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Carnosine supplementation improves glucose control in adults with pre-diabetes and type 2 diabetes: A randomised controlled trial

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Muscle quality

Abstract *Background and aims:* Type 2 diabetes (T2DM) is a major cause of morbidity and mortality globally. Carnosine, a naturally occurring dipeptide, has anti-inflammatory, antioxidant, and anti-glycating effects, with preliminary evidence suggesting it may improve important chronic disease risk factors in adults with cardiometabolic conditions.
Methods and results: In this randomised controlled trial, 43 adults (30%F) living with prediabetes or T2DM consumed carnosine (2 g) or a matching placebo daily for 14 weeks to evaluate its effect on glucose metabolism assessed via a 2-h 75 g oral glucose tolerance test. Secondary outcomes included body composition analysis by dual energy x-ray absorptiometry (DEXA), calf muscle density by pQCT, and anthropometry. Carnosine supplementation decreased blood glucose at 90 min (−1.31 mmol/L; $p = 0.02$) and 120 min (−1.60 mmol/L, $p = 0.02$) and total glucose area under the curve (−3.30 mmol/L; $p = 0.04$) following an oral glucose tolerance test. There were no additional changes in secondary outcomes. The carnosine group results remained significant before and after adjustment for age, sex, and change in weight (all>0.05), and in further sensitivity analyses accounting for missing data. There were no significant changes in insulin levels.
Conclusion: This study provides preliminary support for larger trials evaluating carnosine as a potential treatment for prediabetes and the initial stages of T2DM. Likely mechanisms may include changes to hepatic glucose output explaining the observed reduction in blood glucose without changes in insulin secretion following carnosine supplementation.

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1. Introduction

Type 2 diabetes (T2DM) continues to place an immense burden on healthcare systems globally [1]. The World Health Organization (WHO) estimates that over the last 40 years, the number of people with diabetes has increased from 108 million to 451 million and will increase to 693 million by 2045 [1,2]. This rapid increase has cemented diabetes as a leading cause of mortality [3] and as a major contributor to global burden of disease [4]. Diabetes increases the risk of all-cause mortality by 2−3 times [5] and contributes to as much as 80% of all premature deaths caused by non-communicable diseases [5]. It is also an immense economic burden, with $760 billion USD spent globally on diabetes and its complications in 2019 alone [5]. This is projected to rise to $825 billion by 2030 and $845 billion by 2045 [5].

Due to its significant healthcare and societal burden, effective management of T2DM is critical. The principal aims of management of T2DM are to lower and stabilise blood glucose levels, which decreases the incidence and severity of microvascular and macrovascular complications, improves quality of life, and reduces mortality [6]. More than 120 million people with diabetes worldwide are prescribed metformin as their main therapy, making it the most broadly used glucose-lowering medication [7]. The average cost of Metformin therapy is typically low (approx. USD 10 per month) while other medications used include dipeptidyl peptidase 4 (DPP4) inhibitors, sodium-glucose co-transporter 2 (SGLT2) inhibitors (USD 400 pm), sulfonylureas (USD 5pm), and the glucagon like peptide (GLP-1) receptor agonists (USD 720 pm) [8,9]. These medications are hampered by adverse events, including hypoglycaemic episodes, with resultant sequelae, and some cannot be prescribed in patients with common diabetic comorbidities, such as severe chronic renal disease [10]. This makes the identification of safe, effective, and economically viable [11] means to better control glucose an ongoing priority for researchers.

Chronic hyperglycaemic and oxidative stress produce advanced glycation end products (AGE) and advanced lipid peroxidation end products (ALE), both of which have been implicated in the pathogenesis of diabetes and its complications [12,13]. Carnosine, anserine, and ophidine/balenine, a group of naturally occurring histidine containing dipeptides (HCD), are largely present in the skeletal muscle and brain (olfactory bulb) of mammals [14−19]; with muscle concentrations being several magnitudes larger than in other organs [20]. Carnosine has several physiological activities in humans; being a potent antioxidant, carnosine directly scavenges reactive carbonyl species (RCS) [19] and also activates the Nrf2 pathway thereby hindering the formation of AGEs and ALEs. It also exerts anti-inflammatory action by reducing levels of pro-inflammatory cytokines [19], and has anti-glycating effects [15,20−27]. HCD supplementation also improves glycemic control and insulin levels in adults with prediabetes and diabetes [28−31], and may improve insulin resistance by modulating insulin signaling pathways commonly effected in individuals with T2DM [19]. Carnosine also has a positive effect on the blood lipidome by maintaining levels of trihexosylceramide and phosphatidylcholine and free cholesterol levels [29,32].

Carnosine has been shown to improve glycemic control, at least in part, via anti-AGE action by preventing the formation of cross-linked proteins by methylglyoxal (MG) and neutralizing it [26,33,34]. Free sugars undergo a series of biochemical changes [35] resulting in their oxidisation after which they covalently bond to proteins. These modified proteins form permanent cross-linkages with oxidised sugars and other proteins over time to form AGEs, which through activation of immune processes, contribute to worsening hyperglycemia [34,36]. Furthermore, carnosine's strong anti-oxidant effect enhances the removal of advanced lipid peroxidation end products (ALEs) [37]. ALEs are generated from polyunsaturated fatty acids by reactive oxygen species which create destructive carbonyl species such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), both of which have been linked to the pathophysiology of T2DM [38,39]. Carnosine's primary amine (β-alanine) and its imidazole ring provide its carbonyl quenching ability [40], which effectively removes MDA [30] and MG from in vivo tissue sites [41] preventing oxidative stress and creation of further detrimental reactive oxygen species. The strong antioxidant effect of carnosine has additional implications in the context of diabetes management. The management of chronic inflammation and oxidative stress is a crucial factor in the reduction of diabetic complications, which represents the most significant burden of T2DM.

However, studies investigating the role of carnosine in glucose metabolism are limited by small numbers of participants, frequently treated concomitantly with a variety of glucose-lowering medications as well as a lack of randomized placebo-controlled studies of participants with overweight or obesity. Therefore, our aim was to evaluate the effect of a 14-week carnosine supplementation on glucose metabolism (oral glucose tolerance test) in prediabetes and T2DM. Furthermore, using dual energy x-ray absorptiometry (DEXA) to measure body composition and peripheral quantitative computed tomography (pQCT) to measure intramuscular fat, we aimed to determine the effects of carnosine on adiposity measurements. We hypothesise that oral carnosine supplementation for 14 weeks would improve glucose metabolism and reduce insulin levels in patients with prediabetes or T2DM compared to placebo.

2. Methods

2.1. Study design

The study was a parallel-group double-blinded randomised control trial (RCT). Informed written consent was provided by all participants before commencing the trial. The trial was registered at clinicaltrials.gov (NCT02917928, 28/09/16), and was conducted according to the Standardized Protocol Interventions: Recommendations for Interven-

tional trials [42] and followed the CONSORT guidelines [43]. The study was approved by Monash Health ethics committee (Ref: 16061 A). and conforms to the Declaration of Helsinki [44].

2.2. Sample size

The sample size of the study was calculated using the G-Power software, with blood glucose concentrations via oral glucose tolerance test as the primary outcome. Data from a sub-study in this trial on subjects with overweight or obesity [45], a pilot trial on a cohort of participants with overweight or obesity [28], and from a study of subjects with diabetes by our group; who had mean HbA1c of 7% and fasting glucose of 10 mmol/L was considered in the calculation. A sample size of 22 in each arm was required to detect a 20% change in fasting glucose and absolute change of 0.5 in HBA1c for 80% power, with target recruitment set to 50 participants based on a type I error of 0.05 (two-tailed) [46]. A 20% change in insulin sensitivity was selected based on evidence of similar improvement in insulin sensitivity when treated with troglitazone, a hypoglycemic medication and insulin sensitiser [45].

2.3. Study population

To be eligible, participants were required to be aged 18−70 years. All participants had pre-diabetes or T2DM diagnosed by OGTT (according to the WHO guidelines) and were untreated, or taking only metformin. Participants were excluded if their HbA1c was 8% or higher or had changed their medications in the past 3 months. Participants had to have BMI less than 40 kg/m^2 and a stable body weight (no more than 5 kg above or below the current weight over the last 12 months). Participants were otherwise without diagnosis of any renal, cardiovascular, hematological, respiratory, gastrointestinal, or central nervous system disease, and not pregnant or lactating. Participants were not required to change their diets or routine physical activity but were not included if they consumed more than four standard drinks per week for men and two standard drinks per week for women or if they smoked. Recruited participants were required to remain on the same medications during the 14-week trial. The participant would be discontinued from the study if their HbA1c became greater than 8% and if medication change was stipulated by their medical practitioner at any stage of the study.

2.4. Recruitment, intervention and randomisation

Participants were recruited through advertising via poster, radio, email newsletters and newspapers. Interested participants were first contacted via phone and completed a screening eligibility questionnaire. Potentially eligible participants were then invited for a screening visit, where they were assessed against the inclusion and exclusion criteria, underwent anthropometry and a screening OGTT test at the Clinical Trials Centre of the Monash Health Translational Research Precinct. Eligible participants were randomised to intervention or placebo; the randomisation used block sizes of four by sex, using computer-generated randomisation codes sent to the clinical trials pharmacist by the study statistician, who was blinded to the allocation. The researchers, nurses and other personnel were also blinded to the codes. The codes were only revealed by the clinical trials pharmacist after the last participant had completed the trial. The carnosine group were asked to take 2 g of carnosine orally (Flamma Group, 1 g twice daily including the study days) and the placebo group received an equivalent amount of methylcellulose (1 g twice daily) for 14 weeks. This treatment duration and dosage were based on our pilot study and data from a previous meta-analysis [47,48]. The carnosine powder supplied was laboratory tested independently to ensure purity, and free of adulterants. Medication was dispensed in indistinguishable capsules and containers to ensure participants and researchers were blinded to the group allocation. Participants were asked to inform the trial doctor of any adverse events as they arose, and the trial doctor followed up with all participants at the end of the trial to discuss their experience. Participants were asked to return the treatment containers at the end of the study to assess treatment compliance.

2.5. Outcomes

The primary outcome was plasma glucose level on a 2-h oral glucose tolerance test (OGTT). Secondary measures included HbA1c, insulin levels, body composition, calf muscle density, and anthropometric parameters. All outcomes were measured at baseline and repeated after 14-weeks of intervention.

2.6. Anthropometric, biochemical and body composition assessment

The study physician collected participants' medical history in person and performed a physical examination. This included collecting anthropometric data using a digital scale (Tanita BWB-600) and standing stadiometer, and vital signs collected via Omron digital blood pressure monitor (Model: BBP-742). Waist and hip measurements were measured using a measuring tape at the midpoint between the upper iliac crest and the lowest rib, and at the widest part of the buttocks. Participants' bloods were collected after an overnight fast of at least 10-h using aseptic methods, and analysed by the National Association of Testing Authorities accredited Monash Health pathology service, which operates an automated core laboratory at Monash Medical Centre Clayton.

Whole-body dual-energy x-ray absorptiometry (DEXA; Hologic Discovery A, Hologic, USA) was used to estimate fat and lean mass. Prior to the DEXA scan, participants were asked to empty their bladder, and the DEXA was performed following a 10-h fast to reduce the variability caused by hydration status. The DEXA scanner was calibrated daily with the manufacturer's spine phantom and

the short-term intra-individual coefficient of variation (CV) for lean and fat mass was 1.60% and 2.67%, respectively. All DEXA scans were analysed by the same investigator.

A single 2.5 mm transverse peripheral quantitative computed tomography (pQCT) scan (Stratec XCT3000, Stratec Medizintechnik GmbH, Pforzheim, Germany) at a speed of 20 mm/s and voxel size of 0.8 mm was obtained at 66% of the tibial length of the non-dominant leg. The length of the tibia was determined by measuring the distance between the prominence of the medial malleolus and the tibial plateau. A planar scout view of the distal tibia was used to locate scan sites, and reference lines were placed parallel to the distal joint surface of the tibia. Calf muscle density (mg/cm^3; density of tissue within the muscle compartment after removal of subcutaneous fat and bone) was estimated using manufacturer's algorithms and software (version 6.2). Scans were analysed using a smoothing filter (F03F05) at a threshold of 41 mg/cm^3. The device was calibrated daily using the manufacturer's phantom and the short-term intra-individual CV for muscle density was 1.2%. All pQCT scans were analysed by one individual. Adverse events while participating in the 14-week intervention period were recorded in the progress notes as they arose and were reviewed with the chief investigator at the start of every year while preparing the trial progress reports.

2.7. Glucose and insulin assessment

Participants completed a 2-h OGTT following overnight fasting. The OGTT drink containing 75 g of glucose was consumed within 5 min after which blood was collected aseptically into tubes (Serum Separation Tubes – SST II) at 30-min intervals (0, 30, 60, 90, 120 mins). After centrifugation, the serum samples were stored at −80 °C until analysis. Serum samples at all the time points were measured for glucose and insulin, with the values also used to calculate the area under the curve (AUC) by the trapezoidal method. The homeostatic model assessment of HOMA-IR (insulin resistance), HOMA-β (steady state β-cell function %) and HOMA-S% (Insulin sensitivity), and Matsuda Index (ISI) were also calculated as described previously [28,46,49,50].

2.8. Statistical analysis

Statistical analysis was performed with the statistical software JASP (v0.17.3), and SPSS (v29.0.0.0). Parametric data were reported as mean and standard deviations and non-parametric data were reported as median and interquartile range. Skew was assessed by Shapiro–Wilk testing, with skewed data logarithmically transformed with satisfactory transformation assessed by evaluation of residual plots. Continuous variables were analysed using Analysis of Covariance (ANCOVA) adjusted for baseline values. Additional ANCOVA was performed with diabetes (prediabetes vs T2DM) status, obesity status (BMI >30) or metformin intake as covariates to evaluate interaction effects. All tests were two-tailed, and the alpha level of significance was set at 0.05. The primary analysis was performed on a complete case protocol; however, an additional sensitivity analysis was performed with multiple imputation on missing cases. Missing data was imputed with predictive mean matching, with a total of five imputations modelled. The resulting data sets were analysed as above, with the pooled mean and standard error of the imputations, and the range of the resulting p-values reported.

3. Results

A total of 88 participants were assessed for eligibility, with 49 eligible for enrolment and randomisation. A total of 43 participants (13 women and 30 men) completed the trial at 14 weeks (23 placebo and 20 carnosine). Of 43, 22 participants were assessed as pre-diabetic and 21 as diabetic based on their screening OGTT. Five participants were lost to follow-up (two placebo, three carnosine), and one additional participant was withdrawn after initiating treatment with an additional medication following randomisation (Fig. 1). Baseline participant data is presented in Table 1.

3.1. Effect of carnosine supplementation on obesity measures and intramuscular adipose tissue

After the 14-week intervention, we detected an increase in BMI of 0.74 kg/m^2 ($p = 0.048$) in the carnosine group compared to placebo; but this did not remain significant when adjusting for diabetes status, obesity status or by metformin intake, and did not show any interaction effects with these variables. The carnosine group increased 2.04 kg ($p = 0.04$) in body weight compared to the placebo group, but this did not remain significant after adjusting for obesity or metformin status and displayed no interaction effects. There were no differences in waist circumference or waist-to-hip ratio, body fat percentage (BF%), fat mass and fat free mass or intramuscular adipose tissue between the groups (all $p > 0.05$) (Table 2).

3.2. Effect of carnosine supplementation on glycemic measures

Carnosine supplementation reduced glucose levels at 90-min (mean difference (MD) −1.60 mmol/L, $p = 0.018$) and 120-min ((MD) −1.40 mmol/L, $p = 0.016$), as well as glucose AUC ((MD)−3.30 mmol/L, $p = 0.04$) compared to placebo controls (Table 2, Fig. 2, Fig. 3). Carnosine supplementation had no direct effect on Matsuda ISI. Further analysis revealed a significant interaction effect on ISI with treatment group and gender ($p = 0.018$); when controlling for gender, carnosine increased Matsuda Index by 4.51 but this was borderline non-significant ($p = 0.06$); females in both groups had a higher ISI ((MD) 6.3; $p = 0.008$) compared to males in both groups. A smaller interaction effect by treatment group and obesity status on ISI was also present ($p = 0.04$). There were no changes in any

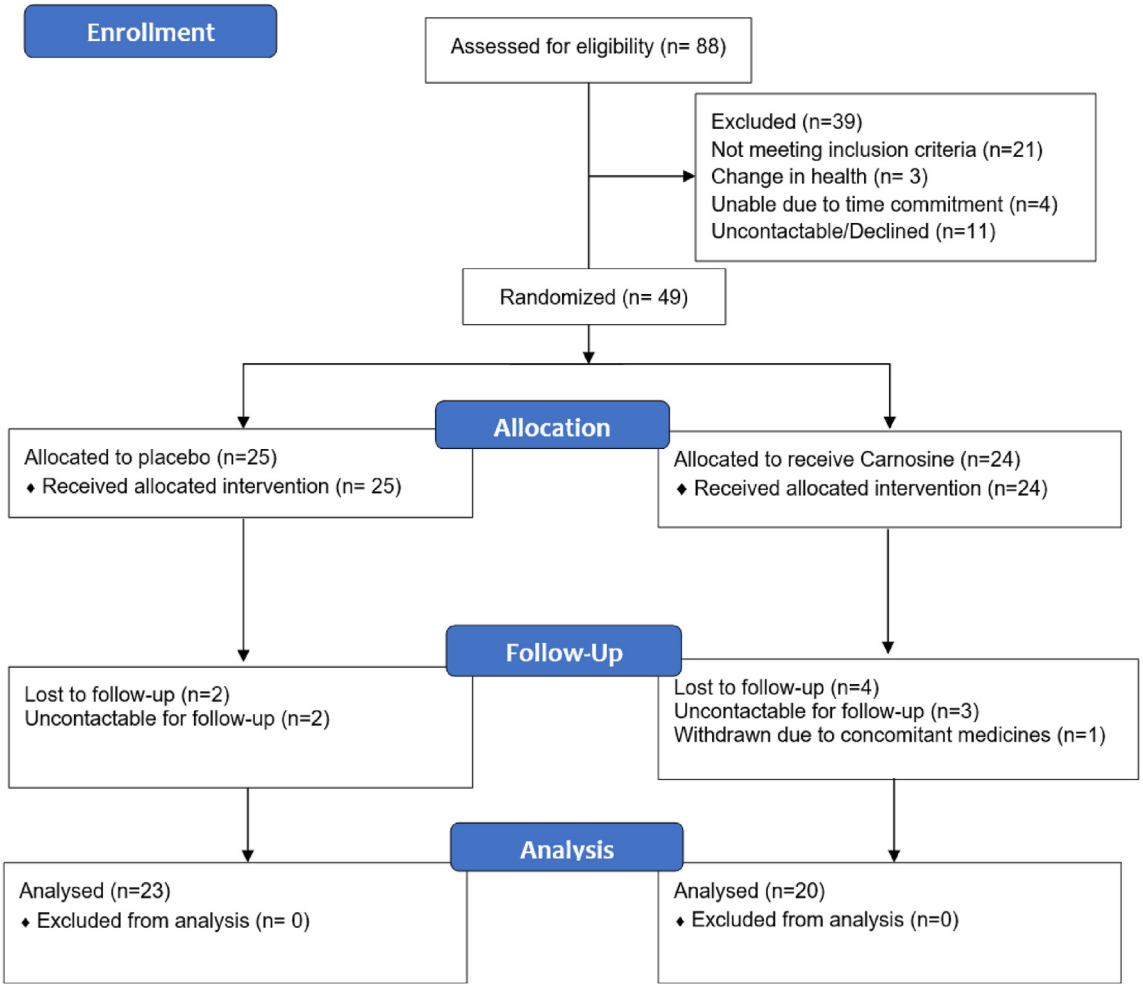

Figure 1 Study flow diagram.

other glucose or insulin measures, insulin AUC, HOMA-S, HOMA-β or HOMA-IR (all $p > 0.05$) (Table 2). There was no interaction between changes in any of the variables and diabetic status, obesity status or by metformin intake. The glucose results remained consistent in sensitivity analysis evaluating the effect of missing data imputation (Table 3).

Carnosine supplementation did not result in any adverse events, and the only reported adverse event being pain and mild bruising at the site of venipuncture in one participant. All participants were followed up monthly by the researchers to ensure compliance; as not all participants returned their supplement containers, the self-reported compliance was excellent.

4. Discussion

In our randomized controlled study, we employed the use of OGTT for its benefits in being easily administrable and its ability to specifically estimate glucose tolerance over a physiological test meal. We showed that carnosine supplementation for 14 weeks reduced 90 and 120-min glucose levels after OGTT, and reduced glucose AUC; and when controlling for gender or obesity status, increased Matsuda insulin sensitivity Index (ISI). There were otherwise no significant changes to body composition, nor other glucometabolic measures. Given carnosine is safe, cost effective [11], and available over the counter in most countries, the reductions in glucose levels make carnosine an attractive agent for the management of pre-diabetes and diabetes, and warrants further large-scale trials to evaluate its long-term effects in the prevention and treatment of T2DM.

In this trial, the reduction of 3.3 mmol/L in glucose AUC following a OGTT represents a 7.2% decrease in glucose response, which is likely to provide clinically significant outcomes in both preventing transition from pre-diabetes and in diabetic complications, particularly given 40% of the cohort was already using metformin [51]. In the Diabetes Prevention Program Outcomes Study (DPPOS), participants with pre-diabetes who returned to normoglycemia had 56% lower risk of diabetes than those who remained pre-diabetic [52]. Similarly, our recent meta-analysis pooling 23 interventional studies showed improvement in glycemic control for participants with both type 1 and T2DM, following carnosine supplementation leading to improvement of fasting glucose ((MD) [95% CI] = −0.6 mmol/L

Table 1 Baseline characteristics of participants.

	Placebo (n = 23)		Carnosine (n = 20)	
Age – years, (median [IQR])	52 [28.4–65.3]		53.8 [30.1–66.3]	
Female, (n [%])	7 [30 %]		6 [30 %]	
Pre-diabetic/Diabetic	11/12		11/9	
Treated with Metformin	9		8	
	Mean	SD	Mean	SD
Weight (kg)	81.8	14.5	88.4	23.8
Height (cm)	169.0	10.3	172.3	12.3
Waist Circumference (cm)	99.0	8.8	103.3	14.7
Hip Circumference (cm)	102.5	7.9	105.7	15.1
Glucose - 0mins (mmol/L)	6.4	1.1	6.4	1.1
Glucose –30mins (mmol/L)	9.6	1.3	10.3	2.3
Glucose –60mins (mmol/L)	12.1	2.1	13.0	2.4
Glucose - 90mins (mmol/L)	12.3	2.7	13.3	3.5
Glucose - 120mins (mmol/L)	11.9	3.0	12.0	3.5
Glucose AUC	43.2	7.5	45.8	9.792
Insulin - 0 min (mIU/ml)	9.7	5.7	14.6	19.3
Insulin - 30 min (mIU/ml)	32.7	30.3	34.4	17.3
Insulin - 60 min (mIU/ml)	41.8	26.9	67.5	58.0
Insulin - 90 min (mIU/ml)	56.1	42.3	96.5	92.0
Insulin - 120 min (mIU/ml)	70.9	64.5	104.0	111.8
Insulin AUC	159.1	113.3	257.5	216.6
HbA1c (%)	6.7	0.8	6.5	0.6
HOMA-IR	2.7	1.7	4.1	5.3
HOMA-S	51.7	37.6	61.4	101.8
HOMA- β	80.8	63.6	118.8	173.3

[−1.1, −0.1], p = 0.03 and HbA1c (MD [95% CI] = −0.5% [−0.4,-0.6], p < 0.001) [48]. In the meta-analysis, sensitivity analyses eliminating studies in which fasting glucose was elevated at baseline, still favored supplementation with carnosine [48], aligning with the results from the present study. We and others also highlighted that there was insufficient data to evaluate whether carnosine supplementation improved glucose following OGTT [48,53], although some showed glucose lowering effects in people with diabetes [29]. While our findings of improved glucose control after OGTT align with these findings, our study did not show changes in HbA1c levels or fasting glucose [30,54]. This is potentially due to differences in participants, with our study investigating people with pre-diabetes and well-controlled T2DM. Additionally, the 14-week intervention in this study may have been too short to cause an appreciable change in HbA1c, which typically requires longer to decrease. While the evidence provided in this study has demonstrated an effect of carnosine supplementation on glucose concentrations, the mechanisms underpinning this are unclear. The glucose-lowering effect was in the absence of an alteration in insulin concentrations, as well as indirect measures of insulin sensitivity, which further highlighted the differences in baseline insulin levels between the placebo and treatment groups (2.7 mIU/ml vs 4.1mIU/ml); is a possible reason as to why a

Table 2 Baseline and follow-up values and changes in anthropometric, body composition and metabolic measures after 14 weeks of supplementation.

	Placebo (n = 23)		Carnosine (n = 20)		Between group mean change[b]	Between group p-value
	Baseline±SD	Follow-up±SD	Baseline±SD	Follow-Up±SD		
Weight (kg)	81.8 ± 14.5	81.5 ± 14.7	88.4 ± 23.8	89.5 ± 23.9	−2.04	0.04
BMI (kg/m^2)	28.5 ± 3.7	28.4 ± 3.6	29.8 ± 4.9	30.2 ± 4.8	−0.74	0.05
Waist Circumference (cm)	99.0 ± 8.8	96.87 ± 7.8	103.3 ± 14.7	103.8 ± 16.0	−1.59	0.2
Waist to hip ratio (WHR)	0.97 ± 0.06	0.97 ± 0.07	0.98 ± 0.1	0.97 ± 0.08	0.01	0.6
Fat mass (kg)	28.5 ± 7.0	28.0 ± 7.26	33.4 ± 11.7	32.8 ± 10.9	−0.47	0.3
Body fat (%)	35.6 ± 6.7	35.13 ± 6.9	37.01 ± 7.9	36.8 ± 7.6	−0.31	0.4
Fat free mass (kg)	51.8 ± 11.7	51.9 ± 11.7	53.1 ± 13.8	53.2 ± 12.7	−0.27	0.5
Calf muscle density (mg/cm^3)[a]	86.0 ± 2.9	85.5 ± 4.0	86.3 ± 5.04	86.2 ± 5.04	−0.74	0.2
Glucose - 0 min (mmol/L)	6.4 ± 1.1	6.3 ± 1.2	6.4 ± 1.1	6.2 ± 1.3	0.08	0.7
Glucose –30 min (mmol/L)	9.6 ± 1.3	9.6 ± 2.2	10.3 ± 2.3	10.2 ± 2.3	0.02	1.0
Glucose –60 min (mmol/L)	12.1 ± 2.1	11.6 ± 2.7	13.0 ± 2.4	12.1 ± 2.7	0.42	0.5
Glucose - 90 min (mmol/L)	12.3 ± 2.7	12.2 ± 2.9	13.3 ± 3.5	11.7 ± 3.05	1.31	0.02
Glucose - 120 min (mmol/L)	11.9 ± 3.0	11.9 ± 3.0	12.0 ± 3.5	10.6 ± 4.1	1.60	0.02
Glucose AUC	43.2 ± 7.5	43.03 ± 9.4	45.8 ± 9.8	42.4 ± 9.9	3.19	0.04
Insulin - 0 min (mIU/ml)	9.7 ± 5.7	9.1 ± 4.6	14.6 ± 19.3	10.3 ± 6.8	0.06	1.0
Insulin - 30 min (mIU/ml)	32.7 ± 30.3	35.5 ± 42.1	34.4 ± 17.3	34.2 ± 19.6	4.03	0.5
Insulin - 60 min (mIU/ml)	41.8 ± 26.9	42.6 ± 30.9	67.5 ± 58.0	58.9 ± 64.3	4.25	0.7
Insulin - 90 min (mIU/ml)	56.1 ± 42.3	52.8 ± 39.2	96.5 ± 92.0	91.3 ± 104.4	3.59	0.7
Insulin - 120 min (mIU/ml)	70.9 ± 64.5	62.6 ± 42.8	104.0 ± 111.8	68.1 ± 65.0	13.9	0.1
Insulin AUC	159.1 ± 2.7	165.1 ± 123.6	257.5 ± 216.6	217.0 ± 197.0	31.63	0.2
HbA1c (%)	6.7 ± 0.8	6.72 ± 1.14	6.5 ± 0.6	6.7 ± 1.1	−0.18	0.4
HOMA-IR	2.7 ± 1.7	2.52 ± 1.35	4.1 ± 5.3	2.7 ± 1.6	0.11	0.8
HOMA-S (%)	51.7 ± 37.6	51.9 ± 29.1	61.4 ± 101.8	161.4 ± 501.1	−116.2	0.3
HOMA- β	80.8 ± 63.6	86.2 ± 108.4	118.8 ± 173.3	140.1 ± 217.8	−26.2	0.7
ISI	3.6 ± 1.23	4.8 ± 2.3	4.7 ± 3.9	6.7 ± 9.7	−0.48	0.8

BMI (body mass index); HOMA-IR (homeostatic model assessment – insulin resistance); HOMA-S% (homeostatic model assessment –insulin secretion); HOMA- β (homeostatic model assessment – beta cell function); AUC (area under the curve). All analysis between group were also adjusted for diabetic status, obesity status, metformin use, age, and sex.

[a] n = 25; Placebo (n = 13) & Carnosine (n = 12).

[b] Mean change in Placebo group – Mean change in Carnosine group.

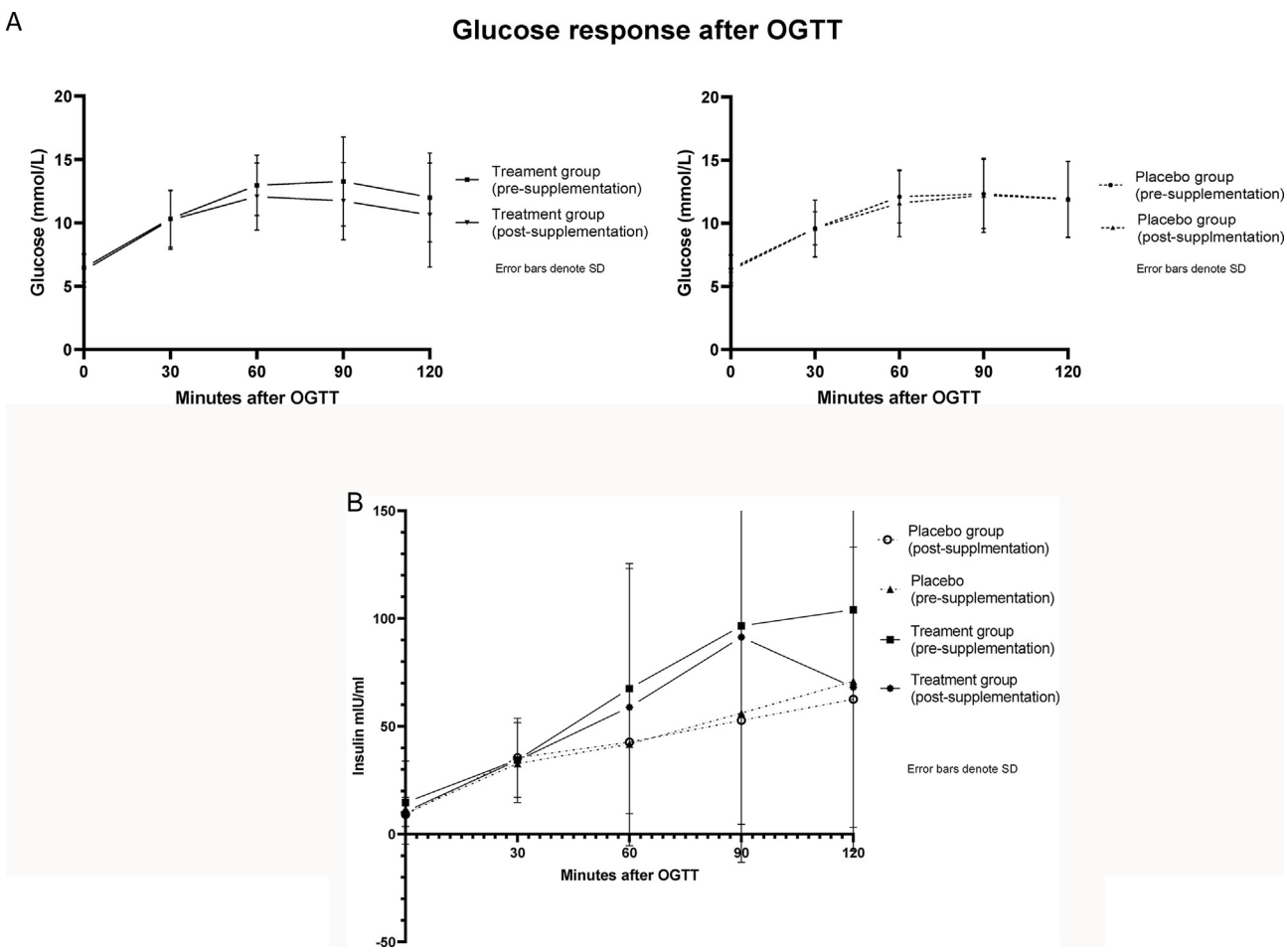

Figure 2 Glucose levels (A) and Insulin level (B) after oral glucose tolerance test at baseline and after 14-week intervention by intervention group.

significant change in insulin at 14 weeks was not detected. This may indicate decreased hepatic glucose output as a potential mediator of the effect seen in this trial.

Carnosine is rapidly hydrolyzed by serum carnosinase to its constituents β-alanine and histidine. Carnosine's poor bioavailability in humans is highlighted by the fact

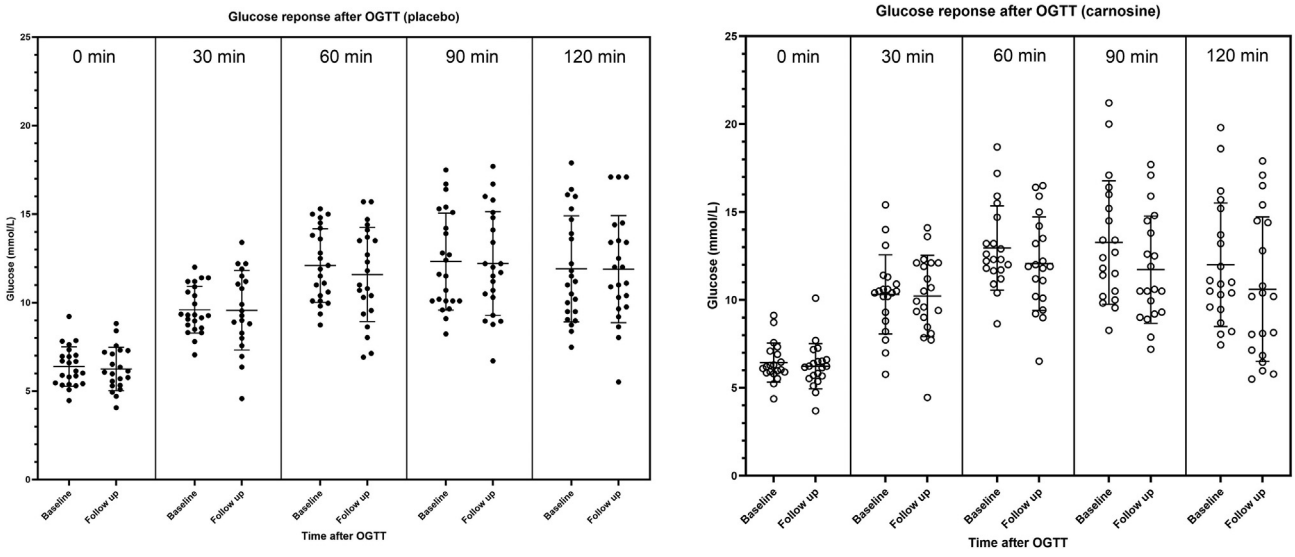

Figure 3 Glucose response after OGTT per participant. Bars denote Mean and SD.

Table 3 Sensitivity analysis of glucose and insulin results, through multiple imputation of missing results, and resulting ANCOVA. Five imputations were used with pooled means and standard errors, and the resulting p-value ranges provided.

Variable	Pooled imputed mean estimates (Std Error)		Range of imputed p-values
Glucose - 0 min (mmol/L)	*Placebo*	6.23 (0.96)	0.19–0.97
	Carnosine	6.21 (0.71)	
Glucose −30 min (mmol/L)	*Placebo*	10.25 (0.63)	0.25–0.99
	Carnosine	9.86 (0.55)	
Glucose −60 min (mmol/L)	*Placebo*	11.90 (0.80)	0.18–0.88
	Carnosine	11.66 (0.55)	
Glucose - 90 min (mmol/L)	*Placebo*	12.69 (0.46)	0.010–0.018
	Carnosine	11.31 (0.41)	
Glucose - 120 min (mmol/L)	*Placebo*	12.05 (0.53)	0.010–0.023
	Carnosine	10.56 (0.48)	
Glucose AUC	*Placebo*	44.06 (1.03)	0.024–0.055
	Carnosine	41.12 (1.05)	
Insulin - 0 min (mIU/ml)	*Placebo*	9.68 (1.12)	0.68–0.96
	Carnosine	9.80 (1.20)	
Insulin - 30 min (mIU/ml)	*Placebo*	33.52 (9.68)	0.08–0.76
	Carnosine	21.78 (13.68)	
Insulin - 60 min (mIU/ml)	*Placebo*	49.03 (9.65)	0.34–0.86
	Carnosine	45.69 (9.33)	
Insulin - 90 min (mIU/ml)	*Placebo*	63.82 (10.24)	0.18–0.81
	Carnosine	77.44 (11.55)	
Insulin - 120 min (mIU/ml)	*Placebo*	69.08 (7.94)	0.086–0.82
	Carnosine	63.15 (11.17)	
Insulin AUC	*Placebo*	185.32 (19.84)	0.42–0.99
	Carnosine	181.87 (21.61)	

that individuals with CNDP1 genotype characterised by low serum carnosinase activity are often afflicted by carnosinemia [55], however, carnosinemia can also be induced by increasing intake [55]. Several clinical trials studies [21,56–60] have utilised doses of carnosine ranging from 0.5 to 2g/day of oral supplement with good efficacy. Despite the inherent activity of serum carnosinase, high dosage carnosine supplementation does seem to create measurable biochemical, cognitive, and metabolic changes in clinical trials in both adults and children compared with placebo [21,56–60]. While some studies [61,62] have shown that carnosine in low (450 mg) and high doses (4 g) is undetectable in plasma soon after ingestion, others have indicated detectable levels of carnosine for up to 5 h after ingestion [63]. Furthermore, early evidence suggests that carnosine maybe transported to intracellular spaces in its dipeptide form before being hydrolysed to its constituents; indicating a rate-limiting delay in absorption and elevation of plasma levels compared with β-alanine absorption [64]. Recently, Oppermann et al. [65] demonstrated in vitro that erythrocytes can prevent enzymatic degradation of carnosine by bi-phasic uptake to a saturation point of 100 mM per erythrocyte. This may be the reason carnosine exhibits physiological abilities despite the understanding of its rapid degradation by serum carnosinase; even being excreted in urine 5 h after ingestion [65]. Furthermore, carnosine in erythrocytes increased intracellular ATP production in 70% of the samples, possibly indicating its scavenging of excess glyceraldehyde-3-phosphate, preventing glycation of glyceraldehyde-3-phosphate dehydrogenase, therefore displaying an antiglycating effect of benefit to erythrocyte glycolysis [65,66]. Previously, Chaleckis et al. demonstrated that carnosine is RBC-enriched while acetyl carnosine is mainly in plasma and that increasing age declines the levels of both metabolites in blood, therefore, indicating why several studies supplementing carnosine have showed physiological improvements in elderly cohorts [67]. Together, this evidence suggests that bioavailability of carnosine in humans is highly dependent on the variations in serum carnosinase activity produced by the polymorphism of the CNDP1 genotype inherited and erythrocyte saturation by the chronic ingestion of high doses.

Our study found a increase of 0.74 kg/m^2 in BMI in the carnosine group ($p = 0.045$) after 14-week supplementation compared to the placebo; with significant change in weight (2.04 kg; $p = 0.04$) between the groups. We found no change in waist circumference or waist-to-hip ratio after carnosine supplementation, in line with previous studies of similar cohorts [28,68]. Liu et al. [54] reported in one trial that carnosine as part of a combined supplement with cinnamon and chromium increased lean mass, which is in contrast to our trial. Although, our study cohort was largely overweight rather than obese and several participants had been pre-treated with metformin and/or diet control and exercise, which could explain the lack of effect observed in these measures. Another meta-analysis [48], also found a significant 3.5 cm decrease in waist circumference, findings not seen here. A prior RCT [69] compared β-alanine (BA) supplementation only ($n = 8$) (the rate-limiting precursor to carnosine and a preferred method to increase muscle carnosine levels by circumventing serum carnosinase activity), creatine only ($n = 8$) and BA plus creatine ($n = 7$) or placebo ($n = 7$) for 28 days along with a

structured exercise routine in a group of physically active females [69]. They found that despite addition of exercise, there were no reductions in body weight, fat mass, body fat and fat-free mass [69] in any cohort; findings echoed in another trial of β-alanine and exercise combined [70]. Another recent meta-analysis of 426 participants found no effect of supplementation on any measure of body composition, and no interaction effects resulting from resistance training, endurance training or combined training [68]. This data, and ours provides evidence that neither carnosine nor β-alanine supplementation seems to affect body composition directly, with or without complimentary exercise in adults without obesity. However, it should be noted that most of these studies were in athletes who are less likely to show substantial changes in body composition. In our trial, participants taking carnosine did not increase ISI, but our further analysis showed significant interactions between carnosine treatment and gender on ISI ($p = 0.02$) and carnosine treatment and obesity on ISI ($p = 0.04$). When controlling for gender, carnosine supplementation increased ISI by 4.51, but this did not reach significance ($p = 0.06$). The ISI index is a measure of both hepatic and peripheral insulin sensitivity [50] and our results demonstrate that both gender and obesity status effect insulin sensitivity. In our findings, females in both treatment groups with obesity had higher ISI than males with obesity from both groups. This is line with other results which suggest that males have higher intramuscular adipose tissue than females and that higher IMAT results in poorer glycemic control [71,72]. A recent meta-analysis showed in children and adolescents with obesity or overweight that fat-free-mass influenced fasting plasma insulin levels [73]. This may indicate that intramuscular adipose tissue (IMAT) as a measure of muscle quality is of more importance than muscle mass alone [73]. This agrees with earlier research which indicates that high IMAT is associated with reduced insulin sensitivity, glucose tolerance and HOMA-IR [71,74–77]. It is also of interest that individuals with T2DM do have higher levels of intramuscular adipose tissue [78] than non-diabetic individuals. Further trials which supplement carnosine in individuals with obesity may better elucidate an effect of carnosine on body composition.

The findings of the current study agree with previous work demonstrating that carnosine has glucose-lowering effects in individuals with impaired glucose tolerance, however, our data was unable to demonstrate any changes in insulin levels as found in other similar trials [28,79], possibly due to the large number of participants with diabetes being treated with metformin in our cohorts, or the fact that we used indirect measurements for insulin sensitivity and secretion. We have shown previously that carnosine supplementation leads to increases in insulin sensitivity and secretion in individuals with overweight and obesity [28]. Future studies will need to employ gold-standard euglycemic-hyperinsulinemic clamp and intravenous glucose tolerance test to accurately evaluate carnosine's effect on these measures.

4.1. Strengths, limitations, and future direction

While this study was methodologically robust, there are some limitations. We used surrogate measures of insulin sensitivity and secretion (ISI, HOMA-IR, HOMA-S and HOMA-β) which are not as sensitive as the gold-standard method. Our analysis showed that the baseline insulin level for the carnosine group was 29.8% higher than the baseline insulin for the placebo group. This random characteristic as well as missing OGTT and insulin data for a few participants (Table 2) may have further obscured the detection of a significant change in insulin levels after 14 weeks, even though the carnosine group lowered HOMA-IR scores to greater degree than the placebo group (change of −1.4 mIU/ml vs −0.18 mIU/ml), although this result was not statistically significant. The higher insulin level in the carnosine group at baseline may have been a reason a larger therapeutic change was evident after treatment. A limitation of the study was that compliance with supplementation was assessed by participant self-reporting as a few participants failed to return empty supplement containers. We assumed compliance as excellent based on those who returned the containers and by case notes collected by researchers during monthly participant follow-ups. Moreover, the trial was limited by a smaller sample size due to the difficulty in recruiting participants with pre-diabetes and T2DM treated with metformin only and furthermore by COVID-19 pandemic which halted the trial for several months. Another limitation was the short follow-up period, which left questions surrounding treatment durability unanswered.

5. Conclusion

A 14-week regimen of carnosine supplementation reduced glucose concentrations following OGTT in patients with pre-diabetes and diabetes but had no effect on insulin concentrations or obesity measures. While this makes carnosine a promising candidate in the management of T2DM, further randomised controlled trials of carnosine supplementation for cardiometabolic conditions are warranted, particularly in the light of carnosine's additional protective benefits to cardiovascular and inflammatory outcomes relevant to diabetes [48,80].

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Declaration of competing interest

The authors report no conflicts of interest. Flamma Group provided the carnosine for the intervention, but then had no input on the design, conduct, analysis, or reporting of the study.

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