Study protocol

KETOsis, immune Function and metabolic Adaptation in response to Short-Term fasting in critical illness (KETO-FAST): A translational substudy of the FAST-ICU cluster-randomized cross-over trial.

Version history: Version 1.4.1

2026-04-14: updated informed consent procedure for participating centers in Sweden.

2026-06-19: sampling volumes updated (total volume unchanged).

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Background

Over the last 10-15 years, large randomized controlled trials of intensive care unit (ICU) patients have found either no benefit or signs of harm from providing full early feeding in relation to estimated energy expenditure [1]. The optimal energy delivery during the acute phase of critical illness remains to be determined. Although current guidelines recommend early hypocaloric nutrition, there is no high-quality evidence supporting this approach over no feeding during the first 3-7 days in the ICU [2].

In healthy humans, starvation results in ketone body production from beta oxidation of fatty acids in the liver [3]. Ketone bodies are efficient fuel sources for the heart and brain [4], but also have beneficial metabolic effects on immune cells [5]. In addition, they act as signal molecules that induce reparative pathways like autophagy [6]. Together with other cellular adaptations activated by nutrient restriction [7], this provides a rationale for why early energy provision may be harmful in critical illness. Post-hoc analyses of two trials investigating early versus delayed parenteral nutrition (PN) found a statistically significant interaction between the beneficial effects of withholding PN and ketosis in children but not adults [8, 9]. Notably, plasma 3-hydroxybutyrate concentrations were modest in adult patients, raising uncertainty as to whether clinically relevant starvation ketosis was achieved in this population.

Despite extensive clinical trial data on early nutrition in critical illness, the physiological effects of short-term starvation in ICU patients remain incompletely characterized, particularly with respect to circulating ketone concentrations and associated cellular responses. The concurrent metabolic alterations in critical illness [10] and hormonal

therapies such as exogenous insulin and corticosteroids likely attenuate the normal starvation response observed in healthy volunteers.

The FAST-ICU (**F**easibility **A**nd **S**afety of delaying nu**T**rition in **I**ntensive **C**are **U**nits) study is a cluster-randomized crossover trial that will address the safety aspects of complete calorie restriction during the first 72 hours in ICU, with incidence of hypoglycemia as the primary outcome. The trial will include 10-15 ICUs and approximately 2000 patients and has received primary ethical approval from the sponsor's site (University of Tartu, Approval 406/T-33). Participating ICUs will be allocated to two consecutive treatment periods in random order, consisting of:

A. no enteral/parenteral nutrition or glucose-containing maintenance fluids for the first 72 hours in ICU, or
B. standard care, including early enteral nutrition and maintenance glucose according to local practice.

Monitoring of blood glucose and insulin administration is performed according to local protocols, and there are no restrictions on patients where oral intake is possible.

As a large proportion of participants are expected to have no nutritional intake for 72 hours solely due to treatment allocation, this trial represents an ideal opportunity to study the adaptive responses to short-term starvation in critically ill patients. This will provide essential translational data to support the design of future RCTs investigating the patient-centred benefits of early calorie restriction in critical illness.

Aim

The aim of this pre-planned, nested sub-study is to provide a comprehensive characterization of changes in substrate utilization, metabolic pathways, activation of autophagy and immune cell function in in patients exposed to delayed nutrition compared with patients receiving standard care within the FAST-ICU trial.

Hypotheses

In patients admitted to the ICU, short-term starvation for 72 hours induces measurable increases in circulating ketone concentrations and associated changes in metabolic and cellular pathways related to autophagy and immune cell function.

Methods

Study participants and settings

This is a pre-planned nested sub-study of the FAST-ICU (**F**easibility **A**nd **S**afety of delaying nu**T**rition in **I**ntensive **C**are **U**nits) cluster-randomized cross-over trial. All ICUs participating in the main trial will be invited to participate. At designated centers, all patients enrolled in FAST-ICU will also participate in the sub-study. Inclusion and exclusion criteria for FAST-ICU are found in the main study protocol.

For reference values from non-critically ill subjects, 10 healthy volunteers will be recruited to perform a 72 hour fast while providing blood samples as described below.

Interventions

The following blood sample will be drawn once daily from an arterial catheter in situ at 8:00 +/ 4 hours up to 72 hours after ICU admission:

- 2 x 2 ml blood sample in EDTA tube – plasma ketone concentration, plasma samples for metabolomics and proteomics analyses, leukocyte differential.
- 0.5 ml blood sample for point-of-care ketone analyzer (where available).

On the final sampling day (day 3 or 4 in ICU depending on admission time) between 07:00 and 11:00, three additional blood samples are drawn;

- 2.5 ml blood sample in PAXgene™ blood RNA tubes (PreAnalytiX)
- 2 ml blood sample preserved with Cytodelics™ stabilizer (Cytodelics).
- 2 ml blood sample in serum separator tube (yellow top) – serum sample for assessment of in vitro metabolic cellular regulation

Following standard processing procedures appropriate to each sample type and collection tube, including centrifugation where applicable, blood samples will be aliquoted and stored at −80 °C until laboratory analyses. All samples will be handled according to standardized protocols to minimize pre-analytical variability and freeze–thaw cycles.

Laboratory analyses

Circulating ketone body concentrations, including β-hydroxybutyrate (3-HB) and acetoacetate, will be quantified in plasma samples using validated assays.

Targeted metabolomic analyses will be performed at the Swedish Metabolomics Center (Umeå, Sweden). The metabolomic panel will focus on predefined metabolic pathways relevant to fasting and critical illness, including ketogenesis, fatty acid oxidation, amino acid metabolism, glycolysis, and tricarboxylic acid (TCA) cycle activity. Metabolite quantification will be conducted using mass spectrometry–based platforms with isotope-labeled internal

standards to enable accurate normalization, batch correction, and comparison across samples.

To investigate circulating signals involved in cellular metabolic regulation, functional in vitro assays will be conducted using cultured cells incubated with patient serum samples. These experiments will assess serum-mediated effects on key cellular processes associated with starvation, including autophagy induction, mitochondrial activation, and oxidative metabolism.

Markers of autophagy in peripheral blood leukocytes will be assessed at both transcriptional and protein levels by RNASeq and Western blotting.

Immune cell phenotype and functional status will be evaluated using multiparameter flow cytometry. This analysis will characterize major immune cell subsets as well as markers of activation, exhaustion (immune checkpoints), metabolic state, and functional capacity relevant to starvation and critical illness. Standardized staining protocols, compensation controls, and predefined gating strategies will be applied to ensure reproducibility and comparability across samples.

Outcomes

The primary outcome is plasma ketone body concentrations (β-hydroxybutyrate and acetoacetate). The primary estimand is the between-group difference in plasma ketone concentrations across the first 72 hours of ICU admission. Secondary outcomes include targeted metabolomic pathway measures, serum-induced cellular responses in vitro (autophagy and mitochondrial activation), leukocyte autophagy markers (transcriptomic and protein expression), and immune cell phenotypes and functional markers assessed by flow cytometry.

Statistical analysis

Baseline characteristics will be summarized by treatment group using mean (standard deviation) or median (interquartile range) for continuous variables and counts (percentages) for categorical variables. Distributions of biochemical and omics-derived variables will be visually inspected and, where appropriate, log-transformed prior to analysis.

The effect of delayed nutrition versus standard care on ketone body concentrations over time will be analyzed using linear mixed-effects models, accounting for repeated measurements within individuals. Fixed effects will include treatment group, time since ICU admission, and their interaction. A random intercept for patient will be included. Cluster effects at the ICU level will be accounted for using appropriate cluster-level methods,

including random effects or robust standard errors. Results will be presented as estimated marginal means or ratios (for log-transformed outcomes) with 95% confidence intervals.

Targeted metabolomic data will be analyzed using multivariable regression or mixed-effects models at the metabolite and pathway level. Given the exploratory nature of these analyses, control for multiple testing will be performed using false discovery rate (FDR) methods. In vitro functional assay outcomes, leukocyte autophagy markers, and flow cytometry–derived immune cell measures will be analyzed using similar mixed-effects modeling approaches where repeated measures are present; otherwise, group comparisons will be performed using appropriate parametric or non-parametric tests.

Missing data are expected due to early ICU discharge, death, or logistical sampling constraints. Mixed-effects models inherently account for missing-at-random data. No imputation is planned for omics or functional outcomes. The extent and reasons for missing data will be reported descriptively.

Pre-specified sensitivity analyses may include adjustment for relevant covariates such as sex, age, insulin administration, corticosteroid exposure, severity of illness, and presence of sepsis. Exploratory subgroup analyses may be performed based on metabolic or treatment-related factors but will be interpreted cautiously.

Analyses will be conducted using R version 4.4.1 or later. A two-sided p-value <0.05 will be considered statistically significant for the primary outcome; secondary and exploratory analyses will emphasize effect sizes and confidence intervals rather than strict significance thresholds.

Sample size considerations

The sample size for this nested sub-study is based on the primary biochemical endpoint, plasma β-hydroxybutyrate (3-HB) concentration during the first 72 hours of ICU admission. Assuming a standard deviation of 1.0 mmol/L, a two-sided significance level of 0.05, and 90% power to detect a clinically relevant between-group difference of 0.5 mmol/L, approximately 110–120 evaluable patients would be required when accounting for repeated measurements within individuals. To allow for missing samples due to early discharge, death, or logistical constraints, a 20% inflation was applied. In addition, a modest inflation was included to account for the cluster-randomized design of the parent trial. Based on these considerations, the minimum target sample size for the substudy is approximately 160 patients. This sample size is expected to provide adequate precision for secondary and exploratory analyses, including targeted metabolomics, cellular functional assays, and immune phenotyping, which are hypothesis-generating in nature.

Ethics and governance

The primary ethical application will be submitted to the Swedish Ethical Review Authority, requesting approval for inclusion using a deferred consent procedure when capacity for informed consent is impaired. Family members or relatives will be consulted regarding study participation in accordance with Swedish legislation.

For participating centers outside of Sweden, local ethical approval is required with either deferred consent for sampling or a waiver of informed consent.

All blood samples will be drawn from arterial lines in situ, amounting to a maximum total volume of 26 ml.

All laboratory analyses will be performed at Karolinska University Hospital or Karolinska Institutet (Stockholm, Sweden), KU Leuven (Leuven, Belgium) and the Swedish Metabolomics Centre (Umeå, Sweden). Material Transfer Agreements will be drafted between all parties before transportation. The sponsor will be responsible for overall data governance and sample stewardship, in accordance with applicable data protection and biobanking regulations.

External funding is required and will be applied for by the primary investigator. In case of insufficient funding for all analyses, the investigators will agree through consensus on which of the samples for secondary analyses to omit or postpone.

References

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