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Ketone body effects on Network stability and Resilience in Humans (KeNeReHum): study protocol for a single-center, randomized, double-blind, crossover trial in healthy adults

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Study protocol

Keywords: Stress Resilience, Ketone bodies, ß-hydroxybutyrate, metabolic intervention, resting-state fMRI, Brain Network Stability, Blood-brain-barrier, healthy adults, Crossover trial

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Title {1a}

Ketone body effects on Network stability and Resilience in Humans (KeNeReHum): study protocol for a single-center, randomized, double-blind, crossover trial in healthy adults

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Abstract

Background:
Stress is a major risk factor for psychiatric disorders, including depression and anxiety. Resilience, the maintenance of mental health despite stressor exposure, varies substantially between individuals, yet the neurobiological mechanisms remain poorly understood. Emerging evidence links stress-related psychopathology to metabolic dysregulation such as hyperglycemia and insulin resistance, suggesting shared underlying mechanisms. Resting-state brain network stability has been associated with preserved cognitive acuity and can be modulated by metabolic factors. The ketone body β-hydroxybutyrate (BHB), produced endogenously during fasting and ketogenic diets, has been shown to enhance brain network stability in young adults compared to glucose. However, the relationships between individual resilience capacity, baseline network properties, and metabolic responsiveness remains unknown.

Methods:
This monocentric, randomized, double-blind crossover trial will recruit 60 healthy adults (20–45 years) from the Longitudinal Resilience Assessment (LORA) cohort, which provides outcome-based resilience measures (stressor reactivity scores) from 5-8 years of longitudinal assessments. Participants will receive a single oral dose of BHB monoester (395 mg/kg) and a calorically matched glucose solution on two separate visits, with the order randomized 1:1. At baseline on both visits, resting-state functional MRI (rs-fMRI) and arterial spin labeling (ASL) will be performed to assess brain network stability and blood-brain barrier permeability. Following ingestion, rs-fMRI will be repeated 30 minutes post-dose to capture acute metabolic effects. The primary outcome is the difference in brain network stability between BHB and glucose conditions. Secondary outcomes examine whether individual differences in resilience moderate these metabolic effects and relate to baseline network and blood-brain barrier measures.

Discussion:
This study uniquely combines an acute metabolic challenge with longitudinal resilience phenotyping to test whether brain network stability and blood-brain barrier function are associated with resilience. The availability of outcome-based resilience measures allows direct examination of whether individual differences in resilience relate to baseline network properties and metabolic responsiveness. If confirmed, findings could inform mechanistic models linking metabolism, neural dynamics, and stress resilience, and guide development of novel metabolic intervention strategies to promote resilience and prevent stress-related disorders.

Trial registration: The study is registered in the German Clinical Trials Register (DRKS), DRKS00038898. Registered 12 January 2026.

Keywords

Stress Resilience, Ketone bodies, ß-hydroxybutyrate, metabolic intervention, resting-state fMRI, Brain Network Stability, Blood-brain-barrier, healthy adults, Crossover trial

Structured summary {1b}

Item	Description
Primary Registry and Trial Identifying Number {4}	German Clinical Trials Register (DRKS) DRKS-ID: DRKS00038898 Registration date: 12 January 2025 URL: https://www.drks.de/search/de/trial/DRKS00038898/details
Secondary Identifying Numbers	n/a; the study was only registered in the DRKS, but not in other WHO primary or partner registries.
Source(s) of Monetary or Material Support	This study is funded by the *European Research Area Network NEURON* (ERA-NET NEURON) under the transnational research project “Ketone Body Signalling in Resilience Promotion”, with national co-funding provided by the Federal Ministry of Education, Research and Technological Progress (BMFTR), Germany (grant number: 01EW2403B). The funding bodies had no role in the design of the study, collection, analysis, interpretation of data, or in writing the manuscript.
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Role of sponsor and funder {3c}	There are no conflicting agreements with other sponsors or funders that affect the collection, management, analysis, interpretation or dissemination of the results.
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Public Title	Effects of Ketone Bodies on Network Stability and Resilience in Humans
Scientific title	Ketone body effects on Network stability and Resilience in Humans (KeNeReHum): study protocol for a single-centred, randomised, double-blind crossover trial

Countries of Recruitment	Germany
Health Condition(s) or Problem(s) Studied	Metabolic effects on brain network stability and their association with stress resilience
Intervention(s)	β-Hydroxybutyrate (BHB) ketone ester, Glucose comparator
Key Inclusion and Exclusion Criteria	***Inclusion:*** • Age: 20-45 years • LORA cohort membership • Self-reported good general health without diagnosed chronic diseases affecting brain function or metabolism • Ability to understand the study information, provide informed consent, and comply with study procedures ***Exclusion:*** ***Metabolic and dietary exclusions***: • Current adherence to or adherence within the last 6 months following a low-carbohydrate or ketogenic diet • Diagnosed diabetes mellitus (type 1 or 2) or other metabolic disorders • Regular use of dietary supplements containing ketone bodies, or other ketogenic compounds • BMI <18.5kg/m² (underweight) or BMI >29.9kg/m² (obesity) ***Psychiatric and neurological exclusions:*** • Current diagnosis of a psychiatric disorder requiring treatment, OR lifetime diagnosis of psychotic disorder, bipolar disorder, or other severe persistent mental disorder; exception: history of adjustment disorder or acute stress reaction is permitted • Current use of psychotropic medication • Known history of substance use disorder (alcohol, illicit drugs, or prescription medications) • Known history of traumatic brain injury or diagnosed neurological disorder ***Cardiovascular and hematological exclusions:*** • Diagnosed cardiovascular diseases • Known bleeding disorders • Skin conditions on hands or fingers that would prevent capillary blood measurements ***MRI safety exclusions:*** • Current pregnancy (confirmed or suspected) • Any MRI contraindication as determined by standardized safety screening, including but not limited to: - Cardiac pacemaker or implantable cardioverter-defibrillator (ICD) - Cochlear implants or other electronic implants - Ferromagnetic implants, clips, or foreign bodies - Severe claustrophobia preventing MRI completion ***Other exclusions:*** • Participation in high-performance sports • Current participation in another interventional clinical trial, or participation in a drug trial within three months prior to enrollment • Current severe mental or physical stress

Study Type	The study is a randomized, double-blind, crossover neuroimaging trial. Each participant will complete two study visits, receiving both, the β-hydroxybutyrate (BHB) and the glucose drink on separate visits in a randomized order. Allocation to the order of administration will follow a 1:1 ratio. The study is designed to test the acute effects of BHB versus glucose on resting-state brain network stability, and to investigate the relationship between these metabolic effects and individual differences in stress resilience.
Date of First Enrollment (planned)	January 2026
Sample Size	60
Primary outcome(s)	Acute metabolic effects on brain network stability
Key Secondary outcome(s)	1a: Association between resilience and baseline brain network stability 1b: Association between resilience and blood–brain barrier permeability 2: Resilience as moderator of metabolic responsiveness 3: Test–retest reliability of neuroimaging measures (baseline brain network stability and blood-brain barrier permeability)
Ethics Review	Ethics Committee of the Landesärztekammer Rheinland-Pfalz (Ethics Committee of the State Medical Association of Rhineland-Palatinate, Germany; approval number 2018-13545_16)
Individual Trial Participant Data sharing statement	De-identified individual participant data, including MRI, questionnaire, and biological outcome data, may be shared with approved research collaborators for scientific purposes, including meta-analyses, following study completion and publication. Prior to sharing, all directly identifying information will be removed, MRI data will be defaced, and data will only be shared in accordance with participant consent, institutional policies, GDPR, and ethical approvals.

Protocol version {2}

Approved protocol version 2, 11 July 2025 by the local ethics committee.

Introduction

Background and rationale {9a}

Scientific background

Stress is a major risk factor for mental disorders, including depression and anxiety. Accumulating evidence demonstrates bidirectional links between stress-related psychopathology and metabolic dysregulation: stress is associated with hyperglycemia, insulin resistance, and low-grade inflammation [1], while high-glycaemic diets may increase the risk of depression and anxiety [2,3]. Moreover, type 2 diabetes may accelerate cognitive decline through cerebral glucose hypometabolism [4]. These observations point to shared pathophysiological mechanisms and raise the possibility that metabolic interventions could promote mental health and stress resilience.

Not all individuals exposed to stress develop psychopathology—a phenomenon termed stress resilience. Recent evidence suggests that resilience emerges from the integration of multiple neurobiological systems across behavioral, neurophysiological, cellular, and molecular levels [5]. This multi-system view implies that

effective resilience-promoting interventions may need to target multiple mechanisms simultaneously. Understanding these mechanisms is critical for developing preventive strategies, yet the neurobiological substrates linking metabolism and stress resilience remain poorly characterized.

Dietary interventions such as intermittent fasting, calorie restriction, and ketogenic diets have been shown to promote overall health and, improve cognitive function [6]. A shared metabolic feature of these interventions is the elevation of plasma β-hydroxybutyrate (BHB) levels, a ketone body produced in the liver through fatty acid oxidation when glucose availability is reduced [6]. Under these conditions, a metabolic state known as ketosis develops, in which ketone bodies, particularly BHB, partially replace glucose as the main fuel for the body and brain. Although glucose is typically considered the brain's primary energy source, BHB is metabolically more efficient yielding more ATP per unit oxygen consumed [7]. This increased efficiency may be particularly relevant for energy-demanding processes such as the maintenance of stable neural network activity. Successful stress adaptation requires a well-coordinated, stable neural network activity to prevent transitions to pathological allostatic states. Such stabilization processes are energetically costly, adding to the brain's already high baseline energy demands [8]. Recent meta-analytic evidence indicates that ketogenic diets are associated with small to moderate reductions in depressive symptoms, although effects on anxiety remain inconclusive [9]. Complementary mechanistic work suggests that ketone-based interventions may modulate neuroenergetic and neuroinflammatory pathways implicated in stress-related psychopathology [10] further supporting their relevance for resilience-focused approaches.

Emerging evidence suggests two potential neurobiological substrates linking metabolism to resilience: brain network stability and blood-brain barrier (BBB) integrity. Animal studies indicate that individuals with a less permeable BBB—limiting entry of inflammatory mediators—show greater stress resilience, and that BBB function is sensitive to metabolic state [11,12]. These findings suggest that metabolic interventions affecting both energy availability and BBB function could influence resilience capacity.

Mujica-Parodi and colleagues [13] demonstrated that brain network stability, measured using resting-state fMRI (rs-fMRI), represents a modifiable biomarker of brain health. In their crossover trial, baseline-corrected brain network stability was significantly higher following acute administration of a BHB ketone monoester compared with an isocaloric glucose bolus (Cohen's dz=0.53) in young healthy adults. Similarly, endogenous BHB production induced by a ketogenic diet increased network stability compared to a standard diet in young adults. These findings suggests that ketone bodies can compensate for network destabilization associated with glucose hypometabolism. Notably, network stability positively correlated with cognitive performance. Network stability has been shown to decrease with age, correlating with impaired cognition, and is accelerated under conditions such as type 2 diabetes [13]. These findings suggests that BHB can acutely modulate brain network dynamics even in young adults without metabolic impairment. However, whether these metabolic effects on brain networks relate to individual differences in stress resilience, operationalized through longitudinal, outcome-based measures, remains unknown.

Bidirectional relationships between metabolism, brain networks, and resilience

The relationship between metabolism, brain network dynamics, and resilience is likely bidirectional. On the one hand, stable and efficiently organized neural networks may constitute a neurobiological substrate supporting adaptive stress responses. Individuals with greater intrinsic network stability show better cognitive performance [13] and less vulnerability to perturbation [14], suggesting that their neural systems may be more capable of maintaining functional integrity under stress.

On the other hand, resilience-related characteristics may themselves shape brain network properties. Individuals who have successfully adapted to stressors over time may develop more stable functional network configurations through experience-dependent plasticity. This view is consistent with longitudinal accounts of resilience as an outcome of dynamic adaptation [15], rather than a fixed trait.

Furthermore, metabolic responsiveness, which reflects the capacity of neural systems to adapt to changes in energy substrate availability, may also vary between individuals and relate to resilience. Analogous to physiological stress reactivity in cardiovascular or endocrine systems, individuals may differ in how their brain networks respond to metabolic challenges such as ketone or glucose administration. Supporting this view, recent multimodal work shows that switching from glucose to ketone metabolism can stabilize large-scale network dynamics via ion-channel–mediated mechanisms [16]. As ketone utilization depends on efficient substrate transport into the brain, interindividual differences in blood–brain barrier function may further contribute to variability in these neuroenergetic responses. If more resilient individuals exhibit stronger BHB-induced stabilization or reduced destabilization under glucose, this would suggest that metabolic flexibility of brain network dynamics represents a modifiable substrate of resilience.

While the present study cannot disentangle causality among these processes, it provides a unique opportunity to examine associations between resilience, baseline network characteristics, and acute metabolic responsiveness, thereby offering mechanistic insights into how metabolism may shape individual differences in stress adaptation.

To empirically evaluate these considerations, the present study leverages the LORA (Longitudinal Resilience Assessment) cohort, in which participants have been prospectively followed for 5-8 years with repeated assessments of stressor exposure and mental health, yielding validated outcome-based resilience measures (stressor reactivity scores) [17,18]. This unique resource enables us to test two complementary hypotheses about the relationship between metabolism, brain networks, and resilience:

1. **Acute metabolic modulation affects network stability** (replication of Mujica-Parodi and colleagues [13]): We hypothesize that BHB increases brain network stability in healthy adults compared to glucose.
2. **Individual resilience capacity relates to brain network characteristics:** We hypothesize that resilience is associated with (a) greater baseline network stability, (b) lower BBB permeability, and (c) differential metabolic responsiveness of brain networks.

If confirmed, these findings would suggest that (1) metabolic state acutely modulates brain network dynamics, and (2) brain network stability and BBB function represent neurobiological characteristics associated with resilience capacity. Together, this would indicate that metabolic interventions targeting network stability may offer a modifiable pathway for resilience promotion and prevention of stress-related disorders.

Explanation for the choice of comparator {9b}

Rationale for β-hydroxybutyrate (BHB):

β-Hydroxybutyrate is an endogenous ketone body produced by hepatic fatty acid oxidation during periods of reduced carbohydrate availability (e.g., fasting, prolonged exercise, ketogenic diet). It serves as an alternative energy source for the brain and other organs when glucose is limited [6]. Exogenous administration of ketone monoesters can rapidly and safely elevate blood BHB levels to nutritional ketosis levels (~0,5 to 5 mmol/L [19]) without requiring dietary restriction [20].

Importantly, Mujica-Parodi and colleagues demonstrated that acute administration of a BHB ketone ester significantly increased brain network stability in healthy young adults (Cohen's dz=0.53) compared to an isocaloric glucose bolus [13]. This acute, reversible metabolic modulation of brain network dynamics provides a unique experimental tool to test whether brain network stability represents a neurobiological substrate of resilience.

Rationale for glucose as comparator:

Glucose was selected as an active comparator (rather than placebo) for several critical methodological reasons:

1. ***Caloric matching:*** The glucose solution will be matched to the BHB ester for caloric content (expected $\mu_{cal} \approx 125$ per dose), controlling for potential confounding effects of energy intake on brain activity.
2. ***Mechanistic contrast***: Glucose represents the brain's default fuel source and the metabolic state most participants typically experience. Comparing BHB to glucose (rather than fasting) isolates the specific effects of ketone metabolism versus standard glucose metabolism.
3. ***Replication of prior work***: This comparator choice directly replicates the experimental design of Mujica-Parodi et al. [13], enabling direct comparison of results.

In summary, the BHB vs. glucose comparison provides a rigorous test of whether acute metabolic shifts can modulate brain network stability, while controlling for caloric intake.

Objectives {10}

Primary objective: **Replication of acute metabolic effects on network stability**

The primary objective is to test whether acute administration of exogenous β-hydroxybutyrate (BHB), compared to an isocaloric glucose bolus, modulates resting-state brain network stability in healthy adults

(<45 years). Based on prior work [13], we hypothesize that brain network stability will be increased after BHB administration compared to glucose. Network stability will be quantified using a dynamic functional connectivity approach described by Mujica-Parodi and colleagues [13]. This study will also assess robustness of these in an independent sample.

Secondary objectives

Secondary Objective 1: Baseline associations between resilience, brain networks, and blood-brain barrier function
We will investigate cross-sectional associations between outcome-based resilience (SR scores) and two neurobiological measures at baseline:

1 a) Resilience and network stability

Hypothesis: Higher resilience (lower SR scores) is associated with greater baseline brain network stability.

Rationale: Stable neural network dynamics may support adaptive stress responses and cognitive performance. Individuals with greater network stability show better cognitive performance and reduced vulnerability to age-related decline [11]. Furthermore, computational models suggest that stable network configurations provide greater resilience to perturbations and facilitate flexible task-dependent reconfiguration [21–23].

1 b) Resilience and blood-brain barrier (BBB) integrity

Hypothesis: Higher resilience is associated with lower BBB permeability (lower kw values), indicating greater barrier integrity.

Rationale: Preclinical studies demonstrate that reduced BBB permeability, limiting neuroinflammatory signaling into the central nervous system, is associated with stress resilience in rodent models [11,12]. Conversely, chronic stress induces BBB breakdown and neurovascular pathology in susceptible animals [9]. This objective tests whether similar associations between BBB integrity and resilience are observable in humans.

Secondary Objective 2: Resilience as a moderator of metabolic responsiveness
We will test whether resilience moderates the acute metabolic effects of BHB versus glucose on brain network stability.

Hypothesis: Individual differences in resilience will moderate the metabolic effects of BHB versus glucose on network stability. The direction of this moderation is not specified, as individual differences may influence network responsiveness in either direction.

Rationale: If resilience relates not only to baseline neural properties but also to dynamic network responses to metabolic challenge, this would support the idea that resilience involves flexible, state-dependent neural adaptations. This moderation is exploratory regarding directionality. Importantly, this cross-sectional design does not permit causal inference about whether resilience drives metabolic responsiveness or vice versa; longitudinal and interventional studies are required to establish causality.

Secondary Objective 3: Test-retest reliability
As a methodological aim, we will assess the test-retest reliability of baseline brain network stability and BBB permeability measures across the two study visits (separated by ~2 weeks), providing important information on the stability of these putative resilience biomarkers.

Exploratory Objectives

Exploratory analyses will examine associations between cognitive and affective processing styles (e.g., Vividness of Visual Imagery Questionnaire [VVIQ] [24,25], Perceived Positive Appraisal Style Scales [PASS; process-focused and content-focused]) [26], stress-related variables contributing to the SR score (e.g., General Health Questionnaire-28 [GHQ-28] [27,28], Life Events [29], Mainz Inventory of Microstressors

[MIMIS]) [30], dietary patterns (Skala zum Ernährungsverhalten [SEV]) [31], physical fitness (International Fitness Scale [IFIS]) [32], and the primary and secondary outcomes.

In addition, exploratory analyses will examine associations between affective and impulsive regulation dimensions and the primary and secondary outcomes. These dimensions are assessed by four additional self-report questionnaires: the Aggression Questionnaire (AQ) [33,34], measuring physical aggression, verbal aggression, anger, and hostility; the Barratt Impulsiveness Scale-11 (BIS-11) [35,36], assessing motor, attentional, and non-planning impulsivity; the Difficulties in Emotion Regulation Scale (DERS) [37], assessing clinically relevant difficulties in emotion regulation; and the State-Trait Anger Expression Inventory (STAXI) [38,39], capturing state and trait anger as well as anger expression styles. These constructs are theoretically linked to resilience-related individual differences: recent evidence suggests that ketone body levels correlate with aggression-related traits [40], and that emotion dysregulation mediates associations between impulsivity, stress reactivity, and aggressive behavior [e.g., 40–45]. Incorporating these measures allows for a more comprehensive characterization of affective and cognitive regulatory processes that may underlie individual differences in resilience and metabolic responsiveness.

These analyses are exploratory and hypothesis-generating and will inform future mechanistic studies on resilience-related individual differences.

Methods: Patient and public involvement, and trial design

Patient and public involvement {11}

Patients and the public were not involved in the design, conduct, or reporting of this trial. The study was designed by the research team based on existing scientific literature and prior experimental work.

Trial design {12}

The study is a randomized, double-blind, crossover neuroimaging trial. Each participant will complete two study visits, receiving both, the β-hydroxybutyrate (BHB) and the glucose drink on separate visits in a randomized order. Allocation to the order of administration will follow a 1:1 ratio. The study is designed to test the acute effects of BHB versus glucose on resting-state brain network stability, and to investigate the relationship between these metabolic effects and individual differences in stress resilience.

Methods: Participants, interventions and outcomes

Trial setting {13}

All MRI assessments and study procedures will be conducted at the Neuroimaging Center (NIC) of the University Medical Center of the Johannes Gutenberg-University Mainz in Germany.

Participants will complete online questionnaires remotely via the institution’s secure LimeSurvey platform prior to the first study visit.

Study drinks (β-hydroxybutyrate [BHB] and glucose solutions) will be prepared and administered by trained study personnel according to the randomization list.
The glucose will be obtained from the hospital pharmacy, whereas the BHB monoester (ΔG®, TΔS Ltd., UK) will be sourced from the commercial supplier. All study materials will be stored under controlled conditions at the study site until use.

Characteristics of the people who are needed for the trial

Characteristic	The people we would expect to see included
Age	We will recruit participants from the ongoing Longitudinal Resilience Assessment (LORA) cohort. In this cohort, mean age at baseline (completed in July 2019) was 28.59 (SD 7.96) with a range of 18-50 years [18]. An age range up to 45 years is selected for this trial because prior work suggests that metabolic modulation affects brain network stability in healthy young adults (Mujica-Parodi et al., 2020).
Sex	The trial aims to recruit equal numbers of participants who self-report as female and male participants (approximately 50% each) in order to obtain a balanced sample. In the LORA cohort at baseline, 65.9% of participants self-reported as female and 34.1% male [18].
Gender	As biological sex is not assessed separately in this study, sex/gender will be recorded based on self-report. A balanced distribution is intended to account for known sex-/ gender-related differences in brain network organization [e.g., 33–35] and in stressor exposure and coping styles [15,50,51].
Race, ethnicity and ancestry	In accordance with European data protection regulations and established practice in Germany, race and ethnicity will not be assessed using predefined racial categories. Instead, proxy indicators of migration background will be collected, including primary language(s) spoken at home. Perceived ethnic minority status will be assessed by self-report. These variables will be reported descriptively in line with PRO-EDI guidance.
Socioeconomic status	Socioeconomic status will be approximated using educational attainment as a proxy measure, and subjective social status (SSS) will be assessed using the McArthur Scale [44]). This is relevant given evidence linking socioeconomic factors to brain network organization [52] and resilience-related outcomes [53]. Given that the educational level in the LORA cohort is rather high [18], we anticipate correspondingly high values.
Geographic location	Participants are recruited in the Mainz region, Germany, and are expected to predominantly reside in urban or suburban areas. The study is coordinated and conducted at a single academic center located in Mainz, Germany.
Other characteristics relevant to the trial	Participants are selected from the LORA cohort based on availability of longitudinal stressor reactivity (SR) scores, an outcome-based measure of resilience [17]. As SR scores are calculated using a residualization approach and are sample-dependent, no population-level expectations are specified.

Eligibility criteria for participants {14a}

Inclusion criteria

Participants must meet all of the following criteria:

- **Age**: 20-45 years at time of enrollment
- **LORA cohort membership**: Active participation in the Longitudinal Resilience Assessment (LORA) study with available long-term stressor reactivity (SR) scores from longitudinal assessments
- **Health status**: Self-reported good general health without diagnosed chronic diseases affecting brain function or metabolism

- **Cognitive capacity**: Ability to understand the study information, provide informed consent, and comply with study procedures
- **Language**: Sufficient German language proficiency to complete questionnaires and understand study instructions

Exclusion criteria

Participants meeting any of the following criteria will be excluded:

Metabolic and dietary exclusions:

- Current adherence to or adherence within the last 6 months following a low-carbohydrate or ketogenic diet (≤30% of energy from carbohydrates or <100g/day [54])
- Diagnosed diabetes mellitus (type 1 or 2) or other metabolic disorders
- HbA1c ≥6.5% (indicating prediabetes or undiagnosed diabetes)
- Regular use of dietary supplements containing ketone bodies, or other ketogenic compounds
- BMI <18.5kg/m² (underweight) or BMI >29.9kg/m² (obesity)

Psychiatric and neurological exclusions:

- Current diagnosis of a psychiatric disorder requiring treatment, OR lifetime diagnosis of psychotic disorder, bipolar disorder, or other severe persistent mental disorder; exception: history of adjustment disorder or acute stress reaction is permitted
- Current use of psychotropic medication (e.g., antidepressants, antipsychotics, mood stabilizers, anxiolytics)
- Known history of substance use disorder (alcohol, illicit drugs, or prescription medications)
- Known history of traumatic brain injury or diagnosed neurological disorder

Cardiovascular and hematological exclusions:

- Diagnosed cardiovascular diseases
- Known bleeding disorders
- Skin conditions on hands or fingers that would prevent capillary blood measurements (e.g., severe eczema, psoriasis, open wounds)

MRI safety exclusions:

- Current pregnancy (confirmed or suspected)
- Any MRI contraindication as determined by standardized safety screening, including but not limited to:
    - Cardiac pacemaker or implantable cardioverter-defibrillator (ICD)
    - Cochlear implants or other electronic implants
    - Ferromagnetic implants, clips, or foreign bodies
    - Severe claustrophobia preventing MRI completion

Other exclusions:

- Participation in high-performance sports (>10 hours training per week) that might significantly alter metabolic parameters
- Current participation in another interventional clinical trial, or participation in a drug trial within three months prior to enrollment
- Current severe mental or physical stress (e.g., acute grief, major life crisis, acute illness) that would interfere with study participation or data quality, as judged by the investigator

Eligibility criteria for sites and those delivering interventions {14b}

All study procedures will be conducted at the study site with appropriate infrastructure to ensure participant safety and data quality. Eligibility criteria for the study site include access to facilities for the standardized preparation of the study beverages, as well as on-site MRI facilities suitable for structural and functional imaging.

Individuals delivering the interventions and conducting study procedures must be appropriately trained and instructed. Study beverages will be prepared and administered by trained study personnel in accordance with standardized operating procedures to ensure consistency and protocol adherence. Capillary blood

sampling will be performed by personnel trained in capillary blood measurements and hygiene procedures in line with applicable safety regulations

MRI examinations will be conducted by trained and experienced technical staff. Prior to the MRI assessment, all participants will undergo a standardized medical briefing and MRI-specific safety screening, which will be performed by a qualified physician. Only personnel meeting these eligibility criteria will be involved in study procedures.

Who will take informed consent? {32a}

Prior to giving their informed consent, potential study participants receive two information sheets by email: (1) a study information brochure providing s and (2) an MRI information brochure outlining MRI procedures, safety considerations, and MRI-specific inclusion and exclusion criteria. The informed consent process follows a two-stage procedure to ensure clear separation between medical (MRI-related) and study-specific information and responsibilities.

Stage 1: MRI safety consent (by physician)

A physician, who is not necessarily otherwise involved in the study, conducts a one-on-one MRI safety briefing, verifies MRI eligibility using a standardized checklist, and obtains written informed consent for the MRI component. This consent remains valid for up to four weeks; if the first study visit occurs later, MRI eligibility will be reverified by the physician.

Stage 2: Study-specific consent (by investigator)

Separately from the MRI safety briefing, a study investigator (PI or a delegated, trained investigator) conducts the study-specific informed consent process. This includes detailed information on the study aims, procedures, voluntary participation, data protection, potential risks or discomforts, and financial compensation.

Participants are given ample time and opportunity to ask questions. A minimum reflection period of 72 hours is provided between receipt of study information and signing of the consent form to ensure informed decision-making. Written informed consent is obtained prior to any study-related procedure, and each participant receives a signed copy for their records.

Day-of-visit verification

On each study visit, MRI safety criteria are re-checked by the study staff using a standardized checklist before the participant enters the scanner. If new contraindications have emerged, an MRI physician will be consulted before proceeding.

Additional consent provisions for collection and use of participant data and biological specimens {32b}

The informed consent document includes detailed provisions governing the collection, storage, and potential secondary use of participant data. Participants are informed that their pseudonymized study data, including MRI and questionnaire data, may be shared with research partners for scientific purposes, both within and outside the European Union (EU) and European Economic Area (EEA). The consent form explicitly addresses the possibility of data transfer to countries without an EU adequacy decision or equivalent data protection safeguards, and participants must provide explicit agreement to these conditions as a prerequisite for study participation. Prior to data sharing, all directly identifiable information is removed. In particular, facial features are removed from MRI data to prevent any risk of re-identification. Any published results or shared datasets will be made available in fully anonymized form, ensuring that individual participants cannot be identified.
Capillary blood samples are collected solely for immediate measurement of glucose, β-hydroxybutyrate, and HbA1c and are not stored for future research or secondary analyses. No biological specimens are stored or used for ancillary studies, genetic analyses, or biobanking. Accordingly, no additional consent provisions for the storage or secondary use of biological specimens apply.

Intervention and comparator

Intervention and comparator description {15a}

Experimental interventions

Intervention A: β-Hydroxybutyrate (BHB) ketone ester

- **Product:** (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (ΔG®, https://www.deltagketones.com)
- **Dosing:** Weight-adjusted to 395 mg/kg bodyweight, calculated individually
- **Preparation:** Diluted in 50–100 mL water (adjusted for palatability), flavoured with gentian tincture
- **Administration:** Consumed within 5 minutes on an empty stomach (fasted ≥12 h) immediately after baseline MRI. Participants are asked to rinse their mouths with water after administration to minimize potential effects of the taste during the scans.
- **Temperature:** Room temperature (~20–22 °C)
- **Expected effect:** Blood BHB rises from ~0.1 mmol/L to 2–4 mmol/L within ~30 minutes

Intervention B: Glucose comparator

- **Product:** Pharmaceutical-grade D-glucose (dextrose monohydrate)
- **Dosing:** Weight-adjusted to match caloric content of BHB ester (~125 kcal; grams calculated individually)
- **Preparation:** Dissolved in 50–100 mL water, flavoured with gentian tincture
- **Administration & temperature:** Identical to BHB intervention
- **Expected effect:** Blood glucose rises from ~80–100 mg/dL to ~140–180 mg/dL within ~30 minutes, normalizing over 60–90 minutes

Blinding and masking

- Both drinks have a comparable bitter-sweet taste
- Study staff prepare the drinks, based on the randomization list
- Drinks are delivered in sealed containers labeled only with participant ID, visit number and date of preparation
- Staff conducting MRI and outcome assessments are blinded to drink identity

Study visit procedures and timeline

Pre-visit instructions

Participants are asked to:

- Fast ≥12 hours (water allowed)
- Avoid alcohol and strenuous exercise for 24 hours
- Refrain from smoking for at least 1 hour prior to the study visit
- Maintain regular sleep schedule
- Take usual medications (except those excluded per protocol)

Visit timeline (~120 minutes)

Arrival and preparation

- Check compliance with pre-visit instructions
- Verify MRI safety and eligibility
- Capillary blood measurement #1: HbA1c (visit 1 only), baseline glucose and BHB
- Participant enters MRI scanner

Baseline MRI (Visits 1 and 2)

- Participant positioning in the MRI tomograph (~5 min)
- localizer (~1 min)
- Resting-state fMRI (~10 min)
- Arterial Spin Labeling (ASL) (~10 min)

Intervention and waiting period

- Participant exits scanner
- Beverage administration (BHB or glucose) consumed within 5 minutes

- 30-minute waiting period in comfortable seating
- Capillary blood measurement #2 at ingestion + 30 min: glucose, BHB
- Participant re-enters MRI scanner

Post-intervention MRI

- Visit 1 (structural scans included):
  - Participant positioning in the MRI tomograph (~5 min)
  - localizer (~1 min)
  - Resting-state fMRI (~10 min)
  - T1-weighted structural MRI (~5 min)
  - T2-weighted structural MRI (~3 min)
- Visit 2 (no structural scans):
  - Participant positioning in the MRI tomograph (~5 min)
  - localizer (~1 min)
  - Resting-state fMRI (~10 min)

Post-assessment and discharge

- Participant exits scanner
- Capillary blood measurement #3 at ingestion + 60 min: glucose, BHB
- Adverse events and tolerability assessment
- Participant is discharged

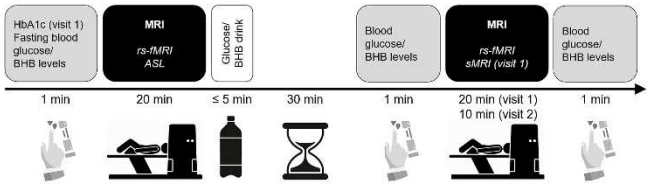


Figure 1. Detailed timeline of procedures during study visits 1 and 2. *Visit 1 includes structural MRI sequences (T1, T2) for coregistration purposes. Both visits include baseline and post-intervention resting-state fMRI (rs-fMRI) and baseline-only arterial spin labeling (ASL). Capillary blood measurements are taken at three timepoints per visit (baseline, post-intervention [ingestion+30 min], end [ingestion+60 min]) to track metabolic changes. Arm A receives BHB on visit 1 and glucose on visit 2; Arm B receives the opposite order.*

Criteria for discontinuing or modifying allocated intervention/comparator {15b}

Each intervention consists of a single oral administration of either the BHB ketone ester or the calorically matched glucose solution. Ketone ester drinks have been shown to be generally well-tolerated [20]. However, interventions or further participation can be discontinued at any time based on predefined safety or participant-related criteria.

Criteria for discontinuing the intervention (same visit)

Immediate discontinuation will occur if any of the following apply:

1. **Allergic reaction or anaphylaxis** – appearance of urticaria, angioedema, bronchospasm, or hypotension requiring medical attention.
2. **Severe gastrointestinal distress** – persistent severe nausea, vomiting, or abdominal pain preventing further participation.
3. **Signs of cardiovascular distress** – severe dizziness, syncope, or palpitations reported by the participant or observed by study staff that prevent safe continuation of the study procedures.
4. **Metabolic abnormalities** – blood glucose < 50 mg/dL, > 200 mg/dL, fasting blood glucose > 126 mg/dL [55] or BHB > 8.0 mmol/L.
5. **Neurological symptoms** – sudden confusion, severe headache, or visual disturbances.

6. **MRI-related issues** – claustrophobia, panic attack, or other conditions preventing safe scanning.
7. **Participant request** – the participant withdraws consent for any reason, consistent with the principle of voluntary participation.

Criteria for discontinuing study participation (between visits)

Study participation will be discontinued if:

1. A serious adverse event (SAE) attributable to study procedures occurs.
2. Pregnancy is detected at safety screening before the second visit.
3. A new medical diagnosis or medication arises that meets exclusion criteria (e.g., diabetes, psychotropic medication).
4. A protocol deviation compromises safety or data validity (e.g., noncompliance with fasting requirements).
5. Investigator judgment indicates that continuation would be unsafe or the data would be invalid.

Procedures following discontinuation

If participation is discontinued due to adverse events or safety concerns:

- The participant will be medically evaluated by a study physician before leaving the site.
- The reason for discontinuation will be documented in the case report form (CRF) and source records.
- Data collected up to that point will be retained and analyzed unless the participant requests deletion (see section {32b}).
- In case of an adverse event–related discontinuation, a follow-up contact within 48 hours will assess symptom resolution.
- Participants discontinuing early will receive prorated compensation according to completed study parts.

Strategies to improve adherence to intervention/comparator {15c}

While the study interventions (BHB and glucose administration) are single-dose and directly observed, ensuring complete adherence requires attention to multiple study components. The following strategies will be implemented:

Pre-visit adherence (fasting and preparation)

Challenge: Participants must fast for ≥12 hours and avoid alcohol and strenuous exercise for 24 hours before each visit.

Strategies:

- *Written instructions*: Detailed pre-visit checklist provided via email before each appointment, with bullet-pointed reminders

- *Reminders*: Participants will be contacted the day before each visit reminding participants to begin fasting and avoid alcohol and strenuous exercise for 24 hours

- *Verification*: Adherence to fasting and preparation instructions will be verified via a checklist on visit day. Non-compliance will be documented and may lead to rescheduling if it would compromise data quality

Visit attendance adherence

Challenge: Participants must attend two visits separated by 1-4 weeks (2 weeks on average)

Strategies:

- *Confirmation and reminders:* Participants will receive an email confirmation immediately after scheduling, and a reminder 24 hours before the visit.
- *Make-up sessions:* If a participant cancels or misses the second visit, one make-up session will be offered within four weeks of the initial visit, provided safety and eligibility criteria remain met.
- *Attendance documentation:* For each visit, participants will sign a visit documentation sheet confirming attendance and completion of study procedures.
- Compensation: Full compensation will be provided only upon completion of both study visits. Participants who attend only one visit will receive partial compensation corresponding to the procedures completed.

Questionnaire completion adherence

***Challenge*:** Participants are required to complete seven online questionnaires (total duration ~30 minutes) prior to visit 1.

***Strategies*:**

- *User-friendly platform:* Questionnaires will be administered via LimeSurvey with a mobile-responsive design, progress indicator, and save-and-resume option.
- *Modular structure*: Questionnaires are divided into short sections to minimize fatigue and allow completion in multiple sessions.
- *Reminder system:* Participants will be reminded via email 3 days and 1 day before visit 1 if questionnaires remain incomplete.
- *Alternative option:* In case of technical difficulties, questionnaires can be completed on-site (online completion preferred for data quality).
- *Incentive:* Completion of all questionnaires is required for visit 1 to proceed; compensation is contingent upon full data submission.

MRI compliance

Challenge: Participants must remain still during MRI sequences and tolerate scanner environment.

Strategies:

- *Pre-scan orientation:* Before the first MRI session, participants receive a detailed explanation of the scanning procedure, including sequence duration, expected noise levels, and communication options to reduce anxiety

- *Comfort measures*: Padding, blankets, and headphones provided; participants positioned carefully to minimize discomfort

- *Communication:* Intercom system allows continuous communication; participants reminded they can use squeeze ball to signal need to stop at any time

- *Motion monitoring:* The scan operator will perform continuous visual monitoring of the participant's position to provide feedback ("please try to hold very still") if excessive motion detected

Monitoring and documentation of adherence

All adherence measures will be documented.

Concomitant care permitted or prohibited during the trial {15d}

This study involves an acute, single-dose intervention, and therefore concomitant care restrictions are limited to factors that could affect metabolic or neurophysiological outcomes.

Prohibited concomitant care

Participants are not permitted to:

- Take exogenous ketone supplements (ketone salts, esters, medium-chain triglycerides) or follow ketogenic or very low-carbohydrate diets (<100 g/day) during or within 6 months prior to the study period.
- Use psychoactive medications (e.g., antidepressants, antipsychotics, anxiolytics, stimulants) or illicit drugs within four weeks prior to study visits.
- Consume alcohol within 24 hours or caffeine on the morning of each visit (fasted state).
- Participate in other interventional clinical trials or have received investigational drugs within the last three months.

Permitted concomitant care

- Stable maintenance medications for chronic conditions (e.g., thyroid replacement, asthma inhalers, non-sedating antihistamines) are permitted if the dosage has been stable for ≥ 3 months.
- Hormonal contraception is permitted and recorded.
- Common supplements (multivitamins, vitamin D, omega-3, calcium, iron) are allowed at standard doses.
- Occasional use of paracetamol or ibuprofen is acceptable if not taken within 24 hours prior to scanning.
- Routine dietary and physical activity habits should be maintained; high-intensity athletic training (>10 h/week) and significant dietary changes are discouraged.

Documentation

All medications, supplements, and relevant lifestyle factors will be documented at each visit in a standardized medication log, including dose, indication, duration, and any changes since the last visit.

Ancillary and post-trial care {34}

This is a minimal-risk neuroimaging study in healthy participants involving acute administration of nutritional ketone bodies (food supplement, EU classification) and glucose. Both substances have well-established safety profiles [20]. The study involves no long-term interventions, and no post-trial care is anticipated or required for the study interventions themselves.

Management of incidental MRI findings:

Although the MRI scans are acquired solely for research purposes, all images will be reviewed by a physician from the Department of Neuroradiology at the University Medical Center Mainz, and a written radiology report will be prepared. Occasionally, anatomical variants or abnormalities may be detected that could indicate underlying medical conditions.
If such incidental findings are identified, the participant will be contacted by a member of the study team to provide the written report either in person or, after prior telephone consultation, by mail. Participants who do not wish to be informed personally may instead authorize the transmission of this information to a physician of their choice.

Outcomes {16}

This section specifies all study outcomes including the measurement variable, analysis metric, method of aggregation, and time point for each outcome.

Primary outcome

Outcome: Resting-state network stability.

Measurement variable: Whole-brain functional connectivity patterns derived from Blood Oxygenation Level Dependent (BOLD) signal measured using rs-fMRI.

Operationalization: Network stability will be quantified following the approach of Mujica-Parodi and colleagues [13]. ROI-level fMRI time series will be divided into non-overlapping time windows of 24 seconds ("snapshots"). For each snapshot, a whole-brain correlation matrix will be computed. Network instability will be defined as the norm of the difference between correlation matrices across all pairs of snapshots for a given τ (time duration [in units of 24 s] over which persistence is calculated), with lower values indicating greater stability.

Analysis metric: The primary outcome is the differential change in network stability from baseline (pre-bolus) to post-intervention (post-bolus) between the BHB and Glucose conditions. The difference of the baseline-corrected values will be tested by a paired *t*-test and quantified by testing the two-way Intervention (BHB vs. Glucose) × Time (baseline vs. post-bolus) interaction effect using a Linear Mixed Model (LMM) (see section {27a} for details).

Method of aggregation: Individual stability scores (scalar norm); aggregated by LMM.

Time points: Measurements at baseline and post-intervention (approximately 30-45 minutes after bolus administration) on visit 1 and visit 2

Rationale: The primary hypothesis posits that exogenous BHB administration will increase neural network stability compared to the isocaloric glucose control.

Secondary outcomes

Secondary Outcome 1a: Association between resilience and baseline brain network stability

Outcome: Cross-sectional association between SR score and baseline brain network stability.

Measurement variable: SR score and baseline network stability (mean across baseline of both visits).

Operationalization: The SR score is computed as the standardized residual from a linear regression of mental health symptoms (GHQ-28 total score [27,28] on stressor exposure (MIMIS daily hassles (based on [56,57]) [30] + LEC major life events (adapted from [29]) across multiple assessment waves in the LORA cohort [17,18]. Higher SR scores indicate higher stressor reactivity (i.e., lower resilience), whereas lower SR scores indicate better maintenance of mental health under stress (i.e., higher resilience). Long-term SR scores will be derived from longitudinal LORA data collected prior to this intervention study.

Analysis metric: LMM will be used to test the association between the SR score and baseline network stability, adjusting for relevant covariates (e.g., age, sex, BMI). Model assumptions (e.g., linearity, homoscedasticity, and normality of residuals) will be checked. If substantial violations occur, robust alternatives will be applied (e.g., transformed outcomes, robust mixed models, or generalized linear models appropriate for the distribution of the data).

Method of aggregation: Individual-level values; one SR score and one mean baseline stability value per participant.

Time points: SR score from prior LORA assessments; baseline network stability averaged across both visits.

Hypothesis: We expect a positive association, such that higher resilience (lower SR score) is associated with greater network stability.

Secondary Outcome 1b: Association between resilience and blood–brain barrier permeability

Outcome: Cross-sectional association between SR score and blood–brain barrier (BBB) permeability.

Measurement variable: Water exchange rate (kw, in min^{-1}) from blood to brain tissue, estimated from arterial spin labeling (ASL) MRI data. Higher kw indicates greater BBB permeability (lower barrier integrity).

Operationalization: ASL data will be analyzed using a two-compartment exchange model fitted to the ASL signal time course to derive voxel-wise kw values. BBB permeability will be summarized as mean kw across gray matter (and exploratory ROI-level analyses).

Analysis metric: LMM will be used to test the association between the SR score and mean whole-brain kw (or regional kw in hypothesis-driven ROIs), adjusting for relevant covariates (e.g., age, sex, BMI). Model assumptions (e.g., linearity, homoscedasticity, and normality of residuals) will be checked. If substantial violations occur, robust alternatives will be applied (e.g., transformed outcomes, robust mixed models, or generalized linear models appropriate for the distribution of the data).

Method of aggregation: Individual-level mean kw values averaged across both baseline assessments (visit 1 and visit 2); one SR score per participant.

Time points: Baseline on both study visits; SR scores from prior LORA assessments.

Hypothesis: Based on preclinical evidence [11,12], we hypothesize a negative correlation, such that higher resilience (lower SR score) is associated with lower BBB permeability (lower kw values, indicating greater barrier integrity).

Secondary Outcome 2: Resilience as moderator of metabolic responsiveness

Outcome: Interaction between stressor reactivity (SR) score and metabolic condition (BHB vs. glucose) on brain network stability change.

Measurement variable: SR score (as defined above) AND the fMRI-derived network stability metric (scalar norm), measured at baseline and post-intervention

Operationalization: The operationalization of network stability and SR score follows the procedures detailed under the primary and secondary outcomes.

Analysis metric: Linear Mixed Model (LMM) analysis testing the three-way interaction term (Intervention × Time × SR score). This term assesses whether the differential change in network stability from baseline to post-bolus (Intervention × Time) is moderated by the continuous SR score.

Method of aggregation: Individual-level SR score (one continuous value per participant). The primary aggregation of the network stability measures (Scalar Norm) is performed on the group level within the Linear Mixed Model (LMM) to account for the repeated-measures design. For descriptive purposes, median-split groups (higher vs. lower resilience) will be compared.

Time points: SR score from prior LORA assessments; fMRI-derived network stability at baseline and post-intervention.

Note: No directional hypothesis is specified; the moderation analysis is exploratory regarding whether higher or lower resilience predicts stronger metabolic responsiveness.

Secondary Outcome 3: Test–retest reliability of neuroimaging measures

Outcome: Test–retest reliability of baseline brain network stability and BBB permeability.

Measurement variable: Intraclass correlation coefficient (ICC) for network stability and BBB permeability (kw) between visit 1 and visit 2 baseline measurements.

Analysis metric: ICC(2,1) for absolute agreement.

Method of aggregation: Group-level ICC across all participants.

Time points: Baseline assessments on visit 1 and visit 2 (interval: 2 weeks on average).

Rationale: Reliability estimates will assess the temporal stability of brain network stability and BBB permeability, informing their potential as trait-like resilience biomarkers.

Exploratory outcomes

Outcome: Associations between cognitive/affective processing styles, impulsive regulation dimensions, lifestyle factors, and primary or secondary outcomes.

Measurement variables:

- **Perceived Positive Appraisal Style Scale (PASS)** [26]: Assesses the tendency to appraise stressful situations positively (content- and process-focused scales). Higher scores reflect more positive appraisal styles.
- **Aggression Questionnaire (AQ)** [33]: Assesses four components of aggression: physical aggression, verbal aggression, anger, and hostility. Items are rated on a 5-point Likert scale; higher scores indicate greater aggression.
- **Barratt Impulsiveness Scale-11 (BIS-11)** [35]: Assesses three facets of trait impulsivity: motor impulsiveness, attentional impulsiveness, and non-planning impulsiveness. Higher scores indicate greater impulsivity.
- **Difficulties in Emotion Regulation Scale (DERS)** [37]: Assesses clinically relevant difficulties in emotion regulation across six facets: non-acceptance, goals, impulse, awareness, strategies, and clarity. Higher scores indicate greater emotion dysregulation.
- **Trait Anger Scale and Anger Expression/Control Scales of the State-Trait Anger Expression Inventory (STAXI)** [38]: Assesses trait anger (disposition to experience anger), and anger expression styles (anger-in, anger-out, anger control).
- **Vividness of Visual Imagery Questionnaire (VVIQ)** [24,25]: Measures vividness of mental imagery across 16 items (sum score; higher = more vivid imagery).
- **Skala zum Ernährungsverhalten (SEV)** [31]: Assesses dietary patterns and eating behavior.
- **International Fitness Scale (IFIS)** [32]: Self-reported physical fitness across five domains (Likert-scaled).

Analysis metric: Exploratory correlation and regression analyses.

Method of aggregation: Individual questionnaire scores; associations with primary and secondary outcomes will be examined in hypothesis-generating analyses.

Time points: Questionnaires completed online before visit 1.

Rationale: Exploratory analyses will identify cognitive, affective, behavioral, and lifestyle correlates of neural resilience markers. No specific hypotheses are preregistered; multiple-comparison corrections will be applied.

Manipulation checks and covariates

Capillary blood measurements (manipulation checks):

- **β-Hydroxybutyrate (BHB):** Confirms ketosis induction. Measured via fingertip capillary sample using ketone test strips at pre-intervention, ingestion + 30 min, and ingestion + 60 min. Expected: BHB increase (to ~2–4 mmol/L) after ingestion; stable in glucose condition.
- **Blood glucose:** Confirms expected post-glucose increase; monitored for safety. Measured at same time points.
- **HbA1c:** Measured at visit 1 for screening (inclusion criterion: HbA1c < 6.5%).

Additional covariates (included in models where appropriate):

- Age, sex, BMI
- Education, subjective social status (SSS) (McArthur Scale [58])
- Self-reported physical fitness (IFIS)
- Dietary patterns (SEV)

MRI data acquisition

All MRI data will be acquired on a 3 Tesla Siemens MAGNETOM Prisma scanner at the Neuroimaging Center, University Medical Center Mainz.

- **Structural imaging:** High-resolution T1-weighted scan (MPRAGE) for anatomical reference and registration.

- **Resting-state fMRI:** Acquired at baseline and post-intervention on both visits. Participants rest with eyes open, fixating on a crosshair.
- **Arterial Spin Labeling (ASL):** Acquired at baseline on both visits to estimate cerebral perfusion and BBB permeability (kw).

Quality control and preprocessing:
Resting-state fMRI data will undergo automated quality assessment using MRIQC [59] prior to preprocessing, providing quantitative image quality metrics (e.g., signal-to-noise ratio, temporal signal-to-noise ratio, framewise displacement, and spatial smoothness). Outputs and summary reports will be visually inspected to identify potential artifacts or outlier scans.

Preprocessing will then be performed using fMRIPrep [60], which implements standardized and reproducible workflows based on *NiPype*. The pipeline includes skull stripping, slice-timing correction, motion correction, susceptibility distortion correction, co-registration to the individual T1-weighted image, and normalization to MNI152 space. Functional data will be denoised using Automatic Removal of Motion Artifacts based on Independent Component Analysis (ICA-AROMA) for motion artifact removal. Framewise displacement (FD) will be computed for each volume; time points with FD > 0.5 mm will be censored from subsequent analyses.

Output quality (e.g., co-registration accuracy, head motion traces, normalization) will be visually inspected using the automatically generated fMRIPrep HTML reports.

Harms {17}

Ketone esters have been extensively studied in humans and exhibit a well-established safety profile. Reported side effects are generally mild and transient, primarily consisting of gastrointestinal discomfort (e.g., nausea, bloating), and occurring predominantly at higher doses or with rapid ingestion (e.g., [20,61,62]). No serious adverse events have been reported in controlled human studies. The ketone monoester used in this trial ((R)-3-hydroxybutyl (R)-3-hydroxybutyrate) is commercially available and classified as a food supplement.

Glucose is the primary physiological energy substrate and is routinely administered in clinical and research settings (e.g., oral glucose tolerance testing) without serious adverse effects. In this study, both substances are administered as single, calorically matched doses, limiting exposure to a short duration and minimizing the risk of accumulation or long-term effects.

Additional anticipated burdens relate to study procedures. MRI assessments are non-invasive. Potential discomfort may include transient claustrophobic sensations or scanner noise, which are mitigated by participant monitoring, hearing protection, and the option to terminate the scan at any time. Capillary blood sampling for glucose, β-hydroxybutyrate, and HbA1c measurements is minimally invasive and may cause brief local discomfort or skin irritation; the risk of infection is minimized through sterile procedures.

All adverse events (AEs) and serious adverse events (SAEs) will be systematically assessed at each study visit and may also be reported spontaneously by participants between visits. Events will be evaluated by a study physician with regard to severity and causal relationship to the intervention. Participants may be withdrawn from the study if deemed necessary for safety reasons, without prejudice. Data collected up to the time of withdrawal may be retained for analysis in accordance with the informed consent and applicable data protection regulations. SAEs will be reported immediately to the principal investigator and the ethics committee in accordance with regulatory requirements. All recorded harms will be documented and reported in trial publications.

Participant timeline {18}

Timeline overview

Pre-enrollment phase: Participants are active members of the LORA cohort with longitudinal resilience data collected over 5-8 years prior to this study.

Enrollment phase:

- Email invitation sent to eligible LORA participants
- Telephone screening, consent discussions
- MRI safety screening, informed consent

- Online questionnaire completion (GHQ-28, MIMIS, Life events checklist, PASS, AQ, BIS-11, DERS, STAXI, VVIQ, SEV, IFIS)

Experimental phase (see section {15a}):

Participants receive both interventions in a crossover manner, such that the intervention administered at visit 2 differs from that at visit 1:

- Visit 1 (Week 0): Baseline MRI + Intervention: BHB or glucose + post-intervention MRI (+ assessment of blood BHB and glucose levels and HbA1c)
- Washout period: 1-4 weeks
- Visit 2 (Week 1-4): Baseline MRI + Intervention: glucose or BHB + post-intervention MRI (+ assessment of blood BHB and glucose levels)

Post-study:

- Follow-up for adverse events if applicable
- Dissemination of results (after study completion)

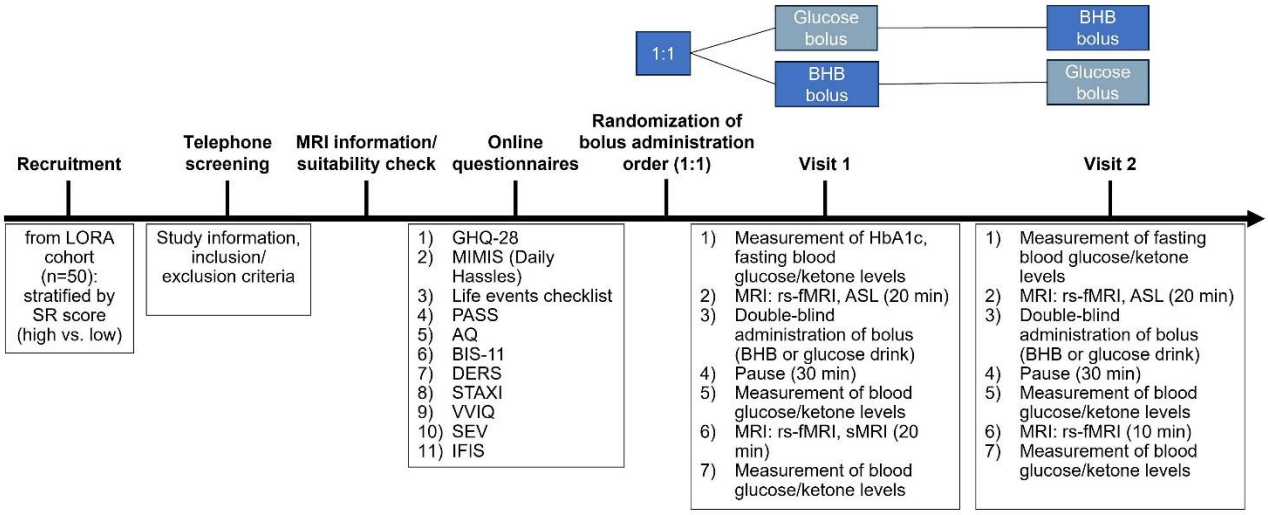


Figure 2. Study timeline and procedural flow. *This figure provides an overview of the study timeline from recruitment through completion of both experimental visits. Participants are recruited from the LORA cohort and stratified by resilience level (high vs. low SR scores) before undergoing telephone screening and MRI suitability assessment. After completing the online questionnaire battery, participants are randomized in a 1:1 ratio to one of two intervention sequences (BHB on Visit 1 followed by glucose on Visit 2, or vice versa). Each visit includes baseline capillary blood measurements, resting-state fMRI and ASL imaging, administration of the assigned drink under double-blind conditions, a 30-minute waiting period, post-intervention imaging, and subsequent blood sampling. Structural MRI (T1- and T2-weighted) is acquired only during Visit 1*.

Table 1. *SPIRIT Schedule of Enrollment, Interventions, and Assessments*

	TRIAL PERIOD		
	Enrolment	**Post-randomization/experimental phase**	
Time point	**Prior to visit 1**	**Visit 1 (Week 0)**	**Visit 2 (Week 1-4)**
ENROLLMENT:			
Eligibility screen	X		
MRI eligibility check	X	X	X
Informed consent	X		
Randomization	X		
INTERVENTION/ COMPERATOR:			
First substance (BHB or		X	
Second substance (glucose or			X

ONLINE ASSESSMENTS	X		
Demographic data	X		
GHQ-28	X		
MIMIS	X		
Life events checklist	X		
PASS	X		
AQ	X		
BIS-11	X		
DERS	X		
STAXI	X		
VVIQ	X		
SEV	X		
IFIS	X		
ON-SITE ASSESSMENTS			
HbA1c (capillary blood)		X	
Glucose (capillary blood)		X	X
BHB (capillary blood)		X	X
Imaging data acquisition:			
rs-fMRT		X	X
ASL		X	X
T1		X	
T2		X	

Sample size {19}

A total of 120 individuals of the LORA cohort will initially be screened for eligibility. Based on prior experience with similar neuroimaging protocols, we anticipate that approximately 50% of screened individuals will meet all inclusion criteria and complete both study visits. Therefore, 60 participants will be enrolled, with the aim of obtaining complete datasets from at least 50 participants after accounting for potential attrition due to technical problems, withdrawal of consent, or other reasons) (Figure 3).

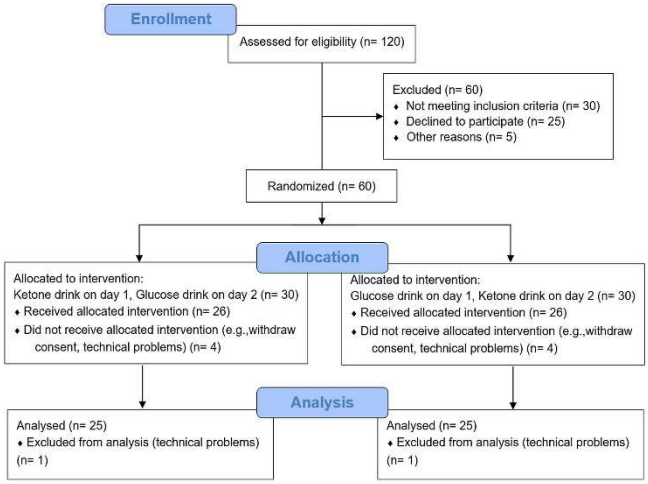


Figure 3. *CONSORT flow diagram showing participant recruitment, randomization, and retention throughout the KeNeReHum study. Numbers shown are projected estimates based on anticipated dropout rates of ~17% (10 of 60 randomized). Final numbers will be reported in the main study publication.*

The primary confirmatory hypothesis is that exogenous β-hydroxybutyrate (BHB) increases resting-state brain network stability in healthy adults compared to glucose. Based on the findings of Mujica-Parodi and colleagues [13], the change in network stability after administration of a calorically matched glucose versus

D-β-hydroxybutyrate (D-βHB) ketone ester bolus was associated with a medium within-subject effect size (Cohen's *dz* = 0.53).

A power analysis conducted using G*Power (Version 3.1.9.7) indicated that, with a power of 0.95, a total of 41 participants are required to detect this effect with a one-tailed paired *t*-test at α = 0.05. To account for potential dropouts and to allow sufficient data for exploratory analyses addressing secondary hypotheses, we aim to recruit 50 participants who meet all inclusion criteria and complete both study visits.

Recruitment {20}

Participants will be recruited from the LORA (Longitudinal Resilience Assessment) cohort, an ongoing longitudinal study examining mechanisms of stress resilience in healthy adults. The LORA cohort currently includes approximately 270 active participants in Mainz who have been followed for 5 to 8 years with regular assessments of stressor exposure and mental health, yielding validated outcome-based resilience measures (SR scores) [18].

Rationale for recruitment from LORA:

1. *Pre-existing resilience data*: LORA participants have longitudinal SR scores, enabling examination of how resilience moderates metabolic effects on brain networks (primary study objective)

2.*Well-characterized sample*: Extensive phenotyping (demographics, mental health history, lifestyle factors) facilitates covariate control and reduces screening burden

3. *High retention*: LORA participants have demonstrated long-term commitment to research, predicting good retention in this follow-up study

Assignment of interventions: randomisation

Sequence generation: who will generate the sequence {21a}

Participants will be randomly assigned to one of two intervention sequences with a 1:1 allocation ratio using a computer-generated randomization list created in R (Version 4.5.1) with a documented seed to ensure reproducibility. In sequence A, participants will receive β-hydroxybutyrate (BHB) monoester on visit 1 and glucose on visit 2. In sequence B, participants will receive glucose on visit 1 and BHB monoester on visit 2.

The complete randomization list, including block sizes and allocation sequence, was generated prior to study initiation by the principal investigator (PI) who is not involved in participant recruitment and data acquisition and has no contact with participants. The randomization list is stored as a password-protected file on a secure institutional server with restricted, role-based access control. Only designated unblinded beverage preparation staff have access to the treatment allocation for each randomization number.

Sequence generation: type of randomisation {21b}

Randomization employs a permuted block design with variable block sizes (2 and 4) in randomized order to ensure balanced allocation while minimizing predictability of treatment assignment. Randomization is stratified by stressor reactivity (SR) status (high vs. low), the hypothesized key moderator, to ensure balanced distribution across sequences within each stratum and to support adequate power for secondary analyses. Details of the blocking structure and randomization sequence are not accessible to personnel involved in participant enrollment, neuroimaging assessments, or outcome data collection.

Allocation concealment mechanism {22}

Allocation concealment is ensured through a two-list system with strictly separated access rights to maintain trial double-blinding:

- *Recruitment list*: Contains sequential IDs stratified by SR status (high vs. low) with no intervention sequence information. This list is accessible to recruitment and study staff.
- *Allocation list*: Contains the mapping of study IDs to intervention sequences (visit 1 and visit 2 beverage assignments). Access is restricted exclusively to unblinded beverage preparation staff who are not involved in participant assessments.

Upon enrolment, participants are assigned the next sequential study ID from the recruitment list according to their SR stratum. The beverage preparation staff then consults the allocation list to prepare the appropriate beverages in identical, opaque containers labelled only with the study ID and visit number. Study personnel conducting assessments and participants remain blinded to treatment allocation throughout the study.
All list access and ID assignments are logged. The lists are stored on a secure institutional server with restricted access.

Implementation {23}

Implementation of randomization:

1. ***Sequence generation:***
    - The allocation sequence is generated centrally using a computer-generated list.
    - Randomization is stratified by SR score (negative vs. positive) with block randomization to ensure balanced allocation.
2. ***Participant enrolment:***
    - Eligible participants are enrolled by the study coordinator (or delegate) after verification of inclusion/exclusion criteria and obtaining informed consent.
3. ***Assignment to interventions:***
    - A trained study team member, not involved in assessments, prepares the intervention solutions according to the participant's allocation sequence from the sealed envelopes.
    - This ensures that both the participants and the data collection staff remain blinded to the assigned intervention.

Assignment of interventions: blinding

Who will be blinded {24a}

This is a double-blind study. The following parties will be blinded to intervention allocation:

Participants: Participants will not be informed which drink (BHB or glucose) they receive on each visit.

Study personnel conducting assessments and handing out intervention: MRI operators, study staff performing MRI data acquisition, obtaining capillary blood measurements, handing out the drinks (BHB or glucose) will remain blinded throughout data collection.

Outcome assessors: All personnel involved in MRI data preprocessing and quality control will be blinded to intervention allocation.

Parties not blinded:

Only the staff preparing the study drinks (necessary for safety and correct preparation) and the PI have access to the randomization list. These individuals are not involved in data acquisition (no contact with participants).

Blinding assessment: At the end of visit 2, participants will be asked whether they have any impression or belief regarding which drink they received on each visit. Responses will be recorded to assess effectiveness of blinding but will not be used for outcome analysis.

How will be blinding be achieved {24b}

Blinding will be achieved by ensuring that the β-hydroxybutyrate monoester and the calorically matched glucose solution are indistinguishable in taste, appearance, and mode of administration. Both drinks have comparable bitter taste profiles, as they will be flavored with gentian tincture to mask taste differences. They are prepared using identical procedures and presented in non-transparent containers to prevent visual identification. These measures ensure effective blinding of both participants and investigators involved in the conduct of the study visits

Procedure for unblinding if needed {24c}

Sealed envelopes corresponding to each participant's randomization number contain the assigned intervention. All study staff has access to these envelopes. The envelopes are only to be opened if knowledge of the administered substance is essential for immediate diagnostic or therapeutic decisions. Unblinding is therefore strictly limited to urgent medical circumstances.

Data collection and management

Plans for assessment and collection of outcomes {25a}

Data types and sources

Neuroimaging data:

- Resting-state fMRI: BOLD signal acquired using a multiband multi-echo echo-planar imaging (EPI) sequence
- Arterial Spin Labeling (ASL): Pseudo-continuous ASL (pCASL) with 3D GRASE readout
- Structural MRI (visit 1 only): T1- and T2-weighted sequences for coregistration and for radiological review by a neuroradiologist

Biological measures:

- Capillary blood measurements at three timepoints per visit (baseline, ingestion + 30 min, ingestion + 60 min) for:
    - β-Hydroxybutyrate (BHB) concentration (mmol/L)
    - Blood glucose concentration (mg/dL)
    - HbA1c (%) (visit 1 only, baseline screening measure)
- Measured using Go-Keto Blood Glucose and Ketone Monitoring System (Vital20 BV) and A1CNow®+ (PTS Diagnostics)

Questionnaire data:

- Administered via LimeSurvey platform (hosted at University Medical Center Mainz)
- Completed remotely before Visit 1
- Data automatically validated for completeness and range checks

Quality assurance procedures

MRI data quality:

- Real-time motion monitoring during acquisition with feedback to participants
- Immediate post-scan visual quality check by MRI operator
- Automated quality assessment using MRIQC (Esteban et al., 2017), generating quantitative metrics (SNR, tSNR, framewise displacement)
- Visual inspection of MRIQC reports by trained researcher before inclusion in analysis
- Preprocessing with fMRIPrep (Esteban et al., 2019) with automated generation of quality control reports
- Exclusion criteria: mean framewise displacement >0.5 mm, excessive motion artifacts, scanner artifacts

Blood measurement standardization:

- All capillary measurements performed by trained personnel using standardized technique according to manufacturer specifications, using sterile single-use lancets.
- Study personnel will wear disposable gloves during the procedure and/or clean their hands before measurements.
- Fingertips of participants will be cleaned and dried before measurements.
- Following each measurement session of one participant, all equipment and work surfaces will be thoroughly disinfected.
- Used lancets will be disposed of immediately in sharps containers

Questionnaire data quality:

- Restricted input format and value range in LimeSurvey
- Missing data alerts before submission
- Manual review of completed questionnaires by study coordinator
- Follow-up contact for clarification if responses appear inconsistent

Training and standardization

- All MRI operators trained on standardized positioning protocol
- Study staff trained on capillary blood measurement technique
- Standard operating procedures (SOPs) documented and accessible to all study personnel

Instruments: Reliability and validity

General Health Questionnaire-28 (GHQ-28):

- Validated German version [28]
- Internal consistency: Cronbach's $\alpha = 0.92$ [28]

Mainz Inventory of Microstressors (MIMIS):

- Developed and validated in German population [30]
- Good ecological validity in daily life assessments

Perceived Positive Appraisal Style Scale (PASS):

- Recently validated [26]
- Two scales: content-focused and process-focused positive appraisal
- Internal consistency: PASS-content: $\alpha = 0.87$; PASS-process: $\alpha = 0.78$ [26]

Aggression Questionnaire (AQ):

- Validated German version [34]
- Internal consistency: Cronbach's $\alpha = 0.62–0.82$ [34]
- Test-retest reliability: $r = 0.73$ [33]

Barratt Impulsiveness Scale-11 (BIS-11):

- Validated German Version [36]
- Internal consistency: Cronbach's $\alpha = 0.69–0.82$ [35,36]
- Widely used in clinical and non-clinical samples; factorial structure validated cross-culturally

Difficulties in Emotion Regulation Scale (DERS):

- Validated German version [63,64]
- Internal consistency: Cronbach's $\alpha = 0.91–0.95$ for the total score; $\alpha \geq 0.80$ for the subscales [37,63,65]

State-Trait Anger Expression Inventory (STAXI):

- Validated German version [39]
- Good internal consistency across subscales ($\alpha = 0.73–0.93$) [39]

Vividness of Visual Imagery Questionnaire (VVIQ):

- German version validated [25]

- High internal consistency with ω=.85 for the total scale (ω=.91 at retest) and subscale consistencies ranging from ω=.75 to .88.
- Test-retest reliability: r_{tt} = 0.68 [25]

Skala zum Ernährungsverhalten (SEV):

- Validated German eating behavior scale [31]
- Good structural validity and responsiveness

International Fitness Scale (IFIS):

- Self-reported fitness across 5 domains [32]
- Validated against objective fitness measures
- test–retest reliability in an adolescent sample: weighted κ-coefficient for overall fitness = 0.65 [32]

Plans to promote participant retention and complete follow-up {25b}

To minimize dropout and ensure complete data collection, the study implements several strategies: participants receive clear pre-visit instructions regarding fasting, activity, and questionnaire completion; they will be contacted prior to visits as a reminder; makeup sessions are offered if a visit is missed. Compliance with pre-visit instructions and questionnaire completion is checked at each visit. Participants are provided with comfort measures during MRI scanning and ongoing support from study staff. All collected data up to the point of discontinuation will be retained and included in analyses unless participants explicitly request deletion. This approach is expected to maximize retention and ensure high-quality, complete follow-up data.

Data management {26}

All study data will be managed in accordance with institutional policies, Good Clinical Practice (GCP), and the General Data Protection Regulation (GDPR) requirements.

Data entry and flow:

- **MRI data:** Acquired in DICOM format on a Siemens 3T Prisma scanner and transferred via secure network to the Neuroimaging Center (NIC) repository. Data will be pseudonymized, converted to BIDS format, and checked for completeness.
- **Questionnaire data:** Collected via LimeSurvey (GDPR-compliant installation at University Medical Center Mainz) and stored in database hosted on institutional servers. Range and plausibility checks will be performed.
- **Biological data:** HbA1c, blood glucose and BHB values are entered into the electronic database.
- **Adverse events:** Documented on AE forms.
- **Long-term resilience measure (SR score):** Longitudinal data provided by the LORA study

Database management and data quality:
The electronic study database is hosted on institutional servers protected by role-based access control and password authentication. Automated range and consistency checks are implemented for all numerical and categorical variables (e.g., physiological parameters, questionnaire scores).

Oversight:
Data management is coordinated by the study coordinator under the supervision of the principal investigator. A detailed Data Management Plan (DMP) describing data flow, validation procedures, and database specifications is filed separately and available upon request.

Confidentiality {33}

All participant data will be handled in accordance with the EU General Data Protection Regulation (GDPR), the German Federal Data Protection Act (BDSG), and institutional data protection policies.

Pseudonymization and separation of identifiers:
Each participant is assigned a unique study identification code prior to any data transfer or analysis. Personal identifiers (e.g., name, contact information, consent forms) are stored separately from study data in a secure master linking file, encrypted and stored on a password-protected server accessible only to the principal investigator (PI) and designated study coordinator. The separation of identifiers is maintained for the entire study duration in accordance with GDPR and BDSG requirements.

Data storage and security:
All electronic study data (MRI, questionnaire, biological, and CRF data) are stored on secure institutional servers within the University Medical Center Mainz IT infrastructure, behind institutional firewalls. Data are further protected by role-base access control. Paper-based source documents (e.g., consent forms, CRFs) are stored in locked cabinets in restricted-access rooms. Regular backups are performed according to institutional policy.

Access control:
Access to pseudonymized data is granted only to authorized study personnel directly involved in data analysis or monitoring. All database access requires personal login credentials.

Data sharing and external access:
Pseudonymized datasets may be shared with approved research collaborators for secondary analyses only if participants have provided explicit consent, including for transfer outside the EU/EEA. Before sharing, all directly identifying information is removed, and MRI data are defaced to prevent re-identification. No biological specimens are stored or shared beyond immediate analysis.

Statistical methods

Statistical methods for primary and secondary outcomes {27a}

Primary Outcome Analysis

The primary hypothesis posits that exogenous β-hydroxybutyrate (BHB) increases resting-state brain network stability compared to an isocaloric glucose bolus. Network stability will be quantified following Mujica-Parodi and colleagues [13]. For each participant, whole-brain functional connectivity matrices will be computed across non-overlapping 24-second time windows, and network instability will be defined as the scalar norm of differences between matrices, with lower values indicating greater stability. A one-tailed paired *t*-test will be performed on within-subject difference scores: Δ_participant = (BHB_post - BHB_baseline) - (Glucose_post - Glucose_baseline). The rational for the paired *t*-test as primary analysis is three-fold: 1) This approach directly replicates the analysis strategy of Mujica-Parodi et al. [11], enabling comparison of effect sizes across studies 2) Sample size (n=50) was determined based on paired *t*-test assumptions (Cohen's dz=0.53, power=0.95, α=0.05). 3) The paired *t*-test is robust to violations of distributional assumptions with moderate sample sizes and provides a transparent, interpretable effect size.

To comprehensively account for the repeated-measures crossover design and the dependency across time points, we will perform a confirmatory analysis using a Linear Mixed Model (LMM). The LMM structure will explicitly test for the differential effect of the intervention across time by including the following fixed effects: Intervention (BHB vs. Glucose), Time (Baseline vs. Post-Bolus), and the Intervention × Time interaction effect. This interaction effect represents the primary hypothesis (i.e., the difference in change scores between conditions). Subject ID will be included as a random intercept to model the within-subject correlation. The model will also test for potential period and sequence effects (order of intervention) as fixed effects.

If both, the paired *t*-test and the LMM Intervention × Time interaction, are significant and in the expected direction, this provides strong evidence for the primary hypothesis. If results diverge (e.g., *t*-test significant

but LMM non-significant after covariate adjustment), this indicates potential confounding that will be explored and reported transparently.

Secondary Outcome Analysis

Secondary outcomes include cross-sectional associations between SR and baseline network stability, and BBB permeability, the moderating effect of stressor reactivity (SR) on metabolic effects and test-retest of neuroimaging measures. The secondary hypotheses will be examined using appropriate statistical methods tailored to each specific outcome.

Secondary Outcomes 1a and 1b (Cross-sectional Associations), i.e., the baseline associations between resilience, network stability, and BBB permeability will be assessed using separate LMMs. The model will include the SR score as a fixed effect and Subject ID as a random intercept to account for the two baseline measurements per participant, controlling for relevant covariates.

Secondary Outcome 2, i.e., the moderation effect of the stressor reactivity (SR) score on the intervention will be tested by including the SR score as a fixed effect and assessing the three-way interaction term (SR score × Intervention × Time) within the primary LMM structure.

Secondary Outcome 3, i.e., test-retest reliability of the baseline neuroimaging measures will be evaluated using the Intraclass Correlation Coefficient (ICC(2,1)).

Sensitivity and Exploratory Analysis

As a direct sensitivity analysis for replication and comparison with previous literature (Mujica-Parodi et al., 2020), simple paired *t*-tests will be performed on the calculated difference scores (Δ=Post−Baseline) between the two conditions. Exploratory analyses will employ correlations and LMMs to investigate associations between metabolic markers, questionnaire data, and the primary/secondary neural outcomes. Appropriate correction for multiple comparisons (e.g., False Discovery Rate, FDR) will be applied to all exploratory analyses.

Exploratory analyses will examine associations of cognitive, affective, impulsive and lifestyle factors, including positive appraisal style (PASS), aggression (AQ), impulsivity (BIS-11), difficulties in emotion regulation (DERS), anger expression (STAXI), visual imagery vividness (VVIQ), dietary behavior (SEV), and self-reported fitness (IFIS)—with primary and secondary outcomes. Covariates such as age, sex, BMI, and baseline metabolic measures (HbA1c, fasting glucose, BHB) will be included in models as appropriate (e.g., if they significantly influence the outcome or the primary interaction effect). Capillary blood measurements of BHB and glucose at three timepoints per visit will serve as manipulation checks to confirm the expected metabolic responses to the glucose boluses.

All analyses will be conducted using standard statistical software (e.g., R or Python), with alpha set at 0.05 for the primary outcome. Multiple comparison corrections will be applied for secondary and exploratory analyses where relevant.

Who will be included in each analysis {27b}

Primary and secondary analyses will follow an intention-to-treat (ITT) approach, including all randomized participants with at least one evaluable post-intervention assessment. Protocol deviations (e.g., incomplete intervention, technical failures) will be documented, but participants will remain in the analysis population where outcome data are available.

How missing data will be handled in the analysis {27c}

Missing data are expected primarily due to technical issues (e.g., MRI artifacts, failed physiological measurements) and are assumed to be missing completely at random (MCAR). No imputation will be

performed for missing outcome values. Sensitivity analyses may be conducted to assess the impact of missing data on the primary outcome.

Methods for additional analyses (e.g. subgroup analyses) {27d}

Exploratory subgroup analyses will be performed to investigate potential moderating factors. **Sex differences** will be assessed by including a Condition × Sex interaction term for the primary outcome (network stability) in linear mixed models. If this interaction is significant, descriptive stratified analyses for males and females will be conducted. This analysis is exploratory, motivated by potential sex differences in ketone metabolism and stress resilience.

Resilience subgroups will be defined using a median split of SR scores, yielding high- and low-resilience groups. Descriptive analyses will examine the effect of BHB versus Glucose within each subgroup and visualize the Condition × Resilience interaction. The primary analysis of resilience as a continuous moderator is addressed in Secondary Outcome 1; these subgroup analyses are exploratory and hypothesis-generating.

No other prespecified additional or adjusted analyses are planned.

Interim analyses {28b}

No formal interim analyses planned.

Protocol and statistical analysis plan {5}

The full study protocol, including the pre-specified statistical analysis plan, is published with this manuscript and will be publicly accessible upon publication.
The statistical analysis plan is fully integrated into the Methods section of the protocol and specifies all primary and secondary outcomes, sample size considerations, randomization procedures, and planned statistical analyses. No separate standalone statistical analysis plan has been prepared.

Any future amendments to the protocol or statistical analysis plan will be documented, dated, and reported transparently in accordance with SPIRIT and journal requirements.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {3d}

The study will be coordinated by the Department of Psychiatry and Psychotherapy at University Medical Center Mainz, which serves as the central hub for overall study management, regulatory compliance, and data coordination.

A Trial Steering Committee (TSC) will provide oversight throughout the study. The TSC includes the principal investigator and co-investigators. Responsibilities of the TSC include monitoring study progress, reviewing protocol deviations, ensuring adherence to Good Clinical Practice, overseeing data quality and integrity, and making decisions regarding necessary study modifications. The TSC will meet quarterly, with additional meetings scheduled as needed.

The operational study team will manage the day-to-day conduct of the trial, including participant recruitment, scheduling of study visits, protocol adherence, safety monitoring, data collection, and quality assurance. The team will meet weekly to coordinate tasks and resolve operational issues.

Composition of the data monitoring committee, its role and reporting structure {28a}

A formal independent Data Monitoring Committee (DMC) is not planned for this study. This decision is justified by the study design: single-center, investigator-initiated, involving healthy adult participants, with a non-therapeutic, low-risk metabolic intervention (BHB vs. glucose). Safety risks related to study procedures

are minimal. Ongoing safety monitoring, including review of adverse events and protocol deviations, will be conducted by the Trial Steering Committee. The principal investigators retain the authority to suspend or terminate the study if safety concerns arise.

Frequency and plans for auditing trial conduct {29}

The study's recruitment progress, protocol adherence, and data quality will be reviewed monthly by the trial steering committee. No formal independent auditing is planned, but any deviations or issues identified will be addressed promptly by the study team.

Protocol amendments {31}

Any major changes to the protocol that affect the conduct of the study, including changes to the study design, sample size, procedures, or objectives, will require a formal amendment to the study protocol. Formal amendments to the protocol will be formally submitted to the responsible local Ethics Committee. Major amendments to the protocol will only be implemented after approval by the Ethics Committee.

Dissemination policy {8}

Results will be published in peer-reviewed scientific journals and presented at national and international conferences. All dissemination will comply with institutional and ethical guidelines.

Discussion

This study uniquely combines an acute metabolic challenge with longitudinal resilience phenotyping to test whether brain network stability and blood-brain barrier function are associated with resilience and responsive to metabolic modulation. The availability of outcome-based resilience measures allows direct examination of whether individual differences in resilience relate to baseline network properties and metabolic responsiveness. If confirmed, findings could inform mechanistic models linking metabolism, neural dynamics, and stress resilience, and guide development of metabolic intervention strategies to promote resilience and prevent stress-related disorders. While this is a single-site study conducted at University Medical Center of the Johannes Gutenberg University Mainz, it is embedded within the international KetoResi consortium, which investigates ketogenic signaling in resilience across multiple species and experimental modalities. The consortium integrates rodent models, molecular and cellular analyses, advanced neuroimaging, and human cohort data to identify mechanisms underlying stress resilience. Regular consortium meetings and shared protocols ensure methodological consistency across the translational pipeline, while the current human study operates independently for data collection purposes. This embedding strengthens the study by allowing mechanistic hypotheses to be tested across species, facilitating back-translation of findings from humans to preclinical models and vice versa.

The study's translational approach is a key strength. Functional connectivity assessed via resting-state fMRI provides a sensitive, non-invasive measure of network stability, while arterial spin labeling MRI enables assessment of blood–brain barrier function. The integration of neuroimaging, metabolic manipulation, and behavioral phenotyping allows exploration of mechanistic links between ketone metabolism, network stability, and resilience. If our hypotheses are confirmed, the study will provide foundational evidence supporting BHB as a potential resilience-promoting intervention, informing future translational and clinical research.

Operationally, the study is feasible given prior data showing measurable effects of ketone administration on network stability. The crossover design increases statistical efficiency and controls for inter-individual variability. Limitations include the relatively small sample size, which may limit power for exploratory analyses, and the single-dose intervention, which does not address long-term effects. The healthy adult population (<45 years) may also limit generalizability to clinical or older populations.

In conclusion, this proof-of-concept study offers a unique opportunity to link metabolic interventions with brain network dynamics and resilience in humans, embedded in a larger translational research framework.

The findings will inform subsequent studies aimed at developing targeted strategies to enhance stress resilience and improve mental and metabolic health, contributing to translational, preventive, and clinical neuroscience.

Trial status

The current version of the study protocol is version 2 (11 July 2025). Recruitment has not yet started and is planned to begin in January 2026. Participant enrollment is expected to be completed in April 2027.

Abbreviations

Acronym	Description
AE	adverse events
ASL	Arterial Spin Labeling
AQ	Aggression Questionnaire
BBB	blood brain barrier
BDSG	German Federal Data Protection Act
BHB	β-hydroxybutyrate
BIS-11	Barratt Impulsiveness Scale-11
BMI	body mass index
BOLD	Blood Oxygenation Level Dependent
CRF	case report form
DERS	Difficulties in Emotion Regulation Scale
DMC	Data Monitoring Committee
FD	framewise displacement
FDR	false discovery rate
EEA	European Economic Area
EPI	echo-planar imaging
(f)MRI	(functional) magnetic resonance imaging
GHQ-28	General Health Questionnaire-28
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
ICA	Independent Component Analysis
ICA-AROMA	ICA-based Automatic Removal of Motion Artifacts
ICC	Intraclass correlation coefficient
ICD	implantable cardioverter-defibrillator
IFIS	International Fitness Scale
ITT	Intention to Treat
LEC	Life Event Checklist

LHC	Life History Calendar
LMM	Linear Mixed Model
LORA	Longitudinal Resilience Assessment
MCAR	Missing completely at random
MIMIS	Mainz Inventory of Microstressors
NIC	Neuroimaging Center
PASS	Perceived Positive Appraisal Style Scale
pCASL	Pseudo-continuous ASL
rs-fMRI	resting-state fMRI
SEV	Skala zum Ernährungsverhalten
SOP	Standard operating procedure
SR score	Stressor reactivity score
STAXI	State-Trait Anger Expression Inventory
TSC	Trial Steering Committee
(t)SNR	(temporal) signal-to-noise ratio
VVIQ	Vividness of Visual Imagery Questionnaire

Declarations

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Authors' contributions {3a}

- **AS**: Principal Investigator; conceived the study idea, acquired funding, initiated the study design, provides overall project supervision, oversees project administration, ensures execution and completion of the project, including data curation and methodology oversight, drafts manuscripts with contributions from all authors.
- **JG**: PhD student; responsible for data collection and investigation procedures, performs statistical analyses under supervision of FUF, drafts study protocol with AS.
- **FUF**: Supervises planned statistical analyses and provides methodological guidance.
- **FMD, DT**: Study physicians; provide clinical expertise, responsible for participant safety and management of adverse events.
- **OT**: Provides clinical expertise.
- **RK**: Supervises imaging procedures related to blood-brain barrier assessment and respective analyses.
- **AH**: Supervises laboratory analyses, including capillary blood measurements and related pharmaceutical expertise.
- **All authors**: Contributed to protocol refinement and approved the final version; will contribute to manuscript preparation and approve final versions arising from this study as applicable.

Sources of funding and other support {7a}

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Availability of data and materials {6}

Only members of the study team will have access to the final trial dataset until completion of data analysis and publication. De-identified data may be shared within the KetoResi consortium for secondary analyses in accordance with institutional and ethical regulations. After publication of the main results, de-identified individual participant data and related documentation may be made available in a suitable public data repository upon reasonable request, in line with FAIR data principles and institutional policies.

Ethics approval and consent to participate {30}

The study has been approved by Ethics Committee of the Landesärztekammer Rheinland-Pfalz (Ethics Committee of the State Medical Association of Rhineland-Palatinate, Germany; approval number 2018-13545_16). A protocol amendment (Amendment 15, Version 3) was submitted to the Ethics Committee on 30 January 2026 and approved on 12 March 2026. The amendment adds four exploratory self-report questionnaires (AQ, BIS-11, DERS, STAXI) and does not affect the study design, interventions, primary or secondary outcomes, or statistical analysis plan. The study is registered in the German Clinical Trials Register (DRKS; registration ID: DRKS00038898). Written, informed consent to participate will be obtained from all participants prior to inclusion in the study. All procedures will be conducted in accordance with the Declaration of Helsinki and relevant institutional and regulatory requirements.

Consent for publication

Not applicable. The study will not include identifiable personal data, images, or videos of participants. Written informed consent for participation (including data use for publication in anonymized form) will be obtained from all participants. A model consent form can be provided upon request.

Competing interests {7b}

The authors declare that they have no competing interests.

Authors’ information (optional)

n/a

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