NEED FOR FEED

DETERMINING METABOLIC PHASES OF CRITICAL ILLNESS

Hanneke Pierre
Franciscus Xaverius
Moonen

Propositions

1. Peripheral blood mononuclear cell mitochondrial respiration is a promising proxy marker for sepsis severity and outcome.
   (this thesis)

2. There is cause for optimism about solving the issue of malnutrition in critically ill patients, in spite of the findings in this thesis.
   (this thesis)

3. The use of body mass index as a measure of health is misleading in most contexts.

4. The COVID-19 pandemic exposed significant inefficiencies and inequities in the scientific publishing system, demonstrating the need for reform.

5. Transitioning away from eating animals is a vital step towards a more ethical, sustainable, and health-conscious future.

6. Lunch breaks should be mandatory and regulated for health care workers.

Propositions belonging to this thesis, entitled
Need for Feed: Determining metabolic phases of critical illness
Hanneke Pierre Franciscus Xaverius Moonen
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Need for Feed

Determining metabolic phases of critical illness

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Need for Feed: Determining metabolic phases of critical illness

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Thesis

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Chapter 1

General introduction and outline of the thesis

Metabolism is the term used to describe all chemical reactions in the human body needed to sustain life. A way to categorise metabolic processes is by distinguishing between anabolism and catabolism. Anabolism comprises pathways by which macromolecules (such as proteins) are synthesised from molecular building blocks (such as amino acids), using energy in the process. Catabolism, in turn, involves processes in which macromolecules are broken down to generate building blocks and energy for other uses (Figure 1A). The predominant energy donor is adenosine triphosphate (ATP), produced in the mitochondrion, an organelle found in all human cells except mature erythrocytes. Mitochondrial respiration is the metabolic process that converts energy stored in macromolecules to ATP (Figure 1B). During all metabolic processes, some energy is lost as heat. We require nutritional substrate in the correct dose and composition to fuel our metabolic processes continuously.

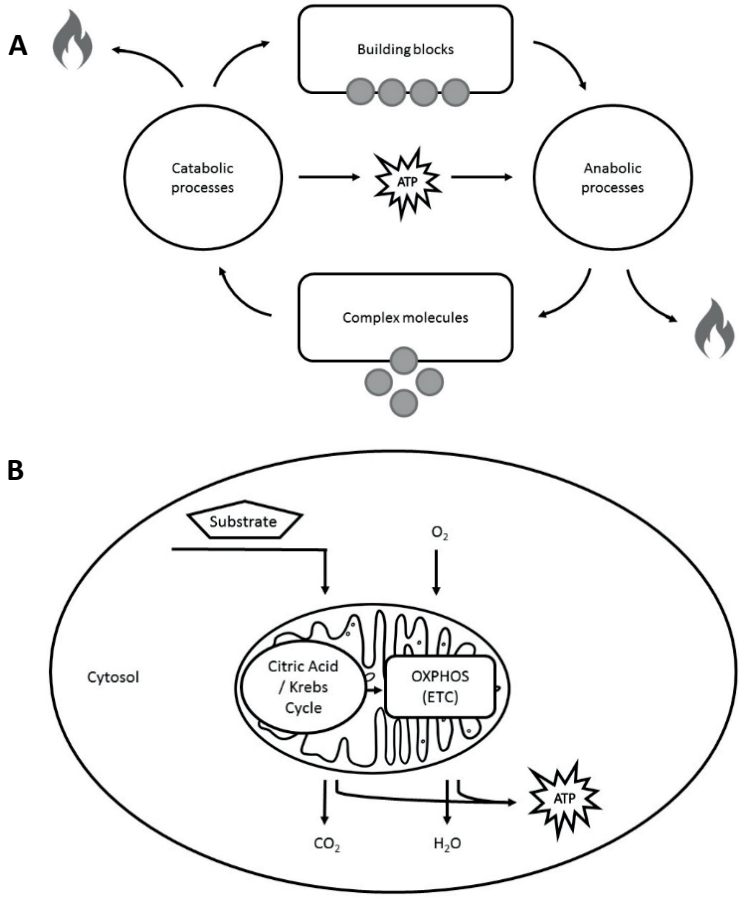

Figure 1. A. Metabolic homeostasis: a state in which catabolic and anabolic processes are in balance. B. Mitochondrial respiration: the conversion of substrate to energy (and byproducts) in the presence of oxygen (O_2) through the citric acid, or Krebs cycle and oxidative phosphorylation (OXPHOS) using the electron transport chain (ETC).

In health, anabolism and catabolism are in balance (homeostasis), thereby enabling life, growth and procreation. In critical illness, this balance is initially disturbed, resulting in a net catabolic state. As a result, various tissues in the body are broken down to release energy reserves and keep up with the catabolic demand (1).

Likely, these metabolic changes are, in part, signs of dysfunction due to the illness. However, it is increasingly assumed that some changes may be an adaptive response of the body to prioritise vital functions and prevent further damage and death. For instance, resistance to anabolic signals, including insulin, is thought to occur in order to prioritise the delivery of energy substrates to vital tissues and processes over the insulin-dependent organs, mainly fat and muscle (1).

Still, the catabolic phase depletes the body's protein reserves, leading to a staggering loss of muscle mass in intensive care unit (ICU) patients. In the past, nutrition support for the critically ill was thought to be aimed at maximal protein and energy provision to attenuate or reverse this catabolic response. However, several clinical trials have failed to prove an unequivocal benefit of 'aggressive' nutrition strategies during the early phase of critical illness, and a few have even shown increased morbidity and mortality in patients receiving high-caloric feeds (2-4,5). Three to five days after the initial assault, ICU patients appear to tolerate and require increased exogenous substrate again, indicating that as the body goes through different phases of critical illness and restoration, the metabolism also changes, altering dietary requirements (6,7). Although post-ICU studies are rare, it is likely that the need for substrate further increases during convalescence as the body switches to anabolism and tries to rebuild what was lost (8).

Thus, not only do critically ill patients require the provision of sufficient substrate, but the wrong quantities or composition at the wrong time could cause significant harm.

It is currently unknown how metabolic phases and subsequent changes in nutritional requirements can best be determined in individual ICU patients. Therefore, nutritional prescriptions are based on static predictive equations based on population-based estimations. Generalised equations are inherently unable to consider all individual differences and the changing composition and requirements of the body throughout the course of the disease. The lack of accuracy of these equations poses significant clinical and scientific challenges, as both caloric under- and overfeeding are associated with worse outcomes of critical illness (9). Furthermore, it is known that despite our current best nutritional therapy practices, patients lose significant amounts of muscle mass and function, which they regain slowly and, in many cases, incompletely during convalescence. Incomplete recovery results in a significant personal, healthcare and societal burden (10-12). The current project aims to shed light on the different metabolic phases and

subsequent nutritional needs in critical illness and explore methods to determine these in the individual patient.

CHANGES IN BIOCHEMISTRY DURING EARLY CRITICAL ILLNESS

Mitochondrial function has been proven to be severely impaired during the early phase of critical illness, with reduced biogenesis, increased reactive oxygen species generation, and decreased ATP synthesis by up to 50 % (22-26). This mitochondrial dysfunction has been linked to increased severity of disease, multiple organ failure syndrome, and worsened long-term outcomes (22,24,26-28). Theoretically, cell death pathways should be activated in this ATP-insufficient environment. However, upon closer examination, there appears to be a paradoxical lack of permanent damage in the organs of survivors (29). These combined findings have led to the theory that the altered mitochondrial function during critical illness reflects a state of adaptive metabolic-bio-energetic downregulation rather than bio-energetic failure (25,29,30).

As described before, early aggressive feeding in the critically ill may paradoxally increase morbidity and mortality (18,19). In contrast to the healthy situation, the increased mobilisation of the body's energy reserves in the net catabolic state of critical illness cannot be abolished by the administration of exogenous substrates. Therefore, meeting all metabolic demands with nutritional substrate in the critically ill results in a surplus (21). In health, an excess of substrate may not lead to immediate damage. However, the acute phase of critical illness may put an insurmountable demand on mitochondria in adaptive hibernation (2,3,25,30). The fact that recovering ICU patients appear to require and tolerate an increased exogenous substrate after 3–5 days may, in turn, reflect an upregulated mitochondrial function.

CHANGES IN ENERGY METABOLISM DURING CRITICAL ILLNESS AND CONVALESCENCE

The total energy humans spend during a specific period is called Total Energy Expenditure (TEE). The TEE can be subdivided into resting energy expenditure (REE), reflecting the energy used to support life in a resting state, and physical activity-related energy expenditure (13-15). During the first phase of critical illness, REE will closely reflect TEE because there is minimal physical activity (16,17). The energy a human spends can be used as a proxy for the energy they require from nutritional substrates. Therefore, it is useful to derive energy expenditure from measurable clinical parameters.

In the current absence of knowledge on the reactivation of the mitochondria, the ESPEN guidelines recommend gradually advancing to target during the first week, not meeting REE before the first 72 hours, to avoid overnutrition (6). After that, patients are usually fed to a target set by predictive equations based on (estimated, lean) body weight. However, there is ample evidence that current REE-predictive equations are inaccurate and lead to under- or overfeeding (6,16,17). Retrospective data show that medical ICU patients receiving inadequate nutrition during the first ICU week (<50% of predicted calorie/protein needs) demonstrated higher mortality compared with patients receiving adequate nutrition delivery (>80% of calorie/protein needs) (39, 40). Therefore, nutritional guidelines recommend using indirect calorimetry (IC) to determine REE in critically ill mechanically ventilated patients as a proxy for nutritional needs (6,7).

Indirect calorimetry determines REE by measuring oxygen consumption (VO_2, in L/min) and carbon dioxide production (VCO_2, in L/min) and subsequently calculates REE according to the adjusted Weir's equation based on the caloric values of the oxidation of one litre of O_2 metabolising a fat and carbohydrate mixture (15,41). Studies describing the course of measured energy expenditure during critical illness and especially during convalescence are rare (42). Uncovering a typical course of energy expenditure throughout critical illness and convalescence could significantly change nutritional guidelines in the ICU and post-ICU settings.

CHANGES IN BODY COMPOSITION DURING CRITICAL ILLNESS AND CONVALESCENCE

Profound changes in body composition often accompany critical illness as the prolonged catabolic phase erodes lean body mass. The subsequent loss of physical fitness, called ICU-acquired weakness, is an important determinant and predictor of disability and quality of life in post-ICU recovery (11). The provision of dietary protein is a well-known anabolic stimulus that promotes and maintains muscle mass in both healthy and clinical settings (43). In this light, optimising nutritional support, especially protein provision during ICU admission, is a promising, easy-to-use tool to preserve muscle mass and improve functional outcomes after ICU discharge. However, the effect of dietary protein to attenuate the catabolic state has yet to be established in clinical trials. Currently, nutritional protein targets are based on estimated fat-free mass (FFM) or lean body mass (LBM), which is assumed to represent skeletal muscle (6). In practice, FFM is almost always estimated based on total body weight. As this leads to significant over- and underestimation in obesity or underweight, dieticians use corrected body weight. However, this method still does not incorporate differences between tissues, tissue- and body weight changes caused by overhydration, as is common in ICU patients.

Bioelectrical impedance analysis (BIA) is a validated, non-invasive, bedside applicable method for assessing body composition. It measures the opposition to an alternating current passing through body compartments (resistance) and the delay in conduction by membranes (reactance). BIA uses these measurements to accurately estimate the contribution of various tissues to the (segmental) body weight. BIA is not yet widely implemented in the ICU, partly because the interpretation of some results is complicated in case of altered hydration status, as is commonly encountered amongst the critically ill (44). However, if interpreted correctly, BIA results have a unique potential to provide real-time insight into the changes in body composition during ICU stay.

Aside from the use of FFM, BIA can provide several other clinically valuable measures. In contrast to BMI, multi-frequency BIA can provide insight into fat distribution. Furthermore, the phase angle derived from measured reactance and resistance is a clinically important bioimpedance parameter related to nutritional status and a predictor of outcomes in several diseases.

OUTLINE OF THE THESIS

Part I focuses on (adaptive) mitochondrial function changes under acute disease-induced metabolic stress conditions. In **Chapter 2**, we provide a comprehensive review of the role of mitochondrial dysfunction during critical illness and convalescence, addressing the potential of nutritional therapies to restore mitochondrial functioning. In **Chapter 3**, we perform a prospective cohort study with matched controls to investigate how mitochondrial function in peripheral blood mononuclear cells progresses in the first week after ICU admission in septic ICU patients.

Part II of this thesis explores patterns in energy metabolism during critical illness and convalescence determined by indirect calorimetry. **Chapter 4** provides an overview of indirect calorimetry use in the ICU and the post-ICU period, covering recent evidence and practical IC use considerations. In **Chapter 5** we perform repeated indirect calorimetry measurements in mechanically ventilated ICU patients at regular intervals until hospital discharge. This prospective observational study compares measured REE during ICU and post-ICU hospital stays among critically ill patients.

The final **Part III** concentrates on changes in body composition during critical illness and convalescence assessed by bioelectric impedance analysis. **Chapter 6** is a review that discusses the potential clinical applications of BIA and explores caveats and solutions to its use in the intensive care setting. In **Chapter 7,** we perform an observational cross-sectional cohort study aiming to to assess the body composition of COVID-19 patients admitted

to the ward or the ICU and identify associations with the severity of disease. **Chapter 8** encompasses a prospective observational study which aims to assess the correlation between baseline phase angle and 90-day adverse outcome of COVID-19, in addition to the derived BIA parameters of body composition. Furthermore, we explore the value of adding phase angle to other baseline clinical characteristics readily available at hospital admission and aid in predicting the disease course.

The need for prolonged invasive ventilation puts COVID-19 pneumosepsis survivors at a high risk of developing ICU-acquired weakness (ICUAW) and the associated post-intensive care syndrome. The RECOVID study, which is detailed in **Chapter 9**, focuses on retrospectively comparing the physical recovery of COVID-19 and non-COVID pneumosepsis ICU survivors during post-ICU hospitalisation.

The provision of dietary protein is a well-known anabolic stimulus that promotes and maintains muscle mass in both healthy and various clinical settings. Correct estimation of nutritional protein requirements during illness remains a topic of much discussion, and several international nutrition guidelines yield many different methods. Using routinely measured lean body mass by BIA is likely a better protein dosing method than equations based on the admission weight or (adjusted) body mass index only. However, in situations that do not allow for routine body composition measurements, such as the unprecedentedly hectic circumstances of the recent COVID-19 pandemic, it is advantageous to search for predictive formulas that agree with BIA-measured FFM in COVID-19 patients. These formulas could simplify protein dosing in vulnerable groups of patients under challenging circumstances. **Chapter 10** describes a post-hoc analysis of the studies described in Chapters 8 and 9, in which we use BIA data to evaluate the accuracy of five more commonly used equations to estimate protein requirements. In addition, we explore what these methods mean for evaluating protein adequacy in the ICU by looking at actual protein provision in our subgroup of ICU patients.

Lastly, in **Part IV**, we discuss the relationship between the findings in each chapter, their implications for clinical practice, and future research directions in this area.

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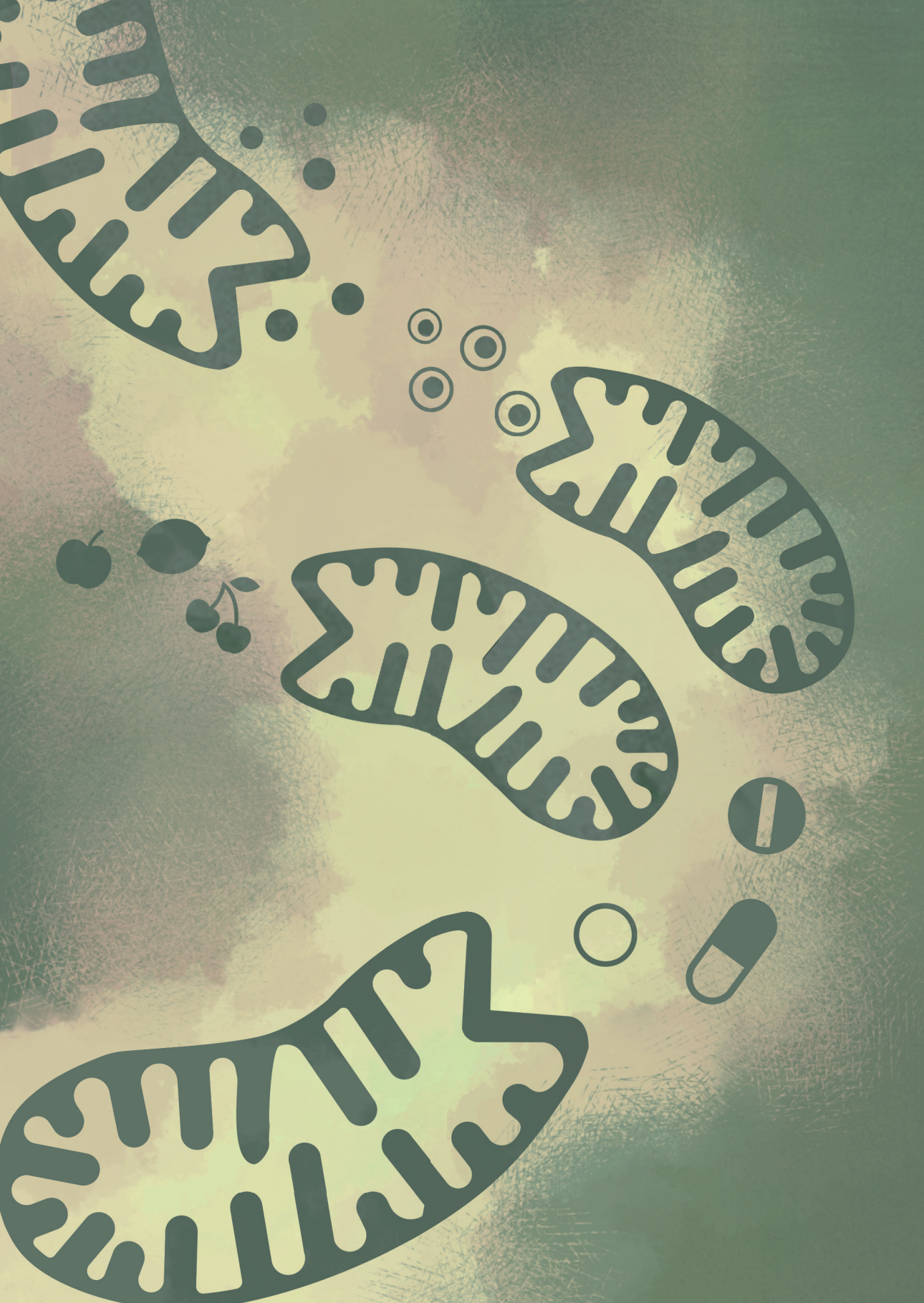

Part I

Biochemical changes during early critical illness

Chapter 2

Mitochondrial dysfunction in critical illness during acute metabolic stress and convalescence: consequences for nutrition therapy

Moonen HPFX, Van Zanten ARH

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ABSTRACT

Purpose of review

Mitochondrial dysfunction is associated with increased morbidity and mortality during and after critical illness. The concept of adaptive mitochondrial metabolic-bio-energetic downregulation rather than bioenergetics failure during the acute phase of critical illness has gained traction. As mitochondria are not able to utilize substrate during adaptive hibernation and aggressive feeding induces further harm, this condition has consequences for nutrition therapy.

Recent findings

Meeting resting energy expenditure in early critical illness is associated with enhanced oxidative stress and attenuation of autophagy, as is hyperglycemia. The negative effect of early high protein administration remains unclear, whereas fat appears bio-energetically inert. Although antioxidant micronutrients are essential to mitochondrial function, high-dosage studies of single vitamins (C and D) failed to show benefit. Convalescence probably requires increased micronutrient and macronutrient administration to aid anabolism and restore mitochondrial function, although robust data on requirements and actual intake are lacking.

Summary

Optimal nutrition therapy in the early phase of critical illness should avoid overfeeding and preserve (adaptive) mitochondrial function. Micronutrient supplementation probably requires a strategic cocktail instead of a high dosage of a single nutrient. Focus on identification of distinct metabolic phases to adapt nutrition during and after critical illness is essential.

Key points

- Mitochondria downregulate their metabolism in the acute phase of critical illness likely in an attempt to avoid cell death, preventing adequate utilization exogenous nutrients.
- Meeting resting energy expenditure in critically ill patients during the early phase is associated with harm, most likely because of increased oxidative stress and attenuation of autophagy.
- The optimal nutrition therapy in the early phase of critical illness requires a balance between sufficient exogenous macronutrients, probably combined with a strategic micronutrients cocktail and avoiding the mitochondrial damage induced by overfeeding.
- Clinical markers should be identified to monitor the metabolic phases of critical illness to target more precisely when nutrition substrates can be utilized.

- Convalescence requires increased substrate administration enhance anabolic recovery, although robust data on mitochondrial behaviour, nutritional requirements, and actual intake are lacking.

INTRODUCTION

Mitochondria are known as the cell's powerhouse, because of their role in adenosine triphosphate (ATP) production through the oxidative phosphorylation (OXPHOS) of macronutrients. Through various other pathways, they play a pivotal role in cell function and survival (1).

Mitochondrial function is proven to be severely impaired during the early phase of critical illness, with reduction of biogenesis, increased reactive oxygen species (ROS) generation, and decreased ATP synthesis up to 50% (2,3,4,5). Mitochondrial dysfunction has been linked to increased severity of disease, multiple organ failure syndrome (MODS), and long-term outcome, making it an important target for therapeutic strategies (2,3,5–7). However, a paradoxical lack of permanent damage in the organs of survivors has led to a theory of adaptive mitochondrial metabolic-bio-energetic downregulation rather than bio-energetic failure (4,8–10). This concept combined with negative outcomes observed in large early-phase nutrition studies suggest aggressive feeding in the acute phase of critical illness puts an unjust demand on mitochondria at a time of adaptive hibernation, inadvertently leading to further damage (4,9,11,12). The question is raised what the optimal strategy is to feed the mitochondria during different metabolic phases of critical illness.

Mitochondrial function in health and disease

Mitochondria are essential to a wide range of cellular functions, including ATP-production, calcium homeostasis, apoptosis, autophagy, and cellular signaling by the release of ROS (13,14). The mitochondrial DNA (mtDNA) proves relatively susceptible to mutations and deletions, particularly under the influence of ROS, warranting a set of tightly self-regulated repair mechanisms (13,15).

Mitochondrial network function is maintained by a delicate balance of fission, fusion biogenesis, and autophagy (Figure 1) (15,16). In the case of disturbances in mitochondrial bioenergetics, various signaling routes allow cross-talk between mitochondria and the nucleus, triggering mitochondrial biogenesis (14). Defective mitochondria can become toxic by excessive production of ROS, which at low levels is used for signaling, but in excess may lead to apoptosis. Mitochondria with mutant mtDNA can merge with other mitochondria through fusion, thereby diluting the damage. Fission, in contrast, is used to create new mitochondria, but also contributes to quality control by enabling the sequestration of damaged mitochondrial parts through mitophagy (16,17).

Autophagy is a mechanism that compensates for nutrient depletion or copes with cellular stress by recycling cellular components, including damaged organelles or macromolecules, to produce amino acids and fatty acids that can be used in OXPHOS (1,17). Autophagy is essential to recover from critical illness (18). However, when excessively induced, autophagy can trigger apoptosis (19). The autophagy process is highly regulated through multiple signaling pathways, several of which involve ROS. As the prime source of ROS, mitochondria emerge as critical mediators in autophagy regulation (1). Conversely, mitochondrial dysfunction could impair autophagy pathways.

Disturbances in mitochondrial functions are associated with severity of disease, short-term and long-term complications of critical illness. ATP depletion and oxidative stress in skeletal muscle are associated with shock severity (3). Significant differences in mitochondrial bioenergetics between eventual survivors and nonsurvivors within 24 h of ICU admission were found (3). Recently, variations in mtDNA were shown to be associated with the development of or protection from delirium during sepsis (20). The strongest association with mitochondrial dysfunction was found in prolonged ICU-acquired weakness (2,5). A murine postsepsis model showed that prolonged muscle weakness was associated with ongoing mitochondrial dysfunction and oxidative damage, even after recovery of muscle mass. Thus, it is likely that mitochondrial bioenergetics are impaired long after sepsis itself has resolved (21). Skeletal muscle biopsies harvested from MODS patients showed a twofold decrease in mitochondrial content (22). Furthermore, ICU patients had a ~50% reduction of the ability of skeletal muscle to synthesize ATP compared with healthy controls, (2). Sepsis survivors had higher muscle ATP levels than eventual nonsurvivors (23).

Mechanisms of mitochondrial dysfunction

Historically, hypoxia was assumed to be the major contributor to mitochondrial dysfunction (24). However, studies in patients with sepsis have shown normal or elevated tissue oxygen levels, as opposed to depletion (8,10). There seems to be a decrease in oxygen utilization for cellular respiration rather than oxygen delivery (2,4,10,25). In patients with sepsis, a reduced oxygen utilization by 22–42% was found, compared with healthy volunteers (26). ATP-levels in organs and skeletal muscle of patients who died from critical illness were significantly lower than in survivors and controls (3,13).

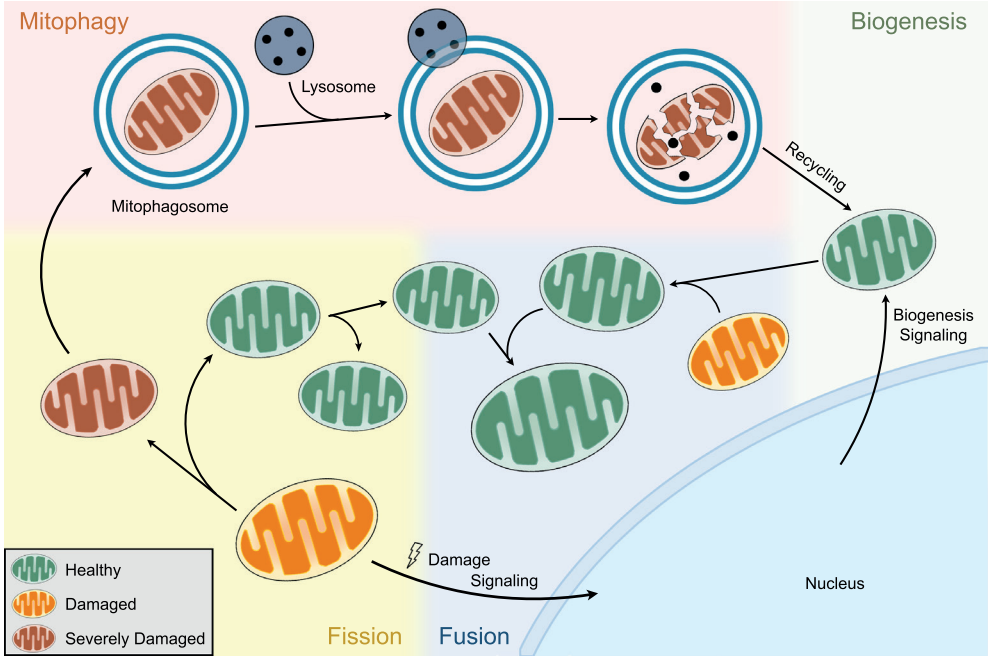

Figure 1. Overview mitochondrial rescue mechanisms.

Mitochondrial network function is maintained by a delicate balance of biodynamics. Through fission, one mitochondrion can split into two independent organelles. Alternatively, a mitochondrion can sequester damaged elements and salvage organelle function. Fusion is the opposite dynamic process, through which two healthy mitochondria can form one larger structure. Additionally, a damaged mitochondrion can fuse with a healthy counterpart, to dilute the damage and preserve function. Mitophagy selectively removes damaged or excess mitochondria, mediated by the formation of a mitophagosome and subsequent merging with a lysosome. Cellular components can then be recycled. In case of imbalances in the mitochondrial network, various signaling routes allow cross-talk between mitochondria and the nucleus, triggering mitochondrial biogenesis through transcription and translation of both nuclear and mitochondrial genes. Image created by C.S. Redshaw for this publication.

OXPHOS dysfunction could explain the inability to maintain ATP levels despite adequate oxygen delivery, leading to increased glycolysis and paradoxically increased lactate levels found even after adequate resuscitation (4,10,13). The question is what causes the inhibition of OXPHOS in the absence of hypoxemia. The hibernation theory provides an alternative explanation by adaptive downregulation rather than bio-energetic failure. The perpetuation of cellular processes in the absence of sufficient ATP to fuel them, as during the increased metabolic demand of early critical illness, eventually leads to apoptosis. Nevertheless, little evidence of cell death is found in organs of critically ill patients and regenerative capacity during convalescence is often remarkable (6,8). It is therefore hypothesized that under great duress in the early phase of critical illness, mitochondria prioritize certain processes, opting to sustain cell life at the expense of functionality (4,8). This strategy decreases ATP utilization, thereby maintaining ATP levels above a critical

threshold and deferring cell death, at the cost of MODS. In keeping with this theory, there is a gradual reduction in oxygen utilization during the early phase of critical illness, in some instances comparable to that of healthy individuals (8). Several factors can further contribute to OXPHOS compromise, including excessive inflammatory mediators, alteration in thyroid hormone functions, reduced mitochondrial protein production, and the uncoupling of fat metabolism (4). Furthermore, critical illness induced hyperglycemia can increase glycolysis, which produces mitotoxic byproducts inhibiting respiratory chain function (10).

Increased ATP demand and concurrent inability to keep up ATP production can overburden mitochondria, leading to hypercalcemia, increased levels of ROS, and other deleterious radicals (4,13). When ROS outnumber antioxidants, the ensuing oxidative stress leads to further damage to the electron transport chain and the mtDNA, creating a vicious circle of mitochondrial damage and ROS production (24). ROS and calcium overload can increase membrane pore permeability in mitochondria, causing mitochondrial products such as mtDNA to leak into the circulation, acting as danger-associated molecular patterns and contributing to MODS (4,27). Opening of the membrane permeability transition pore because of ATP depletion, loss of mitochondrial membrane potential, and synergistic deleterious effect of ROS and calcium can trigger apoptosis (9,13).

Mitochondrial form and function changes in critical illness suggest that mitochondrial rescue mechanisms fail. Preliminary research suggests fission and fusion are upregulated in some tissues during critical illness, although upregulation did not affect mtDNA content and appeared insufficient to restore mitochondrial function (28). Mitochondrial biogenesis response was shown in skeletal muscle taken on day 1–2 in ICU survivors, but not in nonsurvivors, suggesting biogenesis upregulation is associated with survival (23). In contrast, a reduction in mitochondrial density was shown after the onset of sepsis, alluding that although upregulated, biogenesis may be insufficient to maintain homeostasis (27).

Implications for nutrition therapy

Mitochondrial downregulation has consequences for nutrition therapy in different metabolic phases of critical illness. A significant impediment remains that there is no means to identify transition into the next metabolic phase or mitochondrial function itself in the clinical setting (9,27,29). Lambell et al.(29) propose to adhere to three phases of illness: acute, acute late, and recovery. However, this is probably an oversimplification at a time where no biomarkers are available.

Older ICU nutrition guidelines supported early aggressive feeding to meet resting energy expenditure (REE) and thus preventing malnutrition and muscle loss. However, clinical studies have failed to prove an unequivocal benefit of early nutrition and several

prospective randomized clinical trials showed significant harm (nutritrauma) (11,12,30,31). This led to the theory that meeting REE when mitochondria are reduced in both number and capacity inflicts damage, as substrates cannot be utilized and attempting to do so further harms mitochondria (4,30,32). Additionally, muscle protein synthesis uses ATP when ATP production is limited and in high demand, further threatening cell survival (4). Research indicates that a substantial macronutrient deficit during the early phase of critical illness reduces rather than increases the incidence of muscle weakness and allows weakness to recover faster (33). The early phase of critical illness is characterized by insulin resistance and massive mobilization of calorie reserves, which can provide 50–75% of glucose needed (29,34). This process is not suppressed by exogenous nutrition (34,35,36). McKeever et al.(37) found that meeting REE during the first 7 days of ICU stay was associated with increased oxidative stress. Another possible mechanism of harm is through the inhibition of autophagy. Fasting leads to more efficient activation of autophagy (33). Reversely, feeding appears to inhibit autophagy, though in vitro research suggests there might be different phenotypes of autophagy flux implicating that early nutrition does not block autophagy directly, but rather attenuates the beneficial effect of starvation in some patients (19,30,33). Nevertheless, there likely is some need to feed. Optimal nutrition therapy in the early phase of critical illness could be the balance between sufficient exogenous support, preserving gut integrity, while avoiding inducing nutritrauma (30,37,38).

It is unclear which macronutrient is responsible for the main, beneficial or harmful, effects of caloric support. Notably, most studies investigating the effect of caloric intake did not standardize protein intake, possibly introducing bias (39). Protein is of special interest, as it is required for muscle synthesis and the initial catabolic response leads to a reduction in muscle mass up to 1 kg/day during the first 10 days of ICU stay (40). However, data on protein administration in the early phase of critical illness are conflicting. One study assessing the influence of amino acids administration at either 0.8 or 1.2 g/kg on handgrip strength found no difference at ICU discharge (41). Conversely, observational studies showed that additional protein was associated with a reduction in morbidity and mortality (35,42,43). Retrospective analyses found very high-protein intake within the first three days was associated with increased mortality (42,44). Future research should address the optimum timing and dosing of protein and calories individually (29,39). For now, a gradual increase in protein and calorie provision to a target of 1.3 g/kg/day and 70–100% of REE in the early phase is recommended (Figure 2) (45,46).

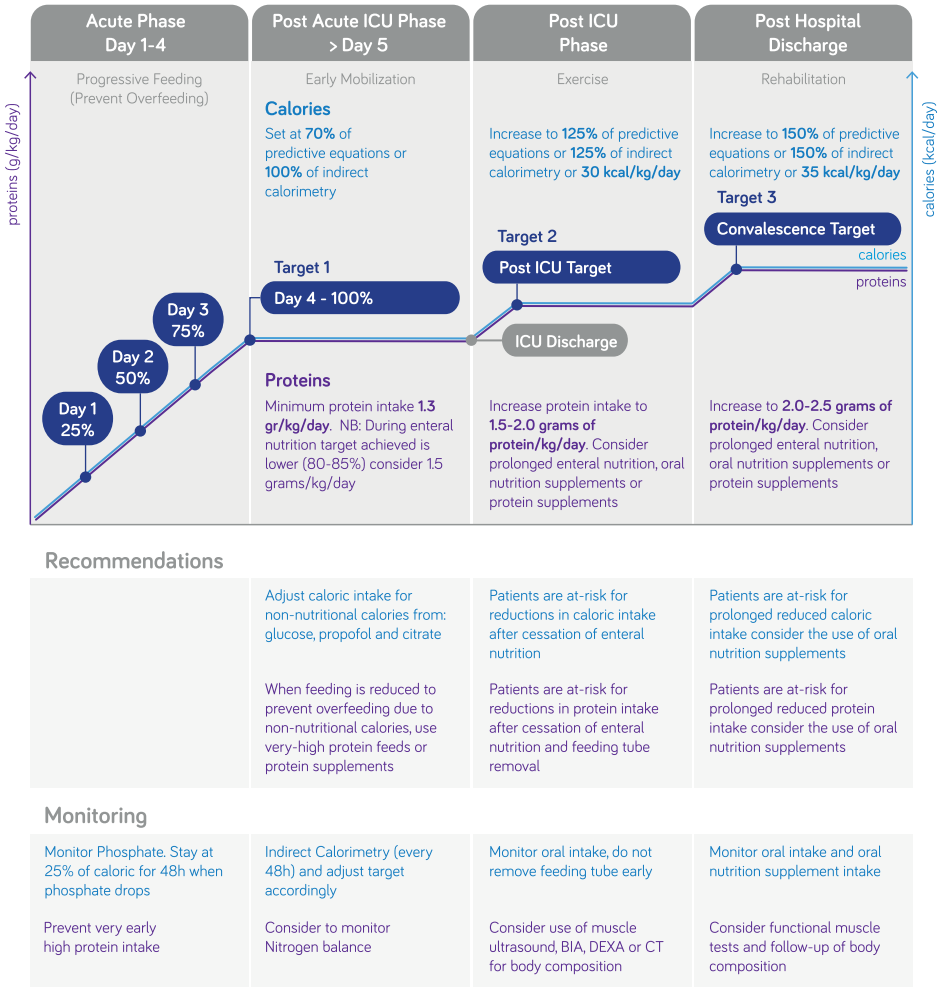

Figure 2. Practical approach to provide proteins and calories during the phases of critical illness and convalescence as proposed by Van Zanten et al.

During the first 3 days, calories and proteins are gradually progressed to target 1 on day 4 in steps of 25% daily increase. Target 1 is 1.3 g/kg/day for proteins and for calories 70% of calculated targets or 100% of target when measured by indirect calorimetry. Target 2 should be met during chronic critical illness and after ICU discharge on general wards. For target 2, calories are increased to 125% of predictive equations or indirect calorimetry or 30 kcal/kg/day and for proteins 1.5–2.0 g/kg/day should be targeted. After hospital discharge, target 3 recommends a higher caloric target (150% of predictive equations or 35 kcal/kg/day) and a higher protein intake of 2.0–2.5 g/kg/day. g/kg/day, grams of proteins per kilogram per day; kcal/day, total kilocalories per day; BIA, bioelectrical impedance analysis; DEXA, dual-energy X-ray absorptiometry; CT, computed tomography scanning. Reproduced with permission from (46).

In critical illness patients receive fatty acids both in the form of nutritional and nonnutritional substrates, adding 29–43% to the caloric intake in enteral and 50% in parenteral feed (47). Puthucheary et al.(32) explored the relationship between muscle

mass loss in early critical illness and the bioenergetic status and found that changes in intramuscular ATP content and skeletal muscle mass are unrelated to the quantity of lipids delivered. This suggests that the lipid component of enteral and parenteral nutrition may be bioenergetically inert. Recent guidelines advise intravenous lipid should not exceed 1.5 g lipids/kg/day (45).

Carbohydrates are the preferred substrate for the production of energy, but in critical illness they may worsen stress-induced hyperglycemia (45,48). Endogenous glucose production is increased in critical illness and this is not abolished when nutrients and insulin are administered (24,45). The EAT-ICU trial found that patients receiving early goal-directed nutrition had severe hyperglycemia and received higher doses of insulin as compared to those in the control group (49). Hyperglycemia induces an increase of mitochondrial oxygen consumption, mitochondrial ROS production and calcium levels in pancreatic cells (50). Moreover, in patients with hyperglycemia, liver cells showed insufficient autophagy and more pronounced mitochondrial abnormalities (19). Nutrition guidelines recommend the amount of glucose in parenteral nutrition or carbohydrates in enteral nutrition administered to ICU patients should not exceed 5 mg/kg/min (45).

Adequate micronutrient levels are essential for mitochondrial function as specific micronutrients play crucial roles in energy metabolism and ATP-production. Recent reviews by Wesselink et al.(24) and Berger (51) outlined the multitude of possible targets to correct imbalances (Figure 3). Among these is vitamin C, the most potent water-soluble antioxidant, which when deficient could lead to increased ROS and impaired OXPHOS (24,51). In experimental sepsis models, intravenous vitamin C reduces organ injury and improves survival (52). However, in a randomized clinical trial, high-dose vitamin C infusion compared with placebo did not significantly reduce organ failure scores, although a significant reduction in 28-day all-cause mortality and decreased ICU and hospital length-of-stay (53). A recent trial on the effects of a cocktail of vitamin C, thiamin (vitamin B1), and steroids compared with placebo was negative on all endpoints (54). Vitamin D deficiency is associated with increased oxidative stress and altered activity of antioxidant enzymes in skeletal muscle (55,56). Skeletal muscle cells treated with metabolized vitamin D showed increased respiration and ATP generation (55,56). Nevertheless a recent early high-dose vitamin D study showed no advantage over placebo concerning 90-day mortality or other, nonfatal outcomes among critically ill, vitamin D–deficient patients (57). In addition, low plasma levels might not adequately reflect low total body stores because of redistribution, incorporation, body fluid redistribution and protein binding and optimal dosages are not known (58). The disappointing results of clinical trials despite promising theoretical advantage might be in part explained by the fact that antioxidants act synergistically. Clinically beneficial supplementation could require the right antioxidant cocktail instead of a high dose of a single micronutrient (51,58).

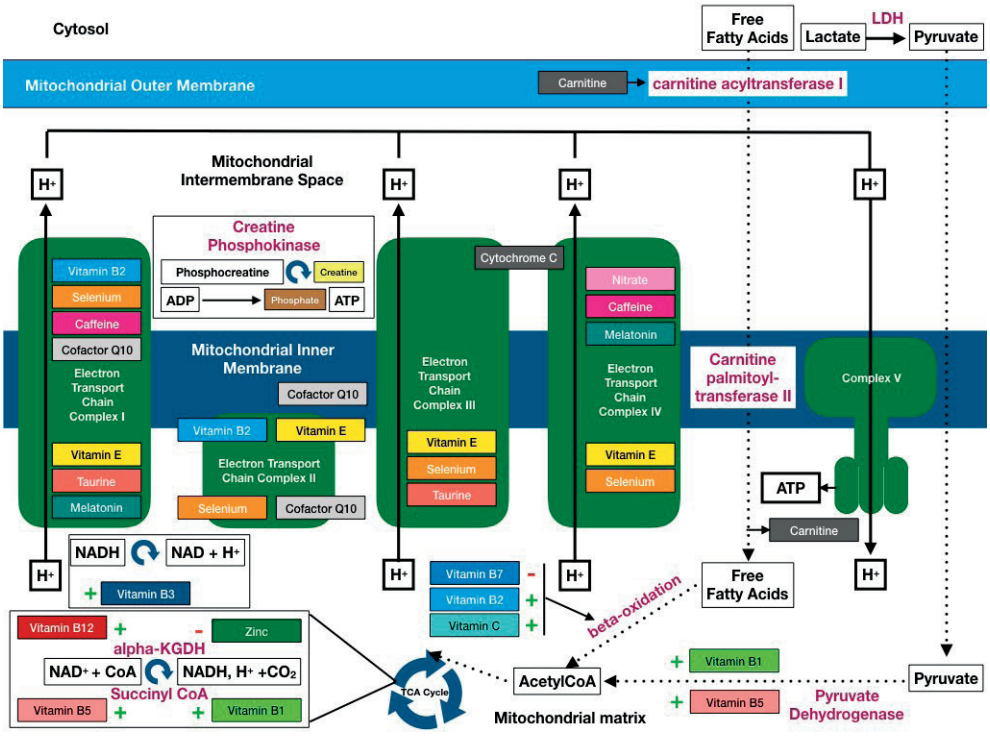

Figure 3. Overview of relevant nutrients in bio-energetic mitochondrial processes.

Several nutrients are involved in the formation of acetyl CoA, which is essential in energy production as it is the starting point of the TCA cycle. Thiamine (vitamin B1) is essential for the conversion of pyruvate to acetyl-coA. Furthermore, high levels of zinc were found to inhibit the glycolysis and TCA cycle. Carnitine is essential in beta-oxidation of free fatty acids. In addition to the formation of acetyl CoA, several nutrients have an direct effect on the TCA cycle. Pantothenic acid (vitamin B5) is the precursor of CoA. Vitamin B 12 is an essential cofactor in the formation of succinyl-CoA, an important metabolite of the TCA cycle. In addition, several nutrients influences the activity of the electron transport chain. Niacin (vitamin B3) is the precursor of NADþ, which has a crucial role in the formation of NADH, which on turn plays a crucial role in the electron transport chain. Complex I and IV activity is decreased during critical illness, but several nutrients positively affect complex I and IV performance. Complex I and IV may be stimulated by selenium, caffeine and melatonin. Complex I and II are also stimulated by CoQ10. Taurine depletion is associated with impaired activity of complexes I and III. Whether the effect of vitamin E on the complexes I and IV is stimulating or inhibiting has not yet been revealed. Nitrate probably inhibits complex IV activity. Riboflavin (vitamin B2) is an important building block for complexes I and II and involved in fatty acid oxidation in the TCA cycle. a-KGDH, alpha-ketoglutarate dehydrogenase; ATP, adenosine triphosphate; CoA, coenzyme A; CO_2, carbon dioxide; CoQ, coenzyme Q; NAD(h), nicotinamide adenine dinucleotide (reduced); PDH, pyruvate dehydrogenase; Vit, vitamin. Reproduced with permission from [24].

A rebound increase in metabolism occurs during the chronic phase of critical illness (8). Indirect calorimetry studies during the recovery phase of critical illness are rare. However, the limited information available suggests a marked increase in metabolic needs. Total Energy Expenditure could increase as much as ~1.7-fold above REE (59). Retrospective

data show that medical ICU patients receiving inadequate nutrition during the first ICU week (<50% of predicted calorie/protein need) demonstrated higher mortality compared with patients receiving adequate nutrition delivery (>80% of calorie/protein needs) (35,60). The ESPEN guidelines recommend to gradually advance to target during the first week, not meeting REE before the first 48 h to avoid overnutrition (45).

Little is known about caloric requirements during post-ICU hospital stay and convalescence. The patient likely enters an anabolic state in which significant calorie and protein delivery is required to restore lost muscle mass. Considering that the average post-ICU patient is older and often frail, it may be assumed that anabolic resistance is relatively common (46). In the absence of conclusive data, therefore, an intake of 1.5–2.5 g/kg/day of proteins should be considered (46). However, observational studies showed that one week postextubation oral intake failed to exceed 50% of daily energy and protein requirements and energy and protein intake in the post-ICU hospitalization period is markedly less than measured energy requirements (61,62). There is a need for prospective studies assessing the course of the energy requirements throughout critical illness and convalescence, the barriers to adequate oral intake and accurate assessment of nutrition intake.

Conclusion

Optimal nutrition therapy in the early phase of critical illness could be finding the balance between sufficient exogenous micronutrients while avoiding mitochondrial damage by overfeeding at a time when the nutrition substrates cannot be utilized. Micronutrient supplementation likely requires an optimal cocktail instead of a high dosage of a single nutrient. It is not known when mitochondria reactivate, but ICU patients appear to require and tolerate increased exogenous substrate after 3–5 days. Further research is needed to identify metabolic phases in the individual patient and to an estimate nutritional needs both during ICU stay and thereafter adequately.

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Chapter 3

Progression of peripheral blood mononuclear cell mitochondrial function during the early phase of sepsis in Intensive Care Unit patients

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Submitted

ABSTRACT

Background

Sepsis is a leading cause of ICU admission and is associated with high rates of multiorgan failure and mortality. Altered mitochondrial function is an essential component of the early sepsis syndrome. However, its progression over time in peripheral blood mononuclear cells (PBMCs), essential mediators of the initial inflammatory response, is thus far unclear.

Aim

To investigate the progression of mitochondrial respiration in peripheral blood mononuclear cells (PBMCs) in the early phase of sepsis in ICU patients.

Methods

A single-centre prospective observational cohort study was conducted in sepsis patients and compared with age- and sex-matched controls. Patients with comorbidities known to affect mitochondrial function were excluded. We measured mitochondrial function using functional respirometry measurements (Oroboros O2K) in PBMCs thrice during the first week of ICU admission. Secondary endpoints included the associations between mitochondrial function and (I) sepsis severity and (II) clinical outcomes, including 3-month mortality.

Results

Basal and ATP-linked respiration and coupling efficiency were increased in sepsis patients (n=25) compared to matched controls (n=26) at all time points. No differences in maximal respiration (evoked by CCCP injection) were detected. Increased basal respiration was associated with 3-month mortality (HR 3.794, 95%CI 1.018-14.149, p=0.047). No differences were observed in other secondary outcomes.

Conclusion

PBMC mitochondria were shown to have an increased respiratory rate during the first week of sepsis. Moreover, a progressive increase in mitochondrial respiration was negatively associated with 3-month survival.

BACKGROUND

Sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to infection, is a primary reason for admission to an Intensive Care Unit (ICU) (1). Sepsis often contributes to (multi)organ failure and is associated with an average 30-day mortality of up to 35% of septic shock cases, accounting for about 20% of all global deaths in 2017 (2,3). Sepsis survivors are at an increased risk of post-hospital discharge morbidity, mortality and a markedly reduced quality of life, which may last years after hospital discharge (4,5). A lack of known therapeutic targets partly explains these poor clinical outcomes.

There is increasing evidence for the role of altered mitochondrial function in the pathogenesis of sepsis-associated multiple organ dysfunction syndrome (6-8). The primary function of the mitochondria is to produce adenosine triphosphate (ATP), the universal energy donor in the cell Mitochondrial respiration is the set of metabolic reactions and processes requiring oxygen at one of the final steps of the oxidative phosphorylation system (OXPHOS) in mitochondria to convert the energy stored in macronutrients to ATP (9-11) (Figure 1). For example, pyruvate, derived from the breakdown of glucose, is converted into Acetyl CoA which subsequently goes into the TCA cycle to produce energy NADH and $FADH_2$ (Figure 1B). Both NADH and $FADH_2$ serve as crucial electron carriers for the OXPHOS where electrons are transported to molecular oxygen through four multiprotein complexes (Figure 1C). This results in a proton gradient across the inner mitochondrial membrane. The energy from this gradient drives the FoF1 ATP-synthase to synthesize ATP. Therefore, mitochondrial respiration can be used as a marker to assess the primary function of mitochondria (Figure 1D). A decreased mitochondrial respiration has been demonstrated in various cells in septic ICU patients, including muscle tissue and blood platelets (7-10,12-16). However, in contrast to these results, studies that measured mitochondrial function in peripheral blood mononuclear cells (PBMCs), which play an essential role in the initial (hyper)inflammatory response that hallmarks sepsis, have resulted in conflicting results.

Human peripheral blood mononuclear cells (PBMCs) are isolated from peripheral blood and identified as any blood cell with a round nucleus (i.e. lymphocytes, monocytes, natural killer cells and dendritic cells) (17). Several studies reported a decreased mitochondrial function in PBMCs during sepsis (7,18), while others reported the opposite, namely an increased mitochondrial function (6,19). One study even reported an increased mitochondrial respiration, but concomitantly, an increased mitochondrial uncoupling leading to reduced ATP-linked respiration (20). Methodological differences, such as varying control groups and respiration mediums, might explain the inconsistency in the results of these studies. For example, the presence of plasma in the medium could influence the results, as suggested by the effects of incubating healthy cells in plasma of septic

patients on mitochondrial respiration of PBMCs, as shown by Belikova and co-workers (6). In addition, control groups were different, including, amongst others, critically ill postoperative patients (7) and non-septic patients with an infection (18).

Furthermore, in two of the mentioned studies, only one measurement was performed in each patient, which does not create insight into the progression of mitochondrial function in PBMCs during ICU stay (7,18). This limitation is unfortunate since performing multiple measurements during ICU stay may reveal time-dependent effects of sepsis on mitochondrial function in PBMCs and its association with clinical outcomes. Although Sjövall *et al.* have performed multiple measurements during ICU stay, no correlations between the time-dependent changes in mitochondrial function during ICU stay and 3-month mortality were found (19). On the contrary, Japiassú *et al.* reported a positive association between increased mitochondrial dysfunction and clinical outcomes, including organ failure and hospital mortality (7).

Rationale

We set out to fill several knowledge gaps based on previously reported studies. To be able to investigate whether mitochondrial derangements originate from the PBMCs themselves, we opted to resuspend the PBMCs in a standardized medium, not plasma. Secondly, studies assessing the potential time-dependent effects of sepsis on mitochondrial function in PBMCs are lacking. Therefore, we performed repeated mitochondrial respiration measurements during the first week of ICU stay. Lastly, we calculated correlations between clinical outcomes and mitochondrial function changes to reveal potential time-dependent associations between mitochondrial function and clinical outcomes.

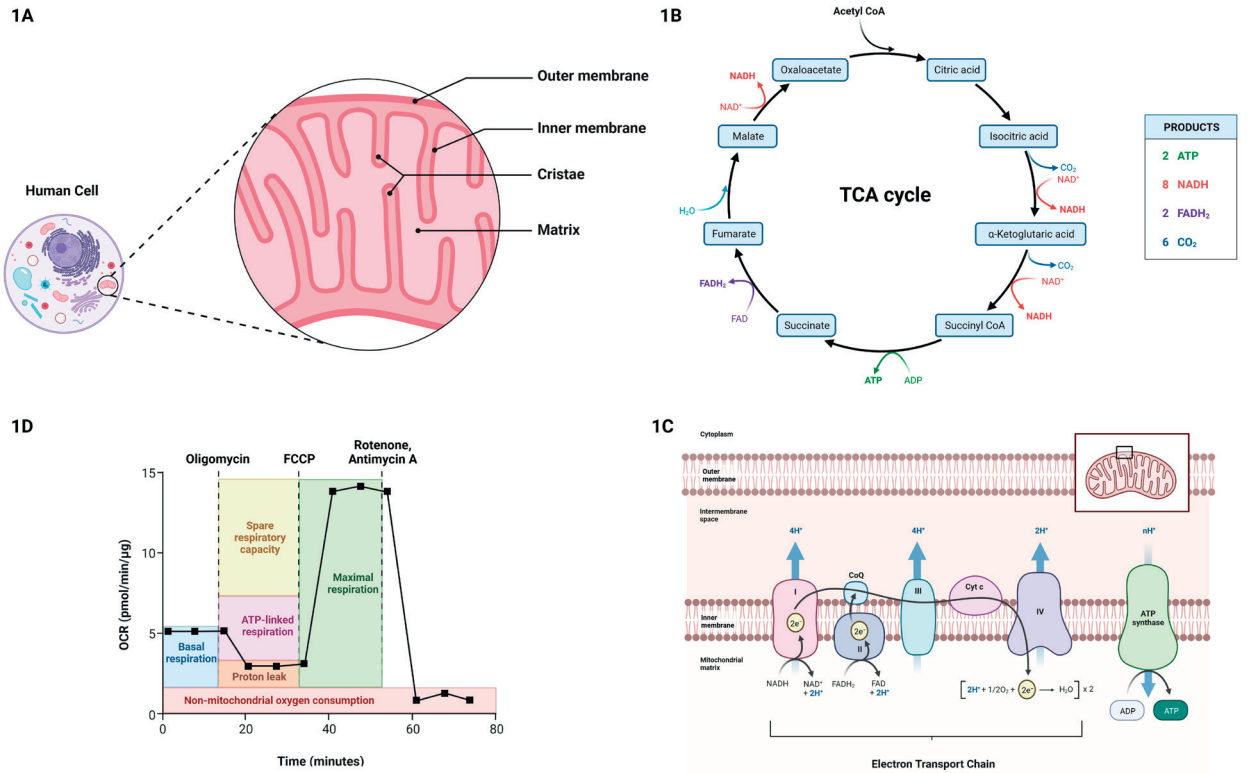

Figure 1. Schematic overview of ATP production in a mitochondrion via the process of the citric acid cycle and oxidative phosphorylation

A. Mitochondria are organelles found in most human cells, the primary function of which is to generate energy in the form of adenosine triphosphate (ATP) through respiration. **B.** The tricarboxylic acid cycle, also known as Krebs cycle, consumes acetate (in the form of acetyl-CoA) and water and reduces NAD+ to NADH, releasing carbon dioxide. The NADH generated by the citric acid cycle is fed into the oxidative phosphorylation (electron transport) pathway. **C.** The electron transport chain (ETC) in the cell is the site of oxidative phosphorylation (OXPHOS). The NADH and succinate previously generated in the citric acid cycle are oxidized, releasing the energy of O2 to power the ATP synthase. **D.** Schematic of the contribution of the key parameters of OXPHOS to the mitochondrial oxygen consumption rate over time after addition of mitochondrial inhibitors. Created with Biorender.

MATERIALS AND METHODS

Study design and setting

A prospective, observational single-centre cohort study with an age- and sex-matched control group was conducted at Gelderse Vallei Hospital (ZGV, Ede, The Netherlands) between January 1, 2018, and January 27, 2023. Due to the severe acute respiratory coronavirus 2 (SARS-CoV-2) pandemic, study inclusions were temporarily halted between March 14, 2020, and October 1, 2020. PBMC measurements were performed at Wageningen University and Research (WUR, Wageningen, The Netherlands).

Study participants

Patients (aged ≥18 years) admitted to the ICU with sepsis and/or septic shock were eligible for inclusion. Patients were enrolled after signing the informed consent by the patient or legal representative. According to the Third International Consensus Definitions, sepsis was defined as a new life-threatening organ dysfunction caused by a dysregulated host response to microbiologically confirmed or clinically suspected (supported by laboratory or radiology findings) infection, as identified by an increase in the sequential organ failure assessment (SOFA) score of ≥2 points. Septic shock was defined as the need for vasopressors to maintain a mean arterial pressure of ≥65 mmHg and serum lactate levels >2 mmol/L (>18 mg/dL) in the absence of hypovolaemia (1).

The control group was recruited from metabolically healthy short-stay hospitalised and outpatient clinic patients, individually matched for age and sex (21).

Patients from the sepsis and control groups were excluded from participation in the case of:

- Urosepsis (ICU patients only);
- Transfer from another ICU (ICU patients only);
- Serum haemoglobin level <5,5 mmol/L;
- Current hemodialysis or continuous renal replacement therapy;
- An expected survival of less than six months due to pre-existent underlying conditions (e.g., end-stage cancer);
- Treatment with chemo-, immune- and/or radiotherapy within the past 12 months;
- A significant event leading to hospitalisation within the previous six months;
- History of solid organ or bone marrow transplant;
- History of drug abuse;
- Family history of mitochondrial disease(s);
- Treatment with any investigational agent in the previous 12 months;
- Treatment with *systemic* corticosteroids or other immunosuppressive medications for active autoimmune disease involving the lung, heart, liver, small or large intestine, or neuromuscular system within three months prior to ICU admission;

- Pregnancy;
- Diabetes Mellitus type I or II (pre-ICU-admission where applicable);
- COPD GOLD stage III or IV or other severe respiratory disorders (FEV1 <30% and FEV1/FVC < 0.7) (pre-ICU admission where applicable);
- Any stage of acute or chronic renal failure (pre-ICU admission, where applicable);
- Any stage of acute or chronic liver failure (pre-ICU admission, where applicable);
- Consumption of >25 grams of ethanol daily (>2.5 alcoholic beverages/day);
- Not able to understand the Dutch language;
- Current participation in intervention research.

Study objectives

The primary study objective was to investigate the progression of mitochondrial respiratory function in PBMCs in septic ICU patients during the first week of ICU admission. Secondary objectives were to investigate the association between mitochondrial respiratory function and (I) sepsis severity and (II) clinical outcomes, including ICU-, hospital and 3-month mortality, length of ICU and hospital stay (LOS) and duration of mechanical ventilation.

Data collection

This study used PBMCs to measure mitochondrial respiratory function.

Sepsis group

Arterial blood samples were collected at three time points via an indwelling arterial access: day 1-2 (24-48h), day 3-4 (72-96h) and day 5-6 (120-144h) after ICU admission (indicated with T1, T2 and T3), respectively. A maximum of 70 mL of whole blood was collected per time point.

Control group

The control group underwent blood sampling by venepuncture with a vacutainer once during their visit to the outpatient clinic or short-stay hospitalisation. No physical tests were performed in this group.

PBMC isolation, washing and counting

Blood samples for PBMC isolation were collected in sodium citrate buffered cell preparation tubes containing a ficoll solution and centrifuged at 1000g for 30 minutes at room temperature. Next, PBMCs were resuspended in warm (37°C) 10mL of Hank Balanced Salt Solution and centrifuged at 400g for 10 minutes at room temperature. The supernatant was then removed, and this washing step was repeated twice. After washing, the resulting PBMC pellet was resuspended in 1 mL of warm (37°C) Seahorse XF base medium supplemented with 2 mM glutamine and 25 mM glucose. The PBMCs were counted using the Cellometer auto T4, and cell viability was assessed by mixing

10 µL of cells with 10 µL acridine orange and propidium iodide stain. PBMCs were then immediately used for high-resolution respirometry.

High-resolution respirometry

Two to five million live PBMCs were injected into a chamber of the Oroboros O2K (Oxygraph-2k Oroboros Instruments, Innsbruck, Austria). The chamber volume was set to 2mL and filled with Agilent Seahorse XF Base medium supplemented with 25 mM glucose and two mM glutamate, and the pH was set to 7.4. The temperature within the chamber was set to 37°C, stirring speed to 750 rotations per minute. Oxygen concentration is continuously measured, recorded and used to calculate oxygen flux per one million live PBMCs using DatLab Software 4.3 (Oroboros Instruments, Innsbruck, Austria) (Figure 1).

After injection of the PBMCs, the basal respiration was recorded first. Second, oligomycin (2.5 µM) was added, which induced a state in which respiration is primarily to compensate for proton leakage. Third, carbonyl cyanide m-chlorophenylhydrazone (CCCP) was added repeatedly (20 nM) until maximum mitochondrial respiration was reached. Fourth, the complex I inhibitor rotenone and the complex III inhibitor, antimycin A, were added (0.5 µM and 2.5 µM, respectively) to determine non-mitochondrial respiration. Each step of the function profiling test was recorded after respiration had stabilised. Additionally, three parameters were calculated. ATP-linked respiration was calculated by subtracting leak respiration from basal respiration. Coupling efficiency was calculated by dividing ATP-linked respiration by basal respiration. Spare respiratory capacity was calculated by subtracting basal respiration from the maximal respiration.

Additional data sources

Data collection from the electronic medical record systems MetaVision® (iMDsoft, Tel Aviv, Israel) and NeoZIS® (MI Consultancy, Katwijk, The Netherlands) included baseline patient characteristics (including disease severity scores), laboratory values and outcome parameters, such as duration of mechanical ventilation and length of ICU and hospital stay.

Study size

Japiassú *et al.* previously studied maximal mitochondrial oxygen consumption in PBMCs of septic ICU patients (n = 20) and critically ill postoperative patients (n = 18) (7). Oxygen consumption was significantly reduced in the septic ICU patient group compared to the control group (5.60 ± 2.0 nmol O_2/min/10^7 cells versus 9.89 ± 3.8 nmol O_2/min/10^7 cells, respectively, $p<0.01$). Assuming altered mitochondrial function (measured as mitochondrial oxygen consumption) during sepsis develops linearly, the expected difference (effect size) between the measurements at the three different time points is similar to 50% of the observed differences by Japiassú et al. Therefore, to achieve a power

of 0.95 with a two-sided significance level of 0.05, 30 subjects per group were needed (as calculated with G*power, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany).

When three valid measurements from an ICU subject could not be obtained due to withdrawal of consent, death, early ICU discharge or technical problems, patients were not matched with a control subject. Moreover, additional patients were included consecutively until complete measurements were obtained in 30 patients. Separate measurements from patients who did not have three complete consecutive measurements were included in the first analyses. The Cox regression models and ANOVA analyses also included patients with valid measurements at T1 and T3.

Statistical analyses

Data verification was conducted manually. Descriptive statistics were performed for demographic and clinical data of all patients and the primary outcome. Normality was assessed numerically and graphically. Continuous values were reported as means with standard deviations (SD; parametric data) or medians with interquartile ranges [IQR; non-parametric data]. Discrete data were presented as proportions (%). Differences in baseline characteristics and clinical outcomes between the sepsis and control group were assessed using the independent samples t-test, Wilcoxon rank sum, Wilcoxon signed rank, chi-squared, or Fisher's exact tests where appropriate.

Secondary outcomes were evaluated using uni- and multivariable Cox proportional hazards regression models or ANOVA analyses where appropriate. Multivariable Cox regression analyses were performed using the Enter and Forward Stepwise Wald methods.

Based on literature and clinical relevance, the variables age, sex, body mass index (BMI), acute physiology and chronic health evaluation II (APACHE II), SOFA and modified nutrition risk in critically ill (mNUTRIC) scores were analysed in regression analyses. Variables were dichotomised (using the median) in case of non-linearity, with the outcome parameter assessed by visual inspection of boxplots.

Finally, all samples' PBMC lymphocyte-monocyte ratios (LMR) were calculated. Their changes over time and differences between survivors and non-survivors were evaluated. Moreover, correlation with parameters of mitochondrial function was assessed using Kendall's Tau-b.

Multicollinearity was assessed using the variance inflation factor (VIF); a value below two was considered acceptable.

IBM SPSS statistics 27 (I.B.M. Corp, Armonk, NY, USA) was used for all analyses and figures representing statistics. Only two-sided analyses were used. P-values ≤0.05 were considered statistically significant.

Ethical approval

The study was approved by the Medical Ethical Committee of Wageningen University (METC-WUR, which was incorporated in the METC Oost-Nederland in 2021, dossier no. 2021-13011) and the assessment Committee for Scientific Research of ZGV (dossier no. 1801-004). The protocol was registered in the Netherlands Trial Register (number NTR6969) and was made available through the International Clinical Trial Registry Platform (NL5918).

RESULTS

Informed consent was obtained from 47 septic patients and 30 age- and sex-matched controls (Figure 2). One sepsis patient was excluded from analyses and further measurements after the withdrawal of consent.

The septic patients were predominantly male (n=33, 72%) and had a mean age of 68 (SD 13) years of age, with a mean BMI of 27 (SD 6) kg/m^2. The primary type of sepsis was pneumosepsis (n=24, 52%), followed by abdominal sepsis (n=17, 37%). At baseline, patients had the following clinical scores: mNUTRIC 5 [IQR 3-6], SOFA 8 [7-10] and APACHE II 18 (14-22). The control patients were matched and subsequently predominantly male as well (n=22, 73%) and had a mean age of 71 (SD 15) years, which was not different from the sepsis group (p=0.3).

Study measurements

Six patients were excluded from further study participation due to death (one patient) or failure of the first measurement (five patients). Mitochondrial respiration data were collected from 40 patients, of whom ten patients were discharged to the general ward before the second (T2, n=3) or third (T3, n=7) measurement could be performed. The other 30 patients completed the measurements at all three consecutive time points for which age (± 2 years) and sex-matched controls were sought. When reviewing the obtained data after study completion, single respirometry measurements in five patients were discarded as they did not meet quality standards (T1 n=1, T2 n=1, T3 n=2 and T1-3 n=1). Measurements failed in four controls (13 %) and partially failed (no reaction to CCCP, rendering basal respiration and proton leak useable) in one patient.

The clinical patient characteristics at the time of blood samplings are shown in Table 1.

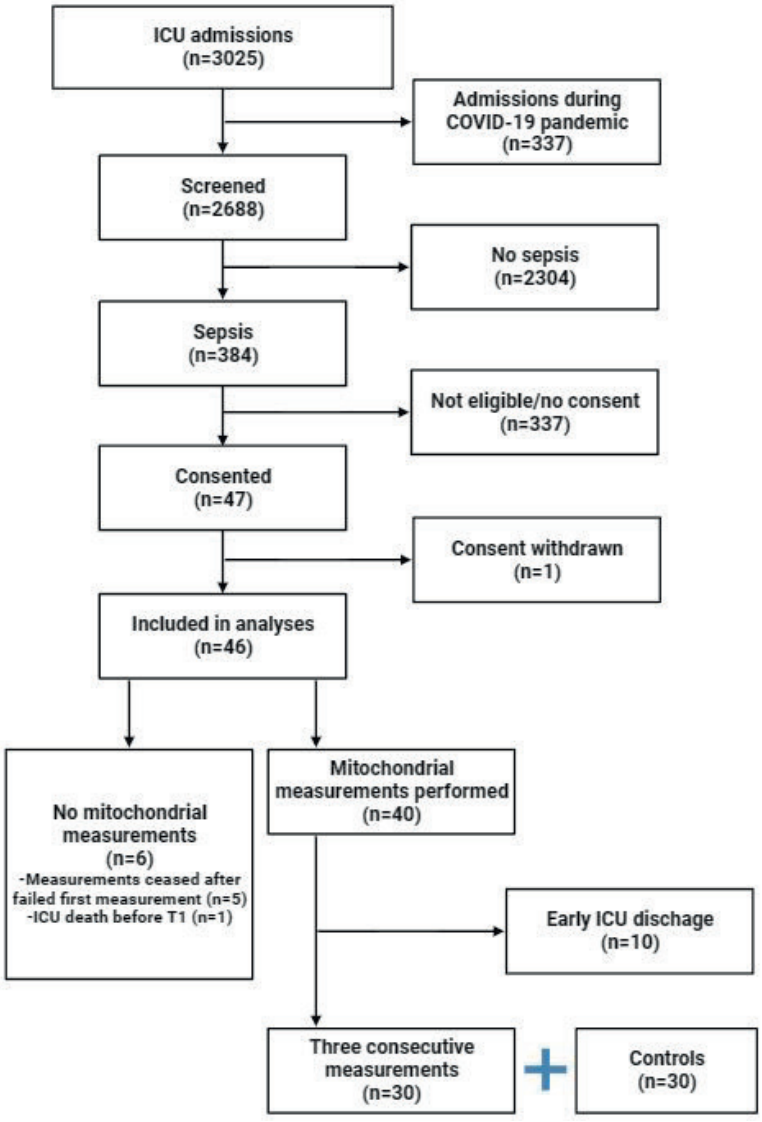

Figure 2. Study flow chart

ICU = intensive care unit. Created with Biorender.com.

Mitochondrial function over time in septic patients and controls

Basal respiration and ATP-linked respiration were significantly increased in patients with sepsis compared to controls within the first week of ICU admission (median 4.27 [IQR 2.70-6.07] versus 2.24 [1.67-3.58] and 2.84 [1.32-4.04] versus 1.37 [0.75-2.02], respectively), as shown in Figure 3 (and eTable 1). No significant change in any other respiratory parameter was observed over time in the PBMCs of the entire sepsis group, as depicted in measurements T2 and T3 (all paired comparisons with T1 $p>0.05$).

Survivors versus non-survivors

All-cause 3-month mortality in the sepsis cohort (n=40) was 35% (n=14). Five (36%) of the deceased patients died in the ICU, seven (50%) in the ward and two after hospital discharge (14%). Compared to the surviving patients (n=31), the non-survivors (n=15) were older (77 (SD 10) versus 63 (SD 13) years of age, $p<0.001$), had higher APACHE II (median 20 [IQR 17-26] versus 15 [12-20], $p=0.008$] and mNUTRIC scores (6 [IQR 6-7] versus 4 [3-5], $p<0.001$) at ICU admission. No significant differences were found in other baseline characteristics.

Regarding biochemical parameters, survivors (results obtained for n=26) had higher serum insulin levels (20.0 [IQR 11.4-36.3] versus 8.4 [4.6-20.5], $p=0.014$) than non-survivors (results obtained for n=12) at T1, although they tended to have less insulin supplementation ($p=0.066$). No statistically significant differences in other laboratory parameters were found (Table 2).

In high-resolution respirometry, mitochondrial proton leak was significantly lower in non-survivors compared to survivors (1.14 [IQR 0.86-1.97] versus 2.04 [IQR 1.28-2.90] nmol O_2/min/10^7, $p=0.048$) at T1. Moreover, a significant increase in basal and ATP-linked respiration was observed over time in non-survivors compared to survivors, as shown in Figures 4 and 5 (e-Table 2).

Table 1. Patient and control characteristics at time of the blood samplings.

	Controls (n=26)	Sepsis patients (n=40[a])								
		T1 (n=38)		p-value[b]	T2 (n=35)		p-value[c]	T3 (n=28)		p-value[d]
Clinical parameters										
SOFA score	n.a.	6 [4-8]		n.a.	4 [3-8]		<0.001*	5 [3-7]		<0.001*
Lactate (mmol/L)	n.a.	1.2 [0.8-1.7]		n.a.	0.9 [0.8-1.2]		0.002*	0.8 [0.6-1.2]		<0.001*
Leukocytes (*10^9/L)	6.5 [5.7 – 8.0]	13.6 [10.0-18.9]		<0.001*	12.7 [9.1-16.3]		0.189	12.2 [9.4-19.1]		0.388
PMBC LMR	4.6 [3.3-5.6]	2.0 [1.1-3.0]		0.010*	2.0 [1.5-3.3]		0.465	2.0 [1.3-3.0]		0.670
CRP (mg/L)	0	249 [148-323]		<0.001*	120 [77-214]		<0.001*	109 [58-204]		<0.001*
Ureum (mmol/L)	6.3 [5.5 – 7.1]	10.7 [7.8-19.5]	n=26	0.002*	11 [5.5-25]	n=20	0.844	10.1 [7.5-15.8]	n=18	0.529
Creatinine (umol/L)	74 [62 – 87]	84 [60-145]		0.024*	72 [52-117]		0.002*	60 [46-112]		<0.001*
Cortisol (nmol/L)	395 [311 – 472]	724 [546-1568]		<0.001*	676 [418-1005]		<0.01*	592 [431-725]		<0.001*
Insulin (mmol/L)	19 [9.4 – 18.5]	18 [8.8-30]		0.280	21 [12-35]		0.523	11.5 [7.7-31.5]		0.755
Insulin supplementation (IU)	n.a.	0 [0-19]		n.a.	0 [0-24]		0.449	0 [0-26]		0.234

a. Unless stated otherwise due to missing variables. b. T1 compared to the control group; p-values were calculated using the Wilcoxon signed rank test c. T1 compared to T2, p-values were calculated using the Wilcoxon signed rank test; d. T1 compared to T3, p-values were calculated using the Wilcoxon signed rank test; * p-value <0.05.

Definitions: T1, day 1-2 (24-48h); T2, day 3-4 (72-96h); T3, day 5-6 (120-144h) after ICU admission; PBMC, peripheral blood mononuclear cell; LMR, lymphocyte-monocyte ratio; CRP, C-reactive protein; IU, international units; n.a., not applicable; SOFA, Sequential Organ Failure Assessment. All values are reported as medians with interquartile ranges.

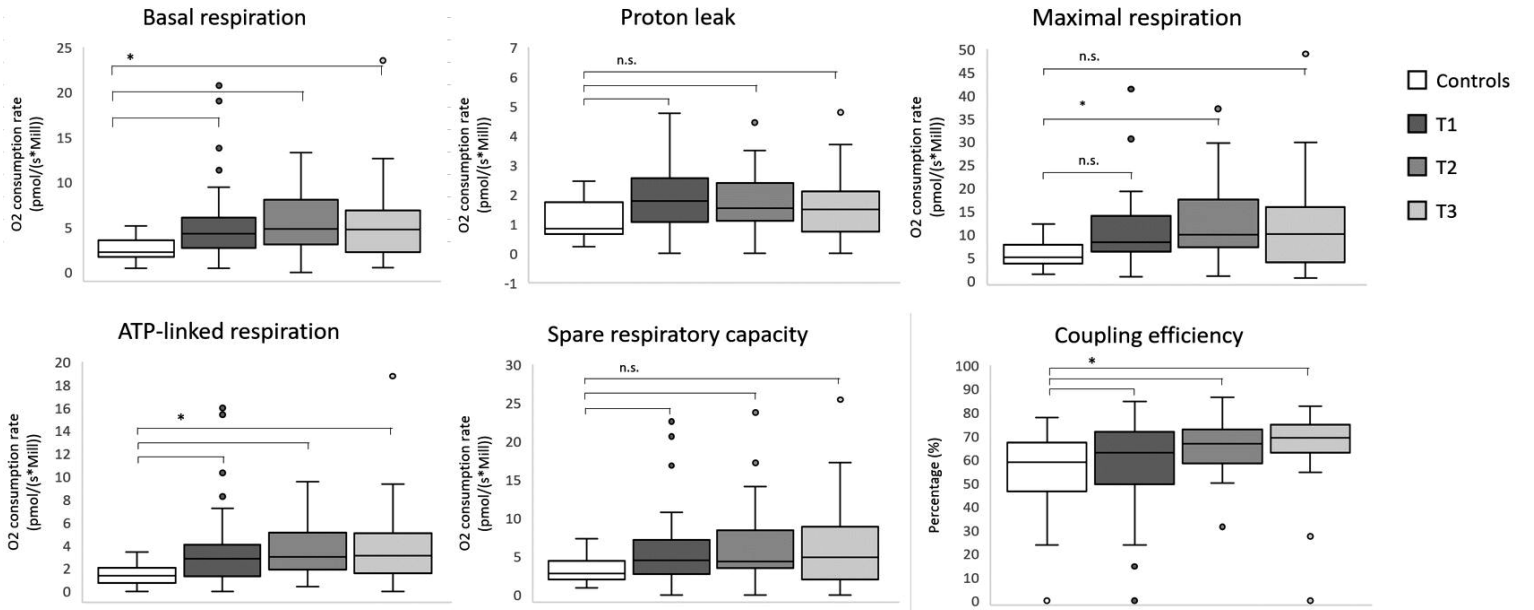

Figure 3. Progression of mitochondrial respiratory parameters during the first week of ICU admission in septic patients and controls.

T1, day 1-2 (24-48h); T2, day 3-4 (72-96h); T3, day 5-6 (120-144h) after ICU admission; n.s. = not statistically significant different; * = p-value <0.05.

Table 2. Survivors (n=26) [a] versus all-cause 3-month non-survivors (n=14)[a]

	T1			T2			T3		
	survivors (n=26)	non-survivors (n=12)	p-value	survivors (n=22)	non-survivors (n=13)	p-value	survivors (n=15)	non-survivors (n=13)	p-value
Clinical parameters									
SOFA score	6 [3-8]	7 [5-8]	0.6	3 [2-7]	5 [4-6]	0.3	4 [2-8]	5 [3-5]	0.9
Lactate (mmol/L)	1.3 [0.8-1.725]	1.2 [1.0-1.6]	0.9	0.9 [0.8-1.3]	1.0 [0.7-1.3]	0.9	0.8 [0.5-1.2]	0.9 [0.7-1.2]	0.5
Leukocytes (*10^9/L)	14.3 [8.5-19.0]	12.8 [10.5-16.8]	1.0	12.5 [9.1-16.3]	13.2 [8.2-16.6]	1.0	12.1 [9.5-17.7]	14.9 [9.2-22.6]	0.3
PBMC LMR	2 [1.1-3.1]	1.6 [1.0-3.9] n=9	1.0	2.3 [1.5-3.6] n=21	1.8 [1.2-2.4]	0.3	2.0 [1.7-3.5]	2.0 [1.1-2.5]	0.2
CRP (mg/L)	252 [169-323]	236 [137-318]	0.63	140 [74-214]	112 [87-280]	0.9	109 [70-168]	97 [35-212]	0.8
Ureum (mmol/L)	10.3 [6.5-18.2]	11.4 [9.1-25.2]	0.3	10.8 [5.1-14.4]	16.4 [8.8-32.6]	0.3	10.8 [6.6-24.7]	9.4 [7.9-14.4]	0.5
Creatinine (umol/L)	84 [57-188]	95 [75-132]	0.9	70 [52-114]	66 [52-124]	0.7	64 [46-137]	53 [44-109]	0.4
Cortisol (nmol/L)	674 [560-1397]	1398 [525-1959]	0.2	549 [418-1005]	697 [583-1062]	0.5	617 [368-761]	601 [506-768]	0.6
Insulin (mmol/L)	20.0 [11.4-36.3]	8.4 [4.6-20.5]	0.014*	20 [12-35]	17.5 [7.9-40.3]	0.8	12.5 [9.1-25.8]	13.0 [5.7-35.8]	1.0
Insulin supplementation (IU)	0 [0-0.75]	9 [0-38]	0.066	0 [0-1]	0 [0-45]	0.3	0 [0-0.75]	0 [0-31]	0.3
Insulin supplementation (IU)**	32 [5-55]	36 [28-54]	0.5	41 [8-95]	45 [38-87]	0.6	50 [13-59]	36 [21-57]	0.8

a. Unless stated otherwise due to missing variables. Definitions: T1, day 1-2 (24-48h); T2, day 3-4 (72-96h); T3, day 5-6 (120-144h) after ICU admission; Abbreviations SOFA, Sequential Organ Failure Assessment; PBMC, peripheral blood mononuclear cell; LMR, lymphocyte-monocyte ratio; CRP, C-reactive protein; IU, international units; ATP-linked respiration = basal respiration minus proton leak; SRC = spare respiratory capacity; maximal respiration minus basal respiratory capacity; coupling efficiency = ATP-linked respiration divided by basal respiration. All values are reported as medians with interquartile ranges. P-values were calculated using the Mann-Whitney U test; * p-value <0.05.

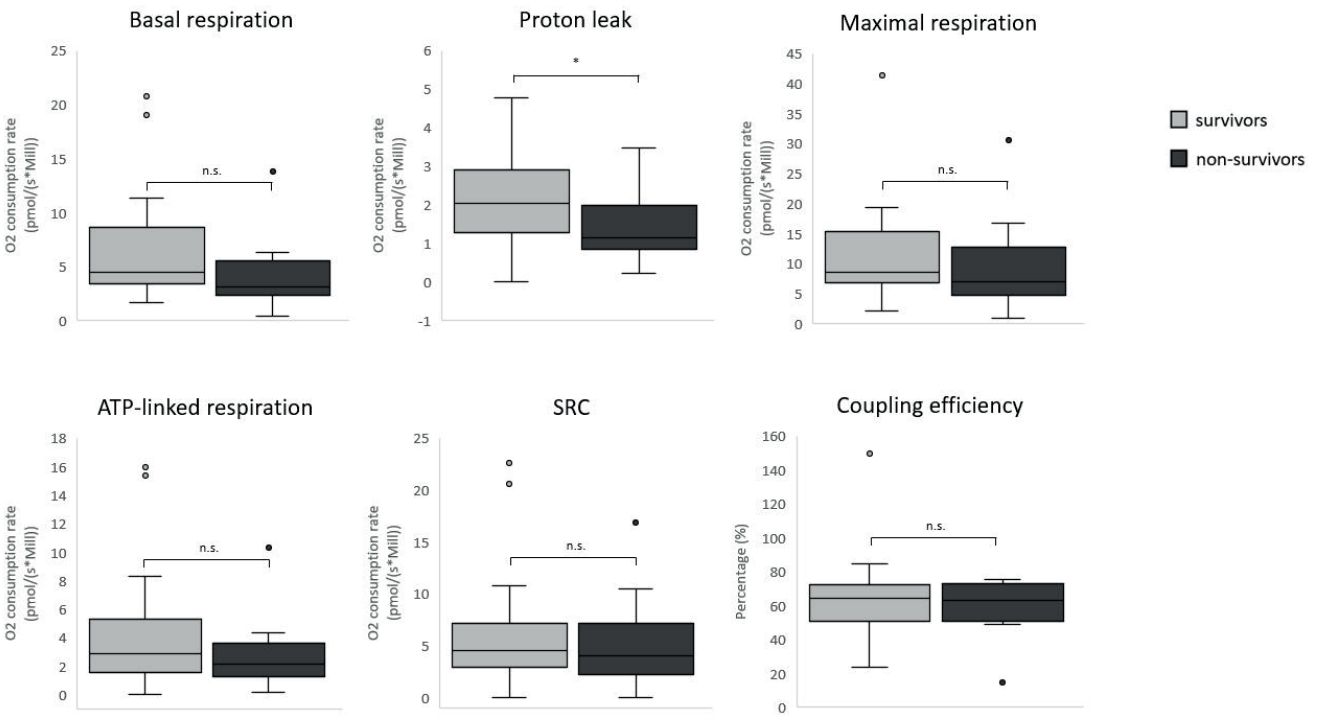

Figure 4. Comparison of mitochondrial respiratory parameters at T1 of sepsis survivors versus non-survivors.
T1, day 1-2 (24-48h) after ICU admission; n.s. = not statistically significant different; * = p-value <0.05.

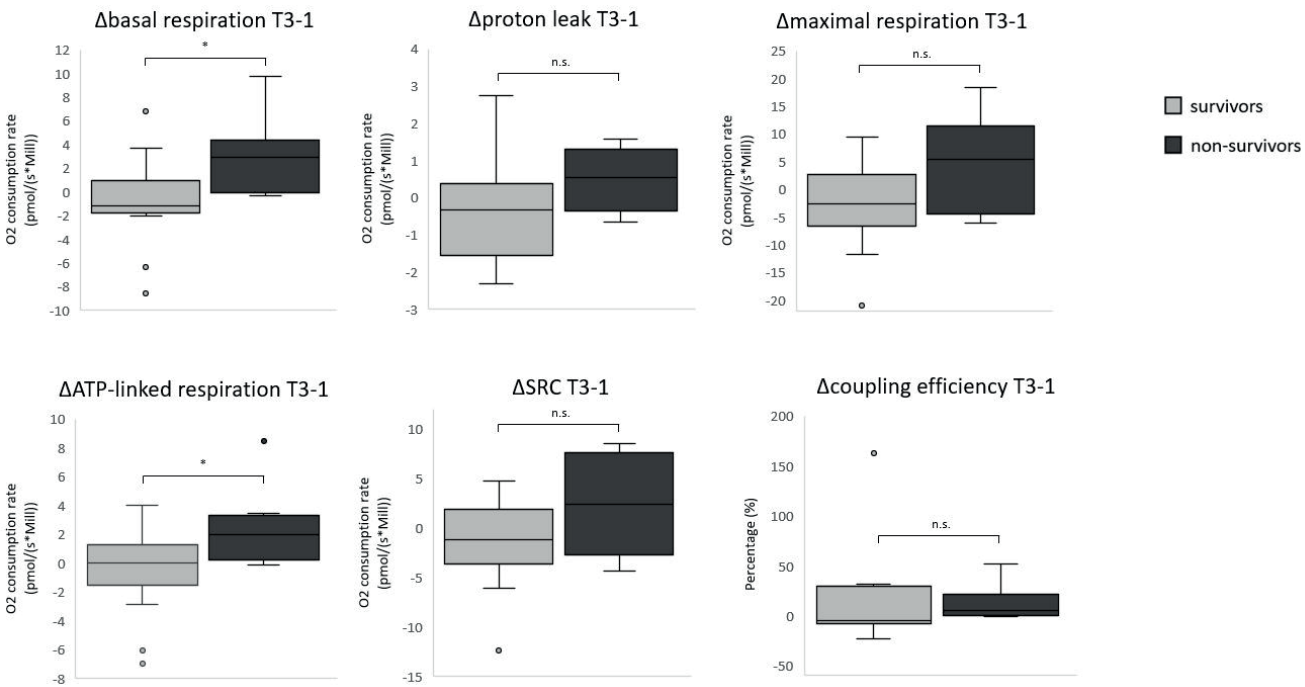

Figure 5. Comparison of the progression of mitochondrial respiratory parameters during the first week of ICU stay of sepsis survivors and non-survivors. T1, day 1-2 (24-48h) after ICU admission; n.s. = not statistically significant different; * = p-value <0.05.

Cox regression

The variables age and mNUTRIC were omitted in the final regression models because of their overlap (and visual correlation) with the APACHE II score. Measured mitochondrial respiration parameters were intercorrelated (all p<0.01); therefore, only basal respiration was used in the final model. None of the mitochondrial respiratory parameters were correlated with the SOFA score. The deltas of these two parameters over time were entered into the final model (ΔSOFA and Δbasal respiration as calculated by T3 minus T1). In the final Cox regression multivariable model, Δbasal respiration (≥0.07 nmol O_2/min/10^7 from T1 to T3) was associated with the primary endpoint of 3-month mortality (HR 3.8, 95%CI 1.0-14.1, p=0.047) (see Table 3). The VIF was <2 for the variables in this final model.

Table 3. Univariable and multivariable Cox regressions for the association of primary endpoint 3-month mortality, baseline and clinical characteristics and mitochondrial respiratory function (n=40).

	Univariable	p-value	Multivariable	p-value
	HR (95%-CI)		HR (95%-CI)	
A. 3-month mortality (n=14)				
Sex (female)	1.060 (0.332-3.385)	0.9	0.707 (0.164-3.051)	0.6
BMI (>25.7)	1.040 (0.364-2.967)	0.9	1.400 (0.381-5.139)	0.6
APACHE II on admission (>18)	2.029 (0.703-5.859)	0.2	1.926 (0.509-7.289)	0.3
delta SOFA T3-1 (≥-2)	0.863 (0.303-2.463)	0.8	1.038 (0.218-3.832)	0.9
delta basal T3-1 (>0.068**)	3.041 (0.886-10.431)	0.08	3.794 (1.018-14.149)	0.047*
B. ICU mortality (n=5)				
Sex (female)	1.633 (0.182-14.639)	0.7	Difficulties with multivariable regression due to low number of deaths	
BMI (>25.7)	1.578 (0.263-9.460)	0.6		
APACHE II on admission (>18)	2.139 (0.356-12.861)	0.4		
delta SOFA T3-1 (≥-2)**	0.223 (0.025-1.998)	0.2		
delta basal T3-1 (>0.068**)	118 (0.007-2.121*10^6)	0.3		
C. Hospital mortality (n=7)				
Sex (female)	1.291 (0.349-4.775)	0.7	0.979 (0.183-5.228)	0.9
BMI (>25.7)	1.028 (0.331-3.192)	1.0	1.554 (0.389-6.207)	0.5
APACHE II on admission (>18)	1.490 (0.480-4.630)	0.5	1.232 (0.288-5.273)	0.8
delta SOFA T3-1 (≥-2)**	0.61 (0.197-1.959)	0.4	0.916 (0.232-3.627)	0.9
delta basal T3-1 (>0.068**)	2.112 (0.565-7.901)	0.3	2.341 (0.577-9.494)	0.2

T1 = day 1-2 (24-48h), T2 = day 3-4 (72-96h) and T3 = day 5-6 (120-144h) after ICU admission. Delta was calculated as: (mitochondrial parameter at T3 minus T1). Abbreviations: HR; hazard ratio, 95%-CI; 95%-confidence interval, BMI; body mass index, APACHE II; Acute Physiology And Chronic Health Evaluation, SOFA; sequential organ failure assessment, basal; basal respiration (measured in nmol O2/min/10^7). * p-value <0.05. ** including negative values (=a decrease in SOFA score or basal respiration, respectively, over time). Multivariable Cox regression analyses were performed using the Enter and Forward Stepwise Wald methods.

Other secondary outcomes

Only two patients needed a tracheostomy to wean from mechanical ventilation. An overview of the duration of mechanical ventilation and ICU and hospital LOS for survivors and non-survivors is summarised in e-Table 4. There were no statistically significant differences between both subgroups in these outcomes. In addition, delta basal respiration was not associated with the secondary outcomes, as shown in e-Table 5.

Lymphocyte-monocyte ratio

The PBMC LMR was lower in sepsis patients than in controls. No change over time (T1-T3) was noted, nor were any differences between survivors and non-survivors (Tables 1-2). Moreover, parameters of mitochondrial function did not correlate with the LMR, except LMR and proton leak on T3 (CC -0.267, p=0.05) (e-Table 5).

DISCUSSION

In this prospective observational study, we found a significant increase in basal and ATP-linked respiration and coupling efficiency in sepsis patients compared to controls within the first week of ICU admission. This observation contrasted our hypothesis, as we did not demonstrate a decrease in PBMC mitochondrial respiration during sepsis. Moreover, a more significant increase in basal and ATP-linked respiration was observed during the first week of ICU stay in non-survivors compared to survivors ($p<0.05$), although these respiration parameters were not statistically different at baseline measurements. This progression of basal respiration was associated with the primary endpoint of 3-month mortality after correction for relevant covariates. Therefore, the current results suggest that the upregulation of basal respiration may serve as a proxy marker for sepsis severity and outcomes.

Our findings are consistent with those of Sjövall *et al.* and Belikova *et al.*, who also found that basal mitochondrial respiration in PBMCs was significantly increased within the first 48 hours of ICU admission (6,19). In addition, Sjövall *et al.* demonstrated a progressive increase in basal and maximal respiration during the first week of sepsis patients compared to healthy controls (19). Strikingly, they observed no differences between surviving and non-surviving patients at any point in time. Both their inclusion and mortality rates were lower than in the present study, which may have led the study to be underpowered for differentiation between survivors and non-survivors. However, their article does neither report the original data nor p-values for the comparisons, so this claim cannot be substantiated.

In contrast with our findings, Jang *et al.* (studying mitochondrial respiration of PBMCs in 10 septic patients measured once shortly after presentation to an emergency department), Japiassú *et al.* (studying mitochondrial respiration of PBMCs in 20 patients during the first 48 hours of septic shock) and Garrabou *et al.* (studying mitochondrial respiration in 19 septic patients, time of measurement not mentioned) observed a significant reduction of ADP-linked respiration in permeabilized PBMCs of septic patients compared to controls (7,8,18). In addition, in the study of Japiassú *et al.*, a significant reduction was observed in ADP-linked respiration in non-surviving sepsis patients compared with the postoperative controls without sepsis (5.60 versus 9.89 nmol O_2/min/10^7, respectively, $p < 0.01$). Survivors demonstrated a 2.9x increase in ATP-linked respiration after one week (7). Contrastingly, we did not observe a significant change in respiratory function over time in survivors. Instead, we found a significant increase in basal and ATP-linked respiration in non-survivors during the first week of ICU stay.

These contradictory observations may be due to methodological variety. A clear difference between the abovementioned studies is the composition of the control group, which may influence the outcomes of comparisons between sepsis and control groups. In the current study, we chose to include sex- and age-matched controls since those are two factors known to influence mitochondrial respiratory function, which has not been done in other studies besides the study of Garrabou *et al.* (8). Moreover, we selected metabolically healthy age- and sex-matched controls visiting the outpatient clinic. In contrast, Japiassú *et al.* included critically ill postoperative ICU patients, whereas Jang *et al.* chose to use three unmatched control groups of younger, older and infected (but not septic) patients (7,18). It is intriguing that Sjövall *et al.* and our study still proved an increase in mitochondrial function parameters in PBMCs of septic patients, even though healthy controls were included (19).

Secondly, exclusion criteria differ between mentioned studies. We excluded many common comorbidities known to affect mitochondrial respiratory function (such as diabetes mellitus and COPD), which allowed us to exclude the potential confounding effect of these comorbidities. Such exclusion criteria were not reported in other studies.

Thirdly, the time at which the PBMCs were collected and measured respiration differed between studies. The timing of blood collection is not described by Garrabou *et al.* (8). Jang *et al.* collected blood samples from patients with sepsis or septic shock upon presentation to the emergency department (18), whereas our measurements and those of Japiassú *et al.* commenced within 48 hours of ICU admission (7). These may be very different (metabolic) time points in a patient's journey. Furthermore, our cohort was slightly older than those of Jang and Garrabou and their coworkers (68 vs 63, resp. 64

years of age), and although SOFA scores were similar in all studies, it is unknown whether all patients in the Garrabou and Jang cohorts required ICU admission.

Fourthly, the methods of respiration measurement vary in comparison with current literature. The current study resuspended PBMCs in a standardized medium, not plasma. This was similar to Japiassú *et al.* (7). On the contrary, Sjövall and coworkers used the patient's plasma (19). However, mitochondrial function in PBMCs is altered by plasma, as was demonstrated by Belikova co-workers (6). Consequently, it is difficult to disentangle the effects of sepsis on plasma content from the effect of sepsis on mitochondria in PBMCs per se. Strikingly, in the current study, decreased mitochondrial respiration was, in fact, not visible. This approach revealed that the mitochondria of PBMCs are not dysfunctional and capable of improving respiratory function. In addition, this suggests that if a worsened respiratory function is observed in PBMCs of septic patients, this is perhaps more likely to originate from potential dysregulating components present in plasma. In addition, in some studies, PBMCs were permeabilized, while we used non-permeabilized PBMCs for respiratory measurements (7,8). It could be hypothesized that this explains the differences with our results. However, since both Sjövall and Jang *et al.* have performed their measurements in both permeabilized and non-permeabilized PBMCs and found consistent results between those experiments, this is unlikely to explain the contrasting results with our studies.

Lastly, it can be hypothesized that the differences in respiratory function of PBMCs belonging to the different groups of our study are caused by a shift in PBMC composition rather than a shift in mitochondrial function per se. In humans, PBMC cell ratios vary across individuals, but typically, lymphocytes are in the range of 70–90 %, monocytes from 10 to 20 %, while dendritic cells are rare, accounting for only 1–2 % (17). In our cohort, the PBMC lymphocyte-monocyte ratio (LMR) was lower in sepsis patients than in controls. This lower count is to be expected, as a lower LMR is associated with systemic inflammation (25). However, parameters of mitochondrial function did not correlate with the LMR, nor did the LMR change over time, and also not if more specific survivors versus non-survivors were compared (Table 2). We only found a significant correlation between proton leak and LMR on T3 (e-Table 5). A similar correlation, or trend, was not found at any other time point or concerning another parameter. Therefore, we caution against interpretation at this time, as it goes beyond the scope and likely the power of the current study. However, it could still be of interest to measure mitochondrial function in distinct cell populations in future studies and should perhaps be considered when leukocytes are used as bioenergetic biomarkers (26).

Although we could not identify consistent methodological differences among all the studies mentioned, combining these methodological differences can contribute to the contrasting results.

Previous studies, although few, measuring mitochondrial function in muscle tissue of different origins have consistently reported a lower activity of mitochondrial complexes and a lower ATP content, concomitant with an altered expression of genes involved in regulating mitochondrial dynamics (15,22,23). In the current study, mitochondrial function was measured in PBMCs. PBMCs are easily and non-invasively obtained. However, PBMCs are important cells during inflammation and systemic infection and may have a different metabolic response to sepsis compared to other tissues directly involved in multiorgan failure, such as the liver and muscles. Based on the results of the current study and in comparison to studies performed using skeletal muscle biopsies, PBMCs do not necessarily reflect the decrease in mitochondrial function, which is reported elsewhere in the body. Indeed, Jeger *et al.* reported that results from previous animal and clinical studies investigating mitochondrial function in several tissue types during sepsis are heterogeneous, reporting increased and decreased mitochondrial oxygen consumption (24). Although speculative, these differences in mitochondrial functioning between tissues may reflect differences in their role during sepsis.

Thus, the increased basal and ATP-linked respiration as found in the current study may reflect an increased ATP demand of PBMCs during human sepsis, as a result of an activation of the immune system to combat the underlying infection. Clinical relevance of this increase is suggested by the higher increase in basal and ATP-linked respiration over time (i.e., between T1 and T3) in non-survivors, compared to survivors. Still, pathophysiological interpretation of this difference between survivors and non-survivors is precarious. The higher increase over time may, partially, be due to a lower basal and ATP-linked respiration in PBMC's at T1 of non-survivors compared to survivors, although this was not statistically significant. Thus, a delayed up-regulation of PBMC activation in PBMCs of non-survivors compared to survivors, cannot fully be excluded but, in view of the lack of statistical significance, is highly speculative. Still, disregarding possible differences at T1, a higher increase in both respiration parameters over time could reflect differences in the development of the infection, or indicate immune dysfunctioning. This study does not provide clarity in this respect, especially since immune mechanisms during sepsis are complex, consisting of simultaneous hyperinflammation and immune suppression. Future studies, however, will take these hypotheses into account.

Progress and issues

We encountered several issues that delayed the study's progression beyond the expected inclusion period. In more sepsis patients than anticipated in advance, we could not

perform all three measurements, primarily due to patients succumbing to their disease. Furthermore, not all measurements were successful. Inclusions were temporarily halted during the SARS-CoV-2 pandemic, as the university laboratory was closed during lockdowns. Inclusion of controls proved more difficult than anticipated due to the extensive list of exclusion criteria (mainly diabetes mellitus and COPD). Including older control patients was incredibly challenging, as they more often had comorbidities or refused the burden of participation.

Strengths

The consecutive measurements at three moments during the first week of ICU admission with fixed intervals are considered a strength of this study. This enabled us to better understand the progress of mitochondrial respiration during the first week of ICU admission in septic patients. In addition, the extensive list of exclusion criteria based on common comorbidities (e.g., diabetes mellitus and COPD) known to affect metabolism and mitochondrial function is a unique strength of this study, as this allowed us to exclude potential confounding effects of these comorbidities.

Limitations

The current study is limited by its single-centre design. However, as the samples needed to arrive at the laboratory in a fresh state, the hospital's proximity to a university laboratory equipped with an Oroboros was an essential condition for this study. Secondly, the possible effects of administered medication on mitochondrial function may represent an unaccounted confounder. Thirdly, multivariate Cox regression analyses found an association between the delta basal respiration and ICU and 3-month mortality, not the severity-of-disease score APACHE II. This may be due to additional confounding factors, which were not accounted for (residual confounding) or multicollinearity, although the VIF was low (<2).

Future directions

Further research is needed to elucidate the role of mitochondria in the sepsis pathophysiology. First, more extensive multicentre trials are needed to consolidate the current study's findings. It would be interesting to measure mitochondrial respiratory function in various other tissues in parallel to create more insight into the potential role of PBMCs as a proxy marker for mitochondrial respiratory function in other tissues. Furthermore, new studies investigating the progression of mitochondrial function over time in several tissues are warranted, including (progression of) gene expression involved in oxidative phosphorylation subunits and mitochondrial biogenesis.

CONCLUSION

This study demonstrated a higher basal and ATP-linked respiration in PBMCs within the first week of ICU admission in sepsis patients compared to their healthy matched controls. In addition, a progressive increase of basal and ATP-linked mitochondrial respiration in PBMCs during the first week of ICU stay was negatively associated with 3-month mortality.

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SUPPLEMENTAL FILES

e-Table 1. Mitochondrial function over time in septic patients (n=40) and controls (n=26).

	Controls (n=26)	Sepsis patients (n=40[a])						
		T1 (n=38)		p-value[b]	T2 (n=35)	p-value[c]	T3 (n=28)	p-value[d]
basal respiration, nmol O_2/min/10^7	2.24 [1.67-3.58]	4.27 [2.70-6.07]		0.014*	4.83 [3.09-8.06]	0.9	4.73 [2.23-6.89]	0.5
proton leak, nmol O_2/min/10^7	0.84 [0.67-1.74]	1.79 [1.07-2.56]		0.2	1.54 [1.12-2.39]	0.6	1.51 [0.74-2.12]	0.9
maximal respiration, nmol O_2/min/10^7	5.05 [3.73-7.79]	8.32 [6.19-13.95]	n=37	0.053	9.90 [7.13-17.52]	0.9	10.03 [3.90-15.85]	0.6
ATP-linked respiration, nmol O_2/min/10^7	1.37 [0.75-2.02]	2.84 [1.32-4.04]	n=37	0.006*	3.01 [1.90-5.08]	0.7	3.11 [1.55-5.03]	0.1
SRC, nmol O_2/min/10^7	2.77 [2.04-4.41]	4.47 [2.74-7.13]	n=37	0.1	4.34 [3.49-8.44]	1.0	4.86 [1.96-8.89]	0.8
coupling efficiency (%)	59.9 [46.8-67.4]	63.5 [50.5-72.4]	n=37	0.048*	66.6 [58.4-72.7]	0.5	69.9 [63.0-74.8]	0.051

a. Unless stated otherwise due to missing variables. b. T1 compared to the control group; p-values were calculated using the Wilcoxon signed rank test c. T1 compared to T2, p-values were calculated using the Wilcoxon signed rank test; d. T1 compared to T3, p-values were calculated using the Wilcoxon signed rank test; * p-value <0.05.

Definitions: T1, day 1-2 (24-48h); T2, day 3-4 (72-96h); T3, day 5-6 (120-144h) after ICU admission; ATP-linked respiration = basal respiration minus proton leak; SRC = spare respiratory capacity; maximal respiration minus basal respiratory capacity; coupling efficiency = ATP-linked respiration divided by basal respiration. All values are reported as medians with interquartile ranges.

e-Table 2. Mitochondrial function over time in survivors (A; n=26) and non-survivors (B; n=14).

	T1				T2				T3			
	survivors (n=26)		**non-survivors (n=12)**	**p-value**	**survivors (n=22)**		**non-survivors (n=13)**	**p-value**	**survivors (n=15)**	**non-survivors (n=13)**		**p-value**
basal respiration	4.52 [3.43-8.63]		3.16 [2.37-5.58]	0.2	4.40 [3.01-8.38]		4.99 [3.15-7.89]	0.8	3.76 [2.13-6.95]	4.80 [2.48-6.94]		0.9
proton leak	2.04 [1.28-2.90]		1.14 [0.86-1.97]	0.048*	1.54 [1.01-2.09]		1.54 [1.33-2.88]	0.4	1.42 [0.79-2.14]	1.74 [0.64-2.27]		0.6
maximal respiration	8.49 [6.81-15.35]	n=25	6.93 [4.68-12.75]	0.2	8.28 [7.08-17.90]		10.69 [5.05-17.64]	0.6	9.20 [3.89-15.13]	10.86 [2.87-16.86]		0.9
ATP-linked respiration	2.89 [1.58-5.33]	n=25	2.16 [1.27-3.59]	0.3	2.89 [1.83-5.61]		3.01 [1.82-5.32]	0.9	3.11 [1.53-5.57]	3.11 [1.87-4.95]		0.9
SRC	4.56 [2.92-7.13]	n=25	4.00 [2.20-7.17]	0.5	4.03 [3.39-8.61]		6.14 [2.18-9.85]	0.6	4.64 [1.89-8.25]	6.06 [1.24-9.92]		0.7
coupling efficiency (%)	64.0 [50.5-72.3]	n=24	62.7 [50.4-73.1]	1.0	67.2 [60.5-72.7]	n=21	62.9 [53.5-71.1]	0.2	68.7 [61.7-74.6]	71.3 [67.0-75.4]	n=12	0.4

A.

Survivors	T1 versus T2 p-value	T1 versus T3 p-value
basal respiration	0.2	0.3
proton leak	0.073	0.3
maximal respiration	0.4	0.3
ATP-linked respiration	0.6	0.7
SRC	0.6	0.3
coupling efficiency (%)	0.2	0.4

B.

Non-survivors	T1 versus T2 p-value	T1 versus T3 p-value
basal respiration	0.2	0.033*
proton leak	0.2	0.1
maximal respiration	0.4	0.075
ATP-linked respiration	0.2	0.004*
SRC	0.4	0.1
coupling efficiency (%)	0.6	0.008*

Definitions: T1, day 1-2 (24-48h); T2, day 3-4 (72-96h); T3, day 5-6 (120-144h) after ICU admission; ATP-linked respiration = basal respiration minus proton leak; SRC = spare respiratory capacity; maximal respiration minus basal respiratory capacity; coupling efficiency = ATP-linked respiration divided by basal respiration. P-values were calculated using the Wilcoxon signed rank test; * p-value <0.05.

e-Table 3. Patient outcomes compared between survivors (n=26) and non-survivors (n=14).

	Survivors	Non-survivors	p-value
ICULOS, days	8 [6-25]	9 [7-16]	0.6
HLOS, days	20 [11-30]	15 [10-24]	0.3
Duration of ventilation, days	4 [1-15]	5 [2-9]	1.0

Abbreviations: ICULOS, intensive care unit length of stay; HLOS, hospital length of stay. All values are reported as medians with interquartile ranges. P-values were calculated using the Mann-Whitney U test.

e-Table 4. ANOVA for the association of secondary endpoints, baseline and clinical characteristics and mitochondrial respiratory function (n=40).

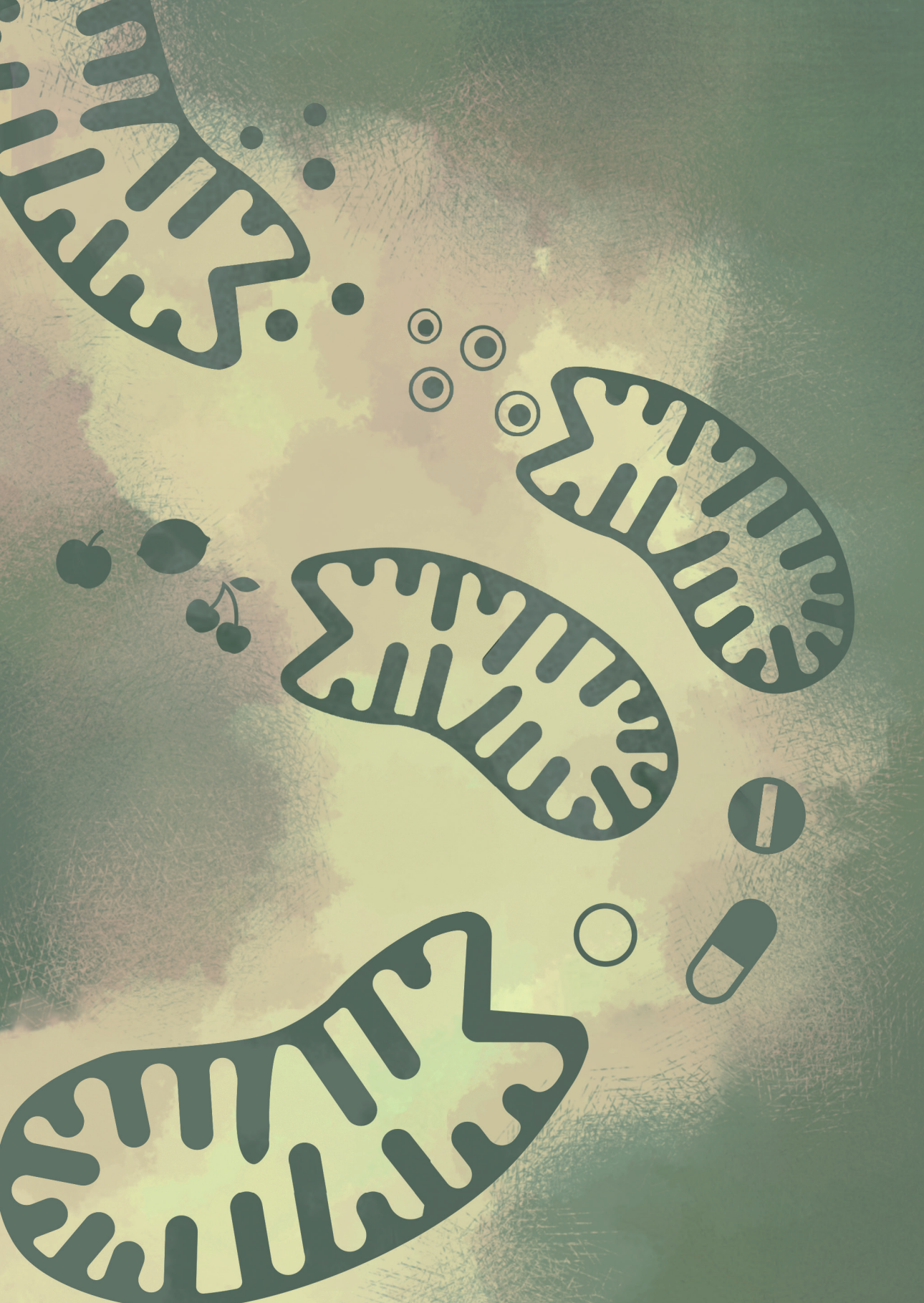
	beta	SE	p-value
A. Duration of mechanical ventilation			
Sex (female)	0.923	4.825	0.9
BMI (>25.7)	-5.209	3.846	0.19
APACHE II on admission (>18)	6.702	3.995	0.109
delta SOFA T3-1 (≥-2)	3.198	4.369	0.5
delta basal T3-1 (>0.068**)	-4.417	4.065	0.3
B. ICU length of stay			
Sex (female)	-0.677	5.059	0.9
BMI (>25.7)	-2.357	4.033	0.6
APACHE II on admission (>18)	6.039	4.189	0.16
delta SOFA T3-1 (≥-2)	5.118	4.581	0.3
delta basal T3-1 (>0.068**)	-4.402	4.262	0.3
C. HOS length of stay			
Sex (female)	1.851	6.736	0.8
BMI (>25.7)	-9.363	5.369	0.097
APACHE II on admission (>18)	7.342	5.577	0.2
delta SOFA T3-1 (≥-2)	1.331	6.099	0.8
delta basal T3-1 (>0.068**)	-6.356	5.675	0.3

T1 = day 1-2 (24-48h), T2 = day 3-4 (72-96h) and T3 = day 5-6 (120-144h) after ICU admission. Delta was calculated as: (mitochondrial parameter at T1 minus T3). Abbreviations: SE; standard error, 95%-CI; 95%-confidence interval, BMI; body mass index, APACHE II; Acute Physiology And Chronic Health Evaluation, SOFA; sequential organ failure assessment, basal; basal respiration (measured in nmol O_2/min/10^7). * p-value <0.05. ** including negative values (=a decrease in SOFA score or basal respiration, respectively, over time).

e-Table 5. Correlations between parameters of mitochondrial function with the LMR in sepsis patients at various time points.

	T1 (n=35) LMR 2.0 [1.1-3.0]		T2 (n=34) LMR 2.0 [1.5-3.3]		T3 (n=28) LMR 2.0 [1.3-3.0]	
	Correlation coefficient	p-value	Correlation coefficient	p-value	Correlation coefficient	p-value
basal respiration	0.126	0.3	-0.139	0.3	-0.180	0.2
proton leak	0.161	0.2	-0.110	0.4	-0.267	0.05*
maximal respiration	0.042	0.7	-0.157	0.2	-0.164	0.2
ATP-linked respiration	0.005	1.0	-0.150	0.2	-0.148	0.3
SRC	-0.018	0.9	-0.170	0.2	-0.124	0.4
coupling efficiency (%)	-0.056	0.7	-0.069	0.6	0.041	0.8

Definitions: T1, day 1-2 (24-48h); T2, day 3-4 (72-96h); T3, day 5-6 (120-144h) after ICU admission; ATP-linked respiration = basal respiration minus proton leak; SRC = spare respiratory capacity; maximal respiration minus basal respiratory capacity; coupling efficiency = ATP-linked respiration divided by basal respiration; LMR = lymphocyte-monocyte ratio. P-values were calculated using the Kendall's Tau-b test; * p-value <0.05.



Part II

Changes in energy metabolism during critical illness and convalescence

Chapter 4

Energy expenditure and indirect calorimetry in critical illness and convalescence: current evidence and practical considerations

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ABSTRACT

The use of indirect calorimetry is strongly recommended to guide nutrition therapy in critically ill patients, preventing the detrimental effects of under- and overfeeding. However, the course of energy expenditure is complex, and clinical studies on indirect calorimetry during critical illness and convalescence are scarce. Energy expenditure is influenced by many individual and iatrogenic factors and different metabolic phases of critical illness and convalescence. In the first days, energy production from endogenous sources appears to be increased due to a catabolic state and is likely near-sufficient to meet energy requirements. Full nutrition support in this phase may lead to overfeeding as exogenous nutrition cannot abolish this endogenous energy production, and mitochondria are unable to process the excess substrate. However, energy expenditure is reported to increase hereafter and is still shown to be elevated 3 weeks after ICU admission, when endogenous energy production is reduced, and exogenous nutrition support is indispensable. Indirect calorimetry is the gold standard for bedside calculation of energy expenditure. However, the superiority of IC-guided nutritional therapy has not yet been unequivocally proven in clinical trials and many practical aspects and pitfalls should be taken into account when measuring energy expenditure in critically ill patients. Furthermore, the contribution of endogenously produced energy cannot be measured. Nevertheless, routine use of indirect calorimetry to aid personalized nutrition has strong potential to improve nutritional status and consequently, the long-term outcome of critically ill patients.

BACKGROUND

The optimal quantity and timing of nutrition support for critically ill patients has long been debated. In the past, nutrition guidelines supported early aggressive feeding to meet estimated energy expenditure (EE), aimed at the prevention of malnutrition and muscle loss. However, clinical studies have failed to prove an unequivocal benefit of early high-dose nutrition support, and several prospective randomized clinical trials showed significant harm, including increased hyperglycemia, hepatic steatosis, and mortality (1–5). In contrast, undernourishment is also common in ICU and post-ICU patients due to both prescription inadequacy and failure to reach the nutrition target (6–12). A negative energy balance in critically ill patients is associated with increased morbidity, including increased length of hospital stay, infections, organ failure, prolonged mechanical ventilation, and even mortality (2, 13). Although there is a clear understanding that over- and underfeeding are associated with worse outcome, optimization of nutrition support is impeded by a lack of insight into the variable nutritional needs of critically ill patients during ICU stay and convalescence, both on a group and individual level (1, 8, 14). The available evidence indicates numerous factors that may lead to significant daily variations in EE in and between critically ill patients (1, 15, 16). Therefore, individualized real-time nutrition therapy is the next step toward optimal patientcare (1, 15, 17–21). Indirect calorimetry (IC) is considered the gold standard to measure caloric needs in critically ill patients at bedside, and its use has been strongly recommended by the recent European Society for Clinical Nutrition and Metabolism (ESPEN) and American Society for Parenteral and Enteral Nutrition (ASPEN) guidelines (1, 16, 18, 22).

This narrative review aims to provide a detailed summary of current evidence on the course of energy expenditure and the use of IC in critically ill patients in the ICU and during the post-ICU hospital stay. We include practical aspects of the use of IC and implications for nutrition therapy.

ENERGY EXPENDITURE

Total energy expenditure (TEE) is defined as the total amount of energy humans need to function. TEE can be subdivided into basal energy expenditure (BEE, or basal metabolic rate; BMR), diet-induced thermogenesis (DIT, or thermic effect of feeding; TEF), and physical activity-related energy expenditure (AEE). BEE and DIT combined, represent the resting energy expenditure (REE, or resting metabolic rate; RMR), which is defined as all energy requirements involved in the body's basal metabolism to maintain vital functions while inactive (Figure 1) (23–25). REE can be measured by IC and in sedentary, healthy

subjects, accounts for about two-thirds of TEE (23). In critically ill patients, REE will closely reflect TEE because of minimal physical activity (8, 19).

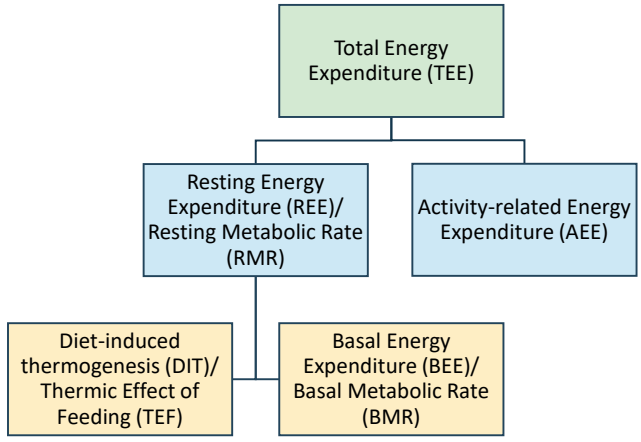

Figure 1. Components of energy expenditure

Energy expenditure during critical illness

Metabolic response to critical illness is complex and has been a subject of research and debate for decades (26).

Historical concepts

In 1942, Sir Cuthbertson, described the metabolic response to traumatic stress as occurring in an ebb phase and a flow phase (Figure 2) (26, 27). The ebb phase lasted minutes to hours after the initial insult and was thought to be characterized by a decline in body temperature and oxygen consumption, aimed at reducing posttraumatic energy depletion (26). After this brief phase of hypometabolism, Sir Cuthbertson and others recognized a significant increase, or "flow," in metabolism, called traumatic inflammation, or hypermetabolism (28–31). Hypermetabolism was thought to result from persistent catabolism, the systemic breakdown of lean tissue mass, and a rise in O_2 consumption to produce endogenous energy substrates to meet the high energy requirements during critical illness (1, 2). This increased catabolism leads to depletion of lean body mass, a syndrome which has been referred to as "autocannibalism" and feedings strategies were aimed at halting this process by satisfying the metabolic flow with substrate. The hypermetabolic phase was thought to end when the healing process began, with metabolism then reverting to the anabolic state (32).

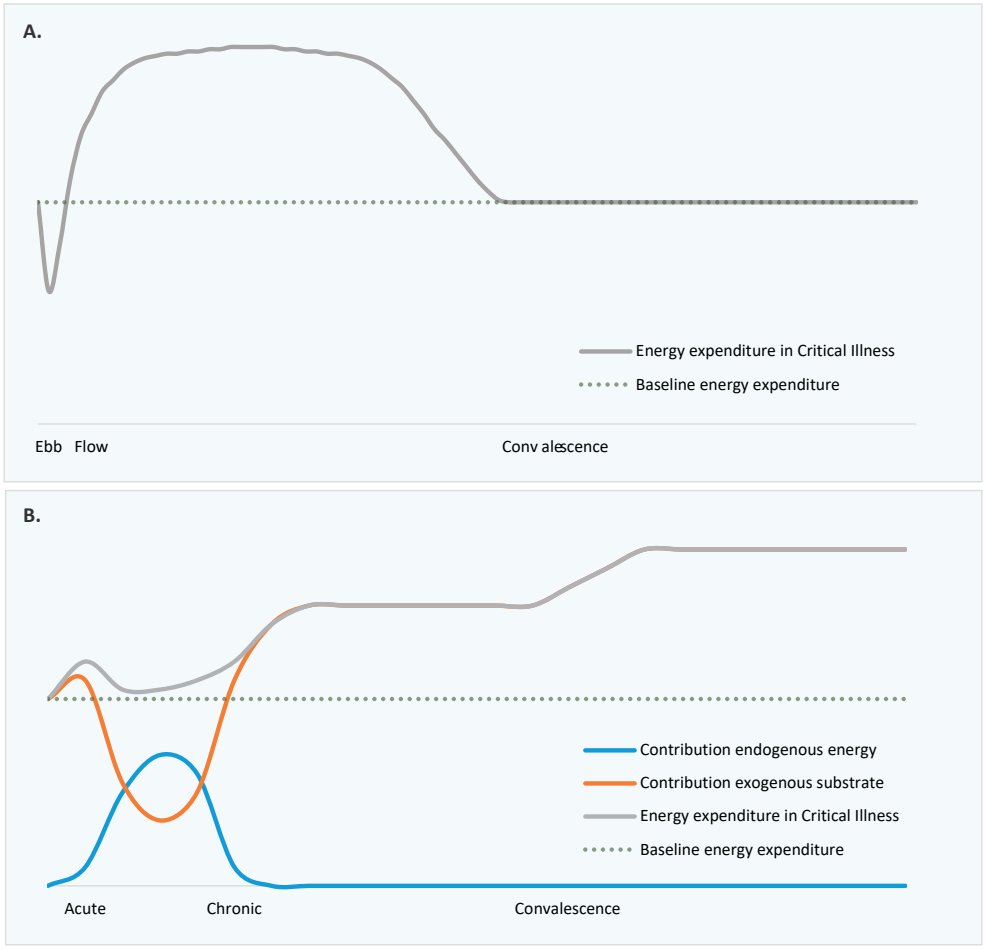

Figure 2. Progressing concepts of energy expenditure in critical illness. a Historical concept of energy expenditure in critical illness. b Current understanding of energy expenditure in critical illness and the contribution of various energy sources.

Current understanding

Cuthbertson's theory is still frequently cited; however, clinical trials have failed to identify a clear course of energy expenditure in all critically ill patients (33). In addition, early aggressive feeding strategies have not had the desired and expected effect. The reality appears more complex and omnifarious than the theory.

The described ebb phase has not been clearly identified in vivo, and its clinical relevance is debatable because of its briefness. Besides, there is usually, and logically, an emphasis on hemodynamic, rather than metabolic stabilization and nutrition support during this phase

of critical illness (34). In line with the flow theory, it is known that the release of catabolic hormones such as norepinephrine, cortisol, and glucagon increases gluconeogenesis, glycogenolysis, mobilization of free fatty acids, and muscle proteolysis in the acute phase of critical illness (2, 17, 35, 36). In addition, increased metabolism has been shown in several diseases, although patterns are highly variable, and the degree of increase from normal REE may reflect the severity of the metabolic response to the injury (1). However, hypermetabolism does not always characterize the initial phase of critical illness, as several studies show that during the first days, oxygen consumption can fall to near-baseline levels (37–39) (Figure 2). This phenomenon is hypothesized to be the result of a decrease in mitochondrial function as an adaptive strategy of metabolic hibernation to prevent cell death by energy substrate overloading at a time when mitochondria cannot keep up with energy demand (40). In patients with sepsis, a reduced oxygen utilization by 22–42% was found, compared with healthy volunteers (41). A higher REE in severe sepsis patients has been associated with higher mortality, further adding to the notion that the metabolic downregulation might be sometimes adaptive rather than a sign of malfunction (42).

Regardless of the rate of metabolism, some unique metabolic changes occur in the acute phase of critical illness, which helps explain the counterintuitive effects of early aggressive feeding. As metabolism is decreased, and catabolism has the upper hand, exogenous nutrient and insulin administration have been shown not to abolish endogenous glucose production (18, 43). Therefore, the endogenous energy production is likely near sufficient to meet energy demand during this phase (30, 44). As a result, full nutrition support may result in overfeeding (15, 17, 18). To reflect this, current nutrition guidelines recommend a gradual increase in caloric intake during the first 3-5 days after ICU admission to avoid overfeeding (18, 22).

After several days, REE increases again, and as endogenous energy production is simultaneously reduced, the risk of underfeeding increases (15, 45, 46). This might be considered the chronic metabolic phase of critical illness. An increase in REE has been demonstrated in both surgical and medical ICU patients, and a maximum REE is found around the ninth or tenth day after ICU admission (34, 38, 47–49). Clinical data on the course of EE during the recovery or convalescence phase of critical illness is scarce and usually derived from studies with small sample size. When available, measured REE is still significantly elevated several weeks after ICU admission, as has been shown in burns, trauma, and sepsis patients, including very recently in COVID-19 (50–52). However, in serial measurements in twelve patients during the post-ICU hospitalization period, Ridley et al. showed significant individual variability in measured EE (11). During this phase, TEE is likely to once again increase above REE, due to increased physical and mental activity, as the focus of treatment is moved toward rehabilitation. Ideally, the patient enters a recovery phase with enhanced anabolism, requiring more substrate. In contrast, the

persistent inflammation, immunosuppression, and catabolism syndrome (PICS) may arise in some (9, 18, 28). Metabolically, PICS is characterized by a persistent catabolic state and hormonal disruption leading to anabolic resistance and inflammation-induced cachexia (53).

Thus, different metabolic phenotypes arguably require a different and individualized nutritional approach. In addition, many individual and iatrogenic factors might cause metabolic requirements to be highly variable among patients as well as over time, making them hard to predict (1, 51, 54). Although they are not the same, regularly measured REE could be a useful proxy for real-time energy requirement in this vulnerable group of patients.

Table 1 summarizes factors influencing energy expenditure, including specifics of the underlying disease and its treatment, anthropometrics, nutritional status, (in)activity, and environment during and after critical illness.

Table 1. Factors affecting energy expenditure in critical illness

↑ Energy Expenditure	↓ Energy Expenditure
• Caucasian Ethnicity	• Female Sex
• Overfeeding	• Older Age
• Physical Exercise, Agitation	• ↓ Lean Body Mass
• ↑ Minute Volume	• Prolonged Fasting, Underfeeding
• Hyperthermia	• Paralysis, Coma
• Hyperthyroidism	• ↓ Minute Volume
• Metabolic Acidosis	• Hypothermia
• Stress (cortisol, glucagon, norepinephrine)	• Hypothyroidism
• Systemic Inflammation, Sepsis	• Metabolic Alkalosis
• Burns	• Medication: β-blockers, Sedatives, Muscle relaxants

Adapted from (1, 8, 19, 25, 55). Symbols: ↑, increase(d); ↓, decrease(d)

INDIRECT CALORIMETRY

If and when the transition into different metabolic phases occurs in individual patients, it is still unidentifiable in clinical practice. Because of not only the high variability between patients, but also during the disease in the individual patient, regular measurements of EE by IC could provide a better target for nutrition therapy in the subsequent phases of disease and convalescence (17, 23).

Indirect calorimetry in theory

IC measures respiratory gas exchange to estimate energy metabolism. On a cellular level, metabolism entails the production of adenosine triphosphate (ATP), with carbon dioxide (CO_2) and water as by-products, by consuming oxygen (O_2) and burning substrates such as glucose, free fatty acids, and amino acids. As the energy produced equals the energy consumed, IC measuring O_2 consumption and CO_2 production represents real-time energy metabolism (24, 30). Direct calorimetry, in contrast, measures heat production and, therefore, energy production directly, but this method is not feasible in clinical practice, as it requires the patients to be measured inside an insulated chamber (23, 24).

IC determines REE by measuring oxygen consumption (VO_2, in L/min) and carbon dioxide production (VCO_2, in L/min) and subsequently calculates REE according to the adjusted Weir's equation, based on the caloric values of the oxidation of 1 L of O_2 metabolizing a fat and carbohydrate mixture (25, 56). The original Weir equation includes urinary nitrogen measurement content representing protein oxidation. However, IC uses an adjusted version based on the Haldane transformation, which assumes that nitrogen is physiologically inert, and therefore, the volume of inspired nitrogen must equal the volume of expired nitrogen. This adjustment excludes the need for urinary measurements, which improves feasibility and introduces only a small error up to 1-2% occurs in final REE calculation (24, 25, 30, 57).

$$REE\ (kcal/day) = 1.44x\ ([VO_{2\ (mL/min)}x\ 3.94] + [VCO_{2(mL/min)}x\ 1.11])$$

Furthermore, IC calculates a respiratory quotient (RQ) during measurement, i.e., the CO_2-production to O_2-consumption ratio (19, 25):

$$RQ = VCO_2/VO_2$$

The RQ is an indicator of the composition of substrate use. It indicates which macronutrients are being metabolized, as different energy pathways are used. A human RQ of 1.0, 0.8, and 0.7 represents glucose, protein, and fat oxidation, respectively (23, 25, 30, 58). The physiological range of the RQ is 0.67-1.3; therefore, it can also be used as a quality indicator of the measurement adequacy (59–61). The approximate respiratory quotient of a mixed oral diet is 0.8.

Indirect calorimetry devices

IC measurements can be performed by using the ventilation circuit in mechanically ventilated patients for gas sampling, or by using a canopy hood or face mask in spontaneously breathing patients to analyze their in- and expired air (Figs.3, 4) (19).

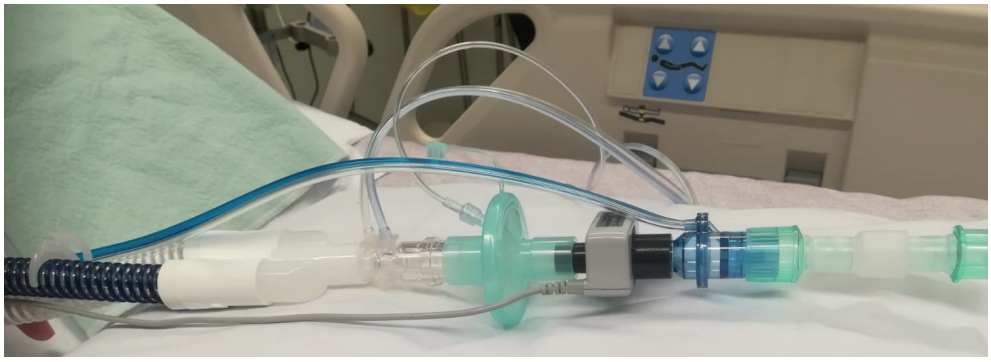

Figure 3. Insertion of a disposable flowmeter into the patient circuit of a mechanic-ventilation system (QNRG®, Cosmed, Italy)

Figure 4. Use of a flow-dilution canopy hood to measure gas exchange in a spontaneously breathing patient (QNRG®, Cosmed, Italy)

Many different devices are available (28). The Deltatrac® (Datex, Finland) was the most validated metabolic monitor and frequently used until sales were discontinued (62–64). Several other devices have made it to the market, each with its limitations. The Quark RMR® (Cosmed, Italy), E-COVX® (Datex-Ohmeda, Finland), CCM Express® (Medgraphics, USA), and Vmax® (Vyaire, USA) were shown to be equal or inferior to the Deltatrac on

several aspects (Table 2) (16, 64–67). In addition to these stand-alone devices, some mechanical ventilators have integrated IC functions, but its use has not yet been validated (63). Lastly, some devices are small, and handheld, such as the Fitmate® (Cosmed, Italy) or MedGem® (Microlife, USA), but have not been validated in critically ill patients (1, 11, 65). In order to overcome all disadvantages of the devices mentioned above. The Q-NRG® (Cosmed, Italy) has been developed by a task force of medical experts from the European Society of Intensive Care Medicine in the international calorimetry study initiative (ICALIC) project. It is the only device tested against mass spectrometry for accuracy during inspired fraction of oxygen (FiO_2)—settings ranging from 0.21 to 0.70 and can be used in both mechanically ventilated and spontaneously breathing patients (16, 19, 62, 68).

Table 2. Overview of comparative studies of IC devices in mechanically ventilated patients [16, 64–67]

	Q-NRG®	Deltatrac®
Deltatrac® (Datex, Finland)	- (No) significant difference in measured REE(16) - Measurements using Q-NRG® significantly faster(16)	
QUARK RMR® (Cosmed, Italy)	- Significant difference in measured REE (p = 0.038) - Measurements using Q-NRG® significantly faster(16)	- No significant difference in mean REE (p=0.166) - No significant differences EE, VCO_2 and VO_2(66) - Significant difference in RQ (P <0.0001)not favoring Deltatrac®, due to measurement values outside the physiological range(60) - Overestimation of VO_2 and VCO_2 by QUARK RMR®(67)
Vmax® (Vyaire, USA)	- Significant difference in measured REE (p < 0.001) - Measurements using Q-NRG® significantly faster(16)	- No significant difference in REE (p = 0.8) is not reliable enough in a clinical research setting(65)
E-COVX® (Datex-Ohmeda, Finland)	- No significant difference in measured REE (p = 0.165) - No significant difference in the duration of measurement	- Overestimation of VO_2 and VCO_2 by E-COVX®(67)
CCM Express® (Medgraphics, USA)	_	- Significant difference in mean REE (p <0.0001) - Significant difference in RQ (p <0.0001) - Significant differences in RQ and VO_2 and VCO_2(p < 0.0001)(66)

Obtaining reliable results

Even with an accurate device, many aspects have to be taken into account to ensure a reliable measurement and a valid interpretation of the results, especially when they consequently lead to an adjustment in nutrition therapy. Because an IC measurement is always a snapshot representation of a continuously changing metabolic state, it is essential to ensure as much of a steady state as possible and practical during the measurement procedure, so that momentary changes in the patient's condition do not

overly influence the interpretation of the baseline EE (7, 30, 69). Furthermore, several conditions potentially influence the measurement itself by altering the gas flow (25).

Steady-state measurement

Many situations influence a patient's steady-state (Table1), and a patient should ideally not experience mental or physical stress, be physically active, or be fed shortly preceding or during the measurement (19). We discuss several points of attention when performing IC in the intensive care setting.

The use of organ support devices for continuous renal replacement therapy (CRRT) and extracorporeal membrane oxygenation (ECMO) are everyday in the ICU setting. The influence of CRRT on REE is controversial (1, 70). Theoretically, heparin-CRRT influences VCO_2 measurements because an individual, unknown amount of CO_2, is influenced by exogenous bicarbonate administration. It may thereby alter the outcome of IC measurement, although others reported that this difference might not be significant (1, 23, 71). Continuous venovenous hemofiltration (CVVH) using citrate anticoagulation in the predilution mode, might affect REE in three ways. O_2 and CO_2 are exchanged in the CRRT circuit, theoretically affecting the Weir formula. Additionally, CRRT induces heat loss and immunologic activation. Lastly, calorie-containing molecules are exchanged within the filter, in addition to citrate itself (65). The most recent study by Jonckheer et al. (72) in 10 critically ill ventilated patients treated with CVVH found that CO_2 alterations due to CVVH are of no clinical importance, so no correction factor for REE is needed with or without CVVH. In contrast with previous recommendations suggesting initiation of IC only several hours after cessation of CVVH, Jonckheer et al. recommend performing IC measurements during CVVH, as CVVH does not seem to alter metabolism.

So far, guidelines lack specific recommendations on nutrition support for ECMO patients. Retrospective data show that underfeeding during ECMO is still prevalent, mainly due to interruptions and poor gastric motility (73). Additionally, ECMO delivers O_2 in addition to removing CO_2, making reliable IC calculation and interpretation even more complicated (19). De Waele et al. (74) proposed to insert consecutively obtained, individual IC measurements of the native and the artificial lung in the adjusted Weir equation to retrieve a measured REE composite as follows:

$$REE_{composite} = 1.44 \times ([3.94 \times VO_{2total}] + [1.11 \times VCO_{2total}])$$

$$\text{With } VO_{2total} = VO_{2native\ lung} + VO_{2ECMO}$$

$$\text{With } VO_{2native\ lung} = VE \times [Fi_{O2} - Fi_{O2}]$$

And $VO_{2ECMO} = [Fi_{O2ECMO} \times VI_{ECMO}] - [Fe_{O2ECMO} \times VE_{ECMO}]$

And $VCO_{2total} = VCO_{2native\ lung} + VCO_{2ECMO}$

With $VCO_{2native\ lung} = [Fe_{CO2} \times VE_{native\ lung}] - [Fi_{CO2} \times VE_{native\ lung}]$

And $VCO_{2ECMO} = [Fe_{CO2ECMO} \times VE_{ECMO}] - [Fi_{cO2ECMO} \times VE_{ECMO}]$

Wollersheim et al. (75) propose a similar equation combining traditional IC measurements of the native lung with calculations based on pre-membrane and post-membrane oxygenator blood gas analyses allowing for simultaneous measurements of lung and ECMO device.

$VO_{2ECMO} = [O_{2BGApost} - O_{2BGApre}] \times$ *ECMO blood flow*

$VCO_{2ECMO} = [CO_{2BGApre} - CO_{2BGApost}] \times$ *ECMO blood flow*

However, these small studies' results require further validation in a larger ECMO patient cohort with different gas flow management (76).

At least 30-60 min preceding IC measurement, no medication alterations should be carried out (8, 63). Sedatives and analgesics may cause reductions in VO_2 and REE (4, 13). Neuromuscular blocking agents also affect the EE, although the effect is small (8, 24, 28, 77, 78). A recent study with continuous infusion of cisatracurium showed a significant reduction in EE measured with the VCO_2 method, although the clinical relevance is presumed to be minor, and in most patients no reductions in caloric prescription are necessary (78). Furthermore, the administration of vasopressors increases REE, whereas specific β-blockers are contradictory reported to decrease REE (8, 35, 77, 79). However, the effect of low-dose cardio-specific β-blockers is negligible (75). Consequently, IC measurements should be repeated as significant dose changes regarding levels of sedation or hemodynamic support are made (24).

From a mechanistic point of view, patients receiving bolus nutrition or orally fed patients should be fasted for at least 5 h before performing IC to obtain a stable measurement (63, 80). However, this is often undesirable and unfeasible in clinical ICU practice (11, 63, 80). In the case of continuous (par)enteral feeding, DIT has minimal effect on IC, if the infusion rate is not altered 1 h before or during measurement (8, 28).

Physical activity, including all body movements related to stress, such as agitation, seizures, shivering, invasive procedures, and unstable analgesia or sedation, can alter EE (19, 81).

Ideally, a patient should rest up to 20 min before IC takes place (76). As this is often difficult, if not impossible to achieve in the ICU setting, these conditions may introduce error into the measurement if they do not resemble the patient's steady state. Physiotherapy or active mobilization should be avoided 2 h before measurements. Endotracheal tube suction should be avoided within 20 min before and during measurements (63). Ventilator settings should not be changed for 60 to 120 min before or during the IC measurement, as the patient needs to adjust to the new settings and therefore, might not be completely stable and at rest (8, 28, 77).

Body temperature variations of more than 1 °C before IC measurement, make results less reliable (28, 68, 78). Some authors report an increase in REE caused by fever, whereas therapeutic hypothermia is associated with a decrease in REE; however, not all studies report similar findings (81).

Gas collection

The ventilation mode may unjustly influence measured EE by directly affecting the measured gas flow used for calculation (15, 82, 83). As the device uses the amount of inspired and expired N_2 as a control to define the amount of inspired and expired oxygen and carbon dioxide, the amount of N_2 will be too low to get a reliable result, when the fraction of inspired oxygen is too high. Patients with an $FiO_2 > 0.6$ cannot be measured accurately by most devices, although the Q-NRG can measure REE in mechanically ventilated patients with a FiO_2 up to 0.7 (6, 28, 63). Consequently, the use of nitric oxide also influences IC measurements (1, 23, 28). Moreover, fast respiratory rates (> 35/min) lead to difficulty in the gas analysis (23, 30). Patients with unspecified amounts of air leakage, such as an uncuffed tracheostomy cannula, endotracheal tube cuff leaks, tracheal-esophageal fistulae, subcutaneous emphysema, or chest tube drainages should be excluded from IC measurements, as the gas collection is unreliable (8, 19, 23). Additionally, an error could be induced by air leakage, instable FiO_2 or expiratory flow, compressed volume, and air trapping in patients with high positive end-expiratory pressure, i.e., PEEP> 10 cmH_2O. Lastly, although the use of a canopy or hood makes measurements possible in spontaneously breathing patients with or without non-invasive ventilation, supplemental O_2 cannot be adequately measured or incorporated into the equations (1, 84).

Although the precautions mentioned earlier aim to ensure a measured EE that reflects real caloric need as closely as possible, it is essential to realize that IC, unless performed continuously, always extrapolates measurements obtained from a short period and therefore never fully accounts for the variation of EE during 24 h (85). IC measurements should ideally be repeated every 2 to 3 days if feasible and whenever a patient's clinical condition or treatment changes significantly, thereby possibly influencing EE (17, 19, 86–88).

Practical considerations

No standardized protocol for performing IC is available (7). However, it stands to reason that the metabolic monitor should be calibrated, connected, and operated correctly, and the technical ranges of the specific device should not be exceeded (54, 63). IC devices are not resistant to moisture, and therefore, humidity in the circuit connected to the mechanical ventilator should be prevented as much as possible by the use of the correct filters, pointing all sample lines upwards, postponing nebulization until after the measurement, and performing timely endotracheal suctioning (although as mentioned before, not within 20 min before measurement, to avoid agitation) (23).

A period of gas exchange in which VO_2 and VCO_2 vary by less than 5% over 5 min or 10% over 10 min should be chosen for calculations, although newer devices may do this automatically (8, 25, 80). Measuring EE in spontaneously breathing and conscious patients could bring difficulties in accepting a canopy hood or face mask because of agitation, claustrophobia, or nausea (1, 61).

IC devices use various disposables at the patient circuit designed for one-time use only, such as flowmeters, filters, adapters, and sampling lines or, alternatively, a canopy hood, to ensure maximum hygiene. The device itself should be completely disinfected after each use. Nevertheless, connection of the IC device to a ventilation circuit requires a brief disconnection of the circuit, resulting in the release of aerosols. Therefore, care should be taken that connection of an IC device to the ventilation circuit of a patient with a disease that is transmittable through aerosols, such as COVID-19, is performed by personnel wearing protective garments and, when possible, takes place in a negative pressure room. Some guidelines advise against the use of IC in COVID-19 patients owing to potential aerosol exposure and therefore infection risk to healthcare providers (89), although others emphasize its value and offer practical guidelines to ensure optimal safety (90).

Alternatives to indirect calorimetry

Several alternatives are used in research and clinical practice to estimate EE in situations where IC is not available or feasible.

Predictive equations to estimate energy expenditure

Predictive equations estimate a patient's energy expenditure using anthropometry and vital parameters to estimate EE. All equations are unreliable, as EE is affected by many individual factors unaccounted for in the formulas (1, 7, 19, 31, 55, 91–95). When comparing the results of predictive equations to those of IC, many discrepancies are found (18). Consequently, the use of predictive equations alone is likely to lead to under- and overfeeding (51). Nutritional guidelines discourage the use of these equations and

advise never to administer more than 70% of the caloric need calculated based on these equations during the first week of ICU stay to prevent overfeeding (18, 22).

Ventilator VCO_2 to estimate energy expenditure

Methods to calculate energy expenditure (EE) based on CO_2 measurements (from the mechanical ventilator, or the pulmonary arterial catheter) have been proposed as a surrogate to IC. The $EEVCO_2$-method uses VCO_2 obtained from the mechanical ventilator or pulmonary artery catheter and a fixed RQ value of 0.86 to substitute VO_2, for mechanically ventilated critically ill patients based on the enteral nutritional products most used in the ICU setting (96):

$$RQ = VCO_2/VO_2$$

$$VO_2=VCO_2/RQ \text{ , with } RQ=0.86$$

The Weir's equation is then adjusted as follows (18, 96):

$$EEVCO_2 \text{ (kcal/day)} = 1.44 \times (3.941 \times [VCO_2\text{(mL/min)}/0.86] + 1.11 \times VCO_2\text{(mL/min)}),$$

simplified:

$$EEVCO_2 \text{ (kcal/day)} = VCO_2 \text{ (ml/min)} \times 8.19$$

Still, the use of a fixed RQ may lead to inaccuracies because of fluctuating substrate use. Applying the food quotient (FQ), or nutritional RQ, instead, may, in part, solve this inaccuracy (19, 30). The approach assumes that the RQ value is equal to the FQ, i.e., the estimated RQs resulting from the oxidation of different energy substrates from nutrition therapy and non-nutritional calorie sources. The RQ is 1.0, 0.7, and 0.8 for carbohydrates, fat, and protein, respectively, enabling calculation of an individual FQ based on the composition of the administered energy sources (both nutritious and non-nutritious):

$$FQ= [fat\% \times 0.7] + [protein\% \times 0.8] + [carbohydrates\% \times 1.0]$$

Whenever relevant, other energy sources with different RQs can be added to the formula, such as in the case of citrate CVVH, where $RQ_{citrate}$ = 1.33. Subsequently, the estimated RQ in the adjusted Weir's equation is substituted for the calculated FQ. Nevertheless, the use of the FQs may be considered unreliable in patients in a catabolic state, as endogenous substrate utilization cannot be estimated by intake. In addition, the $EEVCO_2$ method has consistently been shown to be inferior to IC (97, 98). However, the technique has been proven to be more accurate than predictive equations (18, 91, 96).

IC GUIDED NUTRITION

Despite guideline recommendations to use IC in critically ill patients, the superiority of IC-guided nutritional therapy has not yet been unequivocally proven in randomized clinical trials (15, 86, 99). Even though it was confirmed that IC-guided nutrition support improves a patient's nutritional status, the only significant benefit to outcome proven by RCTs is a significant decrease of nosocomial infections (46, 100–102). Controversy exists concerning its effect on morbidity, mortality, and the length of hospital stay (18).

Associations with clinical outcome

The pilot Tight Calorie Control Study (TICACOS) (29) suggested a 60-day mortality improvement in patients receiving higher caloric IC-guided nutrition than standard care, despite an increased length of ventilation and ICU-stay seen in this group. The subsequent TICACOS international study (103) showed that the use of an IC-guided nutritional goal yielded higher energy and protein delivery, compared with a nutritional goal based on predictive equations, with a trend toward lower mortality. However, overall results were insignificant. Covering 100% of repeated IC-derived REE from the first day of ICU in the EAT-ICU trial (104) did not affect the physical quality of life, infectious complications, or mortality at 6 months as compared to standard nutrition.

There are several possible explanations for these discrepancies. In the EAT-ICU trial, the defined nutritional goal of both protein and calories was largely met; however, the target was set only according to a median of two measurements per patient. For some patients, this meant that energy prescriptions were stationary after extubation, possibly underfeeding some at this stage. Conversely, aiming at covering 100% of measured REE in the early phase might have conferred overfeeding by exogenous nutrient overload in a phase when the endogenous substrate is mostly sufficient to meet REE. Zusman et al. (14) describe a U-shaped curve correlation between the percentage of calories delivered compared to measured EE and mortality in ICU patients, where both under- and overfeeding have harmful effects, and the beneficial effect lies in the middle of the curve. Therefore, caloric outliers on opposing sides of the curve might dilute any significant beneficial results. These observations further underline the need for studies addressing the effect of personalized IC-guided nutrition therapy based on repeat measurements, continued through various metabolic phases of illness and convalescence.

The question remains whether calories delivered to patients during the acute phase of their critical illness should match measured or estimated EE despite the ongoing endogenous nutrient release, which is not suppressed by feeding and remains immeasurable (85). Furthermore, the effect of non-nutritional calories, including propofol, glucose, and citrate, should be taken into account when determining the target exogenous energy

dosage (17). Nutrition guidelines recommend to gradually advance to target during the first week, not meeting REE before the first 48 h to avoid overfeeding (18, 22).

An additional complexity in the interpretation of nutritional trials is the varied amount of protein delivered. The TITACOS studies were not protein targeted, and the amount of protein was determined by the rate of EN or parenteral nutrition provided. This resulted in patients receiving protein below the recommended levels. Current nutritional theory hypothesizes that not the caloric value, but the amount and timing of protein provided is most essential to influence the course of the disease, although the effect might not be the same in all types of critical illness (18, 105–108). Therefore, results might reflect caloric overfeeding, early protein overdosing, late protein underfeeding, or a combination of these aspects. Future research should address the optimal timing and dosing of protein and calories individually.

Respiratory quotient

Aside from energy expenditure, the IC derived RQ provides several theoretical applications, as the RQ indicates which macronutrients is mainly being metabolized. Underfeeding, which promote the use of endogenous fat stores, decreases the RQ, whereas carbohydrate metabolism increases RQ. However, studies in both adult ICU and pediatric burn patients found low sensitivity and specificity of IC derived RQ as an indicator of over- or underfeeding (60, 109). Nevertheless, McClave et al. did show that increases in RQ correlated to increasing respiratory rate and decreasing tidal volume, suggesting that patients developed shallow, rapid respirations in response to increases in the measured overall RQ. Indeed lowering dietary fat guided by RQ can decrease VCO_2 and thereby breathing effort in patients with obstructive lung disease, although the applications in the ICU setting are limited. More recently, several smaller studies found a correlation between (course of) RQ and outcome in critically ill patients, suggesting a potential prognostic use of RQ (110, 111). Nevertheless, even if these patterns of substrate utilization could be reliably identified in larger populations, it remains unclear whether they can and should be influenced to improve outcome. Due to paucity of guiding evidence, it is currently advised that the clinical use of RQ is restricted to a marker of test validity to confirm measured RQ values are in physiologic range, and perhaps a rough estimation of respiratory tolerance of feeding (60).

All taken into account, the association of IC use with important clinical outcomes needs to be further explored before definitive conclusions about its use in the intensive care unit can be drawn. A recent systematic review and meta-analysis by Tatucu-Babet et al. (99) identified 4060 articles on the effect of IC-guided nutrition and clinical outcomes and found only 4 single-center, randomized controlled trials with 396 patients included in the analysis. All 4 studies reported higher receipt of energy close to the measured energy

expenditure by IC compared to the predictive equation arm. However, when combined, no association between IC-guided energy delivery and hospital mortality was found, leading the authors to conclude that it is yet too early for widespread implementation of IC in clinical practice.

Convalescence

No formal guidelines on calories and protein intake are available for the convalescence phase of critical illness. However, as patients likely enter a more physically active and anabolic phase with an increased TEE, it is assumed that a significant protein and calorie delivery is necessary to restore muscle mass and quality of life (17, 50). Furthermore, studies imply that nutrition delivery largely fails to reach nutritional goals in the post-ICU hospitalization phase, although very few studies set goals according to regular IC measurements (11). Recent retrospective data shows that PICS patients are prone to worse long-term outcomes and lower survival when fed with current evidence-based protocol nutrition (53). It has been suggested that high levels of protein, amino acids, and anabolic adjuncts such as insulin, might aid in overcoming anabolic resistance in PICS. This is primarily extrapolated from cancer cachexia and burns research, and mechanistic studies are lacking (112, 113). There is an urgent need for prospective studies measuring EE in the recovering critically ill and analyzing actual nutrition delivery and the effect on long-term outcome in different metabolic phenotypes.

CONCLUSION

Energy expenditure appears highly variable among critically ill patients and in individual patients during various phases of illness. As a consequence, critically ill patients are at considerable risk of under- or overfeeding during ICU and post-ICU hospital stay, when rough and static estimates are used. The most recent international guidelines recommend regular indirect calorimetry to measure energy expenditure as a proxy for caloric requirement in ICU patients. However, the superiority of IC-guided nutritional therapy has not yet been unequivocally proven in randomized clinical trials and further research is urgently warranted. Nevertheless, IC has strong theoretical potential to improve nutritional status and consequently, the long-term outcome of critically ill patients in the various metabolic phases of critical illness. Increased knowledge of practical use and theoretical benefits of IC among clinicians can contribute to more widespread and routine use, thereby promoting research opportunities and real-time targeted and personalized nutrition therapy.

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Chapter 5

Resting energy expenditure measured by indirect calorimetry in mechanically ventilated patients during ICU stay and post-ICU hospitalization: A prospective observational study

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ABSTRACT

Purpose

The metabolic course during and after critical illness is unclear. We performed repeated indirect calorimetry (IC) measurements during ICU- and post-ICU hospitalization to determine resting energy expenditure (REE).

Methods

Prospective observational design. In ventilated ICU patients, IC measurements were performed every three days until hospital discharge. Measured REE as predicted by the Harris-Benedict equation (HBE-REE) and 25 kcal/adjusted body weight/day (25-REE) were compared.

Results

In 56 patients (38% females, 71(13)years, BMI 29(27;31)kg/m^2), 189 ICU IC measurements were performed. Measured REE did not differ from HBE-REE at ICU admission, but was lower than 25-REE. Measured REE was increased compared to baseline on ICU-admission-day four (29(29;30)kcal/kg/day; mean difference 3.1(1.4;4.9)kcal/kg/day, $p < 0.001$) and thereafter during ICU admission. During post-ICU ward stay, 44 measurements were performed in 23 patients, showing a higher mean REE than during ICU stay (33(31;35) kcal/kg/day; mean difference 2.6(1.2;3.9)kcal/kg/day, $p < 0.001$). The REE in the ICU and ward was >110% of HBE-REE from day four onwards.

Conclusions

Critically ill mechanically ventilated patients were shown to have a resting energy expenditure (REE) > 110% of predicted REE on ICU admission day four and thereafter. Indirect calorimetry measurements suggest that the mean energy requirements during post-ICU hospitalization are higher than those in the ICU.

INTRODUCTION

Over the past decade, the increase in ICU survivors has been associated with an increase in long-term sequelae, such as reduced physical strength and quality of life (1, 2, 3). Adequate timing and dosing of nutritional therapy are expected to be key factors in improving quality of life after critical illness. The gold standard for estimating energy requirements is measuring energy expenditure with indirect calorimetry (4,5). However, clinical studies have thus far failed to identify a straightforward course of energy expenditure in the critically ill (6,7).

Evidence shows that endogenous energy production puts patients at risk of overfeeding during the early phase of critical illness when covering 100% of energy expenditure through exogenous nutrition. This is pragmatically avoided through a gradual increase in caloric intake in all patients during the first 3–5 days after ICU admission (4,5). After the initial assault, ICU patients appear to tolerate and require increased exogenous substrate (8). The Coronavirus Disease 2019 (COVID-19) pandemic has led to a surge in longitudinal indirect calorimetry studies that suggest that these patients go through a prolonged hypermetabolic phase during their ICU stay (9, 10, 11).

Whereas data are emerging on the metabolic profile during mechanical ventilation, the course of resting energy expenditure after extubation is less clear since indirect calorimetry in non-ventilated patients is more cumbersome than in ventilated patients as this requires the application of an airtight canopy. Therefore, indirect calorimetry studies during the recovery phase of critical illness are rare and small (12,13). Theoretically, most ICU survivors should experience anabolic upregulation to replace lost muscle mass and quality. On the other hand, persistent inflammation, immunosuppression, and catabolism syndrome may arise in some patients, resulting in anabolic resistance and inflammation-induced cachexia (7). Knowledge of these metabolic patterns is fundamental to optimizing post-ICU nutrition.

Objectives

We aimed to describe the course of resting energy expenditure (REE) during and after critical illness by performing repeated indirect calorimetry measurements in patients during ICU- and post-ICU hospital stay. We hypothesized that resting energy expenditure would remain at the predicted value during the initial phase, followed by an increase to a hypermetabolic level, defined as a resting energy expenditure (REE) > 110% of REE predicted by the Harris-Benedict equation (HBE), with another increase during the anabolic phase of convalescence (7).

MATERIAL AND METHODS

This study had a prospective observational design. The ethics committee of the Gelderse Vallei Hospital approved the RECOVER-energy ICU study (dossier no. 2002–007), and the protocol was registered in the Netherlands Trial Register (number NL8907).

Setting

The study was performed in the ICU and (subsequently) the general wards of the Gelderse Vallei Hospital, a regional teaching hospital in Ede, the Netherlands. The hospital is equipped with 400 beds and two adult ICU units, with a combined capacity of 18 beds. Indirect calorimetry measurements using the Q-NRG® metabolic monitor (Cosmed, Italy) are performed every three days as part of standard care in all mechanically ventilated ICU patients. Indirect calorimetry measurements are performed according to local protocols based on recommendations and technical specifications of the manufacturer. Per our local protocol, personal energy targets are first calculated by the computerized nutrition protocol based on the Food and Agricultural Organization and World Health Organization (FAO/WHO) formula (14) (Supplemental File 1). Actual enteral/parenteral nutritional and nonnutritional (i.e. propofol, glucose, etc) energy and protein provision are automatically calculated hourly by our electronic medical record systems MetaVision® (iMDsoft, Tel Aviv, Israel) based on pump speed, passed time and (duration of) interruptions, if any. Oral nutrition is currently not incorporated, as it cannot be done automatically and oral nutrition is not usually a substantial contribution to the total nutritional intake in ICU patients. A progressive feeding strategy towards 100% of the calculated energy targets at admission day four using continuous enteral feed is used (D1 25%, D2 50%, D3 75%, D4 100%). Thereafter energy targets are automatically adjusted when indirect calorimetry output is entered into the computer system, accounting for nonnutritional calories. For the purposes of this study, energy targets were calculated by the Harris-Benedict Equation and the commonly used weight-based eq. 25 kcal/kg/day (see Reporting of REE, below). Post-extubation Q-NRG canopy measurements were not yet routinely performed at the time of the study. These were therefore considered study measurements. Written informed consent for performing of all study measurements and additional data collection was obtained from the patients or legal representatives.

Participants

Between August 2020 and February 2021, all patients requiring mechanical ventilation upon admission were screened for eligibility if they were expected to be in the ICU for >48 h after inclusion and in the hospital's general ward after ICU discharge. Patients were excluded in cases where the clinical characteristics during the first 24 h of ICU admission could theoretically interfere with the obtaining of a reliable baseline indirect calorimetry measurement according to literature and our local protocol (7). These exclusion criteria

were; a positive endexpiratory pressure exceeded 12 cmH_2O or the fraction of inspired oxygen exceeded 0.7, possible air leaks, such as through chest drains, pneumothoraces, tracheoesophageal fistulae or subcutaneous emphysema. No extracorporeal gas exchange systems are in use at our ICU.

Indirect calorimetry measurements

Resting energy expenditure as measured by indirect calorimetry was the main outcome measure of our study. Measurements were performed within 24 h after intubation and every three days thereafter, using the QNRG metabolic monitor in ventilator mode, as per ICU standard of care. After extubation, the measurements were performed with the Q-NRG in canopy mode. After two post-ICU ward measurements, the frequency was adjusted to once per week. The following parameters were recorded at every measurement: REE, RQ, VO_2 and VCO_2. Measurements were considered valid if patients were in a physiological steady-state, defined as a period of five minutes of <10% variation in oxygen consumption (VO_2) and carbon dioxide production (VCO_2). To achieve this, patients and staff were instructed to avoid strenuous physical effort, endotracheal tube suction, ventilator setting changes, bolus feeding or consumption of an oral meal two hours before the scheduled measurement. Furthermore, the respiratory quotient (RQ) had to be within the physiological range of 0,67-1,2. If (steady-state) measurements could not be performed, or if a patient received non-invasive oxygen therapy, peritoneal- or hemodialysis or -filtration, or continuous renal replacement therapy, measurements were delayed a maximum of 24 h or, thereafter, skipped. The Q-NRG metabolic monitor displays measured REE in Kcal/day. To facilitate comparability between subjects throughout a range of body mass indices, REE was divided by measured lean body mass (LBM) as measured by bioelectric impedance analysis (BIA), resulting in Kcal/kg/day.

Bioelectric impedance measurements

BIA body composition measurements with the InBody S10® (InBody Co., Ltd., Seoul, Korea) are routinely performed upon admission in all ICU patients. This multi-frequency, segmental impedance analyzer requires height, weight, and sex as input parameters. Height and weight as measured upon ICU admission were used. When circumstances did not allow measurements, height was entered as provided by the patient or representative. BIA measurements were performed in a supine position with reusable electrodes attached to the left and right thumb and middle finger and both ankles. The measurements typically took 3 to 5 min.

Data sources

The researchers obtained the included patients' baseline characteristics and clinical parameters prospectively from the electronic medical record systems MetaVision® (iMDsoft, Tel Aviv, Israel) and NeoZIS® (MI Consultancy, Katwijk, The Netherlands). In

addition to the study measurements, other parameters that were prospectively recorded were age, sex, weight, height, BMI, comorbidities (including type 2 diabetes, hypertension, and chronic obstructive pulmonary disease), admission diagnosis, admission Barthel index, admission Acute Physiology And Chronic Health Evaluation (APACHE) II score, admission Nutrition Risk in Critically Ill (NUTRIC) score, ICU length of stay (ICULOS), hospital length of stay (HLOS) and ward length of stay (LOS). Reasons for exclusion and cessation were collected retrospectively if retrievable.

Reporting of REE

To assess metabolic status, a comparison between measured REE and REE as predicted by the HBE (1919) (HBE-REE) and REE as predicted by the commonly used weight-based eq. 25 kcal/kg/day (25-REE) were used.

The height HBE-REE was calculated as follows:

Men: 66.473 + (13.7516*weight in kilograms) + (5.0033*height in centimeters)-(6.755*age in years)

Women: 655.0955 + (9.5634*weight in kilograms) + (1.8496*height in centimeters)-(4.6756*age in years)

Based on the ESPEN guideline (5), the 25-REE, used actual body weight in BMI ≤ 25 kg/m^2 and adjusted body weight in BMI > 25 kg/m^2, based on the patient's height calculated to BMI 25 kg/m^2 with an addition of 20% of the excess weight (actual body weight-adjusted body weight) to account for the metabolic demand of their increased adipose tissue and muscle. For all calculations using actual body weight, weight as measured upon ICU admission in kilograms was used.

Hypermetabolism was defined as an elevated resting energy expenditure (REE) > 110% of REE as predicted by the HBE (11,15,16). As there are no definitions of hypermetabolism based on the ratio of measured versus REE as predicted by the 25 kcal/kg/day, these ratios were not calculated.

Study size

To our knowledge, no prior prospective studies with continuous indirect calorimetry measurements post-ICU discharge were available upon which to base a formal power calculation. A previous study performed 23 successful post-ICU indirect calorimetry measurements in 12 critically ill patients (13). To at least match this number of tests in our prospective design, we continued inclusions until 23 patients had at least one ward measurement.

Statistical methods

Descriptive statistics were performed for the demographic and clinical data of all patients. The quantile-quantile plots were visually assessed for the normality of the distribution of continuous data. When inconclusive, a significant Kolmogorov-Smirnov test was considered indicative of abnormal distribution. Continuous values are reported as mean (95% confidence interval) or median (interquartile range), and discrete data are presented as numbers (%). Differences between subcohorts were assessed using independent samples t-tests for continuous data or chi-squared tests for categorical data. Mann–Whitney U tests or Fisher's exact tests were used when test assumptions were not met. Nondichotomous categorical data are compared using an analysis of variance. For comparisons, the results of all ward measurements were bundled into a grand mean by dividing the means of the measurements per subject by the number of subjects. Paired samples t-tests were used to compare REE at different time points in the ICU and the ward and to compare measured REE to predicted REE. To preclude bias, all paired samples t-tests used analysis-by-analysis exclusion for subjects with missing values. Thus, when comparing REE at two time points, only the REE measured in the subjects who underwent both measurements were used to calculate both means and their comparison. The assumption of normality for the distribution of the differences was met. Equal variances were assumed if Levene's test was not significant. IBM SPSS Statistics 27 (I.B.M. Corp, Armonk, NY, USA) was used for all analyses and figures. Only two-sided analyses were used. P-values ≤0.05 were considered statistically significant. P-values are reported to a single significant figure unless $0.2 \geq P \geq 0.01$, in which case two significant figures are shown. The error bars in the figures represent the 95%- confidence interval of the mean of the repeated measures and were adjusted for the between-subject variability (17).

RESULTS

Between August 2020 and February 2021, 56 patients were included, of whom 23 had at least one post-ICU ward measurement (Flow chart Supplemental Figure 1). The characteristics of these patients are described in Table 1.

Table 1. Patient characteristics at ICU admission. [a]

	All patients n=56 [b]		Patients with ward measurements n=23 [b]		Patients without ward measurements n=33 [b]		P-value
Demographics							
Age, years	71 [13]		69 [18]		71 [12]		1.0 [c]
Females, %	21 (38%)		9 (39%)		12 (36%)		1.0 [d]
Actual body weight, kg	88 (82;94)		88 (79;98)		88 (80;97)		1.0 [d]
Lean body mass, kg	63 (60;67)		63 (57;69)		63 (59;68)		1.0 [e]
Body mass index, kg/m^2	29 (27;31)		29 (27;32)		29 (26;31)		.7 [e]
Comorbidities							
Diabetes Mellitus type 2	15 (27%)		4 (17%)		11 (33%)		0.2 [d]
Cardiovascular disease	38 (68%)		16 (70%)		22 (67%)		1.0 [d]
COPD/Asthma	11 (20%)		4 (17%)		7 (21%)		1.0 [d]
Admission diagnosis							
Surgical	11 (20%)		5 (22%)		6 (18%)		0.7 [f]
Medical	45 (80%)		18 (78%)		27 (82%)		
COVID-19	23 (41%)		8 (35%)		15 (46%)		0.3 [d]
Sepsis	14 (25%)		6 (26%)		8 (24%)		1.0 [d]
Baseline clinical scores							
Barthel score	20 [0]	n=53	20 [0]	n=21	20 [0]	n=32	0.08 [c]
NUTRIC score	4 [3]		5 [4]		4 [2]		0.5 [c]
SOFA score	7 [4]		8 [3]		7 [4]		0.5 [c]
APACHE-II	19 [8]		18 [5]		19 [9]		1.0 [c]

[a] Data are displayed as mean (95%-confidence interval), median [interquartile range] or rate (percentage of total) [b] Unless stated otherwise due to missing data. [c] Mann-Whitney U test [d] independent samples t-tests [e] Fisher's exact test [f] ANOVA. Abbreviations: ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; COVID-19, Coronavirus disease 2019; NUTRIC, nutrition risk in critically ill; SOFA, sequential organ failure assessment; APACHE, acute physiology and chronic health evaluation.

The course of REE in the ICU

During ICU admission, 189 indirect calorimetry measurements were performed (13 in canopy mode), 100 of which were in the subcohort that subsequently also had ward measurements (n = 23). Figure 1 shows the mean course of REE in the ICU in absolute kcal/day and relative to the LBM (kcal/kg/day) for the first four weeks of ICU admission. REE on the ICU admission day was 26 (24-27) kcal/kg/day (Supplemental Table 1). On day four, REE had increased significantly to 29 (29–30) kcal/kg/day (mean difference 3.1 (1.4–4.9) kcal/kg/day, $p < 0.001$). Mean REE was not significantly different between days 4 and 7 or subsequent ICU measurements (Supplemental Table 2).

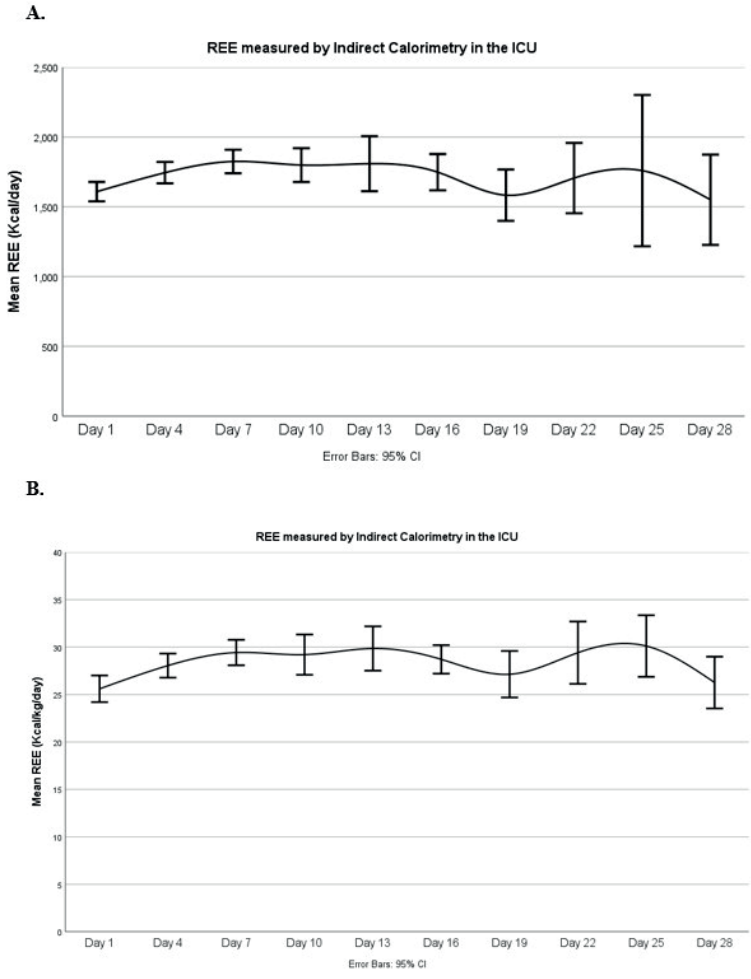

Figure 1. Mean course of REE in the ICU in absolute kcal/day (A) and relative to the Fat-Free Mass in kcal/kg/day (B) on measurement days during the first four weeks of ICU admission (n=56).

REE in the ICU compared to Post-ICU

The length of stay in the ICU was a median of 12 [14] days in the total cohort and 13 [17] days in the subcohort in whom ward measurements could be performed (Table 2). Figure 2 shows where measurements were performed.

Table 2. Outcome measures [a]

	All patients n=56	Patients with ward measurements n=23 [b]
Length of stay		
ICU length of stay, days	12 [14]	13 [17]
Hospital length of stay, days	21 [22]	27 [26]
In hospital mortality		
ICU	14 (25%)	N.a.
Ward	8 (14%)	3 (13%)

[a] Data are displayed as median [interquartile range] or rate (percentage of total). Abbreviations: ICU, intensive care unit

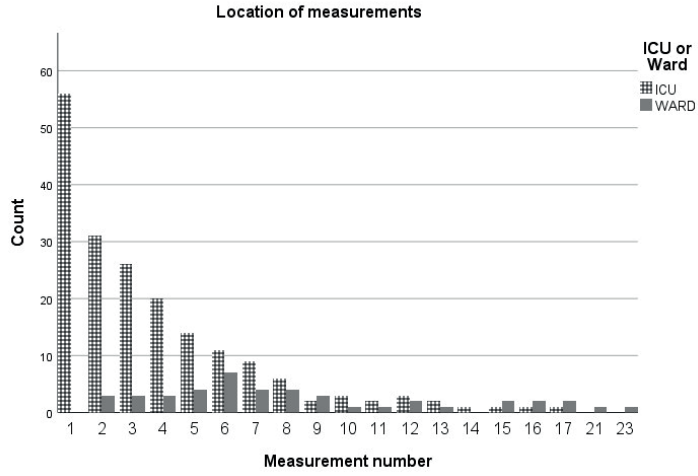

Figure 2. Visual representation of the location where measurements were conducted per measurement number (n=233).

After ICU discharge, a total of 44 measurements could be performed on the ward. The median time between ICU discharge and the first ward measurement was 3 [1] days (Table 3). The mean REE of all ward measurements was 2032 (1869–2195) kcal/day and 33 (31–35) kcal/kg/day. Figure 3 shows the course of REE in the ICU of the subcohort that had ward measurements relative to the mean measure of REE on the ward. The mean REE of the ward measurements was significantly higher than the mean REE during ICU stay in the subcohort, both absolutely (mean difference 289 (145–433) kcal/day, p = 0.003) and when related to LBM (mean difference 2.6 (1.2–3.9) kcal/kg/day, p< 0.001).

Table 3. Overview of measurements performed in the ward.

Measurement number	1	2	3	4	5
Ward week	Week 1		Week 2	Week 3	
Ward day [a]	3 [1]	6[3]	11[2]	16[2]	19
Number of measurements performed	19	15	4	5	1

[a] As ward measurements often had to be postponed longer than 24 hours due to logistical issues, median [interquartile range] based on the actually performed measurements is shown instead of scheduled times.

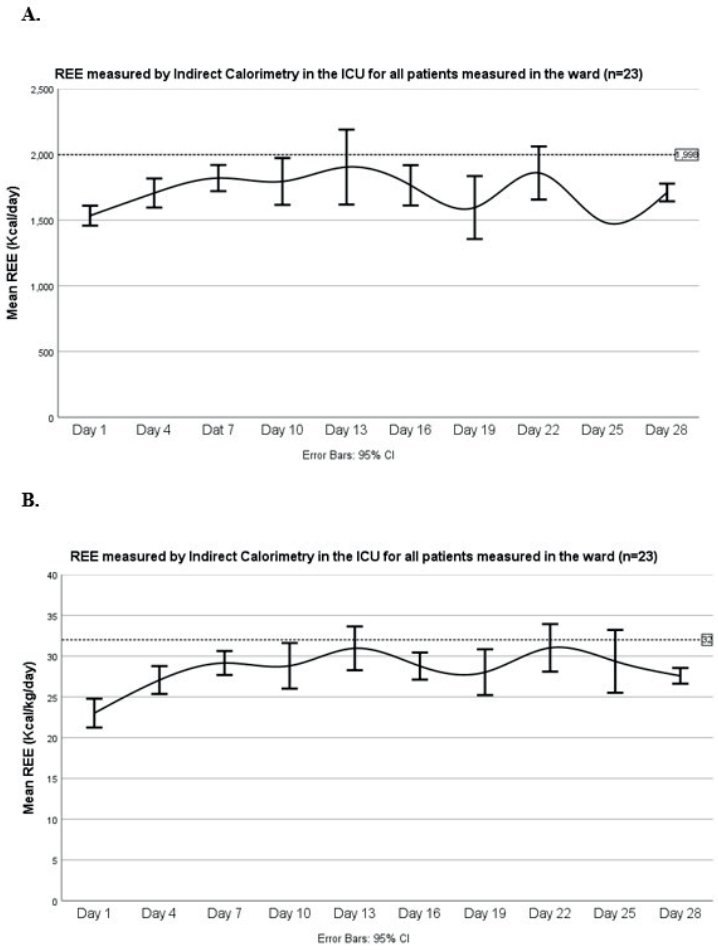

Figure 3. Mean course of REE in the ICU of the subcohort that had ward measurements, relative to the mean measure of REE on the ward, in absolute kcal/day (A) and relative to the Fat-Free Mass in kcal/kg/day (B), on measurement days during the first four weeks of ICU admission (n=23).

Measured vs. predicted REE

The HBE-REE was lower than the measured REE on ICU days 4, 7, 10 and 16 but not on the others (Table 4). The 25-REE was higher on the ICU admission day, but not on the others (Table 4). In the ward, the measured REE was significantly higher than the predicted HBE-REE (mean difference 402 (280–524) kcal/day, p < 0.001), and the 25-REE (mean difference 106 (12,201) kcal/day, p = 0.028).

Hypermetabolism

In addition to being significantly higher than the predicted HBE-REE, the mean REE was >110% of the HBE-REE on ICU days 4, 7, 10 and 16 (Table 4). The grand mean REE on the ward was 126% (118–134) of the mean HBE-REE.

Table 4. Comparing REE as measured by indirect calorimetry and the REE as predicted by the Harris-Benedict equation and the rule of 25 kcal/kg (adjusted body weight)/day during ICU admission [a].

	REE, Kcal/day		**Difference**	**95%-CI for the difference**		**t**	**df**	**P-value**	**Ratio (95%-CI)**
				Lower	**Upper**				
Day 1 n=56	**25- REE**	1914	305	213	398	6.6	55	**<0.001**	n.a.
	IC- REE	1608	-30	-125	65	-0.6	55	0.5	98 (92, 105)
	HBE-REE	1639							
Day 4 n=31	**25- REE**	1931	73.8	-21.5	169	1.6	30	0.062	n.a.
	IC-REE	1857	204	93	314	3.8	30	**<0.001**	113 (106, 120)
	HBE-REE	1653							
Day 7 n=26	**25- REE**	1906	10	-131	152	0.2	25	0.8	n.a.
	IC-REE	1895	260	136	384	4.3	25	**<0.001**	117 (108, 125)
	HBE-REE	1635							
Day 10 n=20	**25- REE**	1955	7.8	-176	192	0.1	19	0.5	n.a.
	IC-REE	1947	227	39	415	2.5	19	**0.021**	115 (103, 128)
	HBE-REE	1720							
Day 13 n=14	**25- REE**	1973	-33	-339	273	-0.2	13	0.8	n.a.
	IC-REE	2006	262	-13	537	2.1	13	0.060	115 (100,131)
	HBE-REE	1744							
Day 16 n=11	**25- REE**	1996	47	-158	252	0.5	10	0.6	n.a.
	IC-REE	1948	240	26	455	2.5	10	**0.032**	115 (102, 128)
	HBE-REE	1708							
Day 19 n=9	**25- REE**	2019	170	-130	470	1.3	8	0.2	n.a.
	IC-REE	1849	81	-246	427	0.5	8	0.602	106 (85, 127)
	HBE-REE	1768							
Day 22 n=6	**25- REE**	1964	15	-442	4722	0.1	5	1.0	n.a.
	IC-REE	1948	235	-304	775	1.1	5	0.3	116 (80, 153)
	HBE-REE	1713							
Day 25 n=2	**25- REE**	1992	-129	-2802	2544	-0.6	1	0.6	n.a.
	IC-REE	2122	336	-309	980	6.6	1	0.9	119 (110, 128)
	HBE-REE	1786							

Table 4. Continued

	REE, Kcal/day		Difference	95%-CI for the difference		t	df	P-value	Ratio (95%-CI)
				Lower	Upper				
Day 28 n=3	**25- REE**	2110	225	-497	947	1.3	2	0.3	n.a.
	IC-REE	1885	12	-1020	996	0.05	2	1.0	103 (43, 161)
	HBE-REE	1873							

[a] Paired samples t-test with analysis-by-analysis exclusion for subjects with missing values. Abbreviations: REE, resting energy expenditure; CI, confidence interval; df, degrees of freedom; ICU, intensive care unit; IC, indirect calorimetry; HBE, Harris-Benedict equation.

Achievability

During the study period, 150 mechanically ventilated ICU patients were screened for eligibility, of whom 56 could be included. Reasons for exclusion were planned transfers, no possibility to perform indirect calorimetry measurements within 24 h (due to logistical issues or patient characteristics), refusal of consent or a moribund status. Patients had a combined ICU-LOS of 802 days, meaning that (802/3=) 267 measurements should have been performed according to protocol. By this calculation, the success rate of indirect calorimetry measurements in the ICU was 71% (189/267). However, the combined ICU-LOS post-extubation was 273 days, and thus 91 (297/3) canopy measurements should have been performed in ICU. This meant the success rate of the canopy measurements in the ICU was 14% (13/91), whereas 100% (176) of the invasive ventilation indirect calorimetry measurements were performed.

Thirty-nine patients had a three-day post-ICU ward-LOS, meaning at least one ward measurement could have been performed. However, only 23 had a ward measurement, meaning that 59% (23/39) of patients discharged to the general ward could be included in the subgroup analysis. With 44/88, the success rate of canopy ward measurements in this subgroup was 50%.

DISCUSSION

This prospective observational study shows that the resting energy expenditure of critically ill mechanically ventilated patients increased to >110% of their predicted REE on day four after ICU admission. Another significant increase in REE was seen during post-ICU hospitalization. The findings in ICU were in line with previous studies showing persistent hypermetabolism in various categories of ICU patients (9, 11, 12, 18). The associated increased energy consumption is likely to escalate the risk of underfeeding when nutritional targets are aimed at predicted rather than measured REE. REE measured

within 24 h of ICU admission was not higher than predicted by the HBE, but significantly lower than REE as predicted by the ESPEN recommended rule of 25 kcal/kg/day. One explanation for this finding may be that patients usually receive a higher dose of neuromuscular blocking agents at admission than thereafter. However, although several studies suggest that continuous infusion of neuromuscular blocking agents is associated with a significant reduction in REE, the magnitude of the effect is small, and insufficient to describe the variation observed between admission and day 4 REE in our cohort (19). An alternative or additional explanation for the low- to-normal initial REE may be found in the hypothesis of metabolic hibernation, which suggests that in a state of extreme stress, oxygen utilization is reduced as an adaptive strategy to prevent cell death by mitochondrial energy substrate overloading (20,21). There is still little knowledge on the reactivation mechanisms of mitochondria and their time-course, but as recovering ICU patients appear to require and tolerate increased exogenous substrate after 3–5 days, the time at which we found an increased REE compared to baseline, this might reflect upregulated mitochondrial function (21).

In addition to the ICU measurements, we performed the highest number of indirect calorimetry measurements in post-ICU patients reported to date. The results showed another significant increase in REE compared to the mean measured REE in the ICU, and > 110% of the mean HBE-REE. Similar studies are lacking, but a nested cohort study by Ridley et al. (13) conducted 23 post-ICU indirect calorimetry measurements in 12 patients and did not see an increased REE relative to the predicted REE in the post-ICU phase. One possible explanation for this discrepancy might be that only 12 individuals had been measured in Ridley's cohort, impeding power. Another hypothesis is that the higher cohort might have increased the height of REE, as some studies have shown that these patients groups are more likely to develop a prolonged state of hypermetabolism in the ICU, although others found conflicting results (9,22,23). Furthermore, our cohort was of higher age (69 years vs. 59 years) and had a lower percentage of male patients (61% vs. 83%), which may have had an effect (22). One study found that young female burn patients had higher levels of anabolic hormones, associated with a lower ICU stay and decreased duration of persistent hypermetabolism (24). Future studies may focus on the course of REE during convalescence in specific ICU patient groups. Theoretically, the switch from the mechanical ventilation mode to the canopy mode for the indirect calorimetry measurements after extubation may have influenced the outcome of the measurements. However, the same device was used for the mechanical ventilation measurements, and a previous study found excellent inter-unit variability and accuracy of the Q-NRG indirect calorimeter in canopy dilution mode in spontaneously breathing adults (25).

Achievability

During this study, we performed 100% of all scheduled measurements during invasive mechanical ventilation, indicating the high achievability of using this technique. It should be noted that at the time of the study, indirect calorimetry measurements using the Q-NRG ventilator mode were standard care. This means that our ICU nurses are familiar with the device and can quickly and adequately perform measurements with little instruction or encouragement from the study team.

However, post-extubation, the success rate of performance of indirect calorimetry measurements according to protocol declined steeply to 14% in the ICU. Furthermore, only 23 of our 56 patients had ward measurements, with a subsequent success rate of 50% for their scheduled measurements. Ridley et al. reported that they could conduct post-ICU ward measurements in 12 out of 56 patients in their study, with a success rate of 32% for the scheduled measurements. Our higher numbers are likely explained by indirect calorimetry measurements being the main focus of this study - rather than a nested substudy - with a dedicated study team that had the skills, time and dedication to attempt these measurements. Nevertheless, many canopy measurements could not be performed, especially during post-extubation ICU stay. Two prior canopy studiesfound that the most common reasons that measurements were not performed were because the patient declined, was agitated, or depended on nasal oxygen supplementation (13,26). Although we did not formally register the reason for failure to perform a measurement, we can report that the most common reasons that measurements could not be performed in the ICU were the use of supplementary oxygen and agitation due to delirium. In the ward, the most common reasons were the patient's decline and the use of supplementary oxygen. In addition, measurements were not carried out if a patient was moribund. Because of these difficulties, measurements had to be rescheduled in the ward more often than in the ICU, leading to a more significant deviation in measurement intervals (Table 3).

Our achievability findings highlight the limitations of the canopy mode for indirect calorimetry in (post-) ICU patients. Nevertheless, when measurements can be performed, the results are exciting and may lead us towards better nutritional care for patients during post-ICU convalescence. We, therefore, encourage other researchers to continue this line of research.

Clinical implications

In this section we reflect on the expected clinical implications of our results to bridge the gap between research and clinical practice. Of course, our results are descriptive exploratory findings, that should always be interpreted cautiously.

We found that critically ill mechanically ventilated patients had a higher measured REE than predicted by the HBE ICU admission day four and thereafter. Endogenous energy production is not expected to play a significant role after day four when HBE-REE significantly underestimated actual REE. Thus, our findings suggest that aiming nutritional targets at HBE-REE rather than measured REE would lead to underfeeding. The rule of 25 kcal/kg/day overestimated the measured REE at ICU admission, but proved to be accurate thereafter during ICU stay. This suggests that when indirect calorimetry is unavailable, 25 kcal/kg/day using ABW may be used in the ICU setting, as is currently advised by the ESPEN guidelines (5). However, a recent meta-analysis contradicts this, and we therefore cannot support the use of 25/kcal/kg ABW/day based on our results alone (27). The risk of further underfeeding increases during post-ICU ward stay, as another increase in REE was seen compared to both predictive methods, and it is known that most patients do not even meet the lower estimates of their energy targets during this time (13,28). Therefore, we recommend aiming caloric targets at measured REE after the initial progressive feeding protocol in the ICU and, when feasible, in the ward. Although the mean REE was not significantly different between measurements after ICU admission, the increase in the standard deviation of the measurements over time suggests a considerable variation in REE between subjects. This can likely be explained by the many variables influencing energy expenditure during and after critical illness, including specifics of the underlying disease and its treatment, nutritional status and (in)activity (7). Consequently, when guiding caloric nutrition in the individual patient, we advise indirect calorimetry measurements to be repeated every threedays and whenever a patient's clinical condition or treatment changes significantly.

Considerations and limitations

This study describes the highest number of indirect calorimetry measurements in post-ICU patients to date. We performed most of the indirect calorimetry measurements per protocol despite the difficulties mentioned above regarding achievability. Nevertheless, several limitations must be considered when interpreting these data. To our knowledge, no prior prospective studies with continuous indirect calorimetry measurements post-ICU discharge were available upon which to base a formal power calculation. Instead, we chose to match the number of tests in only previous study (13) that performed post-ICU indirect calorimetry measurements in our prospective design. This meant we continued inclusions until 23 patients had at least one ward measurement. Future studies should include a power calculation now that more data has become available. The rate of patient inclusion was relatively slow. It took eight months to reach our intended sample size, as we were able to include just over a third of the eligible patients (Flow chart Supplemental Figure 1). Reasons for exclusion varied, but were not collected for each patient, which we would recommend for future studies. A PEEP of >12 mmHg upon ICU admission was set as an exclusion criterion according to our local protocol, to minimize the risk of air leaks which

can theoretically compromise the reliability of indirect calorimetry measurements. Based on the specifications of the Q-NRG device, future studies can likely omit this precaution, increasing inclusion rates by limiting the exclusion criteria.

After inclusion, indirect calorimetry measurements could not always be performed according to schedule for various reasons. Although an attempt was always made to reschedule measurements within 24 h, this still meant that intervals between measurements were not exactly the same in and between patients, especially in the ward (Table 3). Furthermore, the number of measurements decreased over time as patients were discharged, declined further participation or deceased. This led to an increasing confidence interval, complicating the interpretation of the variance. Based on our findings, future studies will be better able to incorporate the expected dropout into their intended sample size. The ward measurements were converted into a grand mean to facilitate comparisons while adjusting for variations in timing and the low number of measurements at later time points,. This is similar to the study by Ridley (13). Nevertheless, we have to consider the possibility that the patients who were discharged to the ward but did not have any ward indirect calorimetry measurements were sicker and, therefore, would have had a different metabolic profile than those in a good enough condition to continue the study. Although patients who did have ward measurements were not different from patients who did not have ward measurements at baseline (Table 1), their course of illness in the ICU likely did vary, as illustrated by the fact that ICU mortality was 25% in the total cohort. However, our numbers were too low to attempt multivariate comparisons between subgroups within this relatively heterogeneous group of patients, such as between survivors and nonsurvivors. This provides an important angle for future research.

Furthermore, combining measurements into a grand mean precludes the deduction of changes in the course of REE during ward stay and between-subject variations. Future studies can better address the issues by incorporating our achievability findings.

We aimed to provide an overview of the course of energy expenditure during different phases of critical illness. ICU discharge to the general ward was considered an important event in the timeline, as it indicates a clinical state that no longer requires continuous organ support and monitoring. However, we recognize that the timing of ICU discharge can depend on more factors than solely the stage of illness, such as the burden of care and even ICU and ward capacity and may, therefore not be the optimum indicator of the next phase of illness. Other indicators could be explored in the future, such as biochemical inflammation markers or clinical scores such as SOFA. Similarly, stages of illness are not always linear, as clinical events such as superinfections and aspiration may cause patients to regress to a more severely ill state after previous improvement. In the present study, great care was taken to ensure that qualified personnel took all measurements under optimal,

steady-state conditions. However, the size of our population did not enable within-subject comparisons of REE to ensure that all variations in REE were physiologically plausible. More extensive prospective studies should be designed to consider covariates that are known to influence REE such as medication, temperature and nutrition, providing an even clearer perspective on the metabolic developments in critical illness and convalescence.

The use of the canopy for post-extubation measurements in ICU patients that our design required was new, labour-intensive and had not yet proven its achievability prior to our study. To ensure a reasonable inclusion rate, relatively broad inclusion criteria were chosen. Now that experience has been obtained with this study design, we can plan to undertake future studies focusing on more specific ICU patient groups. The study protocol was prospectively registered in the Netherlands Trial Register (number NL8907). However, the projected study size was mistakenly entered as 30, even though the protocol that was approved by the ethical committee read 24, as intended. This could not be amended upon discovery, as the Netherlands Trial Register has since been discontinued and no longer allows alterations to previously registered forms.

Conclusion

Critically ill mechanically ventilated patients had a normal resting energy expenditure at ICU admission, which increased to >110% of the predicted REE on day four. Another increase in mean energy consumption was seen during post-ICU ward stay. To prevent underfeeding, caloric targets during and after critical illness should be individualized using repeated indirect calorimetry measurements. Nevertheless, postextubation indirect calorimetry measurements in canopy mode were proven to have significant limitations in both the ICU and the ward, which must be considered in clinical practice and future research.

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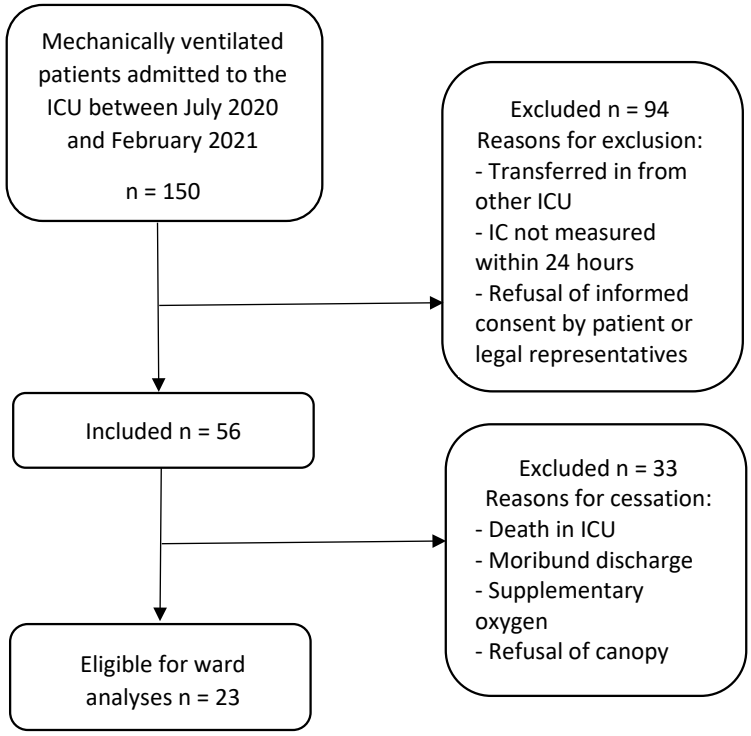


Supplemental Figure 1. Participant flow chart

Abbreviations: ICU, intensive care unit; IC, indirect calorimetry

Supplemental File 1. Local protocol for the calculation of personal energy targets in ICU patients.

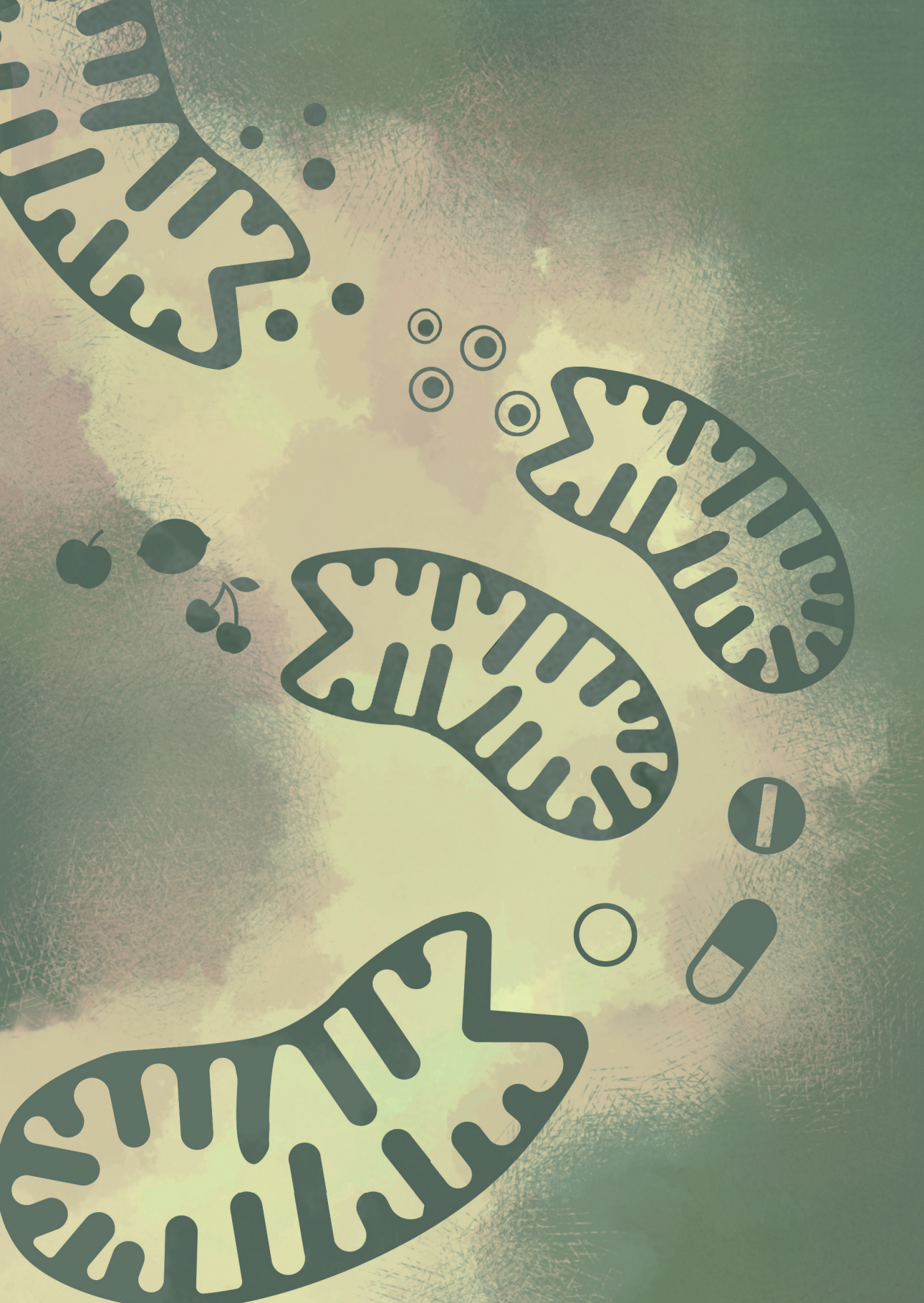
Men
18-30 years of age: 15,4 * weight (kg) – 27 * height (m) + 717
30-60 years of age: 11,3 * weight (kg) – 16 * height (m) + 901
> 60 years of age: 8,8 * weight (kg) + 1128 * height (m) - 1071

Women
18-30 years of age: 13,3 * weight (kg) + 334 * height (m) + 35
30-60 years of age: 8,7 * weight (kg) – 25 * height (m) + 865
> 60 years of age: 9,2 * weight (kg) + 637 * height (m) - 302

Energy needs during controlled ventilation
- BMI ≤ 27 REE + 20% addition
- BMI > 27-30 REE + 20% addition, recalculated for a BMI of 27
- BMI ≥ 30 60-70% of REE + 20% addition, recalculated for a BMI of 27

Spontaneous ventilation or no ventilation
- BMI ≤ 27 REE + 30% addition
- BMI > 27-30 REE + 30% addition, recalculated for a BMI of 27
- BMI ≥ 30 60-70% REE + 30% addition, recalculated for a BMI of 27

Whenever REE can be measured reliably through Indirect Calorimetry, this target is then used.



Part III

Changes in body composition during critical illness and convalescence

Chapter 6

Bioelectric impedance analysis for body composition measurement and other potential clinical applications in critical illness

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PURPOSE OF REVIEW

Insight into body composition is of great value in the ICU. Bioelectric impedance analysis (BIA) is the most applicable bedside technique. However, bioimpedance has not been validated in the critically ill, and the interpretation of the measurements poses challenges. This review discusses the potential clinical applications of BIA and explores caveats and solutions to its use in the intensive care setting.

Recent findings

A correlation is repeatedly found between raw impedance parameters, fluid ratios, overhydration, and adverse outcome of critical illness. However, cut-off and reference values remain elusive. Experience with BIA-guided fluid management in the ICU is limited. BIA-derived muscle mass appears a promising biomarker for sarcopenia, correlating well with CT-analysis. Body cell mass and fat-free mass provide potential use in estimation of metabolic rate, protein requirements and pharmacokinetics. Several methods of reducing bias in BIA parameters in critical illness require validation.

Summary

There are currently too many uncertainties and discrepancies regarding interpretation of bioimpedance in critical illness, to justify therapeutic consequences. However, there are several promising areas of research, concerning some of the most urgent clinical problems in intensive care, emphasizing the need to evaluate further the use and interpretation of bioimpedance in the intensive care setting.

Key points

- Knowledge of real-time body composition has a strong potential for prognostication, personalized nutrition, fluid therapy, and medication management.
- Regular body composition measurements are feasible in the intensive care setting with bioelectrical impedance analysis.
- Raw parameters and water parameters from multifrequency bioelectric impedance devices appear reliably interpretable in the critically ill population.
- Derived bioelectric impedance body composition parameters rely on body water distribution assumptions, which might not be valid in the critically ill population.
- There are currently no validated reference values for the critically ill population regarding derived bioelectric impedance body composition parameters.

INTRODUCTION

Body composition describes the relative contribution of fat, muscle, bone and water to an individual's body volume. In the ICU, real-time knowledge of body composition is advantageous to the individualization and optimization of fluid balances, nutrition regimes and medication dosing. Several body composition techniques are available, based on assumptions of weight (hydrostatic weighing), water content (isotope dilution), volume (air displacement plethysmography), energy attenuation (Dual-Energy X-Ray Absorptiometry; DXA), and imaging techniques like computer tomography (CT) and MRI. Although extensively validated, all techniques have limitations when applied during critical illness, because of costs, impracticality or radiation exposure.

Bioelectric impedance analysis (BIA) is quick, noninvasive and relatively inexpensive, making it ideal for bedside use. However, BIA assumes static ratios, most notably a fixed hydration of tissues, which often do not apply to critically ill patients, making interpretation less straightforward. Nevertheless, it is worth exploring potential applications, as BIA currently seems the most feasible body composition measurement technique in the ICU.

Angles for future research will be indicated throughout this manuscript with an asterix (∗) and are summarized in Table 1.

PRINCIPLES OF BIOIMPEDANCE ANALYSIS

Impedance is the vector analysis of resistance, the opposition to flow of a current, and reactance, the opposition to a current change because of a material's capacitance. When an electrical current is sent through the body, tissues present varying resistance levels. Electrolyte-rich body water is highly conductive; therefore, muscles, having a higher water content, will encounter less resistance than relatively anhydrous tissues, such as fat. Conversely, reactance increases proportionally to cell numbers and their integrity, because of membrane capacitance.

Single-frequency BIA devices (SF-BIA) use a single frequency (usually 50 kHz) to measure impedance. However, low-frequency currents will not penetrate cell membranes, and thus will only measure extracellular water (ECW) impedance. Total body water (TBW) is then estimated through proportional equations. High-frequency currents will go through cells. This impedance reflects combined ICW and ECW : TBW (Figure 1).

Table 1. Suggestions for future research angles concerning bioelectric impedance analysis in critical care

Caveats *	Evidence
Input parameters such as body height and weight are difficult to measure accurately in the ICU setting	Proxy measurements, such as ulna length, can be used to estimate height (40).
	Ideal Body Weight (IBW) cannot replace measured body weight as BIA input parameter (49).
	The Biasioli equation to calculate Total Body Weight (TBW) is based upon height but not weight and can be used to avoid the need for weighing (9;50).
BIA is not validated in patients undergoing large and swift hydration shifts	Changes in TBW determine changes in Phase Angle (PhA) during ICU days 1-3, suggesting that overhydration (OH) significantly influences PhA (50).
	BIA might be most reliable at ICU admission (before fluid resuscitation) or after ICU discharge when hydration status has stabilized (14).
	Altered BIA raw parameters due to hydration shifts do not devaluate their prognostic value (50).
Overhydration distorts the normal distribution of water in the intra- and extracellular space that is used to obtain derived BIA parameters and BIVA	A decline in PhA is related to the hydration score (TBW/FFM x 100%), while Body Cell Mass (BCM) and muscle mass (MM) decrease, suggesting that OH is mainly related to the extracellular compartment (50).
	Decrease of MM might be underestimated, as in case of muscle edema, FFM estimates might overestimate MM, as a constant FFM hydration of 0.73 is usually assumed (33;41;51). This is likely less problematic with (multifrequency) MF-BIA or BIS, where TBW can be measured (4).
	Interstitial edema is interpreted by BIVA as a state of OH, even if there is a state of relative intravascular hypovolemia (24).
	Derived values might be recalculated to a normalized ECW/TBW-ratio, analogous to dialysis BIA-software (20).
Ascites, pleural effusion and urine retention theoretically influence BIA-measurements	Segmental BIA can distinguish apparent trunk OH due to peritoneal dialysate, without influencing the extremities' measurements (52).
	In cirrhosis patients, PhA is positively correlated with CT-derived MM, irrespective of ascites' presence (53).
Changing tissue electrolyte concentrations might influence raw BIA parameters	In chronic kidney disease patients, a 20% increase in Na+ as measured by 23Na-MRI, leads BIS to overestimate ECW by 1.2-2.4 liters, due to lower extracellular resistance (54).
Fever might influence BIA measurements by reducing ECW	In ambulant Influenza persons, individuals (T ≥ 37.1°C) show a tendency toward greater reactance and PhA than afebrile individuals (55).
BIA could interfere with electrical implants, leading manufacturers to advise against use whenever one is present	Multiple studies show that BIA could be safely performed in patients with ICDs (56-59). The same has not been researched for other electrical implants.

BIA, bioelectric impedance analysis; BIVA, bioelectrical impedance vector analysis; FFM, fat-free mass; ICD, implantable cardioverter defibrillator.

Multifrequency BIA devices (MF-BIA), therefore, provide a more direct portrayal of water compartments, making them more reliable in case of altered hydration status or electrolyte imbalances. Bioimpedance spectroscopy (BIS) applies a more extensive frequency range than MF-BIA. The increase in information obtained from BIS potentially improves predictive power. However, it still requires extrapolation based on population references. Superiority of BIS to the SF-BIA and MF-BIA techniques has not been proven in nonhealthy populations (1–3,4).

The phase angle (PhA) shows the relationship between reactance and resistance (Figure2).

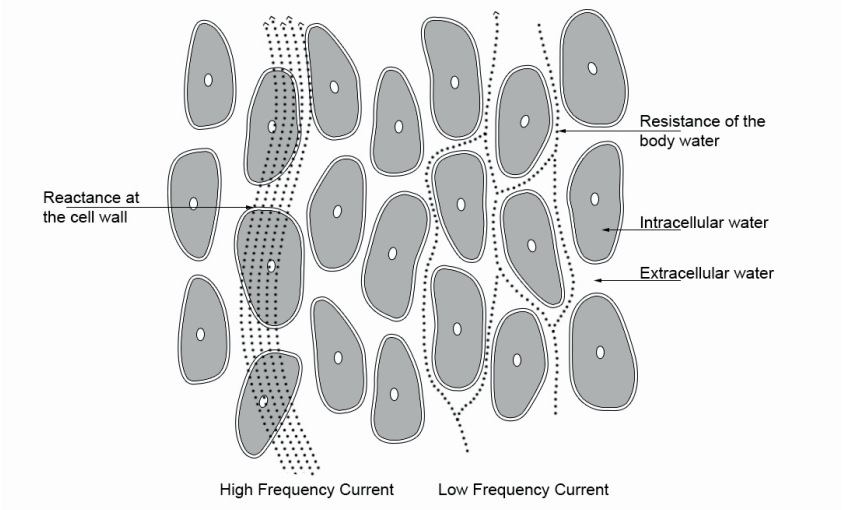

Figure 1. Low-frequency currents will not penetrate cell membranes, and as such will measure extracellular water impedance. High-frequency currents will go through cells, at which point the impedance reflects total body water (TBW).

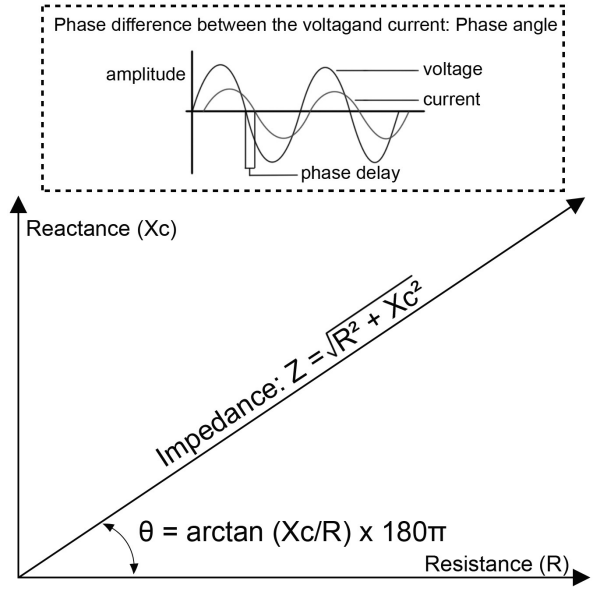

Figure 2. When an electric current passes a cell membrane, reactance causes a time delay, creating a phase shift between voltage and current. The phase angle describes this difference between the voltage and the current. A high-phase angle is, therefore, consistent with large quantities of intact cell membranes and body cell mass.

The greater the number of cell membranes the signal has to pass through, the greater the reactance, and therefore, the PhA. Thus, a large PhA is consistent with a large body cell mass (BCM) relative to ECW, as seen in healthy individuals, whereas ICU patients tend to have a lower PhA. A PhA greater than 6 is assumed normal in health, although PhA varies with sex (men↑) and age (↓because of loss of fat-free mass; FFM), and should ideally be related to a reference population, or converted to standardized PhA (SPhA) before comparing across populations (5,6). PhA measured at 50 kHz is most frequently used, and most reference data are available for this frequency, as this is the frequency at which both resistance and maximum reactance are best measured (7,8).

Bio-electrical impedance vector analysis (BIVA) represents impedance as a vector of reactance and resistance in an x-y plot referring to reference population's tolerance ellipses (Figure3).

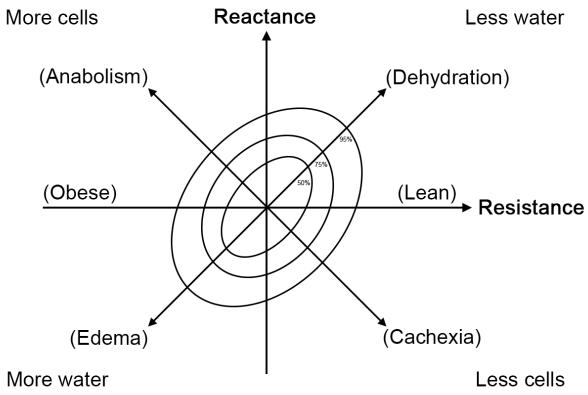

Figure 3. Bioelectric impedance vector analysis relates the length and direction of the phase angle to that of a reference population, enabling a visual interpretation of the clinical relevance of the raw bioelectric impedance analysis values.

BIVA allows simultaneous interpretation of direction (phase) and length of the impedance vector; through which changes in tissue hydration and BCM can be appreciated, independent of regression equations, or body water.

Derived parameters

Reactance, resistance, impedance and PhA, are often referred to as 'raw' BIA parameters, that is, not reliant upon empirical modeling. BIA defines the water volumes using impedance and body height, upon which other body composition parameters are based. Earlier BIA devices regarded the body as one cylinder and extrapolated impedance measured on one side of the body. However, this simplification overlooks possible asymmetry and the proportional difference between the trunk and the limbs. Segmental BIA (SM-BIA) devices consider the body as five separate cylinders and use electrodes on all limbs, improving accuracy (Figure 4).

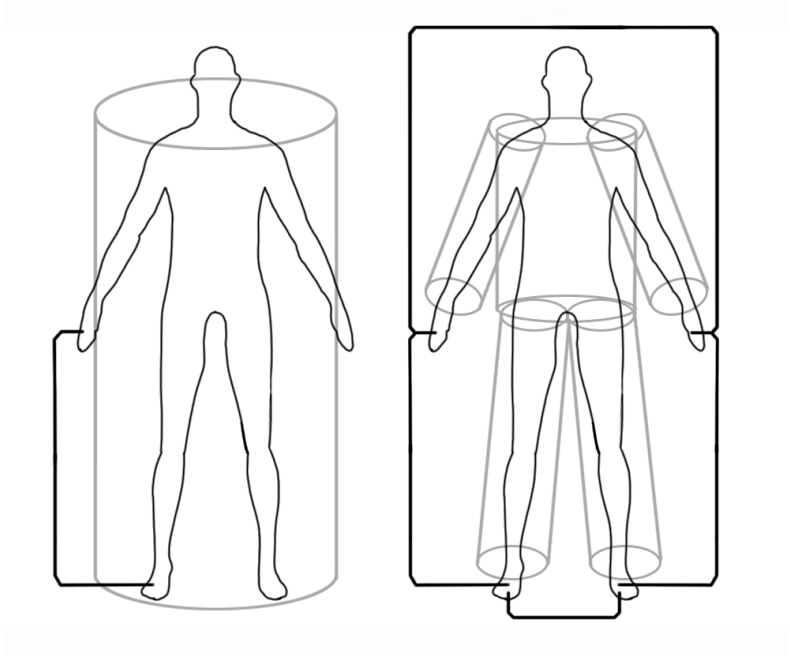

Figure 4. Earlier bioelectric impedance analysis devices regarded the body as one cylinder, calculating body water volumes based on whole-body impedance and body height. Segmental BIA devices consider the body as five separate cylinders and use electrodes on all limbs, improving accuracy. BIA, bioelectric impedance analysis.

Various body composition parameters are derived from thereon, using regression analyses with multiple variables obtained through reference measurements. Figure 5 provides an overview of the relationship between several frequently used parameters. SM-BIA can provide additional values, such as the appendicular skeletal muscle mass (ASMM), the sum of the four limbs' muscle masses.

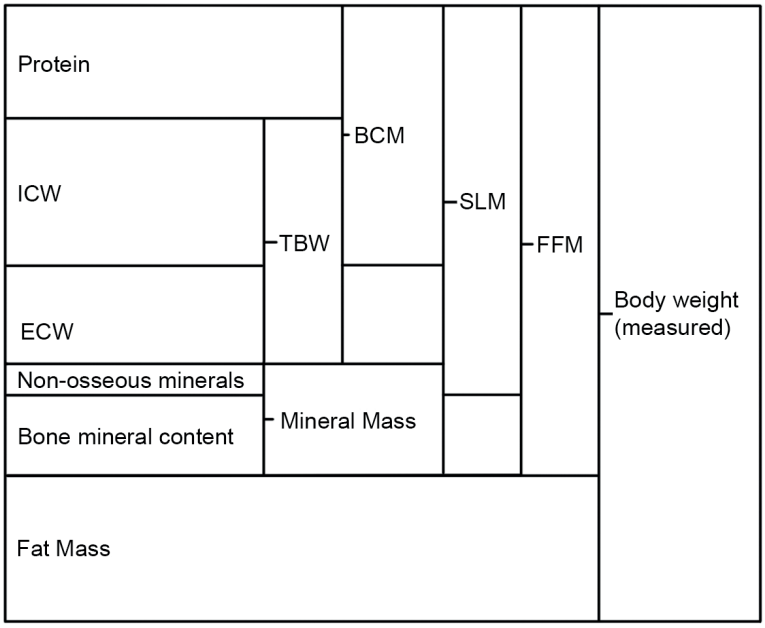

Figure 5. Overview of the relationship between several frequently used derived body composition parameters, based on a multicompartment body composition model. Definitions may vary slightly between sources and device manufacturers.

OUTCOME PREDICTION WITH BIOELECTRIC IMPEDANCE ANALYSIS

Several raw and BIA-derived body composition parameters have been validated as mortality and morbidity predictors in various patient groups and are now being researched as predictors of critical illness outcome (9,10).

Raw Parameters

Diminished cell count, membrane integrity and altered hydration status in critical illness can lead to changes in reactance and resistance, thereby decreasing PhA compared with healthy individuals (11,12). Decreased PhA at ICU admission has been associated with hospital, 28-day, 90-day and 12-month mortality (6,13–16). Concordantly, PhA improved over the first 5 days of ICU stay in ICU survivors, while decreasing significantly in nonsurvivors (17). Furthermore, negative correlations have been observed between admission PhA and the length of ward stay, ICU stay and hospital stay, mechanical ventilation duration the APACHE-II score, and recently with the severity of disease of coronavirus disease 2019 (COVID-19) (15,16,18–20).

However, the cut-off values for the predictive value of PhA vary across these studies∗. The heterogeneity of the ICU populations studied might in part explain these discrepancies. A study comparing sepsis patients with other critically ill patients found that PhA was negatively correlated with the APACHE-II score only in the nonsepsis group (21). Additionally, in addition to the acute changes because of the current illness, PhA inherently also reflects poor underlying health, muscle wasting and frailty, which are independently associated with outcome.

One study using Segmental Multifrequency BIA (SMMF-BIA) found that impedance, reactance and PhA showed more predictive power for mortality than the SAPS, APACHE-II and SOFA severity scoring systems. Similarly, the landmark Phase Angle Project showed that a combined multivariable score improved the discriminative power in predicting mortality, compared with PhA alone (22)∗.

Hydration parameters

Overhydration in ICU patients is positively correlated with adverse outcomes but current methods to assess volume status (in-bed weighing, cumulative fluid balance (CFB), central venous pressure) have their limitations. Marked BIVA-OH on the first 5 days after ICU admission was shown in ICU and 60-day nonsurvivors (19,23,24). Notably, BIVA predicted mortality better than CFB (23).

Fluid distribution can also be assessed by BIA-derived ECW/TBW ratio. A healthy ECW/TBW ratio varies slightly between sources and device manufacturers but ranges from 0.36 to 0.40. An ECW/TBW ratio of more than 0.40 is considered indicative of overhydration of the extracellular compartment∗. ECW/TBW-ratio is higher among ICU nonsurvivors and correlates with a longer mechanical ventilation duration (25). Slobod et al. found that a SF-BIA ECW/TBW-ratio greater than 0.39 on ICU-day 1, associated with an increased number of ventilation days, independent of the APACHE-II score (26). In CRRT ICU patients, a cut-off for SMMF-BIA ECW/TBW-ratio of 0.413 predicted 28-day mortality, with 71.4% sensitivity and 70.6% specificity (27).

On the basis of the assumption that excess volume accumulates primarily as ECW, the quantity of overhydration can be calculated as the difference between expected ECW, based on the euvolemic ECW/TBW ratio, and the measured ECW (28,29). On ICU days 1 and 3, BIS-OH (>1l) associated significantly with hospital mortality in 140 ICU patients with 23 nonsurvivors. Day 3 volume status correlated with the duration of ventilation and ICU stay. More ICU-free and ventilator-free days were observed among patients with normal hydration status on day 3 (OH <1 to 1 l) (30). We showed increased SMMF-BIA-OH, and ECW/TBW ratio were associated with mortality in COVID-19 (20).

Muscle mass

Determining muscle mass is essential in distinguishing the sarcopenic, from the nonsarcopenic obese, as the former are at higher risk of adverse outcome in the ICU. Furthermore, rapid wasting of muscle mass is a major clinical conundrum, as it is a strong independent predictor for morbidity, mortality, physical functioning and quality of life. PhA is often considered a proxy for LBM. Indeed, two studies found that low BIA/BIS-PhA corresponded to low CT- muscle mass (CT-MM) and muscle density in the critically ill (31,32). Additionally, BIA provides several derived muscle parameters, including FFM, soft lean mass (SLM), LBM, SMM, SMM index (SMI) and segmental values. Two groups studied agreement between CT-MM and BIA-SMM in the ICU. One used the SMM automatically generated by the SMMF-BIA software, and found a high correlation, regardless of patients' sex, or edema status (33). Another group calculated SMM, ASMM and total muscle mass based on raw SF-BIA measurements, using three different equations, and found that although the BIA and CT measurements correlated significantly, the agreement was low, with increasing overestimation of muscle mass by BIA at higher CT-MM. However, BIA did correctly identify patients with low CT-MM (31). Therefore, BIA might be clinically useful to identify sarcopenic patients at risk for adverse outcome. However, there was a time difference between the BIA and CT evaluation in these studies, potentially inducing bias. Furthermore, increased muscle mass in ICU patients should not be interpreted as muscle mass of good quality, as intramuscular edema will be classified as muscle mass by both BIA and CT analysis. However, a recent pilot-study comparing CT-MM at ICU admission and BIS-FFM adjusted for overhydration, using an algorithm developed for dialysis patients, found significant correlations and good agreements between the two techniques (32)∗. The unadjusted BIS-FFM correlated with CT-MM but performed poorly in classifying muscularity status (32).

NUTRITION MANAGEMENT

Critically ill patients are at increased risk of malnutrition. Several BIA parameters can potentially provide information on nutrition status and requirements.

Body cell mass

BCM is the metabolically active part of FFM, in contrast to bone and ECW. As such, a decrease of BCM resulting from critical illness is a marker for malnutrition. Logically, increased ECW is associated with a lower BCM/FFM-ratio. A study comparing BIA measurements before and after hemodialysis in AKI patients (mean weight loss 3.8 kg), suggested hydration shifts have little effect on the BCM measurement, theoretically making it more reliable in critically ill patients (34,35)∗.

Raw parameters

PhA inherently reflects BCM. In 89 ICU patients, a PhA less than less than 5.5° showed an accuracy of 79% in identifying patients at high nutrition risk (NUTRIC score ≥5) (19). In renal replacement therapy patients, a PhA cut-off of 4.6° has been shown to predict malnutrition, defined by protein-energy wasting (36,37). A study comparing the accuracy of BIVA, versus the definition according to ESPEN in hospitalized patients, in predicting malnutrition, found that BIVA might be the superior method (38).

Fat-free mass

Assessment of muscularity by BIA is recommended by the Global Leadership Initiative on Malnutrition (GLIM) (39). A prospective study among 60 ventilated ICU patients found that a cumulative energy-deficit during ICU stay was independently associated with loss of BIS-FFM between inclusion and ICU discharge, as well as with ICU-acquired weakness (40). In a retrospective post hoc analysis, including this study, these associations disappeared (41). However, raw parameters remained related to muscle weakness (41)∗.

FFM is closely related to energy expenditure, and some BIA devices offer options to estimate basal metabolic rate (BMR), using FFM-based equations (e.g. Cunningham, or Katch-McArdle). However, based upon derived FFM, these calculations are subject to caveats and have proven to be inferior to indirect calorimetry in several populations, albeit still more accurate than weight-based equations (42,43).

Potentially, BIA-FFM could facilitate targeted protein dosing. Protein targets are usually set to measured actual weight or calculated FFM∗. However, these methods do not incorporate changes in body composition and weight gain because of overhydration, as such masking the decrease of FFM during ICU stay.

FLUID MANAGEMENT

BIA is commonly used in dialysis patients to guide fluid management by calculating dry weight goals (11,44). Likewise, in critical illness PhA, ECW/TBW ratio and overhydration could be used to monitor the effect of fluid management strategies. A prospective, clinician-blinded study was conducted to assess the feasibility and validity of BIVA as a measure of hydration in critically ill patients. The study showed that clinicians blinded to the BIVA results, achieved a mean CFB that was concordant with the prior BIVA classification (i.e. positive for patients' BIVA classified as dehydrated, negative for overhydrated patients and neutral for normally hydrated patients), proving feasibility (45). Moreover, directional BIVA changes correlated with directional changes in fluid balance. However, the study showed that vector length increased in parallel with 2.4 L fluid loss, suggesting BIVA might

be insensitive to smaller changes (45). The effect of BIVA/BIA-guided fluid management on patient-centered outcomes has not yet been researched∗.

GLOMERULAR FILTRATION RATE AND PHARMACOKINETICS

Adequate dosing of renally excreted drugs is challenging in critically ill patients because of changes in kidney function. Most equations to estimate glomerular filtration rate are based on serum creatinine measurement. However, significant limitations arise when these formulas are applied to patients with altered body composition, like low muscle mass (9,10–13). A Dutch group recently developed and validated a formula to predict creatinine/urea clearance based on 24 h urine collection (currently the gold-standard in ICU) using serum creatinine and MF-BIA-BCM and ECW/TBW ratio, with good results (46)∗.

BIA also provides interesting theoretical ways for pharmacokinetic characterization and medication dosing through real-time appreciation of the changing body composition and volumes of distribution (47). However, no recent attempts for predictive pharmacokinetic models using BIA in the ICU have been published∗.

CAVEATS

One main drawback of BIA is the incorporation of reference population values, for all but the raw parameters, which might not apply to the individual patient or population. Although they are validated against standard methods (usually MRI and DXA), the exact equations used by BIA software are rarely released by manufacturers, impairing judgment of applicability (48)∗. Several other caveats impair routine use of BIA in the ICU, such as use of inexact input parameters, the lack of ICU reference and cut-off values, and the possible bias introduced by a rapidly changing clinical status. Evidence regarding other considerations to use and interpretation of BIA in the ICU setting are summarized in Table 2.

Table 2. Caveats to the use and interpretation of bioelectric impedance analysis with potential clinical relevance to the ICU setting*

Subject	Research Angle
Internal validity	Influence of overhydration and rapid hydration shifts on BIA measurements
	Influence of overhydration and rapid hydration shifts on predictive value of BIA parameters
	Influence of body temperature on BIA measurements
	Influence of osmotic shifts on BIA measurements
External validity	Reference values for BIA measurements in (subgroups of) critically ill patients
	Cut-off values for outcome predictive qualities of BIA measurements in (subgroups of) critically ill patients
	Validation of overhydration adjustment of derived parameters in (subgroups of) critically ill patients
Safety	Possible interference of BIA electrical current with electrical implants other than internal ICDs
Clinical use	Development and validation of predictive scoring systems including raw BIA parameters for (subgroups of) critically ill patients
	Assessment of predictive qualities of BIA measurements for malnutrition
	Development and validation of BIA-derived metabolic rate equations with gold-standard methods
	External validation of method to predict glomerular filtration rate based on BIA-derived body cell mass (BIA-eGFR)
	Pharmacokinetic models using BIA-eGFR and effect on outcome parameters
	Pharmacokinetic models using BIA-derived body composition and effect on outcome parameters
	Development and validation of equation for protein dosing to BIA-FFM and effect on outcome parameters
	Exploring options to calculate derived BIA parameters omitting body weight and possibly height
	Effect of BIVA/BIA-guided fluid management on ICU patient-centered outcomes

Abbreviations: BIA, bioelectric impedance analysis; BIVA, bioelectrical impedance vector analysis; ECW, extracellular water; FFM, fat-free mass; ICD, implantable cardioverter defibrillator; OH, overhydration.

CONCLUSION

There are several promising areas of BIA research concerning some of the most urgent clinical problems in intensive care. A correlation is repeatedly found between raw impedance parameter, fluid ratios, overhydration and adverse outcomes in critical illness. BIA-derived muscle mass appears a promising biomarker for sarcopenia, as it correlates well with CT-analysis. BCM and fat-free mass provide potential use in estimation of metabolic rate, glomerular filtration rate, protein needs and pharmacokinetics. Contrastingly, experience with BIA-guided fluid management is still limited and suggested methods of reducing bias in BIA-measurements in the critically ill require validation. There

are currently too many uncertainties and discrepancies regarding the interpretation of BIA measurements in critical illness to justify large therapeutic consequences, emphasizing the need for further evaluation of the use and interpretation of bioelectric impedance in the ICU setting.

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Chapter 7

Association of bioelectric impedance analysis body composition and disease severity in COVID-19 hospital ward and ICU patients: The BIAC-19 study

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ABSTRACT

Background

The current severe acute respiratory syndrome coronavirus 2 pandemic is unprecedented in its impact. It is essential to shed light on patient characteristics that predispose to a more severe disease course. Obesity, defined as a BMI>30 kg/m^2, is suggested to be one of these characteristics. However, BMI does not differentiate between fat mass and lean body mass, or the distribution of fat tissue. The aim of the present study was to assess the body composition of COVID-19 patients admitted to the ward or the ICU and identify any associations with severity of disease.

Methods

We performed an observational cross-sectional cohort study. Bioelectric impedance analysis was conducted amongst all confirmed COVID-19 patients admitted to the ward or ICU of our hospital in the Netherlands, between April 10 and 17, 2020. Body water measurements and derived values were recalculated to dry weight, using a standard ratio of extracellular water to total body water of 0.38. Data were compared between the ward and ICU patients, and regression models were used to assess the associations between baseline characteristics, body composition, and several indicators of disease severity, including a composite score composed of mortality, morbidity, and ICU admission.

Results

Fifty-four patients were included, of which 30 in the ward and 24 in the ICU. The mean age was 67 years (95%-CI 64–71), and 34 (63%) were male. Mean BMI was 29.7 (95%-CI 28.2–31.1) kg/m^2 and did not differ between groups. Body composition values were not independently associated with disease severity. In multiple logistic regression analyses, a low phase angle was associated with COVID-19 severity in the composite score (OR 0.299, p = 0.046).

Conclusion

We found no significant associations between body composition, including fat mass, visceral fat area, and fat-free mass, and disease severity in our population of generally overweight COVID-19 patients. A lower phase angle did increase the odds of severe COVID-19. We believe that factors other than body composition play a more critical role in the development of severe COVID-19.

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2, SARS-CoV-2, has rapidly spread across countries and continents since its first appearance in late 2019, causing high incidences of Corona Virus Disease-2019 (COVID-19). Rates of intensive care (ICU) admission are 10.9% among hospitalized COVID-19 patients (1). ICU patients with severe COVID-19 typically receive prolonged invasive mechanical ventilation with a reported median of 18 days and a case-fatality rate up to 49% (2,3). Studies describing characteristics of COVID-19 patients requiring hospitalization or ICU admission show these factors correlate with advanced age, male sex, high body mass index (BMI), and several obesity-related comorbidities (4, 5, 6, 7). Among COVID-19 ICU patients, obesity is frequently encountered. Increased disease severity with increasing BMI has been shown (8). High BMI is a risk factor for hospitalization in persons <60 years of age, a group otherwise less severely affected (9). Parallels can be drawn with other viral infections, such as H1N1 influenza, that disproportionally impact obese individuals (6,10,11).

A hypothesis is that obese individuals have decreased respiratory volumes and lung compliance due to abdominal obesity (6,10,11). However, adipose tissue may also serve as a reservoir for SARS-CoV-2, predisposing obese individuals to a higher or more persistent viral load (12, 13, 14, 15). As men are more often and more severely affected by COVID-19 compared with women, despite a lower incidence of obesity, it could be hypothesized that not merely fat percentage, but the location of fat tissue is essential, as men are more prone to visceral fat accumulation (16). A possible parallel is found in hepatitis C, where visceral obesity is associated with a higher viral load (17).

Addressing which factors influence susceptibility to a severe course of COVID-19 is essential to aid in prevention, earlier treatment, and organization of health care. As such, BMI as a distinguishing factor is inadequate, as it does not differentiate between different tissues, nor the fat tissue distribution. Several studies have demonstrated that high BMI was associated with lower mortality in critically ill, pneumonia, and Acute Respiratory Distress Syndrome (ARDS) patients (18, 19, 20, 21). These findings could be explained by the lack of discriminatory power of BMI to differentiate between body fat and lean mass and the higher absolute amount of lean mass in non-sarcopenic obesity, compared with individuals with an ideal-weight BMI (22). Indeed, in critically ill patients, it has been shown that BMI is not an independent predictor of mortality when corrected for muscle area (23).

Bioelectrical impedance analysis (BIA) is a validated, non-invasive method for assessing body composition. It measures the opposition to an alternating current passing through body compartments (resistance) and the delay in conduction by membranes (reactance).

BIA uses these measurements to estimate the contribution of various tissues to the (segmental) body weight accurately and provides markers for cellular integrity (phase angle). BIA is not yet widely implemented in the ICU, partly because the interpretation of some results is complicated in case of altered hydration status, as is common amongst the critically ill (24). However, methods to calculate dry body weight values have been described for dialysis patients, where fluid overload is prevalent (25,26). The ratio between extracellular and total body water, as an indicator of hydration status, is easy to use, intuitive and validated as a predictor of survival in these patients (27,28). This provides a theoretical justification to apply this technique to ICU patients.

In this study, we aimed to measure BIA body composition amongst COVID-19 patients in the ward and the ICU to uncover associations between body composition and the course of the disease.

METHODS

Study setting

This cross-sectional observational cohort study was performed at Gelderse Vallei Hospital, a University-affiliated teaching hospital in Ede, The Netherlands. The hospital has two ICU units, with 12 and 5 beds, respectively. During the COVID-19 pandemic two additional ICU units were opened in the operating theatre, adding to a total of 29 ICU beds. Between April 10 and 17, 55 SARS-CoV-2 patients were admitted in our hospital. Nationally, on April 13th 2020, a total of 26.551 people had tested positive for SARS-CoV-2, of whom 8729 had been hospitalized, and 2351 had been admitted to ICU (29, 30). Up until that day a cumulative total of 2823 SARS-CoV-2 positive people had died, of whom 494 in ICU (30). At the time of the study, the hospital did not participate in any COVID-19 related clinical trials.

Study design and participants

All adult patients (aged ≥18 years) with SARS-CoV-2 infection, confirmed by real-time reverse transcriptase-polymerase chain reaction assay (RT-PCR) of nasal and pharyngeal swabs, or strong clinical suspicion of COVID-19 in addition to radiological CORADS classification score ≥3, in spite of negative initial RT- PCR (conform standard practice in the Netherlands at that time), were eligible for inclusion. Exclusion criteria were pregnancy, presence of electrical implants such as pacemakers or implantable cardioverter defibrillators, wounds or skin damage at the designated electrode sites, or inability to maintain posture during the measurement (i.e., 5 min). The institution's ethics board approved this study. Written informed consent was obtained from all patients or their legal representatives. The study was registered in the Netherlands Trial Register (number NL8562).

BIA measurements

BIA measurements were conducted by a trained researcher between April 10–17th 2020. Body composition measurements were performed with the InBody S10® (InBody Co., Ltd., Seoul, Korea). This multi-frequency, segmental impedance analyzer requires height, weight, and sex as input parameters. The number of days from hospital admission to BIA measurement was recorded. The most recent measured weight was used. In the ICU, all patients are weighed daily on a bed with incorporated weighing scale. Ward patients are weighed upon admission and from thereon daily when feasible. For BIA measurements, the most recent weight was used. Height as recorded upon hospital admission was used. Measurements were performed in a seated or supine position with reusable electrodes attached to the left and right thumb and index finger and both ankles. The measurements typically took 3–5 min.

The InBody S10 uses segmental impedance and reactance at multiple frequencies to determine total body water (TBW), (segmental) extracellular water (ECW), and the individual ECW/TBW-ratio (Figure 1). High-frequency currents pass through the TBW, whereas low-frequency currents cannot penetrate cell membranes and flow exclusively through the ECW. Henceforth, it uses validated methods to estimate fat-free mass (FFM), (segmental) soft lean mass (SLM), mineral mass, bone mineral content (BMC), percentage body fat (PBF), visceral fat area (VFA), (segmental) skeletal muscle mass (SMM) and protein mass, in addition to several ratios. Furthermore, individual reference ranges based on sex, age, length, and weight for some values, based on ideal body composition, are provided. In addition, a 50 kHz phase angle (PA) is deduced. PA shows the relationship between reactance and resistance and is regarded a biological marker of cellular health. In health, high cell mass volume and robust cell membranes cause delayed signals and thereby a higher phase angle, whereas ICU patients tend to have a low phase angle (≤5°).

To correct for iatrogenic over- or dehydration of the extracellular compartment, leading to over- or underestimation of derived values, estimated values derived from ECW were recalculated to dry, or euhydrated weight, using a standardized ECW/TBW of 0.38 (reference value for healthy persons).

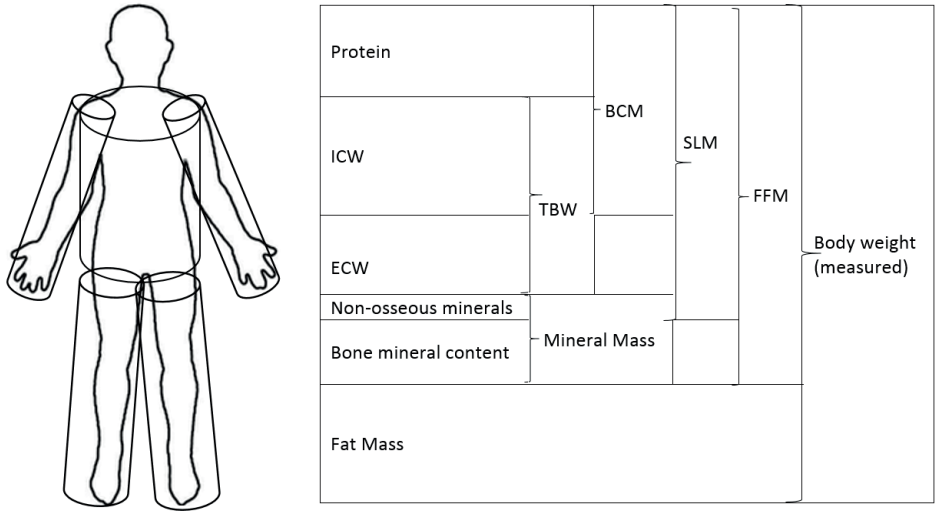

Figure 1. Body composition principles whereupon bioelectric impedance analysis derived estimations are based.

Abbreviations: ICW, intracellular water; TBW, total body water; ECW, extra-cellular water; BCM, body cell mass; SLM, soft lean mass; FFM, fat-free mass; SMM, skeletal muscle mass; SMI, skeletal muscle mass index.

Data collection

Demographic, radiological and clinical data were collected from local electronic medical record systems MetaVision® (iMDsoft, Tel Aviv, Israel) and NeoZIS® (MI Consultancy, Katwijk, The Netherlands). The recorded data included: age, sex, co-morbidities, clinical scores, laboratory results, radiological scores, limited treatment plans (LTP), and body temperature. Cumulative fluid balance (CFB) on the BIA measurement day was recorded whenever available in ICU patients. CFB is not recorded routinely in ward patients in our hospital.

The 28-day outcomes, including ICU- and hospital free days, complications, organ support free days (e.g., ventilation-free days and vasopressor-free days), and mortality were recorded. Expected complications were thrombo-embolic events, renal failure and delirium. Other complications were considered when occurring twice or more in the study population. Furthermore, total ICU and hospital length of stay (ICU LOS resp. HLOS), and hospital discharge destination were recorded.

Disease severity was defined in multiple ways. First, ICU admission for severe COVID-19 was considered. Additional parameters were 28-day mortality and complications. A composite score of ICU-admission and complications, including mortality, was created.

A score of 1 indicated that at least one of the criteria was met, while a score of 0 was assigned to those patients without ICU admission and complications. For the ICU patient group, ventilation-free days and vasopressor-free days, were considered continuous outcome measures related to disease severity.

Statistical analysis

Continuous values are reported as mean (95%-CI) for normally distributed data or median (IQR) for non-normally distributed data. Discrete data are presented as numbers (%). Differences between ICU and ward groups were assessed using independent samples t-tests for continuous data or Chi-squared tests for categorical data. When test assumptions were not met, Mann–Whitney U tests or Fisher's exact tests are used. A sensitivity analysis was performed with the same tests, excluding patients with LTP, waiving ICU admission.

Simple regression analysis was performed for associations between baseline characteristics and disease severity, and for BIA values and disease severity. For binary outcomes, binary-logistic regression was used. Continuous outcomes were univariately analyzed by Poisson regression. When assumptions for Poisson regression were not met, negative binomial regression was used. Multiple logistic regression analysis was performed for BIA values with a p-value ≤0.10 in simple regression analysis. Continuous outcomes were univariately analyzed by Poisson regression. Other covariates were age, sex, SOFA-score and time between admission and measurement.

IBM SPSS statistics 26 (IBM Corp, Armonk, NY, USA) was used for all analyses. Only two-sided analyses were used. P-values <0.05 were considered statistically significant.

RESULTS

Between April 10 and April 17, 2020, 55 patients were eligible for inclusion (Figure 2). One patient declined participation; none were excluded. Four (ward) patients (7%) were confirmed by strong clinical suspicion and CORADS ≥3 despite negative initial RT-PCR (not repeated), all others had a positive initial RT-PCR. Two patients were briefly (<24 h) admitted to the ICU and did not receive any specialized care before discharge; therefore, they were analyzed in the ward-group. In total, 54 patients (mean age 67 (95%-CI 64–71); 34 males (63%), admitted to the ward (n = 30) or ICU (n = 24) underwent BIA measurements. All the included patients were white of Western European descent. The mean BMI of all patients was 29.7 (95%-CI 28.2–31.1), with no significant difference between ward and ICU patients (Table 1). Upon hospital admission ICU patients compared with ward patients had higher SOFA scores (6(IQR 5–7) vs. 2(IQR 2–3); p < 0.001) and serum creatine kinase (CK) (n = 47;

174(IQR 117–423 vs. 97(IQR 45–139), p = 0.002). No other significant differences between groups in baseline characteristics were found.

Dry weight body composition of all patients is shown in Table 2 . No logistical or physical barriers were encountered in performing BIA measurements. Time from ICU admission to measurement was 10(IQR 3–15) days. Median SOFA score on the measurement day was significantly higher in ICU patients than in ward patients (3(IQR 1–5) resp. 1(IQR 1–2), p = 0.009). ICU patients had a mean PaO2/FiO2 ratio of 218 (95% CI 177–260) on their measurement day, eight (33%) were on vasopressors. The median CFB was 3.95 (IQR 1.60–6.47) liters on the day of measurement, as recorded in 19 ICU patients.

Body composition of ward and ICU patients did not differ significantly concerning dry TBW, fat mass, percentage body fat, and VFA. ICU patients had significantly higher FFM, SLM, SMI, and water measures, including fluid overload, ECW-ratio (ECW/TBW). The phase angle was significantly lower in the ICU group. Table 2 (31, 32, 33).

The 28-day outcome measures are summarized in Table 3 and largely favor ward patients. Thirteen of the 30 ward patients (43.3%) had LTPs. A sensitivity analysis excluding LTP patients did not yield any different findings (Supplemental Tables 1-3). All patients who died in the ward (5(16.7%)) had LTPs preventing ICU admission.

ICU patients had a median of 21(IQR 16–23) vasopressor-free days, and 13(IQR 8–17) invasive ventilation-free days, 12 patients (50%) were ventilated in the prone position. Ten (41.6%) ICU patients received insulin therapy, and one patient (4.2%) required renal replacement therapy. The HLOS for ward patients was 8(95%-CI 5–12), and 26(95%-CI 20–38) days for ICU patients ($p < 0.01$). For ICU patients, the ICU LOS was 18(95%-CI 12–32) days.

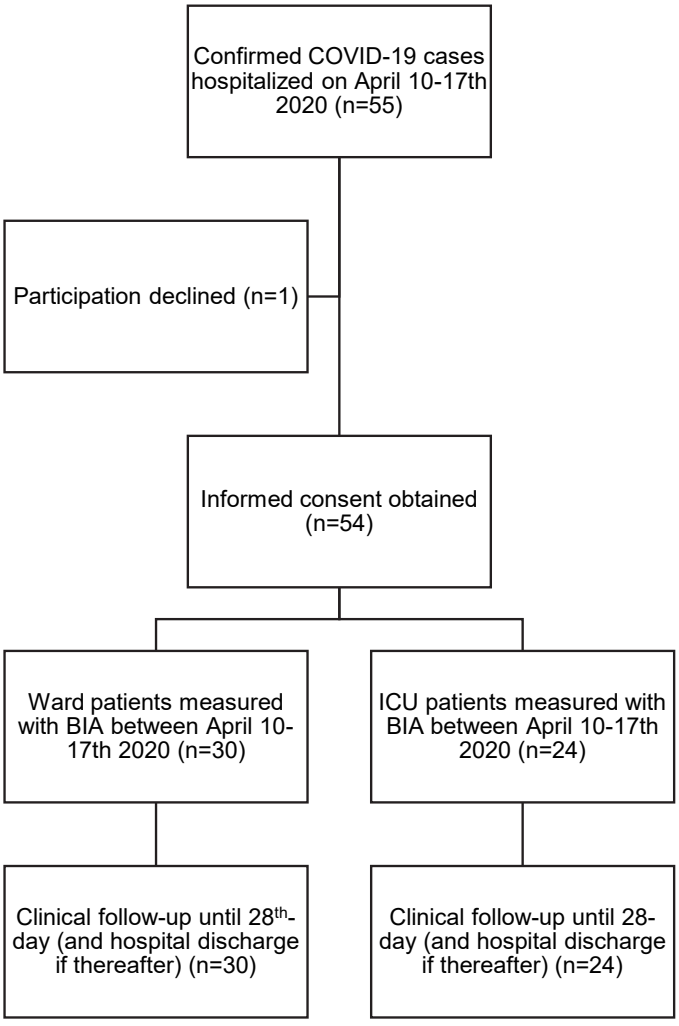

Figure 2. Study flow diagram

Table 1. Patient characteristics upon hospital admission[a].

	All Patients (n=54) [b]	Ward patients (n=30)	ICU patients (n=24)	p- value
Age, mean (95%-CI), years	67 (64 - 71)	69 (64 - 74)	66 (61 - 70)	0.268
Males, no.(%)	34 (63 %)	17 (57%)	17 (71 %)	0.284
Co- morbidities				
Diabetes, no.(%)	14 (25.9 %)	8 (26.7 %)	6 (25.0 %)	0.890
Hypertension, no.(%)	18 (33.3 %)	13 (43.3 %)	5 (20.8 %)	0.081
Chronic lung disease, no.(%)	14 (25.9 %)	9 (30.0 %)	5 (20.8 %)	0.445
Coronary artery disease, no.(%)	5 (9.3 %)	3 (10.0 %)	2 (8.3 %)	1.000
Clinical scores				
Barthel index	20 [19 – 20]	20 [18 – 20]	20 [20 – 20]	0.171
Frailty score	2 [1 – 3]	3 [1 – 5]	2 [1 – 3]	0.305
APACHE II score	12 [9 – 15]	11.5 [9 – 16]	13 [10 – 14]	0.530
SOFA score	4 [2 – 6]	2 [2 – 3]	6 [5 – 7]	**<0.001**
Laboratory results				
Hemoglobin, mmol/L (n=53)	8.8 [7.7 – 9.6]	8.8 [7.7 – 9.6]	8.4 [7.7 – 9.6]	0.929
Leukocytes, 10^9/L (n=53)	8.8 [6.3 – 11.0]	8.4 [6.3 – 10.7]	9.2 [6.2 – 11.8]	0.775
Thrombocytes, 10^9/L (n=53)	230 [183 – 314]	226 [193 – 283]	261 [166 – 320]	0.520
Ferritin, µg/L (n=12)	1300 [812 – 2208]	1021 [397 – 1998]	1470 [894 – 2410]	0.308
Triglycerides, mmol/L (n=5)	1.7 [1.1 – 2.3]	0.8 (-)	1.8 [1.4 – 2.4]	0.157
C-reactive protein,mg/L	126 [72 – 212]	106 [72 – 192]	164 [67 – 235]	0.413
Serum creatinin, µmol/L(n=52)	81 [67 – 116]	81.5 [66 – 111]	80.5 [68 – 126]	0.811
Ureum, mmol/L (n=53)	6.9 [5.4 – 10.7]	6 [5.5 – 12.1]	7.3 [5.0 – 9.8]	0.886
Creatinine Kinase, U/L (n=47)	117 [59 – 292]	97 [45 – 139]	174 [117 – 423]	**0.002**
D-dimer, mcg/ml (n=9)	3.27 [1.98 – 9.54]	9.67 (-)	2.56 [1.48 – 2.56]	0.079

Abbreviations: ICU, Intensive Care Unit; CI, confidence interval; APACHE II, Acute Physiology And Chronic Health Evaluation II; SOFA, sequential organ failure assessment. Subheadings and significant results $p < 0.05$ are in bold. [a]Data are presented as median and interquartile range unless otherwise reported. [b]Unless otherwise reported, due to missing data.

Table 2. Dry weight body composition and characteristics on measurement day[a].

		All patients (n=54)	Ward patients (n=30)	ICU patients (n=24)	p- value
Timeline					
Time from hospital admission to measurement, median (IQR), days		4 (2 – 11)	3 (2 – 4)	11 (6 – 19)	**0.000**
Clinical characteristics					
SOFA score, median [IQR]		2 [1 – 4]	1 [1 – 2]	3 [1 – 5]	**0.009**
Temperature, °C		37.3 (37.0 – 37.5)	37.1 (36.8 – 37.5)	37.4 (37.0 – 37.8)	0.323
Physical characteristics	**Reference value (SE)**				
Height, cm	171.5 (0.3) [b]	174 (171 - 177)	173 (169 - 177)	176 (171 - 180)	0.449
Weight, kg	78 (0.5) [b]	90.3 (84.9 – 95.6)	87.8 (79.8 – 95.8)	93.3 (86.1 – 100.5)	0.310
BMI, kg/m^2	26.5 [b]	29.7 (28.2 – 31.1)	29.3 (27.0 – 31.5)	30.2 (28.4 – 32.0)	0.518
Dry weight BIA-values	**Reference range/ reference value (SE)**				
Dry weight, median [IQR], kg [d]	78 (0.5) [b]	85.6 [74.7 – 101.0]	84.3 [72.4 – 97.8]	91.0 [77.3 – 105.2]	0.240
Fat Mass, kg	9.1 – 17.7 [c]	29.7 (25.9 – 33.6)	31.40 (25.4 – 37.4)	27.64 (22.8 – 32.5)	0.338
Percentage Body Fat (PBF), % [d]	10.0 – 20.0 [c]	32.8 (29.6 – 36.0)	35.0 (30.4 – 39.5)	30.1 (25.5 – 34.7)	0.135
Fat Free Mass (FFM), kg [d]	51.0 –62.3 [c]	59.2 (55.4 – 63.1)	55.5 (50.7 – 60.3)	63.9 (57.8 – 70.0)	**0.028**
Soft Lean Mass (SLM), kg [d]	48.1 –58.7 [c]	55.9 (52.3 – 59.5)	52.4 (47.9 – 56.9)	60.3 (54.6 – 66.1)	**0.028**
Total Body Water (TBW), ℓ	37.4 – 45.8 [c]	44.7 (41.8 – 47.6)	41.5 (38.1 – 45.0)	48.7 (44.0 – 53.3)	**0.013**
Intracellular Water (ICW), ℓ	23.2 – 28.4 [c]	26.9 (25.2 – 28.7)	25.2 (23.0 – 27.4)	29.1 (26.3 – 31.9)	**0.027**
Extracellular Water (ECW), ℓ	14.2 – 17.4 [c]	17.8 (16.6 – 18.9)	16.3 (15.1 – 17.6)	19.6 (17.7 – 21.5)	**0.004**
Visceral Fat Area (VFA), cm^2	< 100 [d]	155.2 (136.1 – 174.2)	162.9 (133.6 – 192.2)	145.5 (121.3 – 169.7)	0.368
Skeletal Muscle mass Index (SMI), kg/m^2 [d]	6.77 – 8.37 [e]	8.0 (7.6 – 8.4)	7.5 (7.1 – 8.0)	8.6 (7.9 – 9.2)	**0.006**
Fluid overload, ℓ	0 [d]	1.3 (1.0 – 1.6)	0.9 (0.6 – 1.2)	1.8 (1.3 – 2.2)	**0.001**
ECW/TBW, ℓ	0.36-0.39 [d]	0.40 (0.39 – 0.40)	0.39 (0.39 – 0.40)	0.40 (0.40 – 0.41)	**0.015**
50kHz Whole Body Phase Angle, °	5.6- 6.5 [f]	4.5 (4.2 – 4.8)	4.8 (4.4 – 5.2)	4.1 (3.8 – 4.5)	**0.017**

Abbreviations: ICU, Intensive Care Unit; SE, standard error; BMI, Body Mass Index; IQR, interquartile range. Subheadings and significant results $p < 0.05$ are in bold. [a]Data are presented as mean and 95% - confidence interval unless otherwise reported. [b]Population reference values for men and women in the age range 65–75 years, based on Dutch public records of 2019 [33]. [c]Whenever available, a population mean of the personalized minimal and maximal ideal measurements provided by the Inbody S10 device were given for each body composition value. [d]Values that are derived from TBW or ECW were recalculated with a healthy ECW/TBW of 0.38 to obtain dry weight values. Healthy reference value or range as provided by Inbody. [e]Mean SMI for healthy white women resp. men ages 67 years as shown by Lee et al. [31]. [f]Pooled mean phase angle for healthy white women resp. men aged 59–69 years in a meta-analysis by Mattiello [32].

Table 3. 28-day outcome and discharge destination[a].

	All Patients (n=54)	Ward patients (n=30)	ICU patients (n=24)	p- value
Length of stay				
Hospital-free days, median [IQR], days	13 [2 – 21]	21 [16 – 23]	2 [0 – 8]	**0.000**
Complications [b]				
Total	28 (51.9%)	10 (33.3%)	18 (75.0%)	**0.002**
Mortality	8 (14.8%)	5 (16.7%)	3 (12.5%)	0.720
Thrombo-embolic event [c]	13 (24.1%)	3 (10.0%)	10 (41.7%)	**0.007**
Renal failure [d]	1 (1.9%)	0 (0.0%)	1 (4.2%)	0.444
Delirium	13 (24.1%)	2 (6.7%)	11 (45.8%)	**0.001**
Other complications [e]	4 (7.4%)	0 (0.0%)	4 (16.7%)	**0.034**
Hospital discharge destination				
Other hospital	2 (4%)	0 (0%)	2 (8%)	0.193
Private home	29 (54%)	21 (70%)	8 (33%)	**0.007**
Rehabilitation facility/nursing home	17 (31%)	5 (17%)	12 (50%)	**0.009**
In-hospital death [f]	6 (11%)	4 (13%)	2 (8%)	0.682

Abbreviations: ICU, intensive care unit; IQR, interquartile range; no., number. Subheadings and significant results $p < 0.05$ are in bold. [a]Data are presented as median no. of patients (%) unless otherwise reported. [b]Percentages do not add to 100% as some patients had multiple complications. [c]Comprised of stroke, pulmonary embolism and deep venous thrombosis.

[d]Renal failure was only scored when requiring new renal replacement therapy. [e]Only pressure sores following prone ventilation were recorded. [f]Some patients died within 28-days, but after hospital discharge to elsewhere.

Predictive modeling

Thirty-four patients met the criteria of the composite outcome score, while 20 were not admitted to the ICU and had no complications. Simple regression analysis for BIA values showed several associations (Supplemental Table 4). Table 4 summarizes the odds ratios derived from the multiple regression analysis, with age, sex, SOFA score at admission and days between hospital admission and measurement used as covariates.

None of the BIA values, including fat mass, VFA, and FFM, was significantly associated with being admitted to the ICU. More fluid overload, higher ECW/TBW-ratio, and decreased PA were associated with increased risk for mortality. The composite outcome score yielded a significant inverse association with PA (OR 0.299, $p = 0.046$).

Table 4. Multiple regression analysis of dry weight BIA values for different outcome variables[a].

BIA variables	Odds ratio [b]	p-value
Outcome: ICU admission [c]		
Fat Free Mass (FFM/LBM), kg	1.135	0.214
Soft Lean Mass (SLM), kg	1.144	0.212
Skeletal Muscle mass Index (SMI)	4.524	0.087
Total Body Water (TBW), ℓ	1.225	0.126
Intracellular Water (ICW), ℓ	1.324	0.207
Extracellular Water (ECW), ℓ	1.808	0.078
Fluid overload, ℓ	45.408	0.059
ECW/TBW	Infinite	0.089
50kHz Whole Body Phase Angle, °	0.000	0.130
Outcome: Mortality [c]		
Fluid overload, ℓ	3.608	**0.043**
ECW/TBW	Infinite	**0.028**
50kHz Whole Body Phase Angle, °	0.208	**0.025**
Outcome: Complications [c]		
Total Body Water (TBW), ℓ	1.068	0.318
Extracellular Water (ECW), ℓ	1.199	0.248
Fluid overload,ℓ	1.558	0.226
ECW/TBW	Infinite	0.218
50kHz Whole Body Phase Angle, °	0.413	0.061
Outcome: Composite score [c]		
Percentage Body Fat (PBF), %	0.911	0.119
Fat Free Mass (FFM/LBM), kg	1.050	0.411
Soft Lean Mass (SLM), kg	1.053	0.413
Total Body Water (TBW), ℓ	1.070	0.383
Intracellular Water (ICW), ℓ	1.103	0.447
Extracellular Water (ECW), ℓ	1.205	0.315
Fluid overload (FO), ℓ	1.817	0.325
ECW/TBW	Infinite	0.249
50kHz Whole Body Phase Angle, °	0.299	**0.046**
Outcome: Vasopressor-free days [d]		
Percentage Body Fat (PBF), %	1.004	0.173
Visceral Fat Area (VFA), cm^2	1.000	0.350

Abbreviations: BIA, bioelectric impedance analysis; ICU, intensive care unit; kHz, kilohertz. Subheadings and significant results $p < 0.05$ are in bold. [a]BIA values were entered in a regression model with the specified outcome variable and the covariates age, sex, SOFA score at admission and days between hospital admission and measurement. [b]The odds ratio represents the expected increase in the outcome measure upon an increase of 1 unit of the relevant BIA variable. [c]BIA values entered in a multiple logistic regression model. [d]BIA values entered in a Poisson regression model, ICU patients only (n = 24).

DISCUSSION

We measured body composition by BIA in COVID-19 patients admitted to general wards and the ICU. Although the average patient was overweight, we did not find differences in BMI, nor significant associations between fat mass, fat distribution (visceral fat localization), or fat-free mass (representing the lean body mass) and the severity of disease. However, a lower phase angle at 50 kHz was associated with an increase in disease severity, reflected by the need for ICU admission, morbidity and mortality.

Multiple studies have shown an association between increased BMI and hospital and ICU admission, mechanical ventilation, and mortality in COVID-19 patients (2, 8, 34, 35, 36). Obesity-related co-morbidities are prevalent amongst individuals infected by COVID-195, 7. In 2016, the World Health Organization estimated that 39% of all adults worldwide were overweight (BMI>25 kg/m^2), and 13% were classified as obese (BMI>30 kg/m^2) (37). However, BMI does not incorporate the quantity and distribution of different tissues and is a crude estimation of body composition. Previously, this has led to misinterpretations due to ethnic variability in body ratios, proportion of subcutaneous versus visceral fat, and contribution of muscle mass to body weight (38).The latter is likely the explanation behind the obesity-mortality-paradox, as it suggested obesity is protective and associated with greater survival (22, 23). By measuring BIA body composition, we used a more precise method to study associations between increased body fat mass and visceral fat area and disease severity in COVID-19 patients.

Remarkably, we did not find differences in body composition between COVID-19 ward and ICU patients. Patients had a mean BMI of 29.7 kg/m^2 and a mean age of 67 years. Our cohort comprised 63% men, in keeping with other studies showing higher hospitalization rates amongst men (1, 3). According to national data, in 2019 the average BMI of persons between the age of 65 and 75 years in the Netherlands was 26.5 kg/m^2. Our cohort is more overweight than the general population (33). This trend of higher BMI and hospitalization is concordant with other publications (7,9). Although ICU patients showed a higher BMI (30.2 kg/m^2) compared with ward patients (29.3 kg/m^2), this difference was not significant. This contrasts with findings of retrospective studies that showed correlations between increased BMI and ICU admission for COVID-19 (39).

Expectedly, we found that the SOFA-score, an indicator of disease severity, was strongly associated with ICU-admission. Additionally, plasma CK at hospital admission was significantly increased in the eventual ICU-group. This was also observed in a cohort of COVID-19 patients in Wuhan, China (40). Other possible biomarkers upon hospital admission such as ferritin, CRP, and D-dimer were not-significantly more elevated in ICU

patients, although the number of observations were low. No other baseline characteristics were associated with ICU-admission.

Our data demonstrate the potential of PA as a predictor for disease severity in COVID-19 patients. PA has been repeatedly proven to predict morbidity and mortality in other patient groups (41, 42, 45). We found no correlations between body composition values and disease severity. A single-center, retrospective study assessing the association between computer tomography-based measurements of visceral fat area and COVID-19 severity found a positive correlation (46). However, this study was conducted among 30 patients, of whom only 13 were admitted to the ICU. Disease severity was defined as need for ICU admission and invasive mechanical ventilation, and morbidity and mortality were not considered. Thus, both the power and comparability to our study are limited.

We encountered no issues in performing BIA measurements during this study. Nevertheless, we recognize that logistical (time, training) or physical barriers (pulse oximeters, bandages) can potentially complicate (routine) BIA measurements, especially in a busy ICU environment. Other bedside techniques to evaluate muscle mass and quality are available, but come with their own limitations. Muscle ultrasound can be useful to measure muscle quality, quantity and the pennation angle, but performing ultrasound reliably requires training and practice and is potentially susceptible to intra- and inter-observer variability (43, 44). BIA is quick and requires only basic training, adding to the feasibility of use of the technique in the ICU. However, interpretation of the results must be done with caution as discussed below.

Limitations and considerations

Under challenging conditions, we were able to assess body composition in COVID-19 patients. However, there are several limitations. Firstly, the sample size of 54 can be considered small. In-hospital mortality was low (n = 8, 14.8%). Therefore, this cohort may yield insufficient power. A composite score was created to decrease the chance of type 1 error and to obtain a more encompassing definition of the disease course. Despite a robust retrospective basis for the visceral fat distribution hypothesis, we could not find associations between fat mass or distribution and disease severity. If our results prove reproducible, this could influence the direction of research for obesity and COVID-19.

Secondly, 13(43%) of our ward patients had LTP, waiving ICU admission regardless of disease severity. However, sensitivity testing, yielded no different results. Therefore, we consider that potential selection bias for ICU admission based on perceived health or projected survival chances did not strongly influence our main findings.

Additionally, the most recently measured weight was used as input for the Inbody S10. Daily weight measurements were not always feasible in the ward patients during the pandemic. However, in this study population, the weight measurement was taken less than 48 h prior to the BIA measurement. We therefore do not suspect this to be of significant influence on the results of this study.

Lastly, the cross-sectional nature of the BIA measurements complicates inter-individual comparability. As both disease and treatment influence body composition, mainly through loss of LBM and increase of fluid overload, differences in time-to-measurement are an important consideration (47, 48). In particular, for ICU patients, in whom fluid overload is common and has prevented BIA measurements from becoming a standard of practice. However, we circumvented this problem by using a multi-frequency BIA device (InBody S10), to enable differentiation between various fluid compartments (28, 49, 50), adjustment for the time from hospital admission to measurement, and recalculation of body composition to dry weight to reduce the effect of overestimating LBM by fluid overload. We recognize that under ideal circumstances, serial BIA measurements provide more details on the course of loss of muscle mass and fluid overload in critically ill patients.

The method to recalculate BIA parameters to dry weight is based on the assumption that absolute fluid overload is defined as the difference between the measured ECW and the expected ECW under physiological conditions, in which ECW/TBW is estimated to be 0.38 (25, 26, 28, 51, 52, 53). This method has not been validated in ICU patients; however, it is incorporated in the 'dialysis mode' of the device, a validated method to determine dry weight in dialysis patients. This adjustment method is based on the assumption that fluid overload is exclusively located in the extracellular compartment and questions have been raised as to whether this is an oversimplification in prolonged or severe edema (54). Likely, there is some muscle cell swelling (ICW) in severe edematous states, leading to overestimation of muscle mass (25, 54). This may explain trends towards higher FFM, SLM, and SMI in the ICU group. Nevertheless, no substantial impact of cell swelling on fat mass and visceral fat area can be expected. Therefore, we are confident that main findings persist. Moreover, additional calculations to estimate fluid overload are useful, as various studies have shown correlations between BIA derived fluid overload and outcome of disease (49, 55, 56). The PA is calculated directly from reactance and resistance and is therefore less directly influenced by fluid overload, although theoretically, rapid fluid shifts can contribute to cell damage and, therefore, decrease PA (22). In practice, PA indeed seems to vary with hydration. However, it remains a good indicator of clinical outcome, as fluid overload itself also negatively impacts outcome of disease, as was suggested by our findings (41, 42, 57).

INTERPRETATION

We assessed body composition using BIA in COVID-19 patients and compared ward and ICU patients. Our cohort was overweight, although this was not related to disease severity. Interestingly, we found no significant associations between fat mass or distribution, or fat-free mass and the severity of illness as reflected by ICU admission, complications or mortality. A low phase angle increased the odds of morbidity and mortality in COVID-19 patients. Cautious interpretation of BIA values is warranted in critically ill patients and correction for fluid overload should be performed. Nevertheless, we have shown that BIA measurements in COVID-19 patients are feasible and provide new levels of insight into body composition and phase angle beyond classical anthropometric data such as BMI. As we did not find associations between body composition and disease severity in COVID-19, we believe that other factors may play a more critical role in the development of severe COVID-19.

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SUPPLEMENTAL TABLES

Supplemental Table 1. Patients characteristics upon hospital admission (full TP ward patients only) [a]

	Full TP ward patients (n=17) [a]	ICU patients (n=24) [a]	p- value
Age, mean (CI), years	61 (56 - 65)	66 (61 - 70)	0.131
Males, no.(%)	11 (65%)	17 (71 %)	0.678
Comorbidities			
Diabetes, no.(%)	3 (17.6 %)	6 (25.0 %)	0.711
Hypertension, no.(%)	5 (29.4 %)	5 (20.8 %)	0.714
Chronic lung disease, no.(%)	5 (29.4 %)	5 (20.8 %)	0.714
Coronary artery disease, no.(%)	0 (0.0 %)	2 (8.3 %)	0.502
Clinical scores			
Barthel index, median[IQR)	20 [20 - 20]	20 [20 - 20]	0.048
Frailty score, median [IQR]	1 [1 - 3]	2 [1 - 3]	0.163
APACHE II score, median[IQR]	10 [7 – 15]	13 [10 – 14]	0.184
SOFA score, median [IQR]	2 [2 – 2]	6 [5 – 7]	**<0.001**
Laboratory results			
Hemoglobin, mmol/L	9.1 [8.6 – 9.9]	8.4 [7.7 – 9.6]	0.223
Leukocytes, 10^9/L	8.3 [6.3 – 9.6]	9.2 [6.2 – 11.8]	0.290
Trombocytes, 10^9/L	230 [188 – 297]	261 [166 – 320]	0.587
Ferritin, mcg/L (n=11)	921[222 - 2290]	1470 [728 - 3080]	0.414
Triglycerides, mmol/L (n=5)	0.8 [-]	1.8 [1.4 – 2.4]	0.157
C-reactive protein, mg/L	107 [74 – 198]	164 [67 – 235]	0.435
Serum creatinin, mcmol/L	70 [63 – 93]	81 [68 – 126]	0.209
Ureum, mmol/L	5.7 [5.0 – 7.5]	7.3 [5.0 – 9.8]	0.223
Creatinine Kinase, U/L (n=37)	97 [49 – 201]	174 [117 – 423]	**0.008**
D-dimer, mcg/ml (n=8)	9.45 [-]	2.56 [1.48 – 2.56	0.275

[a] Data are presented as median and interquartile range unless otherwise reported. [b] Unless otherwise reported, due to missing data. Abbreviations: TP, treatment plan;ICU, Intensive Care Unit; CI, confidence interval; APACHE II, Acute Physiology And Chronic Health Evaluation II; SOFA, sequential organ failure assessment.

Supplemental Table 2. Dry weight body composition and characteristics on measurement day (full TP ward patients only) [a]

	Full TP ward patients (n=17)	ICU patients (n=24)	p- value
Timeline			
Time from hospital admission to measurement, median (IQR), days	3 (2 – 8)	11 (6 – 19)	**0.001**
Clinical characteristics			
SOFA score, median [IQR]	1 [1 – 2]	3 [1 – 5]	**0.027**
Temperature, °C	37.2[36.7 – 37.6]	37.4 [37.0 – 37.8]	0.420
Physical characteristics			
Height, cm	176[171 - 181]	176 [171 - 180]	0.792
Weight, kg	88.0[83.5– 98.9]	94.0[80.0 – 105.9]	0.863
BMI, kg/m^2	28.1[26.2 – 32.5]	29.4[26.6 – 32.2]	0.721
Dry weight BIA-values			
Dry weight, median [IQR], kg [b]	87.6[82.8 – 99.3]	91.0 [77.3 – 105.2]	0.576
Fat Mass, kg	30.3(20.0 – 39.5)	26.5(19.9 – 36.6)	0.349
Percentage Body Fat (PBF),% [b]	34.0 (28.1 – 39.8)	30.1 (25.5 – 34.7)	0.055
Fat Free Mass (FFM), kg	60.7(54.6 – 66.8)	63.9 (57.8 – 70.0)	**0.015**
Soft Lean Mass (SLM), kg	57.3(51.5 – 63.1)	60.3 (54.6 – 66.1)	**0.015**
Total Body Water (TBW), ℓ	45.0(40.6 – 49.5)	48.7 (44.0 – 53.3)	**0.009**
Intracellular Water (ICW), ℓ	27.6(24.8 – 30.3)	29.1 (26.3 – 31.9)	**0.015**
Extracellular Water (ECW), ℓ	17.4(15.7 – 19.1)	19.6 (17.7 – 21.5)	**0.008**
Visceral Fat Area, cm^2	154.9 (98.4 – 214.1)	144.8(99.6 – 200.1)	0.329
Skeletal Muscle mass Index (SMI), kg/m^2	8.0(7.5 – 8.5)	8.6 (7.9 – 9.2)	**0.015**
Fluid overload, ℓ	0.5(0.2 – 0.9)	1.8 (1.3 – 2.2)	**0.011**
ECW/TBW, ℓ	0.39(0.38 – 0.39)	0.40 (0.40 – 0.41)	**0.012**
50kHz Whole Body Phase Angle, °	5.5(5.1 – 5.8)	4.1 (3.8 – 4.5)	**0.011**

[a] Data are presented as mean and 95% - confidence interval unless otherwise reported. [b] Values that are derived from TBW or ECW were recalculated with a healthy ECW/TBW of 0.38 to obtain dry weight values. True measured values are included in the appendix.[c]Whenever available, a population mean of the personalized minimal and maximal ideal measurements provided by the device were given for each body composition value. Abbreviations: TP, treatment plan; ICU, Intensive Care Unit; BMI, Body Mass Index; IQR, interquartile range.

Supplemental Table 3. 28-day outcome and discharge destination (full TP ward patients only) [a]

	Full TP ward patients (n=17)	ICU patients (n=24)	p- value
Length of stay			
Hospital-free days, median [IQR], days	13 [2 – 21]	2 [0 – 8]	**<0.001**
Complications [b]			
Total	3(17.6%)	18 (75.0%)	**<0.001**
Mortality	0 (0.0%)	3 (12.5%)	0.254
Thrombo-embolic event [c]	2 (11.8%)	10 (41.7%)	**0.038**
Renal failure [d]	0 (0.0%)	1 (4.2%)	1.000
Delirium	1(5.9%)	11 (45.8%)	**0.006**
Other complications [e]	0 (0.0%)	4 (16.7%)	0.128
Hospital discharge destination			
Other hospital	0 (0%)	2 (8%)	0.502
Private home	16 (67%)	8 (33%)	**<0.001**
Rehabilitation facility/nursing home	1 (6%)	12(50%)	**0.003**
In-hospital death [f]	0 (0%)	2 (8%)	0.502

[a] Data are presented as median no. of patients (%) unless otherwise reported. [b] Percentages do not add to 100% as some patients had multiple complications. [c] Comprised of stroke, pulmonary embolism and deep venous thrombosis. [d] Renal failure was only scored when requiring new renal replacement therapy. [e] Only pressure sores following prone ventilation were recorded. [f] Some patients died within 28-days, but after hospital discharge to elsewhere. Abbreviations: ICU, intensive care unit; IQR, interquartile range; no., number.

Supplemental Table 4. Results simple regression of BIA values [a] and outcome with p<0.10 [b]

Exposure	Compared groups		p-value
Outcome: ICU admission	**Ward (n=30)**	**ICU (n=24)**	
Fat Free Mass (FFM/LBM), kg	55.5 (50.7 – 60.3)	63.9 (57.8 – 70.0)	0.100
Soft Lean Mass (SLM), kg	52.4 (47.9 – 56.9)	60.3 (54.6 – 66.1)	0.099
Skeletal Muscle mass Index (SMI)	7.5 (7.1 – 8.0)	8.6 (7.9 – 9.2)	**0.025**
Total Body Water (TBW), ℓ	41.5 (38.1 – 45.0)	48.7 (44.0 – 53.3)	**0.037**
Intracellular Water (ICW), ℓ	25.2 (23.0 – 27.4)	29.1 (26.3 – 31.9)	0.094
Extracellular Water (ECW), ℓ	16.3 (15.1 – 17.6)	19.6 (17.7 – 21.5)	**0.011**
Fluid overload, ℓ	0.9 (0.6 – 1.2)	1.8 (1.3 – 2.2)	**0.002**
ECW/TBW	0.39 (0.39 – 0.40)	0.40 (0.40 – 0.41)	**0.002**
50kHz Whole Body Phase Angle, °	4.8 (4.4 – 5.2)	4.1 (3.8 – 4.5)	**0.001**
Outcome: Mortality	**Alive at 28-days (n=46)**	**Deceased at 28-days (n=8)**	
Fluid overload, ℓ	1.1 (0.5 – 1.7)	2.1 (1.2 – 2.5)	**0.032**
ECW/TBW	0.40 (0.39 – 0.40)	0.41 (0.40 – 0.42)	**0.024**
50kHz Whole Body Phase Angle, °	4.7 (4.4 – 5.0)	3.5 (3.0 – 4.0)	**0.027**
Outcome: Complications	**No complications (n=26)**	**Complications (n=28)**	
Total Body Water (TBW), ℓ	45.1 (41.5 – 48.7)	44.4 (39.7 – 49.0)	0.085
Extracellular Water (ECW), ℓ	17.7 (16.2 – 19.2)	17.9 (16.0 – 19.7)	**0.043**
Fluid overload, ℓ	0.7 (0.2 – 1.6)	1.4 (1.0 – 2.2)	**0.027**
ECW/TBW	0.39 (0.39 – 0.40)	0.40 (0.40 – 0.41)	**0.021**
50kHz Whole Body Phase Angle, °	5.1 (4.7 – 5.4)	4.0 (3.7 – 4.3)	**0.004**
Outcome: Composite score	**No complications (n=20)**	**Complications (n=34)**	
Percentage Body Fat (PBF), %	34.8 (29.1 – 40.6)	31.6 (27.6 – 35.7)	0.097
Fat Free Mass (FFM/LBM), kg	58.2 (52.9 – 63.4)	59.9 (54.4 – 65.3)	0.096
Soft Lean Mass (SLM), kg	54.9 (49.9 – 59.8)	56.5 (51.4 – 61.7)	0.096
Total Body Water (TBW), ℓ	43.4 (39.6 – 47.2)	45.5 (41.4 – 49.6)	**0.047**
Intracellular Water (ICW), ℓ	26.4 (24.0 – 28.8)	27.2 (24.7 – 29.7)	0.098
Extracellular Water (ECW), ℓ	17.0 (15.5 – 18.4)	18.3 (16.6 – 19.9)	**0.019**
Fluid overload, ℓ	0.7 (0.1 – 1.5)	1.3 (1.0 – 2.2)	**0.011**
ECW/TBW	0.39 (0.39 – 0.40)	0.40 (0.40 – 0.41)	**0.008**
50kHz Whole Body Phase Angle, °	5.1 (4.7 – 5.6)	4.1 (3.8 – 4.4)	**0.002**
Outcome: Vasopressor-free days	**Odds ratio**		
Percentage Body Fat (PBF), %	1.012		**0.035**
Visceral Fat Area, cm^2	1.002		**0.092**

[a] BIA values were corrected for age and sex. [b] Ventilator-free days did not yield a p<0.10 with any of the BIA values and was therefore not included in this table.

Chapter 8

Bioelectric impedance body composition and phase angle in relation to 90-day adverse outcome in hospitalized COVID-19 ward and ICU patients: The prospective BIAC-19 study

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ABSTRACT

Background & aims

Gaining insight into readily obtainable baseline characteristics that allow prediction of adverse outcome in COVID-19 aids both treatment and healthcare planning. Bioelectric impedance (BIA) Phase Angle (PhA) is correlated with outcome in a multitude of diseases and may be of added value in predicting adverse outcome of COVID-19. We aimed to associate baseline body composition parameters with 90-day adverse outcome of COVID-19 including ICU-admission and to explore the added predictive value of baseline PhA.

Methods

We performed a prospective observational study, conducting BIA amongst COVID-19 patients within 24 hours of hospital admission, with a follow-up of 90 days. Data were compared between ward-only and ICU-patients. Regression models were used to assess the associations between baseline characteristics, body composition and 90-day adverse outcome, including a composite outcome score of morbidity, ICU-admission, and mortality. An ROC-curve was used to explore the added predictive value of PhA to other clinical parameters at baseline for the prediction of adverse outcome.

Results

One-hundred-and-fifty patients were included. Mean age was 68 (66–70) years, 67% were male. Forty-one (27%) patients were admitted to ICU and 77 (51%) met the criteria of the composite outcome score. In multiple regression, PhA was independently, inversely correlated with risk of ICU-admission (OR .531, p = .021), complications (OR .579, p = .031), hospital length of stay (OR .875, p = .037) and the composite outcome score (OR .502, p = .012). An ROC-curve showed that the incorporation of PhA in a composite risk-score improved the discriminative power for the composite outcome from poor to fair, compared to individual predictors (AUC 0.79 (95% CI 0.71–0.87)).

Conclusion

BIA measurements including Phase Angle are independently correlated with an adverse outcome of COVID-19. Interpretation of Phase Angle can be a valuable addition to risk assessment of adverse outcome of COVID-19 at hospital admission.

INTRODUCTION

Since their appearance in late 2019 SARS-CoV-2 and the related Corona Virus Disease-2019 (COVID-19) have challenged healthcare infrastructure worldwide. Much scientific effort has gone into uncovering baseline characteristics that allow for adverse outcome prediction and thereby estimation of healthcare requirements, such as intensive care unit (ICU) capacity. Ideally, risk-scores are composed of measurements and characteristics that are readily available and correlate with outcome from an early stage disease development.

Obesity is suggested to be a predictive characteristic. However, in a previously published cross-sectional observational cohort study amongst 54 hospitalized COVID-19 patients we found no associations between body mass index (BMI), fat mass, visceral fat area (VFA) and other body compositions parameters as measured by bioelectric impedance analysis (BIA) and adverse outcome of COVID-19 once patients are hospitalized (1). Interestingly, we did find that Phase Angle (PhA) was inversely related to the odds of adverse outcomes at 30 days, similar to findings in a variety of other diseases.

The PhA reflects the relationship between the reactance and resistance (together called impedance) of the body. These electrical properties can be measured with a BIA device, by attaching four electrodes to the extremities and conducting a brief measurement, similar to obtaining an electrocardiogram. Phase angle is regarded a biological marker of cellular health, as high cell mass volume and robust cell membranes cause delayed signals and thereby a higher PhA. A PhA greater than 6 is assumed healthy, although the normal range varies with sex and age. Diminished cell count, membrane integrity and altered hydration status in critical illness leads to a decreased PhA, which has been shown to correlate with increased mortality, length of ward-, ICU- and hospital-stay, duration of mechanical ventilation and APACHE-II score in various diseases (2).

Recently, Cornejo-Pareja et al. (3) showed that a PhA <3.95° at hospital admission was a significant predictor of 90-day mortality risk independent of age, sex, BMI, and comorbidities in their cohort of 127 hospitalized COVID-19 patients.

As PhA is quick and easy to obtain in virtually all patients, it can be a valuable addition to other clinical parameters in assessing an individual's risk of severe course of disease, if these initial findings can be solidified.

With this prospective continuation of our research, we aim to assess the correlation between baseline PhA and 90-day adverse outcome of COVID-19, in addition to the derived BIA parameters of body composition. Furthermore, we explore the value of

the addition of PhA to other baseline clinical characteristics that are readily available at hospital admission, and that aid in the prediction of the disease course.

MATERIALS & METHODS

This prospective observational study was performed at Gelderse Vallei Hospital, a teaching hospital in Ede, The Netherlands. The hospital has two ICU units, with a combined capacity of 18 beds. Thirty-eight general ward COVID-19 beds were available. Early dexamethasone administration was protocol in all COVID-19 patients. Between October 1st and November 19th of 2020, the ICU units participated in the REMAP-CAP trial, after which tocilizumab (RoActemra®) became standard of care for COVID-19 in February 2021 (4). The hospital did not participate in other interventional trials during the study period.

Study design and participants

A cross-sectional version of the BIAC-19 study was conducted amongst 54 hospitalized COVID-19 patients between April 10th, and 17th, 2021, of which the results have been published previously (1). When the second 'wave' of COVID-19 hospital admissions in the Netherlands commenced in October 2020, the ethics board approved a restart of the BIAC-19 with a prospective design. Written informed consent was obtained from all patients or their legal representatives. The study protocol is registered in the Netherlands Trial Register (number NL8562).

Between October 12 and February 10 2021, all patients aged 18 years or above, admitted to the hospital on weekdays with COVID-19 symptoms and who proved PCR-confirmed SARS-CoV-2 positive within 24 hours after hospital admission, were eligible for inclusion. Patients were not considered if they were admitted outside working hours, as the researchers were not present to perform the BIA measurements within 24 hours. In addition, patients were not included if a current SARS-CoV-2 infection was not confirmed within 24 hours after admission, nor if they had been transferred in from another hospital. Exclusion criteria were pregnancy, presence of electrical implants, wounds or skin damage at the designated electrode sites, or inability to maintain posture for 5 minutes.

Patients previously included in the cross-sectional analyses, who had their measurement within 24 hours of hospital admission, were reconsidered for the current analysis.

BIA measurements

BIA measurements were conducted by trained researchers with the InBody S10® (InBody Co., Ltd., Seoul, Korea). This multi-frequency, segmental impedance analyzer requires height, weight, and sex as input parameters. Height and weight as measured upon admission

were used. When circumstances did not allow measurements, height as provided by the patient or representative was entered. BIA measurements were performed in supine position with reusable electrodes attached to the left and right thumb and middle finger and both ankles. The measurements typically took 3–5 min.

The InBody S10 measures impedance at multiple frequencies and determines a 50 kHz whole body Phase Angle (PhA). Furthermore, segmental measurements are used to calculate total body water (TBW) and (segmental) extracellular water (ECW). Henceforth, the software uses validated methods to estimate fat-free mass (FFM), soft lean mass (SLM), mineral mass, bone mineral content (BMC), percentage body fat (PBF), VFA, skeletal muscle mass (SMM), body cell mass (BCM) and protein mass, in addition to several ratios and segmental values. Fluid overload (FO) was calculated by subtracting a recalculated ECW based on a normal ECW/TBW ratio of 0.380 from the measured ECW (i.e., OH = ECWmeasured – ((ICW × 0.380)/0.620)), a method that is used in dialysis patients (1,2).

Data collection

Demographic and clinical data were collected from local electronic medical record systems MetaVision® (iMDsoft, Tel Aviv, Israel) and NeoZIS® (MI Consultancy, Katwijk, The Netherlands). The recorded data included: age, sex, co-morbidities, clinical scores, laboratory results, limited treatment plans (LTP; such as do not resuscitate or no ICU-admission orders), treatments and outcome measures.

Whenever included patients were transferred to another hospital within the same admission period, outcome data were provided upon request by the treating physician of that hospital.

Disease severity scoring

Admission sequential organ failure assessment (SOFA) scores were calculated based on the parameters available from the emergency room (ER) records. As no patients had mechanical ventilation upon admission, fraction of inspired oxygen was calculated based on oxygen delivered by nasal cannula, where open-mouth breathing was presumed for all patients (5). Missing values were presumed normal, i.e., 0 points added to the patient's SOFA-score.

As SOFA-score is traditionally used in the ICU and not readily available in the ER, respiratory rate (RR) was recorded as an alternative indication of disease severity in COVID-19.

Outcome measures

Adverse outcome was defined in multiple ways. First, ICU-admission for severe COVID-19, and 90-day mortality and other complications were considered. Expected complications

were thrombo-embolic events, renal failure, and delirium. Other complications were considered when occurring twice or more in the study population. Additionally, a composite outcome score of ICU-admission and complications, including 90-day mortality, were created. A score of 1 indicated that at least one of the criteria was met, while a score of 0 was assigned to those patients without ICU-admission and complications.

Furthermore, hospital length of stay (HLOS), ICU-LOS and hospital discharge destination was recorded. For the ICU-patient group, duration of ventilation and vasopressor use were considered continuous outcome measures related to disease severity.

Statistical analysis

Normality of the distribution of continuous data was visually assessed by the quantile–quantile plots. Continuous values are reported as mean (95% bias-corrected accelerated bootstrap confidence intervals (95%-BCa CI)), discrete data are presented as numbers (%). Patients who had to be admitted to the ICU were compared to ward-only patients. Differences were assessed using independent samples t-tests for continuous data or Chi-squared tests for categorical data. When test assumptions were not met, Mann–Whitney U tests or Fisher's exact tests were used. For non-binary categorical data (i.e., discharge destination) analysis of variance was used.

Predictive modeling

Simple regression analysis was performed for associations between baseline characteristics, body composition and outcome of disease. For binary outcomes, binary logistic regression was used. When conditions for linearity of the logit were not met, transformation was performed. Continuous outcomes were univariately analyzed by negative binomial regression with estimated overdispersion. In all analyses regarding BIA values, age and sex were added into the model to correct for systematic population differences. For binary outcomes that included ICU-admission, patients with an LTP waiving ICU-admission were excluded from the analyses. For outcomes relating to ICU stay, only ICU-patients were considered.

Multiple logistic regression analysis was performed with an enter method for binary outcomes and BIA values with a p-value ≤0.10 in simple regression analysis. For continuous outcomes negative binomial regression was used. Unstandardized beta's (B) with their 95% BCa CI are presented. The adjusted odd ratio (Exp(B)) with its 95%-CI is expressed for a 1-point increase in the predictor. Nagelkerke's R-squared was used to interpret goodness of fit of the logistic regression models. Covariates were age, sex and SOFA-score. Analyses were repeated with RR per minute as a substitute for SOFA-score.

We computed a composite predictive risk-score for the composite outcome score, including sex, age, PhA and RR adjusted for their multiple logistic regression odds-ratios. The risk-score was used in ROC analysis with nonparametric distribution assumption to visually compare its discriminative power for the composite outcome score to the continuous predictors alone. The PhA was inverted (1-PhA), as it alone was inversely related to outcome. The discriminative power of the AUC was classified as follows: 0.90 ≤ AUC ≤1.0, excellent; 0.80 ≤ AUC <0.90, good; 0.70 ≤ AUC <0.80, fair; 0.60 ≤ AUC <0.70, poor; 0.50 ≤ AUC <0.60, failure.

IBM SPSS statistics 27 (IBM Corp, Armonk, NY, USA) was used for all analyses. Only two-sided analyses were used. P-values ≤0.05 were considered statistically significant. P-values are reported to a single significant figure unless 0.2 ≥ P ≥ 0.01, in which case two significant figures are shown.

RESULTS

Between October 10, 2020, and February 11, 2021, 486 patients with PCR confirmed COVID-19 were admitted to our hospital. Of these, 179 patients were screened for inclusion, BIA measurements or PCR could not be performed within 24 h of admission (Figure 1). One patient declined participation; 28 patients were excluded because of contraindications. Five patients from the previous cross-sectional study were eligible for the prospective analysis based on the revised inclusion criteria (1). In total, 150 COVID-19 patients were measured and analyzed.

All the included patients were white of Western-European descent. Forty-one (27%) patients eventually had to be admitted to the ICU. Table 1 summarizes baseline characteristics and measurements, and compares those of eventual ICU-patients to ward-only patients. At admission, eventual ICU-patients had higher SOFA-scores, RR, CRP and CK levels, and lower CFS-scores and lymphocytes, than ward-only patients.

The unstandardized body composition parameters, including PhA, are shown in Table 2 (6, 7, 8). The 90-day outcome is summarized in Table 3 . Of the 11 (10%) ward-only patients who died, seven (70%) had LTP's preventing ICU-admission.

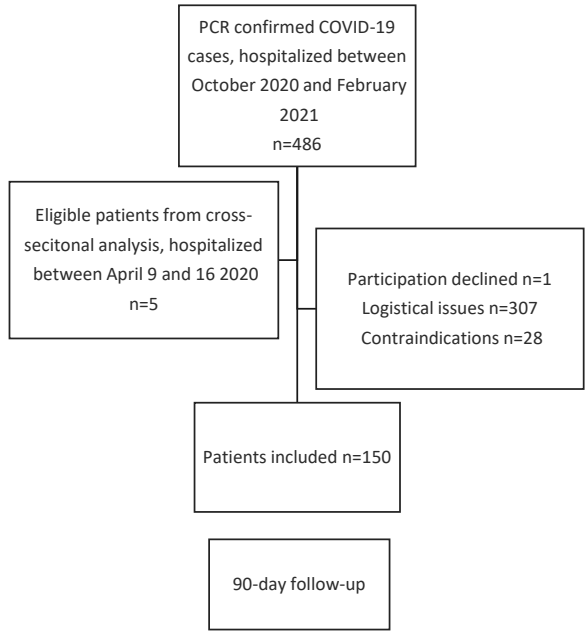

Figure 1. Study flow diagram

Table 1. Patient characteristics upon hospital admission[a].

	All Patients (N=150)[b]	**Ward patients (n=109)[b]**		**ICU patients (n=41)[b]**		**P- value [d]**
Age, years	68 (66-70)	67 (65-70)		68 (66-71)		.6
Males	100 (67%)	69 (63%)		31 (76%)		.2
Co- morbidities						
Diabetes	40 (27%)	28 (26%)		12 (29%)		.7
Hypertension	60 (40%)	45 (41%)		15 (37%)		.7
Astma/COPD	32 (21%)	23 (21%)		9 (22%)		>.9
Cardiovascular Disease	43 (29%)	34 (31%)		10 (24%)		.6
Overweight, BMI 25-30 kg/m^2	67 (45%)	50 (46%)		17 (42%)		.7
Obesity, BMI >30 kg/m^2	50 (33%)	35 (32%)		15 (37%)		.7
Clinical scores						
Clinical Frailty score	3 (3)	3 (3)		2 (2-3)		**.005**
SOFA score	3 (3)	3 (2-3)		4 (4-5)		**<.001**
Respiratory Rate, / minute	25 (24-26)	23 (22-24)	n=106	29 (27 -31)	n=41	**.001**
Temperature, °C	36.7 (36.6-36.9)	36.8 (36.6 -36.9)	n=108	36.7 (36.5 -36.9)	n=41	.6
Laboratory results						
Hemoglobin, mmol/L	8.6 (8.4-8.7)	8.6 (8.4-8.8)	n=108	8.5 (8.2-8.8)	n=41	.5
Leukocytes, 10^9/L	8.1 (7.6-8.6)	7.8 (7.2-8.5)	n=108	8.6 (7.4-9.8)	n=41	.3

Table 1. Continued

	All Patients (N=150)[b]	Ward patients (n=109)[b]		ICU patients (n=41)[b]		P- value [d]
Lymphocytes, 10^9/L	1.1 (1.0-1.2)	1.1 (1.0-1.2)	n=106	0.9 (0.7 – 1.1)	n=41	**.046**
Thrombocytes, 10^9/L	226 (211-240)	232 (216-250)	n=108	212 (186-237)	n=41	.2
Ferritin, µg/L	1501 (997-2206)	586 (80-1100)	n=4	1637 (1066-2421)	n=27	.2
Triglycerides, mmol/L	1.8 (1.5-2.1)	2.0	n=1	1.8 (1.4-2.2)	n=17	.8
C-reactive protein, mg/L	117 (104-129)	99 (87-111)	n=109	162 (135-190)	n=41	**.002**
Serum creatinin, µmol/L	100 (90-112)	97 (87-112)	n=107	107 (88-132)	n=41	.49
Blood urea nitrogen, mmol/L	8.6 (7.6-9.6)	8.1 (7.1-9.1)	n=107	9.8 (7.8-12)	n=41	.17
Creatinine Kinase, U/L	272 (207-347)	187 (138-252)	n=99	483 (299-706)	n=19	**.026**
D-dimer, mcg/ml	4.24 (2.23-6.87)	4.06 (1.21-7.80)	n=26	4.43 (1.77-8.19)	n=26	.9
BUN-to-creatinin-ratio	0.86 (0.81-0.91)	0.08 (0.08-0.09)	n=106	0.09 (0.08-0.11)	n=41	.12

Abbreviations: ICU, Intensive Care Unit; COPD, Chronic Obstructive Pulmonary Disease; BMI, body mass index; SOFA, sequential organ failure assessment; BUN, blood urea nitrogen. [a]Data are presented as number (percentage, %) or mean (95% bias-corrected accelerated bootstrapped confidence interval. [b]Unless otherwise reported, due to missing data. [c]P-values <0.05 are regarded as statistically significant and displayed in bold.

Predictive modeling

Seventy-seven (51%) patients met the criteria of the composite outcome score, while 73 (49%) were not admitted to the ICU and had no complications including 90-day mortality. The simple regression analyses for BIA values (incorporating sex and age) showed several associations with outcome parameters (Supplemental Tables 1–8). Multiple regression analysis was performed for outcomes and BIA values with a p-value ≤0.10 in simple regression analysis.

Table 4 summarizes the adjusted odds ratios derived from the multiple regression analyses, with age, sex and admission SOFA-score used as covariates. The composite outcome score yielded a significant inverse association with PhA (OR 0.629, p = .029). Fat-free mass (OR 1.047, p = .033), SLM (OR 1.050, p = .032), TBW (OR 1.066, p = .029), ICW (OR 1.104, p = .041), ECW (OR 1.181, p = .039), BCM (OR 1.072, p = .026) and SMI (OR 1.447, p = .041) were positively correlated with the chance of ICU-admission. Fat mass (OR .969, p = .021) and VFA (OR .995, p = .050) were significantly inversely associated with complications. ECW/TBW ratio (OR infinitely small, p = .048) was significantly associated with duration of vasopressor use. None of the BIA values were independently associated with ICU-LOS or HLOS.

ICU-patients had a mean ICU-LOS of 17 days (95%-BCa CI 13–23), during which 23 (56%) were ventilated for 12 days (95%-BCa CI 7–18), of which 16 (70%) in the prone position,

for three days (95%-BCa CI 1–4). Vasopressors were used in 24 (59%) patients, for five days (95%-BCa CI 3–7).

Table 2. Unstandardized body composition and characteristics on measurement day [a].

		All patients (N=150)	Ward patients (n=109)	ICU patients (n=41)	P-value
Physical characteristics	**Reference value (SE)**				
Height, cm	171 (0.3)[b]	174 (173-176)	174 (173-176)	174 (172-177)	.9
Weight, kg	78 (0.5)[b]	88 (85-91)	88 (84-92)	90 (86-95)	.4
Body Mass Index, kg/m^2	26.5[b]	29 (28-30)	29 (28-30)	30 (28-31)	.4
BIA-values	**Reference range/ reference value (SE)[c]**				
Fat Mass, kg	9.6 – 17.6	30.1 (27.9 – 32.3)	30.7 (28.2-33.5)	28.3 (24.9-31.8)	.3
Percentage Body Fat (PBF), %	12.7 – 22.7	33.2 (31.5 – 35.0)	34.1 (31.9-36.3)	30.8 (28.0-33.7)	.07
Fat Free Mass (FFM), kg	49.5 – 60.6	58.5 (56.3 – 60.7)	57.2 (54.8-60.0)	61.8 (58.6-65.0)	**.026**
Soft Lean Mass (SLM), kg	46.8 – 57.2	55.1 (53.1 – 57.2)	53.9 (51.7-56.4)	58.3 (55.3-61.3)	**.024**
Body Cell Mass (BCM), kg	32.4 – 39.3	37.7 (36.2-39.2)	36.8 (35.2-38.6)	40.0 (37.8-42.0)	**.026**
Total Body Water (TBW), ℓ	36.4 – 44.5	42.9 (41.4 – 44.6)	41.9 (40.1-43.9)	45.5 (43.2-47.9)	**.014**
Intracellular Water (ICW), ℓ	22.6 – 27.6	26.2 (25.3 – 27.4)	25.8 (24.6-27.0)	27.8 (26.4-29.3)	**.030**
Extracellular Water (ECW), ℓ	13.8 – 16.9	16.7 (16.2 – 17.3)	16.4 (15.7-17.1)	17.7 (16.8-18.6)	**.013**
Visceral Fat Area (VFA), cm^2	< 100[d]	154 (144-166)	160 (146-173)	141 (123-160)	.095
Skeletal Muscle mass Index (SMI), kg/m^2	6.77 – 8.37[e]	8.1 (7.8-8.3)	7.9 (7.7-8.2)	8.4 (8.1-8.8)	**.028**
Fluid overload (FO), ℓ	0	0.59 (0.46-0.73)	0.57 (0.41-0.74)	0.64 (0.41-0.86)	.6
ECW/TBW, ℓ	0.36-0.39[d]	0.39 (0.39-0.39)	0.39 (0.39-0.39)	0.39 (0.39-0.39)	**.014**
50kHz Whole Body Phase Angle, °	5.6- 6.5[f]	5.4 (5.2-5.6)	5.4 (5.2-5.7)	5.2 (4.9-5.4)	.14

Abbreviations: ICU, Intensive Care Unit; SE, standard error. P-values <0.05 are regarded as statistically significant and displayed in bold. [a]Data are presented as number (percentage, %), or mean (95% bootstrapped bias correct accelerated confidence interval). [b]Population reference values for men and women in the age range 65–75 years, based on Dutch public records of 2019 [6]. [c]Whenever available, a population mean of the personalized minimal and maximal ideal measurements provided by the Inbody S10 device were given for each body composition value. [d]Healthy reference value or range as provided by Inbody. [e]Mean SMI for healthy white women resp. men ages 67 years as shown by Lee et al. [7]. [f]Pooled mean phase angle for healthy white women resp. men aged 59–69 years in a meta-analysis by Mattiello [8].

Table 3. 90-day outcome and discharge destinations [a].

	All Patients (N=150)	Ward patients (n=109)	ICU patients (n=41)	p- value
Length of stay				
Hospital length of stay, days	11 (10-13)	6 (6-7)	25 (20-30)	**.001**
Complications[b]				
Total	59 (39%)	29 (27%)	30 (73%)	**<.001**
Mortality	18 (12%)	11 (10%)	7 (17%)	.3
Thrombo-embolic event[c]	31 (21%)	13 (12%)	18 (44%)	**<.001**
Renal failure[d]	18 (12%)	8 (7%)	10 (24%)	**.007**
Delirium	15 (10%)	5 (5%)	10 (24%)	**.001**
Lung fibrosis	4 (3%)	1 (1%)	3 (7%)	.06
Hospital discharge destination				
Private home	113 (75%)	92 (84%)	21 (51%)	
Rehabilitation facility/nursing home	22 (15%)	10 (9%)	12 (29%)	**<.001**
In-hospital death	15 (10%)	7 (6%)	8 (20%)	

Abbreviations: ICU, intensive care unit. P-values <0.05 are regarded as statistically significant and displayed in bold. [a]Data are presented as number (percentage, %), or mean (95% confidence interval). [b]Percentages do not add to 100% as some patients had multiple complications. [c]Comprised of stroke, pulmonary embolism and deep venous thrombosis. [d]Renal failure was only scored when requiring new renal replacement therapy.

Table 4. Multiple regression analysis of BIA values for different outcome variables, including age, sex and SOFA score[a].

BIA variables	B (95%BCa CI)	P-value	OR[b]	95% CI for Odds Ratio		Nagelkerke R^2
				Lower	Upper	
Outcome: Composite score[c], n=127 (no LTP)						
PhA	-.463 (-.918, -.141)	**.029**	0.629	0.398	0.996	.324
Outcome: ICU admission[c], n=127 (no LTP)						
PBF, %	-.037 (-.082, -.005)	.061	.963	.921	1.007	.384
FFM, kg	0.46 (.003, .108)	**.033**	1.047	1.000	1.096	.394
SLM, kg	0.48 (-.001, .109)	**.032**	1.050	1.000	1.102	.394
TBW, l	.064 (-.001, .151)	**.029**	1.066	1.002	1.134	.396
ICW, l	.099 (-.004, .243)	**.041**	1.104	.999	1.219	.393
ECW, l	.166 (.002, .403)	**.039**	1.181	1.004	1.389	.395
BCM, kg	.070 (.002, .161)	**.026**	1.072	1.000	1.149	.394
SMI	.370 (-.087, .928)	**.041**	1.447	0.962	2.178	.386
PhA	-.414 (-1.052, .087)	.12	0.661	0.366	1.194	.380
Outcome: Complications[c], N=150						
FM, kg	-.032 (-.060, -0.11)	**.021**	0.968	0.939	0.998	.283
ECT/TBW, l	3.172 (-14.229, 21.262)	.7	23.8	0	Infinite	.250
VFA, cm^2	-.005 (-.010, .000)	**.050**	0.995	0.989	1.000	.275
PhA	-.397 (-.814, -.061)	.065	0.672	0.427	1.057	.274
Outcome: HLOS[d], N=150						
PBF, %	-.010 (-.022, .001)	.071	0.990	0.979	1.001	NA
VFA, cm^2	-.001(-.003, .000)	.11	0.999	0.997	1.000	NA
PhA	-.063 (-.169, .084)	.3	0.939	0.828	1.065	NA
Outcome: ICU LOS[d], n=41 (ICU only)						
SMI	-.076 (-.380, .344)	.6	0.927	0.749	1.148	NA
Outcome: Vasopressor days[d], n=41 (ICU only)						
ECW/TBW, l	-18.006 (-43.532, -.003)	**.048**	Infinitely small	Infinitely small	15.7	NA

Abbreviations: BIA, bioelectric impedance analysis; BCa CI, bias-corrected accelerated bootstrap confidence interval; LTP, limited treatment plan; PBF, percentage body fat; FFM, fat-free mass; SLM, soft lean mass; ICW, intracellular water; ECW, extracellular water; TBW, total body water; BCM, body cell mass; SMI, skeletal muscle index; PhA, 50 kHz Whole body phase angle; FM, fat mass; VFA, visceral fat area; HLOS, hospital length of stay; NA, not applicable; LOS, length of stay. P-values <0.05 are regarded as statistically significant and displayed in bold. [a]BIA values were entered in a regression model with the specified outcome variable and the covariates age, sex and SOFA score at admission. [b]The odds ratio represents the expected increase in the outcome measure upon an increase of 1 unit of the relevant BIA variable. [c]BIA values entered in a multiple logistic regression model. [d]BIA values entered in a negative binominal regression model.

Table 5 shows these analyses with RR as a substitute for SOFA score as indicator of disease severity. Phase angle remains inversely associated with the composite outcome (OR .502, p = .012), and is newly inversely correlated with ICU-admission (OR .531, p = .021), complications (OR .579, p = .031) and HLOS (OR .875, p = .037). Visceral fat area loses its correlation with complications, as does ECW/TBW-ratio with vasopressor use.

A composite predictive risk-score for the composite outcome score was calculated with age, sex, PhA and RR adjusted for their multiple logistic regression odd-ratios (Table 6) as: risk-score = (RR × 0.129) + (Age × 0.027) - (PhA × 0.498) + (0.696 if male). The subsequent ROC (Figure 2) shows that the incorporation of PhA in the composite risk-score improved the discriminative power for the composite outcome as assessed by the AUC from poor (AUC 0.67–0.69) to fair (AUC 0.79 (95% CI 0.71–0.87), compared to individual predictors.

Table 5. Multiple regression analysis of BIA values for different outcome variables, including age, sex and respiratory rate [a].

BIA variables	B (95%BCa CI)	P-value	OR[b]	95% CI for Odds Ratio		Nagelkerke R^2
				Lower	Upper	
Outcome: Composite score[c], n=127 (No LTP)						
PhA	-.689 (-1.219, -.298)	**.012**	0.502	0.281	0.898	.351
Outcome: ICU admission[c], n=127 (No LTP)						
PBF, %	-.031 (-.075, .000)	.132	0.969	0.927	1.013	.299
FFM, kg	.049 (.004, .110)	**.018**	1.050	1.004	1.099	.324
SLM, kg	.051 (.002, .137)	**.017**	1.053	1.004	1.104	.323
TBW, l	.068 (.015, .138)	**.004**	1.070	1.007	1.138	.326
ICW, l	.103 (.024, .218)	**.013**	1.108	1.005	1.223	.321
ECW, l	.181 (.027, .407)	**.011**	1.198	1.020	1.407	.328
BCM, kg	.072 (.012, .157)	**.013**	1.075	1.004	1.151	.322
SMI	.340 (-.029, .910)	**.050**	1.405	0.946	2.086	.307
PhA	-.632 (-.1252, -.267)	**.021**	0.531	0.285	0.989	.327
Outcome: Complications[c], N=150						
FM, kg	-.029 (-.062, -.007)	**.046**	0.971	0.940	1.002	.341
ECT/TBW, l	6.497 (.039, 8.230)	.4	663.0	0.000	infinite	.321
VFA, cm^2	-.004 (-.010, .000)	.13	0.996	0.177	1.004	.333
PhA	-.547 (-1.104, -.167)	**.031**	0.579	0.344	0.973	.354
Outcome: HLOS[d], N=150 (ICU only)						
PBF, %	-.010 (-.024, .003)	.12	0.990	0.977	1.003	NA
VFA, cm^2	-.001 (-.004, .001)	.16	0.999	0.997	1.000	NA
PhA	-.134 (-.279, .007)	**.037**	0.875	0.765	1.001	NA
Outcome: ICU LOS[d], n=41 (ICU only)						
SMI	-.124 (-.440, .336)	.5	0.883	0.710	1.098	NA
Outcome: Vasopressor days, n=41						
ECW/TBW, l	-16.644 (-49.769, 7.651)	.13	Infinitely small	Infinitely small	234.1	NA

Abbreviations: BIA, bioelectric impedance analysis; BCa CI, bias-corrected accelerated bootstrap confidence interval; LTP, limited treatment plan; PBF, percentage body fat; FFM, fat-free mass; SLM, soft lean mass; ICW, intracellular water; ECW, extracellular water; TBW, total body water; BCM, body cell mass; SMI, skeletal muscle index; PhA, 50 kHz Whole body phase angle; FM, fat mass; VFA, visceral fat area; HLOS, hospital length of stay; NA, not applicable; LOS, length of stay. P-values <0.05 are regarded as statistically significant and displayed in bold. [a]BIA values were entered in a regression model with the specified outcome variable and the covariates age, sex and SOFA score at admission. [b]The odds ratio represents the expected increase in the outcome measure upon an increase of 1 unit of the relevant BIA variable. [c]BIA values entered in a multiple logistic regression model. [d]BIA values entered in a negative binominal regression model.

Table 6. Multiple logistic regression of factors associated with the composite score (n = 125) [a].

Variables	B (95%BCa CI)	P-value	OR[b]	95% CI for Odds Ratio		Nagelkerke R^2
				Lower	Upper	
Age, years	.027 (-.022, .076)	.2	1.027	.981	1.076	.351
Sex (male vs female)	-1.190 (-2.240, -.350)	**.019**	.304	.110	.844	
PhA	-.689 (-1.353, -.303)	**.015**	.502	.281	.898	
RR / min	.121 (.045, .254)	**.001**	1.129	1.047	1.217	

Abbreviations: BCa CI, bias-corrected accelerated bootstrap confidence interval; LTP, limited treatment plan; PhA, 50 kHz Whole body phase angle; RR; respiratory rate. P-values <0.05 are regarded as statistically significant and displayed in bold. [a]Analysis does not include patients with an LTP preventing ICU admission. In two patients respiratory rate was not recorded upon hospital admission. [b]The odds ratio represents the expected increase in the outcome measure upon an increase of 1 unit of the relevant BIA variable.

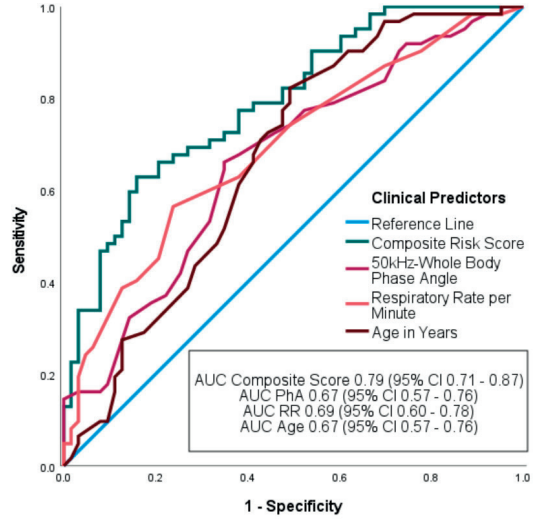

Figure 2. Receiver operating characteristic (ROC) curve of the diagnostic ability of the individual predictors and the composite risk-score in predicting the composite outcome.

DISCUSSION

We aimed to correlate admission BIA body composition with 90-day adverse outcome in 150 COVID-19 patients. After adjusting for age, sex, and disease severity, a lower admission 50 kHz Whole Body Phase Angle at baseline increased the odds of ICU-admission, complications and mortality at 90 days.

Our findings are in line with previous studies, showing correlation between PhA and outcome of disease in multiple patient categories, including COVID-19 patients (1,3,9,10,11,12). Likely, this is explained by the fact that Phase Angle is a reflection the combined effect of premorbid condition, duration and severity of inflammation on cellular quantity and health. However, to ensure interpretation of the association between BIA parameters and outcome of disease independent of severity of disease upon ER presentation, we included SOFA score in our multiple regression analyses and confirmed an independent correlation. Importantly, SOFA score is an ICU instrument and is not routinely calibrated in other settings. Several COVID-19 specific models have been suggested, but none are currently used in our clinical practice. Therefore, we chose to regard respiratory rate as proxy for disease severity. Respiratory rate is a component of the adjusted quick SOFA score and Early Warning Scores that have been validated for use in the ER, and retrospectively related to risk of mortality in elderly COVID-19 patients (13,14,15,16). At baseline, both SOFA score and RR were increased in patients who were eventually admitted to ICU, compared to ward-only patients. Use of RR instead of SOFA score improved the fit of our models whilst increasing significance of the correlation between PhA and all binary outcome parameters.

To explore the added value of baseline PhA to other clinical parameters, a composite risk-score was computed. The addition of PhA improved the discriminative power for the composite of adverse outcome, compared to individual predictors. Based on these results, PhA can and should be considered a valuable component of any future risk-scores concerning COVID-19 and disease course, including ICU-admission. Determination of reference values incorporating age and sex in this population, in order to standardize Phase Angle is the next step in developing an effective and widely applicable risk-score with an effective cut-off value.

Body composition and outcome

The demographics of our cohort are similar to those found in literature (1,3,12). Although the average patient was overweight, body mass index was not different between the ICU and the ward-only group, in concordance with our previous findings (1). This finding further questions the assumption that BMI continues to be related to course of disease in COVID-19 after hospital admission. ICU-patients had increased fat free mass and body water, but lower fat mass, fat percentage and fat area, than ward-only patients. This appears confirmed by the direction of the odds ratios for these parameters and ICU-admission in multiple regression. Nevertheless, the odds ratios for fat, water and lean (fat-free) mass and outcome parameters were each close to one, likely preventing clinical applicability. Similarly, although ECW/TBW was correlated with duration of vasopressor use, the infinitely small odds ratio and its wide 95%-CI negate clinical interpretation based on this sample.

In contrast to our previous cross-sectional research, we did not demonstrate a correlation between fluid overload and adverse outcome. This is most likely explained by the fact that patients had not yet received significant fluid resuscitation, as measurements were performed within 24 hours of hospital admission. Although we previously used correction methods to account for volume overload, we consider baseline measurements as performed in the present cohort methodologically superior.

Strengths and considerations

There are several strengths to this study. We were able to prospectively include 150 proven COVID-19 patients, which to our knowledge forms the largest published BIA COVID-19 cohort to date. This allowed us to confirm the preliminary results of our cross-sectional study in the same study setting, with improved methodology. Our prospective design allowed BIA measurements to be performed it a protocolled manner, within 24 hours of hospital admission. Hereby, the influence of altered hydration status, an important concern in BIA interpretation, can be considered to be negligible (17).

This study is nevertheless subject to several considerations. During the study period, only 150 (31%) of all admitted COVID-19 patients were considered for inclusion, mainly due to logistical issues. To ensure high internal validity of our results, we only included patients with a PCR-proven SARS-CoV-2 infection in whom all measurements could be performed within 24 hours after hospital admission. Due to laboratory logistics and restricted researcher availability, this meant not all patients could be considered. However, there is no reason to suspect this introduced any patient-related selection bias into the current sample. In addition, issues relating power due to the restricted inclusion are unlikely, as the prevalence of the composite outcome score was 51% in the sample.

It is not uncommon that limited treatment plans are agreed upon at admission of patients of advanced age or with relevant comorbidities. These LTPs prevent admission to the ICU even if the severity of the disease would otherwise dictate it. To prevent confounding, we did not include LTP patients in analyses regarding the association between clinical characteristics and ICU-admission, including the composite outcome score. This reduced the sample size for these outcomes, although we do not expect this has let the results to be underpowered. In contrast, the analyses regarding the ICU population only included 41 patients, providing a possible explanation for the insignificant results regarding these outcomes.

CONCLUSION

We assessed admission body composition using BIA in COVID-19 patients and correlated it with 90-day adverse outcome, whilst controlling for age, sex and severity of disease. A low Phase Angle significantly, independently increased the odds of ICU-admission, morbidity and mortality. As PhA is easy and quick to determine, it should be considered as an addition to any baseline clinical risk-score. Determination of reference values incorporating age and sex in this population is the next step in developing an effective and widely applicable risk-score with an effective cut-off value.

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SUPPLEMENTAL TABLES

Supplemental Table 1. 30-day mortality simple binary logistic regression n=150[a]**.**

	Exp(B)	P-value	Transformation
Age, y	1.147	**<.001**	-
Sex	.899	.9	-
DM type 2	1.286	.7	-
COPD/Asthma	1.801	.3	-
CVD	9.562	**<.001**	-
BMI, kg/m^2	.998	>0.9	-
SOFA	1.392	**.065**	-
RR / min	1.077	**.034**	-
CFS	1.579	**.001**	-
ICW, L	.987	.9	-
ECW, L	.978	.8	-
ECW/TBW	3.390	.9	-
TBW, L	.993	.9	-
FO, L	.949	.9	-
FFM, kg	.995	.9	-
FM, kg	.983	.5	-
PBF, %	.985	.6	-
VFA, cm^2	.996	.3	-
SMI, kg/m^2	.827	.5	-
SLM, kg	.994	.8	-
PhA	.651	.3	-
BCM, kg	.992	.9	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 2. 90-day mortality simple binary logistic regression n=150[a].

	Exp(B)	P-value	Transformation
Age, y	1.153	**<.001**	-
Sex	.744	.6	-
DM type 2	1.441	.5	-
COPD/Asthma	1.496	.5	-
CVD	11.900	**<.001**	-
BMI, kg/m^2	1.054	.4	-
SOFA	1.387	**.057**	-
RR / min	1.078	**.027**	-
CFS	1.649	<.001	-
ICW, L	1.019	.8	-
ECW, L	1.031	.8	-
ECW/TBW	1.577	.9	Logarithmic
TBW, L	1.013	.8	-
FO, L	1.033	.9	-
FFM, kg	1.009	.8	-
FM, kg	.997	.9	-
PBF, %	.992	.8	-
VFA, cm^2	.998	.6	-
SMI, kg/m^2	.999	>.9	-
SLM, kg	1.009	.8	-
PhA	.619	.2	-
BCM, kg	1.014	.8	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 3. ICU admission simple binary logistic regression excluding LTP n=127[a].

	Exp(B)	P-value	Transformation
Age, y	1.034	**.057**	-
Sex	.705	.4	-
DM type 2	1.281	.6	-
COPD/Asthma	1.142	.8	-
CVD	.938	.9	-
BMI, kg/m^2	1.054	.15	-
SOFA	2.875	**<.001**	-
RR / min	1.171	**<.001**	-
CFS	.873	.3	-
ICW, L	3.392	**.009**	Square root
ECW, L	5.170	**.007**	-
ECW/TBW	.021	.9	-
TBW, L	2.764	**.006**	-
FO, L	1.229	.5	-
FFM, kg	2.329	**.008**	Square root
FM, kg	.986	.4	-
PBF, %	.966	**.081**	-
VFA, cm^2	.995	.12	-
SMI, kg/m^2	1.494	**.024**	-
SLM, kg	2.391	**.008**	-
PhA	.540	**.032**	-
BCM, kg	2.792	**.009**	Square root

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 4. Complications including 90 day mortality simple binary logistic regression n=150[a].

	Exp(B)	P-value	Transformation
Age, y	3<.001	**<.001**	Square root
Sex	.429	**.021**	-
DM type 2	1.904	**.084**	-
COPD/Asthma	.868	.7	-
CVD	1.130	.7	-
BMI, kg/m^2	.955	.2	-
SOFA	1.451	**.003**	-
RR / min	1.115	**<.001**	-
CFS	1.129	.2	-
ICW, L	.957	.3	-
ECW, L	.940	.3	-
ECW/TBW	7.462	**.004**	Logarithmic
TBW, L	.978	.4	-
FO, L	1.121	.7	-
FFM, kg	.981	.3	-
FM, kg	.974	**.075**	-
PBF, %	.980	.2	-
VFA, cm^2	.996	**.097**	-
SMI, kg/m^2	.854	.4	-
SLM, kg	.980	.3	-
PhA	.643	**.062**	-
BCM, kg	.970	.3	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 5. Composite simple binary logistic regression excluding LTP n=127[a].

	Exp(B)	P-value	Transformation
Age, y	1.064	**<.001**	-
Sex	.367	**.015**	-
DM type 2	1.109	.8	-
COPD/Asthma	.840	.7	-
CVD	.864	.7	-
BMI, kg/m^2	1.001	>.9	-
SOFA	1.897	**<.001**	-
RR / min	1.124	**.001**	-
CFS	1.020	.9	-
ICW, L	1.003	.9	-
ECW, L	1.018	.8	-
ECW/TBW	1316	.3	Logarithmic
TBW, L	1.008	.7	-
FO, L	1.284	.4	-
FFM, kg	1.003	.9	-
FM, kg	.983	.3	-
PBF, %	.982	.3	-
VFA, cm^2	.997	.2	-
SMI, kg/m^2	1.008	>.9	-
SLM, kg	1.003	.9	-
PhA	.507	**.018**	-
BCM, kg	1.003	.9	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 6. HLOS negative binomial regression n=150[a].

	Exp(B)	P-value	Transformation
Age, y	1.983	**.057**	Logarithmic
Sex	9.340	**<.001**	-
DM type 2	.847	.3	-
COPD/Asthma	1.013	.9	-
CVD	1.044	.8	-
BMI, kg/m^2	.997	.8	-
SOFA	1.382	**<.001**	-
RR / min	1.035	**<.001**	-
CFS	.914	**.027**	-
ICW, L	1.023	.1	-
ECW, L	1.035	.2	-
ECW/TBW	.056	.3	Logarithmic
TBW, L	1.015	.1	-
FO, L	.982	.9	-
FFM, kg	1.010	.1	-
FM, kg	.992	.1	-
PBF, %	.989	**.075**	-
VFA, cm^2	.998	**.091**	-
SMI, kg/m^2	1.067	.3	-
SLM, kg	1.011	.1	-
PhA	.871	**.047**	-
BCM, kg	1.016	.1	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 7. ICULOS Negative binomial regression ICU only n=41[a].

	Exp(B)	P-value	Transformation
Age, y	1.015	.4	-
Sex	1.354	.3	-
DM type 2	.642	**.077**	-
COPD/Asthma	1.028	.9	-
CVD	1.251	.4	-
BMI, kg/m^2	.963	.1	-
SOFA	1.146	.2	-
RR / min	1.015	.3	-
CFS	.962	.7	-
ICW, L	.975	.4	-
ECW, L	.946	.3	-
ECW/TBW	<.001	.1	Logarithmic
TBW, L	.982	.4	-
FO, L	.801	.2	-
FFM, kg	.986	.3	-
FM, kg	.986	.2	-
PBF, %	.987	.4	-
VFA, cm^2	.998	.3	-
SMI, kg/m^2	.904	.3	-
SLM, kg	.987	.4	-
PhA	.987	>.9	-
BCM, kg	.983	.4	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 8. Ventilation-days negative binomial regression ICU only n=41[a].

	Exp(B)	P-value	Transformation
Age, y	1.043	.4	-
Sex	1.338	.7	-
DM type 2	.462	.2	-
COPD/Asthma	1.318	.7	-
CVD	1.255	.8	-
BMI, kg/m^2	.923	.2	-
SOFA	1.308	.3	-
RR / min	1.022	.5	-
CFS	1.009	>.9	-
ICW, L	.930	.4	-
ECW, L	.866	.3	-
ECW/TBW	-inf	.3	-
TBW, L	.951	.4	-
FO, L	.668	.4	-
FFM, kg	.962	.4	-
FM, kg	.977	.4	-
PBF, %	.983	.7	-
VFA, cm^2	.997	.6	-
SMI, kg/m^2	.793	.4	-
SLM, kg	.963	.4	-
PhA	.960	.9	-
BCM, kg	.950	.4	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Supplemental Table 9. Vasopression days negative binomial regression ICU only n=41[a].

	Exp(B)	P-value	Transformation
Age, y	1.041	.4	-
Sex	1.216	.8	-
DM type 2	.586	.3	-
COPD/Asthma	.786	.7	-
CVD	1.117	.8	-
BMI, kg/m^2	.951	.4	-
SOFA	1.356	.1	-
RR / min	1.038	.2	-
CFS	1.018	.9	-
ICW, L	.947	.5	-
ECW, L	.868	.2	-
ECW/TBW	-inf	**.088**	-
TBW, L	.958	.4	-
FO, L	.570	.1	-
FFM, kg	.968	.3	-
FM, kg	.979	.3	-
PBF, %	.980	.5	-
VFA, cm^2	.997	.4	-
SMI, kg/m^2	.870	.5	-
SLM, kg	.969	.4	-
PhA	1.188	.7	-
BCM, kg	.963	.5	-

[a] Sex and age were added to all models that included body composition parameters. P-values <0.10 were subsequently entered into multiple regression and are presented in bold. Abbreviations: SOFA, sequential organ failure assessment; RR / MIN, respiratory rate per minute; CFS, clinical frailty scale; COPD, chronic obstructive pulmonary disease; CVD, chronic vascular disease; BMI, Kg/m^2, body mass index; ICW, intracellular water; ECW, extracellular water; TBW, total body water; FO, fluid overload; FFM, fat-free mass; FM, fat mass; PBF, percentage body fat; VFA, visceral fat area; SMI, skeletal muscle index; SLM, soft lean mass; PhA, 50 kHz Whole body phase angle; BCM, body cell mass.

Chapter 9

Physical recovery of COVID-19 pneumosepsis intensive care survivors compared with non-COVID pneumosepsis intensive care survivors during post-intensive care hospitalization: The RECOVID retrospective cohort study

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ABSTRACT

Background

Coronavirus disease 2019 (COVID-19) pneumosepsis survivors are at a high risk of developing intensive care unit (ICU)-acquired weakness (ICUAW) because of high incidence of acute respiratory distress syndrome and the common need for prolonged invasive ventilation. It remains unknown whether regular postpneumosepsis physical rehabilitation strategies are suitable for this extraordinary patient category.

Methods

We retrospectively compared the physical recovery of COVID-19 and non-COVID pneumosepsis ICU survivors during post-ICU hospitalization, defined as the difference in performance on the Medical Research Council Sum-Score (MRC-SS), Chelsea Critical Care Physical Assessment tool (CPAx), and percentage of predicted handgrip strength (POP-HGS). An analysis of covariance model was built using age, sex, Barthel index, body mass index, admission Acute Physiology And Chronic Health Evaluation II score, adequacy of protein delivery during ICU stay, and ward length of stay as covariates.

Results

Thirty-five COVID-19 ICU patients could be compared with 21 non-COVID pneumosepsis ICU survivors. All patients scored ≤48 on the MRC-SS at ICU discharge, indicating ICUAW. When controlling for covariates, COVID-19 patients performed worse on all physical assessments upon ICU discharge, but had improved more at hospital discharge on the MRC-SS ($\eta 2 = 0.214$, $P = .002$) and CPAx ($\eta 2 = 0.153$, $P = .011$). POP-HGS remained lower in COVID-19 patients throughout hospital stay.

Conclusion

COVID-19 ICU survivors are vulnerable to ICUAW, but they show better tendency towards physical rehabilitation than non-COVID pneumosepsis ICU survivors during the post-ICU hospitalization period regarding MRC-SS and CPAx. COVID-19 ICU patients might benefit from early, more intensive physical therapy.

BACKGROUND

Intensive care units (ICUs) worldwide have been confronted with a new and distinct form of pneumosepsis: coronavirus disease 2019 (COVID-19) sepsis. The long-term treatment needs of COVID-19 pneumosepsis survivors are not yet fully appreciated, but the development of acute respiratory distress syndrome and the need for prolonged invasive ventilation puts them at a high risk of developing ICU-acquired weakness (ICUAW) and the associated postintensive care syndrome (1, 2). It has been tentatively suggested that this group might benefit from early mobilization and physical exercise strategies, but this is mostly based on experience in related diseases and expert opinion (3). Results of any randomized controlled trials have not yet been published.

ICUAW is a clinical diagnosis. The Medical Research Council Sum-Score (MRC-SS) and handgrip dynamometry constitute the criterion standard for diagnosis. With MRC-SS, muscle strength is assessed in 12 muscle groups and then individual scores are combined into a sum-score, which yields an overall estimation of motor function. Summed scores below 48 out of 60 and below 36 out of 60 indicate significant and severe weakness, respectively (4). Handgrip strength (HGS) is measured in kilograms and can be converted to a percentage of predicted (POP) score based on reference values to increase comparability. MRC-SS and (POP) HGS are the most well-known methods for assessment in the ICU population. However, it has been suggested that the lesser-known Chelsea Critical Care Physical Assessment tool (CPAx) might provide benefits, especially in the COVID-19 ICU population, as it is a more holistic measurement tool concerning functional recovery and incorporating respiratory functioning (5).

In this retrospective cohort study, we aim to compare the (course of) physical functioning of COVID-19 pneumosepsis survivors to non-COVID pneumosepsis survivors at ICU and hospital discharge based on several physical performance scores. Our results may shed light on the optimum method for assessing physical performance in this large group of patients, as well as help to identify the physical therapy approach they will likely require.

METHODS

Study setting and design

We performed a retrospective cohort study in the ICU of the Gelderse Vallei Hospital, a University-affiliated teaching hospital in Ede, the Netherlands.

All ICU patients at our hospital receive standardized early rehabilitation therapy each weekday from ICU admission to hospital discharge. This is a progressive multistep program

adapted from the program described by Sommers et al (6), and Schweickert et al (7), beginning with passive range of motion exercises, followed by (partially) active exercises and progressive mobilization to the edge of the bed or to a chair, standing, and walking. The content of the daily exercise and mobilization regimen as well as the intensity of the applied interventions are adapted to the patient's cardiorespiratory status, level of wakefulness, cooperation, global muscle strength, and tolerance. Exercise and mobilization interventions are progressively continued on the ward upon ICU discharge.

Energy and protein targets in the ICU are calculated by our computerized nutrition protocol based on the Food and Agricultural Organization (FAO) and World Health Organization (WHO) formulae (8). Protein targets are set according to actual (body mass index (BMI) < 27), corrected (BMI of 27–30; regression to BMI of 27), or ideal body weight (BMI >30; regression to a BMI of 21 in women and a BMI of 22.5 in men) and amount to 1.5 g/kg/day in a BMI of <30, 2.0 g/kg/day in a BMI of 30–40, or 2.5 g/kg/day in a BMI of ≥40. Energy targets are based on calculated resting energy expenditure (REE), with an addition of 20% or 30%, in case of mandatory or spontaneous invasive ventilation, respectively. Targets are adjusted when REE is measured by indirect calorimetry or in case of refeeding syndrome. A progressive feeding strategy towards 100% of targets at admission-day four is used to prevent overfeeding. Actual nutrition and nonnutrition energy and protein delivery are automatically calculated hourly.

Population

Data collected from anonymized records of COVID-19 ICU patients included in the Bioelectric Impedance Analysis in COVID-19 positive patients (BIAC-19) study (Netherlands Trial Register [NTR] NL8562) and the Resting energy expenditure in mechanically ventilated patients in the ICU and during COnV alescence (RECOVER-energy ICU) study (NTR NL8907) were compared with those of a historical cohort of non-COVID pneumosepsis ICU patients, previously collected as part of the RECOVER-energy ICU study and the Mitochondria Intensive Care (MIC) study (NTR NL6969). Inclusion and exclusion criteria of these observational trials can be found in the elsewhere in this thesis. Only patients with polymerase chain reaction–proven COVID-19, or non-COVID pneumosepsis, who had survived the ICU were included in the pooled database to accommodate the research question. Patients transferred from the ICU to a different hospital (COVID-19, n = 5) or discharged to the ward in palliative care (COVID-19, n = 1) were excluded, as physical functioning assessments had not been performed in these cases.

Study parameters

In all prospective studies, physical functioning was assessed with the MRC-SS, CPAx, and measurement of HGS by a trained ICU physiotherapist upon ICU and hospital discharge. An MRC-SS ≤ 48 was considered indicative of ICUAW. HGS was converted to a POP-HGS

based on age and sex using a comparable reference population (9). Other parameters considered were age, sex, BMI, comorbidities (including type 2 diabetes, hypertension, and chronic obstructive pulmonary disease), Barthel index, Acute Physiology And Chronic Health Evaluation (APACHE) II score, Nutrition Risk in Critically Ill (NUTRIC) score, percentage of protein and energy delivered of target during ICU stay, ICU length of stay (ICULOS), hospital length of stay (HLOS), ward length of stay (LOS), duration of mechanical ventilation, and use of neuromuscular blocking agents and immunosuppressive drugs. Steroids were considered if administered continuously or in a singular dose equivalent of ≥100 mg of hydrocortisone. Neuromuscular blocking agents were considered if they were administered continuously for ≥2 h, to exclude anesthetic induction medication.

Statistical analyses

IBM SPSS statistics 27 (IBM Corp, Armonk, NY, USA) was used for all analyses. Continuous values are reported as mean and bias corrected and accelerated bootstrap 95% CI to facilitate comparisons between data with a difference in distribution between the cohorts and to minimize the effect of outliers. Discrete data are presented as numbers (percentages). Normality of the data was visually assessed using the quantile-quantile plot. When inconclusive, the Shapiro-Wilk test was adhered. Differences between groups were assessed using independent samples of t-tests for continuous data or chi-squared tests for categorical data. When test assumptions were not met, Mann-Whitney U tests or Fisher's exact tests were used, respectively. An analysis of covariance (ANCOVA) model was built assessing the association between the admission diagnosis (COVID-19 or non-COVID pneumosepsis) and the difference between physical assessment scores upon ICU and hospital discharge. Empirically, age, sex, and ward LOS were added into the model as covariates. In addition, parameters with a significant difference between the means (COVID-19 vs non-COVID patients) were considered. Nonnormally distributed data were transformed (ward LOS) or categorized (Barthel index and BMI). Bias corrected and accelerated bootstrap partial eta-square ($\eta 2$) was used to estimate effect size; cut-offs were 0.01, 0.06, and 0.14 for a small, medium, or large effect, respectively. Only two-sided analyses were used. P-values <.05 were considered statistically significant. Sensitivity analyses using ICULOS, HLOS, or duration of ventilation as covariates instead of the ward LOS were performed.

Figures representing statistics were made using GraphPad Prism version 8.0.0. for Windows (GraphPad Software, San Diego, CA, USA). The error bars in the figures representing the 95%-CI of the mean of the repeated measures on the physical functioning assessment tests on ICU and hospital discharge were adjusted for the between-subject variability.

RESULTS

The pooled data set included 35 COVID-19 patients (20 BIAC-19 patients and 15 RECOVER-energy patients) admitted to the ICU between March 2020 and 2021 and 21 non-COVID pneumosepsis ICU patients (19 MIC patients and 2 RECOVER-energy patients) admitted between February 2018 and October 2020. Baseline characteristics and outcome measures are summarized and compared in Table 1 and Table 2, respectively. Four (19%) of the non-COVID patients died in the ward, preventing hospital discharge measurements. One COVID patient was not scored on CPAx and HGS upon hospital discharge for reasons unknown.

Table 1. Statistical comparison of baseline characteristics

	COVID-19 Pneumosepsis (n=35)	Non-COVID Pneumosepsis (n=21)	P-value
Baseline Characteristics			
Age, years	67 (64-70)	63 (57-69)	.16
Sex, male	24 (69%)	18 (86%)	.2
BMI, kg/m^2	29 (28-31)	26 (24-28)	**.008**
COPD, (%)	7 (20%)	4 (19%)	1
Hypertension, (%)	9 (26%)	6 (29%)	1
Diabetes Mellitus, (%)	8 (23%)	1 (5%)	.13
APACHE-II score	15 (13-17)	18 (15-22)	**.054**
Barthel-index	20 (19-20)	18 (16-20)	**.041**
NUTRIC score	3 (3-4)	5 (4-5)	**.026**

Note: Continuous values are reported as mean (bias corrected and accelerated bootstrap 95%-CI) and discrete data as numbers (percentages). Differences between groups were assessed using independent samples t-tests for continuous data or chi-square tests for categorical data. When test assumptions were not met, Mann-Whitney U tests or Fisher's exact tests were used, respectively. P-values <.05 for statistical comparisons between cohort means were considered statistically significant and are signified with an asterisk *. Abbreviations: APACHE II, Acute Physiology And Chronic Health Evaluation II; BMI, body mass index; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; NUTRIC, Nutrition Risk in the Critically Ill.

COVID-19 patients performed worse than non-COVID patients on all assessments—except for POP-HGS, which remained lower throughout hospital stay—upon ICU discharge and better at hospital discharge (Figure 1). All patients scored ≤48 on the MRC-SS at ICU discharge, indicating ICUAW. 2 ICUAW had resolved in more COVID-19 than non-COVID patients upon hospital discharge, although not significantly (17 [49%] vs 4 [25%], p =.14).

Table 2. Statistical comparison of outcome and physical functioning of the patients

	COVID-19 Pneumosepsis		Non-COVID Pneumosepsis		P-value
	(n=35)[a]		(n=21) [a]		
ICU Length of Stay, days	22 (18-26)		18 (12-25)		.13
Ward Length of Stay, days	9 (8-11)		9 (6-12)		.17
Hospital Length of Stay, days	32 (26-37)		26 (18-34)		**.033**
Duration of Invasive Mechanical Ventilation, days	17 (13-21)		12 (6-18)		.19
Duration of Neuromuscular Blocking agents, days	3 (2-4)		1 (0-2)		**.001**
Duration of Steroid Use, days	1 (0-1)		1 (1-2)		.17
Protein Delivered of Target, %	78 (62-83)		57 (41-70)		**.018**
Energy Delivered of Target, %	75 (67-81)		59 (43-73)		.070
In-hospital Mortality, (%)	0 (0%)		4 (19%)		**.016**
28-Day Mortality, (%)	0 (0%)		3 (14%)		**.048**
	Physical Functioning Scores				
MRC-SS ICU	36 (34-39)		41 (36-46)		.071
MRC-SS Hospital	47 (45-50)		45 (39-49)	n=16	.15
Delta MRC-SS[b]	11 (9-14)		3 (1-6)	n=16	**<.001**
CPAx ICU	23 (21-25)		31 (25-36)		**.003**
CPAx Hospital	39 (36-42)	n=34	40 (35-45)	n=16	.9
Delta CPAx[b]	16 (14-19)	n=34	10 (6-13)	n=16	**.005**
POP-HGS ICU, %	32 (25-38)	n=34	48 (35-60)	n=20	**.020**
POP-HGS Hospital, %	50 (44-57)	n=34	59 (46-71)	n=16	.4
Delta POP-HGS[b] , %	18 (14-23)	n=34	12 (8-16)	n=15	.12

Note: Continuous values are reported as mean (Bias corrected and accelerated bootstrap 95%-confidence interval), discrete data as numbers (%). Differences between groups were assessed using independent samples t-tests for continuous data or chi-square tests for categorical data. When test assumptions were not met, Mann-Whitney U tests or Fisher's exact tests were used, respectively. P-values <.05 for statistical comparisons between cohort means were considered statistically significant and are signified with an asterisk *. Abbreviations: COVID-19, coronavirus disease 2019; CPAx, Chelsea Critical Care Physical Assessment tool; ICU, intensive care unit; MRC-SS, Medical Research Council Sum-Score; POP-HGS, percentage of predicted handgrip strength. [a] Number of observations, unless otherwise stated, due to missing data. [b] Absolute difference between ICU and hospital discharge.

The association between ICU diagnosis (COVID-19 vs non-COVID) and change (delta) in physical functioning between ICU and hospital discharge was assessed using a univariate linear model (ANCOVA) using age, sex, Barthel index category (normal, 20; reduced, <20), admission APACHE II score, BMI category (normal, 20–24; overweight, 25–29; obese, 30–40), percentage of protein target delivered, and (the square root of) ward LOS as covariates.

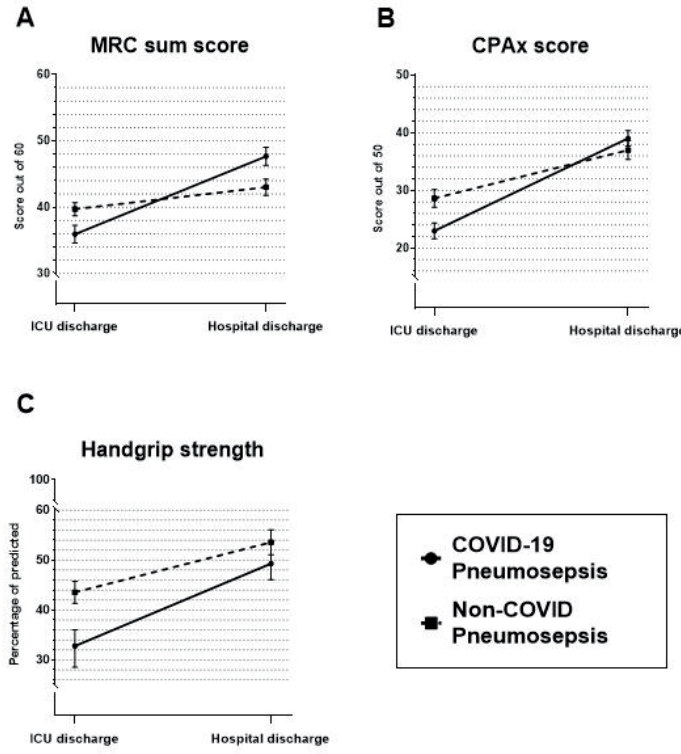

Figure 1.

Comparison of mean (A) MRC sum scores, (B) CPAx scores, and (C) percentage of predicted handgrip strength measurements for COVID-19 sepsis ICU-survivors and non-COVID pneumosepsis ICU-survivors at ICU and hospital discharge. Error bars representing 95%-CI were adjusted for the between-subject variability. COVID-19, coronavirus disease 2019; CPAx, Chelsea Critical Care Physical Assessment tool; ICU, intensive care unit; MRC, Medical Research Council.

The ICU diagnosis was the only covariate with a significant correlation with delta MRC-SS ($F[1, 50] = 5.118$, $p = .002$, partial $\eta 2 = 0.214$) and delta CPAx ($F[1, 49] = 5.496$, $p = .011$, partial $\eta 2 = 0.153$) but not with delta POP-HGS ($F[1, 48] = 0.125$, $p = .6$, partial $\eta 2 = 0.005$). The assumptions for normality of the residuals and equal variances were visually inspected, and they were good.

Separate analyses that used HLOS, ICULOS, or duration of ventilation instead of ward LOS did not challenge the main findings.

DISCUSSION

In accordance with previous research, we observed that COVID-19 ICU patients are prone to ICUAW at ICU and hospital discharge, defined as an MRC-SS of ≤48 (1, 10, 11).

Furthermore, we showed that compared with other pneumosepsis patients, COVID-19 patients scored lower on physical functioning tests upon ICU discharge. Contrastingly, COVID-19 patients showed significantly more improvement of physical functioning on the MRC-SS and the CPAx instruments during post-ICU hospital stay, regardless of baseline characteristics, adequacy of protein administration during ICU stay, and duration of ward LOS. The effect sizes of ICU diagnosis (COVID-19 vs non-COVID pneumosepsis) on delta MRC-SS and delta CPAx, described as partial η2, were large.

MRC-SS is a well-validated, relatively easy bedside method to establish muscle strength, which is sensitive to identify ICUAW, and reliably predicts hospital mortality, days on a ventilator, ICULOS, and HLOS with excellent interrater reliability (12, 13, 14). In our study, ICUAW resolved in more COVID-19 than non-COVID patients during the post-ICU hospitalization period, although not significantly, likely due to lack of power.

Although the MRC-SS is widely used, it is limited in that it focuses solely on assessment of muscle strength. CPAx was developed as a holistic approach to assessing physical functioning, including respiratory function (15). The CPAx is an outcome measure designed to assess 10 domains of physical ability in the post-ICU patient: respiratory function, cough, bed mobility, supine to sitting on the edge of the bed, dynamic sitting, sit to stand, standing balance, transferring from bed to chair, stepping, and grip strength. Use of CPAx is not yet ubiquitous; however, it has been translated into several languages and correlates well to other methods such as MRC-SS (15, 16, 17, 18, 19). Taken together, the advantages of CPAx has experts to advocate for its use specifically in the functional assessment of post-ICU COVID-19 patients(5).

In contrast to the MRC-SS and the CPAx, the change in POP-HGS between ICU and hospital discharge in our study was not different for COVID-19 patients compared with non-COVID pneumosepsis patients. In the past, HGS has been shown to correlate with MRC-SS; however, it has not consistently been shown to predict outcome across the heterogenic ICU population (12). This may be due to the lack of discriminatory power of HGS, as a HGS of 0 kg has previously been shown to be associated with acceptable or even normal MRC-SS measurements (12). In addition, our study may have been underpowered to detect a significant change in HGS.

Limitations and considerations

Our results are subject to the limitations of a retrospective approach. Because of ethical considerations, we were only able to include anonymized records of patients who had previously consented to collection of data in the context of a prospective trial. These trials each had inclusion and exclusion criteria, which may have introduced selection bias into our study. However, our regression model incorporated both empirical covariates

and those that differed on baseline between the cohorts. Thus, we assumed that we have minimized any inclusion bias. Nevertheless, our results require the external validation of a prospective design.

To prevent overfitting of the model in a relatively small sample size, we were not able to add all parameters that differed between the cohorts to the eventual model. We chose not to include NUTRIC score, as it incorporates age, comorbidities, and APACHE II scores, which were already considered separately. Furthermore, we did not consider duration of use of neuromuscular blocking agents at this point. However, as duration of use was longer in the COVID-19 cohort, which performed worse upon ICU discharge, and use of neuromuscular blocking agents is associated with increased muscle weakness, inclusion of this parameter in the model likely would not have changed the direction of our results. We collected several parameters reflecting LOS and duration of therapy, which may differ between a COVID-19 and a non-COVID pneumosepsis ICU cohort and independently influence the outcome of physical therapy tests. Because of multicollinearity, we were not able to add all of these as simultaneously covariates in the ANCOVA model. We chose to use ward LOS, as this best reflects the timespan between the repeated measures. Repeating the analyses with ward LOS substituted with any of the other duration parameters did not change the main results and thus omitting the other variables in the main analysis is unlikely to have biased our results. At this point, delivery of macronutrients is only reliably recorded in the ICU at our hospital, and thus we could not report on adequacy of nutrition on the general ward. In future designs, this parameter might be considered if feasible.

We do not routinely measure muscle strength at hospital, nor ICU admission. However, comparing discharge and admission scores would be very insightful in any prospective trials to come.

CONCLUSION

COVID-19 ICU survivors are a vulnerable group concerning ICUAW, but they show better tendency towards physical rehabilitation than non-COVID pneumosepsis ICU survivors during the post-ICU hospitalization period. COVID-19 ICU patients might therefore benefit from early, more intensive physical therapy. Furthermore, the use of the CPAx yielded similar findings as the MRC-SS in our population, and provides theoretical benefits for use in (post-)ICU COVID-19 patients.

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Chapter 10

Protein requirements and provision in hospitalised COVID-19 ward and ICU patients: Agreement between calculations based on body weight and height, and measured bioimpedance lean body mass

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ABSTRACT

Background

A large proportion of hospitalised COVID-19 patients are overweight. There is no consensus in the literature on how lean body mass (LBM) can best be estimated to adequately guide nutritional protein recommendations in hospitalised patients who are not at an ideal weight. We aim to explore which method best agrees with lean body mass as measured by bioelectric impedance (LBM_{BIA}) in this population.

Methods

LBM was calculated by five commonly used methods for 150 hospitalised COVID-19 patients previously included in the BIAC-19 study; total body weight, regression to a BMI of 22.5, regression to BMI 27.5 when BMI>30, and the equations described by Gallagher and the ESPEN ICU guideline. Error-standard plots were used to assess agreement and bias compared to LBM_{BIA}. The actual protein provided to ICU patients during their stay was compared to targets set using LBM_{BIA} and LBM calculated by other methods.

Results

All methods to calculate LBM suffered from overestimation, underestimation, fixed- and proportional bias and wide limits of agreement compared to LBMBIA. Bias was inconsistent across sex and BMI subgroups. Twenty-eight ICU patients received a mean of 51.19 (95%-BCa CI 37.1;64.1) grams of protein daily, accumulating to a mean of 61.6% (95%-BCa CI 43.2;80.8) of $Target_{BIA}$ during their ICU stay. The percentage received of the target as calculated by the $LBM_{Gallagher}$ method for males was the only one to not differ significantly from the percentage received of $Target_{BIA}$ (mean difference 1.4% (95%-BCa CI -1.3;4.6) p = 1.0).

Conclusions

We could not identify a mathematical method for calculating LBM that had an acceptable agreement with LBM as derived from BIA for males and females across all BMI subgroups in our hospitalised COVID-19 population. Consequently, discrepancies when assessing the adequacy of protein provision in ICU patients were found. We strongly advise using baseline LBM_{BIA} to guide protein dosing if possible. In the absence of BIA, using a method that overestimates LBM in all categories may be the only way to minimise underdosing of nutritional protein.

BACKGROUND

Obesity is a significant independent risk factor for hospitalisation in Coronavirus Disease 2019 (COVID-19) patients (1,2). A large proportion of hospitalised COVID-19 patients, and by extend, ICU patients, are thus overweight. The prevalence of sarcopenic obesity has increased infection rates and morbidity related to COVID-19 (3). A positive correlation between high nutritional risk and adverse clinical outcomes of COVID-19 has been observed (4).

It is suggested that a high protein diet is beneficial during COVID-19 (3), as protein provision may prevent further breakdown of muscle protein for the purpose of gluconeogenesis, and thereby prevent the patient from going into a further catabolic state. Nutrition guidelines advise increasing the protein quantity that is provided as the illness becomes more severe, but vary between prescribing 1.2–2.5 g/kg of protein a day in the intensive care unit (ICU) (5, 6, 7). One study showed that although targets of >1.2 g/kg/day of protein were hard to achieve in COVID-19 ICU patients, a supply of at least 0.8 g/ideal body weight (IBW)/day was already related to lower mortality rates (8).

However, setting protein targets is challenging when patients are not at IBW. Because the overweight (Body mass index (BMI) ≥ 25 kg/m^2), or obese body (BMI≥ 30 kg/m^2) usually contains less protein per kilogram of body weight, the use of total body weight (TBW) likely results in an overestimation of protein needs in overweight and obese persons. Currently, numerous mathematical formulas try to account for variations in body composition (such as between biological sexes) by estimating fat-free or lean body mass (LBM), which is assumed to be the true determinant of protein requirement [9]. It is still unclear which method is superior, which is reflected by discrepancies, or vagueness in recommendations between, and sometimes within, nutritional guidelines, that either suggest multiple methods, or fail to state whether TBM, LBM, or IBW should be used (7, 9, 10, 11). Slight variations in the definitions of fat-free mass (FFM) and LBM between sources further confuse the discussion.

Bioelectric impedance analysis (BIA) is a technique that calculates the volume of body water compartments through the use of measured electric reactance and resistance. The incorporated software then derives LBM through validated regression analyses based on a healthy reference population. BIA derived LBM (LBM_{BIA}) for calculating protein needs has substantial theoretical advantages over mathematical methods regarding body composition (12). In addition, BIA measurements can be performed at the bedside, in contrast to other direct methods such as dual-energy X-ray absorptiometry. However, BIA is not ubiquitously available and can pose challenges related to disinfection when used on

a high volume of patients with a transmittable disease such as COVID-19. Therefore, it is worth exploring the agreement between BIA and commonly used mathematical formulas.

We previously conducted a prospective observational study in which all hospitalised patients for COVID-19 underwent BIA measurements within 24 h of hospital admission (13). The current post-hoc study compares the agreement between LBM_{BIA} and five mathematical methods in estimating LBM in this COVID-19 population. In addition, we retrospectively compare protein provision adequacy in our COVID-19 ICU population based on LBM_{BIA} , to that based on LBM predicted by other methods.

METHODS

For this post-hoc sub-study, baseline data previously collected for the prospective BIAC-19 study were used. The Bioelectric impedance body composition and phase angle concerning 90-day adverse outcome in hospitalised COVID-19 ward and ICU patients: the prospective BIAC-19 study aimed to associate baseline (<24 h of hospital admission) BIA body composition parameters with 90-day adverse outcome of COVID-19 (13). The BIAC-19 study protocol has been registered in the Netherlands Trial Register (number NL8562).

Study setting

The study was performed between April 10th and 17th, 2020, and again between October 10th 2020 and February 11th 2021, at Gelderse Vallei Hospital, a teaching hospital in Ede, The Netherlands. The hospital has two ICU units, with a combined capacity of 18 beds. Thirty-eight general ward COVID-19 beds were available during the study period.

Protein provision ward

Protein targets in the wards are set according to actual (BMI 20–30 kg/m^2) or corrected body weight (BMI <20 kg/m^2 adjusted to 20 kg/m^2; BMI >30 kg/m^2 adjusted to 27 kg/m^2). In addition, the Gallagher method is described in the local protocol. Gallagher et al. developed an equation to calculate percentage body fat through sex, age, BMI, ethnicity and regression models based on the measured (by 4-compartment model (4C) or dual-energy X-ray absorptiometry (DXA)) body fat of 1626 healthy adults with a BMI ≤35 kg/m^2 (14). The Dutch dietary guidelines use a transformation of the original Gallagher formula, to approximate LBM at which protein provision is targeted (15). This method is currently not routinely used in our hospital but is mentioned in the protocols as a potentially superior method (9,16).

Protein provision ICU

Protein targets in the ICU are calculated by our computerized nutrition protocol, and are set according to actual (BMI <27 kg/m^2), corrected (BMI 27–30 kg/m^2; regression to BMI of 27 kg/m^2), or ideal body weight (BMI >30 kg/m^2; regression to BMI 21 kg/m^2 in women and BMI 22.5 kg/m^2 in men), and amount to 1.5 g/kg/day in BMI <30 kg/m^2, 2.0 g/kg/day in BMI 30–40 kg/m^2 or 2.5 g/kg/day in BMI ≥40 kg/m^2. A progressive feeding strategy towards 100% of targets at admission day four is used (10). Actual (par)enteral nutritional and non-nutritional energy and protein provision is automatically calculated hourly. Oral nutrition is currently not incorporated, as it cannot be done automatically and oral nutrition is not usually a substantial contribution to the total nutritional intake in ICU patients.

Study participants

The BIAC-19 study included patients aged 18 years or above admitted to the hospital with COVID-19 symptoms and proved SARS-CoV-2 positive through polymerase chain reaction-test in whom BIA measurements were performed within 24 hours after hospital admission. Exclusion criteria were pregnancy, electrical implants, wounds or skin damage at the designated electrode sites, or inability to maintain posture for 5 minutes.

Patient subgroups for the current study were defined by biological sex (female/male) and BMI category. Normal weight was defined as a BMI <25 kg/m^2, overweight as BMI 25–30 kg/m^2 and obese as BMI>30 kg/m^2.

For the secondary research question addressing protein provision adequacy in the ICU, patients who were admitted to the ICU after transfer to another hospital were excluded, as no ICU nutrition records were available in those cases. In addition, patients who only received oral nutrition were excluded, as protein contents of oral nutrition are not registered.

BIA measurements

Trained researchers conducted BIA measurements with the InBody S10® (InBody Co., Ltd., Seoul, Korea). This multi-frequency, segmental impedance analyser requires height, weight, and sex as input parameters. Height and weight as measured upon hospital admission were used. When circumstances did not allow measurements, height as provided by the patient or their representative was entered. BIA measurements were performed in a supine position with reusable electrodes attached to the left and right thumb and middle finger, and both ankles.

Definition of lean body mass

Inbody regards FFM and LBM as synonyms, defined as TBW minus non-essential storage fat mass (FM), corrected for hydration status through extracellular/total body water ratio (12). In this definition, FFM/LBM includes essential fats, such as those stored in organs, the central nervous system and bone marrow. In other sources TBW minus FM is usually regarded as the LBM, whereas FFM is defined as LBM minus essential body fat. To avoid confusion, we choose to use only the term LBM for TBW minus FM.

Data collection

Demographic and clinical data previously collected for the BIAC-19 study from local electronic medical record systems MetaVision® (iMDsoft, Tel Aviv, Israel) and NeoZIS® (MI Consultancy, Katwijk, The Netherlands) and NeoZIS® (MI Consultancy, Katwijk, The Netherlands) were reused for the current study, i.e., age, sex, ethnicity, height, weight, and protein provision, specifics of the length of stay (LOS) and ventilation in ICU patients.

Lean body mass methods

In addition to measured TBW (kg), four equations for LBM were chosen for comparisons with LBM_{BIA} (kg). The methods aim to approximate IBW (1), adjusted body weight (2/3) or LBM (4), which in all methods is regarded as a proxy for the true determinant of protein requirement: LBM (9). To improve readability, 'LBM' is the term that is used in all equations from hereon.

(1) Adjustment towards a BMI of 22.5, commonly regarded as IBW;

$$LBM_{22.5}\ (kg) = 22.5 * height^2$$

(2) Adjustment towards a BMI of 27.5 in case of obesity (Dutch perioperative guidelines) (17);

$$LBM_{27.5}\ (kg) = 27.5 * height^2 \text{ if } BMI > 30\ kg/m^2$$

(3) Calculation of LBM as stipulated by the ESPEN guidelines on ICU nutrition (10); LBM_{ESPEN} (kg), with

$$\text{Male } IBW_{ESPEN}\ (kg) = 0.9 \times height^2 - 100$$

$$\text{Female } IBW_{ESPEN}\ (kg) = 0.9 \times height^2 - 106$$

(4) The adjusted Gallagher formula for non-Asians (14);

$LBM_{Gallagher}$ (kg), with

Male $LBM_{Gallagher}$ = (0.466 × weight) – (0.00087 × weight x age) + (9.438 × height^2)

Female $LBM_{Gallagher}$ (kg) = (0.24 × weight) – (0.00053 × weight x age) + (10.978 × height^2)

Statistical analysis

Descriptive statistics were calculated for demographics and protein provision in ICU patients. The quantile–quantile plots were visually assessed for the normality of the distribution of continuous data. Continuous values are reported as mean (95% bias-corrected accelerated bootstrap confidence intervals (95%-BCa CI) based on 1000 samples) or median (interquartile range), discrete data are presented as numbers (%). Biological males were compared to female patients. Differences were assessed using independent samples t-tests for continuous data or chi-squared tests for categorical data. When test assumptions were not met, Mann–Whitney U tests or Fisher's exact tests were used.

Agreements between lean body mass methods

We visually checked that the scatter plots showed a monotonic relation between LBM_{BIA} and each method, for all subgroups. Subsequently, a correlation analysis was conducted using Spearman's rank correlation coefficient, as the distribution of the variables was not normal. For this and all subsequent agreement analyses, the normal weight and overweight groups were disregarded when considering the $LBM_{27.5}$ method, as it uses TBW in BMI <30 kg/m^2. As Spearman's correlation only reveals the strength and mean direction of the association but does not reveal information on the presence of a systematic bias, we continued to construct error–standard plots. In this method, the difference or error between two measurements is plotted against the reference or standard method, in this case, LBM_{BIA}. This method was chosen over the Bland–Altman plot, where the difference is plotted against the mean of the two methods, as this can lead to underestimation of proportional bias, and in this case, the LBM_{BIA} method was considered the reference/standard method (Concept illustrated in Supplemental Figure 1). The 95-% Limits of agreements (average difference ± 1.96 standard deviations) with their 95% confidence intervals were calculated and plotted for each comparison. A significant result on a one-sample t-test comparing the mean of the differences to 0 was used to confirm fixed bias whenever visual inspection of the plots was suggestive of one (males and females separately). Where relevant, a sensitivity analysis of the t-test excluding visual outliers was conducted. The presence of proportional bias (i.e. a relationship between the size of the error and size of the reference value) was assessed visually and formally by regressing the difference on the reference value (i.e. LBM_{BIA}) (males and females separately). The assumption for homogeneity of variance for linear regression was confirmed by non-significance of a Levene's test. Proportional bias was considered proven when a relationship was identified (i.e., a significant slope of the regression line).

Protein provision ICU

Protein targets were calculated as 1.3 g/day/LBM and incorporated progressive feeding during the first three days of ICU admission (i.e (1.3 ∗ LBM ∗ duration of admission – first three calendar days) + (0.25 (1.3/24 ∗ duration of the first admission day in hours ∗ LBM) + (0.5 (1.3 ∗ LBM)) + (0.75 (1.3 ∗ LBM)) (note: 1.3 g/kg was chosen as a working example and is not a recommendation. We comment on varying amounts per kilogram between methods in the Discussion section). A Wilcoxon signed-rank test was used to calculate the median difference between the percentages of protein provided to the ICU patients according to target between $Target_{BIA}$ and the other methods. A logarithmic transformation was used to meet the assumption for symmetrical distribution of the differences.

IBM SPSS statistics 27 (I.B.M. Corp, Armonk, NY, USA) was used for all analyses. Only two-sided analyses were used. P-values ≤0.05 were considered statistically significant. P-values are reported to a single significant figure unless 0.2 ≥ P ≥ 0.01, in which case two significant figures are shown.

RESULTS

One-hundred-and-fifty patients were included in the BIAC-19 prospective study and subsequent post-hoc analyses. All the included patients were of white of Western-European descent. Table 1 summarises baseline characteristics and measurements and compares those of biological males and females.

Table 1. Patient characteristics upon hospital admission[a].

	All Patients (N=150)	Males (n=100)	Females (n=50)	P-value
Age, years	68 (66-70)	68 (66-71)	66 (62-71)	.5
Physical characteristics				
Height, cm	174 (173-176)	178 (177-180)	167 (165-168)	**.001**
Weight (TBW), kg	88 (85-91)	91 (87-94)	84 (79-89)	**.031**
Body Mass Index, kg/m^2	29 (28-30)	28 (28-30)	30 (28-32)	**.**11
Normal weight (BMI <24.9)	33 (22%)	21 (21%)	12 (24%)	.4
Overweight (BMI 25-29.9)	65 (43%)	51 (51%)	14 (28%)	.09
Obese (BMI ≥30)	52 (35%)	28 (28%)	24 (48%)	**.019**
LBM_{BIA}, kg	58.5 (56.3 – 60.7)	62.1 (59.9-64.2)	51.1 (48.3 – 54.1)	**.001**
LBM_{BIA} percentage of TBW, %	66.9 (65.2 - 68.7)	69.3 (67.0 – 71.4)	62.0 (59.5 – 64.9)	**.001**

[a]Data are presented as number (percentage, %) or mean (95% bias-corrected accelerated bootstrapped confidence interval). [b] Differences between males and females with a p-value <0.05 are regarded as statistically significantly different and are displayed in bold. Abbreviations: TBW, total body weight; BMI, body mass index; BIA, bioelectric impedance analysis; LBM_{BIA}, lean body mass as measured by BIA.

Agreements between lean body mass methods

All mathematical methods for calculating LBM correlated significantly with LBM_{BIA} at the level of p-value <0.001 (Supplemental Table 1). $LBM_{Gallagher}$ showed the highest correlation coefficient for all subgroups except overweight females, where $LBM_{27.5}$ reached the same coefficient as $LBM_{Gallagher}$.

Figure 1, Figure 2 show the error–standard plots for all methods compared to LBM_{BIA} Visual inspection of the plots suggested a fixed bias for all methods when regarding males and females separately. A one-sample t-test confirmed that the mean value of the difference differed significantly from 0 in all methods, except for the LBM_{ESPEN} for males (−1.7 (95%-BCa CI -3.7; 0.3, p = .096) (Supplemental Table 2). The visual outlier on all plots except LBM_{TBW} discerned herself from the cohort with an LBM% of 80% compared to a mean of 62% (95%-BCa CI 59.5–64.9) for females. A sensitivity analysis excluding this outlier did not change the significance of these findings.

Proportional bias was suspected from visual inspection of all plots except for TBW and confirmed by regressing the difference between the methods and LBM_{BIA}, separately for males and females. A relationship between the error size and the reference value size was confirmed in all methods except TBW (males p = .8; females, p = .087) (Supplemental Table 3).

Protein provision ICU

Forty-one (27%) patients eventually had to be admitted to the ICU. Two ICU patients (5%) were admitted to the ICU after transfer from another hospital, and eleven (27%) only received oral nutrition, which meant that no ICU nutrition records were available in those cases. Consequently, 28 (68%) of the ICU patients could be included in the protein provision ICU sub-analyses (Table 2). ICU patients had a median ICU-LOS of 16 days (IQR 17), during which 21 (75%) patients were ventilated for 14 days (IQR 40), of whom 13 (46%) were in the prone position, for four days (IQR 8).

Patients received 51.19 g (95%-BCa CI 37.1; 64.1) of protein daily during their ICU stay (38.7% (95%-BCa CI 28.5; 48.1) of the target as set by the local protocol. When the protein target was calculated by LBM_{BIA} (including a three-day progression strategy), ICU patients received a mean of 61.6% (95%-BCa CI 43.2; 80.8) of $Target_{BIA}$ during ICU admission. Comparisons with the percentage of target delivered as calculated by the other methods are shown in Table 3. The percentage of protein received of the target as calculated by the $LBM_{Gallagher}$ for males was the only one that did not significantly differ from the percentage received of $Target_{BIA}$ (mean difference 1.4% (95%-BCa CI -1.3; 4.6) p = 1.0).

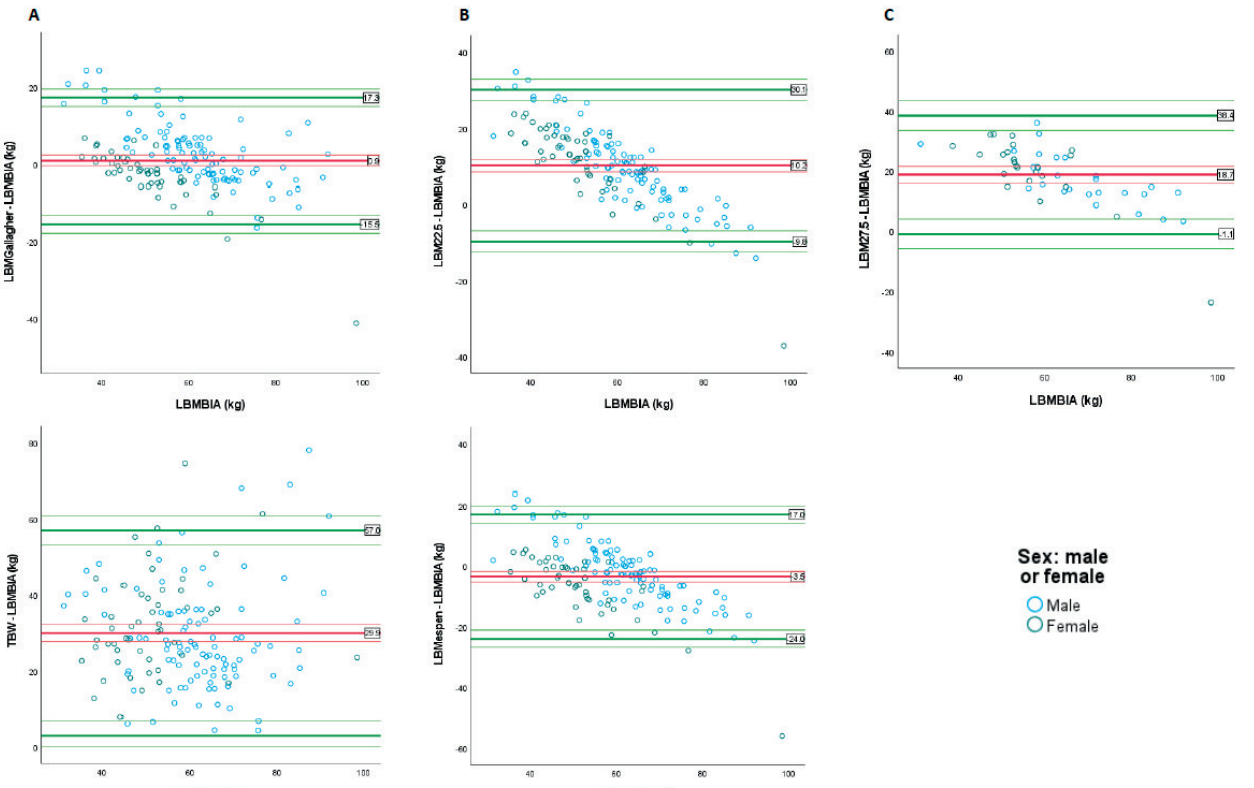

Figure 1. Error–Standard plots comparing the difference (error) in kilograms between LBM_{BIA} and LBM as calculated by the four formulas and LBM_{TBW} (A. LBMGallagher; B. $LBM_{22.5}$; C. $LBM_{27.5}$ (patients with a BMI> 25 kg/m^2); D. LBM_{TBW}; E. LBM_{ESPEN}) to LBM_{BIA} (standard) in kilograms, showing colour grouping for males and females, n = 150 (except $LBM_{27.5}$ where n = 52).

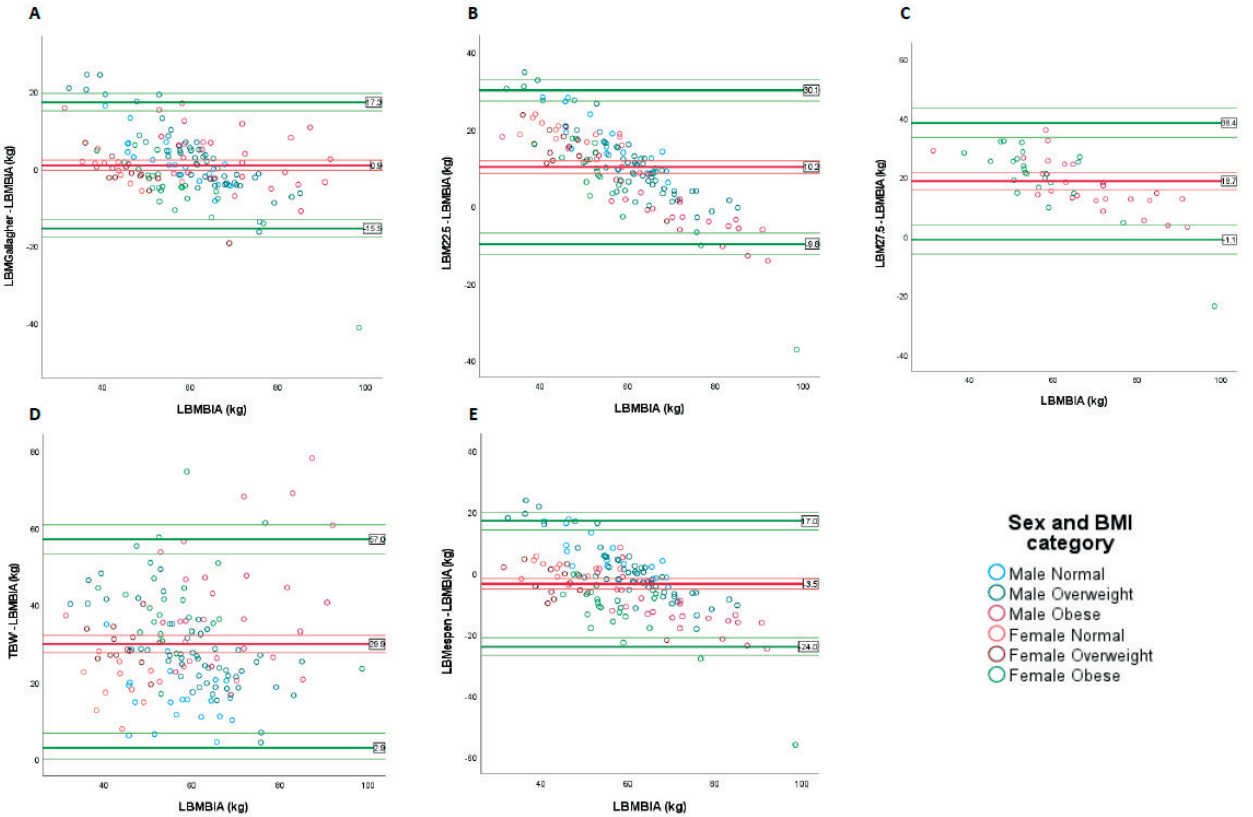

Figure 2. Error–Standard plots comparing the difference (error) in kilograms between LBM_{BIA} and LBM as calculated by the four formulas and LBM_{TBW} (A. $LBM_{Gallagher}$; B. $LBM_{22.5}$; C. $LBM_{27.5}$ (patients with a BMI> 25 kg/m^2); D. LBM_{TBW}; E. LBM_{ESPEN}) to LBM_{BIA} (standard) in kilograms, showing colour grouping for different sex and BMI subgroups, n = 150 (except $LBM_{27.5}$ where n = 52).

Table 2. ICU Patient characteristics upon hospital admission[a].

	All ICU Patients (N=28)	Males (n=20)	Females (n=8)	
Age, years	70 (67 – 73)	71 (67 – 73)	68 (62 – 74)	.5
Physical characteristics				
Height, cm	173 (170 - 177)	177 (173 – 179)	165 (161 – 170)	**.001**
Weight (TBW), kg	88 (84 – 93)	91 (86 – 95)	83 (73 – 92)	.1
Body Mass Index, kg/m^2	29 (28 – 31)	29 (27 – 31)	30 (27 – 33)	.6
Normal weight (BMI <24.9)	5 (18%)	3 (15%)	2 (25%)	.6
Overweight (BMI 25-29.9)	11 (40%)	10 (50%)	1 (12.5%)	.1
Obese (BMI ≥30)	12 (43%)	7 (35%)	5 (62.5%)	.2
LBM_{BIA}, kg	60.8 (57.5 – 63.9)	64.4 (61.5 - 67.7)	52.0 (47.1 – 57.8)	**<.001**
LBM_{BIA} percentage of TBW, %	69.3 (65.9 – 72.6)	71.8 (67.1 – 76.8)	63.1 (59.7 - 66.3)	**.025**

[a]Data are presented as number (percentage, %) or mean (95% bias-corrected accelerated bootstrapped confidence interval).b Differences between males and females with a p-value <0.05 are regarded as statistically significantly different and are displayed in bold. Abbreviations: TBW, total body weight; BMI, body mass index; BIA, bioelectric impedance analysis; LBM_{BIA}, lean body mass as measured by BIA.

DISCUSSION

We aimed to assess which method approximates lean body mass best compared with bioelectric impedance in the hospitalised COVID-19 population. Total body weight and four other common methods were used; regression to a BMI of 22.5 kg/m^2, regression to BMI 27.5 kg/m^2 when BMI>30 kg/m^2, and the equations described by Gallagher and the ESPEN ICU guideline (10,14). Although all methods were correlated with the reference method LBM_{BIA}, we could not identify a mathematical method for calculating LBM that had an acceptable agreement with LBM_{BIA} for males and females across the BMI subgroups.

Although the $LBM_{Gallagher}$ had the smallest overall 95%-CI, this still meant over-and underestimation of the LBM of 16.4 kg. Furthermore, all methods were subject to fixed bias (mean difference deviates from 0) when assessing males and females separately, except the LBM_{ESPEN} for males. All methods except TBW also had proportional bias (association between the difference between measurements and the size of the value measured). The confidence intervals were wide for all methods studied, and visual inspection of the plots suggested that the regression slopes for proportional bias were different per sex/BMI subgroup. We are confident that there is no easy workaround to correct both fixed and proportional bias and make one of the methods agree on an acceptable level with LBM_{BIA} across the whole cohort.

Breaking down the bias

The overestimation of LBM based on TBW (Figure 1, Figure 2 panels D) could be expected, as the fat% is never zero, especially in the current population. Our results show that the size of the overestimation varied widely, although it understandably increased with BMI. The same can be said for $LBM_{27.5}$, as this method essentially presumes a weight equivalent of BMI 27.5 kg/m^2 to be the LBM. For example, a person of 170 cm in height with a BMI of 31 kg/m^2, is presumed to have a LBM of (27.5 ∗ 1.72 =) 79.5 kg on a weight of 89,6 kg, giving him a LBM% of (78.5/89.6 ∗ 100 =) 89%. In reality, excluding the very athletic, most of our patients with a BMI of 31 kg/m^2 will not have a fat% of (100–89 =) 11%. Thus, the $LBM_{27.5}$ method becomes more realistic as actual BMI increases (up to a certain point), explaining the proportional bias that can be seen in Figure 1 panel C. Indeed a previous study compared protein targets considering LBM_{BIA}, TBW and adjusted body weight (ABW) (BMI <20 kg/m^2 adjusted to BMI = 20 kg/m^2 and BMI> 27.5 kg/m^2 adjusted to BMI = 27.5) in 115 hemodialysis patients and concluded that mean protein needs estimated by (adjusted) TBW were higher than those based on LBM_{BIA}, across all BMI categories (P < .01), and most explicitly in obese patients (18). This overestimation occurred eventhough a correction factor in grams/kg was used (LBM_{BIA} ∗ 1.5, whereas (adjusted) TBW ∗ 1.2). A Dutch study comparing protein targets (1.2 g ∗ LBM) set by LBM_{BIA}, ABW (BMI <20 kg/m^2 adjusted to BMI = 20 kg/m^2 and BMI> 30 kg/m^2 adjusted to BMI = 27.5) or TBW in 661 outpatients, showed that ABW estimated LBM_{BIA} correctly (<5% over- or underestimation) in only 33% of their obese patients, whilst LBM_{TBW} estimated between 1% (obese persons) and 33% (underweight persons) correctly (16). These reports are in line with our findings that TBW and regression to a BMI of 27.5 severely overestimated LBM and thereby protein requirements.

Table 3. Comparing the percentage of protein received between the different targeting methods and the TargetBIA (n = 28).

	Males (n=20)[a]							Females (n=8) [a]						
	Percentage of target received			Compared to percentage of $Target_{BIA}$ 61% (95%-BCa CI 39-85)				Percentage of target received			Compared to percentage of $Target_{BIA}$ 61% (95%-BCa CI 24-100)			
Method	Mean	95%-BCa CI		Mean difference	95%-BCa CI		P-value [b]	Mean	95%-BCa CI		Mean difference	95%-BCa CI		P-value [b]
		UL	LL		UL	LL			UL	UL		UL	LL	
$Target_{TBW}$	43	27	59	18.7	11.9	26.0	**<.001**	39	15	65	21.9	7.1	36.3	**.012**
$Target_{Gallagher}$	61	38	83	1.4	-1.3	4.6	1.0	67	26	110	-6.5	-14.3	-0.8	**.012**
$Target_{22.5}$	56	35	77	6.3	3.6	9.0	**.001**	51	20	81	10.3	1.5	21.1	**.012**
$Target_{27.5}$ (n= 7/5)	56.4	20.4	86.4	19.5	7.7	31.2	**.018**	31.5	2.9	65.2	9.3	1.1	17.8	**.043**
$Target_{ESPEN}$	67	42	93	-5.0	-8.9	-1.9	**.001**	75	29	122	-14.0	-26.2	-2.6	**.012**

[a]Unless stated otherwise. [b]Differences with p-values <0.05 are regarded as statistically significantly different and are displayed in bold. Abbreviations: BIA, bioelectric impedance analysis; 95%-BCa CI, 95% bias-corrected accelerated bootstrapped confidence interval; UL; upper limit of agreement; LL lower limit of agreement; TBW, total body weight; ESPEN, European Society for Clinical Nutrition and Metabolism.

The same explanation can be offered for the proportional bias seen in $LBM_{22.5}$. Similar to the $LBM_{27.5}$ method, this method led to more overestimation in females than males (Figure 1 Panel B). Underestimation occurred in more males than females, which is likely the result of the difference in the relationship between TBW and LBM in males and females. Forbes described a semilogarithmic relation between LBM and TBW, with slightly different coefficients for men and women (19). Indeed when we plot TBW and LBM in our cohort (excluding outliers of the mean±2SD), quadratic regression lines for men and women are different, and a common one for both does neither justice (Figure 3). Thus, the same is likely the case for LBM equations.

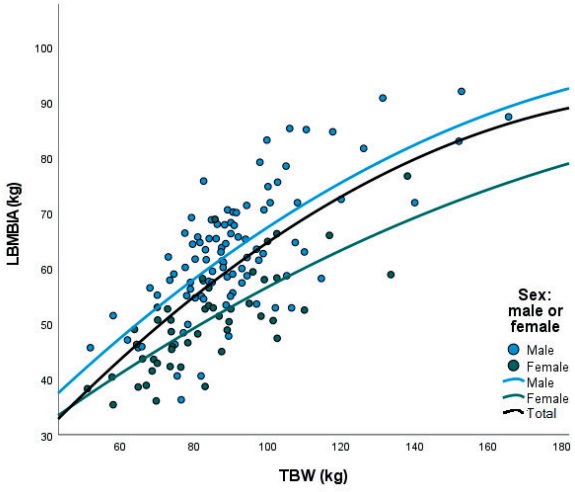

Figure 3. Scatterplot of the relationship between LBM_{BIA} and TBW with fitted quadratic regression lines for men, women and the total cohort, excluding outliers (LBM% men max. 69.3 ± 2 ∗11.4 kg, LBM% women max. 62.0 ± 2 ∗ 9.2 kg), n = 142.

The Gallagher formula and the ESPEN method were the only two LBM equations used that acknowledge the difference in body composition between males and females. Although ESPEN offers no reference for their method, the Gallagher formula uses regression models based on DXA studies (14). As BIA is also validated against DXA, a strong agreement was expected and found (Supplemental Table 1). In addition, $LBM_{Gallagher}$ had the smallest overall 95%-CI. Nevertheless, LBM was often underestimated in women. The previously mentioned Dutch study by Velzeboer et al. (16) found that although LBMGallagher was an improvement over LBM_{TBW} and $LBM_{27.5}$, protein targets set by $LBM_{Gallagher}$ ∗ 1.5 g agreed (<5% over- or underestimation) with LBM_{BIA} ∗ 1.2 g in only 9% (underweight persons) to 54% (obese persons) of the cases. A possible explanation could be differences in body composition between Gallagher's cohort of (white) British and Northern American

volunteers and the Dutch cohorts. Indeed white women had a BMI of 24.5 ± 4.5 kg/m^2 in the Gallagher cohort, compared to a mean BMI of 30 (95%-BCa CI 28–32) kg/m^2 in ours. The LBM_{ESPEN} method was not subject to fixed bias in males, although gross over- and underestimation were still common and only appeared to cancel each other out around a mean of 0 (Figure 1, Figure 2).

Notably, for the female outlier with an LBM% of 80%, underestimation of LBM occurred in all methods except LBM_{TBW}, alluding to the fact that the studied equations may be even less appropriate for non-sarcopenic obese persons.

Protein provision ICU

As a real-world exploration of the subject, a secondary aim of this study was to retrospectively compare actual protein provision adequacy in our COVID-19 ICU population based on LBM_{BIA} to that based on LBM predicted by other methods. There, we found that ICU patients received a mean of 38.7% protein of the local target, or 61.6% (95%-BCa CI 43.2; 80.8) of $Target_{BIA}$ during ICU admission. This discrepancy shows that our local targets overestimated protein requirements by a third. However, proteins were generally underdelivered by either target. Our findings align with findings from other studies proving that adequate protein provision is difficult to achieve in the ICU population, including COVID-19 patients (8,20,21). When comparing the percentage of target delivered as calculated by the other methods to $Target_{BIA}$, all methods except $Target_{Gallagher}$ for males differed significantly. Therefore, using targets set to LBM based on mathematical methods or TBW is likely to lead to significant over-or underdosing of protein in all other groups. This is in line with findings in other patient categories (16,18).

Clinical implications

In practice, it has proven difficult to achieve even low-end protein targets in hospitalised COVID-19 patients (8). This is an urgent issue, as there is reason to assume that a high protein diet is beneficial during COVID-19 (3,4). Therefore, we strongly recommend measuring LBM_{BIA} upon hospital admission (as quickly as possible, to prevent bias through hydration shifts) to guide protein provision.

However, if admission LMB_{BIA} measurements are not feasible, we argue that it is probably safer to accept a certain degree of overestmation rather than underestimation of LBM by formulas, as protein overdoses based on any target have proven less likely to happen than underdosing. Consequently, our results may argue a preference towards the use of $Target_{22.5}$, as it had the lowest overestimation with its entire confidence interval above 0 for both sexes in the ICU cohort (Table 3). Nevertheless, regarding the entire cohort (Figure 1), the use of $LBM_{22.5}$ still led to underestimating LBM in quite a few cases, mostly overweight and obese males. On the other hand, $Target_{27.5}$ and $Target_{TBW}$ have a

confidence interval above 0 for both sexes on the LBM plots of the entire cohort (Figure 1) and regarding targets in the ICU (Table 3). However, this would mean excepting a mean overestimation of LBM of 23.4 kg or 29.9 kg (Figure 1), respectively. It is up to the dietician and clinical to decide whether this is acceptable for their patient.

Although a practical exploration of the subject goes beyond the scope of the current paper, future research could explore the possibility of stratifying methods for estimating LBM according to which works best for which sex/BMI group, if not devising a new universal method based on LBM_{BIA}. Alternatively, the difference between LBM and TBW is sometimes acknowledged through a correction of the amount of protein per kilogram of either (i.e. 1.9 g/kg LBM or 1.5 g/kg TBW) (15,16). However, this correction is based on the assumption of a fixed LBM/TBW ratio, which is an oversimplification that leads to a large error in many individuals (Figure 1). Based on our findings we think it is highly unlikely that a static correction such as the one in the example will improve accuracy of protein targets, and we do not recommend its use without further scientific exploration of the subject.

Limitations

This research is subject to several limitations. No sample size calculation was performed as the data were dependent on the sample size of the mother study, and not all ICU patients could be included in the protein adequacy analyses. The subsequent relatively small cohort size prevented subdividing into BMI categories for these analyses. Segmenting data could be a point of attention for future studies focusing more specifically on protein provision in the ICU.

The formulas used by the Inbody S10 software to calculate the derived BIA parameters (such as LBM) are not publicly available and therefore cannot be provided here. However, Inbody S10 (LBM) calculations are based on regression formulas derived from reference groups, and have independently been validated against other methods such as Dual-Energy X-ray Absorptiometry in peer-reviewed studies in various populations (22, 23, 24). Nevertheless, caution is warranted when applying the results of this study in other populations or BIA devices.

We did not regard underweight persons as a separate category for this study. When regarding BMI 18.5 kg/m^2 as the lower limit of normal weight, the current cohort included three underweight persons (two males with BMI 16 kg/m^2 and 17.3 kg/m^2, one female with BMI 18 kg/m^2), who were grouped in with 30 others in the normal weight category. None of these patients was in the ICU cohort. We do not expect this to have impacted the main findings of this study.

Although we incorporated progressive feeding during the first three days of ICU admission into our targets, accounting for a possibly incomplete first day of admission, we did not account for a possibly incomplete last day. This may lead to an overestimation of the target in the case of ICU discharge early in the day, thereby underestimating the percentage of target provided. As the median ICU-LOS was 16 days, we do regard this possible overestimation as significant. In addition, this bias would be in all methods, therefore not affecting comparisons between methods (and thereby the aim of this study). This study was performed in white, Dutch COVID-19 patients, and results should be interpreted with caution before its results have been confirmed in other populations.

CONCLUSION

We could not identify a mathematical method for calculating lean body mass that had an acceptable agreement with LBM as derived from bioelectric impedance analysis for males and females across all BMI subgroups in our hospitalised COVID-19 population. Consequently, discrepancies were observed when assessing the adequacy of protein provision in ICU patients, who on average only received two-thirds of their protein target as set by BIA. We strongly advise using baseline LBM_{BIA} to guide protein dosing if possible. In the absence of BIA and awaiting a universally applicable method, using a method that overestimates LBM in all categories may be the only way to minimise underdosing of nutritional protein. We emphasise the importance of more research and discussion on this topic.

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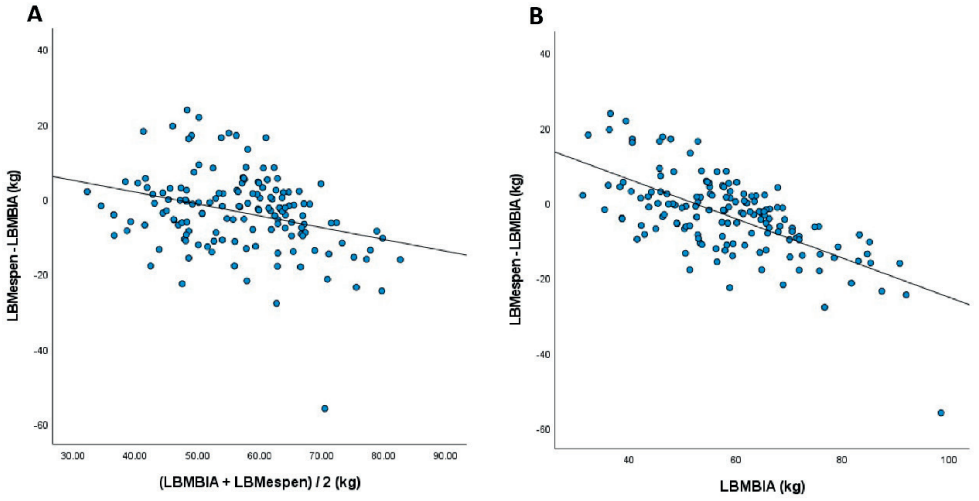
SUPPLEMENTAL FILES



Supplemental Figure 1. Illustrating the difference between A. the Bland–Altman plot and B. error–standard plots.

Supplemental Table 1. Correlations for all methods with LBM_{BIA} for different sex and body mass index categories.

	Spearman's rho [a]					
Method	Males (n=100)			Females (n=50)		
	Normal weight (n=21)	Overweight (n=53)	Obese (n=26)	Normal weight (n=12)	Overweight (n=14)	Obese (n=24)
TBW		.604			.746	
	.662	.485	.724	.650	.732	.550
$LBM_{Gallagher}$		.687			.841	
	.765	.573	.786	.769	.780	.626
$LBM_{22.5}$		.520			.476	
	.683	.560	.703	.723	.726	.342
$LBM_{27.5}$	n.a.		.724	n.a.		.342
LBM_{ESPEN}		.520			.476	
	.683	.560	.703	.723	.726	.342

a All shown correlations were significant at the P < .001 level. Abbreviations: TBW, total body weight; LBM, lean body mass; n.a., not applicable; ESPEN, European Society for Clinical Nutrition and Metabolism.

Supplemental Table 2. One-sample t-test comparing the differences between the methods and LBM_{BIA} to a test value of 0 per sex.

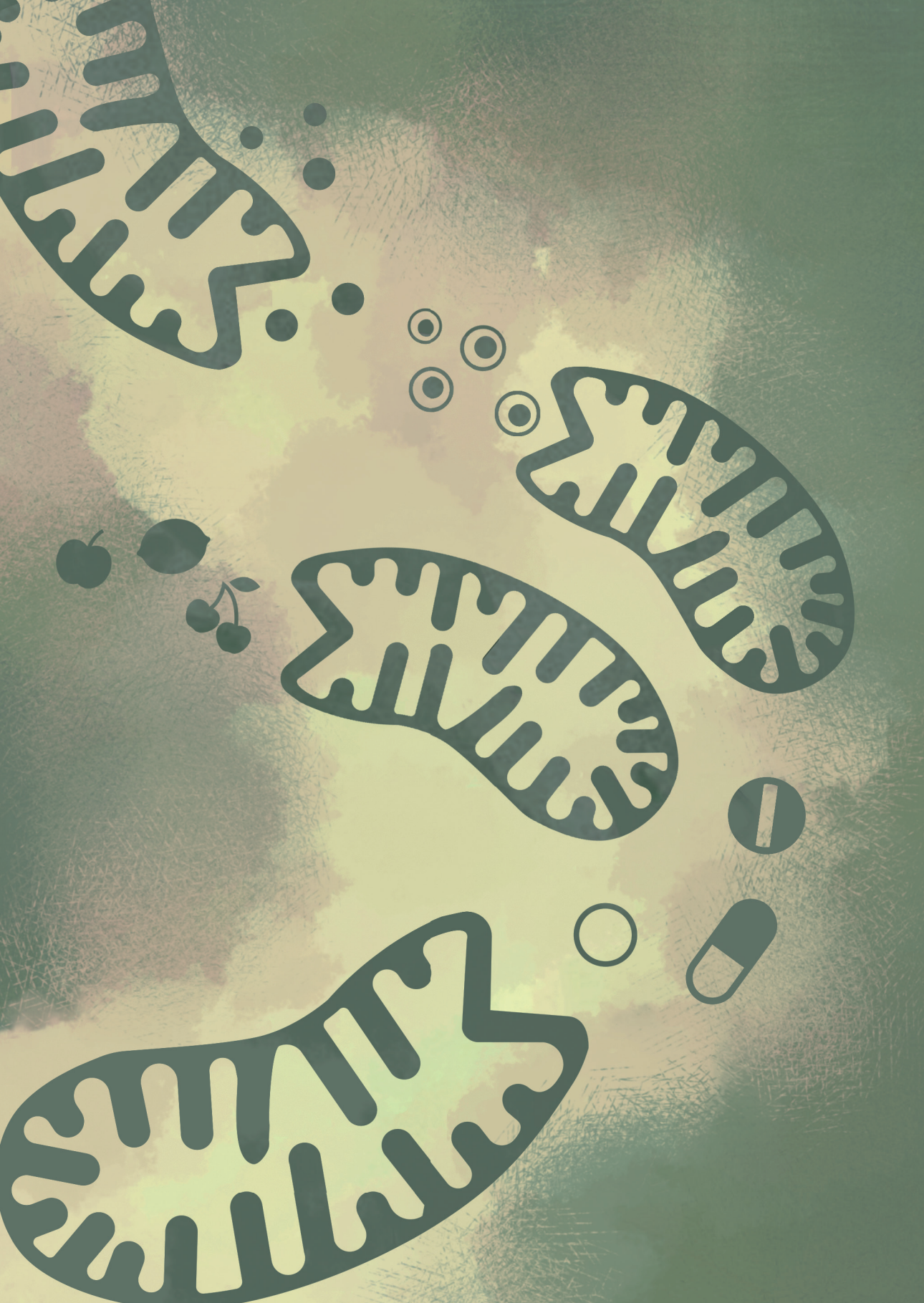
	Test value: 0									
	Males (n=100) [a]					Females (n=50) [a]				
Method	Mean difference	95%-BCa CI		t-statistic	P-value	Mean difference	95%-BCa CI		t-statistic	P-value
		Lower	Upper				Lower	Upper		
TBW	28.6	26.0	31.2	20.5	**.001**	32.6	29.6	35.7	17.4	**.001**
$LBM_{Gallagher}$	3.0	1.5	4.7	3.8	**.001**	-3.3	-5.9	-1.3	-3.1	**.018**
$LBM_{22.5}$	9.5	7.4	11.4	9.3	**.001**	11.6	8.1	14.4	8.1	**.001**
$LBM_{27.5}$ (n= 26/24)	17.2	14.0	20.9	10.4	**<.001**	20.2	14.8	24.3	8.6	**<.001**
LBM_{ESPEN}	-1.7	-3.7	0.3	-1.7	.096	-7.0	-10.5	-4.1	-4.8	**.001**

[a] Unless stated otherwise. Abbreviations: 95%-BCa CI, 95% bias-corrected accelerated bootstrapped confidence interval; TBW, total body weight; LBM, lean body mass; ESPEN, European Society for Clinical Nutrition and Metabolism.

Supplemental Table 3. Linear regression of the difference between the methods and LBM_{BIA}, and LBM_{BIA} per sex.

Method	Males (n=100) [a]		Females (n=50) [a]	
	F	P-value	F	P-value
TBW	.041	.8	3.1	.087
$LBM_{Gallagher}$	91.4	**<.001**	178,9	**<.001**
$LBM_{22.5}$	354.8	**<.001**	166.4	**<.001**
$LBM_{27.5}$ (n= 26/24)	29.7	**<.001**	53.6	**<.001**
LBM_{ESPEN}	245.9	**<.001**	10.6.6	**<.001**

[a] Unless stated otherwise. Abbreviations: TBW, total body weight; LBM, lean body mass; ESPEN, European Society for Clinical Nutrition and Metabolism.



Part IV

General Discussion

Critically ill patients' survival rates have consistently increased over recent decades (1). Nevertheless, despite advancements in current best practices, these patients frequently experience substantial loss of muscle mass and function, which they often recover slowly and incompletely during the convalescent period. Such delayed or incomplete recovery imposes a notable burden on individuals and the society (2-4). Tailored nutritional therapy holds promise in enhancing outcomes for critically ill patients by mitigating catabolic wasting. However, empirical nutritional approaches have not consistently yielded favourable outcomes and, in some instances, have led to adverse effects (5-9). Real-time understanding of the metabolic status and subsequent fluctuations in nutritional requirements of individual patients in the intensive care unit (ICU) would likely enhance the efficacy of interventions. This thesis aims to elucidate the distinct metabolic phases and consequent nutritional demands in critical illness while exploring methodologies for individualised determination. Our findings will be presented from the perspective of a patient's journey.

Emergency Room

> *Jane, a 45-year-old female, arrives at the ER with a high fever, severe shortness of breath, and a productive cough. She reports feeling extraordinarily fatigued and disoriented for the past 24 hours. On examination, she has a temperature of 38.9°C, a heart rate of 120 beats per minute, a respiratory rate of 28 breaths per minute, and a blood pressure of 85/55 mmHg. Her oxygen saturation is 88% on room air. Initial blood tests reveal a leucocytosis and an elevated lactate and c-reactive protein level.*

METABOLISM AND THE ONSET OF CRITICAL ILLNESS

Based on our preliminary findings, it appears likely that Jane is suffering from a respiratory infection. Her concerning vital signs suggest sepsis, necessitating prompt resuscitative interventions to prevent further organ failure. Given the urgent situation, nutritional interventions are not our top priority. However, Jane's metabolic profile has played a part in the development of her illness. In return, the pathology and our interventions will significantly impact her body's function and composition, subsequently affecting nutritional requirements. Therefore, it is prudent to assess Jane's baseline metabolic characteristics.

As discussed in **Chapter 4**, our nutritional needs are contingent upon our physical makeup and activities. Body composition, which encompasses the volume, ratio, and distribution of various metabolically active tissues, is a fundamental determinant. Body mass index (BMI) provides a basic estimate of body composition and metabolic health. While a high BMI, compared to healthy norms, is associated with the development and severity of numerous chronic and acute illnesses, the precise mechanisms remain unclear. In the case of SARS-CoV-2, one hypothesis suggests that obese individuals may have reduced respiratory volumes and lung compliance, while another posits that adipose tissue could act as a reservoir for viruses, contributing to higher viral loads. (10-16). At the start of the Coronavirus Disease-19 pandemic, it was noted that men were more often and more severely affected compared to women, despite a lower incidence of obesity. It was hypothesized that perhaps not merely fat percentage, but the location of fat tissue is essential, as men are more prone to visceral fat accumulation (17).

More than BMI alone is needed to sufficiently elucidate such a hypothesis as it fails to account for tissue distribution. **Chapter 6** introduces bioelectric impedance analysis (BIA) as a more comprehensive technique for assessing body composition. BIA measures resistance and reactance to an alternating current passing through body compartments, providing insights into tissue contributions and cellular integrity. BIA has been instrumental in debunking the Obesity Paradox in cancer patients – the finding that a lower mortality rate is found for overweight or obese people within specific subpopulations - by showing that not the excess fat mass but the associated increased muscle mass in non-sarcopenic individuals has a protective function; a distinction that BMI would be unable to make (18).

Similarly, in **Chapters 7 and 8**, we found that whereas COVID-19 patients admitted to our hospital were generally overweight, body composition values for fat mass and fat distribution as measured by BIA proved not to be independently associated with disease severity. Although our findings did not explain the observed epidemiological differences between severely and less severely affected individuals with COVID-19 as we set out to, it did prove that even under the stressful circumstances of a pandemic, BIA can be used to test pathophysiological theories. In addition, we discovered that a lower phase angle at hospital admission was related to increased disease severity later on. This is likely to be explained by the fact that phase angle reflects the combined effect of premorbid condition, duration, and severity of inflammation on cellular quantity and health. These findings have since been included in several systematic reviews, the outcomes of which corroborate ours (19-21). Thus, even at the onset of critical illness, BIA body composition measurements can provide a valuable marker for risk stratification for the individual patient.

Thus far, the phase angle is the most studied BIA marker. However, as discussed in **Chapter 6**, BIA-derived muscle mass appears to be a promising biomarker for sarcopenia, and body cell and fat-free / muscle mass provide potential use in the estimation of metabolic rate, pharmacokinetics and protein requirements. Dual energy X-ray absorptiometry is the reference method for skeletal muscle mass (SMM) measurements, and the segmental multi-frequency BIA technique has been shown to correlate and agree well in the healthy (22). However, in the ICU setting, computed tomography (CT) scan analysis is often more practical, and therefore more commonly used. Studies have shown, that there is a high correlation between SMM as derived from BIA and CT, although BIA indicated a higher SMM than CT as muscle mass increased (23-24). In general an increase in SMM in ICU patients should not be interpreted as muscle mass of good quality, as intramuscular edema will be classified as muscle mass by both BIA and CT. But these findings show BIA may be more subject to this. However, these studies also observed a stronger correlation between phase angle and CT muscle density compared to CT muscle area, which may in the future help us get an indication of muscle quality (23, 25).

In **Chapter 7**, we used a method to facilitate the interpretation of BIA values in case of overhydration of the extracellular compartment, which was previously not used in the intensive care, but has since been adopted by other researchers (26). A study using a similar adjustment applied to bioimpedance spectroscopy measurement found that it's use improved agreement between BIS- and CT-SMM (25). Future studies could focus on the agreement between adjusted BIA and CT in ICU patients. The use of a hydration adjustment method was unnecessary in the prospective study in **Chapter 8**, as it focussed on a single measurement, which was performed within 24 hours of hospital admission when patients had not yet received significant fluid resuscitation.

Clearly, to assess and, where applicable, correct the changes in body composition throughout disease development, both knowledge of an individual's healthy state and the direction of the changes that occur are necessary. Thus, other BIA parameters will likely become of more value as we commit to measuring our ICU patients early in their disease process to obtain individual baseline values, and when we learn more about the interpretation of BIA results in this population.

Intensive Care

Jane's condition continues to deteriorate in the ER despite initial interventions. She is transferred to the Intensive Care Unit for closer monitoring and more aggressive treatment. In the ICU, she is intubated and placed on mechanical ventilation to support her worsening respiratory distress. In the ICU, Jane receives aggressive treatment for her severe sepsis, including antibiotic treatment and vasopressor support.

MEASURING AND MEETING METABOLIC DEMANDS DURING CRITICAL ILLNESS

Critical illness is marked by the occurrence of vital organ dysfunction and a high risk of imminent death if care is not provided, but it also has a potential for reversibility when treated adequately. As Jane's vital signs stabilise under treatment, we are free to consider what nutrition therapy she will need to sustain and, if possible, improve organ function. Obviously, as a mechanically ventilated patient, Jane cannot consume nutrients orally, nor can she communicate hunger, thirst, or specific cravings. This means that the healthcare team determines the route, quantity and composition of the nutrition she receives.

The extent of this responsibility is underscored by the fact that empiric early aggressive caloric feeding to prevent catabolic wasting, while easily rationalized, was not unequivocally beneficial (5-9). Unlike healthy individuals, the heightened mobilization of the body's energy reserves during critical illness cannot be counteracted solely by administering external substrates (27, 28). Consequently, attempting to fulfil all metabolic requirements through nutritional substrate provision in critically ill patients leads to a surplus, which can paradoxically result in harm (29). It is evident that the metabolic dynamics of the critically ill body diverge significantly from those of healthy individuals. However, the underlying mechanisms driving these alterations remain to be fully elucidated.

As we explain in **Chapter 2**, one recently popularized theory is that during the initial phase of critical illness, mitochondria prioritize essential cellular processes to sustain cell viability, even at the expense of overall functionality. This prioritization serves to conserve ATP levels above a critical threshold, thus postponing cell death (30-32). However, when excess nutrients are introduced, these downregulated mitochondria face an undue burden,

resulting in cellular damage. Evidence from studies examining mitochondrial function in various muscle tissues during early critical illness supports this theory, revealing impaired respiration and ATP production (31, 33-40). Importantly, this dysfunction correlates with disease severity and poorer outcomes, yet survivors typically do not experience permanent organ damage. Their remarkable regenerative capacity during convalescence suggests an adaptive response (31, 32). Notably, recovering ICU patients often exhibit an increased tolerance for and requirement of external substrate after 3–5 days, hinting at a potential upregulation of mitochondrial function.

Our findings in **Chapter 5** support this theory, as repeated indirect calorimetry measurements conducted in mechanically ventilated ICU patients revealed a normal resting energy expenditure upon ICU admission. However, from day four onwards, there was a notable increase in resting energy expenditure levels, surpassing predicted values. These results align with previous studies indicating persistent hypermetabolism across various categories of ICU patients (41-44). Endogenous energy production is expected to play a minor role after day four when the predicted REE significantly underestimated the actual REE (45). Failing to consider this increased need for feed could result in the underfeeding of patients not undergoing indirect calorimetry measurements. Additionally, throughout our study, we observed considerable individual differences in the absolute height of resting energy expenditure. Therefore, consistent with conclusions drawn from the literature reviewed in **Chapter 4**, our research suggests that to tailor caloric nutrition effectively to individual patients, indirect calorimetry measurements should be repeated every three days and whenever there are significant changes in a patient's clinical condition or treatment regimen.

To delve into the biomechanical underpinnings of these clinical observations, we conducted a prospective observational cohort study with matched controls, examining the progression of mitochondrial function in peripheral blood mononuclear cells (PBMCs) during the first week following ICU admission in septic patients (**Chapter 3**). Surprisingly, we discovered an elevation in both basal and ATP-linked respiration in PBMCs from septic patients compared to controls throughout the first week of ICU admission, contrary to our initial expectations. This finding challenges the hypothesis of adaptive downregulation.

However, previous investigations measuring mitochondrial function in various muscle tissues consistently reported diminished activity of mitochondrial complexes and reduced ATP content (39, 46, 47). We postulate that these differences in mitochondrial function across cell types may reflect variations in their roles during sepsis. The heightened basal and ATP-linked respiration observed in our study might signify an increased ATP demand of PBMCs during human sepsis, potentially stemming from immune system activation to combat the underlying infection.

This interpretation gains support from the notable observation that non-survivors exhibited a more pronounced increase in basal and ATP-linked respiration, which correlated with higher 3-month mortality rates. Interestingly, the current results suggest that the upregulation of basal respiration may serve as a proxy marker for sepsis severity and outcomes. To further deepen our understanding, future studies should aim to simultaneously measure and compare mitochondrial respiratory function across various cell types. This approach could provide nuanced insights into the metabolic responses during sepsis.

While caloric feeding is a crucial aspect of nutrition therapy, there is growing interest in the provision of adequate protein, in an attempt to decrease catabolic wasting. Current nutrition guidelines recommend increasing protein quantity as illness severity escalates, yet they lack consistent advice on dosing. Moreover, setting protein targets poses challenges, particularly when patients deviate from the ideal body weight.

Conventional methods often use total body weight to determine protein needs, which may overestimate requirements in overweight or obese individuals, as their bodies typically contain less protein per kilogram. Furthermore, methods relying solely on measured body weight do not account for changes in body composition, such as weight gain due to overhydration, potentially masking decreases in fat-free mass during ICU stay.

As detailed in **Chapter 6**, using bioelectric impedance to measure actual fat-free mass could enhance protein dosing accuracy in the ICU. To validate this hypothesis, we conducted a retrospective study in a COVID-19 context (**Chapter 10**), comparing common methods of protein dosing based on estimated fat-free mass to measurements obtained via bioelectric impedance analysis (BIA). Indeed, we could not identify a mathematical method that exhibited acceptable agreement with BIA across all BMI subgroups in our hospitalised COVID-19 population, leading to protein underdosing in the ICU.

At the time of these studies, we already employed repeated indirect calorimetry measurements to establish individualised caloric targets for our ICU patients. Subsequently, we integrated protein dosing based on baseline-measured fat-free mass into our intensive care protocols. Future investigations will be necessary to establish the association between this level of personalised nutrition therapy and clinical outcomes in our patients.

ICU discharge

Over the course of several weeks in the ICU, Jane's condition gradually improved. Her oxygenation and hemodynamic stability improved, her inflammatory markers decreased, and she no longer required vasopressor support. She was successfully weaned from mechanical ventilation and gradually transitioned to regular medical wards for further recovery.

POST-ICU HOSPITALISATION

If this case had been set in 2020, Jane may have suffered from COVID-19. **Chapter 9**'s findings suggest that as a COVID-19 ICU survivor, Jane would have left the ICU with ICU-acquired weakness (ICUAW), which others have since attributed to the use of neuromuscular blockers and prolonged invasive ventilation (48). However, despite this challenge, we proved she would have had better prospects for physical rehabilitation compared to non-COVID pneumosepsis ICU survivors. Adequate nutrition is crucial to support her rehabilitation process.

Chapter 5 presents the first study to conduct indirect calorimetry measurements after extubation in a prospective setting. Despite the difficulties encountered, the results indicated that patients have a higher energy consumption during their post-ICU ward stay compared to their ICU admission. This higher energy expenditure during recovery aligns with the hypothesis that recovering patients enter an anabolic state, necessitating increased substrate. Importantly, the measured resting energy expenditure (REE) often surpassed predictions, heightening the risk of underfeeding if indirect calorimetry is not used to guide caloric goals. Compounding this risk is the fact that most patients fail to meet even the lower estimates of their energy targets during this period, a finding supported by our research group's post-ICU study (49)

To support Jane's recovery, it would be prudent to set her caloric targets based on repeated energy expenditure measurements, akin to our approach during her ICU stay. This personalised strategy can help mitigate the risk of underfeeding and provide her with the nutritional support necessary for optimal rehabilitation.

Hospital discharge

Jane is discharged from the hospital after a few weeks. She continues to receive follow-up care to ensure the complete resolution of her illness.

LONG TERM RECOVERY

The patient's trajectory through critical illness extends beyond hospital discharge, often involving transitions to step-down units or rehabilitation facilities. This post-hospital phase holds paramount importance for the patient's long-term recovery and overall well-being. Rehabilitation encompasses a spectrum of interventions, including physical and occupational therapy, aimed at restoring function and strength. Its overarching goal is to optimise the patient's physical and psychological health, mitigate potential complications, and facilitate a seamless reintegration into everyday life. While the current thesis did not delve into the post-hospital phase, our research group continues to track our patients throughout convalescence. Future publications will illuminate the metabolic characteristics and nutritional requirements during this pivotal stage of critical illness.

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Summary

The metabolic changes that occur in response to the severe stress that critical illness imposes on the body are increasingly thought of as adaptive strategies to preserve vital tissues and systems rather than defects. Therefore, scientific and medical priorities have progressed from preserving the normal to aiding the adaptation. The timing and nature of these adaptations vary highly between patients, various illnesses, and over time. As there are strong indications that the wrong timing, dose or composition of substrate is not beneficial and sometimes even harmful, it is of immense importance to obtain further insight into the metabolism of the critically ill. The studies making up this thesis, aimed to shed light on the different metabolic phases and subsequent nutritional needs in critical illness and clinical methods to determine these in the individual patient.

PART I CHANGES IN BIOCHEMISTRY DURING EARLY CRITICAL ILLNESS

Mitochondrial function is associated with morbidity and mortality during and after critical illness. However, the concept of adaptive mitochondrial metabolic-bio-energetic downregulation rather than bioenergetics failure during the acute phase of critical illness has gained traction.

In **Chapter 2**, we provided a comprehensive overview of current knowledge of mitochondrial (dys)function during critical illness and convalescence. Studies show that in muscle, mitochondrial function is severely impaired during the early phase of critical illness. However, there is a paradoxical lack of permanent damage in the organs of survivors. This finding, combined with negative outcomes observed in extensive early-phase nutrition studies, has led to the theory that mitochondria undergo an adaptive metabolic-bio-energetic downregulation rather than bio-energetic failure. Not much is known about the implications of these changes for the nutritional needs. The effect of early high protein administration remains unclear, whereas fat appears bio-energetically inert. Although antioxidant micronutrients are essential to mitochondrial function, high-dosage studies of single vitamins (C and D) failed to show benefit. Convalescence probably requires increased micronutrient and macronutrient administration to aid anabolism and restore mitochondrial function, although robust data on requirements and actual intake are lacking. We conclude that optimal nutrition therapy in the early phase of critical illness should avoid overfeeding and preserve (adaptive) mitochondrial function. A scientific focus on identifying distinct metabolic phases to target nutrition during and after critical illness is essential.

In **Chapter 3**, we performed a prospective cohort study with matched controls to investigate how mitochondrial function in peripheral blood mononuclear cells (PBMCs) progresses in the first week after ICU admission in septic ICU patients. We demonstrated a higher basal and ATP-linked respiration in PBMCs within the first week of ICU admission in septic patients compared to their matched controls. In addition, a progressive increase of basal mitochondrial respiration in PBMCs during the first week of ICU stay was negatively associated with 3-month mortality. Based on the results of this study and in comparison to studies performed using skeletal muscle biopsies, PBMCs do not necessarily reflect the decrease in mitochondrial function in septic patients, which has previously been reported elsewhere in other tissues.

PART II CHANGES IN ENERGY METABOLISM DURING CRITICAL ILLNESS AND CONVALESCENCE

The course of energy expenditure during critical illness and convalescence is complex, and clinical studies are scarce. To prevent the detrimental effects of under and overfeeding, bedside calculation of energy expenditure is strongly recommended to guide nutrition therapy in critically ill patients. Indirect calorimetry is the gold standard method for measuring energy expenditure, but it is not yet ubiquitously used in the ICU.

Chapter 4 provides an overview of recent evidence and practical considerations on the application of indirect calorimetry during and after ICU stay. We describe that many individual and iatrogenic factors and metabolic phases of critical illness and convalescence influence energy expenditure. In the first days, energy production from endogenous sources is increased due to a catabolic state and is likely near-sufficient to meet energy requirements. Full nutrition support in this phase may lead to overfeeding as exogenous nutrition cannot abolish this endogenous energy production, and mitochondria cannot process the excess substrate. However, energy expenditure is reported to increase hereafter and is still shown to be elevated three weeks after ICU admission, when endogenous energy production is reduced, and exogenous nutrition support is indispensable. The superiority of IC-guided nutritional therapy has not yet been unequivocally proven in clinical trials, and many practical aspects and pitfalls should be taken into account when measuring energy expenditure in critically ill patients. Furthermore, the contribution of endogenously produced energy cannot be measured. Nevertheless, the routine use of indirect calorimetry to aid personalised nutrition has strong potential to improve nutritional status and, consequently, the long-term outcome of critically ill patients.

In **Chapter 5**, we aim to describe the course of resting energy expenditure by performing repeated indirect calorimetry measurements during ICU and post-ICU hospitalisation.

In a prospective observational design, we performed repeated indirect calorimetry measurements in mechanically ventilated ICU patients within 24 hours of intubation and every three days thereafter. After extubation, the measurements were continued until hospital discharge using the indirect calorimetry canopy mode. Measured REE was compared at different time points in the ICU and the ward. In addition, measured REE was compared to predicted REE. In 56 patients, 233 indirect calorimetry measurements were performed. The measured REE did not differ from the predicted REE at ICU admission. From ICU admission day four, measured REE was increased compared to baseline, indicative of a hypermetabolic state. During post-ICU ward stay, 44 measurements were performed, showing a higher mean REE than during ICU stay. We conclude that critically ill mechanically ventilated patients were hypermetabolic on ICU admission day four and thereafter. Indirect calorimetry measurements suggest energy requirements increase when patients are discharged from the ICU to the general ward.

PART III CHANGES IN BODY COMPOSITION DURING CRITICAL ILLNESS AND CONVALESCENCE

Profound changes in body composition often accompany critical illness as the prolonged catabolic phase erodes lean body mass. The subsequent loss of physical fitness, referred to as ICU-acquired weakness, is an important determinant and predictor of disability and quality of life in post ICU recovery. Therefore, obtaining insight into body composition is of great value in the ICU. Bioelectric impedance analysis (BIA) is the most applicable bedside technique for measuring body composition. However, bioimpedance has not been validated in the critically ill, and the interpretation of the measurements poses challenges.

Chapter 6 reviewed the potential clinical applications of BIA and explored caveats and solutions to its use in the intensive care setting. A correlation was repeatedly found between raw impedance parameters, fluid ratios, overhydration, and adverse outcomes of critical illness. However, cut-off and reference values remain elusive. Experience with BIA-guided fluid management in the ICU is limited. BIA-derived muscle mass appears to be a promising biomarker for sarcopenia, correlating well with CT analysis. Body cell mass and fat-free mass can potentially be used to estimate metabolic rate, protein requirements, and pharmacokinetics. Several methods of reducing bias in BIA parameters in critical illness require validation. There are too many uncertainties and discrepancies regarding the interpretation of bioimpedance in critical illness to justify therapeutic consequences. However, there are several promising research areas concerning some of the most urgent clinical problems in intensive care.

In 2020, severe acute respiratory syndrome coronavirus-2, SARS-CoV-2, rapidly spread across countries and continents, causing high incidences of Corona Virus Disease-2019 (COVID-19) and hospital and ICU admissions. Disease severity was quickly shown to increase with body mass index. However, it was unclear whether fat mass, distribution or a different aspect of body composition was the predisposing factor for a severe course of the disease.

In **Chapter 7,** we aim to assess the body composition of COVID-19 patients admitted to the ward or the ICU and identify any associations with the severity of the disease. We performed an observational cross-sectional cohort study, conducting bioelectric impedance analysis amongst all confirmed COVID-19 patients admitted to the ward or ICU of our hospital at that time. Body water measurements and derived values were recalculated to dry weight using standard extracellular water to total body water ratio. Fifty-four patients were included, of which 30 were in the ward and 24 in the ICU. They were generally overweight, but the mean BMI did not differ between groups. Body composition values proved not to be independently associated with disease severity. In multiple logistic regression analyses, a low phase angle was associated with COVID-19 severity in the composite score. Based on these findings, we concluded that factors other than body composition play a more critical role in developing severe COVID-19.

An unavoidable limitation of the study performed in Chapter 7 was the cross-sectional nature of the BIA measurements, warranting recalculations to dry weight. Under ideal circumstances, serial BIA measurements provide more details on the course of loss of muscle mass and fluid overload in critically ill patients. Therefore, we designed a subsequent prospective observational study.

In **Chapter 8**, we performed a prospective observational study, conducting BIA amongst COVID-19 patients within 24 hours of hospital admission, with a follow-up of 90 days. One hundred and fifty patients were included. In multiple regression, phase angle was independently and inversely correlated with the risk of ICU admission, complications, hospital length of stay, and a composite outcome score. The addition of phase angle improved the discriminative power for a composite of adverse outcomes compared to individual predictors. Based on these results, phase angle can and should be considered a valuable component of any future risk scores concerning COVID-19 and disease course, including ICU admission. In contrast to our previous cross-sectional research, we did not demonstrate a correlation between fluid overload and adverse outcomes. This discrepancy is most likely explained by the fact that patients had not yet received significant fluid resuscitation, further proving the value of early and repeated BIA measurements.

COVID-19 pneumosepsis survivors are at a high risk of developing intensive care unit ICUAW because of the high incidence of acute respiratory distress syndrome and the common need for prolonged invasive ventilation. Whether regular post-pneumosepsis physical rehabilitation strategies suit this extraordinary patient category was unknown.

The RECOVID study, which is detailed in **Chapter 9**, focussed on retrospectively comparing the physical recovery of COVID-19 and non-COVID pneumosepsis ICU survivors during post-ICU hospitalisation. Thirty-five COVID-19 ICU patients could be compared with 21 non-COVID pneumosepsis ICU survivors. All patients had ICUAW upon ICU discharge. However, COVID-19 patients performed worse on all physical assessments upon ICU discharge but had improved more at hospital discharge. Therefore, we assessed that COVID-19 ICU patients might benefit from early, more intensive physical therapy.

Dietary protein is a well-known anabolic stimulus that promotes and maintains muscle mass in both healthy and various clinical settings. A high protein diet is suggested to be beneficial during COVID-19, as protein provision may prevent further breakdown of muscle protein for gluconeogenesis, thereby preventing the patient from going into a further catabolic state. A large proportion of hospitalised COVID-19 patients are overweight. However, there has yet to be a consensus in the literature on how lean body mass (LBM) can best be estimated to adequately guide nutritional protein recommendations in hospitalised patients who are not at an ideal weight. **Chapter 10** described a post-hoc analysis of the studies described in Chapters 8 and 9. In our analysis, we could not identify a mathematical method for calculating LBM that had an acceptable agreement with LBM derived from BIA for males and females across all BMI subgroups in our hospitalised COVID-19 population. Consequently, significant discrepancies were observed when assessing the adequacy of protein provision in ICU patients, who, on average, only received two-thirds of their protein target as set by BIA. Therefore, if possible, we strongly advise using baseline BIA to guide protein dosing.

In **Part IV,** we discuss the conclusions that can be drawn from the findings in this thesis. The metabolism of critically ill patients is remarkably different from that of healthy individuals. While some metabolic traits appear to predispose to particular courses of illness, those illnesses and their treatment conversely impact patients' body composition and metabolic requirements. If nutritional caloric and protein targets are guided by energy requirements and body composition estimations, harmful under- and overfeeding may occur in various phases of critical illness.

Individualised caloric and protein goals based on bedside calorimetry and body composition measurements have the potential to become the backbone of nutrition therapy in the critically ill.

Samenvatting

De metabole veranderingen die optreden tijdens kritieke ziekte worden steeds vaker beschouwd als adaptieve aanpassingen, die vitale weefsels en functies beschermen, in plaats van metabool falen ten gevolge van de ziekte. Met dit inzicht is de prioriteit bij de behandeling van intensive care (IC) patiënten verschoven van het streven naar een zo normaal mogelijke situatie, naar het ondersteunen van de aanpassingen die het lichaam maakt. Het moment en de aard van deze aanpassingen blijken sterk variabel tussen verschillende patiënten, ziekten en over de tijd. Aangezien er sterke aanwijzingen zijn dat de verkeerde timing, dosering of samenstelling van voedingsstoffen de kritiek zieken niet baadt en soms zelfs schaadt, is het van immens belang om meer kennis te vergaren over de verschillende metabole typen en fasen, en hoe we deze kunnen herkennen in de individuele patiënt.

DEEL I VERANDERINGEN IN BIOCHEMIE TIJDENS KRITIEKE ZIEKTE

Mitochondriële functie is geassocieerd met morbiditeit en mortaliteit gedurende en na kritieke ziekte. De theorie dat de vermindering van het mitochondriële energiemetabolisme een adaptief mechanisme is, in plaats van metabool falen door kritieke ziekte, wint aan kracht.

In **Hoofdstuk 2** geven we een overzicht van wat toch nog toe bekend is over mitochondrieel functioneren tijdens en na een kritieke ziekte. Studies tonen aan dat de mitochondriële functie in spieren ernstig verminderd is tijdens de vroege fase van ernstige ziekte. Er is echter een paradoxale afwezigheid van permanente schade in de organen van overlevenden. Deze bevindingen, in combinatie met negatieve resultaten die zijn waargenomen in grootschalige voedingsstudies in de vroege fase, heeft geleid tot de theorie dat mitochondriën een adaptieve metabole en bio-energetische downregulatie ondergaan, in plaats van bio-energetisch falen. Er is nog niet veel bekend over de consequenties van deze veranderingen voor de voedingsbehoefte. Het effect van hoog gedoseerd eiwit in de vroege fase is onduidelijk, terwijl nutritioneel vet bio-energetisch inert lijkt. Hoewel antioxidante micronutriënten essentieel zijn voor mitochondriële functie, laten studies naar hoge dosering van vitamines (C en D) geen voordeel zien. Tijdens de herstelfase na kritieke ziekte hebben patiënten waarschijnlijk een toegenomen behoefte aan micro- en macronutriënten om anabolisme te ondersteunen en mitochondriële functie te herstellen, hoewel robuste data over zowel behoefte als daadwerkelijke inname ontbreken. Wij concluderen dat een optimale voedingsstrategie in de vroege fase van

kritieke ziekte erop gericht moet zijn om schade door overvoeding te voorkomen en (adaptieve) mitochondriële functie te beschermen. Suppletie van micronutriënten moet waarschijnlijk in een strategische combinatie in plaats van een hoge dosering van één bepaalde voedingsstof. Onderzoek naar methoden om afzonderlijke metabole fasen te identificeren is essentieel om voeding tijdens en na kritieke ziekte te optimaliseren.

In **Hoofdstuk 3** beschrijven we de resultaten van een prospectieve cohortstudie met gepaarde controles. De studie beoogde inzicht te verschaffen in het energiemetabolisme van mitochondriën uit mononucleaire cellen uit perifeer bloed van septische patiënten tijdens de eerste week van hun IC-opname. We toonden aan dat septische patiënten een hogere basale en ATP-gekoppelde celademhaling hebben in vergelijking met gepaarde controlepatiënten. Verder bleek een progressieve toename van basale respiratie gedurende de eerste week geassocieerd met drie maanden mortaliteit. Gebaseerd op de resultaten van deze studie concluderen we dat de vermindering van het mitochondrieel energiemetabolisme die eerder werd aangetoond in de verschillende weefseltypen van septische patiënten, niet aantoonbaar is in mononucleaire cellen uit perifeer bloed.

DEEL II VERANDERINGEN IN ENERGIE METABOLISME TIJDENS KRITIEKE ZIEKTE EN HERSTEL

Het beloop van energieverbruik gedurende kritieke ziekte en herstel is complex en er zijn slechts weinig klinische studies die het onderzoeken. Het wordt sterk aangeraden om energieverbruik aan bed te bepalen bij kritiek zieke patiënten, om de schadelijke effecten van over- en ondervoeden te voorkomen. Indirecte calorimetrie is de gouden standaardmethode om energieverbruik te meten, maar het wordt nog niet alom gebruikt op de IC.

Hoofdstuk 4 geeft een overzicht van de actuele kennis en praktische kanttekeningen bij het gebruik van indirecte calorimetriemetingen tijdens en na IC-opname. We beschrijven dat energieverbruik beïnvloed wordt door een veelvoud aan individuele en iatrogene factoren, alsmede de afzonderlijke fasen van kritieke ziekte en herstel. In de katabole fase gedurende de eerste paar dagen lijkt het verbruik van endogene energiebronnen toegenomen, en waarschijnlijk vrijwel afdoende om te voorzien in de totale energiebehoefte. Een hoeveelheid aan exogene voeding die gericht is op het voorzien in de totale energiebehoefte kan in deze fase leiden tot overvoeding, aangezien toediening van exogene voedingsstoffen de endogene energieproductie niet tegen gaat en mitochondriën het overtollige substraat niet kunnen verwerken. Na deze eerste fase, en in elk geval tot drie weken na IC-opname, stijgt het energieverbruik. Aangezien de endogene energieproductie weer is afgenomen, is inname van exogene voedingsstoffen in deze

fase essentieel. Het is nog niet onomstotelijk klinisch bewezen dat een voedingsstrategie gebaseerd op indirecte calorimetrie beter is dan een gebaseerd op schattingsmethoden. Daarnaast moeten menige praktische bezwaren en valkuilen in acht genomen worden, wanneer indirecte calorimetrie wordt toegepast bij kritiek zieke patiënten. Dit is mede aangezien het nog niet mogelijk is om de bijdrage van energie van endogene oorsprong te meten. Toch leidt het toepassen van geïndividualiseerd voedingsadvies, gebaseerd op routinematig verrichtte indirecte calorimetriemetingen potentieel bij aan het verbeteren van de voedingsstatus en daarmee de lange termijn uitkomsten van kritiek ziekte patiënten.

In **Hoofdstuk 5** beoogden we het beloop van energieverbruik tijdens kritieke ziekte te beschrijven door herhaalde indirecte calorimetriemetingen te verrichten gedurende de IC- en post-IC ziekenhuisopname. In een prospectieve observationele studie bij mechanisch geventileerde IC patiënten verrichtten we herhaaldelijke indirecte calorimetriemetingen binnen 24 uur na intubatie en vervolgens elke drie dagen. Na extubatie werden de metingen gecontinueerd tot ziekenhuisontslag. Het energieverbruik in rust werd herhaaldelijk gemeten op de IC en de klinische afdelingen en de resultaten van deze metingen werden vervolgens met elkaar vergleken. Daarnaast werd het gemeten energieverbruik in rust vergleken met het de waarde die door veelgebruikte formules was voorspeld. Er werden 223 indirecte calorimetriemetingen verricht bij 56 patiënten. Bij opname op de IC was het gemeten energieverbruik in rust niet anders dan de voorspelde waarde, maar vanaf de vierde opnamedag was de gemeten waarde hoger dan voorspeld. Dit suggereert een hypermetabole staat. Er werden 44 metingen verricht op de verpleegafdeling na ontslag van de IC. Gedurende dit deel van de ziekenhuisopname was het gemiddelde gemeten energieverbruik in rust hoger dan tijdens de IC-opname. We concludeerden dat onze kritiek zieke, geïntubeerde patiënten vanaf de vierde IC-opnamedag hypermetabool waren. Indirecte calorimetriemetingen laten een toename van het energieverbruik in rust zien wanneer patiënten vanaf de intensive care naar de verpleegafdeling worden ontslagen.

DEEL III VERANDERINGEN IN LICHAAMSSAMENSTELLING TIJDENS KRITIEKE ZIEKTE EN HERSTEL

Kritieke ziekte leidt tot ingrijpende veranderingen in lichaamssamenstelling, onder andere door de afname van de vetvrije massa als gevolg van een langdurige katabole fase. De hieruit volgende IC-verworven spierzwakte is een belangrijke oorzaak en voorspeller voor lichamelijke beperkingen en een verminderde kwaliteit van leven tijdens de revalidatieperiode. Bioelektrische impedantie analyse (BIA) is de meest toepasbare methode om lichaamssamenstelling aan bed te meten. Tot nog toe is het gebruik van

bioelektrische impedantie echter niet gevalideerd bij IC patiënten en de interpretatie van de analyses is uitdagend bij deze groep.

Hoofdstuk 6 beschrijft de mogelijke klinische toepassingen van BIA en exploreert de valkuilen en potentiële oplossingen bij het gebruik van BIA op de IC. Er wordt herhaaldelijk een correlatie gevonden tussen de ruwe impedantie waarden en vochtverdeling, overhydratie en een slechte uitkomst van kritieke ziekte. Toch zijn de afkap- en referentieaarden in de IC populatie nog onduidelijk. De ervaring met het gebruik van BIA bij het reguleren van de vochtverdeling is beperkt. De van BIA afgeleide spiermassameting lijkt een veelbelovende biomarker voor sarcopenie en correleert goed met spiermassaschattingen gebaseerd op computer tomografiebeelden. Lichaamscelmassa en vetvrijemassa zijn potentieel nuttig voor het schatten van metabole activiteit, proteïnebehoefte en farmacokinetiek. Verschillende methoden die de interpretatie van BIA metingen in de IC-populatie betrouwbaarder kunnen maken, moeten worden gevalideerd. Tot die tijd zijn er te veel onzekerheden en discrepanties met betrekken tot de interpretatie van BIA metingen op de IC om er therapeutische consequenties aan te verbinden. Toch zijn er verschillende veelbelovende BIA onderzoekslijnen met betrekking tot de meest prangende klinische uitdagingen die de intensive care geneeskunde kent.

In 2020 brak de Severe Acute Respiratory Syndrome CoronaVirus-2 (SARS-CoV-2) pandemie uit, wat leidde tot een hoge incidentie van Corona Virus Disease-2019 (COVID-19) en toegenomen ziekenhuis- en IC-opnames. Het werd al snel duidelijk dat de ernst van ziekte bij COVID-19 samenhing met overgewicht, maar het was niet duidelijk of het gevaar schuilde in de vetmassa, vetverdeling of een ander aspect van de lichaamssamenstelling.

In **Hoofdstuk 7** beschrijven we de resultaten van een studie naar de associatie tussen lichaamssamenstelling en de ernst van ziekte van opgenomen COVID-19 patiënten. We verrichtten een observationele cross-sectionele cohortstudie waarbij de lichaamssamenstelling van alle opgenomen COVID-19 patiënten met BIA werd gemeten. De lichaamswaterparameters werden herberekend naar drooggewicht, dat werd geschat met hulp van de verhouding tussen het gemeten extracellulair en totaal lichaamswater. Vierenvijftig patiënten werden geïncludeerd, waarvan er 30 op de verpleegafdeling en 24 op de intensive care waren opgenomen. De patiënten hadden overgewicht, maar de gemiddelde Body Mass Index (BMI) was niet verschillend tussen de groepen. De gemeten lichaamssamenstelling parameters bleken niet onafhankelijk geassocieerd met de ernst van ziekte. In multipele logistische regressie analyse was een lage fasehoek wel geassocieerd met ernst van ziekte. Gebaseerd op deze bevindingen concludeerden we dat er andere factoren dan lichaamssamenstelling een belangrijkere rol spelen in het ontwikkelen van ernstige COVID-19.

Een inherente limitatie van de in hoofdstuk 7 beschreven studie was het feit dat de BIA metingen cross-sectioneel werden verricht, waardoor een herberekeningen van de lichaamswater parameters naar drooggewicht noodzakelijk was. Onder ideale omstandigheden geven herhaaldelijke BIA metingen een beter inzicht in spiermassaverlies en de veranderende vochtbalans in kritiek zieke patiënten. Daarom ontwierpen we een prospectieve vervolgstudie.

In **Hoofdstuk 8** verrichtten we een prospectieve observationele studie waarin BIA metingen binnen 24 uur na ziekenhuisopname werden verricht bij COVID-19 patiënten, met een follow-upduur van 90 dagen. Honderdvijftig patiënten werden geïncludeerd. Bij multipele regressie analyse was de fasehoek onafhankelijk omgekeerd gerelateerd aan het risico voor IC-opname, complicaties, opnameduur en een combinatie van deze uitkomsten. Het toevoegen van de fasehoek verbeterde de voorspellende waarde van een risicoscore samengesteld uit voorspellende factoren voor uitkomst van ziekte bij COVID-19, ten opzichte van voorspellende waarde van de individuele factoren. Gebaseerd op deze resultaten kan en moet de fasehoek overwogen worden als een belangrijk onderdeel van elke risicoscore voor het beloop van COVID-19 inclusief IC-opname. In tegenstelling tot ons eerdere cross-sectionele onderzoek liet deze studie geen correlatie tussen overvulling en slechtere klinische uitkomst zien. Dit is waarschijnlijk te verklaren door het feit dat de prospectieve metingen werden verricht vóór de vochtresuscitatie. Dit benadrukt het belang van het vroeg en herhaaldelijk verrichten van BIA metingen bij kritiek zieke patiënten om de interpretatie ervan te bevorderen.

Door het veelvuldig voorkomen van het *Acute Respiratory Distress Syndrome* (ARDS) en de uitzonderlijk langdurige beademingsbehoefte hebben overlevenden van een COVID-19 pneumosepsis een hoog risico op het ontwikkelen van IC- verworven spierzwakte. Het was tot nog toe onbekend of de gebruikelijke revalidatiestrategieën voor post-pneumosepsis patiënten geschikt waren voor deze uitzonderlijke patiëntcategorie. De RECOVID studie die wordt beschreven in **Hoofdstuk 9** vergeleek retrospectief het lichamelijke herstel van pneumosepsispatiënten door SARS-CoV-2 met patiënten met pneumosepsis op basis van andere verwekkers. Vijfendertig COVID-19 patiënten werden vergeleken met 21 anderen. Alle patiënten hadden IC- verworven spierzwakte bij IC ontslag. COVID-19 patiënten deden het slechter bij alle testen voor lichamelijk functioneren bij IC-ontslag, maar waren beter hersteld bij ziekenhuisontslag. Daarom concluderen we dat COVID-19 patiënten mogelijk baat hebben bij vroegere en intensievere fysiotherapie dan andere pneumosepsis IC patiënten.

Voedingseiwitten zijn een bekende anabole prikkel, die spiermassa bevordert en behoudt in zowel gezonde mensen als in verschillende klinische omstandigheden. Er wordt verondersteld dat een hoog-eiwit dieet gunstig is voor COVID-19 patiënten, aangezien

eiwitten de spiereiwitafbraak ten behoeve van gluconeogenese beperken en daarmee een katabole toestand voorkomen. Een groot deel van de opgenomen COVID-19-patiënten heeft overgewicht, maar in de literatuur is geen consensus over hoe de vetvrije lichaamsmassa het beste kan worden geschat bij opgenomen patiënten die niet op hun ideale gewicht zijn, om zodoende een adequaat advies te kunnen geven over de eiwitinname. **Hoofdstuk 10** beschrijft een post-hoc analyse van de in Hoofdstukken 8 en 9 beschreven studies. In deze analyse konden we geen bestaande formule identificeren voor het berekenen van LBM die een acceptabele overeenkomst had met de van BIA afgeleide vetvrije massa. Dit gold voor zowel mannen als vrouwen in al onze BMI-subgroepen in de opgenomen COVID-19-populatie. Als gevolg daarvan werden er aanzienlijke discrepanties waargenomen bij het beoordelen van de adequaatheid van eiwitverstrekking bij IC-patiënten. Gemiddeld ontvingen patiënten slechts tweederde van hun retrospectief met BIA vastgestelde eiwitdoel. Daarom raden we ten zeerste aan om indien mogelijk BIA te gebruiken om eiwitdosering te begeleiden.

In **Deel IV** bespreken we de belangrijkste conclusies die volgen uit de in deze scriptie beschreven onderzoeken. Het metabolisme van kritiek zieke patiënten verschilt opvallend van dat van gezonde mensen. Terwijl sommige metabole kenmerken predisponeren voor een bepaald ziektebeloop, hebben deze ziekten en de behandeling ervan omgekeerd grote invloed op de lichaamssamenstelling en metabole behoeften van patiënten. Als de calorie- en eiwitdoelen afgestemd zijn op geschatte energiebehoefte en de lichaamssamenstelling, kan dit zowel tot schadelijke onder- als overvoeding leiden in verschillende fasen van een ernstige ziekte.

Geïndividualiseerde calorie- en eiwitdoelen, gebaseerd op calorimetrie en metingen van de lichaamssamenstelling aan bed, hebben de potentie om de ruggengraat van voedingstherapie bij kritieke ziekte te worden.

List of abbreviations

ADP	Adenine diphosphate
APACHE II	Acute physiology and chronic health evaluation II
ATP	Adenosine triphosphate
ARDS	Acute respiratory distress syndrome
BCa	Bias-corrected and accelerated bootstrap
BIA	Bioelectric impedance analysis
BMI	Body mass index
CCCP	Carbonyl cyanide m-chlorophenylhydrazone
CI	Confidence interval
CK	Creatinine kinase
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Corona Virus Disease-2019
CRP	C-reactive protein
CT	Computed tomography
ECW	Extracellular water
ER	Emergency room
FEV1	First second forced expiration
FFM	Fat-free mass
FVC	Forced vital capacity
FO	Fluid overload
HLOS	Hospital length of stay
HR	Hazard ratio
ICU	Intensive care unit
ICW	Intracellular water
IQR	Interquartile range
LOS	length of stay
LTP	limited treatment plan
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
mNUTRIC	Modified nutrition risk in critically ill score
PA/PhA	(50 kHz total body) phase angle
PBF	Percentage body fat
PBMC	Peripheral blood mononuclear cell
SARS-CoV-2	Severe acute respiratory syndrome coronavirus-2
SLM	Soft lean mass
SMI	Skeletal muscle mass index
SMM	Skeletal muscle mass

SOFA	Sequential organ failure assessment
TBW	Total body water
TP	Treatment plan
VFA	Visceral fat area
VIF	Variation inflation factor
WUR	Wageningen University and Research
ZGV	Ziekenhuis Gelderse Vallei (Gelderse Vallei hospital)

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About the Author

Hanneke Moonen was born on April 27th 1993 in Landgraaf, The Netherlands. After completing secondary school at the Bernardinuscollege in Heerlen in 2011, she moved to Nijmegen to study Medicine at the Radboud University. Throughout her studies, she actively contributed to the content and quality of the medical curriculum as a student assistant, with an emphasis on acute medicine. Her scientific rotation was entitled 'Student-teacher participation in curriculum development: exploring the perceptions of five stakeholders in a medical curriculum' (Prof. Dr C.R.M.G. Fluit). During her studies, Hanneke developed a strong affinity for surgical specialities. Her final clinical rotations were at the Department of Plastic Surgery at the Radboudumc (Prof. Dr D. Ulrich) and the Departments of Trauma Surgery of the Liverpool Hospital in Sydney, Australia (Dr S. D'Amours & Dr E.C.T.H. Tan) and the Chris Hani Baragwanath Hospital in Johannesburg, South Africa (Prof. F. Plani & Dr E.C.T.H. Tan).

After obtaining her Master of Science in Medicine cum laude in December 2017, she started working as a resident, not in training at the Department of Surgery at Ziekenhuis Gelderse Vallei. Here, she met Professor Dr Arthur van Zanten, who shared his passion for clinical nutrition research with her. Together, they established and performed a collaborative scientific research project between the Department of Intensive Care and the Departments of Nutritional Biology and Human and Animal Physiology of Wageningen University and Research. During this time, Hanneke worked as a resident, not in training, at the Department of Intensive Care between 2019 and 2020 to develop her medical knowledge and skills further. In 2020, she returned to the Department of Surgery at Ziekenhuis Gelderse Vallei, where she was admitted to the surgical training program of the Radboudumc in Nijmegen, which started in January 2022. Hanneke continued working on her research projects during her training, culminating in this thesis.

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