BMJ Open

Effects of supplementation with carnosine and other histidine containing dipeptides on chronic disease risk factors and outcomes: protocol for a systematic review of randomized controlled trials

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-020623
Article Type:	Protocol
Date Submitted by the Author:	13-Nov-2017
Complete List of Authors:	Menon, Kirthi; Monash University School of Public Health and Preventive Medicine Mousa, Aya; Monash University, School of Public Health and Preventive Medicine de Courten, Barbora; Monash University School of Public Health and Preventive Medicine, Monash Centre for Health Research & Implementation
Keywords:	carnosine, chronic disease, protocol, systematic review, randomized controlled trials

Running Title: Carnosine supplementation in the prevention of chronic diseases

Effects of supplementation with carnosine and other histidine containing dipeptides on chronic disease risk factors and outcomes: protocol for a systematic review of randomized controlled trials

Kirthi Menon[a], Aya Mousa[a], Barbora de Courten[a]

[a] Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia.

Corresponding author:

A/Prof Barbora de Courten, MD PhD MPH FRACP
Monash Centre for Health Research and Implementation,
School of Public Health and Preventive Medicine, Monash University
43-51 Kanooka Grove, Clayton
Melbourne, VIC 3168, Australia
Telephone: +61 3 857 22654
Fax: +61 3 9594 7554
Email: barbora.decourten@monash.edu

Author Information:

- **Kirthi Menon** (MCHRI, Melbourne, Australia): kirthi.aravindmenon@monash.edu
- **Aya Mousa** (MCHRI, Melbourne, Australia)**:** aya.mousa@monash.edu
- **Barbora de Courten (**MCHRI, Melbourne, Australia)**:** barbora.decourten@monash.edu

Keywords: Carnosine; chronic disease; protocol; systematic review; randomized controlled trials

Word Count: 2,285
Number of Figures: 0
Number of Tables: 1
Number of References: 49
Number of Supplementary Files: 1

ABSTRACT

Introduction: Ageing of populations globally, coupled with the obesity epidemic, has resulted in the rising prevalence of chronic diseases including diabetes, cardiovascular diseases, cancers, and neurodegenerative disorders. Prevention of risk factors that contribute to these diseases is key in managing the global burden of chronic diseases. Recent studies suggest that carnosine, a dipeptide with anti-inflammatory, anti-oxidative, and anti-glycating properties, may have a role in the prevention of chronic diseases; however the effects of carnosine and other histidine-containing peptides (HCDs) on chronic disease risk factors and outcomes have not been established. We aim to conduct a comprehensive systematic review to examine the effects of supplementation with carnosine and other HCDs on chronic disease risk factors and outcomes and to identify relevant knowledge gaps.

Methods and analysis: Electronic databases including Medline, CINAHL, EMBASE and all EBM will be systematically searched to identify randomized controlled trials (RCTs) and systematic reviews of RCTs, comparing supplementation with carnosine and/or other HCDs versus placebo, usual care or other pharmacological or non-pharmacological interventions. One reviewer will screen titles and abstracts for eligibility according to pre-specified inclusion criteria, after which two independent reviewers will perform data extraction and quality appraisal. Meta-analyses, meta-regression, and sub-group analyses will be conducted where appropriate.

Ethics and dissemination: Ethics approval is not required as this review does not involve primary data collection. This review will generate level-one evidence regarding the effects of carnosine supplementation on chronic disease risk factors and outcomes and will be disseminated through peer reviewed publications and at conference meetings, to inform future research on the efficacy of carnosine supplementation for the prevention of chronic diseases.

PROSPERO registration number: CRD42017075354

Strengths & Limitations:

- This protocol is for the first systematic review to investigate the effects of supplementation with carnosine and other HCDs on chronic disease risk factors and outcomes.
- We employ rigorous international gold-standard methodology and a comprehensive search strategy.
- Findings from our review will provide important evidence on the efficacy of supplementation with carnosine and/or HCDs in chronic disease prevention, and will identify knowledge gaps to guide future research in this area.
- Although we will endeavour to identify grey literature, some unpublished data may be missed and publication bias cannot be ruled out.

INTRODUCTION

The burden of chronic diseases is rapidly increasing in line with the growing obesity epidemic and population ageing [1]. Chronic diseases contribute to 68% of deaths worldwide [2] and 46% of the global burden of disease [1]. In 2012, the four leading causes of chronic disease deaths were cardiovascular diseases (CVD) contributing to 46.2% of deaths, followed by cancers (21.7%), respiratory diseases including asthma and chronic obstructive pulmonary disease (10.7%), and diabetes (4%) [2]. Moreover, obesity, which is a common risk factor for chronic diseases, has already reached unprecedented levels and is continuing to increase at an alarming rate [1]. While lifestyle strategies targeting diet and exercise are effective in reducing obesity and related chronic diseases, these interventions on a population scale are difficult to achieve and maintain in the long-term [1]. Identification of simple and easily scalable interventions is therefore needed to ameliorate the current chronic disease burden.

Supplementation with carnosine (β-alanyl-L-histidine) and other histidine-containing dipeptides (HCDs) such as anserine and *N*-acetylcarnosine, as well as their components (eg: β-alanine) have been proposed as potential strategies for the prevention of chronic diseases [3]. Carnosine, a dipeptide and the "founding member" of the HCD family of soluble peptides, is found naturally in mammalian brain tissue and skeletal and heart muscle [4]. It is synthesised by the enzyme carnosine synthase and degraded by carnosinase [5]. The primary source of carnosine has traditionally been via dietary intake of meat and fish, with varying amounts depending on the type of meat or fish and cooking method [6 7]. However, due to increased production and consumption of processed meats, the amount of carnosine derived from the modern diet is limited [4 5]. In addition, consumption of processed meat has been linked to negative health effects [8 9]. Over-the-counter carnosine supplementation may therefore be a more ideal source of carnosine.

It is thought that carnosine and HCDs may prevent chronic diseases via their anti-inflammatory, anti-oxidative, anti-glycating, anti-ischemic, and chelating properties [10 11].

Cell culture studies have reported that carnosinase content and activity were increased in hyperglycaemia [12], and that polymorphisms in the carnosinase gene (*CNDP1*) predicted progression to end-stage renal disease in patients with type 1 diabetes and diabetic nephropathy [13]. In cancer cell lines, carnosine suppressed tumorigenesis by inhibiting proliferation and inducing apoptosis in human glioblastoma cells as well as colorectal and ovarian carcinoma cells [14-17]. Evidence from animal studies has shown that carnosine supplementation reduced insulin resistance [10] and plasma concentrations of glucose, lipids, and inflammatory markers [11 18], and delayed the development of atherosclerosis [19]. In mouse/rat models, carnosine rescued cognitive decline in Alzheimer's disease [20], and reduced the size of ischemia in various organs including the heart, brain, liver, and kidney [21-25]. Moreover, both carnosine and *N*-acetylcarnosine were shown to delay the development of lens opacification leading to cataracts [26 27].

In humans, observational studies by our group [28 29] and others [30 31] have shown that muscle carnosine content is higher in drug naïve patients with T2DM, and is associated with obesity, insulin resistance, and progressive impairment of glucose tolerance. However, other studies have reported lower muscle carnosine content in patients with T2DM but not T1DM [30]. The primary hypothesis, which is yet to be confirmed, is that carnosine levels increase in an adaptive mechanism to counteract the increased inflammation and oxidative stress present in obesity and T2DM; however, muscle carnosine storage may decline with disease progression or under certain pathological conditions where oxidative stress and glycation are exacerbated [28-30].

Human randomized controlled trials (RCTs) have shown that supplementation with carnosine (0.5 to 2g) or β-alanine (1 to 6g) daily for 1 to 6 months improved a range of outcomes

related to chronic diseases including: cognition and exercise capacity in young and elderly healthy adults [32-36]; physical performance and quality of life in patients with heart failure [37]; glucose metabolism in overweight or obese non-diabetic and prediabetic individuals [38 39]; plasma glucose, haemoglobin A1c (HbA1c), lipid levels, and urinary albumin-creatinine ratio in diabetic nephropathy [40]; and neurological outcomes including balance (foot up and go test) and locomotion (rigidity of extremities and hand movements) in elderly adults and patients with Parkinson's disease [33 41]. In a recent pilot clinical trial by our group [38], we showed that 2g daily of pure carnosine supplementation for 12 weeks reduced insulin resistance, measured by homeostatic model assessment (HOMA), compared with placebo. Similarly, two other RCTs found improved glucose metabolism following supplementation with carnosine combined with chromium and cinnamon [39] or with α-lipoic acid, zinc, and B vitamins [42].

Although current evidence supports a potential role for carnosine and related HCDs in the prevention of chronic diseases, the effect of supplementation with carnosine or HCDs on chronic disease risk factors and outcomes has not been established. Previous systematic reviews [43-45] and meta-analyses [36] have focused only on the effects of carnosine and HCDs on exercise performance, despite a number of newly published studies suggesting a broader role for these dipeptides. We aim to address this knowledge gap by conducting a comprehensive systematic review of RCTs to synthesize current evidence regarding the effects of carnosine and HCD supplementation on chronic diseases risk factors and outcomes.

SYSTEMATIC REVIEW QUESTION

Is carnosine and/or HCD supplementation effective in improving chronic disease risk factors and outcomes compared to placebo, usual care, or other pharmacological or non-pharmacological interventions?

METHODS/ DESIGN

This systematic review will employ rigorous gold-standard methodology and will conform to the reporting standards of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA; Supplementary Table 1). The protocol has been registered on PROSPERO: CRD42017075354.

Eligibility Criteria

The PICO (Population, Intervention, Comparison, Outcomes) framework in Table 1 established *a priori* will be used as a tool for determining the eligibility of articles.

Table 1. PICO for study inclusion

	Participants (P)	**Intervention (I)**	**Comparison (C)**	**Outcomes (O)**
Inclusion	Males and females of any age, ethnicity, geographic area, comorbidities or medication use	Carnosine and related HCDs (beta-alanine, anserine, NAC, etc) supplementation administered in any form (intravenous, intramuscular, or oral), alone (pure) or combined with other intervention/s, of any dosage, and for any duration	Placebo or usual care or any pharmacological or non-pharmacological interventions	All chronic disease risk factors and outcomes including but not limited to metabolic/glycaemic, cardiovascular, and cognitive/ mental health risk factors and outcomes
Exclusion	Studies not in humans	Studies without carnosine and/or HCD supplementation	Studies with no control group	None
Study type	RCTs and systematic reviews of RCTs			
Language	No limit			
Year of publication	No limit			

HCDs: histidine-containing dipeptides; **NAC:** *N*-acetylcarnosine; **RCT:** randomized controlled trial.

Search Strategy

The following electronic databases will be used to identify relevant literature based on the systematic search strategy developed using relevant search terms (Supplementary Table 2):

- MEDLINE via OVID
- MEDLINE in process and other non-indexed citations via OVID
- CINAHL
- EMBASE via OVID
- All Evidence Based Medicine (EBM) Reviews via OVID incorporating:
  - Cochrane Database of Systematic Reviews
  - ACP Journal Club
  - Database of Abstracts of Reviews of Effects
  - Cochrane Central Register of Controlled Trials
  - Cochrane Methodology Register
  - Health Technology Assessment
  - NHS Economic Evaluation Database

Bibliographies of relevant studies as well as systematic reviews identified by the search strategy will be screened for identification of additional studies. Where required data are not presented, the corresponding authors of included studies will be contacted to provide de-identified aggregate data for the purpose of meta-analyses if necessary. Clinical trials registries including the National Institute of Health Clinical Trials Register (https://clinicaltrials.gov/) and the Australian New Zealand Clinical Trials (ANZCTR) registries (https://www.anzctr.org.au) will also be searched to identify unpublished trials and grey literature.

Study Selection

Two reviewers (KM and AM) will review the titles, abstracts, and keywords of every article retrieved by the search strategy using the selection criteria described in Table 1. Disagreement between reviewers regarding the eligibility of articles will be resolved by discussion. Where consensus is not reached, a third reviewer (BdC) will be consulted. Full

text articles will be retrieved for further assessment if the information given suggests that the study meets the selection criteria or if there is doubt regarding the eligibility of the study based on the title and abstract. Any full text articles excluded will be tabulated with reasons for their exclusion.

Quality appraisal of the evidence

Methodological quality of the included studies will be assessed at the study-level by two reviewers (KM and AM) using the Monash Centre for Health Research and Implementation critical appraisal tool [46]. Individual quality items will be investigated using a descriptive component approach which will consider methods of randomisation and allocation of participants to study groups; blinding of participants, investigators and outcome assessors; methods of outcome assessment and reporting; attrition rates; conflicts of interest of authors; presence of pre-specified selection criteria and statistical issues including powering and methods of data analysis. This process will allow each study to be allocated a risk of bias rating. Disagreement among reviewers will be resolved by discussion to reach a consensus.

Data extraction

Data for outcomes of interest based on the selection criteria (Table 1) will be extracted from all the included studies by two reviewers (KM and AM) using a specially developed data extraction form. The data extracted will include general details of the study design and setting; participant characteristics; mean values, standard deviations (SDs), and confidence intervals of the outcomes; point estimates and measures of variability; frequency counts for dichotomous variables; numbers of participants; and intention-to-treat analysis. Both reviewers will check all computed data entries for meta-analysis if applicable.

Grading the body of evidence

Quality of the evidence for the effects of carnosine and/or HCD supplementation on health outcomes will be assessed at the outcome-level and will be graded as high, moderate, low, or very low using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach [47]. Quality of the evidence will be graded by two independent reviewers (AM and KM) based on risk of bias, inconsistence between studies, indirectness, imprecision and publication bias. In line with PRISMA guidelines, quality will be reported at both the study- and outcome-levels where appropriate, and disagreement will be resolved by consensus.

Data Analysis and Synthesis

Data will be presented in summary tables and descriptive text to describe the study populations, interventions, and outcomes of the studies included in the review. Aggregate effect measures will be used for meta-analyses for trials deemed homogenous with regard to participants, interventions, and outcomes. Meta-analyses will be performed using DerSimonian and Laird random effects models in Review Manager V5. Dichotomous results will be presented as relative risks or odds ratios with 95% confidence intervals (CI), while continuous results will be presented as weighted mean difference with 95% CIs. Where outcomes have been measured using different tools or methods, or where results vary substantially, standardized mean differences with 95% CIs will be presented. Statistical heterogeneity will be assessed using the I^2 test where values over 50% will indicate moderate to high heterogeneity. Descriptive analysis will be conducted for studies that are found to be clinically heterogeneous or present insufficient information for pooling. A two-tailed p-value of <0.05 will be considered statistically significant.

Subgroup and Sensitivity Analyses

Subgroup analyses, and where possible, meta-regression will be performed on factors that are assumed to cause variation in the outcomes of interest, and these may include age, gender, body mass index, duration, dose, and route of supplementation, type of carnosine or HCD used, participant disease status, medications, and study duration.

Sensitivity analyses will be performed if indicated to explore the influence of certain factors or studies on the effect size, which will be determined during the review process. Studies contributing to high heterogeneity $I^2 > 50\%$ or those with high risk of bias will be excluded through sensitivity analysis to examine their influence on the results. Where there are sufficient numbers of studies, visual inspection of funnel plots and Egger and Begg [48 49] statistical tests will be used to determine small study effects and publication bias. If applicable, meta-regression and publication bias assessments will be examined on the Comprehensive Meta-analysis software V.3., and p-values <0.05 will be considered statistically significant.

DISCUSSION

Interventions aimed at reducing chronic disease risk factors including obesity, insulin resistance, hypertension, dyslipidaemia, and inflammation, among others, are vital to addressing the growing burden of chronic disease. Although carnosine and HCDs are proposed to have anti-inflammatory, anti-oxidative, and anti-glycating properties, the efficacy of carnosine and HCD supplementation in the prevention of chronic disease risk factors and outcomes has not been established.

Here, we will conduct the first systematic review examining the effects of supplementation with carnosine and/or other HCDs on chronic disease risk factors and outcomes. Using rigorous methodology, pre-specified criteria, and a pre-determined search strategy, this review will synthesize all existing RCT data to establish the effects of carnosine and HCD

supplementation on a broad range of risk factors and outcomes related to chronic diseases including T2DM, CVD, cancers, neurodegenerative disorders, and others. By systematically reviewing and appraising the literature, we will also identify relevant knowledge gaps and uncertainties, thereby providing a platform for future studies in this field. However, although our search will endeavour to identify grey literature, potential publication bias cannot be ruled out as there may be unpublished data not accounted for.

Nevertheless, this review will generate important insights regarding the potential use of carnosine and HCD supplementation for the prevention of chronic diseases. If carnosine and/or HCDs are shown to be effective in reducing chronic disease risk factors and/or outcomes upon review and meta-analysis, this would generate level-one evidence of efficacy with considerable clinical and public health implications.

ETHICS & DISSEMINATION

This study does not require ethical approval as it does not involve primary data collection. Findings from this review regarding the effects of carnosine supplementation on chronic disease risk factors and outcomes will be disseminated through peer reviewed publications and at conference meetings, to inform future research on the use of carnosine supplementation for the prevention of chronic diseases.

ACKNOWLEDGEMENTS

AM is a recipient of the Australian Postgraduate Award Scholarship provided by Monash University. BdC is supported by a National Heart Foundation Future Leader Fellowship (100864).

FUNDING

This study received no specific external funding.

AUTHOR CONTRIBUTIONS

KM developed the search strategy, wrote the first draft of the review protocol, and will contribute to data collection and analysis. AM contributed to the design and scope of the search strategy, revised and edited the manuscript, and will contribute to data collection and analysis. BdC determined the design and scope of the review, revised and edited the manuscript, will supervise the review process, and is the guarantor of the review.

COMPETING INTERESTS

None declared.

REFERENCES

1. WHO. The World Health Report 2002: Reducing Risks, Promoting Healthy Life. Geneva: World Health Organization, 2002.
2. WHO. Global Status Report on noncommunicable diseases 2014. Geneva: World Health Organization, 2014.
3. Xie Z, Baba SP, Sweeney BR, et al. Detoxification of aldehydes by histidine-containing dipeptides: from chemistry to clinical implications. *Chemico-biological interactions* 2013;202(1-3):288-97. doi: 10.1016/j.cbi.2012.12.017 [published Online First: 2013/01/15]
4. Guiotto A, Calderan A, Ruzza P, et al. Carnosine and carnosine-related antioxidants: a review. *Curr Med Chem* 2005;12(20):2293-315. [published Online First: 2005/09/27]
5. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. *Physiol Rev* 2013;93(4):1803-45. doi: 10.1152/physrev.00039.2012 [published Online First: 2013/10/19]
6. Abe H. Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. *Biochemistry (Mosc)* 2000;65(7):757-65. [published Online First: 2000/08/22]
7. Sale C, Artioli GG, Gualano B, et al. Carnosine: from exercise performance to health. *Amino Acids* 2013;44(6):1477-91. doi: 10.1007/s00726-013-1476-2 [published Online First: 2013/03/13]
8. Micha R, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. *Circulation* 2010;121(21):2271-83. doi: 10.1161/circulationaha.109.924977 [published Online First: 2010/05/19]
9. Sinha R, Cross AJ, Graubard BI, et al. Meat intake and mortality: A prospective study of over half a million people. *Archives of Internal Medicine* 2009;169(6):562-71. doi: 10.1001/archinternmed.2009.6
10. Aldini G, Orioli M, Rossoni G, et al. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. *J Cell Mol Med* 2011;15(6):1339-54. doi: 10.1111/j.1582-4934.2010.01101.x [published Online First: 2010/06/04]
11. Lee YT, Hsu CC, Lin MH, et al. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. *Eur J Pharmacol* 2005;513(1-2):145-50. doi: 10.1016/j.ejphar.2005.02.010 [published Online First: 2005/05/10]
12. Riedl E, Koeppel H, Brinkkoetter P, et al. A CTG polymorphism in the CNDP1 gene determines the secretion of serum carnosinase in Cos-7 transfected cells. *Diabetes* 2007;56(9):2410-3. doi: 10.2337/db07-0128 [published Online First: 2007/07/03]
13. Alkhalaf A, Bakker SJL, Bilo HJG, et al. A polymorphism in the gene encoding carnosinase (CNDP1) as a predictor of mortality and progression from nephropathy to end-stage renal disease in type 1 diabetes mellitus. *Diabetologia* 2010;53(12):2562-68. doi: 10.1007/s00125-010-1863-0
14. Iovine B, Guardia F, Irace C, et al. l-carnosine dipeptide overcomes acquired resistance to 5-fluorouracil in HT29 human colon cancer cells via downregulation of HIF1-alpha and induction of apoptosis. *Biochimie* 2016;127:196-204. doi: 10.1016/j.biochi.2016.05.010 [published Online First: 2016/05/29]
15. Iovine B, Oliviero G, Garofalo M, et al. The Anti-Proliferative Effect of L-Carnosine Correlates with a Decreased Expression of Hypoxia Inducible Factor 1 alpha in

Human Colon Cancer Cells. *PLoS ONE* 2014;9(5):e96755. doi: 10.1371/journal.pone.0096755
16. Mikula-Pietrasik J, Ksiazek K. L-Carnosine Prevents the Pro-cancerogenic Activity of Senescent Peritoneal Mesothelium Towards Ovarian Cancer Cells. *Anticancer research* 2016;36(2):665-71. [published Online First: 2016/02/07]
17. Rybakova YS, Kalen AL, Eckers JC, et al. Increased manganese superoxide dismutase and cyclin B1 expression in carnosine-induced inhibition of glioblastoma cell proliferation. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry* 2015;9(1):63-71. doi: 10.1134/S1990750815010096
18. Bao Y, Gao C, Hao W, et al. Effects of Dietary L-carnosine and Alpha-lipoic Acid on Growth Performance, Blood Thyroid Hormones and Lipid Profiles in Finishing Pigs. *Asian-Australas J Anim Sci* 2015;28(10):1465-70. doi: 10.5713/ajas.14.0604 [published Online First: 2015/07/22]
19. Brown BE, Kim CH, Torpy FR, et al. Supplementation with carnosine decreases plasma triglycerides and modulates atherosclerotic plaque composition in diabetic apo E(-/-) mice. *Atherosclerosis* 2014;232(2):403-9. doi: 10.1016/j.atherosclerosis.2013.11.068 [published Online First: 2014/01/29]
20. Herculano B, Tamura M, Ohba A, et al. beta-alanyl-L-histidine rescues cognitive deficits caused by feeding a high fat diet in a transgenic mouse model of Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 2013;33(4):983-97. doi: 10.3233/jad-2012-121324 [published Online First: 2012/10/27]
21. Dursun N, Taskin E, Ozturk F. Protection against adriamycin-induced cardiomyopathy by carnosine in rats: role of endogenous antioxidants. *Biological trace element research* 2011;143(1):412-24. doi: 10.1007/s12011-010-8875-y [published Online First: 2010/10/14]
22. Dobrota D, Fedorova T, Stvolinsky S, et al. Carnosine protects the brain of rats and Mongolian gerbils against ischemic injury: after-stroke-effect. *Neurochemical research* 2005;30(10):1283-8. doi: 10.1007/s11064-005-8799-7 [published Online First: 2005/12/13]
23. Fouad AA, El-Rehany MA, Maghraby HK. The hepatoprotective effect of carnosine against ischemia/reperfusion liver injury in rats. *European journal of pharmacology* 2007;572(1):61-8. doi: 10.1016/j.ejphar.2007.06.010 [published Online First: 2007/07/06]
24. Fujii T, Takaoka M, Muraoka T, et al. Preventive effect of L-carnosine on ischemia/reperfusion-induced acute renal failure in rats. *European journal of pharmacology* 2003;474(2-3):261-7. [published Online First: 2003/08/19]
25. Rajanikant GK, Zemke D, Senut MC, et al. Carnosine is neuroprotective against permanent focal cerebral ischemia in mice. *Stroke* 2007;38(11):3023-31. doi: 10.1161/strokeaha.107.488502 [published Online First: 2007/10/06]
26. Yan H, Guo Y, Zhang J, et al. Effect of carnosine, aminoguanidine, and aspirin drops on the prevention of cataracts in diabetic rats. *Mol Vis* 2008;14:2282-91. [published Online First: 2008/12/17]
27. Mark AB, Yegor EY. Advanced Drug Delivery of N-Acetylcarnosine (N-Acetyl-beta-alanyl-Lhistidine),Carcinine (Beta-alanylhistamine) and L-carnosine (Beta-alanyl-L-histidine) in Targeting Peptide Compounds as Pharmacological Chaperones for Use in Tissue Engineering, Human Disease Management and Therapy: From in vitro to the Clinic. *Recent Patents on Drug Delivery & Formulation* 2010;4(3):198-230. doi: http://dx.doi.org.ezproxy.lib.monash.edu.au/10.2174/187221110793237547

28. de Courten B, Kurdiova T, de Courten MPJ, et al. Muscle Carnosine Is Associated with Cardiometabolic Risk Factors in Humans. *PLOS ONE* 2015;10(10):e0138707. doi: 10.1371/journal.pone.0138707
29. Stegen S, Everaert I, Deldicque L, et al. Muscle histidine-containing dipeptides are elevated by glucose intolerance in both rodents and men. *PLoS One* 2015;10(3):e0121062. doi: 10.1371/journal.pone.0121062 [published Online First: 2015/03/25]
30. Gualano B, Everaert I, Stegen S, et al. Reduced muscle carnosine content in type 2, but not in type 1 diabetic patients. *Amino Acids* 2012;43(1):21-4. doi: 10.1007/s00726-011-1165-y [published Online First: 2011/11/29]
31. Srikanthan P, Singhal A, Lee CC, et al. Characterization of Intra-myocellular Lipids using 2D Localized Correlated Spectroscopy and Abdominal Fat using MRI in Type 2 Diabetes. *Magn Reson Insights* 2012;5:29-36. doi: 10.4137/mri.s10489 [published Online First: 2013/03/09]
32. Hoffman JR, Landau G, Stout JR, et al. beta-Alanine ingestion increases muscle carnosine content and combat specific performance in soldiers. *Amino Acids* 2015;47(3):627-36. doi: 10.1007/s00726-014-1896-7 [published Online First: 2014/12/17]
33. Szczesniak D, Budzen S, Kopec W, et al. Anserine and carnosine supplementation in the elderly: Effects on cognitive functioning and physical capacity. *Arch Gerontol Geriatr* 2014;59(2):485-90. doi: 10.1016/j.archger.2014.04.008 [published Online First: 2014/06/02]
34. Baraniuk JN, El-Amin S, Corey R, et al. Carnosine treatment for gulf war illness: a randomized controlled trial. *Glob J Health Sci* 2013;5(3):69-81. doi: 10.5539/gjhs.v5n3p69 [published Online First: 2013/04/27]
35. del Favero S, Roschel H, Solis MY, et al. Beta-alanine (Carnosyn) supplementation in elderly subjects (60-80 years): effects on muscle carnosine content and physical capacity. *Amino Acids* 2012;43(1):49-56. doi: 10.1007/s00726-011-1190-x [published Online First: 2011/12/07]
36. Hobson RM, Saunders B, Ball G, et al. Effects of beta-alanine supplementation on exercise performance: a meta-analysis. *Amino Acids* 2012;43(1):25-37. doi: 10.1007/s00726-011-1200-z [published Online First: 2012/01/25]
37. Lombardi C, Carubelli V, Lazzarini V, et al. Effects of oral administration of orodispersible levo-carnosine on quality of life and exercise performance in patients with chronic heart failure. *Nutrition* 2015;31(1):72-8. doi: 10.1016/j.nut.2014.04.021 [published Online First: 2014/10/08]
38. de Courten B, Jakubova M, de Courten MP, et al. Effects of carnosine supplementation on glucose metabolism: Pilot clinical trial. *Obesity (Silver Spring)* 2016;24(5):1027-34. doi: 10.1002/oby.21434 [published Online First: 2016/04/05]
39. Liu Y, Cotillard A, Vatier C, et al. A Dietary Supplement Containing Cinnamon, Chromium and Carnosine Decreases Fasting Plasma Glucose and Increases Lean Mass in Overweight or Obese Pre-Diabetic Subjects: A Randomized, Placebo-Controlled Trial. *PLoS One* 2015;10(9):e0138646. doi: 10.1371/journal.pone.0138646 [published Online First: 2015/09/26]
40. Elbarbary NS, Ismail EAR, El-Naggar AR, et al. The effect of 12 weeks carnosine supplementation on renal functional integrity and oxidative stress in pediatric patients with diabetic nephropathy: a randomized placebo-controlled trial. *Pediatric diabetes* 2017 doi: 10.1111/pedi.12564 [published Online First: 2017/07/27]

41. Boldyrev A, Fedorova T, Stepanova M, et al. Carnosine [corrected] increases efficiency of DOPA therapy of Parkinson's disease: a pilot study. *Rejuvenation Res* 2008;11(4):821-7. doi: 10.1089/rej.2008.0716 [published Online First: 2008/08/30]
42. Derosa G, D'Angelo A, Romano D, et al. A Clinical Trial about a Food Supplement Containing α-Lipoic Acid on Oxidative Stress Markers in Type 2 Diabetic Patients. *International Journal of Molecular Sciences* 2016;17(11):1802. doi: 10.3390/ijms17111802
43. Berti Zanella P, Donner Alves F, Guerini de Souza C. Effects of beta-alanine supplementation on performance and muscle fatigue in athletes and non-athletes of different sports: a systematic review. *J Sports Med Phys Fitness* 2017;57(9):1132-41. doi: 10.23736/s0022-4707.16.06582-8 [published Online First: 2016/07/06]
44. Saunders B, Elliott-Sale K, Artioli GG, et al. beta-alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *Br J Sports Med* 2017;51(8):658-69. doi: 10.1136/bjsports-2016-096396 [published Online First: 2016/11/01]
45. Quesnele JJ, Laframboise MA, Wong JJ, et al. The effects of beta-alanine supplementation on performance: a systematic review of the literature. *Int J Sport Nutr Exerc Metab* 2014;24(1):14-27. doi: 10.1123/ijsnem.2013-0007 [published Online First: 2013/08/07]
46. MCHRI. Evidence Synthesis Program templates for critical appraisal and risk of bias (adapted from Critical Appraisal Templates, Centre for Clinical Effectiveness ,Southern Health ,Melbourne,2010): Monash University and Monash Health 2013.
47. Grading quality of evidence and strength of recommendations. *BMJ* 2004;328(7454):1490. doi: 10.1136/bmj.328.7454.1490
48. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50(4):1088-101. [published Online First: 1994/12/01]
49. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *Bmj* 1997;315(7109):629-34. [published Online First: 1997/10/06]

Supplementary Table 1. PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page Number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2 and 6
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	11 – 12
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	11
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3 – 5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	5
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Table 1
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	6 – 7 and Table 1
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Supplementary Table 2

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8 – 9
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7 – 9
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7 – 8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8 and Table 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8 and Table 1
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8 – 9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	9
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	9 – 10
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	9
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9 – 10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8 – 9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

Supplementary Table 2. Sample OVID-MEDLINE search strategy

1. Carnosine/
2. carnosine.mp.
3. beta alanylhistidine.mp.
4. Anserine/
5. anserine.mp.
6. beta alanyl 3 methylhistidine.mp.
7. ophidine.mp.
8. exp beta-alanine/
9. beta alanine*.mp.
10. 3 aminopropionic acid.mp.
11. N-Acetyl-Carnosine.mp.
12. N-Acetyl-L-Carnosine.mp.
13. beta alanyl l histidine.mp.
14. beta-ala-his.mp.
15. l histidine beta alanyl.mp.
16. l alpha alanyl l histidine.mp.
17. histidine.mp.
18. balenine.mp.
19. or/1-18
20. randomi?ed controlled trial.pt.
21. controlled clinical trial.pt.
22. randomi?ed.ti,ab.
23. placebo.ti,ab.
24. clinical trials as topic.sh.
25. randomly.ti,ab.
26. trial.ti.
27. or/20-26
28. exp animals/ not exp humans/
29. 27 not 28
30. Meta-Analysis as Topic/
31. meta analy$.tw.
32. metaanaly$.tw.
33. Meta-Analysis/
34. (systematic adj (review$1 or overview$1)).tw.
35. exp Review Literature as Topic/
36. or/30-35
37. cochrane.ab.
38. embase.ab.
39. (psychlit or psyclit).ab.
40. (psychinfo or psycinfo).ab.
41. (cinahl or cinhal).ab.
42. science citation index.ab.
43. bids.ab.
44. cancerlit.ab.
45. or/37-44
46. reference list$.ab.
47. bibliograph$.ab.
48. hand-search$.ab.
49. relevant journals.ab.
50. manual search$.ab.
51. or/46-50
52. selection criteria.ab.
53. data extraction.ab.
54. 52 or 53
55. Review/
56. 54 and 55
57. Comment/
58. Letter/
59. Editorial/
60. animal/
61. human/
62. 60 not (60 and 61)
63. or/57-59,62
64. 36 or 45 or 51 or 56
65. 64 not 63
66. 27 or 65
67. 19 and 66
68. limit 67 to humans

Research Checklist: PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page Number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2 and 6
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	11 – 12
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	11
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3 – 5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	5
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Table 1
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	6 – 7 and Table 1
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Supplementary Table 2

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8 – 9
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7 – 9
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7 – 8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8 and Table 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8 and Table 1
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8 – 9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	9
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	9 – 10
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	9
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9 – 10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8 – 9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

BMJ Open

Effects of supplementation with carnosine and other histidine containing dipeptides on chronic disease risk factors and outcomes: protocol for a systematic review of randomized controlled trials

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-020623.R1
Article Type:	Protocol
Date Submitted by the Author:	16-Feb-2018
Complete List of Authors:	Menon, Kirthi; Monash University School of Public Health and Preventive Medicine Mousa, Aya; Monash University, School of Public Health and Preventive Medicine de Courten, Barbora; Monash University School of Public Health and Preventive Medicine, Monash Centre for Health Research & Implementation
Primary Subject Heading:	Public health
Secondary Subject Heading:	Nutrition and metabolism, Research methods
Keywords:	carnosine, chronic disease, protocol, systematic review, randomized controlled trials

Running Title: Carnosine supplementation in the prevention of chronic diseases

Effects of supplementation with carnosine and other histidine containing dipeptides on chronic disease risk factors and outcomes: protocol for a systematic review of randomized controlled trials

Kirthi Menon[a], Aya Mousa[a], Barbora de Courten[a]

[a] Monash Centre for Health Research and Implementation, School of Public Health and Preventive Medicine, Monash University, Melbourne, Australia.

Corresponding author:

A/Prof Barbora de Courten, MD PhD MPH FRACP
Monash Centre for Health Research and Implementation,
School of Public Health and Preventive Medicine, Monash University
43-51 Kanooka Grove, Clayton
Melbourne, VIC 3168, Australia
Telephone: +61 3 857 22654
Fax: +61 3 9594 7554
Email: barbora.decourten@monash.edu

Author Information:

- **Kirthi Menon** (MCHRI, Melbourne, Australia): kirthi.aravindmenon@monash.edu
- **Aya Mousa** (MCHRI, Melbourne, Australia)**:** aya.mousa@monash.edu
- **Barbora de Courten (**MCHRI, Melbourne, Australia)**:** barbora.decourten@monash.edu

Keywords: Carnosine; chronic disease; protocol; systematic review; randomized controlled trials

Word Count: 2,285
Number of Figures: 0
Number of Tables: 1
Number of References: 49
Number of Supplementary Files: 1

ABSTRACT

Introduction: Ageing of populations globally, coupled with the obesity epidemic, has resulted in the rising prevalence of chronic diseases including diabetes, cardiovascular diseases, cancers, and neurodegenerative disorders. Prevention of risk factors that contribute to these diseases is key in managing the global burden of chronic diseases. Recent studies suggest that carnosine, a dipeptide with anti-inflammatory, anti-oxidative, and anti-glycating properties, may have a role in the prevention of chronic diseases; however no previous reviews have examined the effects of carnosine and other histidine-containing peptides (HCDs) on chronic disease risk factors and outcomes. We aim to conduct a comprehensive systematic review to examine the effects of supplementation with carnosine and other HCDs on chronic disease risk factors and outcomes and to identify relevant knowledge gaps.

Methods and analysis: Electronic databases including Medline, CINAHL, EMBASE and all EBM will be systematically searched to identify randomized controlled trials (RCTs) and systematic reviews of RCTs, comparing supplementation with carnosine and/or other HCDs versus placebo, usual care or other pharmacological or non-pharmacological interventions. One reviewer will screen titles and abstracts for eligibility according to pre-specified inclusion criteria, after which two independent reviewers will perform data extraction and quality appraisal. Meta-analyses, meta-regression, and sub-group analyses will be conducted where appropriate.

Ethics and dissemination: Ethics approval is not required as this review does not involve primary data collection. This review will generate level-one evidence regarding the effects of carnosine supplementation on chronic disease risk factors and outcomes and will be disseminated through peer reviewed publications and at conference meetings, to inform future research on the efficacy of carnosine supplementation for the prevention of chronic diseases.

PROSPERO registration number: CRD42017075354

Strengths & Limitations:

- This protocol is for the first systematic review to investigate the effects of supplementation with carnosine and other HCDs on chronic disease risk factors and outcomes.
- We employ rigorous international gold-standard methodology including PRISMA guidelines, a PICO framework, and the GRADE approach, and use a comprehensive search strategy.
- Findings from our review will provide important evidence on the efficacy of supplementation with carnosine and/or HCDs in chronic disease prevention, and will identify knowledge gaps to guide future research in this area.
- Although we will endeavour to identify grey literature, some unpublished data may be missed and publication bias cannot be ruled out.

INTRODUCTION

The burden of chronic diseases is rapidly increasing in line with the growing obesity epidemic and population ageing [1]. Chronic diseases contribute to 68% of deaths worldwide [2] and 46% of the global burden of disease [1]. In 2012, the four leading causes of chronic disease deaths were cardiovascular diseases (CVD) contributing to 46.2% of deaths, followed by cancers (21.7%), respiratory diseases including asthma and chronic obstructive pulmonary disease (10.7%), and diabetes (4%) [2]. Moreover, obesity, which is a common risk factor for chronic diseases, has already reached unprecedented levels and is continuing to increase at an alarming rate [1]. While lifestyle strategies targeting diet and exercise are effective in reducing obesity and related chronic diseases, these interventions on a population scale are difficult to achieve and maintain in the long-term [1]. Identification of simple and easily scalable interventions is therefore needed to ameliorate the current chronic disease burden.

Supplementation with carnosine (β-alanyl-L-histidine) and other histidine-containing dipeptides (HCDs) such as anserine and *N*-acetylcarnosine, as well as their components (eg: β-alanine) have been proposed as potential strategies for the prevention of chronic diseases [3]. Carnosine, a dipeptide and the "founding member" of the HCD family of soluble peptides, is found naturally in mammalian brain tissue and skeletal and heart muscle [4]. It is synthesised by the enzyme carnosine synthase and degraded by carnosinase [5]. The primary source of carnosine has traditionally been via dietary intake of meat and fish, with varying amounts depending on the type of meat or fish and cooking method [6 7]. However, due to increased production and consumption of processed meats, the amount of carnosine derived from the modern diet is limited [4 5]. In addition, consumption of processed meat has been linked to negative health effects [8 9]. Over-the-counter carnosine supplementation may therefore be a more ideal source of carnosine.

It is thought that carnosine and HCDs may prevent chronic diseases via their anti-inflammatory, anti-oxidative, anti-glycating, anti-ischemic, and chelating properties [10 11].

Cell culture studies have reported that carnosinase content and activity were increased in hyperglycaemia [12], and that polymorphisms in the carnosinase gene (*CNDP1*) predicted progression to end-stage renal disease in patients with type 1 diabetes and diabetic nephropathy [13]. In cancer cell lines, carnosine suppressed tumorigenesis by inhibiting proliferation and inducing apoptosis in human glioblastoma cells as well as colorectal and ovarian carcinoma cells [14-17]. Evidence from animal studies has shown that carnosine supplementation reduced insulin resistance [10] and plasma concentrations of glucose, lipids, and inflammatory markers [11 18], and delayed the development of atherosclerosis [19]. In mouse/rat models, carnosine rescued cognitive decline in Alzheimer's disease [20], and reduced the size of ischemia in various organs including the heart, brain, liver, and kidney [21-25]. Moreover, both carnosine and *N*-acetylcarnosine were shown to delay the development of lens opacification leading to cataracts [26 27].

In humans, observational studies by our group [28 29] and others [30 31] have shown that muscle carnosine content is higher in drug naïve patients with T2DM, and is associated with obesity, insulin resistance, and progressive impairment of glucose tolerance. However, other studies have reported lower muscle carnosine content in patients with T2DM but not T1DM [30]. The primary hypothesis, which is yet to be confirmed, is that carnosine levels increase in an adaptive mechanism to counteract the increased inflammation and oxidative stress present in obesity and T2DM; however, muscle carnosine storage may decline with disease progression or under certain pathological conditions where oxidative stress and glycation are exacerbated [28-30].

Human randomized controlled trials (RCTs) have shown that supplementation with carnosine (0.5 to 2g) or β-alanine (1 to 6g) daily for 1 to 6 months improved a range of outcomes

related to chronic diseases including: cognition and exercise capacity in young and elderly healthy adults [32-36]; physical performance and quality of life in patients with heart failure [37]; glucose metabolism in overweight or obese non-diabetic and prediabetic individuals [38 39]; plasma glucose, haemoglobin A1c (HbA1c), lipid levels, and urinary albumin-creatinine ratio in diabetic nephropathy [40]; and neurological outcomes including balance (foot up and go test) and locomotion (rigidity of extremities and hand movements) in elderly adults and patients with Parkinson's disease [33 41]. In a recent pilot clinical trial by our group [38], we showed that 2g daily of pure carnosine supplementation for 12 weeks reduced insulin resistance, measured by homeostatic model assessment (HOMA), compared with placebo. Similarly, two other RCTs found improved glucose metabolism following supplementation with carnosine combined with chromium and cinnamon [39] or with α-lipoic acid, zinc, and B vitamins [42].

Although current evidence supports a potential role for carnosine and related HCDs in the prevention of chronic diseases, no previous reviews have investigated the effect of supplementation with carnosine or HCDs on chronic disease risk factors and outcomes. Previous systematic reviews [43-45] and meta-analyses [36] have focused only on the effects of carnosine and HCDs on exercise performance, despite a number of newly published studies suggesting a broader role for these dipeptides. We aim to address this knowledge gap by conducting a comprehensive systematic review of RCTs to synthesize current evidence regarding the effects of carnosine and HCD supplementation on chronic diseases risk factors and outcomes.

SYSTEMATIC REVIEW QUESTION

Is carnosine and/or HCD supplementation effective in improving chronic disease risk factors and outcomes compared to placebo, usual care, or other pharmacological or non-pharmacological interventions?

METHODS/ DESIGN

This systematic review will employ rigorous gold-standard methodology and will conform to the reporting standards of the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA; Supplementary Table 1). A search strategy with key words has been developed (Supplementary Table 2) and the protocol has been registered on PROSPERO: CRD42017075354.

Eligibility Criteria

The PICO (Population, Intervention, Comparison, Outcomes) framework in Table 1 established *a priori* will be used as a tool for determining the eligibility of articles.

Table 1. PICO for study inclusion

	Participants (P)	**Intervention (I)**	**Comparison (C)**	**Outcomes (O)**
Inclusion	Males and females of any age, ethnicity, geographic area, comorbidities or medication use	Carnosine and related HCDs (beta-alanine, anserine, NAC, etc) supplementation administered in any form (intravenous, intramuscular, or oral), alone (pure) or combined with other intervention/s, of any dosage, and for any duration	Placebo or usual care or any pharmacological or non-pharmacological interventions	All chronic disease risk factors and outcomes including but not limited to metabolic/glycaemic, cardiovascular, and cognitive/ mental health risk factors and outcomes
Exclusion	Studies not in humans	Studies without carnosine and/or HCD supplementation	Studies with no control group	None
Study type	RCTs and systematic reviews of RCTs			
Language	No limit			
Year of publication	No limit			

HCDs: histidine-containing dipeptides; **NAC:** *N*-acetylcarnosine; **RCT:** randomized controlled trial.

Search Strategy

The following electronic databases will be used to identify relevant literature based on the systematic search strategy developed using relevant search terms (Supplementary Table 2):

- MEDLINE via OVID
- MEDLINE in process and other non-indexed citations via OVID
- CINAHL
- EMBASE via OVID
- All Evidence Based Medicine (EBM) Reviews via OVID incorporating:
  - Cochrane Database of Systematic Reviews
  - ACP Journal Club
  - Database of Abstracts of Reviews of Effects
  - Cochrane Central Register of Controlled Trials
  - Cochrane Methodology Register
  - Health Technology Assessment
  - NHS Economic Evaluation Database

Bibliographies of relevant studies as well as systematic reviews identified by the search strategy will be screened for identification of additional studies. Where required data are not presented, the corresponding authors of included studies will be contacted to provide de-identified aggregate data for the purpose of meta-analyses if necessary. Clinical trials registries including the National Institute of Health Clinical Trials Register (https://clinicaltrials.gov/) and the Australian New Zealand Clinical Trials (ANZCTR) registries (https://www.anzctr.org.au) will also be searched to identify unpublished trials and grey literature.

Study Selection

Two reviewers (KM and AM) will review the titles, abstracts, and keywords of every article retrieved by the search strategy using the selection criteria described in Table 1. Disagreement between reviewers regarding the eligibility of articles will be resolved by discussion. Where consensus is not reached, a third reviewer (BdC) will be consulted. Full

text articles will be retrieved for further assessment if the information given suggests that the study meets the selection criteria or if there is doubt regarding the eligibility of the study based on the title and abstract. Any full text articles excluded will be tabulated with reasons for their exclusion.

Quality appraisal of the evidence

Methodological quality of the included studies will be assessed at the study-level by two reviewers (KM and AM) using the Monash Centre for Health Research and Implementation critical appraisal tool [46]. Individual quality items will be investigated using a descriptive component approach which will consider methods of randomisation and allocation of participants to study groups; blinding of participants, investigators and outcome assessors; methods of outcome assessment and reporting; attrition rates; conflicts of interest of authors; presence of pre-specified selection criteria and statistical issues including powering and methods of data analysis. This process will allow each study to be allocated a risk of bias rating. Disagreement among reviewers will be resolved by discussion to reach a consensus.

Data extraction

Data for outcomes of interest based on the selection criteria (Table 1) will be extracted from all the included studies by two reviewers (KM and AM) using a specially developed data extraction form. The data extracted will include general details of the study design and setting; participant characteristics; mean values, standard deviations (SDs), and confidence intervals of the outcomes; point estimates and measures of variability; frequency counts for dichotomous variables; numbers of participants; and intention-to-treat analysis. Both reviewers will check all computed data entries for meta-analysis if applicable.

Grading the body of evidence

Quality of the evidence for the effects of carnosine and/or HCD supplementation on health outcomes will be assessed at the outcome-level and will be graded as high, moderate, low, or very low using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach [47]. Quality of the evidence will be graded by two independent reviewers (AM and KM) based on risk of bias, inconsistence between studies, indirectness, imprecision and publication bias. In line with PRISMA guidelines, quality will be reported at both the study- and outcome-levels where appropriate, and disagreement will be resolved by consensus.

Data Analysis and Synthesis

Data will be presented in summary tables and descriptive text to describe the study populations, interventions, and outcomes of the studies included in the review. Aggregate effect measures will be used for meta-analyses for trials deemed homogenous with regard to participants, interventions, and outcomes. Meta-analyses will be performed using DerSimonian and Laird random effects models in Review Manager V5. Dichotomous results will be presented as relative risks or odds ratios with 95% confidence intervals (CI), while continuous results will be presented as weighted mean difference with 95% CIs. Where outcomes have been measured using different tools or methods, or where results vary substantially, standardized mean differences with 95% CIs will be presented. Statistical heterogeneity will be assessed using the I^2 test where values over 50% will indicate moderate to high heterogeneity. Descriptive analysis will be conducted for studies that are found to be clinically heterogeneous or present insufficient information for pooling. A two-tailed p-value of <0.05 will be considered statistically significant.

Subgroup and Sensitivity Analyses

Subgroup analyses, and where possible, meta-regression will be performed on factors that are assumed to cause variation in the outcomes of interest, and these may include age, gender, body mass index, duration, dose, and route of supplementation, type of carnosine or HCD used, participant disease status, medications, and study duration.

Sensitivity analyses will be performed if indicated to explore the influence of certain factors or studies on the effect size, which will be determined during the review process. Studies contributing to high heterogeneity $I^2 > 50\%$ or those with high risk of bias will be excluded through sensitivity analysis to examine their influence on the results. Where there are sufficient numbers of studies, visual inspection of funnel plots and Egger and Begg [48 49] statistical tests will be used to determine small study effects and publication bias. If applicable, meta-regression and publication bias assessments will be examined on the Comprehensive Meta-analysis software V.3., and p-values <0.05 will be considered statistically significant.

DISCUSSION

Interventions aimed at reducing chronic disease risk factors including obesity, insulin resistance, hypertension, dyslipidaemia, and inflammation, among others, are vital to addressing the growing burden of chronic disease. Although carnosine and HCDs are proposed to have anti-inflammatory, anti-oxidative, and anti-glycating properties, the efficacy of carnosine and HCD supplementation in the prevention of chronic disease risk factors and outcomes has not been established.

Here, we will conduct the first systematic review examining the effects of supplementation with carnosine and/or other HCDs on chronic disease risk factors and outcomes. Using rigorous methodology (PRISMA guidelines, PICO framework, GRADE approach), pre-specified criteria, and a pre-determined search strategy, this review will synthesize all

existing RCT data to establish the effects of carnosine and HCD supplementation on a broad range of risk factors and outcomes related to chronic diseases including T2DM, CVD, cancers, neurodegenerative disorders, and others. By systematically reviewing and appraising the literature, we will also identify relevant knowledge gaps and uncertainties, thereby providing a platform for future studies in this field. However, although our search will endeavour to identify grey literature, potential publication bias cannot be ruled out as there may be unpublished data not accounted for.

Nevertheless, this review will generate important insights regarding the potential use of carnosine and HCD supplementation for the prevention of chronic diseases. If carnosine and/or HCDs are shown to be effective in reducing chronic disease risk factors and/or outcomes upon review and meta-analysis, this would generate level-one evidence of efficacy with considerable clinical and public health implications.

ETHICS & DISSEMINATION

This study does not require ethical approval as it does not involve primary data collection. Findings from this review regarding the effects of carnosine supplementation on chronic disease risk factors and outcomes will be disseminated through peer reviewed publications and at conference meetings, to inform future research on the use of carnosine supplementation for the prevention of chronic diseases.

ACKNOWLEDGEMENTS

AM is a recipient of the Australian Postgraduate Award Scholarship provided by Monash University. BdC is supported by a National Heart Foundation Future Leader Fellowship (100864).

FUNDING

This study received no specific external funding.

AUTHOR CONTRIBUTIONS

KM developed the search strategy, wrote the first draft of the review protocol, and will contribute to data collection and analysis. AM contributed to the design and scope of the search strategy, revised and edited the manuscript, and will contribute to data collection and analysis. BdC determined the design and scope of the review, revised and edited the manuscript, will supervise the review process, and is the guarantor of the review.

COMPETING INTERESTS

None declared.

REFERENCES

1. WHO. The World Health Report 2002: Reducing Risks, Promoting Healthy Life. Geneva: World Health Organization, 2002.
2. WHO. Global Status Report on noncommunicable diseases 2014. Geneva: World Health Organization, 2014.
3. Xie Z, Baba SP, Sweeney BR, et al. Detoxification of aldehydes by histidine-containing dipeptides: from chemistry to clinical implications. *Chemico-biological interactions* 2013;202(1-3):288-97. doi: 10.1016/j.cbi.2012.12.017 [published Online First: 2013/01/15]
4. Guiotto A, Calderan A, Ruzza P, et al. Carnosine and carnosine-related antioxidants: a review. *Curr Med Chem* 2005;12(20):2293-315. [published Online First: 2005/09/27]
5. Boldyrev AA, Aldini G, Derave W. Physiology and pathophysiology of carnosine. *Physiol Rev* 2013;93(4):1803-45. doi: 10.1152/physrev.00039.2012 [published Online First: 2013/10/19]
6. Abe H. Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. *Biochemistry (Mosc)* 2000;65(7):757-65. [published Online First: 2000/08/22]
7. Sale C, Artioli GG, Gualano B, et al. Carnosine: from exercise performance to health. *Amino Acids* 2013;44(6):1477-91. doi: 10.1007/s00726-013-1476-2 [published Online First: 2013/03/13]
8. Micha R, Wallace SK, Mozaffarian D. Red and processed meat consumption and risk of incident coronary heart disease, stroke, and diabetes mellitus: a systematic review and meta-analysis. *Circulation* 2010;121(21):2271-83. doi: 10.1161/circulationaha.109.924977 [published Online First: 2010/05/19]
9. Sinha R, Cross AJ, Graubard BI, et al. Meat intake and mortality: A prospective study of over half a million people. *Archives of Internal Medicine* 2009;169(6):562-71. doi: 10.1001/archinternmed.2009.6
10. Aldini G, Orioli M, Rossoni G, et al. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. *J Cell Mol Med* 2011;15(6):1339-54. doi: 10.1111/j.1582-4934.2010.01101.x [published Online First: 2010/06/04]
11. Lee YT, Hsu CC, Lin MH, et al. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. *Eur J Pharmacol* 2005;513(1-2):145-50. doi: 10.1016/j.ejphar.2005.02.010 [published Online First: 2005/05/10]
12. Riedl E, Koeppel H, Brinkkoetter P, et al. A CTG polymorphism in the CNDP1 gene determines the secretion of serum carnosinase in Cos-7 transfected cells. *Diabetes* 2007;56(9):2410-3. doi: 10.2337/db07-0128 [published Online First: 2007/07/03]
13. Alkhalaf A, Bakker SJL, Bilo HJG, et al. A polymorphism in the gene encoding carnosinase (CNDP1) as a predictor of mortality and progression from nephropathy to end-stage renal disease in type 1 diabetes mellitus. *Diabetologia* 2010;53(12):2562-68. doi: 10.1007/s00125-010-1863-0
14. Iovine B, Guardia F, Irace C, et al. l-carnosine dipeptide overcomes acquired resistance to 5-fluorouracil in HT29 human colon cancer cells via downregulation of HIF1-alpha and induction of apoptosis. *Biochimie* 2016;127:196-204. doi: 10.1016/j.biochi.2016.05.010 [published Online First: 2016/05/29]
15. Iovine B, Oliviero G, Garofalo M, et al. The Anti-Proliferative Effect of L-Carnosine Correlates with a Decreased Expression of Hypoxia Inducible Factor 1 alpha in

Human Colon Cancer Cells. *PLoS ONE* 2014;9(5):e96755. doi: 10.1371/journal.pone.0096755

16. Mikula-Pietrasik J, Ksiazek K. L-Carnosine Prevents the Pro-cancerogenic Activity of Senescent Peritoneal Mesothelium Towards Ovarian Cancer Cells. *Anticancer research* 2016;36(2):665-71. [published Online First: 2016/02/07]
17. Rybakova YS, Kalen AL, Eckers JC, et al. Increased manganese superoxide dismutase and cyclin B1 expression in carnosine-induced inhibition of glioblastoma cell proliferation. *Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry* 2015;9(1):63-71. doi: 10.1134/S1990750815010096
18. Bao Y, Gao C, Hao W, et al. Effects of Dietary L-carnosine and Alpha-lipoic Acid on Growth Performance, Blood Thyroid Hormones and Lipid Profiles in Finishing Pigs. *Asian-Australas J Anim Sci* 2015;28(10):1465-70. doi: 10.5713/ajas.14.0604 [published Online First: 2015/07/22]
19. Brown BE, Kim CH, Torpy FR, et al. Supplementation with carnosine decreases plasma triglycerides and modulates atherosclerotic plaque composition in diabetic apo E(-/-) mice. *Atherosclerosis* 2014;232(2):403-9. doi: 10.1016/j.atherosclerosis.2013.11.068 [published Online First: 2014/01/29]
20. Herculano B, Tamura M, Ohba A, et al. beta-alanyl-L-histidine rescues cognitive deficits caused by feeding a high fat diet in a transgenic mouse model of Alzheimer's disease. *Journal of Alzheimer's disease : JAD* 2013;33(4):983-97. doi: 10.3233/jad-2012-121324 [published Online First: 2012/10/27]
21. Dursun N, Taskin E, Ozturk F. Protection against adriamycin-induced cardiomyopathy by carnosine in rats: role of endogenous antioxidants. *Biological trace element research* 2011;143(1):412-24. doi: 10.1007/s12011-010-8875-y [published Online First: 2010/10/14]
22. Dobrota D, Fedorova T, Stvolinsky S, et al. Carnosine protects the brain of rats and Mongolian gerbils against ischemic injury: after-stroke-effect. *Neurochemical research* 2005;30(10):1283-8. doi: 10.1007/s11064-005-8799-7 [published Online First: 2005/12/13]
23. Fouad AA, El-Rehany MA, Maghraby HK. The hepatoprotective effect of carnosine against ischemia/reperfusion liver injury in rats. *European journal of pharmacology* 2007;572(1):61-8. doi: 10.1016/j.ejphar.2007.06.010 [published Online First: 2007/07/06]
24. Fujii T, Takaoka M, Muraoka T, et al. Preventive effect of L-carnosine on ischemia/reperfusion-induced acute renal failure in rats. *European journal of pharmacology* 2003;474(2-3):261-7. [published Online First: 2003/08/19]
25. Rajanikant GK, Zemke D, Senut MC, et al. Carnosine is neuroprotective against permanent focal cerebral ischemia in mice. *Stroke* 2007;38(11):3023-31. doi: 10.1161/strokeaha.107.488502 [published Online First: 2007/10/06]
26. Yan H, Guo Y, Zhang J, et al. Effect of carnosine, aminoguanidine, and aspirin drops on the prevention of cataracts in diabetic rats. *Mol Vis* 2008;14:2282-91. [published Online First: 2008/12/17]
27. Mark AB, Yegor EY. Advanced Drug Delivery of N-Acetylcarnosine (N-Acetyl-beta-alanyl-Lhistidine),Carcinine (Beta-alanylhistamine) and L-carnosine (Beta-alanyl-L-histidine) in Targeting Peptide Compounds as Pharmacological Chaperones for Use in Tissue Engineering, Human Disease Management and Therapy: From in vitro to the Clinic. *Recent Patents on Drug Delivery & Formulation* 2010;4(3):198-230. doi: http://dx.doi.org.ezproxy.lib.monash.edu.au/10.2174/187221110793237547

28. de Courten B, Kurdiova T, de Courten MPJ, et al. Muscle Carnosine Is Associated with Cardiometabolic Risk Factors in Humans. *PLOS ONE* 2015;10(10):e0138707. doi: 10.1371/journal.pone.0138707
29. Stegen S, Everaert I, Deldicque L, et al. Muscle histidine-containing dipeptides are elevated by glucose intolerance in both rodents and men. *PLoS One* 2015;10(3):e0121062. doi: 10.1371/journal.pone.0121062 [published Online First: 2015/03/25]
30. Gualano B, Everaert I, Stegen S, et al. Reduced muscle carnosine content in type 2, but not in type 1 diabetic patients. *Amino Acids* 2012;43(1):21-4. doi: 10.1007/s00726-011-1165-y [published Online First: 2011/11/29]
31. Srikanthan P, Singhal A, Lee CC, et al. Characterization of Intra-myocellular Lipids using 2D Localized Correlated Spectroscopy and Abdominal Fat using MRI in Type 2 Diabetes. *Magn Reson Insights* 2012;5:29-36. doi: 10.4137/mri.s10489 [published Online First: 2013/03/09]
32. Hoffman JR, Landau G, Stout JR, et al. beta-Alanine ingestion increases muscle carnosine content and combat specific performance in soldiers. *Amino Acids* 2015;47(3):627-36. doi: 10.1007/s00726-014-1896-7 [published Online First: 2014/12/17]
33. Szczesniak D, Budzen S, Kopec W, et al. Anserine and carnosine supplementation in the elderly: Effects on cognitive functioning and physical capacity. *Arch Gerontol Geriatr* 2014;59(2):485-90. doi: 10.1016/j.archger.2014.04.008 [published Online First: 2014/06/02]
34. Baraniuk JN, El-Amin S, Corey R, et al. Carnosine treatment for gulf war illness: a randomized controlled trial. *Glob J Health Sci* 2013;5(3):69-81. doi: 10.5539/gjhs.v5n3p69 [published Online First: 2013/04/27]
35. del Favero S, Roschel H, Solis MY, et al. Beta-alanine (Carnosyn) supplementation in elderly subjects (60-80 years): effects on muscle carnosine content and physical capacity. *Amino Acids* 2012;43(1):49-56. doi: 10.1007/s00726-011-1190-x [published Online First: 2011/12/07]
36. Hobson RM, Saunders B, Ball G, et al. Effects of beta-alanine supplementation on exercise performance: a meta-analysis. *Amino Acids* 2012;43(1):25-37. doi: 10.1007/s00726-011-1200-z [published Online First: 2012/01/25]
37. Lombardi C, Carubelli V, Lazzarini V, et al. Effects of oral administration of orodispersible levo-carnosine on quality of life and exercise performance in patients with chronic heart failure. *Nutrition* 2015;31(1):72-8. doi: 10.1016/j.nut.2014.04.021 [published Online First: 2014/10/08]
38. de Courten B, Jakubova M, de Courten MP, et al. Effects of carnosine supplementation on glucose metabolism: Pilot clinical trial. *Obesity (Silver Spring)* 2016;24(5):1027-34. doi: 10.1002/oby.21434 [published Online First: 2016/04/05]
39. Liu Y, Cotillard A, Vatier C, et al. A Dietary Supplement Containing Cinnamon, Chromium and Carnosine Decreases Fasting Plasma Glucose and Increases Lean Mass in Overweight or Obese Pre-Diabetic Subjects: A Randomized, Placebo-Controlled Trial. *PLoS One* 2015;10(9):e0138646. doi: 10.1371/journal.pone.0138646 [published Online First: 2015/09/26]
40. Elbarbary NS, Ismail EAR, El-Naggar AR, et al. The effect of 12 weeks carnosine supplementation on renal functional integrity and oxidative stress in pediatric patients with diabetic nephropathy: a randomized placebo-controlled trial. *Pediatric diabetes* 2017 doi: 10.1111/pedi.12564 [published Online First: 2017/07/27]

41. Boldyrev A, Fedorova T, Stepanova M, et al. Carnosine [corrected] increases efficiency of DOPA therapy of Parkinson's disease: a pilot study. *Rejuvenation Res* 2008;11(4):821-7. doi: 10.1089/rej.2008.0716 [published Online First: 2008/08/30]
42. Derosa G, D'Angelo A, Romano D, et al. A Clinical Trial about a Food Supplement Containing α-Lipoic Acid on Oxidative Stress Markers in Type 2 Diabetic Patients. *International Journal of Molecular Sciences* 2016;17(11):1802. doi: 10.3390/ijms17111802
43. Berti Zanella P, Donner Alves F, Guerini de Souza C. Effects of beta-alanine supplementation on performance and muscle fatigue in athletes and non-athletes of different sports: a systematic review. *J Sports Med Phys Fitness* 2017;57(9):1132-41. doi: 10.23736/s0022-4707.16.06582-8 [published Online First: 2016/07/06]
44. Saunders B, Elliott-Sale K, Artioli GG, et al. beta-alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *Br J Sports Med* 2017;51(8):658-69. doi: 10.1136/bjsports-2016-096396 [published Online First: 2016/11/01]
45. Quesnele JJ, Laframboise MA, Wong JJ, et al. The effects of beta-alanine supplementation on performance: a systematic review of the literature. *Int J Sport Nutr Exerc Metab* 2014;24(1):14-27. doi: 10.1123/ijsnem.2013-0007 [published Online First: 2013/08/07]
46. MCHRI. Evidence Synthesis Program templates for critical appraisal and risk of bias (adapted from Critical Appraisal Templates, Centre for Clinical Effectiveness ,Southern Health ,Melbourne,2010): Monash University and Monash Health 2013.
47. Grading quality of evidence and strength of recommendations. *BMJ* 2004;328(7454):1490. doi: 10.1136/bmj.328.7454.1490
48. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994;50(4):1088-101. [published Online First: 1994/12/01]
49. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *Bmj* 1997;315(7109):629-34. [published Online First: 1997/10/06]

Supplementary Table 1. PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page Number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2 and 6
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	11 – 12
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	11
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3 – 5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	5
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Table 1
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	6 – 7 and Table 1
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Supplementary Table 2

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8 – 9
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7 – 9
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7 – 8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8 and Table 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8 and Table 1
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8 – 9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	9
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	9 – 10
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	9
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9 – 10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8 – 9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

Supplementary Table 2. Sample OVID-MEDLINE search strategy

1. Carnosine/	36. or/30-35
2. carnosine.mp.	37. cochrane.ab.
3. beta alanylhistidine.mp.	38. embase.ab.
4. Anserine/	39. (psychlit or psyclit).ab.
5. anserine.mp.	40. (psychinfo or psycinfo).ab.
6. beta alanyl 3 methylhistidine.mp.	41. (cinahl or cinhal).ab.
7. ophidine.mp.	42. science citation index.ab.
8. exp beta-alanine/	43. bids.ab.
9. beta alanine*.mp.	44. cancerlit.ab.
10. 3 aminopropionic acid.mp.	45. or/37-44
11. N-Acetyl-Carnosine.mp.	46. reference list$.ab.
12. N-Acetyl-L-Carnosine.mp.	47. bibliograph$.ab.
13. beta alanyl l histidine.mp.	48. hand-search$.ab.
14. beta-ala-his.mp.	49. relevant journals.ab.
15. l histidine beta alanyl.mp.	50. manual search$.ab.
16. l alpha alanyl l histidine.mp.	51. or/46-50
17. histidine.mp.	52. selection criteria.ab.
18. balenine.mp.	53. data extraction.ab.
19. or/1-18	54. 52 or 53
20. randomi?ed controlled trial.pt.	55. Review/
21. controlled clinical trial.pt.	56. 54 and 55
22. randomi?ed.ti,ab.	57. Comment/
23. placebo.ti,ab.	58. Letter/
24. clinical trials as topic.sh.	59. Editorial/
25. randomly.ti,ab.	60. animal/
26. trial.ti.	61. human/
27. or/20-26	62. 60 not (60 and 61)
28. exp animals/ not exp humans/	63. or/57-59,62
29. 27 not 28	64. 36 or 45 or 51 or 56
30. Meta-Analysis as Topic/	65. 64 not 63
31. meta analy$.tw.	66. 27 or 65
32. metaanaly$.tw.	67. 19 and 66
33. Meta-Analysis/	68. limit 67 to humans
34. (systematic adj (review$1 or overview$1)).tw.	
35. exp Review Literature as Topic/	

Research Checklist: PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item	Page Number
ADMINISTRATIVE INFORMATION			
Title:			
Identification	1a	Identify the report as a protocol of a systematic review	1
Update	1b	If the protocol is for an update of a previous systematic review, identify as such	N/A
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number	2 and 6
Authors:			
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author	1
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review	11 – 12
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments	N/A
Support:			
Sources	5a	Indicate sources of financial or other support for the review	11
Sponsor	5b	Provide name for the review funder and/or sponsor	N/A
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol	N/A
INTRODUCTION			
Rationale	6	Describe the rationale for the review in the context of what is already known	3 – 5
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)	5
METHODS			
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review	Table 1
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage	6 – 7 and Table 1
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated	Supplementary Table 2

Study records:			
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review	8 – 9
Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)	7 – 9
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators	7 – 8
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications	8 and Table 1
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale	8 and Table 1
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis	8 – 9
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised	9
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)	9
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)	9 – 10
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned	9
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)	9 – 10
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)	8 – 9

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.