CLINICAL TRIAL PROTOCOL

A single-centre, placebo-controlled, cross-over randomized controlled trial evaluating the metabolic effects of a ketone ester food supplement in intensive care patients: The KETOCARE RCT

Acronym: The KETOCARE RCT

Version number: v1.3 – **Date** 17/04/2024

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Sponsor

University Hospitals Leuven (UZ Leuven)

Principal Investigator

Prof. Dr. Greet Van den Berghe

Sub-Investigator

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Prof. Dr. Lies Langouche
Prof. Dr. Jan de Hoon

Confidentiality Statement

The information in this document is strictly confidential and is available for review to Investigators, potential Investigators and appropriate Ethics Committees, Institutional Review Boards or Competent Authorities. No disclosure should take place without written authorization from the Sponsor.

SIGNATURES

Title: A single-centre, placebo-controlled, cross-over randomized controlled trial evaluating the metabolic effects of a ketone ester food supplement in intensive care patients: The KETOCARE RCT

Protocol: The KETOCARE RCT

The undersigned confirm that the above referenced protocol has been acknowledged and accepted, and agree to conduct the Trial in compliance with the approved protocol, and will adhere to: the principles outlined in the requirements for the conduct of clinical trials in the EU as provided for in the EU Clinical Trial Regulation 536/2014 (CTR) and any subsequent amendments thereto, the ICH guidelines, the most recent version of the Declaration of Helsinki, the Belgian law of May 7th 2017 related to clinical trials on medicinal products for human use, the EU General Data Protection Regulation 2016/679 (GDPR), the Belgian law of July 30th 2018 on the protection of natural persons with regard to the processing of personal data, the Belgian Law of August 22nd 2002 on patient rights, and any other regulatory requirements and Standard Operating Procedures (SOPs), as applicable.

The undersigned agree not to disclose the confidential information contained in this document for any purpose other than the evaluation or conduct of the Trial, without prior written consent of the Sponsor.

The undersigned also commit to making the findings of the Trial publicly available through publication and/or other dissemination tools, in accordance with this protocol and applicable regulations, without any unnecessary delay and to provide an honest, accurate and transparent account of the Trial; and to explain any discrepancies or deviations from the approved Trial protocol.

Principal Investigator

.....	.....	.....
Name & Title	Signature	Date

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LIST OF ABBREVIATIONS

Abbreviation	Definition
(e)CRF	(electronic) Case Report Form
AE	Adverse Event
AESI	Adverse Event of Special Interest
AR	Adverse Reaction
CA	Competent Authority
CI	Coordinating Investigator
CM	Concomitant Medication
CSR	Clinical Study Report
CTP	Clinical Trial Protocol
CTIS	Clinical Trial Information System
CTR	EU Clinical Trial Regulation 536/2014
DMP	Data Management Plan
DPA	Data Processing Annex
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
EC	Ethics Committee
EU	European Union
ECG	Electrocardiogram
EoT	End of Trial
FPFV	First Patient First Visit
GCP	Good Clinical Practice (latest version of ICH E6)
GDPR	EU General Data Protection Regulation 2016/679
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council on Harmonisation
IMP	Investigational Medicinal Product
ISF	Investigator Site File
JCI	Joint Commission International
LPLV	Last Patient Last Visit
MAH	Marketing Authorisation Holder
MP	Monitoring Plan
PI	Principal Investigator (Participating Site)
PRO	Patient Reported Outcome
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure

SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
3HHB	R-3-hydroxybutyl-R-3-hydroxybutanoate
EN	Enteral nutrition

FUNDING AND SUPPORT

Funder	Type of Financial or Non-Financial Support
European Research Council (ERC)	Non-commercial financial support by research grant AdvG 2017-785809
Flemish Government Methusalem Program	Non-commercial financial support by research grant METH14/06
TDeltaS	Free delivery of the investigational product

ROLES AND RESPONSIBILITIES

The Principal Investigator (PI) is responsible for the conduct of the Trial at his/her Participating Site, and for protecting the rights, safety and well-being of the Trial participants. As such the PI must ensure adequate supervision of the Trial conduct at the Participating Site. If any tasks are delegated, the PI will maintain a log of appropriately qualified persons to whom he/she has delegated specified Trial-related duties. The PI will ensure that adequate training is provided and documented for all Trial staff, prior to conducting assigned Trial-related activities.

It is the Coordinating Investigator's (CI's) responsibility to supervise the general conduct (e.g. Trial progress, communication, protocol training and support of the participating sites, annual reporting to the Ethics Committee (EC), end of Trial notification(s) and results reporting…) of the Trial. The CI fulfils both Investigator and Sponsor responsibilities, as outlined in International Council on Harmonisation – Good Clinical Practice (ICH-GCP) E6(R2) and applicable regulations.

PI and CI shall each be referred to as «Investigator(s)».

TRIAL SYNOPSIS

Title of clinical Trial («Trial»)	A single-centre, placebo-controlled, cross-over randomized controlled trial evaluating the metabolic effects of a ketone ester food supplement in intensive care patients: The KETOCARE RCT
Protocol Short Title Acronym	The KETOCARE RCT
Sponsor name	University Hospitals Leuven (UZ Leuven)
Coordinating Investigator	Prof. Dr. Greet Van den Berghe
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Contact Phone CI	016/ 34 40 21
Public database nbr	ISRCTN: 13903536
Sub Investigators	Prof. Dr. Jan Gunst (UZ Leuven) Prof. Dr. Lies Langouche (KU Leuven) Prof. Dr. Jan de Hoon (UZ Leuven)
Medical condition or disease under investigation	Adult critical illness
Trial rationale	To study the feasibility, safety and tolerability of enteral ketone ester supplementation to increase circulating ketone levels in adult critically ill patients.
Primary objective	To identify a supplementation dose of enteral ketone esters that is able to increase circulating 3-hydroxybutyrate concentrations in adult crtitically ill patients by at least 0.22 mmol/l.
Secondary objective(s)	To demonstrate that supplementation of enteral ketone esters is safe.
Trial Design	Single-center, placebo-controlled, cross-over randomized controlled trial
Endpoints (Efficacy = E; Safety = S, Tolerability = T)	- An increase in plasma 3-hydroxybutyrate by at least 0.22 mmol/l following administration of enteral 3HHB (E) - Plasma levels of 3HHB and metabolites 3-hydroxybutyrate and 1,3-butanediol over time during (6 hour period) and after the intervention (18 hour period) (E) - Urine levels of 3HHB and metabolites 3-hydroxybutyrate and 1,3-butanediol over time during (6 hour period) and after the intervention (18 hour period) (E) - Blood glucose concentrations during the 48h-study period (E) - Incidence of ketoacidosis during the intervention window and until 18h after stopping study infusion (S - Intolerance for the supplement (including abdominal discomfort or vomiting) (S/T) - Incidence of severe (<40 mg/dl) hypoglycemia during the intervention window and until 18h after stopping study infusion (3HHB or placebo) (S)
Sample Size	16 patients for each study dose completing the 48h study period (maximum of 48 patients completing the 48h study period in total)

Dietary supplement, dosage and route of administration	Enteral administration of a ketone ester supplement (3-HHB) in increasing dosage (25g, 50g, 75g)
Active comparator product	Enteral administration of 5% of glucose
Maximum duration of treatment and Follow Up of a Participant	Duration of intervention: 48 hours. Clinical follow-up until 48 hours after the intervention or until ICU discharge, whatever comes first.
Maximum duration of entire Trial	2 years
Date anticipated First Patient First Visit (FPFV)	1-9-2023
Date anticipated Last Patient Last Visit (LPLV)	1-9-2025

1 Background, Rationale and Risk Assessment

The majority of prolonged critically ill patients develop muscle weakness and wasting during their stay at the intensive care unit (ICU), which hampers recovery and is associated with longer-term morbidity and mortality [1, 2]. Although preventive measures such as aggressive sepsis treatment and early mobilization have shown some benefit, other effective therapeutic or preventive interventions are currently lacking. Providing parenteral nutrition to supplement insufficient enteral nutrition was found unable to prevent this debilitating condition, whereas in contrast, accepting a macronutrient deficit early during critical illness has shown to accelerate recovery and reduce muscle weakness [3, 4]. In pediatric ICU patients, accepting such an early macronutrient deficit increased plasma concentrations of the ketone body 3-hydroxybutyrate, a direct effect that statistically mediated an important part of the outcome benefits [5, 6]. In adult ICU patients, an early macronutrient restriction did also increase plasma concentrations of 3-hydroxybutyrate, but to a much smaller extent than in pediatric patients and without independently mediating the outcome benefit [6]. One could speculate that the lower impact of withholding early parenteral nutrition on ketogenesis may explain the lower effect size of the intervention in adult patients.

Preclinical research by our group further strengthens the **potential clinical benefit of increasing plasma 3-hydroxybutyrate in critically ill patients**. Indeed, in a mouse model of sepsis-induced prolonged critical illness, we demonstrated that exogenous administration of the ketone body 3-hydroxybutyrate, administered as daily bolus injections of a racemic mixture of R,S-3-hydroxybutyrate sodium salt (3HB-Na), significantly attenuated the development of muscle weakness [7]. Subsequent dose-response and toxicity studies revealed that ketone esters are preferred to ketone salts, with a broader therapeutic window [8]. Indeed, the effective dose of 5g/kg/day of the ketone salt 3HB-Na was close to the toxic threshold [9], whereby increased morbidity and mortality by increased doses are likely explained by excessive sodium intake, since ketone esters have a substantially higher threshold for toxicity.

A potentially **safer alternative** for ketone salts is the ketone ester R-3-hydroxybutyl-R-3-hydroxybutanoate (**3HHB**) which is endogenously converted to two 3-hydroxybutyrate molecules [10]. The ketone ester is hydrolyzed to 3-hydroxybutyrate and 1,3-butanediol by esterases that are located in the circulation, in the gastro-intestinal tract, the liver, and other tissues [11]. The butanediol molecule is further metabolized to 3-hydroxybutyrate in the liver by alcohol and aldehyde dehydrogenases [11, 12]. We demonstrated that treatment of septic mice with the ketone ester 3HHB **improved muscle weakness** from a dose of 3.5 g/kg/day onward [13]. Up to a dose of 7 g/kg/day 3HHB, signs of organ damage or liver toxicity were absent. However, septic mice showed a higher peak plasma concentration and slower 3-hydroxybutyrate clearance than healthy control mice, leading to toxicity with a bolus of 14 g/kg/day. **This toxicity of a single bolus was completely avoided by a continuous intravenous infusion of this dose** (i.e. 14 g/kg/day) over 24h, while the protective effect on muscle weakness remained equally present [13]. This preclinical research has led to a patent application PCT/EP2017/081394 which is currently in its national phase.

Mechanistically, we could demonstrate that the muscle protection of 3-hydroxybutyrate treatment was limited to a direct effect on muscle force, whereas the ongoing sepsis-induced muscle atrophy was not affected [7]. The ketone body protection did not appear to be related to its use as an energy substrate, but rather by its effects as a signaling molecule affecting regeneration pathways [7] and by its conversion to cholesterol [14].

The next step in this research is investigating whether ketone esters might prevent or improve debilitating ICU-acquired weakness also in critically ill patients. A first step is to define a well-tolerated and effective dose able to increase the blood 3-hydroxybutyrate in critically ill patients. Oral administration of ketone esters has been extensively tested and found to be safe in healthy adults to induce ketosis, tested for up to 28 days [10, 11, 15].

2 Trial Objectives and Design

2.1 Trial objectives

We were able to demonstrate that treatment of septic mice with the ketone ester 3HHB improved muscle weakness. In order to study this effect in a randomized clinical trial in critically ill patients, we first want to determine a dose of 3HHB that effectively and safely increases plasma 3-hydroxybutyrate concentrations in adult critically ill patients.

To this purpose, we want to test fixed doses of 25 g, 50 g and 75 g of 3HHB in order to reach an increase of 0.22 mmol/l plasma 3-hydroxybutyrate, as was observed with the effective dose of 3HHB in earlier mice studies [13]. The fixed doses of 25 g, 50 g and 75 g that will be tested are based on the human equivalent (0.6 g/kg/day) of the effective and non-toxic dose in rodents (7 g/kg/day) [16]. Sustained ketosis through oral ingestion of the ketone ester 3HHB has previously been shown to be safe in healthy adults, tested at doses between 0.140 and 2.142 g/kg/day for up to 28 days [10, 11, 15].

2.2 Primary Endpoints

The primary efficacy endpoint will be an increase in plasma 3-hydroxybutyrate by at least 0.22 mmol/l following administration of enteral 3HHB.

2.3 Secondary Endpoints

- Plasma levels of 3HHB and metabolites 3-hydroxybutyrate and 1,3-butanediol over time during (6 hour period) and after the intervention (12 hour period)
- Urine levels of 3HHB and metabolites 3-hydroxybutyrate and 1,3-butanediol over time during (6 hour period) and after the intervention (12 hour period)
- Plasma levels of cholesterol (HDL, LDL, total), triglycerides en free fatty acids over time during (6 hour period) and after the intervention (12 hour period)
- Blood glucose concentrations during the 48h-study period
- Incidence of severe (<40 mg/dl) hypoglycemia during the intervention window and until 12h after stopping study infusion (3HHB or placebo)
- Incidence of ketoacidosis during the intervention window and until 12h after stopping study infusion
- Intolerance for the supplement (including abdominal discomfort or vomiting)

2.4 Trial Design

This will be a single center trial, in adult critically ill patients. With a cross-over design (Fig. 1), patients will receive in random order on 2 consecutive days 3HHB and placebo.

Patients will receive the investigational product between ICU day 4 and 14, to avoid the time of increase in endogenous ketogenesis that we have previously documented to be present in the first 2 to 3 days in ICU [6]. The investigational product (3HHB) or placebo will be delivered through the enteral feeding tube.

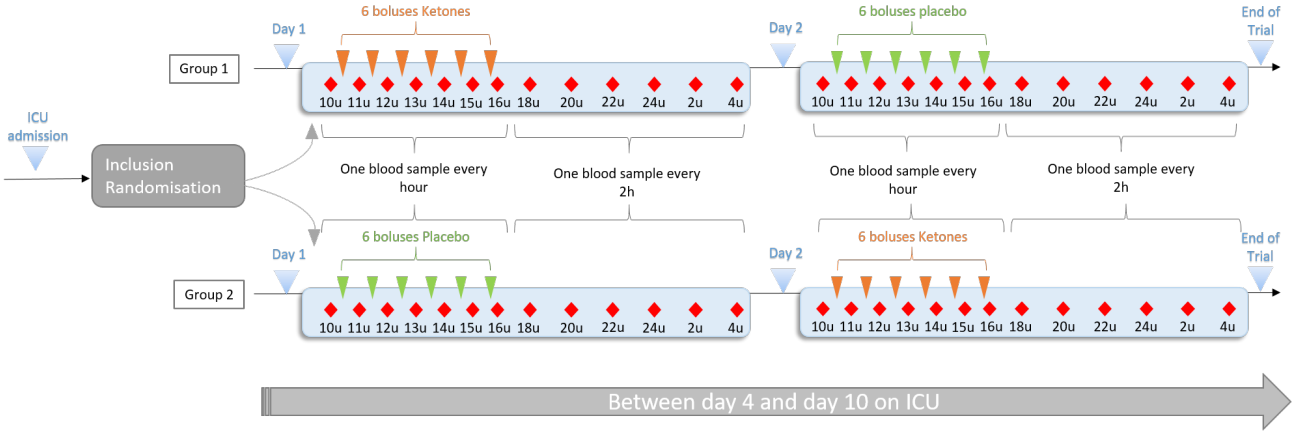

Figure 1: Cross-over design of the trial. In group 1, participants will receive 3HHB on intervention day 1, followed by a placebo on intervention day 2. Group 2 will recieve placebo on intervention day 1 and 3HHB on intervention day 2. Repetitive blood sampling is indicated in red.

Patients are typically started on enteral nutrition (EN) within 48 hours of admission, unless they are able to eat. EN is delivered for 2x10h per day (8am-6pm and 8pm-6am), with 2x2h interruption (6-8am and 6-8pm) to measure gastric residual volume (Fig 2). EN is usually started at a low rate, and increased depending on tolerance (as deducted in part from gastric residual volumes). For this trial, the caloric intake will be kept constant over the 2 intervention days avoiding the influence of caloric intake on outcome measures. Patients with nasogastric tubes who are not receiving enteral nutrition are also eligible for inclusion. This will allow to study ketone absorption and clearance in both fed and unfed state in the critically ill patient.

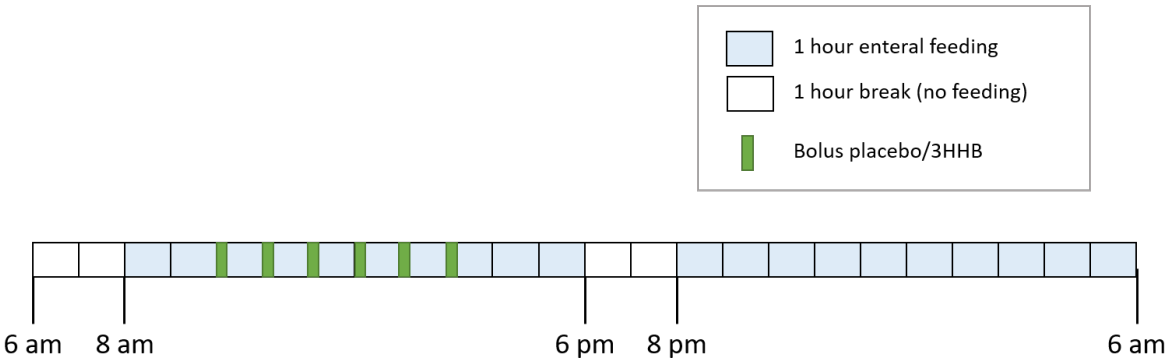

Figure 2: 10 hour feeding scheme. Intermittent administration of 3HHB/placebo (in green) every hour for 6 times.

We will administer 3HHB or placebo via repeated small boluses through the nasogastric feeding tube, as is also done for enterally administered medication. The nasogastric tube will be flushed with a 5% glucose solution prior and after administration of 3HBB or placebo to ensure that the bolus has completely entered the stomach while also avoiding clotting of the nasogastric tube.

The first bolus wil be given at 10 am (after 2 hours of enteral feeding) and this wil be repeated 5 times with one hour interval (6 doses in total). After the last bolus, the patient will be fed for three more hours. The next day, this procedure will be repeated (Fig. 1).

We will first perform this trial with a 25 g dose of 3HHB divided over 6 boluses. The density of 3HHB is 1.07 g/ml, 25 g has a volume of 23.4 ml, so each bolus consist of 3.9 ml 3HHB. Next, we will assess the primary and secondary endpoints in this cohort of patients. If the dose of 25 g is found safe but ineffective for increasing plasma 3-hydroxybutyrate at least with 0.22 mmol/l, a second cohort of patients will be tested with the same cross-over study design but with a higher 3HHB dose (50 g of 3HHB, divided over 6 boluses of 7,8 ml). If this dose is again safe but ineffective, a third cohort of patients will be tested with the same cross-over study design with an increased dose of 75 g of 3HHB, divided over 6 doses of 11.7ml. For each cohort of patients tested (cohort 1 to test 25 g, cohort 2 to test 50 g and cohort 3 to test 75 g),

16 patients will be included. If a patient is not able to complete the 48h study period (e.g. due to consent withdrawal or due to unplanned surgery or other procedures) and this is not related to safety concerns that are directly attributed to the intervention, an extra patient will be included in order not to compromize the statistical power of the study.

2.5 Translational research

Blood glucose and 3-hydroxybutyrate concentrations will be quantified repeatedly using blood gas analysis and point-of-care devices during the 48h trial window.

For this trial, we will collect blood samples at 10±1 am on ICU test day 1, before administration of the first bolus, repeat this every hour, up to 1 hour after the last bolus (4 pm), and then every 2 hours until 12h post final bolus (4 am on ICU test day 2). We will repeat this on test day 2. In total, 26 samples (104 ml blood) will be collected from each patient. To minimize blood waste and reduce infection, we will use a VAMP system for blood sampling. Each sample consists of 1 EDTA tube (purple K2E tube - BD Vactutainer 368861) containing 4 ml of blood. Samples will be stored on ice immediately and processed for further analysis. After processing, samples will be frozen at -20°C and stored at -80°C.

We will collect a urine sample from the 24h urine collection at 8 am ±1 hours at the end of both intervention days. These samples will be processed, frozen and stored at -80°C for further analysis.

As a standard of care, whole blood glucose measurements are performed every 4 hours on arterial blood using a blood gas analyzer in the ICU. Using the left-over of this clinically collected sample, we will also measure 3-hydroxybutyrate using ketone test strips (Nova StatStrip).

2.6 Expected Duration of the Trial

Patients will receive 3HHB or placebo, which will be administered over 6 hours on two consecutive days in ICU (between ICU day 4 and day 14). Repeated measurements will be done on blood and urine during the 48h intervention. The last sample will be taken on the morning following the 2nd intervention day in the ICU. This is the end of the intervention for the individual patient. We will do a clinical follow-up for at least 48 hours after the intervention.

Since we will only include patients who have an expected ICU stay of at least 5 days, we estimate that we will need a 2-4 month screening period. This can be doubled or tripled when we need to test higher doses of ketones.

3 Trial Population / Eligibility Criteria

3.1 Inclusion criteria

Participants eligible for inclusion in this Trial must meet **all** of the following criteria:

- Voluntary written informed consent of the participant or their legally authorized representative has been obtained
- Age >= 18 years
- Patient expected to stay at the ICU for at least 5 days
- The presence of a nasogastric feeding tube

All participants who are considered for Trial participation, per the above criteria will be documented on the Screening Log, including Screen Failures.

3.2 Exclusion criteria

Participants eligible for this Trial must **not** meet any of the following criteria:

- Therapy restriction code
- Patients refusing blood transfusion upon ICU admission will be considered as having a therapy restriction upon admission and will not be included
- Expected to die within 48 hours after screening (= moribund patients)
- No arterial and central venous line, or expected to have one of these lines removed before the end of the study period (= not critically ill enough to be representative for the future target population).
- Contraindication for enteral feeding
- Readmission to the ICU after previous inclusion in the RCT
- Inborn metabolic disease
- Receiving ketogenic diet in ICU
- Underweigt (BMI<20) or admitted with complications due to anorexia nervosa
- Known to be pregnant or lactating
- ICU admission with diabetic ketoacidosis or hyperosmolar hyperglycemic state
- Acute or chronic liver failure
- High glucose need to prevent spontaneous hypoglycemia
- Metabolic acidosis (pH <7.30 and bicarbonate <18mmol/l)
- Patient receives Extracorporeal membrane oxygenation (ECMO)

Participants who meet one or more of the above exclusion criteria must not proceed to be enrolled/randomized in the Trial and will be identified on the Screening Log as Screen Failure.

4 Trial Procedures

4.1 Participant consent and withdrawal of consent

The Trial will be conducted only on the basis of prior informed consent by the Trial participants and/or their legally authorized representative(s). As such, no Trial-related procedures will be conducted prior to obtaining written informed consent from potential Trial participants.

In the majority of patients, we expect that informed consent will have to be obtained from the patient's closest family member or legal representative due to the nature of their illness. As regaining consciousness and mental competence often occurs gradually and slowly in these patients, it is very difficult to determine at what time the patient is able to give a valid informed consent. In addition, recovery can take from several days to several months. Often, patients are only able to give a valid consent after discharge from ICU, at what time the intervention is already terminated. Therefore, we will give an opting out form to the legal representative, who can give it to the patient once he/she is well recovered. With this document, the patient has the possibility to terminate the study intervention (if still applicable) and/or withdraw from further data collection.

The process for obtaining and documenting initial and continued informed consent from potential Trial participants will be conducted in accordance with ICH-GCP E6(R2), applicable regulatory requirements and internal Standard Operating Procedures (SOPs).

All originally signed obtained Informed Consent Forms (ICFs) must be retained/archived in the Trial Master File (TMF) and must not be destroyed (even when a scanned copy is available) before expiration of the legal archiving term as defined in the protocol section entitled "Archiving".

Participants may voluntarily withdraw consent to participate in the Trial for any reason at any time. The participant's request to withdraw from the Trial must always be respected without prejudice or

consequence to further treatment. Consent withdrawal will be documented in the participant's medical record.

Trial data collected before withdrawal can be used in the trial. Results of analyses on samples obtained before withdrawal of consent can also be used. No new trial data or samples will be collected after withdrawal of the participant. No new analysis on samples will be performed after withdrawal.

4.2 Selection of Participants / Recruitment

Patients will be included in the study if they meet all inclusion criteria that are described above (Sub-paragraph 3.1 – Inclusion criteria). Screening for inclusion will be done on working days, and exceptionally, during the weekends or legal holidays depending on the research staff availability. Screening for inclusion will be performed by the principal investigator or delegate members of the research team.

4.3 Randomization Procedure

A blocked randomization scheme with blocks of 4 patients will be used in this study. The code for randomization blinding is stored in the randomization tool, developed in FilemakerPro with restricted access to the database administrators. Randomization will be carried out by dedicated research staff members using a role-based user access.

4.4 Premature discontinuation of Trial treatment

Participants may voluntarily discontinue from Trial treatment and/or prematurely end their participation in the Trial for any reason at any time. In such case the Investigator will make a reasonable effort to document the primary reason for this decision.

The Investigator may also decide at any time during the course of the Trial, to temporarily interrupt or permanently discontinue the Trial intervention if it is deemed that continuation would be detrimental to, or not in the best interest of the participant.

Similarly, the Sponsor, CA/EC can decide to halt or prematurely terminate the Trial when new information becomes available whereby the rights, safety and well-being of Trial participants can no longer be assured, when de integrity of the Trial has been compromised, or when the scientific value of the Trial becomes obsolete and/or unjustifiable.

Circumstances requiring premature treatment interruption or discontinuation of the Trial, include but are not limited to:

- Safety concerns that are directly related to the intervention or unacceptable intolerability
- Trial participation while in violation of the inclusion and/or exclusion criteria
- Pregnancy

In any such case of early Trial termination and/or treatment interruption/discontinuation, the Investigator will continue to closely monitor the participant's condition and ensure adequate medical care and follow-up.

5 Trial product of interest

Food supplement (& company brand name)	**Used within Indication?** (Y or N)	**Route of administration (po,sc,iv,…)**	**Dose/dosage and units**
deltaG Ketone	Not applicable	Enteral (gastric tube)	25g / 50g / 75g

DeltaG Ketones are commercially available as a soluble food supplement (called 'deltaG Ketone Performance'). We will be using pure deltaG Ketone ester as ketone supplement (100% R-3-hydroxybutyl-R-3-hydroxybutanoate, $C_8H_{16}O_4$). This ketone product has been developed at the university of Oxford and was shown to be safe and efficacious in healthy human volunteers [17]. Since 2008, deltaG has been the subject of a wide range of scientific safety studies that led to a Generally Recognized as Safe (GRAS) designation and a U.S. FDA notification. This supplement is also currently being investigated in patients with type 1 diabetes [18], in patients with type 2 diabetes and heart failure [19] and in a KU Leuven trial with athletes [20]. The food supplement will be delivered to us by deltaG.

As described in chapter 2.4 (Trial design), 3HHB and placebo will be administered through the same feeding tube as EN. A syringe with the intervention product will be attached to the tube port and instilled by slowly and steadily pushing on the plunger. A flush of 10 ml 5% glucose will be given after each administration to prevent clotting of the tube. After the last (6^{th}) bolus administration, we will flush with 50 ml 5% glucose. We will also use 5% glucose as placebo.

6 Safety reporting

6.1 Definitions

6.1.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a patient or subject during an experiment, and which does not necessarily have a causal relationship with this treatment.
An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a product, whether or not considered related to the product. Any worsening (i.e., any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE.

6.1.2 Adverse Reaction (AR)

An AR is any untoward and unintended responses to an investigational medicinal product or to an experiment and, when an investigational product is concerned, related to any dose administered.

6.1.3 Serious Adverse Event (SAE)

An SAE is any untoward medical occurrence that results in any of the following:

- Death
- A life-threatening[a] experience
- In-patient hospitalisation or prolongation of existing hospitalisation
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- Important medical events that may be considered an SAE when - based on appropriate medical judgement - they may jeopardise the subject and may require medical or surgical intervention to prevent one of the above outcomes

[a] *The term "life threatening" in the definition of SAE refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe.*

6.2 Adverse Events that do not require reporting

In general, the following should not be reported as AEs:

- Pre-existing conditions, including those found as a result of screening (these should be reported as medical history or concomitant illness).
- Pre-planned procedures, unless the condition for which the procedure was planned has worsened from the first trial-related activity after the subject has signed the informed consent.

The following events are commonly observed and are therefore not considered as adverse events for the purpose of the trial:

Critical illness is a severe and life-threatening condition, whereby serious events are expected to occur. Especially long-stay patients have a particularly high risk of morbidity and mortality. In daily medical practice, all medical events and procedures are recorded in a central electronic patient file. In this clinical study, only those safety parameters that could theoretically be related to the used study fluid will be documented, as well as those complications for which the treating physician judges a potential relationship. Critically ill patients with expected ICU stay of at least 5 days are inherently suffering from multiple medical conditions and are at risk of new complications. Reporting all untoward medical events would not be practically feasible neither relevant for the data. Life-threatening events occurring in the study population such as (but not restricted to) hypotension, hypoxia, coma are all typical manifestations of critical illness. Such events are thus considered as a confirmation that patient selection has been adequate (reflecting a true long-stay ICU population) and thus will not be considered as (serious) adverse event, and will not be recorded neither reported to the sponsor or the ethical committee. Nevertheless, any serious adverse reaction of which the treating physician judges a potential relationship with the used study fluid will be reported to the study sponsor.

Although these events should not be reported to the Sponsor, these should be recorded in the patient's medical notes according to routine practice.

The following events not to be considered as SAEs are:

- Pre-planned hospitalisations unless the condition for which the hospitalisation was planned has worsened from the first trial-related activity after the subject has signed the informed consent.
- Hospitalisation as part of a standard procedure for protocol therapy administration. However, hospitalisation or prolonged hospitalisation for a complication of therapy administration will be reported as an SAE.
- Hospitalisation or prolongation of hospitalisation for technical, practical, or social reasons, in absence of an AE.
- Mortality or 'serious' outcome related to disease progression.

6.3 Recording of adverse events (AEs)

There are no known risks associated with the study-specific intervention (oral ketone administration).
The participant will be asked to report any adverse event related to the study-specific intervention to the study team. These reported events will be documented by the Investigator in the source documents and reported to the Sponsor through the (e)CRF.
The following minimum information should be recorded for each adverse reaction (AR):

- AE description
- start and stop date of the AR
- severity
- seriousness
- causality assessment to the study interventions
- outcome

6.4 Reporting to the Ethics Committee

The sponsor will assess whether any relevant safety information that becomes available during the study should be reported ad hoc to the EC.

The sponsor has the obligation to, once a year throughout the clinical trial (or on request), submit a progress report to the EC containing an overview of all SARs (Serious Adverse Reactions) occurred during the reporting period and taking into account all new available safety information received during the reporting period.

All deaths until 48h after the study period will be reported without delay to the Sponsor (irrespective of whether the death is related to disease progression, study procedure or is an unrelated event). The

sponsor will notify all deaths, as soon as possible after becoming aware, to the Central EC and provide additional information if requested.

7 Statistics and Data Analysis

7.1 Sample Size

The study is powered to detect a 0.22 mM within-patient increase in plasma 3HB with 95% certainty, 80% power, calculated with a paired t-test. The anticipated effect size is 0.786 given that patients not treated with ketone esters have a mean difference of 0.001mM between intervention day 1 and 2 with a SD of 0.28mM (based on prior data from ICU patients) and the anticipated required increase with the administration of ketone esters is 0.22mM (which was the effective increase in the mice studies). If the lower limit of the 95% confidence interval is not above 0.22mM increase, a higher dose will be tested.

Approximately 160 participants will be screened to achieve 16 participants completing the 48h study period or an estimated total of 8 evaluable participants per treatment group.

The screening will be repeated for each dose study. In total a maximum of approximately 480 patients will be screened and a maximum of 3x 16 patients completing the 48h study period will be included.

7.2 Statistical Analysis

For each patient, and for each study day, maximum concentration, time to reach maximum concentration, delta Max (difference between maxium concentration and baseline concentration), area under the curve, elimination rate and elimination half life will be calculated for 3HBB and its metabolites 3-hydroxybutyrate and 1,3-butanediol. Delta Max of 3-hydroxybutyrate and time within range (plasma 3-hydroxybutyrate 0.22mmol/l above baseline value) will be calculated as primary outcome variables. For blood lipid parameters, maximum concentration, time to reach maximum concentration, delta Max (difference between maximum concentration and baseline concentration), and area under the curve will be calculated. Data will be compared with paired t-tests after transforming the data to near-normally distributed values if needed. If the latter is not possible, non-parametrical tests will be used. Within the crossover design, each patient thus serves as his/her own control.
To investigate whether the order of administration of placebo and 3HHB affected the response, the delta Max of the placebo day and the 3HHB day will be assessed with a repeated measures ANOVA in which the grouping variable will be randomization (placebo/3HHB or 3HHB/placebo) and time is the difference between test day 1 and test day 2.If more than one dose will be tested, parameters will also be compared between doses. These data will be compared with standard t-tests after transforming the data to near-normally distributed values if needed. If the latter is not possible, non-parametrical tests will be used. P-values will be considered significant when at or below 0.05 without correction for multiple testing. If a higher dose reached a significant increase in the paired analysis, and also compared to the lower dose, this will further strengthen the conclusion that the lower dose was ineffective while the higher dose was effective. If the higher dose does not reach a statistical difference from the lower dose this might suggest that the lower dose already created a small, but non-statistical increase which was not picked-up in the paired analysis.

The study setup is designed and will continue until 16 participants per tested dose completed the 48h study period. This is the primary analysis (per-protocol-analysis). Data from patients with a shorter study period will be analysed in as a sensitivity analysis to confirm those partial results are in line with the primary data set. Imputation of missing data would not be accurate due to the small data set.

8 Data handling

8.1 General Data handling information

Data collection, handling, processing and transfer for the purpose of this Study will be performed in compliance with applicable regulations, guidelines for clinical studies and internal procedures. It remains the responsibility of the Investigator to check that all data relating to the Study, as specified in the Study protocol, are entered into the electronic Case Report Form ((e)CRF) in accordance with the instructions provided and that the forms are filled out accurately, completely and in a timely manner.

(e)CRFs are provided by the Sponsor for each participant. The Study data will be transcribed from the source records into an (e)CRF by Study Staff.

The (e)CRFs shall under no circumstances capture personal data such as but not limited to the participant or their relative(s) name, home address, contact details, full date of birth medical record number (e.g. UZ Leuven EAD number), social security number etc.

8.2 Study specific data handling information

The data will be stored in a validated Filemaker database with password protection and complete audit trail. Beside account access control on system level by the Active Directory which governs access to all clinical UZ- data, a secured, controlled and restricted role-based user access on file level will be implemented. In the Filemaker® application export and printing privileges can be restricted to a certain role since version 10.
For this project export data export privileges will only be granted to the database administrators. The database will be stored on the central FilemakerPro servers which are managed, maintained and backed up by the UZ Leuven IT department according to the central IT policies (at least 1 daily backup on mirrored servers).

The following data will be collected in the pseudonymized (e)CRF :

- Patient study number

- Informed consent, date of IC, type of IC (patient or legal representative)

- Age

- Sex

- ICU admission date and time

- Height, weight and body mass index (BMI)

- Medical history (diabetes, malignancy, renal replacement therapy, Charlson comorbidity index)

- Baseline characteristics including reason for ICU admission and APACHE II score

- Clinical characterterics from ICU admission until discharge including hemodynamic and respiratory status, renal function, liver function, SOFA score and infections

- ICU length of stay

- ICU mortality

- All medications received by the patients on the ICU before the intervention and on the intervention days will be registered

- Parenteral nutrition (kilocalories, amino acids, lipids and carbohydrates cumulatively administered during intervention window and 1 day prior

- Enteral nutrition (kilocalories, amino acids, lipids and carbohydrates cumulatively administered during intervention window and 1 day prior

-Results from the routine blood gas analyses on the clinical samples will be collected

- Intervention related parameters:
    - Start- and stop date and time of first intervention day
    - Start- and stop date and time of second intervention day
    - Dose of IMP
    - Amount of cycles of IMP administration
- Blood glucose values and ketone values measured at 4h intervals
- Blood 3HHB + metabolites values (3-hydroxybutyrate and 1,3-butanediol) measured in collected blood samples
- Blood lipid values (HDL, LDL, total cholesterol, triglycerides and free fatty acids) measured in collected blood samples
- Urine 3HHB + metabolites values (3-hydroxybutyrate and 1,3-butanediol) measured in collected urine samples
- Parenteral nutrition (kilocalories, amino acids, lipids and carbohydrates cumulatively administered during ICU day 3-4-5-6)
- Enteral nutrition (kilocalories, amino acids, lipids and carbohydrates cumulatively administered during during ICU day 3-4-5-6)
- Biochemical, metabolic, endocrine and inflammatory markers associated with ketone metabolism in blood and urine samples
- Adverse reactions

8.3 Direct Data Access

The Investigator will permit direct access to Trial data and documents for the purpose of monitoring, audits and/or inspections by authorized entities such as but not limited to: the Sponsor or its designees and competent regulatory or health authorities. As such (e)CRFs, source records and other Trial related documentation (e.g. Investigator Site File, the Trial Master File, pharmacy records, etc.) must be kept current, complete and accurate at all times.

9 Ethical and Regulatory Considerations

9.1 Ethics Committee (EC) review & reports

Before the start of the Study, this protocol and other related documents will be submitted for review to the EC for Study authorization. The Study shall not commence until such approvals have been obtained and until other relevant essential Study documents, such as duly signed contract agreements, evidence of adequate Study financing etc. are in place.

9.2 Protocol / GCP compliance

The Study must be performed in accordance with the protocol, current ICH and ICH-GCP guidelines, and applicable regulatory and country-specific requirements. ICH guidelines are an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human participants. Compliance with this standard provides public assurance that the rights, safety, and well-being of Study participants are protected, consistent with the principles that originated in the most recent version of the Declaration of Helsinki, and that the Study data are credible, reliable and reproducible.

9.3 Data protection and participant confidentiality

The Study will be conducted in compliance with the requirements of the EU General Data Protection Regulation 2016/679 (GDPR), and the relevant Belgian laws implementing the GDPR including the Belgian Privacy Act of 30 July 2018 on the protection of privacy in relation to the processing of personal data. Any collection, processing and disclosure of personal data, such as participant health and medical information is subject to compliance with the aforementioned personal data protection laws (cfr. Data Processing Annex (DPA) in Appendix). In case personal data is transferred outside the European Economic Area, safeguards will be taken by the Sponsor to ensure that appropriate protection travels with the data in accordance with the GDPR. (https://ec.europa.eu/info/law/law-topic/data-protection/international-dimension-data-protection/rules-international-data-transfers_en#documents)

Any personal data shall be treated as confidential at all times including during collection, handling and use or processing, and the personal data (including in any electronic format) shall be stored securely at all times and with all technical and organizational security measures that would be necessary for compliance with EU and national data protection legislation (whichever is more stringent). The Sponsor shall take appropriate measures to ensure the security of all personal data and guard against unauthorized access thereto or disclosure thereof or loss or destruction while in its custody.

10 Research Registration, Dissemination of Results and Publication Policy

The Declaration of Helsinki (latest version) and European and Belgian regulations require that every research Study involving human participants be registered in a publicly accessible database before recruitment of the first participant. The CI is responsible for registering the Study.

In addition, the CI will fulfil their ethical obligation to disseminate and make the research results publicly available. As such the CI is accountable for the timeliness, completeness and accuracy of the reports. Researchers, authors, Sponsors, editors and publishers must adhere to accepted guidelines for ethical reporting. Negative and inconclusive, as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in publication.

11 Insurance/Indemnity

In accordance with the Belgian Law relating to experiments on human persons dated May 7, 2004, Sponsor shall assume, even without fault, the responsibility of any damages incurred by a Study Patient and linked directly or indirectly to the participation to the Study, and shall provide compensation therefore through its insurance."

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