (a) and standard (b) group during the study period (P = 0.403).

In both the groups, the actual macronutrient intake was 15–20 % lower than prescribed **(Table 5-5)** on most of the study days. As planned, absolute protein intake was significantly higher in the intervention group, whereby the prescribed target protein supply was not adequately met in both the groups **(Table 5-5 and Figure 5-3)**.
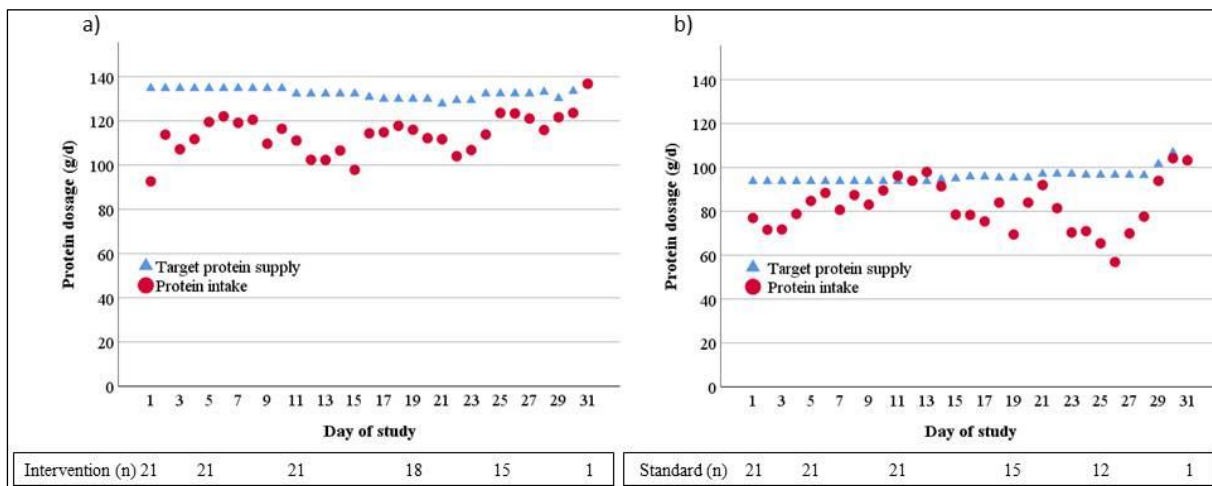
Figure 5-3 Mean target protein supply *vs.* protein intake (g/d) in the intervention (a) and standard (b) group during the study period (P < 0.001).

The prescribed target protein supply and actual intake related to g/kg BW/d during the study period are shown in **Figure 5-4**. Because of the recurrence in catabolic states of critical illness (ebb phase), sepsis, and high levels of serum urea, the protein target of 1.8 g/kg BW/d temporarily had to be reduced in some patients of the intervention group. In general, the prescribed target protein supply (intervention: 1.8 g/kg BW/d *vs.* standard: 1.2 g/kg BW/d) was

not met adequately in both the groups during the study period **(Table 5-5; Figures 5-3 and 5-4, respectively)**.
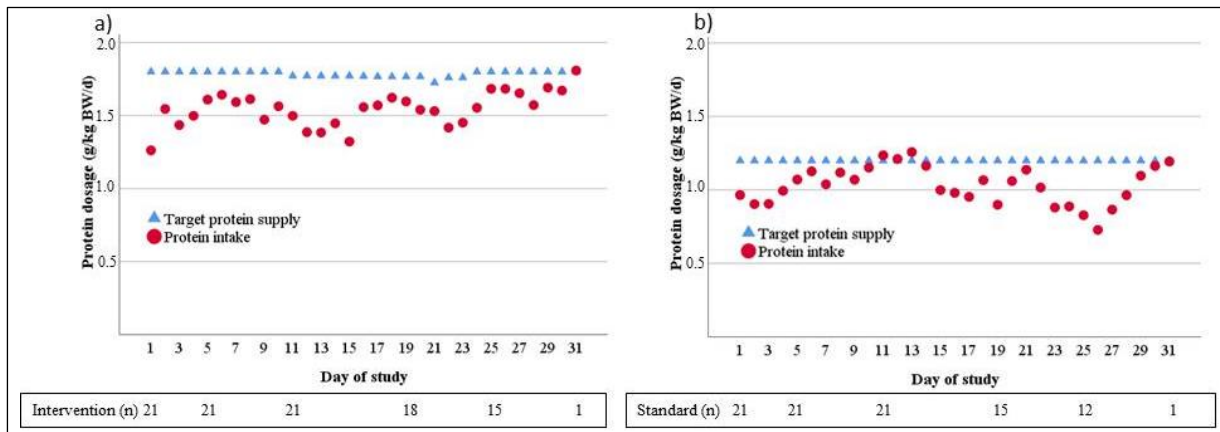
Figure 5-4 Mean target protein supply *vs.* protein intake (g/kg BW/d) in the intervention (a) and standard (b) group during the study period (P < 0.001).
Abbreviation: BW, body weight.

Compared to the intervention care, the standard care provided more carbohydrates and fat. However, prescribed carbohydrate and fat targets were not met adequately in both the groups **(Table 5-5; Figures 5-5 and 5-6, respectively)**.
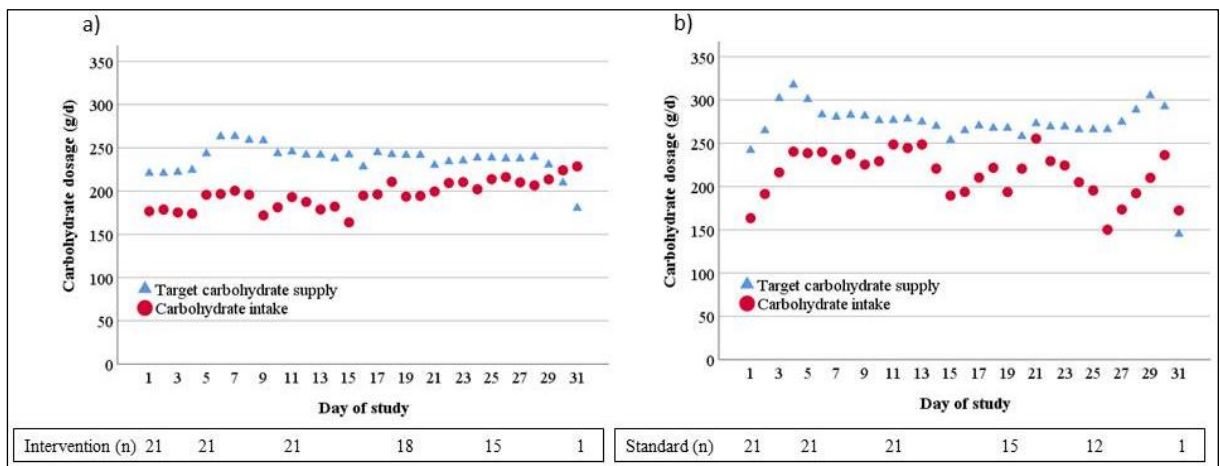
Figure 5-5 Mean target carbohydrate supply *vs.* carbohydrate intake (g/d) in the intervention (a) and standard (b) group during the study period (P < 0.001).
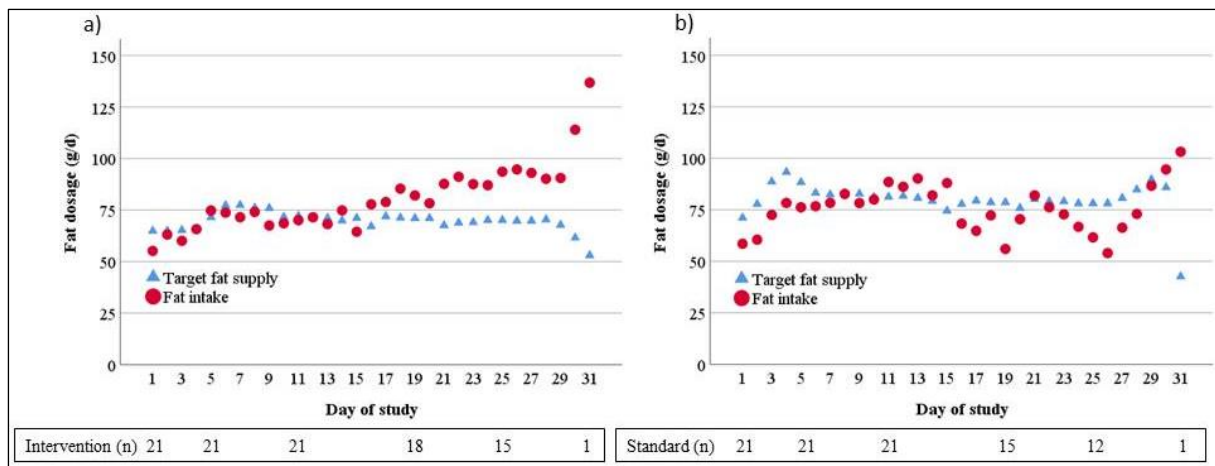
Figure 5-6 Mean target fat supply *vs.* fat intake (g/d) in the intervention (a) and standard (b) group during the study period (P < 0.001).

The discrepancies observed between target energy and nutrient supply, and actual intake occurred due to medical and organizational reasons (e.g., high GRV, inadequate implementation of prescribed nutrition regimes).

In order to meet the prescribed nutrition targets, all patients were fed both via enteral and parenteral route. Because commercial enteral formulas were provided with 'standard' amounts of protein, amino acids were often supplemented parenterally to meet protein targets. The difference in protein intake between the two groups was achieved by individual selection, dosage (ml/h), and combination (e.g., enteral and parenteral nutrition) of commercial products used in routine clinical practice. The percentage distribution of enteral and parenteral energy (E %) relative to total energy intake (100 E %) are shown in **Figure 5-7**; between-group differences were significant for both, the percentage distribution of enteral and parenteral energy intake (P < 0.001).
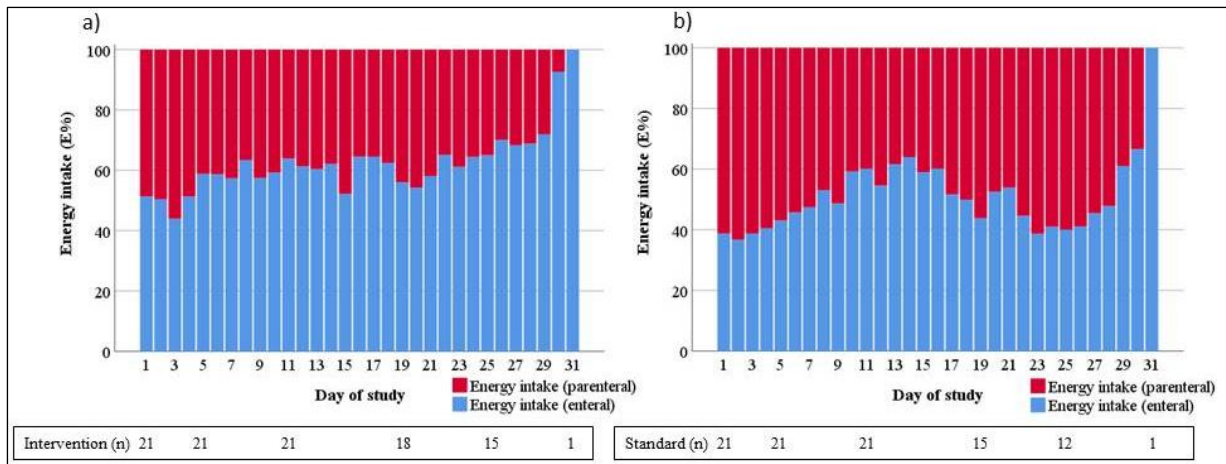
Figure 5-7 Percentage of enteral and parenteral energy intake (E %) in the intervention (a) and standard (b) group during the study period.
Abbreviation: E %, energy percent.

The percentage distribution of protein/amino acids, carbohydrates/glucose and fat/lipids administered via enteral and/or parenteral route as measured by the total intake are shown in **Figures 5-8, 5-9, and 5-10, respectively**. Whereas between-group differences for the proportion of enteral protein and parenteral amino acid intake did not reach significance (both $P = 0.294$), the amount of enteral carbohydrate and lipid intake, and the amount of parenteral glucose and fatty acid intake differed significantly between the groups (all $P < 0.001$).
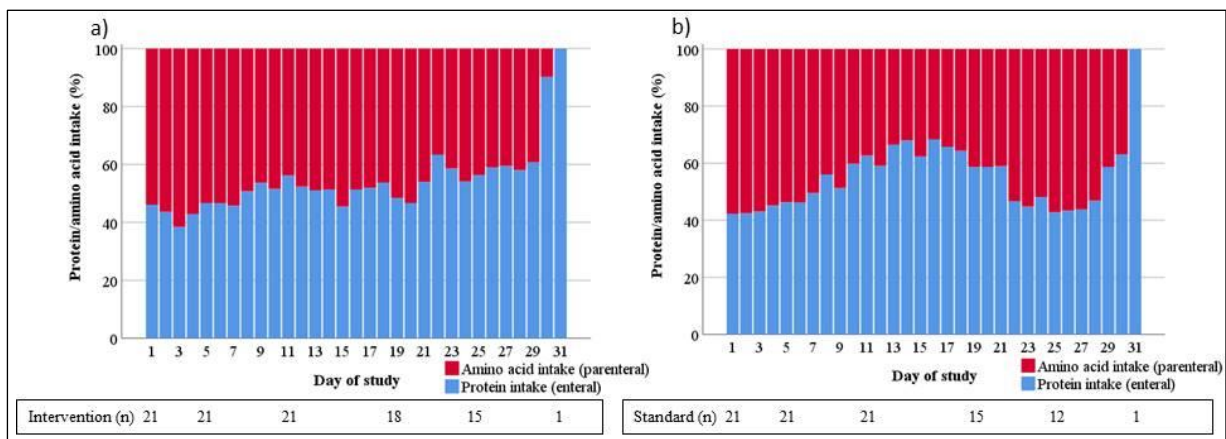
Figure 5-8 Percentage of enteral protein and parenteral amino acid intake (%) in the intervention (a) and standard (b) group during the study period.

Figure 5-9 Percentage of enteral carbohydrate and parenteral glucose intake (%) in the intervention (a) and standard (b) group during the study period.
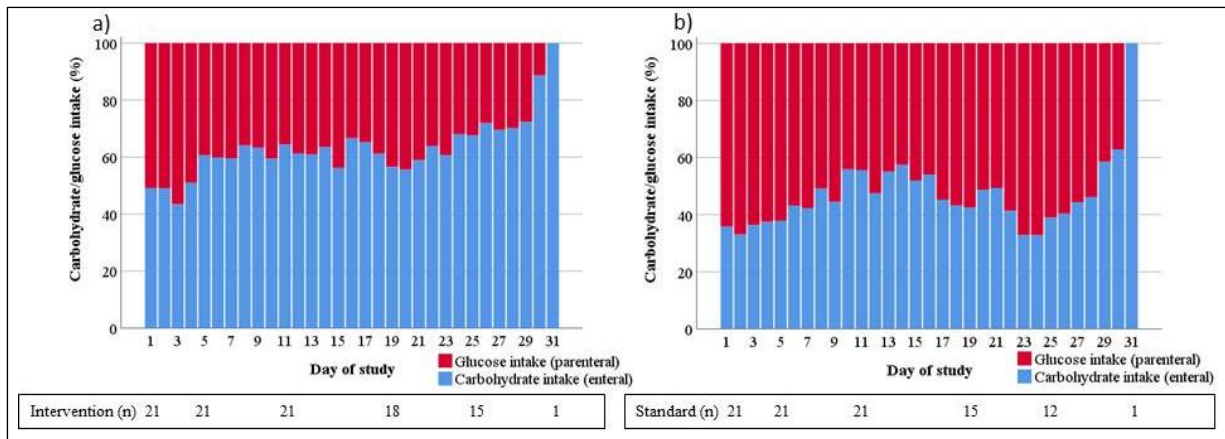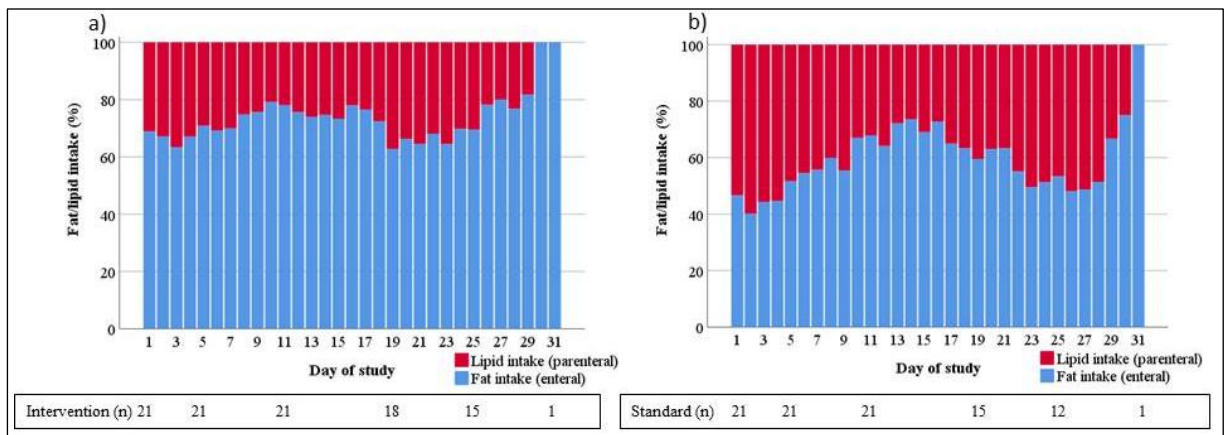

Figure 5-10 Percentage of enteral fat and parenteral lipid intake (%) in the intervention (a) and standard (b) group during the study period.

5.5 Qualitative protein intake

The products used for nutrition therapy showed a wide variety in terms of protein amounts (g/100 ml) and their amino acid profile **(Tables 4-1, 4-2, and 4-3, respectively)**. In addition, protein/amino acid supply depended on the patients' group allocation and oBW, which also lead to heterogeneity in the intake amounts of EAA, conditionally-EAA, and non-EAA.

Throughout the study period, intake of all the EAAs was significantly different (all $P < 0.001$) between the intervention and standard group **(Table 5-6)**.

Table 5-6 Essential amino acid intake during the study period[1].

Essential amino acids	Intervention (n = 21)	Standard (n = 21)	P-values[2]
Lysine			
- g/kg BW/d	0.12 ± 0.05	0.08 ± 0.03	**< 0.001**
- g/d	8.87 ± 2.01	6.68 ± 1.66	**< 0.001**
Threonine			
g/kg BW/d	0.07 ± 0.03	0.05 ± 0.02	**< 0.001**
- g/d	5.24 ± 1.25	3.90 ± 0.92	**< 0.001**
Methionine			
- g/kg BW/d	0.06 ± 0.02	0.04 ± 0.02	**< 0.001**
- g/d	4.22 ± 1.11	3.03 ± 0.68	**< 0.001**
Phenylalanine			
- g/kg BW/d	0.08 ± 0.03	0.05 ± 0.02	**< 0.001**
- g/d	5.76 ± 1.21	4.32 ± 0.99	**< 0.001**
Tryptophan			
- g/kg BW/d	0.02 ± 0.01	0.02 ± 0.01	**< 0.001**
- g/d	1.78 ± 0.40	1.30 ± 0.27	**< 0.001**
Valine			
- g/kg BW/d	0.01 ± 0.04	0.07 ± 0.03	**< 0.001**
- g/d	7.77 ± 1.69	5.87 ± 1.40	**< 0.001**
Leucine			
- g/kg BW/d	0.15 ± 0.06	0.10 ± 0.04	**< 0.001**
- g/d	11.03 ± 2.42	8.26 ± 1.91	**< 0.001**
Isoleucine			
- g/kg BW/d	0.08 ± 0.03	0.06 ± 0.02	**< 0.001**
- g/d	6.23 ± 1.38	4.67 ± 1.09	**< 0.001**

[1]Data shown as mean ± SD throughout all study days; [2]P-values for between-group differences.
Abbreviation: BW, body weight.

For conditionally-EAAs, the intake of the respective substances related to g/kg BW/d differed significantly between the groups, whereas the intake of tyrosine, cysteine, and glutamine related to g/d was the same **(Table 5-7)**.

Table 5-7 Conditionally essential amino acid intake during the study period[1].

Conditionally essential amino acids	Intervention (n = 21)	Standard (n = 21)	P-values[2]
Tyrosine			
- g/kg BW/d	0.05 ± 0.04	0.03 ± 0.03	**< 0.001**
- g/d	3.30 ± 2.10	2.86 ± 1.95	0.485
Cysteine[3]			
- g/kg BW/d	0.005 ± 0.005	0.003 ± 0.003	**< 0.001**
- g/d	0.34 ± 0.27	0.28 ± 0.19	0.384
Histidine			
- g/kg BW/d	0.05 ± 0.02	0.03 ± 0.01	**< 0.001**
- g/d	3.43 ± 0.72	2.55 ± 0.54	**< 0.001**
Arginine			
- g/kg BW/d	0.12 ± 0.06	0.08 ± 0.05	**< 0.001**
- g/d	9.16 ± 3.62	6.21 ± 2.40	**0.003**
Glutamine			
- g/kg BW/d	0.07 ± 0.07	0.05 ± 0.05	**< 0.001**
- g/d	5.11 ± 3.62	4.47 ± 3.32	0.553

[1]Data shown as mean $\pm$ SD throughout all study days; [2]P-values for between-group differences. [3]Calculated only from enteral nutrition, amount of acetylcytein of parenteral amino acid solution not applicable. Abbreviation: BW, body weight.

Throughout the study period, non-EAAs intake differed significantly between the intervention and standard group (all $P < 0.05$), except for serine related to g/d **(Table 5-8)**.

Table 5-8 Non-essential amino acid intake during the study period[1].

Non-essential amino acids	Intervention (n = 21)	Standard (n = 21)	P-values[2]
Glycine			
- g/kg BW/d	0.11 ± 0.07	0.07 ± 0.06	**< 0.001**
- g/d	8.47 ± 4.34	5.53 ± 3.04	**0.015**
Alanine			
- g/kg BW/d	0.11 ± 0.06	0.07 ± 0.04	**< 0.001**
- g/d	8.36 ± 3.39	5.65 ± 2.24	**0.004**
Proline			
- g/kg BW/d	0.12 ± 0.06	0.09 ± 0.04	**< 0.001**
- g/d	8.87 ± 2.55	6.98 ± 2.42	**0.018**
Serine			
- g/kg BW/d	0.07 ± 0.04	0.05 ± 0.03	**< 0.001**
- g/d	4.83 ± 1.75	3.87 ± 1.67	0.076
Glutamic acid			
- g/kg BW/d	0.16 ± 0.07	0.11 ± 0.05	**< 0.001**
- g/d	11.56 ± 3.18	9.00 ± 3.02	**0.011**
Aspartic acid and asparagine			
- g/kg BW/d	0.12 ± 0.05	0.08 ± 0.04	**< 0.001**
- g/d	8.71 ± 2.30	6.66 ± 2.08	**0.004**

[1]Data shown as mean $\pm$ SD throughout all study days; [2]P-values for between-group differences.
Abbreviation: BW, body weight.

Regarding the intake of BCAAs throughout the study period, there were significant differences between the groups, with the same ratio of representatives **(Table 5-9)**.

Table 5-9 Total branched-chain amino acid intake and ratio during the study period.

	Intervention (n = 21)	Standard (n = 21)	P-values[1]
Total BCAA (Valine, Leucine, Isoleucine)			
- g/kg BW/d	0.34 ± 0.13^2	0.24 ± 0.09^2	**< 0.001**
- g/d	25.02 ± 5.47^2	18.79 ± 4.39^2	**< 0.001**
Ratio (Valine : Leucine : Isoleucine)	1 : 1.4 : 0.8	1 : 1.4 : 0.8	–

[1]P-values for between-group differences; [2]Data shown as mean ± SD throughout all study days.
Abbreviations: BCAA, branched-chain amino acids; BW, body weight.

5.6 Skeletal muscle mass

Mean values of baseline (d 0) and follow-up QMLT at the different measurement points did not differ between the groups (**Table 5-10**). Patient-specific averages of all four QMLT measurement points at inclusion (d 0) were 13.5 ± 7.4 mm and 13.4 ± 7.1 mm in the intervention and standard group, respectively (P = 0.967). Follow-up measurements were performed as planned (at study day 16 ± 2: intermediate, intervention, n = 18, standard: n = 20; at study day 28 ± 2: final, intervention n = 15, standard: n = 12).

Table 5-10 QMLT at different time points of the study period[1].

Parameter	Inclusion measurement (d 0)			Intermediate measurement (d 16 ± 2)			Final measurement (d 28 ± 2)		
	Intervention (n = 21)	Standard (n = 21)	P-values[2]	Intervention (n = 18)	Standard (n = 20)	P-values[2]	Intervention (n = 15)	Standard (n = 12)	P-values[2]
Midpoint measurement, right (mm)	15.1 ± 8.5	14.2 ± 7.9	0.696	14.2 ± 8.2	14.2 ± 10.7	0.998	12.6 ± 7.5	13.1 ± 5.0	0.849
Midpoint measurement, left (mm)	14.1 ± 7.8[3]	14.4 ± 7.7	0.896	14.4 ± 7.8	14.1 ± 10.3	0.909	12.7 ± 8.0[3]	14.4 ± 6.7	0.556
Two-thirds measurement, right (mm)	12.2 ± 6.7	12.4 ± 7.3	0.923	12.5 ± 6.8	12.4 ± 9.3	0.957	11.5 ± 7.2	12.6 ± 5.9	0.677
Two-thirds measurement, left (mm)	12.0 ± 7.7[3]	12.4 ± 6.4	0.872	12.3 ± 6.6	12.4 ± 7.2	0.963	11.7 ± 8.0[3]	13.1 ± 7.0	0.638
Mean of all measurement points, right and left (mm)	13.5 ± 7.42	13.4 ± 7.2	0.967	13.4 ± 7.1	13.3 ± 9.2	0.972	12.2 ± 7.52	13.3 ± 6.0	0.667

[1]Data shown as mean ± SD; [2]P-values for between-group differences; [3]In one patient only measurements on the right leg possible.
Abbreviation: QMLT, quadriceps muscle layer thickness.

5.7 Effects of protein quantity on daily changes in skeletal muscle mass

Analysis of time and group effects using linear mixed model procedure showed a significant effect of time on muscle mass degradation (**Table 5-11**). Daily changes in QMLT at the respective measurement points for both the groups and calculated interaction effects (time; group; time x group) are shown in **Table 5-11**. Considering patient-specific averages of all four measurement points, estimated daily QMLT changes were -0.15 ± 0.08 mm and -0.28 ± 0.08 mm in the intervention and the standard group, respectively (time effect, $P < 0.001$; intervention effect, $P = 0.368$; time x intervention effect, $P = 0.242$). Accordingly, a mean decrease in QMLT of -3.4 ± 1.8 mm (30.4 ± 11.7 %) and -5.7 ± 2.5 mm (51.8 ± 21.1 %) within 28 days might be estimated for the intervention and standard group, respectively (time effect, $P = 0.008$; intervention effect, $P = 0.222$; time x intevention effect, $P = 0.17$). At all time and measurement points, absolute values were lower in the intervention group compared to the standard group and did not reach significance. Covariate adjustment (actual protein intake and adequacy) did not change the results (**Tables 5-12 and 5-13, respectively**). Accordingly, in both the groups, there were no effects of carbohydrate intake and adequacy, as well as fat intake and adequacy on daily QMLT changes (all $P > 0.05$).

Table 5-11 Estimated daily changes in QMLT and calculated interaction effects[1].

Parameter	Estimated daily QMLT changes		P-values from linear mixed models		
	Intervention	Standard	Group	Time	Group x Time
Midpoint measurement, right (mm)	-0.19 ± 0.13	-0.36 ± 0.12	0.372	**0.003**	0.349
Midpoint measurement, left (mm)	-0.18 ± 0.10	-0.24 ± 0.10	0.902	**0.005**	0.174
Two-thirds measurement, right (mm)	-0.13 ± 0.08	-0.28 ± 0.08	0.341	**0.001**	0.191
Two-thirds measurement, left (mm)	-0.08 ± 0.06	-0.21 ± 0.06	0.271	**0.002**	0.134
Mean of all measurement points, right and left (mm)	-0.15 ± 0.08	-0.28 ± 0.08	0.368	**< 0.001**	0.242

[1]Data shown as estimation ± SE.
Abbreviation: QMLT, quadricpes muscle layer thickness.

Table 5-12 Estimated daily changes in QMLT and calculated interaction effects; adjusted for actual protein intake[1].

Parameter	Estimated daily QMLT changes		P-values from linear mixed models				
	Intervention	Standard	Group	Time	Protein intake	Group x Time	Group x Protein intake
Midpoint measurement, right (mm)	-0.16 ± 0.14	-0.24 ± 0.13	0.913	**0.012**	0.835	0.350	0.409
Midpoint measurement, left (mm)	-0.15 ± 0.11	-0.25 ± 0.10	0.738	**0.013**	0.866	0.527	0.831
Two-thirds measurement, right (mm)	-0.11 ± 0.09	-0.26 ± 0.08	0.907	**0.004**	0.937	0.198	0.354
Two-thirds measurement, left (mm)	-0.06 ± 0.07	-0.22 ± 0.66	0.320	**0.007**	0.981	0.124	0.932
Mean of all measurement points, right and left (mm)	-0.12 ± 0.09	-0.27 ± 0.08	0.669	**0.002**	0.985	0.227	0.681

[1]Data shown as estimation ± SE.

Abbreviation: QMLT, quadriceps muscle layer thickness.

Table 5-13 Estimated daily changes in QMLT and calculated interaction effects; adjusted for protein adequacy[1].

Parameter	Estimated daily QMLT changes		P-values from linear mixed models					
	Intervention	Standard	Group	Time	Protein adequacy	Group x Time	Group x Protein adequacy
Midpoint measurement, right (mm)	-0.18 ± 0.13	-0.33 ± 0.13	0.749	**0.012**	0.619	0.412	0.762
Midpoint measurement, left (mm)	-0.16 ± 0.11	-0.23 ± 0.11	0.859	**0.014**	0.564	0.649	0.859
Two-thirds measurement, right (mm)	-0.11 ± 0.09	-0.26 ± 0.08	0.944	**0.005**	0.767	0.231	0.408
Two-thirds measurement, left (mm)	-0.07 ± 0.07	-0.21 ± 0.07	0.320	**0.007**	0.806	0.139	0.849
Mean of all measurement points, right and left (mm)	-0.13 ± 0.09	-0.26 ± 0.08	0.698	**0.003**	0.706	0.272	0.760

[1]Data shown as estimation ± SE.

Abbreviation: QMLT, quadriceps muscle layer thickness.

5.8 Effects of individual patient and medical characteristics combined with protein quantity on daily changes in skeletal muscle mass

Effects of sex, time, and group on daily QMLT changes

At two-thirds measurement right, there was significant interaction of group, time, and sex (ß [standard] = 0.490 ± 0.236, P = 0.047). After sex-specific separation (each group men: n = 15; women: n = 6), daily decrease in QMLT was higher in the standard group compared to the intervention group (men [standard]: ß = -0.045 ± 0.09; women [standard]: ß = -0.399 ± 0.250) not reaching significance in both men (P = 0.639) and women (P = 0.130).

For all other measurement points, no interactions between group, time, and sex were observed (all P > 0.05).

However, in all the cases, there was a significant effect of time (all P < 0.05) and, only for midpoint measurement left, sex (ß = 2.046 ± 0.999, P = 0.048).

Effects of height, time, and group on daily QMLT changes

Significant interaction of time and height on daily QMLT changes was observed at midpoint measurement right (ß = 0.016 ± 0.008, P = 0.043) and at midpoint measurement left (ß = 0.015 ± 0.006, P = 0.017).

Additionally, there was a significant interaction of group, time, and height at two-thirds measurement right (ß [standard] = 0.0229 ± 0.009, P = 0.015). When separated for groups, the interaction of time and height reached significance in the intervention group (ß = 2.034 ± 0.614, P = 0.005), but not in the standard group (ß = -0.124 ± 0.578, P = 0.833).

For the mean of all measurement points, a significant effect of group and time (ß [standard] = -0.216 ± 0.100, P = 0.038) as well as of intervention time and height (ß = 0.011 ± 0.005, P = 0.02) on daily QMLT changes was observed.

At two-thirds measurement left, no interactions of group, time, and height were found.

Additionally, in all the cases, there was a significant effect of time on daily QMLT changes (all P < 0.05).

Effects of age, time, and group on daily QMLT changes

Neither for the single measurement points nor the mean of all measurement points, interactions between age, time, and group reached significance (all P > 0.05). But, in all the cases, time alone significantly affected daily QMLT changes (all P < 0.05).

Effects of oBW, time, and group on daily QMLT changes

By examining oBW as additional covariate, significant interaction of time and oBW on daily QMLT changes was observed at midpoint measurement right (ß = 0.018 ± 0.009, P = 0.047) and midpoint measurement left (ß = 0.017 ± 0.007, P = 0.020).

For two-thirds measurement right, interaction of oBW, time, and group was significant (P = 0.019). After separation for groups, interaction of time and oBW reached significance in the standard group (ß = 0.023 ± 0.007, P = 0.007), but not in the intervention group (ß = -0.002 ± 0.007, P = 0.816).

Interaction of group and time (ß [standard] = -0.217 ± 0.101, P = 0.038) as well as of time and oBW reached significance for the mean of all measurement points (ß = 0.013 ± 0.005, P = 0.024).

In contrast, no interactions (all P > 0.05) were found for two-thirds measurement left.

Additionally, in all the cases, intervention time alone affected daily QMLT changes significantly (all P < 0.05).

Effects of baseline QMLT, time, and group on daily QMLT changes

Taking into account baseline QMLT at each measurement point, significant interaction with time and group (ß [standard] = -0.053 ± 0.015, P = 0.002) at midpoint measurement right was observed. After separation for groups, interaction of time and baseline QMLT was significant in the standard group (ß = -0.055 ± 0.011, P < 0.001), but not in the intervention group (ß = -0.001 ± 0.009, P = 0.890).

At midpoint measurement left, there was significant interaction of group, time, and baseline QMLT (ß [standard] = -0.037 ± 0.015, P = 0.018). In the standard group, interaction of time and baseline QMLT was significant (ß = -0.037 ± 0.011, P = 0.003), while no interaction was observed in the intervention group (ß = -0.024 ± 0.010, P = 0.974).

Interaction of group, time, and baseline QMLT reached significance at two-thirds measurement right (ß [standard] = -0.038 ± 0.012, P = 0.002). When separated for groups, significant interaction of time and baseline QMLT was observed in the standard group

(ß = -0.038 ± 0.009, P = 0.001), but not in the intervention group (ß = 0.003 ± 0.007, P = 0.688).

For the mean of all measurement points, significant interaction of group, time, and baseline QMLT (ß [standard] = -0.036 ± 0.011, P = 0.004) was found. In the standard group, interaction of time and baseline QMLT (ß = -0.034 ± 0.008, P = 0.001) reached significance, while there was no interaction in the intervention group (ß = 0.002 ± 0.007, P = 0.738).

No interactions (all P > 0.05) were found at two-thirds measurement left.

In all the cases, a significant effect of time alone on daily QMLT changes was observed (all P < 0.05). Additionally, in the intervention group, baseline QMLT affected daily QMLT changes significantly at midpoint measurement right (ß = -0.238 ± 0.101, P = 0.031) and at two-thirds measurement left (ß = -0.132 ± 0.052, P = 0.015).

Effects of diagnosis, time, and group on daily QMLT changes

Significant interaction of group and diagnosis (ß [standard] = -0.887 ± 0.436, P = 0.49) was found for two-thirds measurement left, whereas diagnosis alone did not affect daily QMLT changes (P = 0.063).

For midpoint measurement right (ß = 0.833 ± 0.319, P = 0.11), midpoint measurement left (ß = 0.648 ± 0.260, P = 0.018), two-thirds measurement right (ß = 0.617 ± 0.240, P = 0.014), and the mean of all measurement points (ß = 0.643 ± 0.220, P = 0.006) a significant effect of diagnosis alone was observed.

After separation for diagnosis, in the case of general surgery (intervention: n = 2; standard: n = 5), between group differences did not reach significance for none of the measurement points (all P > 0.05), whereas only for midpoint measurement right, a significant effect of time was observed (P = 0.035).

For abdominal surgery (intervention: n = 5; standard: n = 5), at two-thirds measurement right, significant interaction of time and group (ß [standard] = -0.622 ± 0.263, P = 0.046) was observed. Additionally, a significant effect of time alone was observed for midpoint measurement right, midpoint measurement left, two-thirds measurement right, and the mean of all measurement points (all P < 0.05).

In the case of ARDS (intervention: n = 3; standard: n = 8), there was a significant effect of group on daily QMLT changes at two-thirds measurement left (ß [standard] = -2.707 ± 1.1564, P = 0.043). For all the other measurement points, a significant effect of time, group, and their interaction did not reach significance (all P > 0.05).

In patients suffering from cardiovascular surgery (intervention: n = 5; standard: n = 2), a significant effect of time alone was proven for all the measurement points (all P < 0.05), whereas group alone and combined with time did not affect daily QMLT changes (all P > 0.05).

As urogenital diseases only occurred in the intervention group (n = 3) and there was only one case of trauma surgery in the standard group (*vs.* n = 3 in the intervention group), between group comparison could not be performed.

Effects of severity of illness, time, and group on daily QMLT changes

Taking TISS and SAPS II, respectively, into account, no interactions with time and group were found (all P > 0.05).

Moreover, no interactions were found between SOFA-score, time, and group at midpoint measurement right, two-thirds measurement right and left, as well as for the mean of all measurement points (all P > 0.05).

However, significant interaction of SOFA-score and intervention time was observed for the midpoint measurement left (ß = -0.066 ± 0.020, P = 0.002).

In all the cases, there was a significant effect of time alone on daily QMLT changes (P = 0.002).

Effects of administration of catecholamines, time, and group on daily QMLT changes

Except for midpoint measurement right (catecholamines x group: ß [standard] = -0.251 ± 0.117, P = 0.041), no significant interactions and no single effects of the number of days with administration of catecholamines, time, and group were observed (all P > 0.05).

Effects of duration of mechanical ventilation, time, and group on daily QMLT changes

No interactions and no single effects were found regarding the interaction of duration of mechanical ventilation, time, and group on daily QMLT changes (all P < 0.05).

Combined effects of individual patient and medical characteristics, time, and group on daily QMLT changes

Combined adjustment for individual patient and medical characteristics (sex, age, height, oBW, baseline QMLT, diagnosis, severity of illness [SAPS II, TISS, and SOFA-score], administration of catecholamines, and duration of mechanical ventilation), time, and group did not affect QMLT changes observed during the study period (all $P > 0.05$).

5.9 Biochemical markers

Routine laboratory diagnosis

The biochemical markers of the patients are highly individual and disease-specific. Therefore, a large heterogeneity of values was observed both within and between subjects during the study period. At baseline, no between-group differences in the respective parameters could be observed **(Table 5-14)**. Contrarily, throughout the study period, the mean values of some biochemical markers (e.g., total protein; urea, serum; creatinine; CK; highest blood glucose; highest and lowest lactate, respectively; and thrombocytes) differed significantly between the intervention and standard group (all $P < 0.05$) **(Table 5-15)**. At the end of the study period **(Table 5-16)**, biochemical parameters did not differ between the intervention and standard group (all $P > 0.05$), except for triglycerides and 25-OHD (both $P < 0.001$). Compared to baseline **(Tables 5-14 and 5-16, respectively)**, there was a statistically significant improvement in albumin (total [overall study collective]: $P < 0.001$; intervention: $P = 0.026$; standard: $P = 0.008$), total protein (total: $P = 0.007$; standard: $P = 0.027$), highest blood glucose (total: $P = 0.003$; intervention: $P = 0.021$), total bilirubin (standard: $P = 0.028$), calcium (total: $P = 0.001$; intervention: $P = 0.002$), and CRP (total: $P = 0.030$; intervention: $P = 0.032$). However, there was an increase in IL-6 (intervention: $P = 0.049$) and a decrease in erythrocytes (total: $P = 0.042$; intervention: $P = 0.013$) and hemoglobin (intervention: $P = 0.013$).

Table 5-14 Biochemical markers at study entry[1].

Parameter	Total	Intervention	Standard	P-values[2]
Albumin (g/l)	24 ± 4[3]	24 ± 4[4]	24 ± 4[5]	0.344
Total protein (g/l)	51 ± 7[6]	51 ± 7[7]	51 ± 7[8]	0.735
Urea, serum (mg/dl)	81 ± 35[9]	83 ± 32[8]	79 ± 39[8]	0.711
Creatinine, serum (mg/dl)	1.3 ± 0.7[9]	1.2 ± 0.7[8]	1.3 ± 0.8[8]	0.867
Creatine kinase (U/l)	99.5 ± 4694.0[10]	1608.0 ± 6048.0[11]	75.0 ± 92.0[12]	0.390
Highest blood glucose/d (mg/dl)	179 ± 43[13]	187 ± 50[14]	172 ± 36[8]	0.288
Lowest blood glucose/d (mg/dl)	126 ± 27[13]	130 ± 30[14]	123 ± 26[8]	0.438
Highest lactate/d, serum (mmol/l)	1.3 ± 0.4[6]	1.3 ± 0.3[7]	1.3 ± 0.5[8]	0.846
Lowest lactate/d, serum (mmol/l)	0.8 ± 0.3[6]	0.8 ± 0.3[7]	0.8 ± 0.3[8]	0.384
Triglycerides (mg/dl)	193 ± 96[15]	211 ± 102[16]	123[17]	0.495
ALT/GPT (U/l)	80 ± 129[9]	94 ± 151[8]	65 ± 105[8]	0.487
AST/GOT (U/l)	90 ± 166[9]	100 ± 209[8]	81 ± 113[8]	0.720
Total bilirubin (mg/dl)	2.1 ± 3.2[6]	1.5 ± 1.7[7]	2.6 ± 4.2[8]	0.304
Calcium (mmol/l)	2.09 ± 0.15[6]	2.06 ± 0.14[7]	2.13 ± 0.17[8]	0.148
$1,25(OH)_2D$ (pg/ml)	10 ± 5[15]	10 ± 5[16]	16[17]	0.341
25-OHD (ng/ml)	10 ± 8[18]	11 ± 9[15]	7.3[17]	0.734
C-reactive protein (mg/dl)	155 ± 120[7]	163 ± 139[12]	146 ± 98[20]	0.756
Procalcitonin (µg/l)	2.3 ± 3.8[6]	2.4 ± 3.4[8]	2.3 ± 4.3[7]	0.882
Interleukin-6 (ng/l)	113 ± 104[4]	151 ± 130[20]	71 ± 38[21]	0.114
Leukocytes (G/l)	15.0 ± 6.8[9]	16.2 ± 5.9[8]	13.8 ± 7.4[8]	0.260
Erythrocytes (G/l)	2.98 ± 0.34[9]	3.00 ± 0.34[8]	2.96 ± 0.35[8]	0.691
Thrombocytes (G/l)	290 ± 178[9]	262 ± 117[8]	318 ± 223[8]	0.316
Hemoglobin (g/dl)	8.7 ± 0.9[9]	8.7 ± 0.9[8]	8.6 ± 1.0[8]	0.671

[1]Data shown as mean $\pm$ SD; [2]P-values for between-group differences; [3]n = 33; [4]n= 17; [5]n =16; [6]n = 41; [7]n = 20; [8]n = 21; [9]n = 42; [10]n = 30; [11]n = 18; [12]n =12; [13]n = 40; [14]n = 19; [15]n = 5; [16]n = 4; [17]n = 1; [18]n = 6; [19]n = 11; [20]n = 9; [21]n = 8.

Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D3; 25-OHD, 25-hydroxyvitamin D3; ALT/GPT, alanine aminotrans-ferase/glutamate pyruvate transaminase; AST/GOT, aspartate aminotransferase/ glutamate oxaloacetate transaminase; U, unit; G, giga.

Table 5-15 Mean values of biochemical markers during the study period[1].

Parameter	Total (n = 42)	Intervention (n = 21)	Standard (n = 21)	P-values[2]
Albumin (g/l)	25 ± 6	25 ± 6	25 ± 5	0.508
Total protein (g/l)	51 ± 8	52 ± 9	50 ± 7	**< 0.001**
Urea, serum (mg/dl)	81 ± 50	91 ± 56	70 ± 42	**< 0.001**
Creatinine, serum (mg/dl)	1.3 ± 1.0	1.2 ± 1.0	1.3 ± 1.1	**0.038**
Creatine kinase (U/l)	1500.4 ± 10617.1	2271.4 ± 1405.7	560.9 ± 2703.7	**0.027**
Highest blood glucose/d (mg/dl)	169 ± 45	172 ± 46	166 ± 43	**0.015**
Lowest blood glucose/d (mg/dl)	119 ± 24	119 ± 24	119 ± 24	0.754
Highest lactate/d, serum (mmol/l)	1.5 ± 1.0	1.6 ± 1.1	1.5 ± 0.8	**0.005**
Lowest lactate/d, serum (mmol/l)	1.0 ± 2.0	1.1 ± 2.7	0.9 ± 0.5	**0.036**
Triglycerides (mg/dl)	196 ± 118	195 ± 119	196 ± 119	0.976
ALT/GPT (U/l)	72 ± 131	66 ± 132	77 ± 130	0.109
AST/GOT (U/l)	104 ± 331	104 ± 361	103 ± 297	0.944
Total bilirubin, (mg/dl)	2.2 ± 5.0	2.1 ± 3.7	2.3 ± 6.1	0.435
Calcium (mmol/l)	2.13 ± 0.20	2.13 ± 0.22	2.14 ± 0.18	0.393
$1,25(OH)_2D$ (pg/ml)	19 ± 15	20 ± 17	18 ± 14	0.560
25-OHD (ng/ml)	14 ± 9	14 ± 8	14 ± 9	0.638
C-reactive protein (mg/dl)	135 ± 114	137 ± 120	132 ± 107	0.710
Procalcitonin (µg/l)	4.2 ± 18.1	4.7 ± 20.8	3.5 ± 14.7	0.207
Interleukin-6 (ng/l)	106 ± 195	120 ± 198	91 ± 191	0.207
Leukocytes (G/l)	14.1 ± 6.8	14.3 ± 6.4	13.8 ± 7.2	0.088
Erythrocytes (G/l)	3.00 ± 0.54	3.01 ± 0.54	2.99 ± 0.55	0.649
Thrombocytes (G/l)	248 ± 143	233 ± 119	264 ± 164	**< 0.001**
Hemoglobin (g/dl)	8.7 ± 1.3	8.7 ± 1.2	8.7 ± 1.4	0.379

[1]Data shown as mean $\pm$ SD; [2]P-values for between-group differences.
Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D3; 25-OHD, 25-hydroxyvitamin D3; ALT/GPT, alanine aminotrans-ferase/glutamate pyruvate transaminase; AST/GOT, aspartate aminotransferase/ glutamate oxaloacetate transaminase; U, unit; G, giga.

Table 5-16 Biochemical markers at the end of the study period[1].

Parameter	Total	Intervention	Standard	P-values[2]
Albumin (g/l)	$27 \pm 5^{3,*}$	$26 \pm 4^{4,*}$	$29 \pm 5^{5,*}$	0.159
Total protein (g/l)	$59 \pm 102^{6,*}$	55 ± 10^{7}	$57 \pm 9^{5,*}$	0.633
Urea, serum (mg/dl)	83 ± 53^{8}	95 ± 61^{9}	71 ± 41^{10}	0.152
Creatinine, serum (mg/dl)	1.2 ± 1.1^{8}	1.0 ± 0.9^{9}	1.3 ± 1.2^{10}	0.360
Creatine kinase (U/l)	44.7 ± 41.7^{11}	48.8 ± 48.4^{12}	36.9 ± 25.7^{13}	0.526
Highest blood glucose/d (mg/dl)	$160 \pm 45^{14,*}$	$163 \pm 42^{15,*}$	158 ± 49^{15}	0.724
Lowest blood glucose/d (mg/dl)	122 ± 32^{14}	121 ± 30^{15}	124 ± 35^{15}	0.756
Highest lactate/d, serum (mmol/l)	1.7 ± 1.5^{14}	1.8 ± 2.0^{15}	1.5 ± 0.9^{15}	0.533
Lowest lactate/d, serum (mmol/l)	1.2 ± 1.3^{14}	1.4 ± 1.7^{15}	1.0 ± 0.9^{15}	0.415
Triglycerides (mg/dl)	102 ± 14^{16}	112^{17}	92^{17}	**< 0.001**
ALT/GPT (U/l)	58 ± 102^{8}	48 ± 27^{9}	71 ± 144^{10}	0.472
AST/GOT (U/l)	62 ± 88^{8}	56 ± 63^{9}	69 ± 110^{10}	0.634
Total bilirubin, gesamt (mg/dl)	2.2 ± 5.9^{18}	3.1 ± 7.8^{10}	$1.4 \pm 3.0^{10,*}$	0.358
Calcium (mmol/l)	$2.2 \pm 0.2^{8,*}$	$2.2 \pm 0.2^{9,*}$	2.2 ± 0.2^{9}	0.795
$1,25(OH)_2D$ (pg/ml)	41^{17}	–	41^{17}	–
25-OHD (ng/ml)	15 ± 8^{16}	9^{17}	20^{17}	**< 0.001**
C-reactive protein (mg/dl)	$91 \pm 88^{10,*}$	$84 \pm 77^{19,*}$	104 ± 110^{20}	0.633
Procalcitonin (µg/l)	1.3 ± 2.1^{18}	1.2 ± 2.0^{10}	1.4 ± 2.3^{10}	0.698
Interleukin-6 (ng/l)	144 ± 404^{9}	$51 \pm 23^{19,*}$	294 ± 651^{13}	0.188
Leukocytes (G/l)	14.1 ± 8.2^{8}	14.0 ± 6.1^{9}	14.3 ± 10.1^{10}	0.909
Erythrocytes (G/l)	$2.9 \pm 0.4^{8,*}$	$2.8 \pm 0.4^{9,*}$	2.9 ± 0.3^{10}	0.189
Thrombocytes (G/l)	233 ± 128^{18}	214 ± 97^{10}	252 ± 154^{10}	0.353
Hemoglobin (g/dl)	8.4 ± 1.0^{8}	$8.1 \pm 1.1^{9,*}$	8.7 ± 0.9^{10}	0.100

[1]Data shown as mean ± SD; [2]P-values for between-group differences. [3]n = 28; [4]n = 16; [5]n = 12; [6]n = 29; [7]n = 17; [8]n = 41; [9]n = 21; [10]n = 20; [11]n = 23; [12]n = 15; [13]n = 8; [14]n = 38; [15]n = 19; [16]n = 2; [17]n = 1; [18]n = 40; [19]n = 13; [20]n = 7; *Statistically significant difference compared to baseline (all P < 0.05).

Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D3; 25-OHD, 25-hydroxyvitamin D3; ALT/GPT, alanine aminotrans-ferase/glutamate pyruvate transaminase; AST/GOT, aspartate aminotransferase/ glutamate oxaloacetate transaminase; U, unit; G, giga.

Analysis of 24-h urine sampling

Because of the great difficulty in collecting 24-h-urine samples in routine clinical practice, urine protein, urine urea, urine creatinine, and nitrogen balance data are not sufficiently valid to perform an analysis that would allow reliable conclusions to be drawn about the possible effects of high-protein supply compared with standard nutritional care.

5.10 Clinical outcome

Nutrition intervention had no effects on ICU LOS and on illness scores, complication rates, and other secondary study outcomes **(Table 5-17)**. However, for the overall study collective (total), there was a statistically significant improvement in the clinical scores (SAPS II: P = 0.013; TISS: P < 0.001; SOFA-score: P = 0.015) compared to baseline **(Tables 5-1 and 5-17, respectively)**. Moreover, in the intervention group, there was a significant improvement in TISS (P = 0.003), while, in the standard group, this effect was observed for all the clinical scores (SAPS II: P = 0.037; TISS: P = 0.001; SOFA-score: P = 0.038).

Table 5-17 Illness scores and clinical outcome parameters at the end of the study period.

Parameter	Total	Intervention	Standard	P-values[1]
SAPS II[2]	37 ± 13[3,4,*]	37 ± 14[3,5]	37 ± 13[3,6,*]	0.978
TISS[2]	14 ± 8[3,4,*]	15 ± 8[3,5,*]	13 ± 7[3,6,*]	0.450
SOFA-score[2]	5 ± 4 [3,7,*]	5 ± 4[3,8]	5 ± 5[3,8,*]	0.616
Study days (d)[2]	25 ± 6[3,7]	26 ± 5[3,8]	24 ± 6[3,8]	0.241
ICU LOS (d)	65 ± 41[3,7]	68 ± 34[3,7]	62 ± 48[3,7]	0.605
Duration of mechanical ventilation (h throughout study period)[2]	777 ± 164[3,7]	797 ± 133[3,8]	758 ± 191[3,8]	0.457
Duration of mechanical ventilation (h throughout ICU stay)[9]	1361 ± 932[3,7]	1372 ± 642[3,8]	1350 ± 1170[3,8]	0.941
Necessity for CRRT during study period[2]	18 (43)[10]	10 (48)[8,10]	8 (38)[8,10]	0.533
Pneumonia during ICU stay[9]	36 (86)[7,10]	19 (90)[8,10]	17 (81)[8,10]	0.378
Wound infections during ICU stay[9]	22 (52)[7,10]	11 (52)[8,10]	11 (52)[8,10]	1.0
Death during ICU stay[9]	15 (36)[8,10]	8 (38)[8,10]	7 (33)[8,10]	0.747

[1]P-values for between-group differences; [2]Data recorded at the end of study period; [3]Data shown as mean ± SD; [4]n = 37; [5]n = 18; [6]n = 19; [7]n = 42; [8]n = 21; [9]Data recorded after ICU discharge/death; [10]Data shown n (%); *Statistically significant difference compared to baseline (all P < 0.05). Abbreviations: SAPS II, simplified acute physiology score II; TISS, therapeutic intervention scoring system; SOFA, sequential organ failure assessment; ICU, intensive care unit; LOS, length of stay; CRRT, continuous renal replacement therapy.

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6 Discussion

6.1 Effects of a medical high-protein intake on the loss of skeletal muscle mass in critically ill patients

Recent clinical observations had suggested a positive association between protein intake and beneficial effects on surrogate outcomes during critical illness (Allingstrup et al. 2017; van Zanten et al. 2018; de Azevedo et al. 2019; Danielis et al. 2019). Therefore, a prospective comparative RCT was conducted to test the hypothesis that higher protein intake (1.8 g/kg BW/d) than current recommendations (1.0 g protein/kg BW/d and 1.2 g amino acids/kg BW/d, respectively [Elke et al. 2018]; 1.3 g protein equivalents/kg BW/d [Singer et al. 2018]) could help maintain or even restore muscle mass in long-term ICU patients. In the context of individualized medical nutrition in the daily clinical routine practice, the study design compared two groups with 'standard' (1.2 g/kg BW/d) or 'high' (1.8 g/kg BW/d) protein supply. Although the study personnel intensively supported the study program, the implementation of the study protocol was occasionally hampered by individual gastrointestinal intolerances and necessary medical interventions (e.g., repeated surgical procedures, transports for diagnostic reasons such as CT and MRI, percutaneous coronary intervention [PCI], etc.). Consequently, the prescribed nutritional targets (e.g., energy controlled nutrition therapy, target protein supply) could not be fully achieved **(Table 5-5; Figures 5-2, 5-3, and 5-4, respectively)**. Nevertheless, as planned, the mean protein supply in the intervention group was 50 % higher than under the standard supply **(Table 5-5; Figures 5-3 and 5-4, respectively)**. This allowed a reliable comparative assessment of protein effects on the study outcomes.

Clinical research showed that ICU patients lose about 10 % of muscle mass during the first 10 days of ICU admission (Puthucheary et al. 2013). Throughout the study period, a significant time-dependent loss of QMLT both in the intervention and standard group without significant differences between groups was observed **(Table 5-11)**. Thus, the results of the present study confirm previous observations regarding the general time-dependent loss of muscle mass during critical illness (Puthucheary et al. 2013; Gamrin-Gripenberg et al. 2018).

Only a few comparative intervention studies have evaluated the effects of quantitative protein delivery on disease-specific muscle wasting in critically ill patients. *Fetterplace et al. (2018)* conducted a clinical trial (n = 60 ICU patients) whose primary objective was to evaluate the use of a volume-based enteral protocol with supplemental protein (initiated 48 hours after ICU admission) to significantly increase actual protein and energy intake in mechanically ventilated

critically ill patients compared with standard care. In line with their hypothesis, the mean protein intake in the intervention group reached 1.20 ± 0.30 g/kg BW/d, while, in the standard group, it was only 0.75 ± 0.11 g/kg BW/d. Thus, it can be assumed that this latter amount might be too low to cover metabolic needs in these patients. The attenuation of muscle loss observed in the intervention group compared with the standard group could be explained by a high protein/muscle breakdown rate in the standard group due to protein undernutrition. In a blinded RCT (n = 119 ICU patients), *Ferrie et al. (2016)* observed a greater forearm muscle thickness (ultrasound measurement defined as secondary objective) when the actual parenteral protein supply within the first seven days in the ICU was slightly increased (intervention: 1.1 ± 0.22 g/kg BW/d; standard: 0.9 ± 0.21 g/kg BW/d). These clinical trials provided moderate amounts of protein that was close to or even below the actual recommendations. Therefore, no conclusions can be drawn about the effects of a high-protein protocol on muscle mass. In contrast, *Nakamura et al. (2020)* conducted a randomized controlled trial (n = 117) to assess the effect of high-protein (1.8 g/kg BW/d) compared to medium-protein supply (0.9 g/kg BW/d) under equal energy intake (target defined per kg BW/d; nutrition therapy initiated within 48 hours after ICU admission) on the loss of femoral muscle volume without (n = 56) and in combination with active early rehabilitation (belt-type electrical muscle stimulation; n = 61). Concurrent to similar clinical trials, including the present study, the protein supply did not reach the target (median of actual intake: 1.5 g and 0.8 g/kg BW/d, respectively). The high-protein supply was associated with a lower loss of muscle mass (-12.9 ± 8.5 %) compared to the medium-protein group (-16.9 ± 7.0 %, P = 0.0059). Interestingly, subgroup analyses revealed that a high-protein supply was only effective when combined with active early rehabilitation. In consequence, the authors concluded that only a combination of 'high' protein supply and physical rehabilitation could help preserve muscle mass in ICU patients (Nakamura et al. 2020). However, because the studies mentioned above focused on high-protein supply in the early phase of critical illness, a comparison of the same with the results of the present study is limited. In general, it can be assumed that the effects of high-protein supply on the loss of muscle mass, which is particularly high in the early period after ICU admission (van Gassel et al. 2020), may be different from those observed in the late phase.

Regarding the time-dependent MPB during critical illness in more detail, *Gamrin-Gripenberg et al. (2018)* performed an observational trial (n = 20) to evaluate skeletal muscle protein and amino acid turnover in long-term ICU patients, without considering specific nutritional treatment protocols. Initially (days 10–20 after ICU admission), high rates of skeletal muscle

protein degradation were observed, whereas the mechanisms attenuated over time and appeared to be eliminated between days 30–40 after ICU admission. The authors concluded that in the later phase of stress metabolism disease-related MPB decreases, while at the same time MPS increases, and the ability of the patient's body to metabolize exogenous substrates may be correspondingly increased (Gamrin-Gripenberg et al. 2018). Thus, it might be hypothesized that high-protein supply in the early phase of critical illness can contribute to minimize MPB, while in the late phase it can support to further trigger MPS, reduce endogenous proteolysis, and preserve muscle mass (Weijs 2014; Davies et al. 2017; Koekkoek et al. 2018). These findings support the approaches of the present study to investigate the effects of high-protein supply on the loss of muscle mass in the later phase of critical illness.

6.2 Effects of individual patient characteristics, medication, medical treatment, and severity of illness combined with a medical high-protein supply on the loss of skeletal muscle mass

In general, the muscle status of the patients can be predicted by their individual characteristics (e.g., sex, age, height, BW, pre-hospital muscle mass, diagnosis) and, additionally, several medical treatment conditions (e.g., need for mechanical ventilation, severity of illness, administration of catecholamines) can trigger the muscle wasting during critical illness as well (Yang et al. 2018). Therefore, the effects of selected physical and medical characteristics of the study patients (sex, age, height, oBW, baseline QMLT, diagnosis, severity of illness, administration of catecholamines, and duration of mechanical ventilation) on daily QMLT changes throughout the study period in the intervention and standard group, respectively, were analyzed as well.

Regarding sex, a higher decrease in QMLT was observed in women of the standard group compared to women of the intervention group not reaching significance, while there was generally no difference in daily QMLT changes in men between the groups. While data of comparative studies directly addressing the influence of sex on the extent of muscle wasting in ICU patients are scarce, *Braunschweig et al. (2014)* observed an increased loss of muscle mass in women compared to men (11 % *vs.* 4 % within 10 days), assessed via CSA of the third lumbar vertebrae by CT. In this context, all patients received a target protein supply of 1.2 g/kg BW/d (Braunschweig et al. 2014). Although in the intervention group of the present study the target protein supply was set ≥ 1.2 g/kg BW/d (protein target: 1.8 g/kg BW/d; actual intake: 1.5 g/kg BW/d), a statistically significant difference between the women and men could not be shown.

In general, research suggests sex-specific differences in pathophysiological and biochemical mechanisms that lead to muscle wasting (Rosa-Caldwell and Greene 2019; Anderson et al. 2017) and overall, the progression of these mechanisms appears to be more rapid in women than in men (Lipes et al. 2013; Callahan et al. 2015). In addition, several structural differences in muscular phenotypes following chronic muscle disuse have been observed between men and women (Callahan et al. 2015). However, details about the exact mechanisms and on the extent to which they are further triggered by critical illness have to be elucidated.

In the present study, results regarding the effect of height on daily QMLT changes are inconsistent between the different measurement points (midpoint measurement right, midpoint measurement left, two-thirds measurement right, two-third measurement left, mean of all measurement points). Thus, a statistically significant interaction of height and time, which indicated higher muscle wasting over time in taller subjects compared with smaller subjects, without an effect of group allocation (high-protein supply *vs.* 'standard' nutritional care), was observed at midpoint measurements left and right. In contrast, significant effect of height on daily QMLT changes at two-thirds measurement right was found in the standard group, but not in the intervention group. Based on these results, on the one hand, it could be assumed that muscle wasting is generally lower in taller patients; on the other hand, this observation seems to be partially valid only for patients of the standard group, which might indicate a potential benefit of larger body size when receiving lower amounts of protein ($\leq$ 1.2 g/kg BW/d). However, no comparative data were found in the literature directly addressing the effect of body size on daily QMLT changes in ICU patients to futher discuss this observation.

The present trial did not demonstrate an interaction of patient age and time to daily QMLT changes. *Wandrag et al. (2019a)* also showed no significant correlation between age and loss of muscle mass (r = -0.04, P = 0.8) when analyzing loss of muscle depth in relation to metabolic and inflammatory markers by estimating patients' protein requirements at 1.2–1.5 g/kg/BW/d. Additionally, *Fetterplace et al. (2018)* observed a non-significant effect of age on QMLT in ICU patients (effect estimate, 0.02 $\pm$ 0.03; 95 % confidence interval [CI], -0.03–0.08; P = 0.44). In contrast, in the study of *Puthucheary et al. (2013)*, which aimed to characterize the pathophysiology of skeletal muscle wasting and impaired MPS and MPB after critical illness, age was associated with $\geq$ 10 % loss of the CSA of the rectus femoris at day 10 after ICU admission (odds ratio [OR], 1.05/year; 95 % CI, 1.01–1.07/year; P < 0.001). *Tanaka and Yamada (2020)* conducted a longitudinal observational study to examine changes in QMLT and potential effects on physical function in septic ICU patients. In this context, a negative

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correlation was found between muscle thickness and age (r = -0.71, P < 0.05) until day 14, so that increasing age of ICU patients seems to be associated with higher loss of skeletal muscle mass (Tanaka and Yamada 2020). In general, the current state of research shows a large heterogeneity in the results regarding the extent of muscle wasting in ICU patients in relation to age. However, in this context, literature regarding the principle mechanisms of age-related sarcopenia can also serve as a solid basis (Larsson et al. 2019). But, based on current data, it remains to be elucidated whether the underlying mechanisms are further triggered by critical illness. In fact, persisting sarcopenia at ICU-admission is identified as a risk factor for prolonged ICU LOS, longer duration of mechanical ventilation, extended rehabilitation, overall complicated medical treatment, and mortality (Moisey et al. 2013; Larsson et al. 2019).

Limited by the clinical conditions at the study setting, it was not possible to obtain data on the actual BW of the patients, therefore a so-called 'oBW' at BMI 25 kg/m² was calculated (see Chapter 4.3). Significant interaction of oBW and time with no treatment effect (high-protein supply *vs.* 'standard' nutritional care) was found at midpoint measurements right and left. Here, higher oBW was generally associated with a decrease in muscle wasting over time. Additionally, at two-thirds measurement right, a significant effect of oBW on daily QMLT changes was found in the standard group only, but not in the intervention group. In this case, muscle wasting decreased with higher oBW. Based on this observation it could be assumed that in patients receiving 'standard' protein supply (actual intake: 1.0 g/kg BW/d) higher oBW might be beneficial to attenuate muscle loss. As studies examining muscle wasting in critically ill patients predominantly consider patient BMI, data regarding the single effect of BW on QMLT changes are scarce. *Fetterplace et al. (2018)* showed a non-significant effect estimate of BMI on QMLT changes of 0.02 ± 0.09 (P = 0.82). However, in contrast to the present trial, the BMI was calculated using the actual BW determined by bed scales. Since in the present study the oBW was calculated assuming a BMI of 25 kg/m² in all patients due to the lack of bed scales, no actual BMI could be determined. Therefore, a comparison of the results of the present study with those of other studies and a valid statement regarding the effects of BW on QMLT changes during the study period are limited.

Generally, research suggests that low skeletal muscle quality and quantity at ICU admission are independent risk factors for mortality (Weijs et al. 2014). In the present study, a higher baseline QMLT was associated with increased muscle wasting in patients receiving standard protein supply (actual intake: 1.0 g/kg BW/d). Thus, these patients lost significantly more muscle mass throughout the study period than patients of the high-protein group (actual intake: 1.5 g/kg

BW/d) with higher baseline QMLT. *Reid et al. (2004)* observed increased muscle wasting in patients with higher initial muscle mass compared with patients with lower muscle mass, regardless of protein and energy intake. *Fetterplace et al. (2018)* observed that a higher baseline QMLT generally was associated with higher QMLT at ICU discharge (effect estimate, 0.61 ± 0.11; 95 % CI, 0.38–0.83; $P < 0.001$). Additionally, after adjusting for baseline QMLT, protein supply of 1.2 g/kg BW/d was associated with an attenuation in muscle loss of 0.22 cm (95 % CI, 0.06–0.38; $P = 0.01$) (Fetterplace et al. 2018). In the present study, daily QMLT changes were not affected by baseline QMLT in the intervention group, while higher baseline QMLT seemed to increase muscle wasting in the standard group. Based on this observation it could be assumed that higher daily protein intake (> 1.0 g/kg BW/d) might potentially help maintain muscle mass in patients with a greater amount of baseline QMLT.

Additionally, previous studies indicate that muscle wasting during ICU stay might be affected by diagnosis and severity of illness. Thus, high SOFA-score, neurologic failure, and MOV have been identified to increase the risk for muscle wasting and development of ICUAW (Schefold et al. 2020). Regardless of the actual protein intake (1.5 g/kg BW/d *vs.* 1.0 g/kg BW/d) the present analysis showed no effects of SAPS II and TISS on the extent of muscle wasting throughout the study period. However, there was significant interaction of SOFA-score and time at several measurement points, indicating a time-dependent increase in muscle wasting with higher actual SOFA-score. Because there are a variety of scores and methods for assessing patient severity of illness and disease status, comparison of the present data with those from previous studies is limited. *Fetterplace et al. (2018)* found no effect of acute physiology and chronic health evaluation (APACHE) III on QMLT changes (effect estimate, 0.02 ± 0.02; 95% CI, -0.02–0.06; $P = 0.44$). Accordingly, *Puthucheary et al. (2013)* did not observe any effects of APACHE II and SAPS II on QMLT changes by day 10. Contrarily, *Tanaka and Yamada (2020)* found a negative correlation between SOFA-score and QMLT by day 14 ($r = -0.72$, $P < 0.05$). Thus, increasing muscle wasting with higher severity of illness cannot be excluded in the present study.

Moreover, the results of the present study suggest that the extent of muscle wasting may vary between different admission diagnoses. In patients undergoing abdominal surgery or with ARDS, daily QMLT changes were significantly affected by the protein intake during the study period (higher daily QMLT changes in the standard group compared with the intervention group; $P < 0.05$), although only at some measurement points. Thus, in both diagnoses (abdominal surgery, ARDS), higher protein intake could be beneficial to minimize loss of

muscle mass during ICU stay. However, data of comparative studies are scarce, so the effect of a high-protein supply on the preservation of muscle mass in various diagnoses still remains questionable. Nevertheless, sepsis, inflammation, MOF, respiratory failure, trauma, and cancer have been identified as independent risk factors for muscle wasting in general (Schefold et al. 2010; Nakanishi et al. 2020; Schefold et al. 2020).

In addition to individual patient characteristics affecting muscle status, research suggests that various medical treatment conditions such as medication (e.g., administration of catecholamines), duration of mechanical ventilation, and overall immobilization are independent risk factors for muscle wasting and development of ICUAW during ICU stay (De Jonghe et al. 2002; Jolley et al. 2016; Wandrag et al. 2019a). As these aspects generally lead to catabolic states and paralyze muscle function, imbalances between muscle protein synthesis and breakdown are triggered, causing marked morphological and neurological changes in muscle structure and function (Nakanishi et al. 2020).

Regarding the patients' medication and respiratory treatment during the study period, no significant effects of catecholamine administration and duration of mechanical ventilation on daily QMLT changes were observed in both the groups in the present study. As several studies identified administration of catecholamines and duration of mechanical ventilation as independent risk factors for muscle wasting and development of ICUAW, modulation of catecholamine support in terms of the amount of specific agents (e.g., adrenaline, noradrenaline, dopamine, dobutamine, etc.) and modulation of ventilator settings (e.g., support of spontaneous breathing) could be predictive factors for the extent of muscle wasting (Thiele et al. 2000; De Jonghe et al. 2002; Nanas et al. 2008; Zambon et al. 2016). Therefore, these last aspects should be integral part of future studies.

In general, demonstrating the effects of individual factors in isolation may be complicated by the fact that muslce wasting during critical illness and the risk of developing ICUAW depend mainly on a combined effect of patients' physical and medical characteristics and may therefore be highly individualized (Yang et al. 2018). In this context, a retrospective data analysis of *Looijaard et al. (2019)* showed that low admission skeletal muscle area (SMA) was significantly more common in men, older, lighter patients with lower BMI, higher severity of illness, and longer hospital stay before ICU admission. Additionally, in patients with low SMA, early (day 2–4) high-protein intake (> 1.2 g/kg BW/d) was associated with a reduction in 60-day mortality. Due to the design of the study, changes in SMA throughout ICU stay were not monitored. Therefore, possible effects of individual patient characteristics on long-term

changes in SMA depending on the timing and dosage of target protein supply remain questionable (Looijaard et al. 2019). *Jaitovich et al. (2019)* showed that greater muscle mass was generally associated with better overall outcomes regardless of sex, although the magnitude of this association differed between men and women. *Joskova et al. (2018)* observed an association of low SMA compared with normal SMA with mortality in women (47.5 % *vs.* 20.0 %, P = 0.008) and in men (32.3 % *vs.* 7.5 %, P < 0.001). It remains unclear, whether a medical high-protein supply help preserve muscle mass and, thus, counteract the effects of individual patient characteristics and various medical treatment conditions that additionally influence muscle wasting. Therefore, future research should focus more precisely on these multi-factor influences on QMLT changes during critical illness combined with high-protein supply. In addition, clinical intervention studies that take into account the consideration of these patient characteristics in group allocation are recommended to further evaluate the impact of nutritive protein levels on QMLT in long-term critical illness.

6.3 Effects of a medical high-protein supply on biochemical markers in critically ill patients

Although there were no relevant differences regarding baseline anthropometric and clinical data between the intervention and standard group **(Table 5-1)**, there was a notable heterogeneity in admission diagnoses and disease pattern **(Table 5-2)**. In general, the severity of illness and the course of the disease are very individual. Consequently, biochemical markers of clinical routine analyzed throughout the study period demonstrated a large heterogeneity both within and between subjects **(Tables 5-14, 5-15, and 5-16, respectively)** and, thus, data interpretation is limited. Generally, there appeared to be an improvement in some parameters at the end of the study period (e.g., albumin [total, intervention, and standard], total protein [total and standard], highest blood glucose [total, intervention], total bilirubin [standard group], calcium [total, intervention], and CRP [total, intervention]), whereas deterioration was observed in other parameters (e.g., IL-6 [intervention], erythrocytes [total, intervention], and hemoglobin [standard]) compared with baseline. At the end of the study period, group differences did not reach significance, except for triglycerides and 25-OHD (both P < 0.05). However, it should be noted that biochemical markers were not analyzed consistently for each patient on all study days (including days of inclusion and end of study period), and no adjustment was made for missing values. Combined with the large heterogeneity of values both within and between subjects, these data are only marginally representative and, thus, no more comprehensive

analysis has been conducted in relation to the study intervention. Therefore, no valid statement can be made about the influence of a medical high-protein supply on the improvement of the patients' biochemical markers in general and the parameters of (muscle) protein metabolism in particular.

Even though research suggests that high-protein nutrition therapy can contribute to improve the nitrogen balance in critically ill patients (Danielis et al. 2019; Kim et al. 2020), current studies show large heterogeneity in the design (e.g., population, protein dosage, route of application [enteral and/or parenteral], measured outcome) and the results of different single biochemical markers (e.g., albumin, CK, CRP, IL-6). Because of the disease state and various medical treatment methods (e.g., CRRT, mechanical ventilation, medication), the response (e.g., metabolic, immunological) reflected by these parameters might be impaired and, thus, their validity is slightly limited. General statements that concern the effects of adequate nutrition support and especially of high-protein supply on biochemical markers in ICU patients cannot be made. In general, there is an urgend need to include new approches (e.g., DNA sequencing, proteomics, metabolomics, microbiome analyses) in routine laboratory analysis to validate biochemical markers that reflect the metabolic and immunological response to nutritional therapy in ICU patients (Stoppe et al. 2020).

However, the present study aimed to adapt the nutrition regimes regularly taking into account the individual disease- and phase-specific alterations in metabolism, monitored by several biochemical markers (e.g., blood glucose, lactate, triglycerides, serum urea). Despite great efforts to adapt the dietary regimes prescribed in the study protocol to individual patient metabolic changes, the challenges already experienced in comparative studies (Binnekade et al. 2005; Zusman et al. 2016; Sharma et al. 2019) in implementing an individual disease- and phase-specific nutrition protocol during critical illness were demonstrated.

As another secondary objective, the present study aimed to investigate the effect of a high-protein supply (actual intake: 1.5 g/kg BW/d) compared to a standard nutritional care (actual intake: 1.0 g/kg BW/d) on nitrogen balance by analyzing 24-h-urine samples collected regularly throughout the study period. However, due to organizational difficulties in samples collection within routine clinical practice, data of urine protein, urine urea, urine creatinine, and nitrogen balance are not reliable. Therefore, it was not possible to perform a data analysis that would allow a valid conclusion on the effects of protein quantity on nitrogen balance in the study patients. The difficulties of 24-h-urine sample collection in routine clinical practice have been widely described in previous studies (Dickerson 2016; Berger et al. 2019; Danielis et al. 2019).

Accordingly, the present study confirmed considerable need for action to optimize the implementation of 24-h-urine sample collections and nitrogen balances in routine clinical practice in order to monitor protein metabolism.

6.4 Effects of a medical high-protein supply on clinical outcome in critically ill patients

All clinical illness scores (SAPS II, TISS, and SOFA-score) improved with time compared to baseline in both the study groups, whereby significance was achieved in the overall study collective and the standard group, while it only applied for TISS in the intervention group **(Tables 5-1 and 5-17, respectively)**. However, between group differences were not significant at the end of the study period. Other clinical parameters, such as ICU LOS, duration of mechanical ventilation throughout the study period and ICU LOS, need for CRRT, and death during the study period, did not differ between the groups as well. In addition, no effect of 'high'- *vs.* 'standard'-protein supply on the occurrence of pneumonia and wound infections during ICU stay were observed **(Table 5-17)**. Thus, increasing protein delivery within an otherwise similar balanced medical nutrition therapy may not result in large improvements in clinical outcome. However, it could be assumed that a protein supply higher than the actual recommendation might be clinically safe and not further detrimental to patient outcome.

In general, comparative studies investigating the effects of different dosages of protein intake on patient clinical outcome show a large heterogeneity in study design (e.g., observational *vs.* interventional, dosage and timing of protein intake, hypo- *vs.* hypercaloric approach, measured outcome parameters) and report inconclusive results (Kutsogiannis et al. 2011; Weijs et al. 2012; Doig et al. 2015; Ferrie et al. 2016). A systematic review and meta-analysis published by *Davies et al. (2017)* indicated that different amounts of protein intake (0.67 ± 0.38 g *vs.* 1.02 ± 0.42 g/kg BW/d) were generally not associated with any effect on overall mortality, ICU and hospital LOS, duration of mechanical ventilation, and occurrence of infections during ICU stay. However, these amounts were obviously lower than those used in the present study and, thus, comparison of the study results is limited. *Song et al. (2017)* performed a cross-sectional observational study (n = 211) to investigate the effect of an adequate protein provision (target: 1.2–1.5 g/kg BW/d) on clinical outcome parameters (e.g., overall mortality, ICU and hospital LOS, duration of mechanical ventilation) in the early phase of critical illness. By achieving > 90 % of the prescribed target protein supply within the first 7 days after ICU admission, patients' clinical outcome was significantly improved compared to patients who did not reach adequate protein intake as defined by the investigators (Song et al.

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2017). In a prospective RCT (n = 138) *de Azevedo et al. (2019)* observed no differences in clinical outcome parameters (ICU LOS, duration of mechanical ventilation, ICU and hospital mortality) in ICU patients randomized either to a high-protein group (2.0–2.2 g protein/kg BW/d) or a control group (1.4–1.5 g protein/kg BW/d). Even though the daily protein intake was higher than in the present study, there was no further benefit or harm to clinical outcome parameters (de Azevedo et al. 2019). Thus, on the one hand, it could be concluded that a protein intake exceeding 1.5 g/kg BW/d, which has been achieved in the intervention group of the present study, might not further improve clinical outcome parameters. On the other hand, it could be hypothesized that high-protein intake might be generally safe as it did not negatively affect patients' outcome (de Azevedo et al. 2019).

However, the latter studies were predominantly concerned with the effect of different protein dosages on clinical outcome parameters administered in the early phase of critical illness. *Koekkoek et al. (2018)* performed a retrospective trial (n = 455 ICU patients) with the primary aim to investigate the optimal timing and dosage (< 0.8 g *vs.* 0.8–1.2 g *vs.* > 1.2 g/kg BW/d) of protein intake on outcome parameters such as need for CRRT, ICU and hospital LOS, mortality (6-month, ICU, hospital), and clinical scores (SOFA-score). Increasing protein intake from < 0.8 g/kg BW/d on days 1–2, to 0.8–1.2 g/kg BW/d on days 3–5, and up to > 1.2 g/kg BW/d from day 5 was associated with the lowest mortality, whereas other outcome parameters (ICU and hospital LOS, need for CRRT, duration of mechanical ventilation, SOFA-score) were not affected. In general, low protein intake (< 0.8 g/kg BW/d) was associated with increased mortality. Consistent with the present study, this trial also showed no significant improvement in most clinical parameters with higher protein intake, but it confirmed that even high-protein intake (> 1.2 g/kg BW/d) did not negatively affect clinical outcome of patients and could improve all-cause mortality, especially when administered in the later phase of critical illness (Koekkoek et al. 2018).

6.5 Nutrition therapy during the study period

As intended by the study protocol, 100 % of measured/calculated EE was aimed to be covered by enteral and/or parenteral nutrition. The rationale for this energy target was that patients were enrolled at approximately day 10 after ICU admission and, thus, the ebb phase of post-aggression metabolism should have been overcome (Singer et al. 2018). In this later phase of critical illness, metabolic functions are expected to be stabilized again leading to improved exogenic substrate utilization and reduced endogenic substrate production (Cuthbertson 1942).

Research suggests that 100 % energy feeding may be beneficial, whereas both hypo- and hypercaloric feeding appear to be associated with an unfavorable outcome (e.g., increased mortality) during this phase of disease (Zusman et al. 2016). Nevertheless, target energy supply could not be covered adequately throughout the study period **(Table 5-5 and Figure 5-2)**, as unpredictable disease-specific incidents (e.g., gastrointestinal intolerances, medical interventions) and organizational reasons (e.g., personnel and temporal difficulties) affected the implementation of the nutrition regimes prescribed in the study protocol.

In practice, target protein supply was not adequately covered by actual intake in both the groups during the study period **(Table 5-5; Figures 5-3 and 5-4, respectively)**. Moreover, achieving the target protein supply with the nutrition products used in routine clinical practice has been very challenging because of the large heterogeneity in their composition and the amounts of macronutrients they contain **(Tables 4-1, 4-2, 4-3, and 4-4, respectively)**. Thus, trying to reach patients' individual protein target using the products given might be reasonable for an inadequate provision of energy, carbohydrates, and fat (under- and overfeeding, respectively) during the study period **(Table 5-5; Figures 5-5 and 5-6, respectively)**. In this context, the discrepancies between target fat supply and actual intake might be caused by the use of a high-caloric enteral nutrition product, especially in the later study days. Although this product allowed providing high amounts of protein, which was in particular important to reach the protein target in the intervention group without SPN, it also contained high amounts of fat partially exceeding the calculated fat target. Thus, it is clear that achieving predefined nutritional goals with commercially available nutrition products is a major challenge in clinical practice.

Throughout the study period, the administration route (enteral, parenteral, or both) was decided daily upon physicians' assessment of the patients' clinical situation. Thus, on average, combined enteral and parenteral nutrition was provided throughout the whole study period, except for the last day **(Figures 5-7, 5-8, 5-9, and 5-10, respectively)**. In addition to medical and disease-specific reasons (e.g., patients' individual tolerance with respect to the dosage administered [ml/h]), this observation could be explained by the fact that the individually targeted energy and nutrient supply could not be sufficiently achieved only with the available enteral products of fixed composition.

In general, in the present study, a protein target of 1.8 g/kg BW/d was defined for the intervention group to exceed current German guidelines, which suggest 1.0 g protein/kg BW/d and 1.2 g amino acids/kg BW/d, respectively, and European guidelines, which recommend

1.3 g protein equivalents/kg BW/d (Elke et al. 2018; Singer et al. 2018). The dosages used in previous trials indicate possible beneficial effects with increasing protein supply on overall clinical outcome (Hoffer and Bistrian 2012; Arabi et al. 2020). However, throughout the study period, a mean protein intake of 1.5 g/kg BW/d was reached in the intervention group, while the standard group received about 1.0 g protein/kg BW/d. Therefore, the intervention group's protein intake was 50 % higher than it was under standard care. Additionally, the protein intake achieved in the intervention group was higher than that achieved in comparative studies investigating the effect of protein supply on muscle wasting (Ferrie et al. 2016; Fetterplace et al. 2018; Nakamura et al. 2020). Nevertheless, it should be noted that no differentiation between enteral and parenteral delivery was made for calculating patients' individual protein supply. Thus, the total protein supply did not consider a surcharge of approximately 17 % for parenteral administration to deliver amounts of protein mass equivalent to those from enteral application, as suggested by *Hoffer (2011)*.

Even though the present study focused predominantly on the effect of protein quantity on preservation of muscle mass, a possible effect of protein quality should not be excluded. Due to the variety of nutrition products used in the present study, there was a large heterogeneity in the amounts and sources of protein, and consequently in the amino acid-mixture **(Tables 4-1, 4-2, and 4-3, respectively)**. In general, the intake (g/kg BW/d) of EAAs, conditionally EAAs, and non-EAAs was significantly higher in the intervention group compared to the standard group **(Tables 5-6, 5-7, and 5-8, respectively)**. Accordingly, BCAA-intake was significantly higher in the intervention group compared to the standard group, whereas the ratio of valine, leucine, and isoleucine was equal in both the groups **(Table 5-9)**. Research suggests that the enrichment of medical nutrition products with EAAs in general, BCAAs in the ratio 1 : 2 : 1 (valine : leucine : isoleucine), HMB, taurine, and creatine (a metabolite of glycine, arginine, and methionine) might be effective to modify muscle protein metabolism and, thus, muscle wasting (Wandrag et al. 2015; Mitchell et al. 2016; Liu et al. 2019; Singer et al. 2020). However, studies to date are highly heterogeneous in aspects such as amino acid-mixtures and dosages used, study population (e.g., surgical diseases, chronic obstructive pulmonary diseases [COPD], chronic heart failure [CHF], sarcopenia, healthy people undergoing bed-rest, etc.), and outcomes measured (e.g., ICU LOS, functional outcomes, body composition, nitrogen balance, protein metabolism, overall quality of life) (Wandrag et al. 2015; Heyland et al. 2019; Wandrag et al. 2019b). Even though no valid recommendation regarding the optimal amount

and mix of amino acids can be made, all the EAAs, conditionally EAAs, and non-EAA should be part of a medical nutrition approach (Elke et al. 2018; Singer et al. 2018).

While the enteral preparations used throughout the study period provided all the EAA, non-EAA, and conditionally EAA, the parenteral amino acid solution did not contain glutamine. In this context, research suggests that parenteral nutrition supplemented with glutamine dipeptides (0.3 g/kg BW/d) within a balanced nutrition approach improves glutamine balance and thereby the clinical outcome in critically ill patients compared to standard parenteral nutrition (Goeters et al. 2002). However, because the patients in the present study received predominantly combined (enteral and parenteral) nutrition therapy, and as the parenteral amino acid solution did not contain glutamine dipeptides, the supply of amino acids in general may have been inadequate to some extent. This may have failed to improve nitrogen and protein metabolism, leading to the hypothesis that patient outcome would have been positively influenced by the use of a parenteral amino acid solution containing glutamine dipeptides (Stehle et al. 2017; Cruzat et al. 2018).

Additionally, by nature of the protein sources, the enteral products used for nutrition therapy in the present study contained taurine, but the exact amounts were not given by the product specifications. Thus, no hypothesis that concerns potential effects of taurine on (muscle) protein metabolism can be proposed for the intervention and standard group, respectively. Moreover, whilst the parenteral amino acid solution used for the nutrition therapy during the study period contained acetylcysteine, the exact amount was not applicable. Consequently, total intake of cysteine during the study period could only be calculated based on the enteral administration and, thus, may be slightly underestimated.

In general, when dietary regimes were implemented in the study setting in routine clinical practice, energy supply was quantified using the Harris-Benedict-equation and modification with disease-specific coefficients. In contrast, for study purposes, IC measurements were performed to determine the patients' actual EE in the present study. As shown in **Tables 5-3 and 5-4, respectively**, data of calculated target energy supply additionally documented from the PDMS throughout the study period differed considerably from the actual EE measured by IC. The weak correlation observed between the respective methods to determine the patients' EE **(Table 5-4)** demonstrates that the estimation by the Harris-Benedict-equation (originally developed for healthy adults) using the BW documented at the PDMS and the 'optimal' BW at BMI 25 kg/m², respectively, might be inadequate in this target group and, thus, IC measurements should be the preferred method. Accordingly, research suggests, that IC is the

most exact method to determine EE in critically ill patients because it can be performed bedridden and does measure the patients' metabolic response directly by covering all the influencing factors due to illness and medical treatment (Rousing et al. 2016). In general, implementation of IC measurements into routine clinical practice was feasible throughout the study period and it should therefore be used as standard practice to ensure adequate calculation of individual energy and nutrient supply (Delsoglio et al. 2019).

6.6 Ultrasound measurement of skeletal muscle mass

In routine clinical practice, QMLT measurement by ultrasound was feasible and easy to perform without much time or patient distress. Nevertheless, conduction of QMLT measurements according to the study protocol was partially impaired due to disease-specific and organizational reasons as well. Thus, in some cases, the measurement of QMLT could be biased by the disease-related position of patients, resulting in minimal differences from the optimal position for QMLT measurements described by *Tillquist et al. (2014)*. In addition, measurements were exceptionally performed by different physicians, which may also be a reason for variations in overall measurement technique and performance. While research suggests a great intra- and interrater reliability for ultrasound measurement of QMLT (Tillquist et al. 2014; Paris et al. 2017), marginal inaccuracies in the QMLT data acquisition throughout the study period cannot be excluded. Additionally, follow-up measurements were not always made at the days prescribed in the study protocol (days 14 and 28 after inclusion) due to organizational reasons. Therefore, comparison of muscle mass content two and four weeks after inclusion might be affected by heterogeneity in the actual timing of the respective measurements **(Table 5-10)**. However, by performing a linear mixed model procedure, changes in QMLT over time could be assessed to compensate for this non-compliance with the study protocol **(Tables 5-11, 5-12, and 5-13, respectively)**.

The protocol for QMLT measurement according to *Tillquist et al. (2014)* used for study purpose can be conducted easily and quickly, and should be implemented standardly in routine clinical practice. If abdominal CT scans have been obtained for other medical reasons, they should also be used to monitor skeletal muscle mass (Looijaard et al. 2018).

6.7 Further strategies to improve muscle status during critical illness

In the present study, possible effects of physical treatment on the loss of muscle mass (QMLT changes), the recovery process, and overall clinical outcome were not examined. But, besides nutrition therapy including an adequate supply of protein/amino acids, research shows that physical therapy during ICU stay is of special importance for preserving muscle mass in critically ill patients (Preiser et al. 2014; Gala et al. 2020; McKendry et al. 2020). In general, physical rehabilitation includes early mobilization (within 72 hours after ICU admission), limb exercising, and alternation of the patients' position during ICU stay (Bein et al. 2015; Jang et al. 2019). Early mobilization aims to attenuate muscle loss, to preserve muscle mass, to optimize peripheral muscle strength, and to improve respiratory, neurological, and cognitive functions. Thereby, clinical outcome parameters such as duration of mechanical ventilation, ICU LOS, incidence of ICUAW, and overall quality of life should be modified specifically (Eggmann et al. 2018; Jang et al. 2019). In this context, type of physical mobilization and timing might influence the effects on muscle mass preservation. Current research suggests that a combination of endurance (bed cycling) and resistance (administration of weights/resistance by therapist) training, electrical stimulation, and physical mobilization that begins very early (within 48 hours) after ICU admission, might be associated with an overall improvement in physical outcome of ICU patients compared to standard manual mobilization by specific handles of the physiotherapist alone (Gerovasili et al. 2009; Bein et al. 2015; Eggmann et al. 2018; Fontes Cerqueira et al. 2018; Hickmann et al. 2018). Hereby, adequate energy and nutrient supply, especially an adequate intake of protein/amino acids, seems to be essential for the effectiveness of physical treatment (Martindale et al. 2017; Phillips et al. 2017). In particular, supplementation of EAAs, conditionally EAAs, or their metabolites (e.g., citrulline, ornithine, HMB) combined with physical activity seems to be effective improving patients' rehabilitation (Jones et al. 2015; Gala et al. 2020; McKendry et al. 2020). Nevertheless, based on previous studies, no valid statement regarding the effects of protein quantity (various dosages) and quality (amino acid profile) combined with various types of physical treatment on outcome parameters such as QMLT changes, incidence of ICUAW, overall physical function, and quality of life in ICU patients can be defined (Wischmeyer et al. 2017). Therefore, these aspects should be focused in future research, especially by differentiating for several types of diagnosis and considering patient baseline and medical characteristics (e.g., severity of illness, duration of mechanical ventilation, medication).

6.8 Strengths and limitations

The strengths of this prospective RCT are its performance under realistic clinical conditions and the definition of individual SOP-guided nutrition protocols. These were adapted within short time intervals to ensure balanced energy and nutrient intake. Earlier technical experience with ultrasound technology under ICU conditions ensured reliable QMLT measurements. Moreover, the results of the previously conducted pilot study could be used to make valid calculations of sample size and power. Nevertheless, it cannot be ruled out that the effect size was likely overestimated: the estimated variation in QMLT expected on the basis of the pilot study (50 %) could not be shown.

Possible limitations included that the study might not be adequately powered for secondary outcome parameters because only the QMLT data obtained in the pilot study were used to perform the power calculation. In addition, there is the possibility that diagnoses at the time of admission vary between the groups **(Table 5-2)**. Difficulties in ensuring the correct locations for QMLT measurements (Tillquist et al. 2014) may have lead to minor inaccuracies and a wider spread of data (SD > 5.3 %), increasing the risk for lower sensitivity of the statistical test (probability of a type 2 error). Moreover, patients' characteristics like diagnosis, height, weight, and QMLT at baseline were not considered in group assignment. A clear limitation is that at the time of admission, bed-scales were not available, and 'optimal' body weights were calculated assuming a BMI of 25 kg/m² and taking into account the measured heights. Indeed, this might have led to some inaccuracies in computing individual nutrition protocols. Moreover, no differentiation between enteral or parenteral delivery was made. Thus, the total protein supply was calculated without considering a surcharge of approximately 17 % for parenteral administration, as suggested by *Hoffer (2011)*. Despite great efforts to implement daily nutrition intake prescribed in the nutrition protocol, energy and nutrient targets could not be fully achieved. Due to several medical and organizational reasons, follow-up measurements of QMLT were not always performed exactly two and four weeks after inclusion. Besides, in some patients, only one follow-up measurement could be performed (no adjustment for missing data). Regarding the biochemical markers monitored throughout the study period, there was a large heterogeneity both within and between subjects, and no adjustment for missing values has been performed. Therefore, interpretation of these parameters is limited. In addition, a reliable collection of 24-h urine samples could not be integrated into routine clinical practice. Thus, 24-h nitrogen excretion could not be calculated as planned. The trial was conducted in a single center, which might restrict its general implications.

7 Conclusion

In conclusion, our RCT did not achieve a statistically significant effect of an increased supply of protein (1.5 g *vs.* 1.0 g/kg BW/d) within a medical nutrition therapy in the late phase of critical illness on the loss of muscle mass in long-term immobilized ICU patients. However, individual patient and medical characteristics (sex, height, oBW, baseline QMLT, diagnosis, severity of illness, administration of catecholamines) were identified as potential risk factors for the extended muscle wasting during ICU stay, whereby there was large heterogeneity between the respective measurement points. The patients' biochemical parameters did not differ between the intervention and standard group at the end of the study period, except for triglycerides and 25-OHD. Compared to baseline, there were significant alterations in some biochemical markers (e.g., albumin, total protein, highest blood glucose, total bilirubin, calcium, CRP, IL-6, erythrocytes, and hemoglobin) at the end of the study period, whereby these effects cannot be strictly assigned to one of the study groups. As patients' biochemical parameters showed a large heterogeneity both within and between subjects throughout the study period, data interpretation is limited. In general, compared to baseline, there was an improvement in the illness scores (SAPS II, TISS, and SOFA-score) in both the groups. However, the high-protein nutrition intervention had no effects on LOS in ICU and on illness scores, complication rates (e.g., occurrence of pneumonia and wound infections), duration of mechanical ventilation, necessity of CRRT, and mortality. Thus, increasing protein delivery within an otherwise similar balanced medical nutrition therapy may not result in large improvements in clinical outcome. Nevertheless, a protein supply higher than actual recommendations (DGEM: 1.0 g protein/kg BW/d and 1.2 g amino acids/kg BW/d, respectively; ESPEN: 1.3 g protein equivalents/kg BW/d) was clinically safe and had no adverse effects on the patients' biochemical markers and clinical outcome.

In general, larger multi-center trials are needed to evaluate whether the observed numerical differences in muscle loss could be a true finding and translate into improved clinical outcomes. Sufficient statistical power should be achieved to evaluate potential effects of protein quantity on biochemical markers and clinical outcome parameters.

8 Outlook

Strategies to counteract the excessive loss of muscle mass generally occurring due to critical illness are of special importance to optimize the recovery process and to preserve the patients' quality of life. Thus, besides medical treatment, a combination of both adequate nutrition and physical therapy seems to be efficient to improve patients' overall outcome and especially their muscle status (Kou et al. 2019). Therefore, on the one hand, future research should investigate the effects of single amino acids and specific amino acid-mixtures on the preservation of muscle mass in more detail, using comparable methods and measuring standardized, comparable outcome parameters. On the other hand, different types of physical treatment (e.g., endurance and resistance training, electrical stimulation, and physical mobilization that begins very early after ICU admission) should be considered for their potential to preserve muscle mass in combination with adequate nutritional therapy. Additionally, the effects of individual patient and medical characteristics (e.g., sex, age, height, weight, baseline QMLT, severity of illness, diagnosis) on the extent of muscle wasting during critical illness should be examined in more detail as well. These approaches should help to generate comparative data, strengthen the evidence in this area, improve the patient outcome in general, and prevent the development of clinically relevant symptoms such as ICUAW (Wischmeyer et al. 2017).

With reference to the data of the present study, a further analysis will examine the effects of protein quality (amino acid profile) on the QMLT changes obtained throughout the study period. Moreover, the quality of the carbohydrate and fat intake during the study period will be examined in more detail. Additionally, nutrition therapy from ICU admission until study inclusion will be analyzed to evaluate the adequacy of energy and nutrient supply in the early phase of critical illness according to the internal SOP, current guidelines, and recent study results. Moreover, CT-scans of the patients are used when available to compare and validate the results of the ultrasound measurements.

9 Summary

Critical illness is characterized by substantial hormone- and cytokine-mediated changes in protein metabolism in various organs leading to both increased breakdown and decreased synthesis rates. In consequence, a considerable and life-threatening loss of muscle mass occurs. Medical therapeutic measures like long-term sedation and mechanical ventilation during the stay in an intensive care unit (ICU) can further enhance this muscle degradation, which can lead to clinically relevant symptoms such as ICU-acquired weakness (ICUAW). If left unabated, these conditions can severely impact the long-term patient outcome.

Besides targeted medication and exercise, a quantitatively higher protein intake (compared to actual recommendations for healthy adults) in critically ill patients might be reasonable to cover disease-specific increase in nitrogen/amino acid requirements and, thus, may contribute to overcome marked loss of functional proteins. Mainly based on observational studies, the European Society for Clinical Nutrition and Metabolism (ESPEN) strongly recommends a daily intake of 1.3 g protein equivalents/kg body weight (BW), while US-American guidelines suggest considerably higher protein quantities (1.2–2.0 g/kg BW/d). Only few randomized controlled trials evaluated the effects of higher (> 1.2 g/kg BW/d) protein/amino acid administration on patient outcome (e.g., morbidity, mortality, length of stay) and selected metabolic markers (e.g., nitrogen balance; urine urea, creatinine, and protein; glomerular filtration rate). But, due to the broad variations in the study designs (e.g., different patient populations, lack of individualized nutrition concepts including adequate energy supply, different route of administration), they report inconclusive results. Therefore, final recommendations for an adequate protein supply in critical illness cannot be made.

The primary aim of the present study was to evaluate whether a daily high-protein intake can significantly contribute to preserve or even regain muscle mass in long-term ICU patients. Secondary aims included the effects of high-protein/amino acid administration on nitrogen balance, frequency of clinical symptoms such as pneumonia and wound infections, and biochemical markers of routine laboratory diagnosis.

A randomized controlled trial was conducted in 42 critically ill patients (age 65 ± 15 years; 12 female; simplified acute physiology score [SAPS] II 45 ± 11; therapeutic intervention scoring system [TISS] 20 ± 7; sequential organ failure assessment [SOFA-] score 7 ± 3). The subjects were randomly assigned to either intervention (1.8 g protein/kg BW/d) or standard (1.2 g protein/kg BW/d) group. Nutrient supply via enteral and/or parenteral nutrition was

calculated based on the individual energy expenditure measured by indirect calorimetry and target protein content. The remaining non-protein energy was given as 60 energy percent (E %) carbohydrates and 40 E % fat; an adequate supply of all the necessary micronutrients was ensured. Muscle changes (quadriceps muscle layer thickness [QMLT]) were observed through sonography at inclusion and during the follow-up period, two (intermediate) and four (final) weeks after inclusion. The measurement points were fixed on two sides at midpoint and two-thirds between anterior superior iliac spine (ASIS) and top of the patella. Sample size and power calculation were based on a pilot study in hospitalized patients (detection limit: 5 % muscle mass changes; n = 42 with 80 % of power). The data were analyzed descriptively wherein chi-squared tests or unpaired two-sample t-tests checked for group differences. Daily changes in muscle mass were estimated using a linear mixed model. The data are shown as the mean ± standard deviation [SD] and estimation ± standard error (SE), respectively. Moreover, selected additional aspects of nutrition therapy (e.g., actual protein intake, protein adequacy) as well as individual patient and medical characteristics (e.g., sex, age, 'optimal' BW at BMI 25 kg/m² [oBW], height, baseline QMLT, disgnosis, severity of illness [SAPS II, TISS, SOFA-score], duration of mechanical ventilation, administration of catecholamines) were tested for affecting the extent of muscle mass loss.

Actual protein intake reached 1.5 ± 0.5 g and 1.0 ± 0.5 g/kg BW/d in the intervention and standard group, respectively. Mean values of all measurement points of QMLT at inclusion (d 0) were 13.5 ± 7.4 mm and 13.4 ± 7.1 mm in the intervention and standard group, respectively (P = 0.967). In both the groups, QMLT decreased over time (P < 0.001), while the estimated mean values of daily QMLT changes were -0.15 ± 0.08 mm (intervention) and -0.28 ± 0.08 mm (standard), without significant between-group differences (intervention effect, P = 0.368; time x intervention effect, P = 0.242). Illness scores and clinical outcome showed no group differences. One by one adjustment for individual patient and medical characteristics sex, age, and severity of illness did not change statistical evaluation (all P > 0.05), whereas for height (height x time, ß = 0.011 ± 0.005, P = 0.02; group x time, ß [standard] = -0.216 ± 0.100, P = 0.038), oBW (time x oBW, ß = -0.013 ± 0.005, P = 0.024; group x time, ß [standard] = -0.217 ± 0.101, P = 0.038), baseline QMLT (group x time x baseline QMLT, ß [standard] = -0.036 ± 0.011, P = 0.004; time x baseline QMLT, ß [standard] = -0.034 ± 0.008, P = 0.001), and diagnosis (ß = 0.643 ± 0.220; P = 0.006) significant effect was observed for the mean of all measurement points. Additionally, for the mean of all measurement points, medical parameters such as duration of mechanical ventilation

and administration of catecholamines did not affect QMLT changes throughout the study period (all P > 0.05). Combined adjustment for all the individual patient and medical characteristics (sex, age, height, oBW, baseline QMLT, diagnosis, severity of illness [SAPS II, TISS, SOFA-score], duration of mechnical ventilation, and administration of catecholamines) did not affect QMLT changes observed throughout the study period (P > 0.05). Moreover, at the end of the study period, biochemical parameters did not differ between the intervention and standard group (all P > 0.05), except for triglycerides and 25-hydroxyvitamin D3 (both P < 0.001). Compared to baseline there was a statistically significant (all P < 0.05) improvement in albumin (total, intervention, and standard), total protein (total and standard), highest blood glucose (total, intervention), total bilirubin (standard), calcium (total, intervention), and C-reactive protein (total, intervention). There was also an increase in interleukin-6 (intervention), and a decrease in erythrocytes (total, intervention) and hemoglobin (standard) (all P < 0.05). Additionally, nutrition intervention had no effects on LOS in ICU and on illness scores, complication rates (e.g., occurrence of pneumonia and wound infections), duration of mechanical ventilation, necessity of continuous renal replacement therapy (CRRT), and mortality. Nevertheless, at the end of the study period, there was an improvement in the clinical scores (SAPS II, TISS, and SOFA-score) compared to baseline, whereby significance was only reached in the overall study collective (all P < 0.05). Because of the great difficulty in collecting 24-h-urine samples in routine clinical practice, nitrogen balance data are not sufficiently valid and do not allow reliable conclusions to be drawn about the possible effects of high-protein supply compared with standard nutritional care.

In this single-center trial an increased supply of protein (1.5 g *vs.* 1.0 g/kg BW/d) within a medical nutrition therapy in the late phase of critical illness did not achieve a statistically significant effect on the loss of muscle mass in long-term immobilized ICU patients. Moreover, several individual patient and medical characteristics (e.g., height, oBW, baseline-QMLT, and diagnosis) were identified as potential risk factors for the extent of muscle wasting during ICU stay. In addition, the patients' biochemical markers and clinical outcome were not negatively affected by a medical high-protein nutrition therapy. Larger multi-center trials are needed to evaluate whether the observed numerical differences in muscle loss could be a true finding and translate into improved biochemical markers and clinical outcomes.

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10 Zusammenfassung

Kritische Erkrankungen sind durch erhebliche Hormon- und Zytokin-vermittelte Veränderungen im Proteinstoffwechsel verschiederner Organe charakterisiert, die zu einem erhöhten Abbau und einer verminderten Syntheserate führen. In Folge dessen kommt es zu einem beträchtlichen und lebensbedrohlichen Verlust von Muskelmasse. Medizinische Behandlungsmaßnahmen, wie lang andauernde Sedierung und maschinelle Beatmung, können den Muskelabbau zusätzlich verstärken und zur Entstehung von klinisch relevanten Symptomen wie der erworbenen Skelettmuskelschwäche (intensive care unit acquired weakness [ICUAW]) beitragen. Bleiben diese Faktoren unberücksichtigt, kann das Outcome der Patienten langfristig stark beeinträchtigt werden.

Neben zielgerichteter Medikation und körperlicher Aktivität kann eine quantitativ hohe Proteinaufnahme (im Vergleich zu aktuellen Empfehlungen für gesunde Erwachsene) bei kritisch Kranken notwendig sein, um den krankheitsspezifisch gesteigerten Stickstoff/Aminosäuren-Bedarf zu decken und somit dem hohen Verlust an Funktionsproteinen entgegenzuwirken. Die Europäische Gesellschaft für klinische Ernährung und Stoffwechsel (European Society for Clinical Nutrition and Metabolism [ESPEN]) empfiehlt eine tägliche Aufnahme von 1,3 g Protein-Äquivalenten/kg Körpergewicht/d, während US-amerikanische Leitlinien sogar wesentlich höhere Aufnahmen von 1,2–2,0 g/kg Körpergewicht/d empfehlen. Im Allgemeinen basieren diese Empfehlungen überwiegend auf den Ergebnissen von Beobachtungsstudien. Nur wenige randomisierte, kontrollierte Interventionsstudien untersuchten den Effekt einer quantitativ hohen Proteinzufuhr (> 1,2 g/kg Körpergewicht/d) auf das Outcome der Patienten (z. B. Morbidität, Mortalität, Aufenthaltsdauer auf der Intensivstation) und verschiedene Stoffwechselparameter (z. B. Stickstoffbilanz; Harnstoff, Kreatinin und Protein im Urin; glomeruläre Filtrationsrate). Aufgrund großer Heterogenität im Studiendesign (z. B. verschiedene Patientenkollektive, Mangel an individualisierten Ernährungskonzepten inklusive einer adäquaten Energiezufuhr, verschiedene Applikationswege) wurden jedoch keine einheitlichen Ergebnisse erzielt. Eine grundsätzliche Empfehlung zur adäquaten Proteinzufuhr bei Intensivpatienten kann daher nicht ausgesprochen werden.

Das primäre Ziel der vorliegenden Studie war es zu untersuchen, ob eine tägliche hohe Proteinaufnahme zum Erhalt bzw. zum Wiederaufbau der Muskelmasse von Intensivpatienten beitragen kann. Sekundäre Ziele beinhalteten die Untersuchung der Effekte einer hohen Protein-/Aminosäurenzufuhr auf die Stickstoffbilanz, Häufigkeit des Auftretens klinischer

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Symptome wie Pneumonien und Wundinfektionen sowie biochemische Parameter der Routinediagnostik im Vergleich zur Standard-Proteinzufuhr.

Es wurde eine randomisierte kontrollierte Studie an 42 Intensivpatienten (Alter: 65 ± 15 Jahre; 12 Frauen; Simplified Acute Physiology Score [SAPS] II 45 ± 11; Therapeutic Intervention Scoring System [TISS] 20 ± 7; Sequential Organ Failure Assessment [SOFA-] Score 7 ± 3) durchgeführt. Geeignete Patienten wurden in eine Interventions- (1,8 g Protein/kg Körpergewicht/d) und Standardgruppe (1,2 g Protein/kg Körpergewicht/d) randomisiert. Die Energiezufuhr erfolgte auf Basis des mittels indirekter Kalorimetrie gemessenen, aktuellen Energieumsatzes. Die Verteilung der nicht-Protein Energie auf Kohlenhydrate und Fette erfolgte im Verhältnis 60 : 40 Energie-Prozent (E %); eine ausreichende Versorgung an Mikronährstoffen war gewährleistet. Das Monitoring der individuellen Energie- und Nährstoffzufuhr erfolgte täglich. Das primäre Outcome, die Dicke des Musculus rectus femoris (quadriceps muscle layer thickness [QMLT]), wurde mittels Sonographie beidseitig an definierten Messpunkten zu Beginn der Studienphase sowie nach zwei (Zwischenmessung) und vier (Abschlussmessung) Wochen gemessen. Die Power-Kalkulation erfolgte auf Basis einer Pilotstudie an stationär behandelten Intensivpatienten (Nachweisgrenze: 5 % Muskelveränderung; n = 42; 80 %-ige Power). Die Auswertung der Daten erfolgte deskriptiv, wobei Gruppenvergleiche mittels ungepaarten zweiseitigen t-Tests bzw. Chi-Quadrat-Tests vorgenommen wurden. Die täglichen Veränderungen des Gehalts an Muskelmasse wurden mittels linear gemischtem Modell geschätzt. Die Daten sind als Mittelwert ± Standardabweichung (standard deviation [SD]) bzw. Schätzer ± Standardfehler (SE [standard error]) dargestellt. Darüber hinaus wurden zusätzliche Aspekte der Ernährungstherapie (z. B. tatsächliche Proteinaufnahme, prozentuale Proteinaufnahme gemessen am Proteinziel [Adäquanz]) sowie verschiedene individuelle Patienten- und medizinische Charakteristika (z. B. Geschlecht, Alter, optimales Körpergewicht bei BMI 25 kg/m², Größe, Baseline-QMLT, Diagnose, Krankheitsschwere, Dauer der maschinellen Beatmung, Catecholamingabe) hinsichtlich ihrer Effekte auf den Verlust von Muskelmasse untersucht.

Die tatsächliche Proteinaufnahme lag bei 1,5 ± 0,5 g und 1,0 ± 0,5 g/kg Körpergewicht/d in der Interventions- bzw. Standardgruppe. Zu Beginn der Studienphase (d 0) bestand zwischen den Gruppen kein Unterschied im Gehalt an Muskelmasse (Mittel aller Messpunkte; Intervention: 13,5 ± 7,4 mm, Standard: 13,4 ± 7,1 mm; jeweils n = 21; P = 0,967). In beiden Gruppen wurde über die Zeit eine signifikante Abnahme des Gehalts an Muskelmasse beobachtet (P < 0,001),

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wobei die tägliche Abnahme -0,15 ± 0,08 mm und -0,28 ± 0,08 mm in der Interventions- bzw. Standardgruppe betrug, ohne dass ein signifikanter Unterschied zwischen den Gruppen bestand (Interventionseffekt, P = 0,368; Zeit x Interventionseffekt, P = 0,242). Hinsichtlich der Krankheitsschwere und des klinischen Outcomes zeigten sich keine Unterscheide zwischen den Gruppen. Bei Adjustierung nach individuellen Patienten- und medizinischen Charakteristika erfolgte keine Veränderung der statistischen Auswertung durch die Faktoren Krankheitsschwere (SAPS II, TISS, SOFA-Score), Geschlecht und Alter (alle P > 0,05), während Größe (Größe x Zeit, ß = 0,011 ± 0,005, P = 0,02; Gruppe x Zeit, ß [Standard] = -0,216 ± 0,100, P = 0,038), optimales Körpergewicht (Zeit x optimales Körpergewicht, ß = -0,013 ± 0,005, P = 0,024; Gruppe x Zeit, ß [Standard] = -0,217 ± 0,101, P = 0,038), Baseline-QMLT (Gruppe x Zeit x Baseline-QMLT, ß [Standard] = -0,036 ± 0,011, P = 0,004; Zeit x Baseline-QMLT, ß [Standard] = -0,034 ± 0,008, P = 0,001) und Diagnose (ß = 0,643 ± 0,220; P = 0,006) einen signifikanten Einfluss auf die Muskelabnahme (Mittel aller Messpunkte) nahmen. Die Muskelabnahme (Mittel aller Messpunkte) wurde nicht durch klinische Parameter wie Dauer der maschinellen Beatmung und Catecholamingabe beeinflusst (alle P > 0,05). Die kombinierte Adjustierung nach allen individuellen Patienten- und medizinischen Charakteristika (Geschlecht, Alter, Größe, optimales Körpergewicht, Baseline-QMLT, Diagnose, Krankheitsschwere, Dauer der maschinellen Beatmung, Catecholamingabe) nahm keinen Einfluss auf die Muskelveränderungen während der Studienphase (P > 0,05). Des Weiteren zeigten sich zwischen der Interventions- und Standardgruppe, mit Ausnahme der Triglyceride und des 25-Hydroxy-Vitamin D3 (beide P < 0,001), keine signifikanten Unterschiede in den biochemischen Parametern am Ende der Studienphase (alle P > 0,05). Im Vergleich zur Baseline wurde eine signifikante Verbesserung (alle P < 0,05) verschiedener biochemischer Marker wie Albumin (Gesamt, Intervention, Standard), Gesamtprotein (Gesamt und Standard), höchste Blutglucose (Gesamt und Intervention), Gesamt-Bilirubin (Standard), Calcium (Gesamt, Intervention) und C-reaktives Protein (Gesamt, Intervention) erreicht. Gleichzeitig ließ sich ein Anstieg des Interleukin-6 (Intervention) und eine Abnahme des Gehalts an Erythrozyten (Gesamt, Intervention) und Hämoglobin (Standard) (alle P < 0,05) beobachten. Darüber hinaus nahm die Ernährungsintervention keinen Einfluss auf die Dauer des intensivmedizinischen Aufenthalts und der maschinellen Beatmung, Komplikationsraten (z. B. Auftreten von Pneumonien und Wundinfektionen), die Notwendigkeit zur Anwendung kontinuierlicher Nierenersatzverfahren sowie die Mortalität. Im Vergleich zur Baseline wurde im Gesamtkollektiv eine signifikante Verbesserung der klinischen Scores (SAPS II, TISS, SOFA-Score) beobachtet (alle P < 0,05). Aufgrund großer Schwierigkeiten bei der

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Durchführung von 24-h-Urinsammlungen im Klinikalltag sind die Daten der Stickstoffbilanz nicht ausreichend valide, um Aussagen über mögliche Effekte einer hohen Proteinzufuhr im Vergleich zur Standard-Therapie zu treffen.

In der vorliegenden Monozenterstudie wurde durch eine hohe Proteinzufuhr (1,5 g *vs.* 1,0 g/kg BW/d) innerhalb eines medizinischen Ernährungskonzepts in der Spätphase einer kritischen Erkrankung kein statistisch signifikanter Einfluss auf den Muskelverlust langliegender Intensivpatienten erreicht. Darüber hinaus wurden verschiedene individuelle Patienten- und medizinische Charakteristika (z. B. Diagnose, Größe, Gewicht, Baseline-QMLT) als potenzielle Risikofaktoren für das Ausmaß des krankheitsbedingten Muskelabbaus während der intensivmedizinischen Behandlung identifiziert. Weiterhin zeigte die hochdosierte Proteingabe keinen negativen Einfluss auf die biochemischen Marker und das klinische Outcome der Patienten. Es bedarf größerer Multizenterstudien, um die beobachteten, zahlenmäßigen Unterschiede im Verlust an Muskelmasse zu bestätigen und eine Verbesserung der biochemischen Marker und des klinischen Outcomes nachzuweisen.

11 References

Abe T, Kearns CF, Fukunaga T. Sex differences in whole body skeletal muscle mass measured by magnetic resonance imaging and its distribution in young Japanese adults. Br J Sports Med. 2003; 37 (5): 436–40. doi: 10.1136/bjsm.37.5.436.

Allingstrup MJ, Esmailzadeh N, Wilkens Knudsen A, Espersen K, Hartvig Jensen T, Wiis J, Perner A, Kondrup J. Provision of protein and energy in relation to measured requirements in intensive care patients. Clin Nutr. 2012; 31 (4): 462–8. doi: 10.1016/j.clnu.2011.12.006.

Allingstrup MJ, Kondrup J, Wiis J, Claudius C, Pedersen UG, Hein-Rasmussen R, Bjerregaard MR, Steensen M, Jensen TH, Lange T, Madsen MB, Møller MH, Perner A. Early goal-directed nutrition versus standard of care in adult intensive care patients: The single-centre, randomised, outcome assessor-blinded EAT-ICU trial. Intensive Care Med. 2017; 43 (11): 1637–59. doi:10.1007/s00134-017-4880-3.

Al-Tarrah K, Jones SW, Moiemen N, Lord JM. Potential role of adipose tissue and its hormones in burns and critically ill patients. Burns. 2020; 46 (2): 259–66. doi: 10.1016/j.burns.2019.01.012.

Anderson LJ, Liu H, Garcia JM. Sex Differences in Muscle Wasting. Adv Exp Med Biol. 2017; 1043: 153–97. doi: 10.1007/978-3-319-70178-3_9.

Arabi YM, Al-Dorzi HM, Sadat M. Protein intake and outcome in critically ill patients. Curr Opin Clin Nutr Metab Care. 2020; 23 (1): 51–8. doi: 10.1097/MCO.0000000000000619.

Aramendi I, Burghi G, Manzanares W. Dysglycemia in the critically ill patient: current evidence and future perspectives. Rev Bras Ter Intensiva. 2017; 29 (3): 364–72. doi: 10.5935/0103-507X.20170054.

B. Braun Melsungen AG. Parenteral Nutrition - Product Information (Product Labels). Melsungen; 2017.

Baldwin CE, Paratz JD, Bersten AD. Diaphragm and peripheral muscle thickness on ultrasound: Intra-rater reliability and variability of a methodology using non-standard recumbent positions. Respirology. 2011; 16 (7): 1136–43. doi: 10.1111/j.1440-1843.2011.02005.x.

Barton RN. Neuroendocrine mobilization of body fuels after injury. Br Med Bull. 1985; 41 (3): 218–25. doi: 10.1093/oxfordjournals.bmb.a072054.

Bein T, Bischoff M, Brückner U, Gebhardt K, Henzler D, Hermes C, Lewandowski K, Max M, Nothacker M, Staudinger T, Tryba M, Weber-Carstens S, Wrigge H. S2e guideline: positioning and early mobilisation in prophylaxis or therapy of pulmonary disorders: Revision 2015: S2e guideline of the German Society of Anaesthesiology and Intensive Care Medicine (DGAI). Anaesthesist. 2015; 64 (Suppl 1): 1–26. doi:10.1007/s00101-015-0071-1.

Bendavid I, Zusman O, Kagan I, Theilla M, Cohen J, Singer P. Early Administration of Protein in Critically Ill Patients: A Retrospective Cohort Study. Nutrients. 2019; 11 (1): 106. doi: 10.3390/nu11010106.

Berger MM, Reintam-Blaser A, Calder PC, Casaer M, Hiesmayr MJ, Mayer K, Montejo JC, Pichard C, Preiser JC, van Zanten, AR. Monitoring nutrition in the ICU. Clin Nutr. 2019; 38 (2): 584–93. doi: 10.1016/j.clnu.2018.07.009.

Bertsch S, Lang CH, Vary TC. Inhibition of glycogen synthase kinase 3beta activity with lithium in vitro attenuates sepsis-induced changes in muscle protein turnover. Shock. 2011; 35 (3): 266–74. doi: 10.1097/SHK.0b013e3181fd068c.

Binnekade JM, Tepaske R, Bruynzeel P, Mathus-Vliegen EMH, de Hann RJ. Daily enteral feeding practice on the ICU: attainment of goals and interfering factors. Crit Care. 2005; 9 (3): R218–25. doi: 10.1186/cc3504.

Bloch S, Polkey MI, Griffiths M, Kemp P. Molecular mechanisms of intensive care unit-acquired weakness. Eur Respir J. 2012; 39 (4): 1000–11. doi: 10.1183/09031936.00090011.

Bodine SC, Stitt TN, Gonzalez M, Kline WO, Stover GL, Bauerlein R, Zlotchenko E, Scrimgeour A, Lawrence JC, Glass DJ, Yancopoulos GD. Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo. Nat Cell Biol. 2001; 3 (11): 1014–9. doi: 10.1038/ncb1101-1014.

Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. Am J Physiol Endocrinol Metab. 2014; 307 (6): E469–84. doi: 10.1152/ajpendo.00204.2014.

Bogorad AM, Lin KY, Marintchev A. eIF2B Mechanisms of Action and Regulation: A Thermodynamic View. Biochemistry. 2018; 57 (9): 1426–35. doi: 10.1021/acs.biochem.7b00957.

Braunschweig CA, Sheean PM, Peterson SJ, Gomez Perez S, Freels S, Troy KL, Ajanaku FC, Patel A, Sclamberg JS.; Wang Z. Exploitation of diagnostic computed tomography scans to assess the impact of nutrition support on body composition changes in respiratory failure patients. JPEN J Parenter Enteral Nutr. 2014; 38 (7): 880–5. doi: 10.1177/0148607113500505.

Braunschweig CL, Levy P, Sheean PM, Wang X. Enteral compared with parenteral nutrition: a meta-analysis. Am J Clin Nutr. 2001; 74 (4): 534–42. doi: 10.1093/ajcn/74.4.534.

Bröer S, Bröer A. Amino acid homeostasis and signalling in mammalian cells and organisms. Biochem J. 2017; 474 (12): 1935–63. doi: 10.1042/BCJ20160822.

Brook MS, Wilkinson DJ, Atherton PJ. Nutrient modulation in the management of disease-induced muscle wasting: evidence from human studies. Curr Opin Clin Nutr Metab Care. 2017; 20 (6): 433–9. doi: 10.1097/MCO.0000000000000413.

Calder PC, Adolph M, Deutz NE, Grau T, Innes JK, Klek S, Lev S, Mayer K, Michael-Titus AT, Pradelli L, Puder M, Vlaardingerbroek H, Singer P. Lipids in the intensive care unit: Recommendations from the ESPEN Expert Group. Clin Nutr. 2018; 37 (1): 1–18. doi: 10.1016/j.clnu.2017.08.032.

Callahan DM, Tourville TW, Miller MS, Hackett SB, Sharma H, Cruickshank NC, Slauterbeck JR, Savage PD, Ades PA, Maughan DW, Beynnon BD, Toth MJ. Chronic disuse and skeletal muscle structure in older adults: sex-specific differences and relationships to contractile function. Am J Physiol Cell Physiol. 2015; 308 (11): C932–43. doi: 10.1152/ajpcell.00014.2015.

Casaer MP, Mesotten D, Hermans G, Wouters PJ, Schetz M, Meyfroidt G, van Cromphaut S, Ingels C, Meersseman P, Muller J, Vlasselaers D, Debaveye Y, Desmet L, Dubois J, van Assche A, Vanderheyden S, Wilmer A, Van den Berghe G. Early versus Late Parenteral Nutrition in Critically Ill Adults. N Engl J Med. 2011; 365 (6): 506–17. doi: 10.1056/NEJMoa1102662.

Compher C, Chittams J, Sammarco T, Nicolo M, Heyland DK. Greater Protein and Energy Intake May Be Associated With Improved Mortality in Higher Risk Critically Ill Patients: A Multicenter, Multinational Observational Study. Crit Care Med. 2017; 45 (2): 156–63. doi: 10.1097/CCM.0000000000002083.

Cruzat V, Macedo Rogero M, Noel Keane K, Curi R, Newsholme P. Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. Nutrients. 2018; 10 (11): 1564. doi: 10.3390/nu10111564.

Curtis E, Litwic A, Cooper C, Dennison E. Determinants of Muscle and Bone Aging. J Cell Physiol. 2015; 230 (11): 2618–25. doi: 10.1002/jcp.25001.

Cuthbertson DP. Post-Shock Metabolic Response. Lancet. 1942; 239 (6189): 433–7. doi: 10.1016/S0140-6736(00)79605-X.

Cuthbertson DP, Zagreb HC. The Metabolic Response to Injury and Its Nutritional Implications. JPEN J Parenter Enteral Nutr. 1979; 3 (3): 108–29. doi: 10.1177/014860717900300302.

Dai Y-J, Sun L-L, Li M-Y, Ding C-L, Su Y-C, Sun L-J, Xue, S-H, Yan F, Zhao, C-H, Wang W. Comparison of Formulas Based on Lipid Emulsions of Olive Oil, Soybean Oil, or Several Oils for Parenteral Nutrition: A Systematic Review and Meta-Analysis. Adv Nutr. 2016; 7 (2): 279–86. doi: 10.3945/an.114.007427.

Danielis M, Lorenzoni G, Azzolina D, Iacobucci A, Trombini O, De Monte A, Gregori D, Beltrame F. Effect of Protein-Fortified Diet on Nitrogen Balance in Critically Ill Patients: Results from the OPINiB Trial. Nutrients. 2019; 11 (5): 972. doi: 10.3390/nu11050972.

Davies ML, Chapple L-AS, Chapman MJ, Moran JL, Peake SL. Protein delivery and clinical outcomes in the critically ill: a systematic review and meta-analysis. Crit Care Resusc. 2017; 19 (2): 117–27.

de Azevedo JRA, Lima HCM, Montenegro WS, Souza SCdC, Nogueira IROM, Silva MM, Muniz NdA. Optimized calorie and high protein intake versus recommended caloric-protein intake in critically ill patients a prospective, randomized, controlled phase II clinical trial. Rev Bras Ter Intensiva. 2019; 31 (2): 171–9. doi: 10.5935/0103-507X.20190025.

De Jonghe B, Sharshar T, Lefaucheur J-P, Authier F-J, Durand-Zaleski I, Boussarsar M, Cerf C, Renaud E, Mesrati F, Carlet J, Raphaël JC, Outin H, Bastuji-Garin S. Paresis acquired

in the intensive care unit: a prospective multicenter study. JAMA. 2002; 288 (22): 2859–67. doi: 10.1001/jama.288.22.2859.

De Waele E, Jakubowski JR, Stocker R, Wischmeyer PE. Review of evolution and current status of protein requirements and provision in acute illness and critical care. Clin Nutr. 2020; S0261–5614 (20): 30707–X. doi: 10.1016/j.clnu.2020.12.032.

De Waele E, Jonckheer J, Wischmeyer P. Indirect calorimetry in critical illness: a new standard of care? Curr Opin Crit Care. 2021; May 14 (online ahead of print). doi: 10.1097/MCC.0000000000000844.

Deane A, Chapman MJ, Fraser RJL, Horowitz M. Bench-to-bedside review: the gut as an endocrine organ in the critically ill. Crit Care. 2010; 14 (5): 228. doi:10.1186/cc9039.

Delsoglio M, Achamrah N, Berger MM, Pichard C. Indirect Calorimetry in Clinical Practice. J Clin Med. 2019; 8 (9): 1387. doi:10.3390/jcm8091387.

Dickerson RN. Nitrogen Balance and Protein Requirements for Critically Ill Older Patients. Nutrients. 2016; 8 (4): 226. doi: 10.3390/nu8040226.

Doig GS, Simpson F, Bellomo R, Heighes PT, Sweetman EA, Chesher D, Pollock C, Davies A, Botha J, Harrigan P, Reade MC. Intravenous amino acid therapy for kidney function in critically ill patients: a randomized controlled trial. Intensive Care Med. 2015; 41 (7): 1197–208. doi: 10.1007/s00134-015-3827-9.

Donoghue V, Schleicher GK, Spruyt MGL, Malan L, Nel DG, Calder PC, Blaauw R. Four-oil intravenous lipid emulsion effect on plasma fatty acid composition, inflammatory markers and clinical outcomes in acutely ill patients: A randomised control trial (Foil fact). Clin Nutr. 2019; 38 (6): 2583–91. doi: 10.1016/j.clnu.2018.12.010.

Duan K, Gao X, Zhu D. The clinical relevance and mechanism of skeletal muscle wasting. Clin Nutr. 2020; 47 (5): 857–67. doi: 10.1016/j.clnu.2020.07.029.

Dumas J-B. Recherches de Chimie organique. In: Gay-Lussac JL, Arago F, ed. Annales de chimie et de physique. Paris: Crochard; 1833. 164–181.

Eggmann S, Verra ML, Luder G, Takala J, Jakob SM. Effects of early, combined endurance and resistance training in mechanically ventilated, critically ill patients: A randomised controlled trial. PLoS ONE. 2018; 13 (11): e0207428. doi: 10.1371/journal.pone.0207428.

Elke G, Schädler D, Engel C, Bogatsch H, Frerichs I, Ragaller M, Scholz J, Brunkhorst FM, Löffler M, Reinhart K, Weiler N. Current practice in nutritional support and its association with mortality in septic patients - results from a national, prospective, multicenter study. Crit Care Med. 2008; 36 (6): 1762–7. doi: 10.1097/CCM.0b013e318174dcf0.

Elke G, Kuhnt E, Ragaller M, Schädler D, Frerichs I, Brunkhorst FM, Löffler M, Reinhart K, Weiler N. Enteral nutrition is associated with improved outcome in patients with severe sepsis. Med Klin Intensivmed Notfmed. 2013; 108 (3): 223–33. doi: 10.1007/s00063-013-0224-4.

Elke G, Wang M, Weiler N, Day AG, Heyland DK. Close to recommended caloric and protein intake by enteral nutrition is associated with better clinical outcome of critically ill septic patients: secondary analysis of a large international nutrition database. Crit Care. 2014; 18 (1): R29. doi: 10.1186/cc13720.

Elke G, van Zanten ARH, Lemieux M, McCall M, Jeejeebhoy KN, Kott M, Jiang X, Day AG, Heyland DK. Enteral versus parenteral nutrition in critically ill patients: an updated systematic review and meta-analysis of randomized controlled trials. Crit Care. 2016; 20 (1): 117. doi: 10.1186/s13054-016-1298-1.

Elke G, Hartl W, Kreymann K, Adolph M, Felbinger T, Graf T, de Heer G, Heller A, Kampa U, Mayer K, Muhl E, Niemann B, Rümelin A, Steiner S, Stoppe C, Weimann A, Bischoff S. Clinical Nutrition in Critical Care Medicine - Guideline of the German Society for Nutritional Medicine (DGEM). Aktuel Ernahrungsmed. 2018; 43 (05): 341–408. doi: 10.1055/a-0713-8179.

Ema R, Wakahara T, Mogi Y, Miyamoto N, Komatsu T, Kanehisa H, Kawakami Y. In vivo measurement of human rectus femoris architecture by ultrasonography: validity and applicability. Clin Physiol Funct Imaging. 2013; 33 (4): 267–73. doi: 10.1111/cpf.12023.

Fernández R, Urbano J, Carrillo Á, Vivanco A, Solana MJ, Rey C, López-Herce J. Comparison of the effect of three different protein content enteral diets on serum levels of proteins, nitrogen balance, and energy expenditure in critically ill infants: study protocol for a randomized controlled trial. Trials. 2019; 20 (1): 585. doi: 10.1186/s13063-019-3686-8.

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 (6): 795–805. doi: 10.1177/0148607115618449.

Fetterplace K, Deane AM, Tierney A, Beach LJ, Knight LD, Presneill J, Rechnitzer T, Forsyth A, Gill BMT, Mourtzakis M, MacIsaac C. Targeted Full Energy and Protein Delivery in Critically Ill Patients: A Pilot Randomized Controlled Trial (FEED Trial). JPEN J Parenter Enteral Nutr. 2018; 42 (8): 1252–62. doi: 10.1002/jpen.1166.

Fontes Cerqueira TC, de Cerqueira Neto ML, Cacau LdAP, Oliveira GU, Silva Júnior WMd, Carvalho VO, de Mendonça JT, de Santana Filho VJ. Ambulation capacity and functional outcome in patients undergoing neuromuscular electrical stimulation after cardiac valve surgery: A randomised clinical trial. Medicine (Baltimore). 2018; 97 (46): e13012. doi:10.1097/MD.0000000000013012.

Forbes GB. Lean Body Mass-Body Fat Interrelationships in Humans. Nutr Rev. 1987; 45 (10): 225–31. doi: 10.1111/j.1753-4887.1987.tb02684.x.

Fresenius Kabi Limited. Enteral Nutrition - Product Information (Folder). Cheshire, United Kingdom; 2016.

Fresenius Kabi Deutschland GmbH. Parenteral Nutrition - Product Information (Product Labels). Bad Homburg; 2017.

Friedrich O, Diermeier S, Larsson L. Weak by the machines: Muscle motor protein dysfunction - a side effect of intensive care unit treatment. Acta Physiol (Oxf). 2018; 222 (1): e12885. doi:10.1111/apha.12885.

Gala K, Desai V, Liu N, Omer EM, McClave SA. How to Increase Muscle Mass in Critically Ill Patients: Lessons Learned from Athletes and Bodybuilders. Curr Nutr Rep. 2020; 9 (4): 369–380. doi: 10.1007/s13668-020-00334-0.

Gallagher D, Heymsfield SB. Muscle distribution: Variations with body weight, gender, and age. Appl Radiat and Isot. 1998; 49 (5-6): 733–4. doi: 10.1016/S0969-8043(97)00096-1.

Gamrin-Gripenberg L, Sundström-Rehal M, Olsson D, Grip J, Wernerman J, Rooyackers O. An attenuated rate of leg muscle protein depletion and leg free amino acid efflux over time is seen in ICU long-stayers. Crit Care. 2018; 22 (1): 13. doi: 10.1186/s13054-017-1932-6.

Gerovasili V, Stefanidis K, Vitzilaios K, Karatzanos E, Politis P, Koroneos A, Chatzimichail A, Routsi C, Roussos C, Nanas S. Electrical muscle stimulation preserves the muscle mass of critically ill patients: a randomized study. Crit Care. 2009; 13 (5): R161. doi: 10.1186/cc8123.

Goeters C, Wenn A, Mertes N, Wempe C, Van Aken H, Stehle P, Bone H-G. Parenteral L-alanyl-L-glutamine improves 6-month outcome in critically ill patients. Crit Care Med. 2002; 30 (9): 2032–7. doi: 10.1097/00003246-200209000-00013.

Gomes MD, Lecker SH, Jagoe RT, Navon A, Goldberg AL. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. Proc Natl Acad Sci USA. 2001; 98 (25): 14440–5. doi: 10.1073/pnas.251541198.

Gordon BS, Kelleher AR, Kimball SR. Regulation of muscle protein synthesis and the effects of catabolic states. Int J Biochem Cell Biol. 2013; 45 (10): 2147–57. doi: 10.1016/j.biocel.2013.05.039.

Gunst J, De Bruyn A, Van den Berghe G. Glucose control in the ICU. Curr Opin Anaesthesiol. 2019; 32 (2): 156–62. doi: 10.1097/ACO.0000000000000706.

Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. Proc Natl Acad Sci USA. 1918; 4 (12): 370–3. doi: 10.1073/pnas.4.12.370.

Heidegger CP, Berger MM, Graf S, Zingg W, Darmon P, Costanza MC, Thibault R, Pichard C. Optimisation of energy provision with supplemental parenteral nutrition in critically ill patients: a randomised controlled clinical trial. Lancet. 2013; 381 (9864): 385–93. doi: 10.1016/S0140-6736(12)61351-8.

Hermans G, Van den Berghe G. Clinical review: intensive care unit acquired weakness. Crit Care. 2015; 19 (1): 274. doi: 10.1186/s13054-015-0993-7.

Hermans G, De Jonghe B, Bruyninckx F, Van den Berghe G. Clinical review: Critical illness polyneuropathy and myopathy. Crit Care. 2008;12 (6): 238. doi: 10.1186/cc7100.

Hermans G, Van Mechelen H, Clerckx B, Vanhullebusch T, Mesotten D, Wilmer A, Casaer MP, Meersseman P, Debaveye Y, Van Cromphaut S, Wouters PJ, Gosselink R, Van den Berghe G. Acute outcomes and 1-year mortality of intensive care unit-acquired weakness. A cohort study and propensity-matched analysis. Am J Respir Crit Care Med. 2014; 190 (4): 410–20. doi:10.1164/rccm.201312-2257OC.

Heyland DK, MacDonald S, Keefe L, Drover JW. Total Parenteral Nutrition in the Critically Ill Patient. JAMA. 1998; 280 (23): 2013. doi: 10.1001/jama.280.23.2013.

Heyland DK, Day A, Clarke GJ, Hough CT, Files DC, Mourtzakis M, Deutz N, Needham DM, Stapleton R. Nutrition and Exercise in Critical Illness Trial (NEXIS Trial): a protocol of a multicentred, randomised controlled trial of combined cycle ergometry and amino acid

supplementation commenced early during critical illness. BMJ open. 2019; 9 (7): e027893. doi:10.1136/bmjopen-2018-027893.

Hickmann CE, Castanares-Zapatero D, Deldicque L, Van den Bergh P, Caty G, Robert A, Roeseler J, Francaux M, Laterre P-F. Impact of Very Early Physical Therapy During Septic Shock on Skeletal Muscle: A Randomized Controlled Trial. Crit Care Med. 2018; 46 (9): 1436–43. doi: 10.1097/CCM.0000000000003263.

Hoffer LJ, Bistrian BR. Appropriate protein provision in critical illness: a systematic and narrative review. Am J Clin Nutr. 2012; 96 (3): 591–600. doi: 10.3945/ajcn.111.032078.

Hoffer LJ. How much protein do parenteral amino acid mixtures provide? Am J Clin Nutr. 2011; 94 (6): 1396–8. doi: 10.3945/ajcn.111.023390.

Hu Z, Wang H, Lee IH, Du J, Mitch WE. Endogenous glucocorticoids and impaired insulin signaling are both required to stimulate muscle wasting under pathophysiological conditions in mice. J Clin Invest. 2009; 119 (10): 3059–69. doi: 10.1172/JCI38770.

Jaitovich A, Khan MMHS, Itty R, Chieng HC, Dumas CL, Nadendla P, Fantauzzi JP, Yucel RM, Feustel PJ, Judson MarcA. ICU Admission Muscle and Fat Mass, Survival, and Disability at Discharge: A Prospective Cohort Study. Chest. 2019; 155 (2): 322–30. doi: 10.1016/j.chest.2018.10.023.

Jang MH, Shin M-J, Shin YB. Pulmonary and Physical Rehabilitation in Critically Ill Patients. Acute Crit Care. 2019; 34 (1): 1–13. doi: 10.4266/acc.2019.00444.

Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. J Appl Physiol. 2000; 89 (1): 81–8. doi: 10.1152/jappl.2000.89.1.81.

Jolley SE, Bunnell AE, Hough CL. ICU-Acquired Weakness. Chest. 2016; 150 (5): 1129–40. doi: 10.1016/j.chest.2016.03.045.

Jones C, Eddleston J, McCairn A, Dowling S, McWilliams D, Coughlan E, Griffiths RD. Improving rehabilitation after critical illness through outpatient physiotherapy classes and essential amino acid supplement: A randomized controlled trial. J Crit Care. 2015; 30 (5): 901–7. doi: 10.1016/j.jcrc.2015.05.002.

Joskova V, Patkova A, Havel E, Najpaverova S, Uramova D, Kovarik M, Zadak Z, Hronek M. Critical evaluation of muscle mass loss as a prognostic marker of morbidity in critically ill

patients and methods for its determination. J Rehabil Med. 2018; 50 (8): 696–704. doi: 10.2340/16501977-2368.

Kim TJ, Park S-H, Jeong H-B, Ha EJ, Cho WS, Kang H-S, Kim JE, Ko S-B. Optimizing Nitrogen Balance Is Associated with Better Outcomes in Neurocritically Ill Patients. Nutrients. 2020; 12 (10): 3137. doi: 10.3390/nu12103137.

Koekkoek WACK, van Setten CHC, Olthof LE, Kars JCNH, van Zanten ARH. Timing of PROTein INtake and clinical outcomes of adult critically ill patients on prolonged mechanical VENTilation: The PROTINVENT retrospective study. Clin Nutr. 2018; 38 (2): 883–90. doi: 10.1016/j.clnu.2018.02.012.

Koekkoek KWAC, van Zanten ARH. Nutrition in the critically ill patient. Curr Opin Anaesthesiol. 2017; 30 (2): 178–85. doi: 10.1097/ACO.0000000000000441.

Koekkoek KWAC, van Zanten ARH. Nutrition in the ICU: new trends versus old-fashioned standard enteral feeding? Curr Opin Anaesthesiol. 2018; 31 (2): 136–43. doi: 10.1097/ACO.0000000000000571.

Kou K, Momosaki R, Miyazaki S, Wakabayashi H, Shamoto H. Impact of Nutrition Therapy and Rehabilitation on Acute and Critical Illness: A Systematic Review. J UOEH. 2019; 41 (3): 303–15. doi: 10.7888/juoeh.41.303.

Kress JP, Hall JB. ICU-acquired weakness and recovery from critical illness. N Engl J Med. 2014; 370 (17): 1626–35. doi: 10.1056/NEJMra1209390.

Kristensen J. Energy expenditure and changes in body composition during exercise-based rehabilitation. J R Army Med Corps. 2013; 159 (1): 30–4. doi: 10.1136/jramc-2013-000012.

Kutsogiannis J, Alberda C, Gramlich L, Cahill NE, Wang M, Day AG, Dhaliwal R, Heyland DK. Early use of supplemental parenteral nutrition in critically ill patients: Results of an international multicenter observational study. Crit Care Med. 2011; 39 (12): 2691–9. doi: 10.1097/CCM.0b013e3182282a83.

Lambell KJ, Tatucu-Babet OA, Chapple L-A, Gantner D, Ridley EJ. Nutrition therapy in critical illness: a review of the literature for clinicians. Crit Care. 2020; 24 (1): 35. doi:10.1186/s13054-020-2739-4.

Langlois PL, D'Aragon F, Hardy G, Manzanares W. Omega-3 polyunsaturated fatty acids in critically ill patients with acute respiratory distress syndrome: A systematic review and meta-analysis. Nutrition. 2019; 61: 84–92. doi: 10.1016/j.nut.2018.10.026.

Laplante M, Sabatini DM. mTOR Signaling. Cold Spring Harb Perspect Biol. 2012; 4 (2): a011593. doi: 10.1101/cshperspect.a011593.

Larsen R, Ziegenfuß T, Mathes A. Beatmung: Indikationen - Techniken - Krankheitsbilder. 6th ed. Berlin, Heidelberg: Springer; 2012.

Larsson L, Degens H, Li M, Salviati L, Lee YI, Thompson W, Kirkland JL, Sandri M. Sarcopenia: Aging-Related Loss of Muscle Mass and Function. Physiol Rev. 2019; 99 (1): 427–511. doi: 10.1152/physrev.00061.2017.

Lheureux O, Preiser J-C. Role of Nutrition Support in Inflammatory Conditions. Nutr Clin Pract. 2017; 32 (3): 310–7. doi: 10.1177/0884533617695242.

Lipes J, Mardini L, Jayaraman D. Sex and mortality of hospitalized adults after admission to an intensive care unit. Am J Crit Care. 2013; 22 (4): 314–9. doi: 10.4037/ajcc2013225.

Lipshutz AKM, Gropper MA. Acquired neuromuscular weakness and early mobilization in the intensive care unit. Anesthesiology. 2013; 118 (1): 202–15. doi: 10.1097/ALN.0b013e31826be693.

Liu J, Klebach M, Visser M, Hofman Z. Amino Acid Availability of a Dairy and Vegetable Protein Blend Compared to Single Casein, Whey, Soy, and Pea Proteins: A Double-Blind, Cross-Over Trial. Nutrients. 2019; 11 (11): 2613. doi: 10.3390/nu11112613.

Looijaard WGPM, Dekker IM, Stapel SN, Girbes ARJ, Twisk JWR, Oudemans-van Straaten HM, Weijs PJM. Skeletal muscle quality as assessed by CT-derived skeletal muscle density is associated with 6-month mortality in mechanically ventilated critically ill patients. Crit Care. 2016; 20 (1): 386. doi: 10.1186/s13054-016-1563-3.

Looijaard WGPM, Molinger J, Weijs PJM. Measuring and monitoring lean body mass in critical illness. Curr Opin Crit Care. 2018; 24 (4): 241–7. doi: 10.1097/MCC.0000000000000511.

Looijaard WGPM, Dekker IM, Beishuizen A, Girbes ARJ, Oudemans-van Straaten HM, Weijs PJM. Early high protein intake and mortality in critically ill ICU patients with low skeletal muscle area and -density. Clin Nutr. 2019; 37 (7): 2192–201. doi: 10.1016/j.clnu.2019.09.007.

Mak RH, Rotwein P. Myostatin and insulin-like growth factors in uremic sarcopenia: the yin and yang in muscle mass regulation. Kidney Int. 2006; 70 (3): 410–2. doi: 10.1038/sj.ki.5001622.

Marik PE, Bellomo R. Stress hyperglycemia: an essential survival response! Crit Care. 2013; 17 (2): 305. doi: 10.1186/cc12514.

Martindale RG, Heyland DK, Rugeles SJ, Wernerman J, Weijs PJM, Patel JJ, McClave SA. Protein Kinetics and Metabolic Effects Related to Disease States in the Intensive Care Unit. Nutr Clin Pract. 2017; 32 (1 Suppl): 21S–9S. doi: 10.1177/0884533617694612.

McCarthy MS, Martindale RG. Immunonutrition in Critical Illness: What Is the Role? Nutr Clin Pract. 2018; 33 (3): 348–58. doi: 10.1002/ncp.10102.

McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, McCarthy MS, Davanos E, Rice TW, Cresci GA, Gervasio JM, Sacks GS, Roberts PR, Compher C. 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 (A.S.P.E.N.). JPEN J Parenter Enteral Nutr. 2016; 40 (2): 159–211. doi: 10.1177/0148607115621863.

McKendry J, Thomas ACQ, Phillips SM. Muscle Mass Loss in the Older Critically Ill Population: Potential Therapeutic Strategies. Nutr Clin Pract. 2020; 35 (4): 607–616. doi: 10.1002/ncp.10540.

Mitchell WK, Wilkinson DJ, Phillips BE, Lund JN, Smith K, Atherton PJ. Human Skeletal Muscle Protein Metabolism Responses to Amino Acid Nutrition. Adv Nutr. 2016; 7 (4): 828S-38S. doi: 10.3945/an.115.011650.

Mizock BA. Alterations in fuel metabolism in critical illness: hyperglycaemia. Best Pract Res Clin Endocrinol Metab. 2001; 15 (4): 533–51. doi: 10.1053/beem.2001.0168.

Moisey LL, Mourtzakis M, Cotton BA, Premji T, Heyland DK, Wade CE, Bulger E, Kozar RA. Skeletal muscle predicts ventilator-free days, ICU-free days, and mortality in elderly ICU patients. Crit Care. 2013; 17 (5): R206. doi: 10.1186/cc12901.

Mourtzakis M, Wischmeyer P. Bedside ultrasound measurement of skeletal muscle. Curr Opin Clin Nutr Metab Care. 2014; 17 (5): 389–95. doi: 10.1097/MCO.0000000000000088.

Mundi MS, Nystrom EM, Hurley DL, McMahon MM. Management of Parenteral Nutrition in Hospitalized Adult Patients. JPEN J Parenter Enteral Nutr. 2017; 41 (4): 535–49. doi: 10.1177/0148607116667060.

Nakamura K, Nakano H, Naraba H, Mochizuki M, Takahashi Y, Sonoo T, Hashimoto H, Morimura N. High protein versus medium protein delivery under equal total energy delivery in critical care: A randomized controlled trial. Clin Nutr. 2020; 40 (3): 796–803. doi: 10.1016/j.clnu.2020.07.036.

Nakanishi N, Takashima T, Oto J. Muscle atrophy in critically ill patients : a review of its cause, evaluation, and prevention. J Med Invest. 2020; 67 (1.2): 1–10. doi: 10.2152/jmi.67.1.

Nanas S, Kritikos K, Angelopoulos E, Siafaka A, Tsikriki S, Poriazi M, Kanaloupiti D, Kontogeorgi M, Pratikaki M, Zervakis D, Routsi C, Roussos C. Predisposing factors for critical illness polyneuromyopathy in a multidisciplinary intensive care unit. Acta Neurol Scand. 2008; 118 (3): 175–81. doi: 10.1111/j.1600-0404.2008.00996.x.

Nicolo M, Heyland DK, Chittams J, Sammarco T, Compher C. Clinical Outcomes Related to Protein Delivery in a Critically Ill Population: A Multicenter, Multinational Observation Study. JPEN J Parenter Enteral Nutr. 2016; 40 (1): 45–51. doi: 10.1177/0148607115583675.

Nienow MK, Susterich CE, Peterson SJ. Prioritizing nutrition during recovery from critical illness. Curr Opin Clin Nutr Metab Care. 2021; 24 (2): 199–205. doi: 10.1097/MCO.0000000000000728.

Paris MT, Mourtzakis M, Day A, Leung R, Watharkar S, Kozar R, Earthman C, Kuchnia A, Dhaliwal R, Moisey L, Compher C, Martin N, Nicolo M, White T, Roosevelt H, Peterson S, Heyland DK. Validation of Bedside Ultrasound of Muscle Layer Thickness of the Quadriceps in the Critically Ill Patient (VALIDUM Study). JPEN J Parenter Enteral Nutr. 2017; 41 (2): 171–80. doi: 10.1177/0148607116637852.

Patkova A, Joskova V, Havel E, Kovarik M, Kucharova M, Zadak Z, Hronek M. Energy, Protein, Carbohydrate, and Lipid Intakes and Their Effects on Morbidity and Mortality in Critically Ill Adult Patients: A Systematic Review. Adv Nutr. 2017; 8 (4): 624–34. doi: 10.3945/an.117.015172.

Pavlickova Aimova P, Hronek M, Zadak Z. The importance and dosage of amino acids in nutritional support of various pathological conditions in ICU patients. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2014; 158 (3): 346–55. doi: 10.5507/bp.2014.034.

Peake SL, Davies AR, Deane AM, Lange K, Moran JL, O'Connor SN, Ridley EJ, Williams PJ, Chapman MJ. Use of a concentrated enteral nutrition solution to increase calorie delivery to critically ill patients: a randomized, double-blind, clinical trial. Am J Clin Nutr. 2014; 100 (2): 616–25. doi: 10.3945/ajcn.114.086322.

Peñuelas O, Muriel A, Frutos-Vivar F, Fan E, Raymondos K, Rios F, Nin N, Thille AW, González M, Villagomez AJ, Davies AR, Du B, Maggiore SM, Matamis D, Abroug F, Moreno RP, Kuiper MA, Anzueto A. Prediction and Outcome of Intensive Care Unit-Acquired Paresis. J Intensive Care Med. 2018; 33 (1): 16–28. doi: 10.1177/0885066616643529.

Phillips SM, Dickerson RN, Moore FA, Paddon-Jones D, Weijs PJM. Protein Turnover and Metabolism in the Elderly Intensive Care Unit Patient. Nutr Clin Pract. 2017; 32 (1 Suppl): 112S-20S. doi: 10.1177/0884533616686719.

Preiser J-C, Ichai C, Orban J-C, Groeneveld ABJ. Metabolic response to the stress of critical illness. Br J Anaesth. 2014; 113 (6): 945–54. doi: 10.1093/bja/aeu187.

Preiser J-C, van Zanten ARH, Berger MM, Biolo G, Casaer MP, Doig GS, Griffiths RD, Heyland DK, Hiesmayr M, Iapichino G, Laviano A, Pichard C, Singer P, Van den Berghe G. Metabolic and nutritional support of critically ill patients: Consensus and controversies. Crit Care. 2015; 19 (1): 35. doi: 10.1186/s13054-015-0737-8.

Puthucheary ZA, Rawal J, McPhail M, Connolly B, Ratnayake G, Chan P, Hopkinson NS, Phadke R, Dew T, Sidhu PS, Velloso C, Seymour J, Agley CC, Selby A, Limb M, Edwards LM, Smith K, Rowlerson A. Acute skeletal muscle wasting in critical illness. JAMA. 2013; 310 (15): 1591–600. doi: 10.1001/jama.2013.278481.

Rasmussen BB, Volpi E. Muscle Biology and mTORC1 Signaling in Aging. In: Cruz-Jentoft AJ, Morley JE, eds. Sarcopenia. Chichester, West Sussex: Wiley-Blackwell; 2012: 20–40. doi: 10.1002/9781118338032.ch3.

Reid CL, Campbell IT, Little RA. Muscle wasting and energy balance in critical illness. Clin Nutr. 2004; 23 (2): 273–80. doi: 10.1016/S0261-5614(03)00129-8.

Reintam Blaser A, Berger MM. Early or Late Feeding after ICU Admission? Nutrients. 2017; 9 (12): 1278. doi: 10.3390/nu9121278.

Richter M, Baerlocher K, Bauer JM, Elmadfa I, Heseker H, Leschik-Bonnet E, Stangl G, Volkert D, Stehle P. Revised Reference Values for the Intake of Protein. Ann Nutr Metab. 2019; 74 (3): 242–50. doi: 10.1159/000499374.

Rooyackers O, Wernerman J. Imaging opens possibilities both to target and to evaluate nutrition in critical illness. Crit Care. 2014; 18 (3): 144. doi: 10.1186/cc13882.

Rosa-Caldwell ME, Greene NP. Muscle metabolism and atrophy: let's talk about sex. Biol Sex Differ. 2019; 10 (1): 43. doi: 10.1186/s13293-019-0257-3.

Rousing ML, Hahn-Pedersen MH, Andreassen S, Pielmeier U, Preiser J-C. Energy expenditure in critically ill patients estimated by population-based equations, indirect calorimetry and CO2-based indirect calorimetry. Ann Intensive Care. 2016; 6 (1): 16. doi: 10.1186/s13613-016-0118-8.

Rugeles S, Villarraga-Angulo LG, Ariza-Gutierrez A, Chaverra-Kornerup S, Lasalvia P, Rosselli D. High-protein hypocaloric vs normocaloric enteral nutrition in critically ill patients: A randomized clinical trial. J Crit Care. 2016; 35: 110–4. doi: 10.1016/j.jcrc.2016.05.004.

Sanada K, Kearns CF, Midorikawa T, Abe T. Prediction and validation of total and regional skeletal muscle mass by ultrasound in Japanese adults. Eur J Appl Physiol. 2006; 96 (1): 24–31. doi: 10.1007/s00421-005-0061-0.

Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. Cell. 2010; 141 (2): 290–303. doi: 10.1016/j.cell.2010.02.024.

Saxton RA, Sabatini DM. mTOR Signaling in Growth, Metabolism, and Disease. Cell. 2017; 168 (6): 960–76. doi: 10.1016/j.cell.2017.02.004.

Schefold JC, Bierbrauer J, Weber-Carstens S. Intensive care unit-acquired weakness (ICUAW) and muscle wasting in critically ill patients with severe sepsis and septic shock. J Cachexia Sarcopenia Muscle. 2010; 1 (2): 147–57. doi: 10.1007/s13539-010-0010-6.

Schefold JC, Wollersheim T, Grunow JJ, Luedi MM, Z'Graggen WJ, Weber-Carstens S. Muscular weakness and muscle wasting in the critically ill. J Cachexia Sarcopenia Muscle. 2020; 11 (6): 1399–412. doi: 10.1002/jcsm.12620.

Schulman RC, Mechanick JI. Metabolic and nutrition support in the chronic critical illness syndrome. Respir Care. 2012; 57 (6): 958–77; discussion 977–8. doi: 10.4187/respcare.01620.

Schwab S, Schellinger PD, Werner C, Unterberg A, Hacke W, ed. NeuroIntensiv. 3rd ed. Berlin, Heidelberg: Springer; 2015.

Schwartz AL, Ciechanover A. Targeting proteins for destruction by the ubiquitin system: implications for human pathobiology. Annu Rev Pharmacol Toxicol. 2009; 49: 73–96. doi: 10.1146/annurev.pharmtox.051208.165340.

Senger D, Erbguth F. Critical-illness-Myopathie und -Polyneuropathie. Med Klin Intensivmed Notfmed. 2017; 112 (7): 589–96. doi: 10.1007/s00063-017-0339-0.

Seymour JM, Ward K, Sidhu PS, Puthucheary Z, Steier J, Jolley CJ, Rafferty G, Polkey MI, Moxham J. Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriceps strength in COPD. Thorax. 2009; 64 (5): 418–23. doi: 10.1136/thx.2008.103986.

Sharma K, Mogensen KM, Robinson MK. Pathophysiology of Critical Illness and Role of Nutrition. Nutr Clin Pract. 2019; 34 (1): 12–22. doi: 10.1002/ncp.10232.

Shepherd S, Batra A, Lerner DP. Review of Critical Illness Myopathy and Neuropathy. Neurohospitalist. 2017; 7 (1): 41–8. doi: 10.1177/1941874416663279.

Siao S-F, Yen Y-H, Yu Y-F, Zong S-L, Chen C-HC. Intensive Care Unit-Acquired Weakness. Hu li za zhi. 2020; 67 (3): 6–13. doi: 10.6224/JN.202006_67(3).02.

Singer P. Protein metabolism and requirements in the ICU. Clin Nutr ESPEN. 2020; 38: 3–8. doi: 10.1016/j.clnesp.2020.03.026.

Singer P, Blaser AR, Berger MM, Alhazzani W, Calder PC, Casaer MP, Hiesmayr M, Mayer K, Montejo JC, Pichard C, Preiser J-C, van Zanten ARH, Oczkowski S, Szczeklik W, Bischoff SC. ESPEN guideline on clinical nutrition in the intensive care unit. Clin Nutr. 2018; 38 (1): 48–79. doi:10.1016/j.clnu.2018.08.037.

Singer P, Glass YD, Kagan I. The best recipe: fat-based, protein-based, single amino acids? Curr Opin Crit Care. 2020; 26 (4): 335–340. doi: 10.1097/MCC.0000000000000735.

Slee AD. Exploring metabolic dysfunction in chronic kidney disease. Nutr Metab (Lond). 2012; 9 (1): 36. doi:10.1186/1743-7075-9-36.

Song G, Ouyang G, Bao S. The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med. 2005; 9 (1): 59–71. doi: 10.1111/j.1582-4934.2005.tb00337.x.

Song JH, Lee HS, Kim SY, Kim EY, Jung JY, Kang YA, Park MS, Kim YS, Kim SK, Chang J, Chung KS. The influence of protein provision in the early phase of intensive care on clinical outcomes for critically ill patients on mechanical ventilation. Asia Pac J Clin Nutr. 2017; 26 (2): 234–40. doi: 10.6133/apjcn.032016.01.

Stehle P, Kuhn KS. Glutamine: an obligatory parenteral nutrition substrate in critical care therapy. Biomed Res Int. 2015; 2015: 545467. doi: 10.1155/2015/545467.

Stehle P, Ellger B, Kojic D, Feuersenger A, Schneid C, Stover J, Scheiner D, Westphal M. Glutamine dipeptide-supplemented parenteral nutrition improves the clinical outcomes of critically ill patients: A systematic evaluation of randomised controlled trials. Clin Nutr ESPEN. 2017; 17: 75–85. doi:10.1016/j.clnesp.2016.09.007.

Stoppe C, Wendt S, Mehta NM, Compher C, Preiser J-C, Heyland DK, Kristof AS. Biomarkers in critical care nutrition. Crit Care. 2020; 24 (1): 499. doi: 10.1186/s13054-020-03208-7.

Sundström M, Tjäder I, Rooyackers O, Wernerman J. Indirect calorimetry in mechanically ventilated patients. A systematic comparison of three instruments. Clin Nutr. 2013; 32 (1): 118–21. doi:10.1016/j.clnu.2012.06.004.

Tanaka K, Yamada T. Ultrasound Measurement of Septic Shock-induced Acute Skeletal Muscle Atrophy in Intensive Care Unit. PM R. 2020; 13 (4): 347–52. doi:10.1002/pmrj.12406.

Thiele RI, Jakob H, Hund E, Tantzky S, Keller S, Kamler M, Herold U, Hagl S. Sepsis and catecholamine support are the major risk factors for critical illness polyneuropathy after open heart surgery. Thorac Cardiovasc Surg. 2000; 48 (3): 145–50. doi: 10.1055/s-2000-9640.

Thomaes T, Thomis M, Onkelinx S, Coudyzer W, Cornelissen V, Vanhees L. Reliability and validity of the ultrasound technique to measure the rectus femoris muscle diameter in older CAD-patients. BMC Med Imaging. 2012; 12 (1): 7. doi: 10.1186/1471-2342-12-7.

Tillquist M, Kutsogiannis DJ, Wischmeyer PE, Kummerlen C, Leung R, Stollery D, Karvellas CJ, Preiser J-C, Bird N, Kozar R, Heyland DK. Bedside Ultrasound Is a Practical and Reliable Measurement Tool for Assessing Quadriceps Muscle Layer Thickness. JPEN J Parenter Enteral Nutr. 2014; 38 (7): 886–90. doi: 10.1177/0148607113501327.

Tourel C, Burnol L, Lanoiselé J, Molliex S, Viallon M, Croisille P, Morel J. Reliability of standardized ultrasound measurements of quadriceps muscle thickness in neurological critically ill patients: a comparison to computed tomography measures. J Rehabil Med. 2020; 52 (3): jrm00032. doi: 10.2340/16501977-2638.

University Hospital of Bonn (UKB). Department of Anesthesiology and Intensive Care Medicine. Standard Operating Procedure for Intensive Care Units. 2nd ed. Bonn; 2016.

University Hospital of Bonn (UKB). Institute of Clinical Chemistry and Clinical Pharmacology. Specifications of the Central Laboratory. Bonn; 2019.

van Gassel RJJ, Baggerman MR, van de Poll MCG. Metabolic aspects of muscle wasting during critical illness. Curr Opin Clin Nutr Metab Care. 2020; 23 (2): 96–101. doi: 10.1097/MCO.0000000000000628.

van Zanten ARH, Petit L, De Waele J, Kieft H, de Wilde J, van Horssen P, Klebach M, Hofman Z. Very high intact-protein formula successfully provides protein intake according to nutritional recommendations in overweight critically ill patients: a double-blind randomized trial. Crit Care. 2018; 22 (1): 156. doi: 10.1186/s13054-018-2070-5.

van Zanten ARH, De Waele E, Wischmeyer PE. Nutrition therapy and critical illness: practical guidance for the ICU, post-ICU, and long-term convalescence phases. Crit Care. 2019; 23 (1): 368. doi: 10.1186/s13054-019-2657-5.

Vanhorebeek I, Latronico N, Van den Berghe G. ICU-acquired weakness. Intensive Care Med. 2020; 46 (4): 637–53. doi: 10.1007/s00134-020-05944-4.

Verma R, Deshaies RJ. A proteasome howdunit: the case of the missing signal. Cell. 2000; 101 (4): 341–4. doi: 10.1016/s0092-8674(00)80843-0.

Wandrag L, Brett SJ, Frost G, Hickson M. Impact of supplementation with amino acids or their metabolites on muscle wasting in patients with critical illness or other muscle wasting illness: a systematic review. J Hum Nutr Diet. 2015; 28 (4): 313–30. doi: 10.1111/jhn.12238.

Wandrag L, Brett SJ, Frost GS, Bountziouka V, Hickson M. Exploration of muscle loss and metabolic state during prolonged critical illness: Implications for intervention? PLoS ONE. 2019a; 14 (11): e0224565. doi: 10.1371/journal.pone.0224565.

Wandrag L, Brett SJ, Frost GS, To M, Loubo EA, Jackson NC, Umpleby AM, Bountziouka V, Hickson M. Leucine-enriched essential amino acid supplementation in mechanically

ventilated trauma patients: a feasibility study. Trials. 2019b; 20 (1): 561. doi:10.1186/s13063-019-3639-2.

Weber-Carstens S, Koch S, Spuler S, Spies CD, Bubser F, Wernecke KD, Deja M. Nonexcitable muscle membrane predicts intensive care unit-acquired paresis in mechanically ventilated, sedated patients. Crit Care Med. 2009; 37 (9): 2632–7. doi: 10.1097/CCM.0b013e3181a92f28.

Weijs PJM, Stapel SN, de Groot,S. D. W., Driessen RH, de Jong E, Girbes ARJ, Strack van Schijndel RJM, Beishuizen A. 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 (1): 60–8. doi: 10.1177/0148607111415109.

Weijs PJM. Fundamental determinants of protein requirements in the ICU. Curr Opin Clin Nutr Metab Care. 2014; 17 (2): 183–9. doi: 10.1097/MCO.0000000000000029.

Weijs PJM, Looijaard WGPM, Dekker IM, Stapel SN, Girbes AR, Oudemans-van Straaten HM, Beishuizen A. Low skeletal muscle area is a risk factor for mortality in mechanically ventilated critically ill patients. Crit Care. 2014; 18 (2): R12. doi: 10.1186/cc13189.

Weinel LM, Summers MJ, Chapple L-A. Ultrasonography to measure quadriceps muscle in critically ill patients: Ultrasonography to measure quadriceps muscle in critically ill patients: A literature review of reported methodologies. Anaesth Intensive Care. 2019; 47 (5): 423–34. doi: 10.1177/0310057X19875152.

Weir JB de V. New Methodes for Calculating Metabolic Rate with special Reference to Protein Metabolism. J Physiol. 1949; 109 (1–2): 1–9. doi: 10.1113/jphysiol.1949.sp004363

Wischmeyer PE, Puthucheary Z, San Millán I, Butz D, Grocott MPW. Muscle mass and physical recovery in ICU: innovations for targeting of nutrition and exercise. Curr Opin Crit Care. 2017; 23 (4): 269–78. doi: 10.1097/MCC.0000000000000431.

Witteveen E, Sommers J, Wieske L, Doorduin J, van Alfen N, Schultz MJ, van Schaik IN, Horn J, Verhamme C. Diagnostic accuracy of quantitative neuromuscular ultrasound for the diagnosis of intensive care unit-acquired weakness: a cross-sectional observational study. Ann Intensive Care. 2017; 7 (1): 40. doi: 10.1186/s13613-017-0263-8.

Wolfe RR. The 2017 Sir David P Cuthbertson lecture. Amino acids and muscle protein metabolism in critical care. Clin Nutr. 2017; 37 (4): 1093–100. doi: 10.1016/j.clnu.2017.12.010.

Wu C, Wang X, Yu W, Tian F, Liu S, Li P, Li J, Li N. Hypermetabolism in the Initial Phase of Intensive Care Is Related to a Poor Outcome in Severe Sepsis Patients. Ann Nutr Metab. 2015; 66 (4): 188–95. doi: 10.1159/000430848.

Yang T, Li Z, Jiang L, Wang Y, Xi X. Risk factors for intensive care unit-acquired weakness: A systematic review and meta-analysis. Acta Neurol Scand. 2018; 138 (2): 104–14. doi: 10.1111/ane.12964.

Zambon M, Beccaria P, Matsuno J, Gemma M, Frati E, Colombo S, Cabrini L, Landoni G, Zangrillo A. Mechanical Ventilation and Diaphragmatic Atrophy in Critically Ill Patients: An Ultrasound Study. Crit Care Med. 2016; 44 (7): 1347–52. doi: 10.1097/CCM.0000000000001657.

Zusman O, Theilla M, Cohen J, Kagan I, Bendavid I, Singer P. Resting energy expenditure, calorie and protein consumption in critically ill patients: a retrospective cohort study. Crit Care. 2016; 20 (1): 367. doi: 10.1186/s13054-016-1538-4.

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