2025, Retos, 62, 341-349

Betaine as an Ergogenic Aid to Improve Muscle Fatigue in Physical Exercise: A Systematic Review of Randomized Clinical Trials

Betaína como ayuda ergogénica para mejorar la fatiga muscular en el ejercicio físico: Una revisión sistemática de ensayos clínicos aleatorizados

*Larissa Torres Fernandes, *Deysimara de Cássia Santos, *Lucas Teixeira Marques, *Danielle Negri Ferreira Neves, **Otávio de Toledo Nóbrega, *Mariana de Melo Cazal, *Carina Carvalho Silvestre, *Ciro José Brito
*Federal University of Juiz de Fora Campus of Governador Valadares (Brazil), **University of Brasilia (Brazil)

Abstract. Objectives: This systematic review aims to investigate the influence of betaine supplementation on muscle fatigue during exercise. Methodology: To this end, the PRISMA guidelines were adopted and the study was registered with PROSPERO (CRD42023469111). Searches were conducted using specific keywords according to the PICOS strategy on March 12, 2024, in the PubMed, Cochrane Library, Web of Science, Embase and SCOPUS databases. Randomized clinical trials (RCTs) in adults of both sexes that used betaine as a supplement, compared to placebo, to improve muscle fatigue during physical exercise were included. Animal studies and studies with concomitant use of betaine and other supplements were excluded. Muscle fatigue was assessed using the one-repetition maximum test and blood lactate during leg press or squat and bench press exercises. The risk of bias of the included studies was assessed using the Cochrane RoB2 tool and the certainty of the evidence using GRADE. Results: Five RCTs were included, in which a total of 93 participants were evaluated; all were male, with 57 individuals allocated to the intervention group with a mean age of 19.18±2.67 years, and 58 individuals in the control group with a mean age of 19.66±2.44 years. Betaine promoted more repetitions until muscle fatigue in the leg press or squat (n = 3) and bench press (n = 2), with reductions in serum lactate in two of these studies. Conclusion: Betaine can be considered as a potential ergogenic resource for improving muscle fatigue, providing a greater number of repetitions in exercises and eliminating lactate in the blood.
Keywords: Betaine, Muscle Fatigue, Exercise, Dietary Supplements, Systematic Review.

Resumen. Objetivos: Esta revisión sistemática investigó la influencia de la suplementación con betaína en la fatiga muscular durante el ejercicio. Metodología: Para ello se ocupó las directrices PRISMA y se registró el estudio en PROSPERO (CRD42023469111). Se realizaron búsquedas utilizando palabras clave específicas según la estrategia PICOS en el 12 de marzo de 2024 en las bases de datos PubMed, Cochrane Library, Web of Science, Embase y SCOPUS. Se incluyeron ensayos clínicos aleatorios (ECA) en adultos de ambos sexos que utilizaron betaína como suplemento, en comparación con placebo, para mejorar la fatiga muscular durante el ejercicio físico. Se excluyeron los estudios en animales y los estudios con uso concomitante de betaína y otros suplementos. La fatiga muscular se evaluó mediante la prueba de una repetición máxima y el lactato sanguíneo durante los ejercicios de leg-press, o sentadilla y press de banca. El riesgo de sesgo de los estudios incluidos se evaluó mediante la herramienta Cochrane RoB2 y la certeza de las pruebas mediante GRADE. Resultados: Se incluyeron cinco ECA, en los que se evaluó a un total de 93 participantes; todos eran varones, con 57 individuos asignados al grupo de intervención con una edad media de 19,18±2,67 años, y 58 individuos en el grupo de control con una edad media de 19,66±2,44 años. La betaína promovió más repeticiones hasta la fatiga muscular en el leg-press o sentadilla (n = 3) y en el press de banca (n = 2), con menores nivels del lactato sérico en dos estudios. Conclusiones: La betaína puede considerarse un potencial recurso ergogénico para mejorar la fatiga muscular, proporcionar un mayor número de repeticiones en los ejercicios y eliminar el lactato en sangre.
Palabras clave: Betaína, Fatiga muscular, Ejercicio, Suplementos Dietéticos, Revisión sistemática.

Fecha recepción: 10-07-24. Fecha de aceptación: 12-10-24
Larissa Torres Fernandes
farm.larissatorresfernandes@gmail.com

Introduction

The use of nutritional supplements as an ergogenic resource promotes improved physical performance. Supplementation can also optimize muscle recovery time, delay fatigue in muscle fibers, and counteract the inhibitory effect of the central nervous system on muscle contraction, resulting in better performance, among other (Waldman, et al. 2023). Betaine (*N-N-N*-trimethylglycine) stands out among ergogenic resources, choline-derived substance that can be found in betaine-rich foods such as whole wheat grains, beets, spinach, invertebrates, whole wheat germ and bran (Kaur, et al. 2019; Arazi, et al. 2022; Machek , et al. 2022; Arumugam, et al. 2021; Dobrijevic, et al. 2023). Betaine can also be produced biologically from the oxidation of dietary choline in the liver and kidneys (Arazi, et al. 2022; Machek, et al. 2022; Dobrijevic, et al. 2023). The average intake of betaine by adults is approximately 100 to 400 mg/day. However, for people who consume mostly whole food products and invertebrates, intake can be between 1 and 2.5 grams (g) per day (Arazi, et al. 2022; Machek, et al. 2022; Arumugam, et al. 2021; Dobrijevic, et al. 2023). As an ergogenic resource, betaine promotes perceived benefits from 7 days and a maximum of 14 days considering the intake of moderate doses (2.5g/day) in active populations (Arazi, et al. 2022; Freitas, et al. 2015; Gao, et al. 2019; Waldman, et al. 2023; Yang, et al. 2020).

The main mechanisms of action attributed to betaine include its function as an osmolyte, protective agent against protein denaturation and methyl group donor (Cholewa, et al. 2019; Gao, et al. 2019; Kaur, et al. 2019; Dobrijevic, et al. 2023). Some studies indicate that chronic consumption of betaine can provide improvements in physical performance, mainly aimed at muscle strength and power in physically active individuals (Ismaeel, 2017; Aguinaga-Ontoso, et al. 2023). In addition, other studies have shown that chronic betaine consumption can improve muscular endurance, modulate endocrine function and reduce body fat percentage (Arazi, et al. 2022; Freitas, et al. 2015; Gao, et al. 2019; Waldman, et al. 2023). At the muscle level, betaine provides greater cellular hydration time, protection against acidotic denaturation of intracellular proteins and contributes to the synthesis of essential metabolic proteins (Cholewa, et al. 2019; Waldman, et al. 2023). In addition, betaine at the muscle level helps to stimulate the modulation of skeletal muscle function, contraction, respiration, mitochondrial biogenesis and improved blood flow (Waldman, et al. 2023). In the studies carried out by Cholewa et al (2014) and Waldman et al (2023), the authors suggest that betaine supplementation can reduce perceptions of muscle fatigue. This is due to betaine's ability to promote increased acetylcholine synthesis in the motor neuron (Cholewa, et al. 2014; Waldman, et al. 2023).

In this perspective, it is known that muscle fatigue is defined as any decrease in muscle power during physical exercise. Muscle fatigue is the main cause of reduced physical performance in exercise practitioners and sports athletes, and can lead to alterations in the performance of a single muscle, imbalance, influence on postural control, and alteration of movement patterns (Verschueren, et al. 2021; Cervantes Hernández , et al. 2022). Some supplements are capable of providing a reduction in muscle fatigue (Ihsan, et al. 2024). In this sense, studies indicate that betaine supplementation may promote reduced muscle fatigue, with an increase in the number of maximum repetitions until fatigue and a decrease in post-exercise serum lactate. However, few studies have investigated the effects of betaine supplementation on muscle fatigue during physical exercise (Arazi, et al. 2022; Hoffman, et al. 2011). Despite the existence of systematic reviews aimed at investigating the effects of betaine supplementation related to health (Zawieja; Zawieja & Chmurzynska, 2019; Gao, et al. 2019; Ashtary-Larky, et al. 2022; Xu, et al. 2023) and physical performance (Cholewa, et al. 2014; Cholewa, et al. 2019) such as strength and power (Ismaeel, 2017; Aguinaga-Ontoso, et al. 2023), there are still gaps regarding its effects on other outcomes, such as muscle fatigue. Consequently, there is no consensus in the literature regarding the relationship between betaine supplementation and improvement in muscle fatigue. Therefore, this systematic review aims to investigate the influence of betaine supplementation on muscle fatigue during physical exercise, presenting evidence on the use of this supplement.

Materials and methods

This is a systematic review, following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses – PRISMA (Page, et al. 2021) (check list in the supplementary material). The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42023469111).

Data sources and search strategies

The search strategy included terms related to "betaine," "fatigue," and "exercise," standardized by the Medical Subject Headings (MeSH) and Health Sciences Descriptors (DeCS), in different combinations according to the search protocols of each database, without time restrictions (supplementary material). The indexed databases used were: Medical Literature Analysis and Retrieval System Online (PubMed/Medline), Excerpta Medica Database (EMBASE), Web of Science, Scopus, and Cochrane Library. The search in the databases was conducted on March 12, 2024. The search strategies used are detailed in the supplementary material.

Study selection and eligibility criteria

The articles were selected from the databases by two reviewers (DNFN and LTF). All articles found were imported into the Rayyan Reference Manager platform. The process of selecting studies to be included in the review was initially conducted by both authors (DNFN and LTF) reading the titles and abstracts. The full texts of the selected articles were subsequently read (DNFN and LTF). The two authors carried out the process in a standardized and independent manner in both phases. In cases of disagreement, a third author (LTM) was contacted to resolve and clarify such discrepancies.

Studies were included if they met the PICOS criteria, as well as the research question: "Does betaine supplementation improve muscle fatigue in exercise practitioners?" The study population consisted of individuals of both sexes, aged 14 to 60 years, who were physical exercise practitioners. The intervention was betaine supplementation during physical exercise. Studies using some form of placebo were adopted for comparison. The outcome established was reduced muscle fatigue during physical exercise. The type of study used in the review was randomized clinical trials (RCTs) in humans, either parallel or crossover. The following exclusion criteria were adopted: animal studies, studies which were not randomized clinical trials, and studies involving concurrent use of betaine and other supplements.

Data extraction

The data collected from the selected studies were organized and presented in a Microsoft Excel® spreadsheet, independently conducted by two reviewers (LTM and DNFN). In the event of discrepancies, a third author (DCS) was responsible for making decisions. The following data were collected: first author's name; year of publication; country; objective; study design; participant descriptions (number of participants, mean age, participant characteristics); intervention (intervention strategy, dose and supplementation period, evaluation parameters, placebo evaluation form); and results. In terms of evaluation, muscle fatigue was investigated using the one-repetition maximum (1-RM) test, blood lactate levels, and the type of exercise performed for upper limbs (bench press) and lower limbs (leg press or squat).

Risk of bias analysis

The risk of bias assessment was conducted for the outcome of muscle fatigue using the Cochrane RoB 2.0 tool (Higgins, et al. 2022). The following types of bias for RCTs were assessed: random sequence generation (selection bias); blinding of participants and personnel (performance bias); blinding of outcome assessment (detection bias); incomplete outcome data (attrition bias); selective reporting (reporting bias); and overall risk of bias. In addition to the aforementioned biases, carryover and washout effects were also evaluated for crossover RCTs (selection bias). Domain assessments and overall risk of bias were classified as (L) low, (U) unclear, and (H) high (Higgins, et al. 2022), according to the Cochrane manual criteria. The overall risk of bias was determined by combining the bias domains. The risk of bias analysis was independently and blindly conducted by two reviewers (DCS, DNFN). In case of discrepancies, a third author (LTM) was responsible for making decisions.

Certainty of Evidence

The certainty of the evidence was assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach. This method enables assessing the evidence for each outcome analyzed based on the following domains: study design; methodological limitations (risk of bias); inconsistency; indirect evidence; imprecision and publication bias. The quality of evidence in GRADE is classified into four levels at the end of the evaluation: high, moderate, low and very low (Schünemann, et al. 2013). The outcome adopted for evaluation in GRADE was muscle fatigue. Two researchers independently addressed the five GRADE domains (DNFN, LTF). In case of disagreement, a third author (LTM) was responsible for the decision. We justified all decisions to lower the certainty rating of any study using footnotes at the end of Table 4. The GRADEpro GDT software (https://gdt.gradepro.org/app/) was used to perform the certainty of the evidence.

Results

Selection and identification of studies

The search strategies across different databases yielded a total of 53 studies. After removing duplicate studies, there were 33 studies remaining. Titles and abstracts of these studies were screened, resulting in the exclusion of 28 articles that did not meet the established criteria (supplementary material), leaving five studies eligible for full-text reading. All of these studies (Arazi, et al. 2022; Hoffman, et al. 2009; Lee, et al. 2013; Machek, et al. 2022; Nobari, et al. 2021) were included in this systematic review, as depicted in the PRISMA flow diagram (Figure 1).

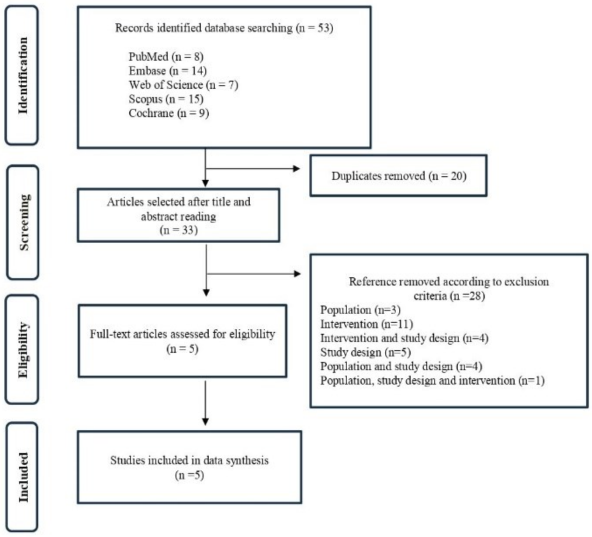

Figure 1. Flow diagram of study selection

Characteristics of studies

The studies included in the review (n = 5) were published between 2009 and 2022, in different countries: two in the United States of America (USA) (Hoffman, et al. 2009; Lee, et al. 2013), one in Iraq (Nobari , et al. 2021), one in Poland (Machek, et al. 2022), and one in Iran (Arazi, et al. 2022). All of them were double-blind RCTs (n = 5), with two being crossover RCTs (n = 2). The total actual sample, without underestimation due to the study design (crossover), involved 93 participants. All participants were men, with 57 individuals allocated to the intervention group, with a mean age of 19.18 ± 2.67 years, and 58 individuals in the control group, with a mean age of 19.66 ± 2.44 years. The intervention period ranged from 14 days to 14 weeks (chronic). The betaine supplementation dose ranged from 2 to 6 g/day, divided into two doses per day in all studies. The main characteristics of the studies are presented in Table 1.

Table 1.
Characteristics of studies

Author, year, country	Objectives	Study design	Participants				Characterization of participants
			N		Average age (years)		
			IG	CG	IG	CG	
Hoffman, et al. 2009, USA	To examine the effectiveness of 15 days of betaine supplementation on muscle endurance, power performance, and fatigue rate in college-aged active men	RCT, double-blind	12	12	20.4 ±1.3	21.4 ±4.7	Male individuals, active in college-age
Lee, et al. 2013, USA	Investigate the ergogenic effects of betaine supplementation on strength and power performance	RCT, Crossover, double-blind	12	12	21±3	21±3	Healthy and recreationally active men
Nobari,	To investigate the effect of betaine	ECR, Double-	14	15	15.5	15.5	Young professional soccer

et al. 2021, Iraq	supplementation on the biomotor abilities of young professional soccer players	blind			±0.3	±0.3	players
Arazi, et al. 2022, Iran	To examine the effects of short-term betaine supplementation on muscle endurance, plasma levels of lactate, testosterone, and cortisol, and the testosterone/cortisol ratio in response to acute resistance exercise	RCT, Crossover, double-blind	10	10	16±1	16±1	Young handball players
Machek, et al. 2022, Polond	To compare the impacts of a potential synergy of blood flow restriction and betaine on leg press performance, lactate concentrations, and exercise-associated biomarkers	ECR, Mixed, double-blind	9	9	23±3	22±1	Young apparently healthy individuals with recreational endurance training

Subtitle: RCT, randomized controlled trial; IG, Intervention Group (betaine supplementation); CG, Control Group (placebo supplementation).

Effects of betaine supplementation on muscle fatigue

Muscle fatigue was assessed in this review using the one-repetition maximum (1-RM) test and blood lactate levels. Among the five studies included, only one directly assessed muscle fatigue (Hoffman, et al. 2009), while four studies indirectly evaluated muscle fatigue (not described as the study objective and not assessed independently)(Arazi, et al. 2022; Lee, et al. 2013; Machek, et al. 2022; Nobari, et al. 2021). Muscle fatigue was assessed in exercises such as bench press, leg press or squat in the studies.

Maximum number of repetitions (1-RM) test

Significant differences were reported in two studies that compared the number of repetitions in bench press exercises between the intervention group (IG) and the control group (CG) and in leg press, between IG and CG after the supplementation period (Arazi, et al. 2022; Nobari, et al. 2021). Only one study reported a significant difference in the number of repetitions solely in the squat exercise, with no significant differences observed in the bench press exercise (Hoffman, et al. 2009). The study by Lee et al. (2013) indicated no significant difference in the total number of repetitions in squats and bench press until muscle fatigue between the IG and CG (Lee, et al. 2013). Finally, one study pointed out that betaine supplementation in IG provided significant effects in performing the 1-RM test in the leg press exercise in terms of exercise and execution time (Machek, et al. 2022), as described in Table 2.

Blood lactate level

Blood lactate levels were assessed in two studies comparing the IG and CG before and after the intervention period (Arazi, et al. 2022; Machek, et al. 2022). Reductions in blood lactate levels were reported in both studies when comparing the IG and CG after betaine supplementation (Table 2).

Table 2.
Characteristics of the interventions and result

Author, year, country	Intervention						Results
	Strategy	Dosage	Assessment points	Duration	Control	Assessment method	
Hoffman, et al. 2009, USA	BET mixed in 240 ml of sports drink	1.25g divided into 2 doses per day	Pre (T1) and 7 days later (T2) and 14 days (T3) after supplementation	14 days	PL	1-RM Bench press and squat	No significant differences were observed in the bench press exercise. The number of repetitions performed in the squat exercise was significantly higher ($p < 0.05$).
Lee, et al. 2013, USA	BET and 300 ml of Gatorade	2.5g divided 2 times a day	Before the ergometric test After the ergometric test	14 days	PL	1-RM Bench press and squat	There were no significant differences in the total number of repetitions of back squats performed at 85% 1-RM until fatigue.
Nobari, et al. 2021, Iraq	BET and 300ml of water, 2 hours before training and 1 hour after training	2g divided into 2 doses per day	P1 pre-season; P2, during the seven weeks following mid-season; and P3, in the week following the end of the season	14 weeks	PL	1-RM Bench press and leg press	There were no significant effects of time on the 1 RM bench press ($p > 0.05$), but there was significance in the group by time interaction ($p = 0.005$). There was significance in time ($p = 0.001$) and group by time ($p < 0.001$) in the leg press.
Arazi, et al. 2022, Iran	BET and warm water after a meal	2.5g divided into 2 doses per day	Pre-supplementation Post-supplementation	14 days	PL	1-RM Bench press and leg press Lactate	There was a significant difference in lactate levels ($p < 0.001$), in the number of repetitions in bench press exercises ($p < 0.001$) and leg press ($p < 0.001$).
Machek,	BET fine white	6g divided into	Blood samples: pre and post-	14 days	PL	1-RM	There were significant

et al. 2022, Poland	powder in transparent gelatin capsules	2 doses per day supplementation (30 minutes and 3 hours)	Post-exercise Bench press and leg press Lactate	effects of exercise condition ((p = 0.003) and time (p < 0.001) for ΔLactate < in lactate levels.

Assessment of risk of bias

According to the Cochrane RoB 2.0 tool, all studies were classified as having some concern, as at least one of the domains was judged to have some concern (U), with no domain classified as high risk of bias (H), as shown in Figure 2 and 3. The crossover studies (n = 2) did not present the participant allocation sequence in the randomization process, possibly due to the proposed experimental design, and were therefore classified in the Random Sequence Generation domain with some concerns (U) (Arazi, et al. 2022; Lee, et al. 2013). The other RCTs presented a low risk of bias (L) in the Random Sequence Generation domain (Hoffman, et al. 2009a; Machek, et al. 2022; Nobari, et al. 2021). Evaluation of carryover and washout effects in crossover RCTs indicated a low risk of bias (L) (Arazi, et al. 2022; Lee, et al. 2013). All studies (n = 5) presented a low risk of bias for Blinding of Participants and Personnel; Blinding of outcome assessment; and Incomplete outcome data (Arazi, et al. 2022; Hoffman, et al. 2009; Lee, et al. 2013; Machek, et al. 2022; Nobari, et al. 2021). Finally, all studies (n = 5) presented some concerns (U) in relation to selective reporting, as there was no evidence that the RCT research protocol was registered in any database by the authors of the included studies (Arazi, et al. 2022; Hoffman, et al. 2009; Lee, et al. 2013; Machek, et al. 2022; Nobari, et al. 2021). A detailed assessment of the risk of bias is provided in the supplementary material.

Article	Selection Bias		Performance Bias	Detection Bias	Attrition Bias	Reporting Bias	Risk of global bias
	Random Sequence Generation	Carryover and washout effects	Blinding of Participants and Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting	
Hoffman, et al. 2009	+	NA	+	+	+	!	!
*Lee, et al. 2013	!	+	+	+	+	!	!
Nobari, et al. 2021	+	NA	+	+	+	!	!
*Arazi, et al. 2022	!	+	+	+	+	!	!
Machek, et al. 2022	+	NA	+	+	+	!	!

Abbreviations: + L, low; ! U, unclear; - H, high; NA, Not applicable

* Crossover RCTs

Figure 2. Risk of bias analysis

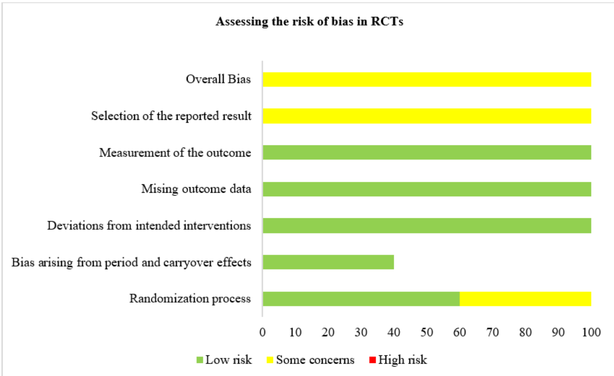

Figure 3. Assessing the risk of bias in RCTs

Certainty of Evidence

The assessment of the certainty of the evidence indicated that there was a degree of uncertainty in the findings regarding the benefit of betaine supplementation in improving muscle fatigue during exercise. The certainty of the evidence was downgraded in the inconsistency (serious) and imprecision (very serious) domains. Table 3 presents a summary of the results, with footnotes explaining the downgrading judgments (decrease in the certainty of the evidence). Therefore, confidence in the estimate of the effect is very limited, in addition to the uncertainties of the results to the detriment of the limitations of the RCTs.

Table 3.
Certainty assessment

Certainty assessment							N° of patients		Effect		Certainty
N° of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Betaine	Placebo	Relative (95% CI)	Absolute (95% CI)	
5	randomised trials	not serious[a]	serious[b]	not serious	very serious[c]	none[d]	57	58	-	see comment	⊕◯◯◯ Very low

a. Although some studies have considered some concerns regarding the randomization sequence (crossover) and protocol registration (all studies), we believe that this would not influence the lowering of the domain.

b. Although the average age and population show that the sample is homogeneous, the intervention period (14 days to 14 weeks) and the dose of betaine supplementation (2 to 6 g/day) varied greatly.

c. The total actual sample, without underestimation due to the study design, involved 93 participants.

d.Although the gray literature was not used as a database, the databases included in the review were those recommended by the Cochrane Handbook for Systematic Reviews of Interventions (PubMed/Medilene Excerpta Medica Database (EMBASE) and Cochrane Library). In addition, in order to broaden the results found, the following databases were included in the review: Web of Science and Scopus.

Discussion

This systematic review aimed to investigate the influence of betaine supplementation on muscle fatigue during physical exercise, presenting evidence on the use of this supplement. A total of five RCTs were included, involving 93 male participants, over an intervention period of 14 days to 14 weeks and with betaine supplementation doses ranging from 2 to 6 g/day. The results of the studies show a possible effect of betaine supplementation on muscle fatigue indirectly through the greater number of repetitions focused on lower limb exercises, whether in the leg press (Arazi, et al. 2022; Nobari, et al. 2021) or squats (Hoffman, et al. 2009). Furthermore, betaine seems to favor the performance of a greater number of repetitions in bench press exercises (Arazi, et al. 2022; Nobari, et al. 2021). In a direct assessment of muscle fatigue, blood lactate levels were reduced after betaine supplementation (Arazi, et al. 2022; Machek, et al. 2022). The main mechanism described for this effect is suggested by Waldman et al. (2023), who propose that betaine increases free choline, resulting in increased acetylcholine synthesis in motor neurons, reducing perceived effort and muscle fatigue (Waldman, et al. 2023). This enables a greater number of repetitions during physical exercise (Arazi, et al. 2022).

Betaine appears to be an effective ergogenic resource for increasing the number of repetitions in leg press exercises during the 1-RM test aimed at muscle fatigue. However, it is important to note that other factors, beyond muscle fatigue, may directly influence the results of the 1-RM test. Previous studies, such as those by Cholewa et al. (2013) and Cholewa et al. (2018), confirm this hypothesis (Cholewa, et al. 2013; Cholewa, et al. 2018). According to Dias et al. (2013), factors such as stretching, recovery intervals between maximum repetition attempts, ambient temperature, and dehydration can impact the execution of the 1-RM test (Dias, et al. 2013). In the study conducted by Hoffman et al. (2009), which directly assessed muscle fatigue through the 1-RM test, participants underwent a prior warm-up with standardized load, followed by 3 to 4 attempts at 1-RM, with a rest period of 3 to 5 minutes between each attempt (Hoffman, et al. 2009). The warm-up may influence the execution of exercises that require knee extension and flexion movements, as is the case with squats. Despite the warm-up, the study by Hoffman et al. (2009) revealed that it was possible to perform a greater number of repetitions in the 1-RM test (Hoffman, et al. 2009). This improvement may be attributed to the standardization of other variables influencing performance, allowing for a more effective execution of the test (Hoffman, et al. 2009). Furthermore, this study was pioneering in investigating the ergogenic effects of betaine on muscle fatigue. While Hoffman et al. (2009) observed an increase in the number of repetitions, Lee et al. (2013) did not find significant differences in their results (Hoffman, et al. 2009; Lee, et al. .2013). This suggests that factors such as individual variability may be influencing the outcomes and need to be further explored in future investigations.

There are some mechanisms attributed to betaine supplementation that indicate its contribution to reducing muscle fatigue. One of these mechanisms is attributed to its action as a protective agent against the denaturation of intracellular proteins. Betaine helps maintain the stability and conformation of proteins, which can protect intracellular muscle proteins against acidotic denaturation, enhancing greater mechanical tension promoted by attenuated metabolic fatigue and concomitantly prolonged contraction (Cholewa, et al. 2018; Willingham, et al. 2020). Additionally, Willingham et al. (2020) point out in their study that betaine can donate a methyl group for the synthesis of essential metabolic proteins such as creatine and carnitine. This mainly occurs during periods of hypertonicity during physical exercise (Willingham, et al. 2020). Other studies address the ability to promote the elevation of nitric oxide levels provided by betaine supplementation, as it is a compound rich in nitrate (Jones, 2013; Pryor, et al. 2017). Jones (2013) describes that the elevation of nitric oxide levels in the blood under physiological stress conditions promotes modulation of skeletal muscle function, including contraction, respiration, mitochondrial biogenesis, and blood flow (Jones, 2013). This results in improved physical performance and delayed muscle fatigue.

Muscle fatigue can be directly quantified through blood lactate (Hernández-Cruz, et al. 2022). Lactate concentrations reflect the difference between the production and elimination rates of lactate (Theofilidis, et al. 2018; Brooks, 2018; Bartoloni, et al. 2024). This difference is mainly due to oxidation occurring in muscle tissue during exertion, representing a significant source of lactate elimination (Brooks, 2018). In this regard, some studies report significant positive correlations between anaerobic power production and plasma lactate (Temfemo, et al. 2011; Zagatto, et al. 2017). Of the studies included in the review, only two assessed blood lactate before and after exercise, demonstrating a reduction in lactate levels even with an increase in the number of repetitions, reflecting the difference in lactate production and elimination rates, as well as a positive effect on exercise time and condition for lactate during the leg press 1-RM test (Machek, et al. 2022). A study by Trepanowski et al. (2011) indicates a lower accumulation of blood lactate induced by exercise during betaine supplementation versus placebo supplementation. The authors suggest that the lower lactate accumulation, associated with betaine's cellular hydration maintenance effect, may have contributed to exercise performance (Trepanowski, et al. 2011). Arazi et al. (2022) speculate that one possible explanation for post-exercise blood lactate levels being lower may be the ability of betaine supplementation to improve mitochondrial respiration, resulting in increased lactate oxidation. This leads to lower blood lactate levels, even with increased anaerobic work (Arazi , et al. 2022). However, in the studies by Waldman et al. (2023) and Apicella et al. (2013), the authors point out a higher number of repetitions without changes in lactate concentrations after betaine supplementation (Apicella, et al. 2013; Arazi, et al. 2022).

The supplementation period and dose can significantly impact the effectiveness of the intervention. In this regard, most scientific evidence indicates that betaine's ergogenic resources are pronounced after 14 to 15 days of supplementation, with moderate doses (2.5g/day) administered to active populations (Arazi, et al. 2022; Gao, et al. 2019; Hoffman, et al. 2009; Waldman, et al. 2023; Yang, et al. 2020). However, Hoffman et al. (2009) stated in their study that improvements in physical performance can be perceived with betaine supplementation from 7 days onwards. Thus, the studies included in this review adopted the supplementation period and dose according to the literature to allow for an ergogenic effect of betaine.

There are some limitations to this systematic review, mainly in relation to the generalization and extrapolation of the results. This is due to the small number of studies in the indexed databases, the small sample size, methodological flaws including some concerns about the risk of bias presented by the studies and the very low level of certainty of the evidence. Another important point is that we did not consider gray literature, which can favor publication bias and a lower balanced view of the evidence. In addition, these studies did not directly investigate betaine concentration in plasma and muscle fatigue, so it is impossible to make affirmative and direct inferences about betaine supplementation and the improvement of muscle fatigue in exercisers. In other words, our confidence in the improvement of muscle fatigue through betaine supplementation is limited. Given the limitations of the study, the results presented should be treated as preliminary findings. Moreover, this systematic review presents inferences that are still speculative and indirect about muscle fatigue using the 1-RM test. As the 1-RM test is not only influenced by supplementation, there are other factors and physiological conditions that can influence the performance of a higher number of repetitions. Therefore, this is the first systematic review to investigate the influence of betaine supplementation on muscle fatigue during exercise.

This review has helped to identify the existing gaps in betaine supplementation and muscle fatigue during exercise, encouraging new studies to investigate mainly the effect of betaine supplementation directly on muscle fatigue. Thus, there is a need for studies that assess blood lactate levels; serum betaine concentrations; that better describe the nutritional control of participants to avoid diet-related biases; studies that investigate the specific mechanisms by which betaine impacts muscle fatigue; studies that describe the standardization of the methods used to perform the 1-RM test; and studies that use diverse populations with different age groups.

Conclusions

In conclusion, a betaine can be considered as a potential ergogenic resource for reducing muscle fatigue in exercisers, especially when it comes to increasing the number of repetitions in the 1-RM test in leg press or squat exercises. However, new randomized clinical trials are needed to directly investigate muscle fatigue. This will allow us to affirm the effects of betaine supplementation on muscle fatigue in exercisers with solid scientific evidence.

References

Aguinaga-Ontoso, I., Guillen-Aguinaga, S., Guillen-Aguinaga, L., Alas-Brun, R., & Guillen-Grima, F. (2023). Effects of Nutrition Interventions on Athletic Performance in Soccer Players: A Systematic Review. *Life*, *13*(6), 1271. https://doi.org/10.3390/life13061271

Apicella, J. M., Lee, E. C., Bailey, B. L., Saenz, C., Anderson, J. M., Craig, S. A. S., Kraemer, W. J., Volek, J. S., & Maresh, C. M. (2013). Betaine supplementation enhances anabolic endocrine and Akt signaling in response to acute bouts of exercise. *European Journal of Applied Physiology*, *113*(3), 793–802. https://doi.org/10.1007/s00421-012-2492-8

Arazi, H., Aboutalebi, S., Taati, B., Cholewa, J. M., & Candow, D. G. (2022). Effects of short-term betaine supplementation on muscle endurance and indices of endocrine function following acute high-intensity resistance exercise in young athletes. *Journal of the International Society of Sports Nutrition*, *19*(1), 1–16. https://doi.org/10.1080/15502783.2022.2041988

Arumugam, M. K., Paal, M. C., Donohue, T. M., Jr, Ganesan, M., Osna, N. A., & Kharbanda, K. K. (2021). Beneficial Effects of Betaine: A Comprehensive Review. *Biology*, *10*(6), 456. https://doi.org/10.3390/biology10060456

Ashtary-Larky, D., Bagheri, R., Ghanavati, M., Asbaghi, O., Tinsley, G. M., Mombaini, D., Kooti, W., Kashkooli, S., & Wong, A. (2022). Effects of betaine supplementation on cardiovascular markers: A systematic review and Meta-analysis. *Critical Reviews in Food Science and Nutrition*, *62*(23), 6516–6533. https://doi.org/10.1080/10408398.2021.1902938

Bartoloni, B., Mannelli, M., Gamberi, T., & Fiaschi, T. (2024). The Multiple Roles of Lactate in the Skeletal Muscle. *Cells*, *13*(14), 1177. https://doi.org/10.3390/cells13141177

Brooks, G. A. (2018). The Science and Translation of Lactate Shuttle Theory. *Cell Metabolism*, *27*(4), 757–785. https://doi.org/10.1016/j.cmet.2018.03.008

Cervantes Hernández, N., Hernandez Nájera, N., & Carrasco Legleu, C. E. (2022). Comparación de pruebas para medir la fatiga muscular en el entrenamiento de atletas hombres de CrossFit: una revisión sistemática (Comparison of tests to measure muscle fatigue in training of male CrossFit athlete: a systematic review). *Retos*, *43*, 923–930. https://doi.org/10.47197/retos.v43i0.89787

Cholewa, J. M., Hudson, A., Cicholski, T., Cervenka, A., Barreno, K., Broom, K., Barch, M., & Craig, S. A. S. (2018). The effects of chronic betaine supplementation on body composition and performance in collegiate females: a double-blind, randomized, placebo controlled trial. *Journal of the International Society of Sports Nutrition*, *15*(1). https://doi.org/10.1186/s12970-018-0243-x

Cholewa, J. M., Wyszczelska-Rokiel, M., Glowacki, R., Jakubowski, H., Matthews, T., Wood, R., Craig, S. A. S., & Paolone, V. (2013). Effects of betaine on body composition, performance, and homocysteine thiolactone. *Journal of the International Society of Sports Nutrition*, *10*, 1–12

Cholewa, J. M., Guimarães-Ferreira, L., & Zanchi, N. E. (2014). Effects of betaine on

performance and body composition: a review of recent findings and potential mechanisms. *Amino acids*, *46*(8), 1785–1793. https://doi.org/10.1007/s00726-014-1748-5

Cholewa, J. M., Newmire, D. E., Rossi, F. E., Guimarães-Ferreira, L., & Zanchi, N. E.

(2019). An overview of betaine supplementation, sports performance, and body composition. Nutrition and Enhanced Sports Performance, 691-706.

Dias, R. M. R., Avelar, A., Menêses, A. L., Salvador, E. P., Silva, D. R. P. da, & Cyrino, E. S. (2013). Segurança, reprodutibilidade, fatores intervenientes e aplicabilidade de testes de 1-RM. *Motriz: Revista de Educação Física*, *19*, 231–242

Dobrijević, D., Pastor, K., Nastić, N., Özogul, F., Krulj, J., Kokić, B., Bartkiene, E., Rocha, J. M., & Kojić, J. (2023). Betaine as a Functional Ingredient: Metabolism, Health-Promoting Attributes, Food Sources, Applications and Analysis Methods. *Molecules (Basel, Switzerland)*, *28*(12), 4824. https://doi.org/10.3390/molecules28124824

Freitas, H. R., Barbosa, M., & Ramos, T. da S. (2015). O papel da suplementação de betaína na atividade física: uma revisão sistemática. *Nutrire*, *40*(2), 246–260. https://doi.org/10.4322/2316-7874.50914

Gao, X., Zhang, H., Guo, X., Li, K., Li, S., & Li, D. (2019). Effect of Betaine on Reducing Body Fat—A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients*, *11*(10), 2480. https://doi.org/10.3390/nu11102480

Hernández-Cruz, G., Estrada-Meneses, E. F., Ramos-Jiménez, A., Rangel-Colmenero, B. R., Reynoso-Sánchez, L. F., Miranda-Mendoza, J., & Quezada-Chacón, J. T. (2022). Relación entre el tipo de ejercicio físico y la fatiga cuantificada mediante VFC, CK y el lactato en sangre (Relationship between physical exercise type and fatigue quantified through HRV, CK, and blood lactate). *Retos*, *44*, 176–182. https://doi.org/10.47197/retos.v44i0.89479

Higgins, J. P. T., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J., & Welch, V. A. (2022). *Manual Cochrane para revisões sistemáticas de intervenções versão 6.3*. Cochrane

Hoffman, J. R., Ratamess, N. A., Kang, J., Gonzalez, A. M., Beller, N. A., & Craig, S. A. (2011). Effect of 15 days of betaine ingestion on concentric and eccentric force outputs during isokinetic exercise. *Journal of Strength and Conditioning Research*, *25*(8), 2235–2241. https://doi.org/10.1519/JSC.0b013e3182162530

Hoffman, J. R., Ratamess, N. A., Kang, J., Rashti, S. L., & Faigenbaum, A. D. (2009). Effect of betaine supplementation on power performance and fatigue. *Journal of the International Society of Sports Nutrition*, *6*, 7. https://doi.org/10.1186/1550-2783-6-7

Ihsan, fadli, Kozina, Z. ., Sukendro, S., Nasrulloh, A. ., Arzhan Hidayat, R. ., & Perdana, satya . (2024). Estrategias nutricionales para una recuperación rápida en el deporte: una revisión de la literatura (Nutritional Strategies for Rapid Recovery in Sport: A Literature Review). *Retos*, *57*, 153–164. https://doi.org/10.47197/retos.v57.105622

Ismaeel, A. (2017). Effects of Betaine Supplementation on Muscle Strength and Power: A Systematic Review. *Journal of Strength and Conditioning Research*, *31*(8), 2338–2346. https://doi.org/10.1519/JSC.0000000000001959

Jones, A. M. (2013). Dietary nitrate: the new magic bullet. *Sports Sci Exch*, *26*(110), 1–5.

Kaur, S., Sharma, N., Vyas, M., Mahajan, R., Satija, S., Mehta, M., & Khurana, N. (2019). A review on pharmacological activities of betaine. *Plant Arch*, *19*, 1021-1034.

Lee, B.-A., Lee, S.-H., & Oh, D.-J. (2013). Effects of peripheral injury in athletes with long-term-exercise participation in modern pentathlons. *Journal of Exercise Rehabilitation*, *9*(5), 481.

Machek, S. B., Harris, D. R., Zawieja, E. E., Heileson, J. L., Wilburn, D. T., Radziejewska, A., Chmurzynska, A., Cholewa, J. M., & Willoughby, D. S. (2022). The Impacts of Combined Blood Flow Restriction Training and Betaine Supplementation on One-Leg Press Muscular Endurance, Exercise-Associated Lactate Concentrations, Serum Metabolic Biomarkers, and Hypoxia-Inducible Factor-1α Gene Expression. *Nutrients*, *14*(23), 5040. https://doi.org/10.3390/nu14235040

Nobari, H., Cholewa, J. M., Castillo-Rodríguez, A., Kargarfard, M., & Pérez-Gómez, J. (2021). Effects of chronic betaine supplementation on performance in professional young soccer players during a competitive season: a double blind, randomized, placebo-controlled trial. *Journal of the International Society of Sports Nutrition*, *18*(1). https://doi.org/10.1186/s12970-021-00464-y

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, n71. https://doi.org/10.1136/bmj.n71

Pryor, J. L., Wolf, S. T., Sforzo, G., & Swensen, T. (2017). The effect of betaine on nitrate and cardiovascular response to exercise. *International Journal of Exercise Science*, *10*(4), 550

Schünemann, H., Brożek, J., Guyatt, G., & Oxman, A. (2013). *The GRADE handbook*. Cochrane Collaboration London, UK

Temfemo, A., Carling, C., & Ahmaidi, S. (2011). Relationship between Power Output, Lactate, Skin Temperature, and Muscle Activity During Brief Repeated Exercises With Increasing Intensity. *Journal of Strength and Conditioning Research*, *25*(4), 915–921. https://doi.org/10.1519/JSC.0b013e3181d680f0

Theofilidis, G., Bogdanis, G. C., Koutedakis, Y., & Karatzaferi, C. (2018). Monitoring Exercise-Induced Muscle Fatigue and Adaptations: Making Sense of Popular or Emerging Indices and Biomarkers. *Sports (Basel, Switzerland)*, *6*(4), 153. https://doi.org/10.3390/sports6040153.

Trepanowski, J. F., Farney, T. M., McCarthy, C. G., Schilling, B. K., Craig, S. A., & Bloomer, R. J. (2011). The Effects of Chronic Betaine Supplementation on Exercise Performance, Skeletal Muscle Oxygen Saturation and Associated Biochemical Parameters in Resistance Trained Men. *Journal of Strength and Conditioning Research*, *25*(12), 3461–3471. https://doi.org/10.1519/JSC.0b013e318217d48d

Verschueren, J., Tassignon, B., Verhagen, E., & Meeusen, R. (2021). The interaction of acute physical fatigue with three traditional functional performance tests and the reactive balance test. *Physical Therapy in Sport*, *49*, 188–195

Waldman, H. S., Bryant, A. R., & McAllister, M. J. (2023). Effects of Betaine Supplementation on Markers of Metabolic Flexibility, Body Composition, and Anaerobic Performance in Active College-Age Females. *Journal of Dietary Supplements*, *20*(1), 89–105. https://doi.org/10.1080/19390211.2021.1973644

Willingham, B. D., Ragland, T. J., & Ormsbee, M. J. (2020). Betaine Supplementation May Improve Heat Tolerance: Potential Mechanisms in Humans. *Nutrients*, *12*(10), 2939. https://doi.org/10.3390/nu12102939

Xu, J., Nie, Z., Qiu, X., Zhang, J., & Han, S. (2023). Effects of betaine supplementation on inflammatory markers: a systematic review and meta-analysis of randomised controlled trials. *International Journal of Food Sciences and Nutrition*, *74*(7), 721–729. https://doi.org/10.1080/09637486.2023.2257906

Yang, M.-T., Lee, X.-X., Huang, B.-H., Chien, L.-H., Wang, C.-C., & Chan, K.-H. (2020). Effects of Two-Week Betaine Supplementation on Apoptosis, Oxidative Stress, and Aerobic Capacity after Exhaustive Endurance Exercise. *Antioxidants*, *9*(12), 1189. https://doi.org/10.3390/antiox9121189

Zagatto, A. M., Miyagi, W. E., Sousa, F. A. de B., & Gobatto, C. A. (2017). Relationship between anaerobic capacity estimated using a single effort and 30-s tethered running outcomes. *PLOS ONE*, *12*(2), e0172032. https://doi.org/10.1371/journal.pone.0172032

Zawieja, E. E., Zawieja, B., & Chmurzynska, A. (2021). Betaine Supplementation Moderately Increases Total Cholesterol Levels: A Systematic Review and Meta-Analysis. *Journal of Dietary Supplements*, *18*(1), 105–117. https://doi.org/10.1080/19390211.2019.1

Datos de los/as autores/as y traductor/a:

Larissa Torres Fernandes	farm.larissatorresfernandes@gmail.com	Autor/a
Deysimara de Cássia Santos	deysimara16@gmail.com	Autor/a
Lucas Teixeira Marques	lucastmarques@yahoo.com	Autor/a
Danielle Negri Ferreira Neves	danielle.negri@ufjf.br	Autor/a
Otávio de Toledo Nóbrega	otnobrega@gmail.com	Autor/a
Mariana de Melo Cazal	mariana.cazal@ufjf.br	Autor/a
Carina Carvalho Silvestre	silvestre.carina@ufjf.br	Autor/a
Ciro José Brito	cirojbrito@gmail.com	Autor/a
Christopher James Quinn	englishconsultingbrazil@gmail.com	Traductor/a