INPLASY

Organic osmolyte supplementation and intracellular fluid dynamics in endurance athletes: a systematic review and meta-analysis protocol

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Silva Junior, MG.

Corresponding author:
MARIO SILVA JUNIOR

mariogomes11316@gmail.com

Author Affiliation:
Independent Researcher, Goiânia, Goiás, Brazil.

ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 7 May 2026 and was last updated on 7 May 2026.

INTRODUCTION

Review question / Objective Does enteral supplementation with organic osmolytes (creatine, betaine, glycerol, taurine) increase intracellular water (ICW), measured by multifrequency bioimpedance spectroscopy or isotope dilution (D2O), in endurance-trained athletes? Is ΔICW associated with improved time to exhaustion, time-trial performance, or reduced neuromuscular fatigue index?

Condition being studied Intracellular fluid (ICW) homeostasis and osmotic cell volume regulation in skeletal muscle during prolonged endurance exercise, and their relationship with neuromuscular fatigue and exercise tolerance in trained athletes.

METHODS

Participant or population Healthy adults ≥18 years classified as endurance athletes (VO2max ≥45 mL/kg/min); cycling, running, triathlon, race walking. Exclusion: sedentary individuals, clinical populations, animal studies.

Intervention Enteral (oral) supplementation with one or more organic osmolytes: creatine monohydrate, betaine (trimethylglycine), glycerol, or taurine — administered in isolation or as a defined combination. Protocols may be acute (single dose or <5 days) or chronic loading (≥5 days). Osmolytes must be the primary experimental variable; co-ingestion with carbohydrates or electrolytes is permitted if the osmolyte contribution is isolable.

Comparator Isocaloric and/or iso-osmotic placebo (e.g., maltodextrin, flavored water) without active osmolyte content; or conventional water/electrolyte hydration protocol without organic osmolyte supplementation. Crossover designs where participants serve as their own control are eligible.

Study designs to be included Randomized controlled trials (RCTs) — crossover or parallel-

group. No language restrictions. Inception to May 2026.

Eligibility criteria INCLUSION: Randomized controlled trials (crossover or parallel-group); healthy adults ≥18 years classified as endurance athletes (VO2max ≥45 mL/kg/min or equivalent); oral osmolyte supplementation as primary intervention; ICW measured by multifrequency bioimpedance spectroscopy (BIS ≥4 frequencies), multifrequency BIA (≥4 frequencies), or isotope dilution (D2O/NaBr); at least one endurance performance metric (TTE, TT, power output, or neuromuscular fatigue index); no language restriction; inception to May 2026. EXCLUSION: Sedentary or clinical populations; intravenous/parenteral administration; single-frequency BIA (50kHz) as sole fluid assessment method; body mass change as sole fluid proxy; uncontrolled multi-ingredient supplements; animal studies; pediatric populations; no control arm.

Information sources PubMed/MEDLINE, EMBASE, Cochrane CENTRAL, Web of Science, SPORTDiscus. Grey literature: ClinicalTrials.gov, WHO ICTRP.

Main outcome(s) PRIMARY OUTCOME 1 — Intracellular water change (ΔICW): absolute (litres) and percentage change from baseline, measured by multifrequency BIS or isotope dilution (D2O/NaBr). PRIMARY OUTCOME 2 — Endurance performance / neuromuscular fatigue: time to exhaustion (TTE, seconds or minutes); time-trial completion time or mean power output (Watts); isometric or isokinetic fatigue index (% force decline). Effect measure: Standardized Mean Difference (SMD) with 95% CI.

Quality assessment / Risk of bias analysis Risk of bias will be independently assessed by two reviewers using the Cochrane Risk of Bias 2 (RoB 2) tool across five domains: (1) randomization process; (2) deviations from intended interventions; (3) missing outcome data; (4) outcome measurement; (5) selection of reported results. Overall judgment: Low risk, Some concerns, or High risk. Disagreements resolved by a third reviewer. Results visualized using the robvis R package. Evidence certainty graded using the GRADE approach via GRADEpro GDT software.

Strategy of data synthesis When ≥2 studies report the same outcome with extractable data, a random-effects meta-analysis will be conducted using the DerSimonian-Laird estimator with Restricted Maximum Likelihood (REML) for Tau^2 estimation, implemented in R (metafor package v4.0).

Effect measures: continuous outcomes (ΔICW, TTE, power output) will be expressed as Standardized Mean Difference (SMD) with 95% CI; binary outcomes (adverse events) as Risk Ratio (RR) with 95% CI.

Heterogeneity will be quantified using: (1) Cochran Q-test (α=0.10); (2) I^2 statistic — interpreted as low (75%); (3) Tau^2 for between-study variance; (4) 95% Prediction Interval to express uncertainty for a new study.

If I^2>50%, pre-specified subgroup analyses become mandatory. When quantitative synthesis is not possible due to clinical or methodological diversity (fewer than 2 studies per outcome), a structured narrative synthesis will be presented following SWiM (Synthesis Without Meta-Analysis) guidelines. Publication bias will be assessed by funnel plot inspection and Egger regression test when ≥10 studies per outcome are available; Trim-and-Fill applied if asymmetry is detected. All R code will be deposited in an open OSF repository upon manuscript submission.

Subgroup analysis Ten pre-specified subgroup analyses (minimum 3 studies per subgroup to conduct meta-analysis; otherwise reported narratively). Subgroup interactions tested by Chi^2 test (p<0.10 threshold).

SG-01 — Osmolyte type: creatine | betaine | glycerol | taurine | combinations (≥2 osmolytes).
SG-02 — Administration timing: acute pre-event (<2h) | chronic loading (≥5 days) | mixed protocol.
SG-03 — Relative dose: low (1.2 g/kg).
SG-04 — ICW measurement method: multifrequency BIS | multifrequency BIA (≥4 freq.) | single-frequency BIA (50kHz) | isotope dilution (D2O/NaBr) | body mass proxy.
SG-05 — Athletic level: recreational (VO2max 65 mL/kg/min).
SG-06 — Sex: exclusively male | exclusively female | mixed.
SG-07 — Sport modality: cycling | running | triathlon/multisport | other.
SG-08 — Environmental condition: temperate (≤26°C) | moderate heat (26–34°C) | extreme heat (>34°C).
SG-09 — Supplementation duration: ≤7 days | 8–21 days | >21 days.
SG-10 — Performance outcome type: TTE | time-trial | sustained power output | neuromuscular fatigue index.

Meta-regression will be conducted for ≥10 studies on continuous moderators: dose (g/day), duration (days), VO2max (mL/kg/min), environmental temperature (°C), and ΔICW as mediator of performance outcomes.

Sensitivity analysis Seven pre-specified sensitivity analyses: (AS-01) exclusion of studies rated High risk of bias overall by RoB 2; (AS-02) restriction to BIS or isotope dilution (D2O/NaBr) studies only; (AS-03) exclusion of crossover studies with inadequate washout period (<14 days for creatine, <7 days for betaine/taurine); (AS-04) fixed-effects model (Mantel-Haenszel) as alternative to random-effects; (AS-05) exclusion of grey literature and conference abstracts; (AS-06) exclusion of studies with exclusive industry funding; (AS-07) restriction to studies with GRADE High or Moderate certainty.

Country(ies) involved Brazil.

Keywords organic osmolytes; intracellular water; creatine; betaine; glycerol; taurine; endurance athletes; meta-analysis; bioimpedance; neuromuscular fatigue.

Contributions of each author

Author 1 - MARIO SILVA JUNIOR.
Email: mariogomes11316@gmail.com