# Study Protocol

## Protein Dosing Strategies in Critically Ill Patients: Comparing Actual Body Weight and Fat-Free Mass Approaches in an Observational Cohort Study

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## Protocol synopsis

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| Study Title | Protein Dosing Strategies in Critically Ill Patients: Comparing Actual Body Weight and Fat-Free Mass Approaches in an Observational Cohort Study |
| Study Objectives | The study is designed to investigate the following research questions:<br>**Primary:**<br>The **primary outcome measure** of this study is **90- day mortality**, comparing patients who received protein based on *total body weight* with those who received protein based on *fat-free mass*. Mortality will be assessed as both short-term mortality (ICU and 30-day mortality) and long-term mortality (90-day mortality).<br><br>**Secondary:**<br>**Secondary outcome measures** include several clinical and nutritional endpoints, all comparing the cohort receiving protein based on *actual body weight* with the cohort receiving protein based on *fat-free mass*:<br>• **ICU and hospital length of stay**, defined as the total duration of ICU and hospital admission in days, measured from ICU admission until discharge.<br>• **Duration of invasive mechanical ventilation (IMV)**, defined as the number of days a patient receives IMV from initiation until discontinuation.<br>• **Urea–creatinine ratio (UCR) during ICU stay**, comparing differences in UCR between both groups throughout the ICU stay.<br>• **Changes in BIA parameters over time**, including changes in fat-free mass and other body composition measures, based on repeated bioelectrical impedance analysis (BIA) measurements during ICU stay.<br>• **Protein and energy intake during ICU stay**, comparing daily and cumulative (first 14 days) protein and energy intake between the two groups.<br>• **Hazard ratio for mortality**, evaluating the relationship between protein intake (based on TBW and FFM) and mortality. This includes actual protein intake per kilogram TBW and FFM, with analysis of days with full intake and patients with an ICU stay >7 days. Covariates will be included to account for potential confounders. The analysis will also be broken down by sex.<br>• Differences in **indirect calorimetry** between the two groups, assessing energy expenditure and comparing results from indirect calorimetry measurements during ICU stay. |
| Study Design | This study is a single-centre, combined retrospective and prospective cohort study conducted at Hospital Gelderse Vallei. Since April 2020, measurement of body composition has been implemented as part of standard care for all patients admitted to the intensive care unit (ICU). Each patient admitted to the ICU undergoes bioelectrical impedance analysis (BIA) upon admission and approximately every third day thereafter (±1 day), using the InBody S10® device (InBody Co., Ltd., Seoul, Korea).<br><br>From July 7th, 2023, protein requirements for ICU patients have been calculated based on fat-free mass rather than total body weight, as part of the standard of care. This study evaluates differences in clinical outcomes resulting from this change in practice. |

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| | All patients are screened for inclusion and exclusion criteria upon ICU admission. BIA measurements are extracted directly from the device. Data on protein intake and clinical outcome measures are retrieved and anonymized from the electronic patient record systems MetaVision® (iMDsoft, Tel Aviv, Israel), Neozis® (MI Consultancy, Katwijk, The Netherlands), and Nexus® (NEXUS AG, Donaueschingen, Germany). |
| Patients | 620 critically ill patients admitted to the ICU (310 with protein dosing based on actual body weight and 310 with protein dosing based on fat free mass) |
| Inclusion Criteria | Patients meeting all of the following criteria are eligible for the study:<br>1. Age ≥ 18 years<br>2. A BIA measurement within 48 hours after ICU admission<br>3. Two BIA measurements during ICU stay |
| Exclusion Criteria | Any patient meeting one or more of the following criteria is not eligible for the study:<br>1. Transfer from another ICU resulting in the first BIA measurement >48 hours after admission to Gelderse Vallei Hospital<br>2. A previous ICU admission within 2 weeks before the current admission<br>3. Contra-indications for BIA measurement:<br>i. Patients with electrical implants such as a pacemaker or ICD<br>ii. Patients who cannot maintain posture for the (5 min) duration of the measurement<br>iii. Patients with wounds or skin damage in the designated electrode sight<br>iv. Pregnancy |
| Study Parameters | <u>The objective parameters to be studied will be:</u><br>• All-cause 90-day mortality<br>• All-cause 30-day mortality<br>• All-cause in-hospital and ICU mortality<br>• Hospital length of stay<br>• ICU length of stay<br>• Post-ICU hospital days<br>• Time-to-discharge alive<br>• Length of mechanical ventilation<br>• Body composition measured through body impedance analysis upon admittance and every third day (+/- 1 day) after that<br>• Urea-to-creatinine ratio during ICU stay<br>• Daily protein intake during ICU stay<br>• Daily energy intake during ICU stay<br>• Protein target during ICU stay<br>• Energy target during ICU stay |
| Other Parameters | **Demographical characteristics**<br>• Age [yrs], race, sex [m/f], weight [kg], height [cm], and weight/height (BMI in kg/m$^2$)<br>• Pre-existing comorbidities<br>• Type of admission (medical, surgical/emergency or elective), covid-19 at admission.<br>• Baseline laboratory results (CRP, albumin, creatinine, blood urea nitrogen (BUN), urea-to-creatinine-ratio<br>**Markers of disease severity and treatment** |

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| | • ICU admission diagnosis<br>• APACHE-II and IV scores<br>• NUTRIC-score<br>• Barthel Score<br>• SOFA-score |
| Study Group | All patients admitted to the ICU will be included according to the in- and exclusion criteria. A total of 620 eligible patients will be included: 310 treated during the ABW dosing period and 310 during the FFM dosing period |
| Study Period | The inclusion period began in April 2020, when BIA (Bioelectrical Impedance Analysis) became part of standard care. A total of 620 patients will be included in the study. One group of patients was fed based on ABW (Actual Body Weight) until July 2023, and a second group was fed based on FFM (Fat-Free Mass) thereafter. Patients for whom obtaining informed consent was not feasible were included retrospectively, while prospective inclusion continued afterward. The study will conclude once a total of 620 patients have been included, followed by a 90-day follow-up period for the final patient |
| Study Description | This study aims to evaluate the effect of two different methods of calculating protein requirements on clinical outcomes and body composition in critically ill patients admitted to the Intensive Care Unit (ICU) at Hospital Gelderse Vallei. Since April 2020, bioelectrical impedance analysis (BIA) has been implemented as standard practice to assess body composition in all ICU patients. In July 2023, the ICU transitioned from protein dosing based on actual body weight to dosing based on fat-free mass. The study compares these two cohorts to determine whether protein dosing based on fat-free mass results in improved clinical outcomes, such as lower mortality, shorter ICU and hospital length of stay, reduced duration of mechanical ventilation, preservation of muscle mass, and lower urea–creatinine ratios. All patients admitted to the ICU will be screened for inclusion and exclusion criteria. Those meeting all inclusion and no exclusion criteria will be enrolled in the study. Data on body composition, protein intake, and clinical outcomes will be collected and analysed. |
| Statistical Analysis | All statistical analyses will be conducted using R 4.4.1 and Rstudio. Continuous variables will be reported as means or medians, when appropriate, including SD, ranges, and 95%CI. Discrete variables will be displayed as proportions. P-values $<0.05$ will be considered statistically significant. Comparisons will be made using appropriate statistical tests. |

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## Abbreviations and Definitions

| Term | Explanation |
|---|---|
| BIA | Bioelectrical Impedance Analysis |
| BMI | Body Mass Index |
| EC | Ethics Committee |
| FFM | Fat-Free Mass |
| IBW | Ideal Body Weight |
| ICU | Intensive Care Unit |
| LBM | Lean Body Mass |
| TBW | Total Body Weight |
| ZGV | Ziekenhuis Gelderse Vallei |

# 1 Introduction

## 1.1 Background

Proteins are essential macronutrients for critically ill patients. Deficiency of proteins can result in a higher rate of hospital-acquired infections, impaired wound healing, and loss of skeletal muscle mass (1,2). On the other hand, adequate protein intake during ICU admission is associated with a higher degree of recovery to independent walking (3), which can most likely be translated into preserved muscle strength at ICU discharge.

Critically ill patients are known to be prone to sarcopenia, which can be defined as generalized and progressive loss of muscle mass and strength. (4) Sarcopenia can be developed due to different factors such as inactivity, inflammation, and nutritional status.(5) The prevalence of sarcopenia is estimated to be approximately 30-70% in patients admitted to the intensive care unit (ICU) and is associated with adverse clinical outcomes and even mortality.(5,6).

There are studies performed that have shown beneficial effects on the loss of muscle mass and muscle protein synthesis induced by the administration of higher dosages of protein.(7) An element of establishing the appropriate dosages of protein is providing insight into the influence of protein provision on this process. This can be carried out by repeatedly measuring the lean body mass (LBM) from ICU admission until discharge.

In recent years, the importance of including body composition in assessing a patient's nutritional status has become increasingly apparent (8,9). Our hypothesis, supported by studies like Velzeboer et al. 2017 (10), is that protein requirements are primarily determined by the fat-free mass (FFM) of the body. However, in actual practice protein goals are often calculated using the actual body weight or estimated ideal body weights with predictive formulas. Predictive formulas cannot take real-time changes in muscle mass or sarcopenic obesity into account, and can differ greatly from the actual lean body mass. This is especially a problem when people are underweight or overweight. (10)

There are several methods to determine the LBM, including dual-energy X-ray absorptiometry (DXA/DEXA), computerized tomography (CT), and magnetic resonance imaging (MRI)[11]. Disadvantages of these techniques include the need for specialized trained personnel, high costs, stationary equipment not available in the ICU itself, the time-consuming process, and the possible involvement of radiation exposure (12, 13). Another method to determine the LBM is using bioelectrical impedance analysis (BIA). BIA is a technique in which a weak current is passed through the body, and the resistance across this current is calculated. The advantage of BIA over other methods is that it is non-invasive, less expensive, and fast(14,1). Moreover, it can be used in the ICU due to its portability(16). These BIA measurements could be an alternative to the before-mentioned techniques to estimate the LBM.

## 1.2 Description of the study

This study is a single-centre combined retrospective and prospective cohort study, performed at hospital Gelderse Vallei. Starting from April 2020, the measurement of body composition of all patients admitted to the intensive care unit in our hospital was implemented as a standard of care. All patients admitted to the intensive care unit receive a bioelectric impedance analysis (BIA) upon admittance and every third day (-/+ 1 day) after that, using the Inbody S10 ® (InBody Co., Ltd., Seoul, Korea).

Every patient will be screened in- and exclusion criteria upon admittance. The BIA measurements will be extracted from the device. The patients' protein intake and various clinical outcome measures will be extracted from the electronic patient record MetaVision ® (iMDsoft, Tel Aviv, Israel), Neozis and Nexus and anonymized. Data will be extracted, and the database will be locked after the protocol publication.

**Rationale for study design**
This study hypothesizes that calculating protein requirements based on fat-free mass rather than actual body weight will result in improved clinical and nutritional outcomes in critically ill patients. Current clinical practice often relies on protein dosing per kilogram of actual body weight, which does not account for variations in body composition such as sarcopenia or obesity. Using fat-free mass provides a more accurate reflection of the metabolically active tissue and may therefore allow for more precise and individualized protein dosing. Prospective data on changes in lean body mass (LBM) among ICU patients—measured using bioelectrical impedance analysis (BIA) while correcting for extracellular fluid—are currently lacking. This study addresses that gap by systematically monitoring body composition throughout ICU admission. The findings may offer valuable insight into muscle mass deterioration during critical illness and support the development of more accurate protein requirement guidelines.

## 2 Study objectives
**Primary:**
The **primary outcome measure** of this study is **90-day mortality**, comparing patients who received protein based on *total body weight* with those who received protein based on *fat-free mass*. Mortality will be assessed as both short-term mortality (ICU and 30-day mortality) and long-term mortality (90-day mortality)**Secondary:**

**Secondary outcome measures** include several clinical and nutritional endpoints, all comparing the cohort receiving protein based on *actual body weight* with the cohort receiving protein based on *fat-free mass*:

- **ICU and hospital length of stay**, defined as the total duration of ICU and hospital admission in days, measured from ICU admission until discharge.
- **Duration of invasive mechanical ventilation (IMV)**, defined as the number of days a patient receives IMV from initiation until discontinuation.
- **Urea–creatinine ratio (UCR) during ICU stay**, comparing differences in UCR between both groups throughout the ICU stay.
- **Changes in BIA parameters over time**, including changes in fat-free mass and other body composition measures, based on repeated bioelectrical impedance analysis (BIA) measurements during ICU stay.
- **Protein and energy intake during ICU stay**, comparing daily and cumulative (first 14 days) protein and energy intake between the two groups.
- **Hazard ratio for mortality**, evaluating the relationship between protein intake (based on TBW and FFM) and mortality. This includes actual protein intake per kilogram TBW and FFM, with analysis of days with full intake and patients with an ICU stay >7 days. Covariates will be included to account for potential confounders. The analysis will also be broken down by sex.
- Differences in **indirect calorimetry** between the two groups, assessing energy expenditure and comparing results from indirect calorimetry measurements during ICU stay.

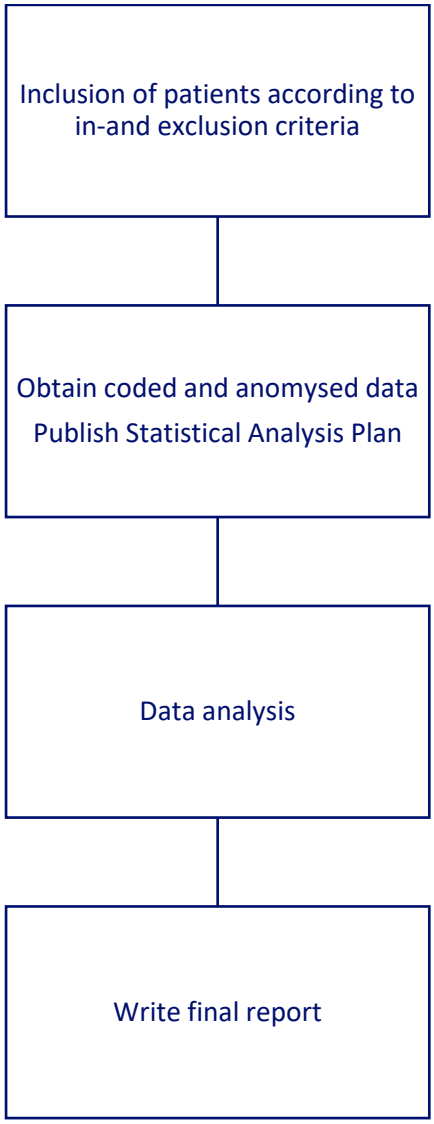
## 3 Study design
This is a combined retrospective and prospective observational cohort study.

### 3.1 Study description
Upon admittance, all patients will be screened according to the in- and exclusion criteria on ICU admission. Patients that meet all the inclusion criteria and none of the exclusion criteria will be included in this prospective observational cohort study. Patients will be enrolled through an opt-out procedure with a leaflet in the admission folder.

Height and weight will be measured and recorded upon admittance by the researchers. Measurements will be performed by ICU nurses following standard of care within 24 hours after admittance to the ICU and repeated every 3 days. The BIA measurements will be extracted from the device. Various baseline variables, daily protein and energy intake (both enteral and oral), and various clinical

outcome measures will be extracted from the electronic patient record MetaVision ® (iMDsoft, Tel Aviv, Israel) and Nexus and anonymized.

### 3.2 Schematic diagram of study design




## 4 Patients

### 4.1 Study population

Critically ill patients admitted to the ICU

### 4.2 Inclusion criteria

Patients meeting all of the following criteria are eligible for the study:

1. Age ≥ 18 years
2. A BIA measurement within 48 hours after ICU admission
3. Two BIA measurements during ICU stay

### 4.3 Exclusion criteria

Any patient meeting one or more of the following criteria is not eligible for the study:

1. Transfer from another ICU resulting in the first BIA measurement >48 hours after admission to Gelderse Vallei Hospital

2. A previous ICU admission within 2 weeks before the current admission

3. Contra-indications for BIA measurement:
   v. Patients with electrical implants such as a pacemaker or ICD
   vi. Patients who cannot maintain posture for the (5 min) duration of the measurement
   vii. Patients with wounds or skin damage in the designated electrode site
   viii. Pregnancy

# 5 Study parameters

## 5.1 Objective study parameters

The objective parameters to be studied will be:

- All-cause 90-day mortality
- All-cause 30-day mortality
- All-cause in-hospital and ICU mortality
- Hospital length of stay
- ICU length of stay
- Post-ICU hospital stay
- Time-to-discharge alive
- Length of mechanical ventilation (invasive and non-invasive)
- Body composition measured through body impedance analysis upon admittance and every third day (+/- 1 day) after that
- Urea-to-creatinine ratio during ICU stay
- Daily protein intake during ICU stay (absolute and per kg IBW and FFM). IBW: TBW is used if BMI < 27; if BMI > 27, weight is calculated for a BMI of 27
- Daily energy intake during ICU stay (absolute and per kg IBW and FFM). IBW: TBW is used if BMI < 27; if BMI > 27, weight is calculated for a BMI of 27
- Protein target during ICU stay
- Energy target during ICU stay

## 5.2 Overview of study parameters and covariates

**Demographical characteristics**

- Age [yrs], race, sex [m/f], weight [kg], height [cm], and weight/height (BMI in $kg/m^2$)
- Pre-existing comorbidities
- Type of admission (medical or surgical and emergency or elective).
- Baseline laboratory results (CRP, albumin, creatinine, blood urea nitrogen (BUN), urea-to-creatinine ratio)

**Markers of disease severity and treatment**

- ICU admission diagnosis
- APACHE-II and IV scores

- Barthel score
- NUTRIC-score
- SOFA-score

**6 Study procedures and assessments**
**6.1 Study procedures**
*6.1.1 Screening*
The inclusion period started on April 2020. Patients for whom obtaining informed consent was not feasible (before BCWO approval) were included retrospectively, while prospective inclusion continued thereafter with opt-out procedure. The study will conclude once a total of 620 patients have been included, followed by a 90-day follow-up period for the final patient.

*6.1.2. Measurements*
BIA measurements will be performed by the ICU nurses within 24 hours of admittance and repeated every third day (+/1 day) after that during ICU admission.

**6.2 Assessments**
*6.2.1 Demographics*
Demographics including date of birth (age) and gender are recorded.

*6.2.2 Anthropometry*
Actual body weight and height will be measured by the researchers. Bodyweight and height are used to calculate the Body Mass Index (BMI, kg/m2).

*6.2.3 Medical history, pre-existing conditions, and concomitant medication*
Relevant medical history and pre-existing conditions are recorded at baseline.

*6.2.4 Diagnosis*
Diagnosis for admission will be recorded at screening.

*6.2.5 Clinical measurements*
BIA measurements will be performed by the ICU nurses within 24 hours and repeated every third day (+/1 day) after that during ICU admission (standard care).

## 7. Statistical analysis plan
7.1 **General considerations**
All statistical analyses will be performed using R version 4.4.1 (or updated version). Categorical data will be presented as counts alongside percentages. Continuous data will be presented as either mean with standard deviation or median with interquartile range [IQR] in case of non-normal distribution. Baseline and outcome differences will be compared using the Chi-Square or Fisher exact test for categorical variables, independent sample t-test for normally distributed continuous data, and Mann-Whitney U test for continuous data with non-normal distributions. Linearity over time will be assessed visually and by comparing model fit between linear and spline-based mixed models. Non-linear models will be chosen if they significantly improve model fit.

**7.2 Primary analysis**

The primary endpoint is 90-day mortality. Mortality will be compared between patients receiving protein prescribed according to total body weight (TBW cohort) and those receiving protein based on fat-free mass (FFM cohort). Unadjusted cumulative survival will be visualized using Kaplan–Meier curves, and group differences will be evaluated using the log-rank test. To estimate independent

associations between protein dosing method and 90-day mortality, multivariable Cox proportional hazards regression models will be fitted. Covariates will be eligible for inclusion only if <10% of their values are missing. Candidate variables will first be screened using univariable Cox regression; variables associated with mortality at $p < 0.20$ will be considered for the multivariable model. Baseline characteristics that differ between groups or are clinically important will also be evaluated and included if they improve model fit.

Potential covariates include age, sex, APACHE IV score, admission category (medical/surgical), COVID-19 status, UCR at admission, and dry fat-free mass (dryFFM). Collinearity will be assessed using Variance Inflation Factors (VIF > 5 indicating concern). Proportional hazards assumptions will be tested using Schoenfeld residuals; if violated, models with time-varying effects or stratified Cox models will be considered. Covariate numbers will follow the one-in-ten rule to limit overfitting. Results will be reported as hazard ratios (HRs) with 95% confidence intervals (CIs), using two-sided $p < 0.05$ as significance threshold.

If substantial baseline imbalance is identified, a sensitivity analysis using propensity scores will be performed with relevant covariates (age, sex, APACHE score, BMI, admission category).

### 7.3 Secondary Endpoint Analyses

#### 7.3.1 Continuous single-measurement outcomes

ICU length of stay, hospital length of stay, and duration of invasive mechanical ventilation will be compared using linear regression. Models will be adjusted for baseline covariates that differ between groups or are clinically relevant (Age, APACHE IV , Covid, dryFFM).

#### 7.3.2 Repeated-measure outcomes (UCR, BIA parameters)

Longitudinal outcomes, including the urea–creatinine ratio (UCR) and dryFFM, will first be visualized graphically to assess temporal patterns. If appropriate, linear mixed-effects models (LMMs) with a random intercept for participants will be used. Time will be included as a categorical or spline-based variable depending on observed non-linearity. Interaction terms between group and time will be tested to evaluate differential evolution across cohorts.

### 7.4 Missing Data

The primary outcome (mortality) is expected to be complete; no imputation will be performed. Secondary outcomes with missingness (e.g., laboratory values, BIA measures, nutritional intake) will be assumed missing at random. If missing data are substantial, multiple imputation sensitivity analyses will be performed.

### 7.5 Additional Analytic Procedures (Study-Specific Specifications)

#### 7.5.1 UCR change-from-baseline (days 1–30)

Daily UCR will be analyzed from day 1 to day 30.

- UCR will be included only up to the initiation of dialysis (CVVH).
- Patients with active gastrointestinal bleeding will be excluded from UCR analyses.
- UCR change will be expressed as percentage change relative to baseline.
- Longitudinal modeling will use linear mixed-effects models; non-linearity will be accommodated via spline functions.
- Sex interactions will be evaluated, and stratified figures will be produced for men and women.

- Analyses will be repeated for all patients and for the non-COVID subgroup.

### 7.5.2 Body weight definitions

- For BMI < 27 kg/m², total body weight (TBW) will be used as recorded.
- For BMI ≥ 27 kg/m², TBW will be adjusted downward to the weight corresponding to BMI 27 to create an ideal body weight (IBW) equivalent for intake calculations.

### 7.5.3 Computation of dry fat-free mass (dryFFM)

Fluid overload will be derived as:
**FO = ECW − (0.38 × ICW / 0.62)**
If FO > 0, this value will be subtracted from FFM to calculate dryFFM.

### 7.5.4 Nutritional intake calculations

Daily nutritional intake will be calculated as the sum of oral and enteral intake.

- Intake will be expressed separately for protein and energy.
- Non-nutritional calories (e.g., from citrate and propofol) will be included in total energy intake.
- Days without recorded nutritional data will be treated as missing.
- Discharge day will be excluded; admission day will be included, with intake target scaled to the actual hours of observation.
- A standardized buildup protocol will be applied, increasing intake by 25% per 24 hours until full target is reached on day 4.
- In case of refeeding syndrome, defined as a decrease in phosphate from >0.70 mmol/L at admission to <0.65 mmol/L within 72 hours without another cause, targets will be reset to 25% for 48 hours, after which increments of 25% per day resume.

### 7.5.5 Protein dosing and intake metrics

No a priori difference in baseline characteristics is expected other than the dosing strategy:

- TBW cohort: prescribed at 1.5 g/kg TBW aiming for 1.3 g/kg (ESPEN) for patients with normal BMI, if BMI >27 IBW is used.
- FFM cohort: prescribed at 1.85 g/kg dryFFM.

Daily intake and cumulative intake will be expressed per kg IBW and per kg FFM.

Separate figures will depict protein and energy intake normalized by TBW (accounting for IBW) and by FFM, stratified by sex.

## 7.6 Baseline and Outcome Tables

### 7.6.1 Baseline characteristics

Baseline tables will include all prespecified binary and continuous variables.

- Continuous variables will be summarized using mean (SD) or median [IQR] depending on distribution.

- Statistical testing: t-tests for normally distributed variables, Wilcoxon tests for non-normal variables, and $\chi^2$ tests for binary variables.
  Baseline tables will be generated for all patients and non-covid patients
- Outliers will not be removed unless reflecting data entry errors.

### 7.6.2 Outcome tables

Binary and continuous outcomes will be summarized similarly and compared between groups. Outcome tables will be produced for all patients and non-covid patients. Outliers will not be removed unless reflecting data entry errors.

## 7.7 Primary outcome subgroup analyses

A separate baseline table will be produced comparing 90-day survivors and non-survivors across all baseline variables. Kaplan–Meier curves will be generated to compare unadjusted survival between cohorts. A final Cox model including age, APACHE IV, UCR, cohort, COVID status, and dryFFM will be fitted, with assessment of collinearity and proportional hazards assumptions.

Adjusted survival curves will be generated from model-based predictions.

## 7.8 Sensitivity analysis: Actual protein intake and 90-day mortality

Among patients with ICU length of stay ≥ 7 days, an analysis will focus on days where ≥100% of the prescribed target was achieved (excluding buildup/refeeding days). Protein intake will be expressed per kg TBW (accounting for IBW) and per kg FFM.

Restricted cubic spline Cox models will be used to evaluate dose-response shapes for protein intake, adjusted for age, APACHE IV, COVID status, and UCR. Sex-stratified figures will be created.

## 7.9 Body composition analyses

### 7.9.1 Temporal evolution of BIA parameters

Figures will be created for:

- All patients
- ICU survivors only

DryFFM percentage change relative to baseline will be analyzed.

- If changes appear linear, linear mixed-effects models will be used.
- If non-linear, spline-based mixed-effects models will be fitted.
- Interaction with cohort will be evaluated.

### 7.9.2 Relationship between TBW and FFM at baseline

Scatterplots with LOESS smoothing will show the association between BIA-measured TBW and dryFFM, with separate curves for men and women and an overall dashed curve. The potential for deriving sex-specific conversion factors to estimate FFM from TBW will be explored.

## 7.10 UCR longitudinal analyses (detailed)

UCR percentage change from baseline will be analyzed from day 1–30. Analyses will be performed for all patients and non-COVID patients.

Mixed-effects spline models will include cohort × time interactions, and additional sex interactions where appropriate. Both percentage change and absolute UCR values may be presented graphically.

### 7.11 Indirect calorimetry (REE) analyses

Resting Energy Expenditure (REE) measured by indirect calorimetry will be analyzed longitudinally using mixed-effects spline models, adjusting for age, sex, and dryFFM. Predicted curves over time will be plotted for each cohort.

## 8 Ethical considerations

### 8.1 Basic principles and regulations

The investigator will ensure that this study is conducted in full conformance with the principles of the 'World Medical Association Declaration of Helsinki' (52$^{nd}$ WMA General Assembly, Edinburgh, Scotland, October 2000, including the Notes of Clarification as added in 2002, Washington, and 2004, Tokyo).

### 8.2 Ethics Committee

This protocol was deemed not subject to the Dutch Medical Research Involving Human Subjects Act (niet-WMO). Therefore, approval from a central ethics committee was not required. The protocol was reviewed and approved by the Hospital Gelderse Vallei internal research review committee (BCWO).

### 8.3 Informed Consent

To minimize the risk of consent bias and reduce the burden on critically ill patients and their families, an opt-out informed consent procedure was implemented. Because it was neither feasible nor realistic to obtain informed consent from patients who had already been admitted to the ICU before the introduction of the opt-out procedure, permission was obtained from the BCWO to retrospectively include and anonymize patients, until the opt-out procedure became fully operational. Following BCWO approval, all newly admitted ICU patients or their legal representatives have been provided with an information leaflet about the study, included in the standard ICU admission folder. This allows them to decline participation at any time, in accordance with the approved opt-out procedure.

### 8.4 Confidentiality of study data

The investigator is responsible for treating study information as confidential. The investigator will ensure that the patient information will not be made publicly available. All patient study records are stored in a coded dataset to maintain the patients' confidentiality.

## 9 Administrative aspects

### 9.1 Source data

Source data are the medical records from MetaVision ® (iMDsoft, Tel Aviv, Israel) and Nexus used in Hospital Gelderse Vallei.

### 9.2 Data handling

The investigator is responsible for the design of the data collection forms.

### 9.3 Documentation and storage

All documents pertaining to the conduct of the study are kept by the investigator for a minimum period of 15 years in the digitalized archive system of the hospital.

## 10 References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