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 randomised controlled trials

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To cite: Menon K, Mousa A, de Courten B. Effects of supplementation with carnosine and other histidine-containing dipeptides on chronic disease risk factors and outcomes: protocol for a systematic review of randomised controlled trials. *BMJ Open* 2018;**8**:e020623. doi:10.1136/bmjopen-2017-020623

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2017-020623).

Received 13 November 2017
Revised 16 February 2018
Accepted 23 February 2018

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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, antioxidative and antiglycating 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, Cumulative Index of Nursing and Allied Health, Embase and all Evidence-Based Medicine will be systematically searched to identify randomised 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 prespecified inclusion criteria, after which two independent reviewers will perform data extraction and quality appraisal. Meta-analyses, metaregression and subgroup 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 and limitations of this study

- This protocol is for the first systematic review to investigate the effects of supplementation with carnosine and other histidine-containing peptides (HCDs) on chronic disease risk factors and outcomes.
- We employ rigorous international gold-standard methodology including Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, a Population, Intervention, Comparison, Outcomes framework and the Grading of Recommendations Assessment, Development and Evaluation 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, antioxidative, antiglycating, anti-ischaemic 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 tumourigenesis 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 ischaemia 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 type 2 diabetes mellitus (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 randomised controlled trials (RCTs) have shown that supplementation with carnosine (0.5–2g) or β-alanine (1–6g) 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, 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 synthesise 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 with 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; see online supplementary table 1). A search strategy with keywords has been developed (see online supplementary table 2) and the protocol has been registered on Prospective Register of Systematic Review Protocols: CRD42017075354.

Eligibility criteria

The Population, Intervention, Comparison, Outcomes (PICO) 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	Men and women 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; PICO, Population, Intervention, Comparison, Outcomes framework; RCT, randomised 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 (see online supplementary table 2):

- Medline via Ovid;
- Medline in process and other non-indexed citations via Ovid;
- Cumulative Index of Nursing and Allied Health;
- Embase via Ovid;
- All Evidence-Based Medicine Reviews via Ovid incorporating:
  - Cochrane Database of Systematic Reviews;
  - American College of Physicians (ACP) Journal Club;
  - Database of Abstracts of Reviews of Effects;
  - Cochrane Central Register of Controlled Trials;
  - Cochrane Methodology Register;
  - Health Technology Assessment;
  - National Health Service 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 deidentified 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 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 article excluded will be tabulated with reasons for its 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 prespecified 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, SDs and CIs 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 V.5. Dichotomous results will be presented as relative risks or ORs with 95% CIs, 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, standardised 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, metaregression 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, antioxidative and antiglycating 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), prespecified criteria and a predetermined search strategy, this review will synthesise 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 on review and meta-analysis, this would generate level-one evidence of efficacy with considerable clinical and public health implications.

ETHICS AND 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).

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

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Detail has been removed from this case description/these case descriptions to ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Provenance and peer review Not commissioned; externally peer reviewed.

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