

TITLE

The effects of oral taurine dose and supplementation period on endurance exercise performance in humans: a meta-analysis

AUTHOR

Waldron, Mark; Tallent, Jamie; Patterson, Stephen D.; et al.

JOURNAL

Sports Medicine

DATE DEPOSITED

9 March 2018

This version available at

<http://research.stmarys.ac.uk/id/eprint/2159/>

COPYRIGHT AND REUSE

Open Research Archive makes this work available, in accordance with publisher policies, for research purposes.

VERSIONS

The version presented here may differ from the published version. For citation purposes, please consult the published version for pagination, volume/issue and date of publication.

Title: The effects of oral taurine dose and supplementation period on endurance exercise performance in humans: a meta-analysis

Running title: Taurine and endurance performance

Authors: ^{1,2*}Dr. Mark Waldron; ¹Dr. Stephen David Patterson; ¹Dr. Jamie Tallent; ¹Dr. Owen Jeffries.

Affiliation:

¹School of Health, Sport and Applied Sciences, St Mary's University, London, TW1 4SX, UK.

²School of Science and Technology, University of New England, Armidale, NSW 2350, Australia.

Corresponding Author:

Dr. Mark Waldron

Tel: +44 2082408246

email: mark.waldron@stmarys.ac.uk

Key points:

- A meta-analysis was conducted to evaluate the effects of isolated oral taurine ingestion on endurance performance and to assess the contribution of i) the dose and ii) the supplementation period to the ergogenic effect.
- Human endurance performance can be improved by a '*small*' magnitude after orally ingesting a single dose of taurine in varying amounts (1 g - 6 g).
- Further research is needed to establish the effects of the oral taurine dose on endurance performance and which populations would benefit most from its supplementation.

Abstract

Background Taurine is central to many physiological processes, some of which are augmented by exogenous supply and have the potential to facilitate endurance performance; however, its independent effects on performance have not been systematically analysed.

Objective To evaluate the effects of isolated oral taurine ingestion on endurance performance and to assess the contribution of i) the dose and ii) the supplementation period to the ergogenic effect.

Methods A search was performed using various databases in September 2017. The studies were screened using search criteria for eligibility. 10 peer-reviewed articles were identified for inclusion. A sub-analysis of time-to-exhaustion (TTE) trials ($n = 7$) was also performed. The effects of i) dose and ii) the acute (single dose) or chronic (> 1 day) supplementation periods were assessed using meta-regression. The doses of taurine ranged from 1-6 g/day and were provided in single doses and for up to two weeks among a range of subjects.

Results Taurine ingestion improved overall endurance performance (Hedges' $g = 0.40$, 95% CI = 0.12 to 0.67, $P = 0.004$), which was similar in TTE trials (Hedges' $g = 0.43$, 95% CI = 0.12 to 0.75, $P = 0.007$). There were no differences between acute or chronic supplementation for the full sample ($P = 0.897$) or the TTE group ($P = 0.896$). The dose of taurine did not moderate its effect on endurance performance ($P > 0.05$).

Conclusion Human endurance performance can be improved by orally ingesting a single dose of taurine in varying amounts (1 g - 6 g).

1. Introduction

Taurine, a sulfur-containing amino acid, is one of the primary ingredients in popular energy drinks [1], as well as most meats and seafood [2]. Taurine is the most abundant free amino acid in mammalian tissue, accounting for 50-60 % of the free amino acid pool [3] and is available to facilitate a variety of biological processes that can support endurance exercise performance. For example, once inside the muscle, taurine assists with sarcoplasmic reticulum Ca^{2+} handling [4,5], with improvements in muscle performance attributed to taurine-facilitated Ca^{2+} handling of both cardiac and skeletal myocytes [3]. An anti-oxidative role has also been ascribed to taurine, which facilitates taurine's stabilising effects in the mitochondrial matrix, thus improving the efficiency of ATP turnover in the muscle cell [6]. Indeed, prevention of taurine uptake in taurine transporter (TauT) knockout mice significantly reduces time to exhaustion [7]. It is thought that one, or a combination of these mechanisms, can enhance endurance performance by increasing the capacity of human muscle over prolonged periods. However, despite the known physiological effects of taurine, less is understood about the efficacy of taurine supplementation for enhancing endurance performance in human participants.

The lack of historical interest in isolated taurine supplementation and human performance is somewhat surprising, given its inclusion in popular energy drinks, and its purported contribution to their ergogenic effects. Indeed, a recent meta-analysis demonstrated that the dosage of taurine, as opposed to caffeine, when co-ingested in energy drinks explained their effects on different types of exercise performance [8]. However, the authors did not include investigations using isolated taurine supplementation, focussing on co-ingested energy drinks alone. This is problematic because this does not remove the potential *in-vivo* interaction

between taurine and other ingredients, such as caffeine, which have been identified in animal models [9]. Furthermore, this approach overlooks research that has investigated higher isolated doses of taurine (4-6 g) compared to that of typical of energy drinks (1-2 g). This is important to understand since individual studies have reported that higher doses (6 g/day for seven days) can increase markers of aerobic performance, such as maximal oxygen uptake ($\dot{V}O_{2\max}$) [10]. Therefore, this study systematically reviewed and meta-analysed all peer-reviewed studies that have provided human subjects with isolated taurine. The aim of the meta-analysis was to determine the effects on endurance performance and the importance of the dose and supplementation period to the ergogenic effects.

2. Methods

2.1 Search strategy

All literature that investigated the effect of taurine on endurance performance was searched and obtained using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines, with a pre-determined search strategy [11]. There was no limit on the status or language of the publication and the final searches were performed in PubMed, Science Direct and SPORTDiscus (EBSCO) on 01.09.17. The search terms used were ‘taurine AND exercise OR taurine AND performance OR ‘taurine AND ‘aerobic’.

2.2 Study selection

Once all of the articles were identified, two reviewers screened the titles and abstracts for inclusion, removing any duplicates. ‘Other sources’ were also identified, such as hard-copies of taurine-related research from conferences or industry studies, all of which were excluded.

The reference lists of the initial papers were reviewed independently by two authors (MW and OJ), which did not reveal any additional papers. The remaining articles were then assessed by MW and OJ against the initial search criteria. To be included in this analysis, the studies must have: i) administered oral taurine to humans of any age, without co-ingestion with other supplements or drugs and ii) evaluate exhaustive protocols > 3-min. Of the remaining papers, some were further removed for the reasons outlined in Figure 1. In part, this included papers that had co-ingested taurine with other supplements, such as caffeine or polyphenols. As endurance performance encompasses a variety of activities, we limited this review to three forms of exhaustive exercise modality. These were: time-to-exhaustion (TTE) trials; time-trials (TT) and power output during closed-loop tasks. Overall effects (i.e. combination of all modalities) and effects of the TTE modality were considered for the eventual analysis. Tests, such as the Bruce protocol or adapted versions and graded exercise tests to exhaustion, were considered as TTE trials based on the commonly reported metric of test duration. Time-trials were converted to speed (m/s) for analysis. We removed any forms of exercise that were non-exhaustive or performed for less than 3-min, based on the fundamental understanding that exercise to exhaustion over this period of time will require predominant contributions from aerobic metabolism, irrespective of the intensity [12].

*******Insert Figure 1 near here*******

2.3 Data extraction and quality assessment

Data were extracted independently by two authors (MW and OJ) and entered into a custom excel spreadsheet. Collected data included: i) characteristics of the sample (sex, health status, age, training status); ii) study design; iii) taurine dose; iv) duration of supplementation; v)

performance outcomes; and vi) bias. Risk of bias was assessed by two authors (MW and OJ) according to Cochrane collaboration guidelines [13]. Where details of the study were unclear, the authors were contacted for information of confirmation of the method. Publication bias was also accounted for by plotting the effect size as a percentage against the standard error (SE) for each study (Figure 3).

2.4 Statistical analysis

Data analyses were performed by one author (MW). Raw data were extracted in the form of a mean, SD and sample size for the meta-analysis. Publicly available software (WebPlotDigitizer, Version 3.12) was used to extrapolate any unreported values from figures to raw mean and SD data. The overall meta-analysis of endurance performance was performed in Statistical Package for the Social Sciences (SPSS, Version 22, IBM Corp., Armonk, NY, USA), including all 10 studies. The effect sizes and inverse-variance weights were calculated according to published equations Lipsey and Wilson [23]. Data were analysed with a fixed-effects model, with heterogeneity assessed using the I^2 statistic. Hedges' g and 95% confidence intervals were used to express the standardised mean differences between taurine and placebo groups across studies. To assess the effects of taurine on TTE, a second meta-analysis was performed on 7 of the studies. Meta-regressions were performed on both the full ($n = 10$) and partial data sets ($n = 7$) using the continuous variable of dose, referring to the daily dose (g/day) of taurine provided to the participants in the study, as the moderating variable. A sub-group analysis was also performed on both datasets based on the type of supplementation regime (acute or chronic). The magnitudes of the effects were assessed based on the thresholds of: 0.2, 0.5 and 0.8 for small, moderate and large, respectively [24]. Statistical significance was set at $P < 0.05$ for all analyses.

3. Results

3.1 Study selection

The initial searches retrieved 3431 articles, plus an additional 24 studies through other sources. These were reduced to 2799 after removal of duplicates. Further screening left 122 full-text articles over. Searches of their reference lists did not reveal any missing papers. On the basis of the inclusion criteria, 112 articles were removed from the 122. Therefore, 10 papers were included in the final meta-analysis and 7 in the TTE analysis (see Figure 1). Effects for the TT and study measuring power output are presented for demonstrative purposes.

3.2 Study characteristics

The characteristics of the 10 included studies are summarized in Table 1. The studies included a total of 116 participants, comprising both males and females of varying age, training and health status. Seven of the studies were cross-over designs, whilst three were independent group designs. The range of taurine that was orally administered across studies was 1 g/day to 6 g/day and this was well-balanced between acute (single dose; $n = 6$) and chronic (multiple doses > 1 day; $n = 4$) supplementation regimes. There were no adverse events noted in any of the studies.

*****Insert Table I near here*****

3.3 Overall meta-analysis

The results of the overall meta-analysis are reported in Figure 2. Overall, there was a small-to-moderate improvement in endurance performance with taurine compared to placebo (Hedges' $g = 0.40$, 95% CI = 0.12 to 0.67, $P = 0.004$). The I^2 statistic demonstrated 29.4% heterogeneity. The meta-analysis of the TTE group ($n = 7$) also showed a small-to-moderate effect of taurine on performance (Hedges' $g = 0.43$, 95% CI = 0.12 to 0.75, $P = 0.007$), with 0% heterogeneity (I^2).

3.4 Sub-group analysis

The sub-group analysis demonstrated no difference ($P = 0.897$) in the effects of taurine on endurance performance between acute (Hedges' $g = 0.39$, 95% CI = 0.13 to 0.64, $P = 0.003$) and chronic (Hedges' $g = 0.42$, 95% CI = 0.07 to 0.75, $P = 0.016$) supplementation for the full sample. The same was true for the TTE group, with no differences ($P = 0.896$) in the effects of taurine on endurance performance between acute (Hedges' $g = 0.45$, 95% CI = 0.11 to 0.77, $P = 0.007$) and chronic (Hedges' $g = 0.42$, 95% CI = 0.07 to 0.75, $P = 0.016$) supplementation.

3.5 Meta-regression

The dose of taurine provided to participants across all ten studies ($b = 0.053$, $P = 0.431$) or the TTE group ($b = 0.0013$, $P = 0.98$) did not moderate the outcome of taurine on endurance performance.

*******Insert Figure 2 near here*******

3.6 Risk of bias

The studies included had a generally low or unclear risk of bias, with one study [10] not counterbalancing or stating any blinding procedures. Similarly, allocation concealment and blinding were unclear in another study [14]. Figure 3 shows that publication bias analysis (standard mean differences and standard error relationship) was symmetrical, with no outliers.

*******Insert Figure 3 near here*******

4. Discussion

The main findings of this analysis were that oral taurine ingestion improved endurance performance by a '*small*' amount (Hedges g 0.4), with marginally larger effects (Hedges g 0.43) found for TTE trials (Figure 2). The secondary sub-group analysis did not demonstrate differences between acute and chronic supplementation regimes, indicating that taurine's effects were not determined by the duration of the supplementation period. Furthermore, there was no influence of the taurine dose on its performance effects, suggesting that doses as low as 1 g or as high as 6 g can be effective in increasing endurance performance. Collectively, these results indicate that endurance performance can be improved to a similar magnitude (*small*) after providing 1 g of taurine in a single oral dose or 6 g for up to two weeks. Despite Hedges' g indicating a *small* effect of taurine on endurance exercise performance, these changes could confer practically or clinically-relevant effects. For example, training-induced improvements in Bruce protocol exercise duration are typically

'large' among heart failure patients (ES ~ 1.1; [25-26]). Increases in time to exhaustion among adults of mixed training status are also 'small' after ingestion other ergogenic aids, such as caffeine (~ 0.4; [27]). The small effect sizes noted herein, therefore, represent a substantial portion of the typical enhancements observed in endurance performance after months of training or are equally ergogenic as caffeine.

The current meta-analysis was based on studies providing isolated doses of taurine and not those co-ingesting with other supplements. Interestingly, our analysis confirms the suggestions of a previous meta-analysis [8], where taurine was assumed to explain the ergogenic effects of energy drinks. Only one study from the current analysis was shared between the two meta-analyses, owing to the number of experimental groups included by Kammerer et al. [18]. It was important to conduct our analysis because it was possible that taurine's effects were dependent on the *in-vivo* interaction with caffeine [9], which could not be ruled out unless studies providing isolated doses of caffeine were independently analysed. Therefore, the combined results of this study and the previous meta-analysis [8], have systematically identified that the potential interactions between caffeine and taurine are not necessary to improve human endurance performance.

The current meta-regression analysis identified that the dose of taurine did not affect the outcome on endurance performance. This is important for potential users of taurine, who are unlikely to incur any additional benefit from consuming large doses. Indeed, coupling this with the finding that single doses were equally effective as chronic loading periods, means that improvements in endurance performance can be achieved without long-term ingestion or the requirement for doses that are closer to the most tolerable upper limit of 10 g/day [28].

Whilst this should be treated as preliminary evidence ($n = 10$ studies), potential taurine users might consider this when designing their supplementation plan for endurance exercise, which will be more cost and time-effective. Further research examining the effects of different doses of taurine on endurance performance among the same participants would add to the current body of literature and corroborate these findings.

The reasons for improvements in endurance performance after oral taurine supplementation are remain speculative; however, recent studies have revealed that acute supplementation can increase lipolysis and reduce the contribution from glycolytic metabolism, thereby altering the fuel utilisation and metabolic efficiency of exercise [29]. Others ascribe the effects of taurine to an anti-oxidative role, which facilitates taurine's stabilising effects in the mitochondrial matrix, thus improving the efficiency of ATP turnover in the muscle cell [6]. However, these effects require corroboration among human participants. Whilst the mechanisms of taurine's action are likely to become clearer in the near future, it is likely that a combination of factors explain its '*small*' effects of endurance performance.

This analysis is not without limitations. Firstly, it is noted that our study selection incorporated findings from both healthy volunteers and heart failure patients, of varying ages. It is possible that the health or training status of the subjects would influence the effects of taurine; however, there was not sufficient data to investigate this hypothesis. As identified in Figure 2, the largest effect sizes were shared between healthy and non-healthy subjects, inferring that this would not have affected the outcome of the analysis. It is, therefore, possible that taurine supplementation can facilitate endurance performance, irrespective of the participants' health status. The number of studies included in this analysis was, however,

quite small, yet homogenous, as indicated by the null effects of the heterogeneity testing. However, it would be useful for more studies to be performed to investigate the effects of taurine on endurance performance, particularly among subjects from different populations. The lower representation of TTs in the overall analysis and the contrast in findings between these studies [17,20] also highlights the need for further research using this model of performance.

5. Conclusion

The implications of this study are that human endurance performance can be improved by orally ingesting taurine of varying doses (1 g - 6 g), with as little as a single acute dose. This can be viewed as a relatively simple and inexpensive way of increasing endurance capacity to improve athletic performance or clinical outcomes. The population that would most benefit from this supplement remains unclear, given the range of subjects used across the 10 selected articles, and future research should also investigate the effects of oral taurine supplementation across participants of varying age, health and training status.

Acknowledgements

No sources of funding were received in the preparation of this article and the authors, Mark Waldron, Stephen Patterson, Jamie Tallent and Owen Jeffries, have no conflicts of interest directly relevant to its contents.

References

1. McLellan TM, Lieberman HR. Do energy drinks contain active components other than caffeine?. *Nut Res.* 2012;70:730-744.
2. Stapleton PP, Charles RP, Redmond HP, Bouchier-Hayes DJ. Taurine and human nutrition. *Clin Nutr.* 1997;16:103-108.
3. Huxtable JR. Physiological actions of taurine. *Physiol Rev.* 1992;72:101-163.
4. Hamilton EJ, Berg HM, Easton CJ, Bakker AJ. The effect of taurine depletion on the contractile properties and fatigue in fast-twitch skeletal muscle of the mouse. *Amino Acids.* 2006;31:273-278.
5. Dutka TL, Lamboley CR, Murphy RM, Lamb GD. Acute effects of taurine on sarcoplasmic reticulum Ca²⁺ accumulation and contractility in human type I and type II skeletal muscle fibers. *J Appl Physiol.* 2014;117:797-805.
6. Hansen SH, Andersen ML, Birkedal H, Cornett C, Wibrand F. The important role of taurine in oxidative metabolism. *Adv Exp Med Biol.* 2006;583:129-135.
7. Ito T, Yoshikawa N, Schaffer SW, Azuma J. Tissue taurine depletion alters metabolic response to exercise and reduces running capacity in mice. *J Amino Acids* 2014. doi: 10.1155/2014/964680
8. Souza DB, Del Coso J, Casonatto J, Polito MD. Acute effects of caffeine-containing energy drinks on physical performance: a systematic review and meta-analysis. *Eur J Nutr.* 2017;56:13-27.
9. Bakker AJ, Berg HM. Effect of taurine on sarcoplasmic reticulum function and force in skinned fast-twitch skeletal muscle fibres of the rat. *J Physiol.* 2002;538:185-194.
10. Zhang, M, Izumi I, Kagamimori S, Sokejima S, Yamagami T, Liu Z, Qi B. Role of taurine supplementation to prevent exercise-induced oxidative stress in healthy young men. *Amino Acids.* 2004;26:203-207.
11. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart LA. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Systematic Reviews*, 4:1
<http://www.systematicreviewsjournal.com/content/4/1/1>
12. Baker JS, McCormick MC, Robergs RA. Interaction among Skeletal Muscle Metabolic Energy Systems during Intense Exercise. *J Nutr Metab.* 2010. doi: 10.1155/2010/905612.
13. Higgins JP, Altman DG. Assessing Risk of Bias in Included Studies, in *Cochrane Handbook for Systematic Reviews of Interventions: Cochrane Book Series* (eds JP Higgins, S Green), John Wiley & Sons, Ltd, Chichester, UK, 2008.
14. Lee HM, Paik IY, Park TS. Effects of dietary supplementation of taurine, carnitine or glutamine on endurance exercise performance and fatigue parameters in athletes. *Korean J Nutr.* 2003;36:711-719.
15. Rutherford JA, Spriet LL, Stellingwerff T. The effect of acute taurine ingestion on endurance performance and metabolism in well-trained cyclists. *Int J Sport Nutr Exerc Metab.* 2010; 20:322-329.
16. Beyranvand MR, Khalafi MK, Roshan VD, Choobineh S, Parsa SA, Piranfar MA. Effect of taurine supplementation on exercise capacity of patients with heart failure. *J Cardiol.* 57:333-337.
17. Balshaw TG, Bampouras TM, Barry TJ, Sparks SA. The effect of acute taurine ingestion on 3-km running performance in trained middle-distance runners. *Amino Acids.* 2013;44:555-561.
18. Kammerer M, Jaramillo JA, Garcia A, Calderon JC, Valbuena LH. Effects of energy drink major bioactive compounds on the performance of young adults in fitness and cognitive tests: a randomized controlled trial. *J Int Soc Sport Nutr.* 2014; 11:44.

19. Milioni F, Malta ES, Rocha LG, Mesquita CA, Zagatto EC. Acute administration of high doses of taurine does not substantially improve high-intensity running performance and the effect on maximal accumulated oxygen deficit is unclear. *Appl Physiol Nutr Metab*. 2016; 41:498-503.
20. Ward R, Bridge CA, McNaughton LR, Sparks SA. The effect of acute taurine ingestion on 4-km time trial performance in trained cyclists. *Amino Acids*. 2016; 48:2581-2587.
21. Ahmadian M, Roshan VD, Ashourpore E. Taurine supplementation improves functional capacity, myocardial oxygen consumption, and electrical activity in heart failure. *J Diet Supp*. 2017;14:422-432.
22. Warnock R, Jeffries O, Patterson S, Waldron M. The effects of caffeine, taurine or caffeine-
taurine co-ingestion on repeat-sprint cycling performance and physiological responses. *Int J Sports Physiol Perform*. 2017;24:1-24.
23. Lipsey MW, Wilson DB. *Practical meta-analysis*. Sage: London, UK, 2001.
24. Rosenthal R, Rosnow RL *Essentials of Behavioral Research: Methods and Data Analysis*. New York: McGraw-Hill, 1984.
25. Arad M, Adler Y, Koren-Morag N, Natanzon S, Sela BA, Ben DI, Shechter M, Schwammenthal E, Freimark D. Exercise training in advanced heart failure patients: discordance between improved exercise tolerance and unchanged NT-proBNP levels. *Int J Cardiol*. 2008;126:114-119.
26. Baraas F, Rilantono L, Diniharini S, Kurniawan I, Christian R, Kusmana D. Effect of short-term low-intensity exercise training on association of oxygen free radicals and nitric oxide production in patients with acute myocardial infarction. *Int J Angiol*. 2013;22:159-164.
27. Doherty M, Smith PM. Effects of caffeine ingestion on exercise testing: a meta-analysis. *Int J Sport Nutr Exerc Metab*. 2004;14(6):626-46.
28. Shao A, Hathcock JN. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. *Regul Toxicol Pharmacol*. 2008;50:376-99
29. De Carvalho FG, Barbieri RA, Carvalho MB, Dato CC, Campos EZ, Gobbi RB, Papoti M, Silva ASR, de Freitas EC. Taurine supplementation can increase lipolysis and affect the contribution from energy systems during the front crawl maximal effort. *Amino Acids*. 2018;50(1):189-198.

Figure legends

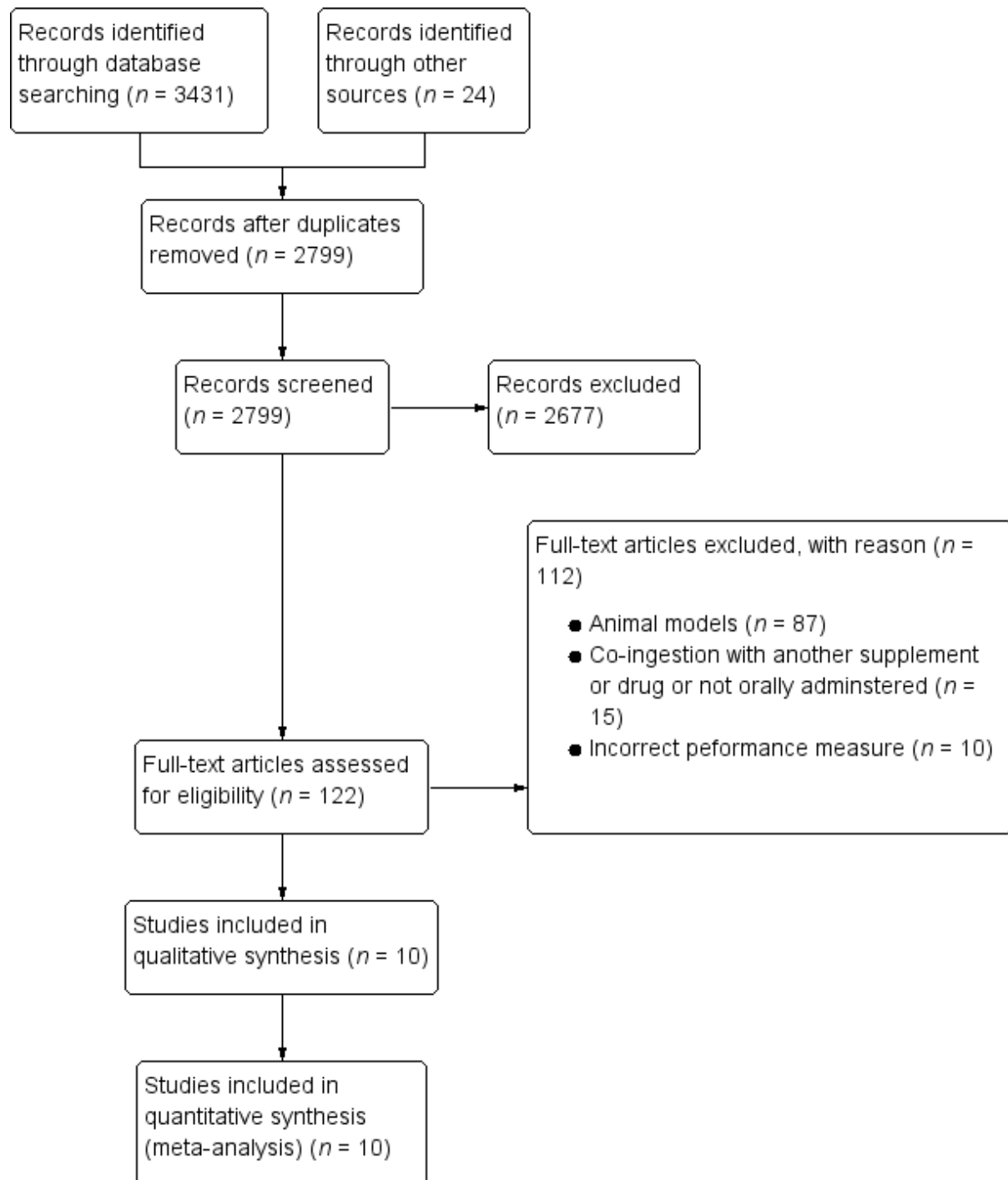


Figure 1. The process and reasons for study selection ($n = 10$) in the final meta-analysis.

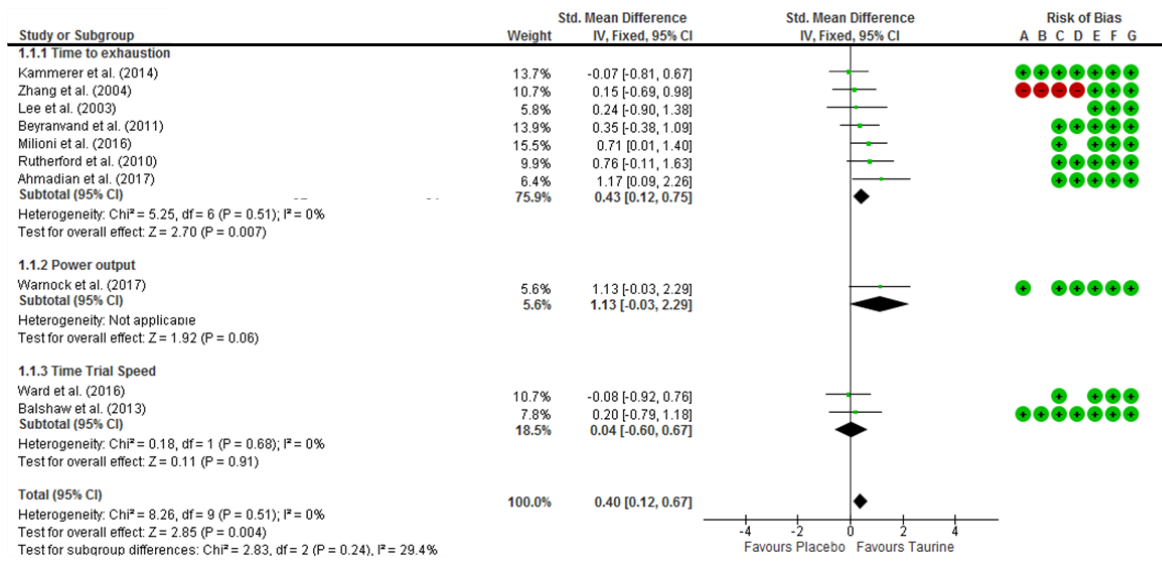


Figure 2. Effect of taurine on endurance performance. Green circles = low risk of bias; red circles = high risk of bias; blank spaces = unclear risk of bias.

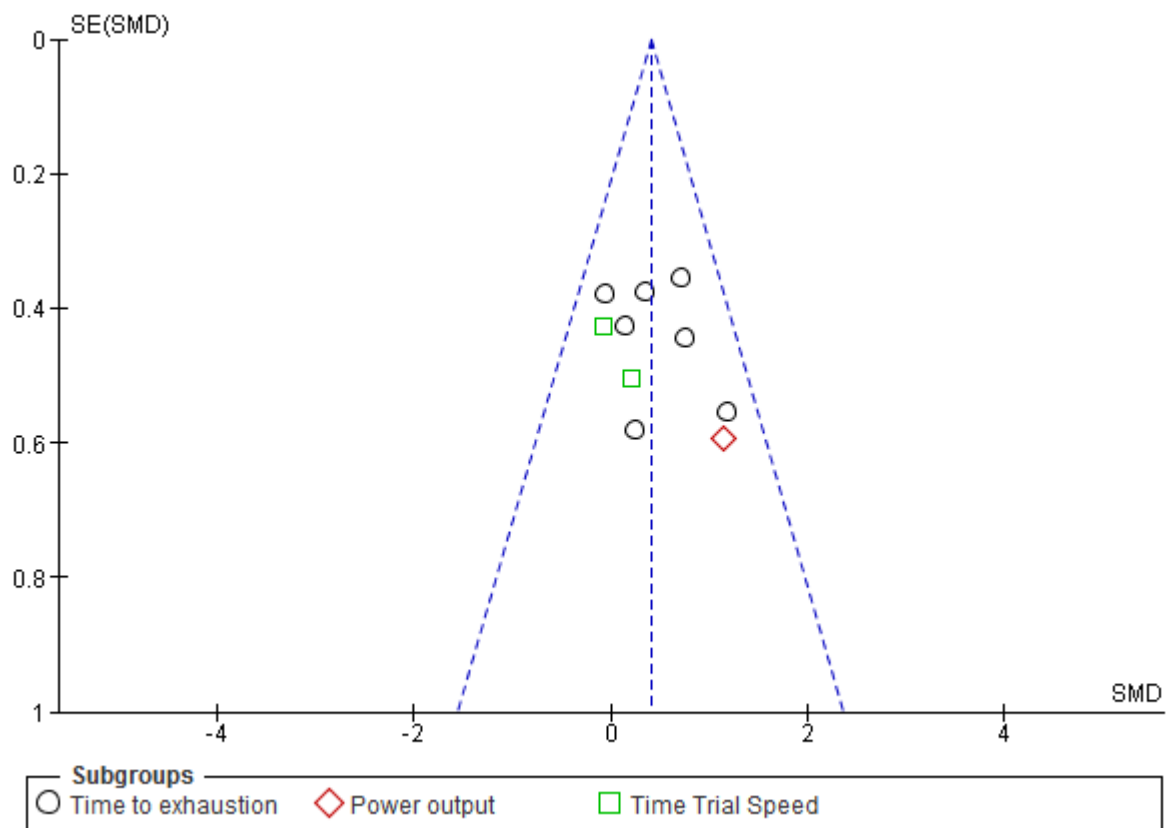


Figure 3. Risk of bias, determined by the relationship between standard mean difference (SMD) and standard error (SE) of the SMD.

Table I. Summary of studies included in the meta-analysis for the effects of taurine on endurance performance ($n = 10$).

Study	Design	Sample	Dose	Duration	Performance type	Outcome
Lee et al. (2003)[14]	Independent groups, single-blind, randomised	Healthy, male college students ($n = 6$ per group). Age 20.2 ± 0.6 vs. 21.2 ± 0.4 years.	4 g /day	14 days	Treadmill running @ 75% $\dot{V}O_{2max}$	8.1% \uparrow in TTE
Zhang et al. (2004)[10]	Crossover (not counterbalanced)	Healthy sedentary males ($n = 11$). Age range 18 - 20 years.	6 g (3 x 2 g/day)	7 days	$\dot{V}O_{2max}$ test cycling	2.6% \uparrow exercise time; 2% \uparrow $\dot{V}O_{2max}$; 3.8% \uparrow power output.
Rutherford et al. (2010)[15]	Crossover, double-blind, randomised	Healthy, endurance-trained males ($n = 11$). Age 27.2 ± 1.5 years.	1.66 g (single dose)	1-h pre-exercise	90-min cycle @ $\sim 65\%$ $\dot{V}O_{2max}$, followed by TT @ 5 kJ/km body mass	NS 4.6% \uparrow in taurine vs. placebo
Beyranvand et al. (2011)[16]	Independent groups, double-blind, randomised	Elderly heart failure patients male and female ($n = 15$ vs. 14). Age 60.9 ± 5.7 vs. 60.3 ± 7.5 years.	1.5 g (3 x 500 mg/day)	14 days	Bruce protocol or adapted Bruce protocol	17.5% \uparrow exercise time in combination of Bruce protocols
Balshaw et al. (2013)[17]	Crossover, double-blind, randomised	Well-trained male middle distance runners ($n = 8$). Age 19.9 ± 1.2 years.	1 g (single dose)	2-h pre-exercise	3 km treadmill TT	1.7% \uparrow TT performance
Kammerer et al. (2014)[18]	Crossover, double-blind, randomised	Healthy male soldiers ($n = 14$). Age 20 ± 1 years	1 g (single dose)	45-min pre-exercise	$\dot{V}O_{2max}$ test treadmill	NS \downarrow 1.2% $\dot{V}O_{2max}$ or 0.6% \downarrow TTE in $\dot{V}O_{2max}$ test
Milioni et al. (2016)[19]	Blinded, crossover, randomised	Healthy, recreationally-trained males ($n = 17$). Age 25 ± 6 years.	6 g (single dose)	1.5-h pre-exercise	TTE @ 110% $\dot{V}O_{2max}$	2.9% NS \uparrow in TTE @ 110% $\dot{V}O_{2max}$
Ward et al. (2016)[20]	Crossover, double-blind, randomised	Healthy, well-trained male athletes ($n = 11$). Age 34.6 ± 11.5 years.	1 g (single dose)	2-h pre-exercise	4 km cycling TT	NS \downarrow 1% in TT performance
Ahmadian et al. (2017)[21]	Independent groups, double-blind, randomised	Elderly heart failure patients (sex not mentioned) ($n = 8$). Age 60.1 ± 5.4 vs. age 60.1 ± 8.3 years.	1.5 g (3 x 500 mg/day)	14 days	Adapted Bruce Protocol	25.7% \uparrow exercise time; 22.6 % \uparrow distance covered
Warnock et al. (2017)[22]	Crossover, single-blind, randomised	Healthy, recreationally-trained males ($n = 7$). Age 20.8 ± 0.9 years.	50 mg/kg body mass (single dose)	1.5-h pre-exercise	3 x Wingate repeated sprint	14% \uparrow mean peak power; 14.3% \uparrow peak power; 9% \uparrow mean power

Note: TT = time-trial; TTE = time-to-exhaustion; NS = non-significant.