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Saadati, Saeede, Kabthymmer, Robel Hussen, Aldini, G, Mousa, Aya, Feehan, Jack and de Courten, Barbora (2023) Effects of carnosine and histidine-containing dipeptides on biomarkers of inflammation and oxidative stress: a systematic review and meta-analysis. *Nutrition reviews*. ISSN 0029-6643

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Effects of carnosine and histidine-containing dipeptides on biomarkers of inflammation and oxidative stress: a systematic review and meta-analysis

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Context: Carnosine and histidine-containing dipeptides (HCDs) are suggested to have anti-inflammatory and antioxidative benefits, but their effects on circulating adipokines and inflammatory and oxidative stress biomarkers remain unclear.

Objectives: The aim of the present systematic review and meta-analysis was to determine the impact of HCD supplementation on inflammatory and oxidative stress biomarkers. **Data Sources:** A systematic search was performed on Medline via Ovid, Scopus, Embase, ISI Web of Science, and the Cochrane Library databases from inception to 25 January 2023. **Data Extraction:** Using relevant key words, trials investigating the effects of carnosine/HCD supplementation on markers of inflammation and oxidative stress, including C-reactive protein (CRP), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), adiponectin, malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), total antioxidant capacity (TAC), and catalase (CAT) were identified. Meta-analyses were conducted using random-effects models to calculate the weighted mean differences (WMDs) and 95% confidence intervals (CIs). **Data Analysis:** A total of 9 trials comprising 350 participants were included in the present meta-analysis. Carnosine/HCD supplementation led to a significant reduction in CRP (WMD: -0.97 mg/L; 95% CI: $-1.59, -0.36$), TNF- α (WMD: -3.60 pg/mL; 95% CI: $-7.03, -0.18$), and MDA (WMD: -0.34 μ mol/L; 95% CI: $-0.56, -0.12$) and an elevation in CAT (WMD: 4.48 U/mL; 95% CI: $2.43, 6.53$) compared with placebo. In contrast, carnosine/HCD supplementation had no effect on IL-6, adiponectin, GSH, SOD, and TAC levels. **Conclusion:** Carnosine/HCD supplementation may reduce inflammatory and oxidative stress biomarkers, and potentially modulate the cardiometabolic risks associated with chronic low-grade inflammation and lipid peroxidation. **Systematic Review Registration:** PROSPERO registration no. CRD42017075354.

Key words: carnosine, histidine-containing dipeptide, inflammation, meta-analysis, oxidative stress.

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INTRODUCTION

Inflammation and oxidative stress are involved in the pathogenesis of a myriad of chronic diseases, including obesity, type 2 diabetes, atherosclerosis and other cardiovascular disorders depression, and chronic liver and kidney disease.^{1–5} Chronic low-grade inflammation is common in obesity, and contributes to endothelial dysfunction and impaired insulin secretion through β -cell dysfunction.^{6,7} Similarly, oxidative stress, characterized by elevated free radicals and reactive oxygen species (ROS), contributes to insulin resistance, micro- and macrovascular diabetes-related complications, as well as impaired glucose tolerance.^{8–11} These processes are interrelated, with inflammation leading to high oxidative stress in a positive feedback loop.¹² Moreover, when the levels of oxidative stress are high, endogenous antioxidants, including catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), may not be able to stop the overproduction of ROS that can harm cellular proteins, lipids, and DNA/RNA, leading to cell death and the development of chronic diseases.¹³

Although anti-inflammatory and antioxidant medications are available, primary prevention methods remain essential to mitigate the growing burden of chronic disease. Previous evidence has demonstrated the anti-inflammatory and antioxidative properties of histidine-containing dipeptides (HCDs) in different chronic conditions.^{14–16} HCDs are a group of soluble peptides and its founding member, carnosine (β -alanine L-histidine), is either produced naturally in the mammalian heart, skeletal muscle, brain tissue, and kidneys or naturally from food, as well as through dietary supplementation.^{14,17} Carnosine has been extensively studied in both animal models^{18–21} and human clinical trials^{22–27} in the context of several disease pathologies.

Carnosine could be an effective strategy for ameliorating oxidative stress via its well-established antioxidant activity, which can be attributed to a direct radical scavenging activity and detoxifying effect towards radical and oxidizing species, metal chelating effects,^{20,28,29} and as recently found, by activating the expression of members of the endogenous antioxidant system (nuclear factor erythroid 2-related factor 2 [Nrf2] pathway).³⁰ Indeed, the administration of 20 mg/100 g of carnosine for 4 weeks was shown to enhance serum levels of glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) in rats with streptozotocin-induced diabetes.³¹ In addition, carnosine supplementation improved the activities of GSH-PX and SOD and decreased malondialdehyde (MDA) formation and ethanol-induced oxidative damage in Wistar rats,³² Sprague–Dawley rats,³³ and Balb/cA mice.³⁴ Carnosine has also been shown to alleviate inflammation through

its direct effects on modulating inflammatory cytokine production in mice.^{35,36}

However, data from human studies are notably inconsistent. While some studies support the use of carnosine for reducing markers of oxidative stress and improving antioxidant status,^{37,38} others suggest that carnosine has no effect on advanced glycation end-products (AGEs) or precursors of advanced lipoxidation end-products (ALEs), such as 4-hydroxynonenal (4-HNE) and MDA.^{39,40} The effect of carnosine on proinflammatory cytokines is also controversial. One gram per day of carnosine supplementation for 12 weeks resulted in decreased levels of tumor necrosis factor α (TNF- α) with no significant effects on interleukin (IL)-6 (IL-6).²³ However, 12-week histidine supplementation in women with obesity suppressed inflammation through reduction in TNF- α and IL-6 levels.⁴¹

The effects of carnosine/HCDs on inflammatory and oxidative stress status have not been previously synthesized. In addition, since the related evidence has had conflicting results, this study aimed to address this knowledge gap by conducting a comprehensive systematic review and meta-analysis of existing randomized controlled trials (RCTs) investigating the effects of carnosine and other HCDs on inflammatory and oxidative stress biomarkers.

METHODS

The protocol for the present review was developed a priori, preregistered on PROSPERO (CRD42017075354), and published previously.⁴² This review conforms to the updated 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.⁴³

Data sources and searches

The electronic databases such as Medline via Ovid, Scopus, Embase, ISI Web of Science, and the Cochrane Library from inception to 25 January 2023 were systematically searched to identify relevant studies. Databases were searched using medical subject headings (MeSH) and non-MeSH terms, which are shown in [Table S1](#) (see the Supporting Information online). No restrictions were applied in terms of language or year of publication. The reference lists of eligible studies were manually searched for the identification of additional studies. Google Scholar was also used to manually search for grey literature (ie, studies not included in scientific databases). The deidentified aggregate data for the purpose of meta-analysis were requested if the necessary data were not reported (maximum of 3 e-mail attempts).

Study selection

A systematic review management platform (Covidence; Veritas Health Innovation Ltd) was used to import all the titles and abstracts of the papers from the searches. Duplicates were subsequently removed, and the remaining articles were checked for potential eligibility. Two independent reviewers (S.S. and R.H.K.) screened the titles and abstracts of each article found during the initial search. Full texts were then retrieved for all papers that seemed to fulfill the inclusion criteria. Any disagreement regarding the eligibility of the studies was resolved by discussion with a third reviewer (A.M.).

Eligibility criteria

Studies that met the selection criteria under a predetermined PICOS (Population, Intervention, Comparison, Outcomes, and Study design) framework, as indicated in Table 1, were considered eligible. In brief, the following eligibility criteria were applied—(1) Participants: males or females of any age, ethnicity, medication use, or comorbidities; (2) Intervention: carnosine or related HCDs (anserine, N-acetylcarnosine [NAC], β -alanine, etc), administered alone (pure) and in any form (oral, intravenous, or intramuscular); (3) Comparison: placebo, any pharmacological or nonpharmacological interventions, or usual care; (4) Outcomes: measurement of any inflammatory and oxidative stress outcomes, including CRP, TNF- α , IL-6, adiponectin, MDA, GSH, SOD, TAC, and CAT; and (5) Study design: only RCTs with crossover or parallel designs and systematic reviews of RCTs were included. Systematic reviews were used to find any additional RCTs not captured by the search, which were then located and screened for eligibility.

The following exclusion criteria were applied: (1) animal or cell culture/experimental studies, (2) studies

using a combination of carnosine and/or related HCDs (β -alanine, anserine, NAC, etc) with other supplements (other combined interventions such as diet and/or exercise were included as long as the intervention was delivered in the same way to both groups), (3) studies without an appropriate control group, (4) studies not assessing the endpoints of interest, and (5) narrative reviews, non-peer-reviewed literature, conference abstracts, letters, editorials, observational studies, and case reports.

Data extraction

Two independent reviewers (S.S. and R.H.K.) extracted data from eligible full-text articles using a predefined data extraction form. Extracted data included the following: first author, study location, study design, publication year, sample sizes of the intervention and control groups, dose, frequency, and duration of the intervention, type of supplement, age, health status, and body mass index of the participants, and the study results (mean or median of baseline, follow-up, or difference between baseline and follow-up values [delta], with standard deviations [SDs], 95% confidence intervals [CIs], standard errors [SEs], or interquartile ranges). Data from crossover trials were extracted only for the first phase. All computed data entries and extracted data for the meta-analysis were cross-checked for accuracy.

Quality assessments

The same independent reviewers assessed the risk of bias in the included studies using the Cochrane Risk of Bias 2.0 tool (RoB 2),⁴⁴ as per the protocol. The randomization and allocation process; the presence of predetermined selection criteria; blinding of participants, investigators, and outcome assessors; dropout rates,

Table 1 PICOS criteria for inclusion of studies

Parameter	Inclusion criteria	Exclusion criteria
Population	Men and women of any age, ethnicity, geographic area, comorbidities, or medication use	Studies not in humans (animal or cell-culture studies)
Intervention	Carnosine and related HCDs (beta-alanine, anserine, NAC, etc) alone (pure) supplementation administered in any form (intravenous, intramuscular, or oral) and of any dosage and for any duration	Studies without carnosine and/or HCD supplementation, or studies that combined carnosine and related HCDs with other supplements (other interventions eg, diet and/or exercise were included if the intervention was delivered in the same way to both groups)
Comparator	Placebo or usual care or any pharmacological or nonpharmacological interventions	Studies with no control group
Outcome	Inflammatory and oxidative stress biomarkers	Studies without outcome of interest
Study design	RCTs in either parallel or cross-over design	Narrative reviews, letters, editorials, non-peer-reviewed studies, conference proceedings

Abbreviations: HCD, histidine-containing dipeptide; NAC, N-acetylcarnosine; PICOS, Population, Intervention, Comparison, Outcomes, Study design; RCT, randomized controlled trial.

statistical power, and analysis methods; outcome assessment and reporting; and author conflicts of interest were all examined as individual quality items. Based on all of these factors, each study received a risk-of-bias grade of either high risk, low risk, or some concerns, and disagreement was resolved by discussion.

The overall certainty of each outcome across included studies was evaluated by 2 independent reviewers (S.S. and A.M.) using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach.⁴⁵ Each outcome was graded as high, moderate, low, or very low based on risk of bias, inconsistency, indirectness, imprecision, and other biases, including publication bias. For the GRADE assessment, risk of bias was assessed using the results of the RoB 2 assessment described above. For inconsistency, visual inspection of forest plots, including the magnitude and direction of effect size estimates, consideration of whether CIs overlapped, and between-study variability were used; these factors were considered in relation to the baseline values and cumulative supplement dose, which could logically explain inconsistency. Variations in the population, intervention, and outcomes of interest were considered for indirectness. Imprecision was rated based on the number of studies for a given outcome and the pooled sample size as well as the width of the CIs.

Data synthesis and statistical analysis

Stata version 17.0 (StataCorp) was used to analyze data. The mean change and SD of the relevant outcomes were used to calculate the overall effect size, which was presented as weighted mean differences (WMDs) and 95% CIs based on a random-effects model. A random-effects model was chosen as there was both significant statistical and clinical heterogeneity in terms of the study methods and population characteristics between the studies. If the SD change was not reported, the SD change was calculated using the formula provided by the Cochrane Collaboration,⁴⁶ which is as follows: $SD = \text{square root} [(SD_{\text{baseline}})^2 + (SD_{\text{final}})^2 - (2R \times SD_{\text{baseline}} \times SD_{\text{final}})]$, where the correlation coefficient (R) = 0.8. In addition, SE was converted to SD using the formula $SD = SE \times (\sqrt{n})$ where SE was reported. The main meta-analysis pooled all studies using carnosine and related HCDs and reporting inflammatory and oxidative stress markers. Descriptive analysis was used for the studies with inadequate information to be pooled for meta-analysis. Statistical heterogeneity was assessed using the I^2 test, with values more than 40% indicating moderate to high heterogeneity and significance determined by the P value for heterogeneity (P_{het}).

Publication bias was evaluated using visual inspection of funnel plot asymmetry and using Egger's regression test.⁴⁷ Sensitivity analyses were conducted where studies with a high risk of bias or having some concerns and studies performed on children were excluded to assess their effects on the overall results. Statistical significance was determined by a 2-tailed P value < 0.05.

RESULTS

Study selection

The process of study selection is depicted in Fig. 1. The primary database search yielded 5507 records. After removing duplicates, 3458 articles remained and were screened by title and abstract, of which 3273 articles were deemed ineligible. The remaining 185 records underwent full-text review. Of these, 173 articles were excluded, due to combined intervention ($n=9$); not having a control group ($n=8$); failure to report the outcome of interest ($n=149$); being an animal study ($n=1$); not using a randomized design ($n=3$); or being a systematic review ($n=3$). The remaining 12 studies proceeded to data extraction; however, 3 studies were removed due to having the same sample of participants; Feng et al⁴¹ and Du et al,⁴⁸ and references 23,37 and 49,50 used 2 reports from the same studies and hence were treated as a single study. Therefore, a total of 9 unique RCTs were included.

Study characteristics

The general characteristics of the included studies are described in Table 2.^{27,37,38,40,41,49,51-53} Trials were published between 2013 and 2022 and were carried out in South Korea,²⁷ Taiwan,⁵¹ Iran,^{37,40} the United States,⁵² Egypt,³⁸ Turkey,⁵³ Slovakia,⁵⁰ and China.⁴¹ Of the 9 RCTs, 3^{27,51,53} had a crossover design and the rest had a parallel design. Four studies were performed in both genders,^{37,38,40,50} while the rest were conducted only either in males^{27,51-53} or females.⁴¹ Two of the 9 studies were in children,^{38,40} with 1 study in children with autism⁴⁰ and the other in children with type 1 diabetes nephropathy.³⁸ The remaining studies were conducted in healthy adults,^{27,51-53} overweight or obese adults,^{41,50} and patients with type 2 diabetes.³⁷ Participants were excluded if they were taking anti-inflammatory medications^{37,41} or any other medication that could affect oxidative stress.^{50,51,53} However, some participants used antihypertensive medication³⁸ and kept their normal dietary intake habit.^{40,52} One study did not report any information on medication use.²⁷ The sample sizes in the studies with adult participants ranged from 5 to 92

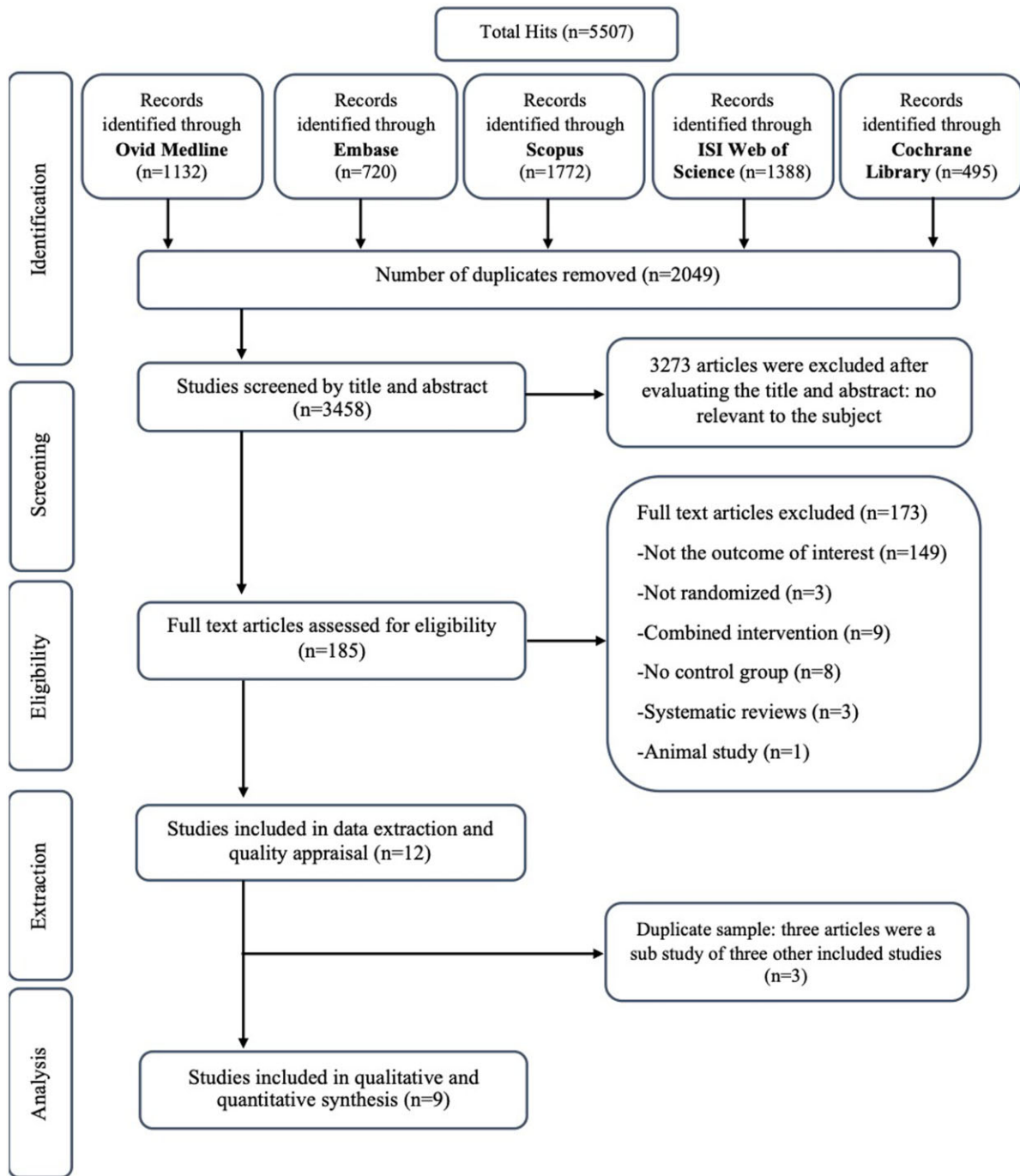


Figure 1 Consolidated Standards of Reporting Trials (CONSORT) diagram of the screening and selection process for the systematic review and meta-analysis of the effects of carnosine and histidine-containing dipeptides on inflammatory and oxidative stress biomarkers.

participants, and the mean age of participants ranged from 20 to 48 years, whereas in the studies of children, sample sizes ranged from 36 to 85 participants, with a mean age of 8.36 to 13.3 years. Four studies supplemented carnosine alone,^{37,38,40,50} 3 used β -alanine alone,^{27,52,53} 1 study used anserine alone,⁵¹ and 1 study used histidine alone.⁴¹ Supplementation doses varied from 0.5 g/d to 12 g/d and 1 study used 15 mg/kg and

30 mg/kg as 2 different arms of the same trial.⁵¹ The duration of supplementation ranged from 1 day to 12 weeks.

Risk-of-bias assessment

Assessments of the methodological quality of the included trials are presented in Fig. 2. Overall, 6 studies

Table 2 Characteristics of studies included in the systematic review of the effects of carnosine/HCDs on inflammatory and oxidative stress biomarkers

Reference	Country	Design, setting	Participants	Sample size (n) and sex	Sample size, n		Frequency/duration	Mean age, y		Mean BMI, kg/m ²		Intervention carnosine/HCD-dose control group	Pooled
					IG	CG		IG	CG	IG	CG		
Jin et al (2022) ²⁷	Korea	Crossover, R, PC, DB	Healthy adults	M (18)	9	9	4 wk	20.78 ± 1.2	20.33 ± 4.36	NR	NR	500 mg/day β-alanine (4 wk) vs placebo	Yes
Alkhatib et al (2020) ⁵¹ (A)(B) ^a	Taiwan	Crossover, R, PC	Healthy adults	M (5)	3	2	24 h	20.9 ± 1.7	20.9 ± 1.7	NR	NR	30 mg/kg anserine vs placebo	Yes
Ghods et al (2018) ⁴⁰	Iran	Parallel, R, PC, DB	Children with autism	M/F (M: 27; F: 9)	18	18	8 wk	8.92 ± 2.74	8.36 ± 2.90	NR	NR	500 mg/day carnosine vs placebo	Yes
Houjehani et al (2018) ²³ /Houjehani et al (2018) ³⁷	Iran	Parallel, R, PC, DB	Adults with T2D	M/F (M: 22; F: 22)	23	21	12 wk	43.0 ± 7.6	40.4 ± 5.1	29.1 ± 5.3	28.3 ± 4.6	1 g/day carnosine vs placebo	Yes
Varanoske et al (2018) ⁵²	USA	Parallel, R, PC, DB	Healthy adults	M (19)	10	9	2 wk	22.4 ± 3	23 ± 3.8	NR	NR	12 g/day β-alanine vs placebo	Yes
Elbarbary et al (2017) ³⁸	Egypt	Parallel, R, PC, DB	Children with T1D nephropathy	M/F (M: 43; F: 42)	43	42	12 wk	12.4 ± 3.4	13.3 ± 2.8	NR	NR	1 g/day carnosine vs placebo	Yes
Belviranli et al (2016) ⁵³	Turkey	Crossover, R, PC, DB	Healthy adults	M (22)	11	11	3 wk	21.7 ± 1.9	21.7 ± 1.9	NR	NR	3.2 g/day β-alanine vs placebo	Yes
Baye et al (2018) ⁴⁹ /De Courten et al (2016) ⁵⁰	Slovakia	Parallel, R, PC, DB	Overweight and obese adults	M/F (M: 18; F: 6)	13	11	12 wk	42 ± 6	43 ± 9	30.4 ± 4.5	32.3 ± 4.6	2 g/day carnosine vs placebo	Yes
Feng et al (2013) ⁴¹ /Du et al (2017) ⁴⁸	China	Parallel, R, PC, DB	Obese adults with metabolic syndrome	F (92)	47	45	12 wk	45 ± 11	47 ± 10	≥ 28	≥ 28	4 g/day histidine vs placebo	Yes

^aA, B was used in multi-arm studies.

Abbreviations: BMI, body mass index; F, female; CG, control group; DB, double-blinded; HCD, histidine-containing dipeptide; IG, intervention group; M, male; NR, not reported; PC, placebo-controlled; R, randomized; T1D, type 1 diabetes; T2D, type 2 diabetes.

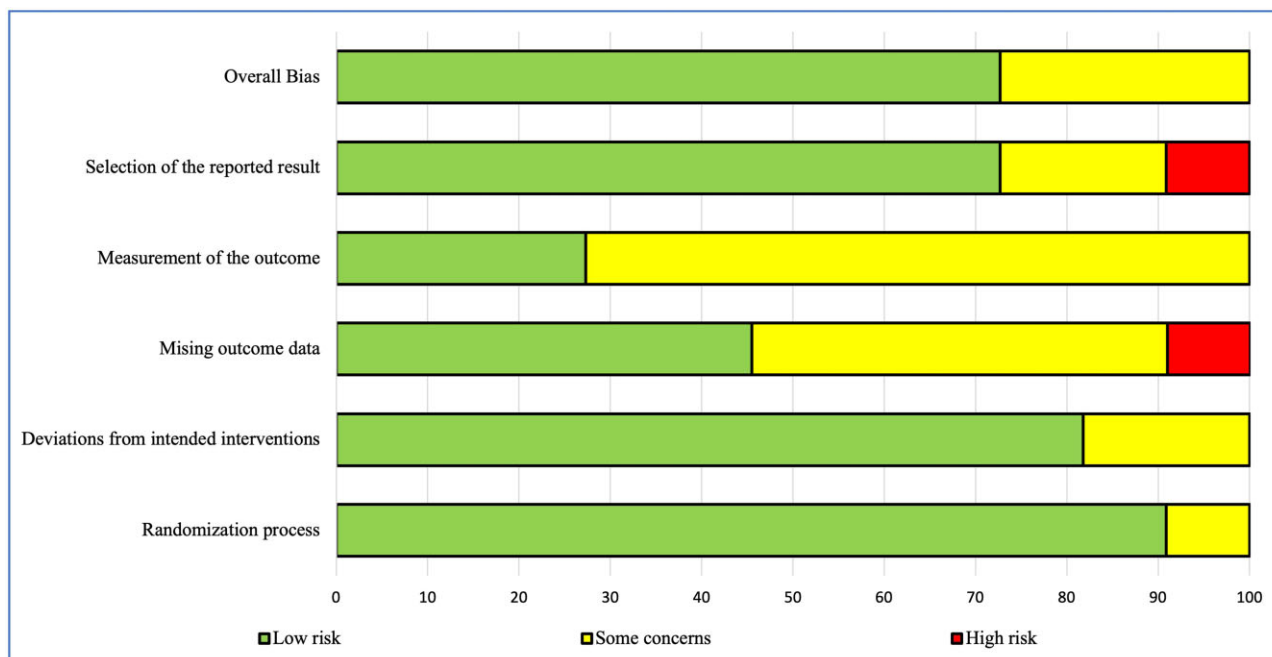


Figure 2 Risk-of-bias summary.

were identified as low risk of bias and 3 studies were considered as having some concerns.

Meta-analysis and sensitivity analysis

All 9 studies were included in the meta-analysis, with a total sample size of 350 participants. The sensitivity analysis also evaluated the effect of each individual study on the total effect size by deleting studies with a high risk of bias or having some concerns and studies performed on children, each study one at a time.

Effect of carnosine/HCD supplementation on inflammatory cytokines and adipokine

Meta-analysis of data from 3 studies ($n = 60$ intervention and 52 placebo) demonstrated that carnosine/HCD consumption led to a significant decrease in CRP concentrations (WMD: -0.97 mg/L; 95% CI: $-1.59, -0.36$; $P = 0.001$; $P_{het} = 0.74$, $I^2 = 0.00\%$) compared with placebo (Fig. 3a).^{41,50,52} All of the studies investigating the effect of carnosine/HCD intake on CRP levels were assessed as having a low risk of bias, except for 1 study,⁵² which was assessed as having some concern of risk of bias. However, when excluding this study in sensitivity analysis, results remained significant.

Pooled data from 3 studies ($n = 68$ intervention and 63 placebo) indicated that TNF- α levels were reduced significantly in those receiving carnosine/HCDs compared with placebo, although with high statistical heterogeneity (WMD: -3.60 pg/mL; 95% CI: $-7.03, -0.18$; $P = 0.03$;

$P_{het} < 0.001$, $I^2 = 96.65\%$) (Fig. 3b).^{27,37,41} The findings from sensitivity analyses showed that the results were nonsignificant after the exclusion of the study with some concern in terms of risk of bias⁴¹ (WMD: -4.83 pg/mL; 95% CI: $-11.13, 1.47$).

There was no significant difference in change in IL-6 between carnosine/HCDs ($n = 78$) and placebo ($n = 72$) in a pooled analysis of 4 studies, with a WMD of -1.50 pg/mL (95% CI: $-3.01, 0.01$; $P = 0.051$) and high heterogeneity ($P_{het} < 0.001$, $I^2 = 84.95\%$) (Fig. 3c).^{27,37,41,52} Two studies were assessed as having moderate risk of bias,^{41,52} while the remaining studies were considered as having a low risk of bias. In sensitivity analysis, results were significant after removing the study with some concern in terms of risk of bias⁵² (WMD: -0.98 pg/mL; 95% CI: $-1.43, -0.53$).

Only 2 studies with a low risk of bias ($n = 50$ intervention and 43 placebo) reported on adiponectin as an outcome measure. Overall, results demonstrated no difference in adiponectin levels with carnosine/HCD supplementation versus placebo, with high heterogeneity (WMD: 0.83 ng/mL; 95% CI: $-0.64, 2.30$; $P = 0.26$; $P_{het} = 0.01$, $I^2 = 86.42\%$) (Fig. 3d).^{41,50} Sensitivity analysis by risk of bias was not possible due to the small number of studies.

Effect of carnosine/HCD supplementation on biomarkers of oxidative stress

A meta-analysis of 5 studies ($n = 132$ intervention and 124 placebo) showed that carnosine/HCD

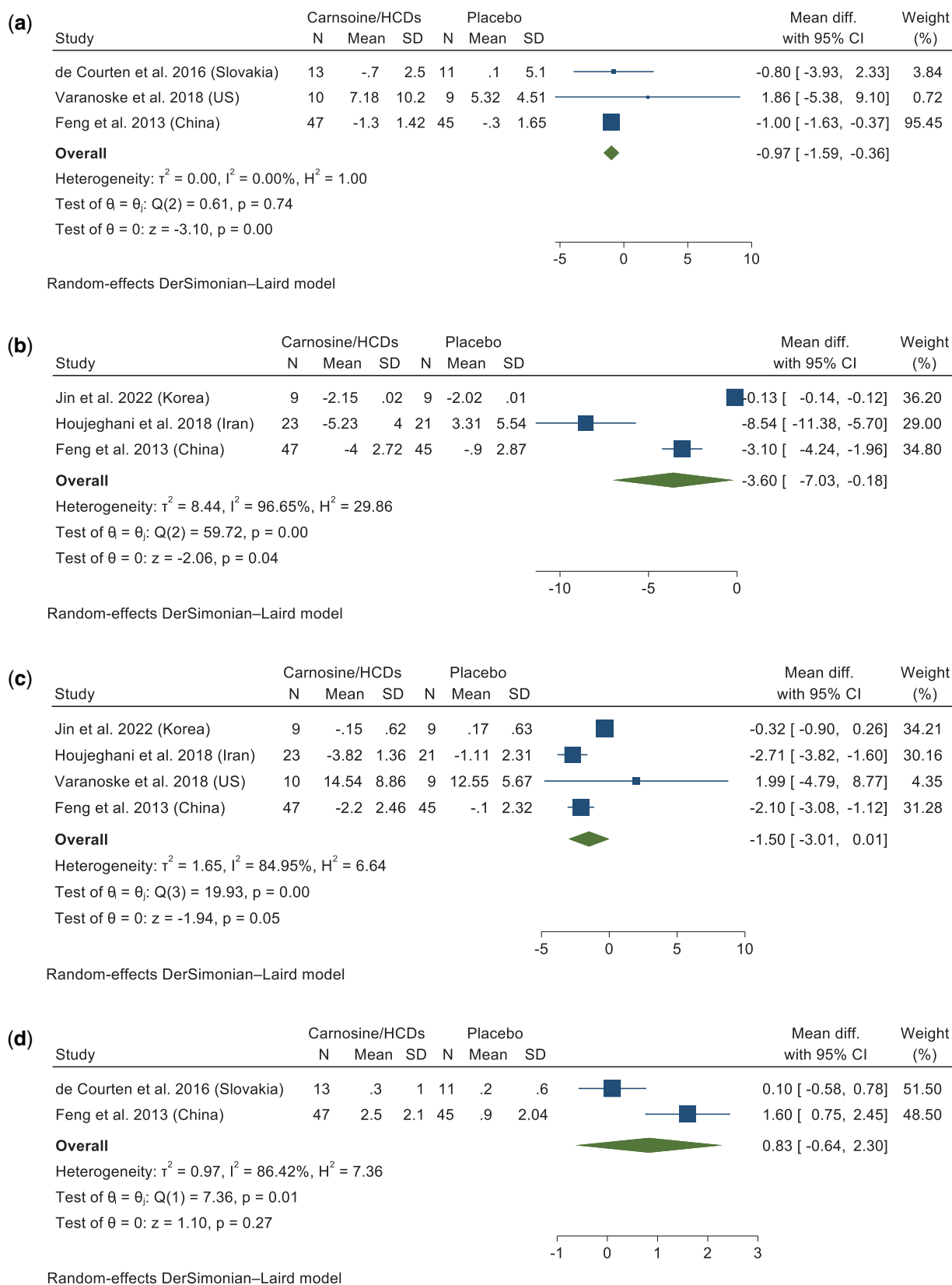


Figure 3 Forest plot showing results of a meta-analysis of the effects of carnosine/HCD supplementation on a) CRP, b) TNF- α , c) IL-6, d) adiponectin. Data are reported as weighted mean differences with 95% CIs. *Abbreviations:* CI, confidence interval; CRP, C-reactive protein; diff, difference; HCD, histidine-containing dipeptide; IL-6, interleukin-6; SD, standard deviation; TNF- α , tumor necrosis factor α .

supplementation significantly decreased MDA levels compared with placebo, with high heterogeneity (WMD: $-0.34 \mu\text{mol/L}$; 95% CI: $-0.56, -0.12$; $P=0.001$; $P_{het} < 0.001$, $I^2 = 86.56\%$) (Fig. 4a).^{37,38,40,41,53} All of the studies investigating MDA as their outcome measure were assessed as having a low risk of bias, except for 1 study that had some concern in terms of risk of bias.⁴¹ In sensitivity analyses, after removing 2 studies (WMD: $-1.44 \mu\text{mol/L}$; 95% CI: $-3.18, 0.29$ ⁴¹; and WMD: $-1.51 \mu\text{mol/L}$; 95% CI: $-3.06, 0.04$ ⁴⁰), which contributed to the high heterogeneity, the results were nonsignificant.

Pooling of 4 studies ($n = 54$ intervention and 47 placebo) for GSH showed no significant difference between the carnosine/HCDs and placebo groups (WMD: $-7.29 \mu\text{mol/L}$; 95% CI: $-38.28, 23.69$; $P=0.64$; $P_{het} = 0.00$, $I^2 = 83.79\%$) (Fig. 4b).^{41,51,53} Two studies were assessed to have concerns in terms of risk of bias^{41,51} and 1 study had a low risk of bias.⁵³ According to sensitivity analyses by risk of bias, only excluding the study with some concern⁴¹ altered the results (WMD: $-1.05 \mu\text{mol/L}$; 95% CI: $-1.81, -0.29$). After removing the study⁵¹ that contributed to heterogeneity, results remained nonsignificant.

There were no significant differences in SOD levels between the carnosine/HCDs and control groups in a pooled analysis of 5 studies ($n = 77$ intervention and 68 placebo) (WMD: 5.16 U/mL ; 95% CI: $-6.18, 16.50$; $P=0.38$; $P_{het} = 0.00$, $I^2 = 94.64\%$) (Fig. 4c).^{37,41,51,53} Except for 2 studies that were assessed to have concerns in terms of risk of bias,^{41,51} the rest had a low risk of bias. The results remained nonsignificant after the exclusion of the studies with concerns of risk of bias for sensitivity analyses.

Pooled data from 2 studies with a low risk of bias ($n = 54$ intervention and 53 placebo) indicated that carnosine/HCD supplementation did not change TAC levels compared with placebo (WMD: -0.46 mmol/L ; 95% CI: $-3.83, 2.92$; $P=0.79$; $P_{het} = 0.11$, $I^2 = 61.84\%$) (Fig. 4d).^{38,53} Sensitivity analysis by the risk of bias was not possible due to the limited number of studies.

The effects of carnosine/HCD consumption on CAT levels were evaluated in 3 studies (1 study with a low risk of bias³⁷ and 2 studies with some concern in terms of risk of bias [$n = 29$ intervention and 25 placebo]). Pooled analysis indicated that CAT concentrations increased significantly following carnosine/HCD intake compared with placebo (WMD: 4.48 U/mL ; 95% CI: $2.43, 6.53$; $P=0.00$; $P_{het} = 0.98$, $I^2 = 0.00\%$) (Fig. 4e).^{37,51} Sensitivity analysis by the risk of bias was not possible due to the small number of studies.

Publication bias

Based on Egger's regression test, there was no indication of publication bias for CRP ($P=0.50$), IL-6

($P=0.17$), TAC ($P=0.10$), or CAT ($P=0.98$), but there were significant results for TNF- α ($P < 0.001$), adiponectin ($P=0.006$), MDA ($P=0.001$), GSH ($P=0.03$), and SOD ($P=0.001$), indicating that the results for these may be affected by publication bias. Visual inspection of funnel plots also confirmed these findings (see Fig. S1 in the Supporting Information online).

GRADE assessment

The GRADE approach was used for the assessment of the certainty of the evidence (see Table S2 in the Supporting Information online). The quality of evidence for CRP was low due to serious indirectness (most of the studies evaluating CRP were conducted in populations with different health statuses) and serious inconsistency (different directions of estimates). For TNF- α , the evidence was of low certainty, downgraded due to serious inconsistency (heterogeneity: $I^2 = 96.39\%$, $P_{het} < 0.001$) and inclusion of studies with some concern in terms of risk of bias. IL-6 and adiponectin levels were ranked as very low certainty due to serious inconsistency (heterogeneity: $I^2 = 82.73\%$ and $I^2 = 86.42\%$, respectively; $P_{het} < 0.001$ and $P_{het} = 0.01$, respectively), imprecision (wide CIs), and an indication of inclusion of studies with some concern in terms of risk of bias. For markers of oxidative stress, GSH and SOD were considered as having very-low-certainty evidence, due to serious inconsistency (heterogeneity: $I^2 = 83.79\%$ and $I^2 = 94.64\%$, respectively; both $P_{het} < 0.001$), serious imprecision (wide CIs), and indication of inclusion of studies with some concern in terms of risk of bias. Certainty of the evidence for MDA was also very low due to serious inconsistency (heterogeneity: $I^2 = 86.56\%$), serious indirectness (analyses included studies that were conducted in children and adults), as well as indication of inclusion of studies with some concern in terms of risk of bias. In addition, the certainty of the evidence for TAC and CAT was also very low due to serious inconsistency (different directions of estimates), serious indirectness (included studies both in healthy adults and individuals with type 2 diabetes), and serious imprecision (small sample size and wide CIs).

DISCUSSION

This is the first systematic review and meta-analysis to examine the effects of carnosine/HCDs on markers of inflammation and oxidative stress. The findings show that carnosine/HCD consumption significantly reduced CRP, TNF- α , MDA, and CAT levels compared with placebo. No differences were found for other biomarkers assessed, including IL-6, adiponectin, GSH, SOD, and TAC.

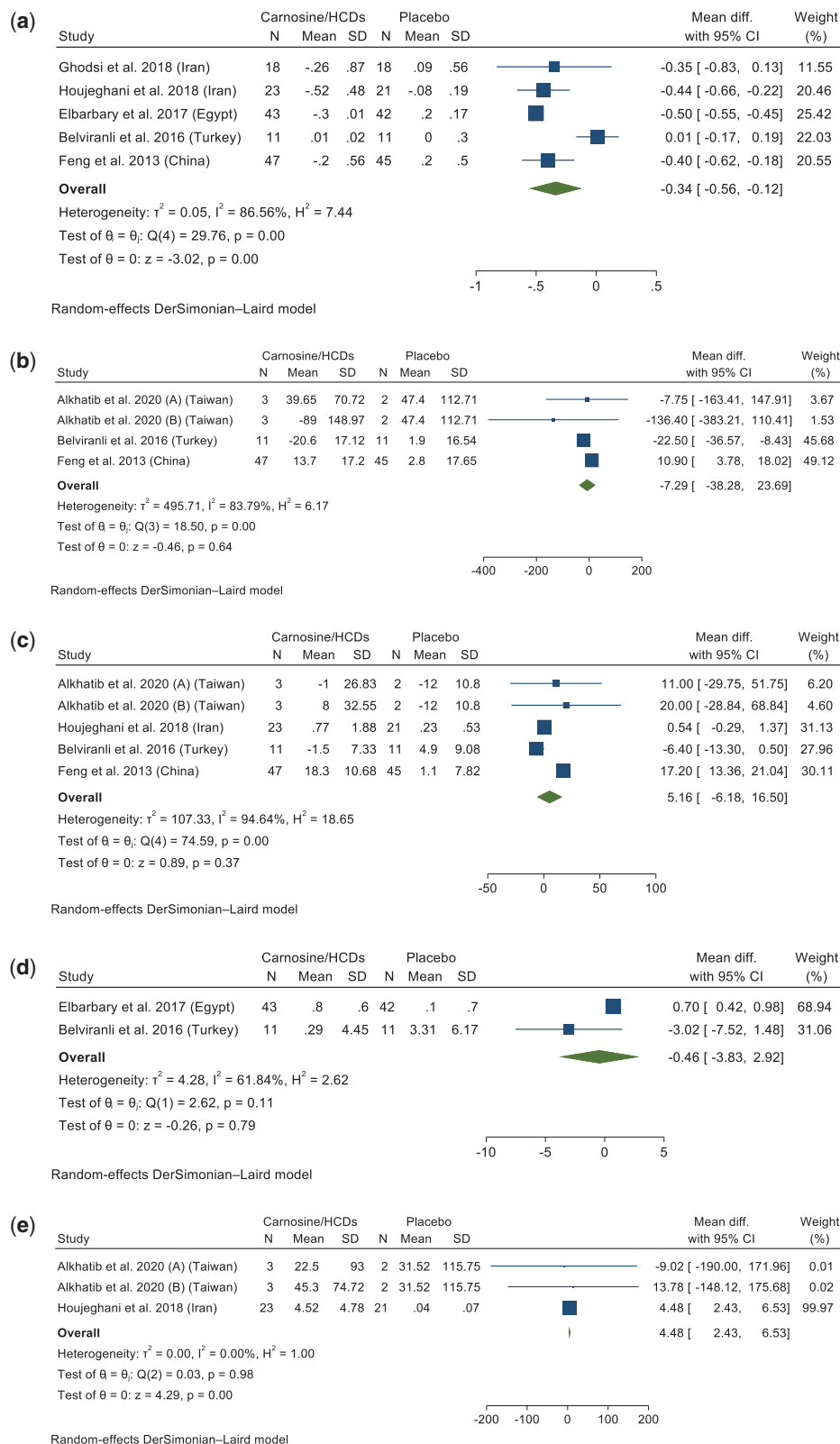


Figure 4 Forest plot showing results of a meta-analysis of the effects of carnosine/HCD supplementation on a) MDA, b) GSH, c) SOD, d) TAC, and e) CAT. Data are reported as weighted mean differences with 95% CIs. *Abbreviations:* CAT, catalase; CI, confidence interval; diff., difference; GSH, glutathione; HCD, histidine-containing dipeptide; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase; TAC, total antioxidant capacity.

The results of this study showed that carnosine/HCD supplementation reduced CRP and TNF- α levels but had no effect on IL-6 and adiponectin levels. Several studies have documented the anti-inflammatory effects of carnosine/HCDs. Recently, a carnosine derivative (resistant to carnosinase) was shown to delay the development of diabetic nephropathy through promotion of renal inflammation in diabetic (*db/db*) mice.⁵⁴ Carnosine also decreased inflammatory molecules, such as CRP and TNF- α levels in Wistar rats,⁵⁵ and reduced apparent nitric oxide (NO) formation, thereby modulating macrophage-mediated inflammatory processes in stimulated murine macrophages.⁵⁶ In addition, 1000 mg/kg per day of carnosine administration for 1 month in rats decreased TNF- α and IL-6 levels.⁵⁷ Apart from the direct effects of carnosine on inflammatory cytokines, carnosine could inhibit AGE and ALE formation and further contribute to the amelioration of inflammation.⁵⁸ No effects on adiponectin were found; however, only 2 studies could be pooled in this analysis. Apart from anti-inflammatory markers of adipose tissue, there is a study showing that 1-week high-dose β -alanine supplementation increases the anti-inflammatory response through elevation of IL-10 concentration during intense military training.⁵⁹ However, it is important to note that there was no study evaluating the effect of carnosine/HCDs on other classic anti-inflammatory markers, such as IL-1 receptor antagonist (IL-1ra). Similarly, only a single study investigated the effectiveness of carnosine supplementation on other adipokines, including serum leptin, resistin, and adiponectin,⁴⁹ indicating that carnosine intake reduced serum leptin and resistin levels in adults with overweight or obesity. Despite these divergent results, this review highlights the lack of available data and the need for further research on the impacts of carnosine on adipose tissue inflammation as well as classic anti-inflammatory markers.

Previous *in vitro* and *in vivo* studies support the potential antioxidant effects of carnosine. In line with the results from the present study, Aydin et al⁶⁰ demonstrated that 250 mg/kg daily (5 days/wk) carnosine supplementation for 2 months significantly reduced MDA levels in oxidative stress-induced rats. Similarly, 2 other studies in rats reported decreased MDA levels and elevated GSH levels following carnosine supplementation of 1000 mg/kg daily for 1 month and 10 mg/kg twice a week for 1 month.^{18,57} *In vitro*, carnosinol, a new carnosine analogue, was shown to increase the activities of SOD and CAT in L6 skeletal muscle cells.¹⁵ The present data agree with the findings for CAT, showing improved CAT levels following carnosine consumption. Carnosine also increased SOD and GSH levels in carbon tetrachloride-treated human lymphocyte

cultures.⁶¹ However, the findings from this study did not show any difference in GSH and SOD levels in carnosine-treated groups compared with placebo, likely due to the different population groups, various methods for assessment of the endpoints, and a small number of included studies in these analyses.

Numerous mechanisms of action regarding the antioxidant properties of carnosine/HCDs have been proposed. A wide range of antioxidant enzymes are involved in neutralizing ROS, including gamma-glutamyltransferase (GGT), Nrf2, GSH, SOD, CAT, glutathione reductase (GSSG-Rd), GSH-Px, and glutathione S-transferase (GST).⁶² Carnosine could increase the expression of the Nrf2 transcription factor, which then leads to upregulation of the vitagenes antioxidant pathway, including heat shock protein 70 (Hsp70), sirtuins (SIRT3), thioredoxin (Trx), gamma-glutamylcysteine synthetase (Gamma-GCs), and heme oxygenase-1 (HO-1).^{63,64} In Sprague-Dawley rats, intraperitoneal administration of carnosine increased TAC by partial improvement in Trx and GSH/GSSG antioxidant systems and subsequently decreased ROS formation.⁶⁵ Additionally, multiple functions of ROS scavenging^{28,29,66,67} and mitochondrial modulation through preservation of expression of proliferator-activated receptor gamma coactivator-1 α (PGC-1 α) and sirtuin3 (crucial regulator of mitochondrial function) were attributed to carnosine and its analogues.¹⁵ Carnosine/HCDs are also proposed to be an effective chelator of heavy metal ions, especially copper and zinc, and consequently aid in detoxification of these toxic elements.⁶⁸

The proposed mechanism for carnosine's anti-inflammatory effect is based on its antioxidant properties. Oxidative stress and inflammation have a close relationship, with each potentially causing the other. Carnosine's antioxidant effects are achieved through direct and indirect mechanisms, with the latter mediated by Nrf2. The ability to activate Nrf2 in oxidative stress conditions or restore its expression is a crucial finding and likely explains many of the benefits associated with carnosine, including its anti-inflammatory action. Furthermore, the fact that carnosine activates Nrf2 independently of electrophilic binding to Keap1 is a significant advantage. Electrophilic Nrf2 activators tend to lack specificity and can affect multiple targets, increasing the risk associated with clinical development.³⁰ There are several unanswered questions regarding the mechanism by which carnosine acts as an Nrf2 activator, particularly with regard to whether it can activate Nrf2 in basal conditions or when Nrf2 response is reduced, as seen in certain pathological conditions including inflammation, and further research is needed to understand these mechanisms.

The present study has several strengths. A rigorous search strategy was used and only included RCTs, with no restrictions on the year of publication. To ensure transparency, a predefined protocol for the systematic review was published⁴² and registered on a public forum. Moreover, this meta-analysis encompasses several inflammatory and oxidative stress parameters and adipokines, enabling us to provide a thorough review of the effects of carnosine/HCD supplementation on a range of biomarkers. However, there were limitations in the included studies. The small participant numbers in most of the included studies (all studies had participant numbers <100) was a main limitation in the literature and, as a result, many of the studies were likely underpowered to detect effects on these outcomes. Moreover, there were a limited number of eligible studies for inclusion in the present systematic review and meta-analysis and most of them investigated the effect of carnosine/HCD supplementation for a shorter period. Importantly, the current study also has some limitations. First, subgroup analysis or meta-regression was not performed due to the limited number of included studies. Second, grey literature, non-English-language manuscripts, and non-peer-reviewed papers were not included. Third, these findings might have been impacted by the heterogeneity of the included trials in terms of the populations studied and the supplementation types, doses, and durations. Coupled with the small number of studies, this precluded further sub-investigations, including examining the effects of carnosine in a dose-dependent manner or stratifying different HCD types or population groups. These factors should be considered in future research. Finally, based on GRADE assessment, most of the outcomes of interest were downgraded to low or very low certainty of evidence. As a result, future studies that are well designed and have a rigorous methodology will be helpful in improving the certainty of evidence for the outcomes of interest.

CONCLUSION

The findings show that carnosine/HCDs may reduce inflammatory biomarkers, including CRP and TNF- α , and oxidative stress markers, such as MDA and CAT, but have no significant effects on IL-6, adiponectin, GSH, SOD, and TAC levels when compared with placebo. However, to strengthen the current body of evidence, larger clinical trials with longer follow-up durations and rigorous methodology to ensure the certainty of evidence of the outcome of interest are necessary.

Acknowledgments

The authors are grateful to Ahmad Alkhatib and Alyssa N. Varanoske for their contribution in providing the requested data.

Author contributions. S.S.: Designed research, screened data, performed quality assessment of studies, undertook study extractions and analyzed data, and wrote the paper; R.H.K.: screened data, performed quality assessment of studies, undertook study extractions and analyzed data, and wrote the paper; G.A.: Contributed to the work's conception, design, data collection, editing, and revising of the manuscript; A.M.: Contributed to conceptualization, quality assessment of studies, editing, and revising of the manuscript; J.F.: Contributed to conceptualization, data interpretation, editing, and revising of the manuscript; and B.d.C.: contributed to conceptualization, data interpretation, and final editing and revising of the manuscript. All authors: Provided intellectual input in line with ICMJE criteria for authorship and read and approved the final manuscript.

Funding. This work received no specific external funding. S.S. and R.K. are the recipients of the Monash Graduate Scholarship.

Declaration of interest. The authors have no relevant interests to declare.

Data availability. Data described in the manuscript will be made available upon request from corresponding author.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Table S1 Sample OVID-MEDLINE search strategy

Table S2 GRADE assessment of the effect of carnosine/HCDs on inflammatory and oxidative stress biomarkers meta-analyses

Figure S1 Funnel plots: a) CRP, b) TNF- α , c) IL-6, d) adiponectin, e) MDA, f) GSH, g) SOD, h) TAC, and i) CAT)

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