Supplementation-Induced Change in Muscle Carnosine is Paralleled by Changes in Muscle Metabolism, Protein Glycation and Reactive Carbonyl Species Sequestering

Martin SCHÖN[1], Ivica JUST[3,4], Patrik KRUMPOLEC[1,3], Pavel BLAŽÍČEK[5], Ladislav VALKOVIČ[3,6,7], Giancarlo ALDINI[8], Chia-Liang TSAI[9], Barbora DE COURTEN[10,11], Martin KRŠŠÁK[3,4,12], Barbara UKROPCOVÁ[1,2]*, Jozef UKROPEC[1]*

** These authors contributed equally to this work.*

[1]Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovak Republic, [2]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic, [3]High Field MR Centre, Department of Biomedical Imaging and Image-Guided Therapy, Medical University of Vienna, Vienna, Austria, [4]Christian Doppler Laboratory for Clinical Molecular MR Imaging, Vienna, Austria, [5]Private laboratory 4vive, Bratislava, Slovak Republic, [6]Department of Imaging Methods, Institute of Measurement Science, Slovak Academy of Sciences, Bratislava, Slovak Republic, [7]Oxford Centre for Clinical MR Research, RDM Cardiovascular Medicine, University of Oxford, Oxford, United Kingdom, [8]Department of Pharmaceutical Sciences, Università Degli Studi di Milano, Milano, Italy, [9]Institute of Physical Education, Health and Leisure Studies, National Cheng Kung University, Taiwan, [10]Department of Medicine, School of Clinical Sciences, Monash University, Melbourne, Australia, [11]School of Health and Biomedical Sciences, RMIT University, Bundoora, Australia, [12]Division of Endocrinology and Metabolism, Department of Internal Medicine III, Medical University of Vienna, Vienna, Austria

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Summary

Carnosine is a performance-enhancing food supplement with a potential to modulate muscle energy metabolism and toxic metabolites disposal. In this study we explored interrelations between carnosine supplementation (2 g/day, 12 weeks) induced effects on carnosine muscle loading and parallel changes in (i) muscle energy metabolism, (ii) serum albumin glycation and (iii) reactive carbonyl species sequestering in twelve (M/F=10/2) sedentary, overweight-to-obese (BMI: 30.0±2.7 kg/m^2) adults (40.1±6.2 years). Muscle carnosine concentration (Proton Magnetic Resonance Spectroscopy; ^{1}H-MRS), dynamics of muscle energy metabolism (Phosphorus Magnetic Resonance Spectroscopy; ^{31}P-MRS), body composition (Magnetic Resonance Imaging; MRI), resting energy expenditure (indirect calorimetry), glucose tolerance (oGTT), habitual physical activity (accelerometers), serum carnosine and carnosinase-1 content/activity (ELISA), albumin glycation, urinary carnosine and carnosine-propanal concentration (mass spectrometry) were measured. Supplementation-induced increase in muscle carnosine was paralleled by improved dynamics of muscle post-exercise phosphocreatine recovery, decreased serum albumin glycation and enhanced urinary carnosine-propanal excretion (all $p<0.05$). Magnitude of supplementation-induced muscle carnosine accumulation was higher in individuals with lower baseline muscle carnosine, who had lower BMI, higher physical activity level, lower resting intramuscular pH, but similar muscle mass and dietary protein preference. Level of supplementation-induced increase in muscle carnosine correlated with reduction of protein glycation, increase in reactive carbonyl species sequestering, and acceleration of muscle post-exercise phosphocreatine recovery.

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Fax +420 241 062 164, e-mail: physres@fgu.cas.cz, www.biomed.cas.cz/physiolres

Key words
Carnosine supplementation • Muscle carnosine concentration • Phosphocreatine post-exercise recovery • Reactive carbonyl species • Protein glycation

Corresponding authors
J. Ukropec, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05 Bratislava, Slovak Republic. E-mail: jozef.ukropec@savba.sk

Introduction

Since 1975, the worldwide prevalence of obesity has nearly tripled and reached pandemic proportions [1], notably due to the lack of effective and accessible preventive and therapeutic strategies. Prediabetes and type 2 diabetes (T2D] represent the most common obesity-associated metabolic disease, and their increasing incidence parallels obesity and sedentary lifestyle epidemics which substantially contribute to the disruption of glucose homeostasis [2]. Lifestyle interventions, based on exercise, nutritional counselling, and weight loss, were found to be effective in T2D prevention [3,4]. Safe and low-cost interventions, with a potential to act in synergy with lifestyle modification to counteract health, social and economic burdens linked to obesity and related metabolic complications, could bring additional benefits for the patients and health care systems.

The potential role of carnosine (β-alanyl-L-histidine) in prevention and treatment of metabolic [5], cardiovascular [5] and neurodegenerative diseases [6] has been identified. Favourable metabolic effects of this dipeptide include lowering chronic inflammation, oxidative stress, advanced glycation and lipoxidation end-products, as well as chelating and pH buffering properties [7]. Large body of evidence from animal and cell studies point at the potential use of carnosine or its precursors to improve glucose metabolism [8-12], and limited evidence from clinical studies shows the beneficial effects on muscle metabolism [13]. In animal studies, carnosine clearly showed a potential to modulate insulin signalling, to improve insulin-stimulated glucose uptake in the muscle, and insulin secretion through decreasing lipid peroxidation in pancreatic beta-cells and capacity to regulate appetite and lipolysis [14]. These all are a plausible mechanisms of carnosine action in reducing the risk of type 2 diabetes (T2D). Previously published results of this study showed that 3-month carnosine supplementation has a potential to improve glucose tolerance in sedentary middle-aged obese individuals with prediabetes [15], that it could induce favorable changes in plasma lipidome, iron metabolism and reciprocal regulation of plasma leptin and resistin [16,17], but its capacity to modulate dynamics of exercise-related muscle energy metabolism has not yet been examined.

Phosphorus and proton Magnetic Resonance Spectroscopy (^{31}P-MRS and ^{1}H-MRS) has been recognized as a reliable non-invasive tool to assess *in vivo* dynamics of human muscle energy metabolism as well as carnosine content [18]. Two human studies to date analyzed skeletal muscle carnosine content in patients with diabetes but reported discrepant results [19,20]. Our group has previously highlighted the positive relationship between muscle carnosine and the impairment of whole-body glucose metabolism in sedentary middle-aged men, with the highest muscle carnosine content found in newly diagnosed drug-naive patients with T2D [20]. However, the effects of carnosine supplementation, on muscle carnosine content and its potential role in modulating muscle energy metabolism and systemic metabolic changes in individuals with the high risk of metabolic disease remain relatively unexplored [1]. Therefore, we analyzed the outcomes of the dynamic ^{31}P-MRS examination in muscle of the patients from our carnosine supplementation study [15] to prove the hypothesis that interrelations between supplementation-induced changes in muscle carnosine, and (i) exercise-related dynamics of muscle energy metabolism, (ii) serum albumin glycation and (iii) reactive carbonyl species sequestering exist and require further investigation.

Methods

Study design and participants

The study was performed from September 2013 to July 2014. Volunteers were recruited from the community *via* newspaper advertisement and the study was performed at the Institute of Experimental Endocrinology, Biomedical Research Center Slovak Academy of Sciences, Bratislava, Slovakia. In the study protocol of the original randomized placebo-controlled clinical trial, which is depicted in Figure 1 and the primary outcomes and sample size calculation were previously published in [15], participants were randomly assigned to receive 2 g carnosine per day (2×1 g) or identically looking placebo for 12 weeks. Adherence to

the intervention was encouraged by weekly telephone contact and anthropometric measurements were performed monthly. Carnosine supplementation was well-tolerated, and the participants did not report any side effects, which corresponds to the evidence from previous studies reporting its favorable safety profile [22]. All the participants were middle-aged healthy overweight or obese (BMI ≥25 kg/m^2), non-diabetic (75 g oGTT; oral glucose tolerance test, ADA criteria 2006) non-smokers who did not participate in any regular structured physical activity (>3 times of >1 h of aerobic and/or strength exercise per week, Physical activity guidelines for adults by World Health Organization) and did not use illicit drugs or take any medications or food supplements. In this report, we present results of the exploratory analysis in a subgroup of 12 individuals receiving carnosine who were willing to undergo dynamic 7T ^{31}P-MRS examination of exercising calf muscles before and after 12-week carnosine supplementation. Placebo group was not measured due to capacity constraints. Results of this report could therefore only have the validity of the single-arm non-controlled interventional trial aimed at depicting interrelations without any inference on causality. This study conforms to the Declaration of Helsinki and all procedures involving patients were approved by The Ethics Committee of the University Hospital Bratislava (06907/2016/HF/4). Written informed consent was obtained from all subjects prior entering the study. Study was registered at Clinicaltrials.gov (NCT02011100).

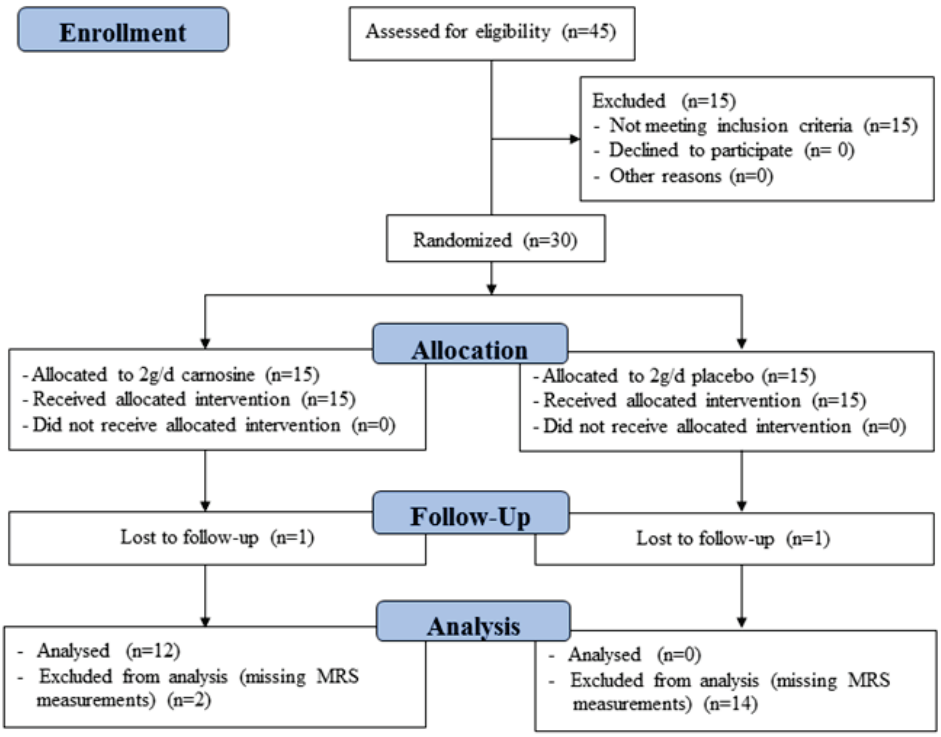

Fig. 1. Flowchart of the study.

Metabolic phenotyping

Body Mass Index (BMI) was calculated, and waist circumference measured. Bioelectric impedance (Omron-BF511, Japan) was used to assess body composition. Blood pressure (Johnson & Johnson Inc., UK) was measured in a sitting position after 30 min rest. Indirect calorimetry (Geratherm Respiratory, Germany) was used to measure resting energy expenditure (REE) and metabolic substrate preference (RQ) during 30 min bed rest at thermal comfort.

Here we present the results of an oral glucose tolerance test (2h-oGTT, 75 g of glucose) performed before intervention, to show that we work with the nondiabetic population.

Serum glucose, insulin, total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides and hsCRP were analysed (Alpha-medical, Slovakia). Urinary and serum levels of carnosine and carnosine-propanal were measured with (LC-ESI-MS) as described in [23]. Serum carnosinase content and activity were measured using a sandwich ELISA [18]. The volume of habitual ambulatory activity was measured during at least three

consecutive days with accelerometers (Lifecorder Plus, Kenz, USA). Protein-preference score was calculated from the validated 72-food-items food preference questionnaire [24].

Magnetic Resonance Spectroscopy

Skeletal muscle carnosine content was measured using ^{1}H-MRS on 7T whole-body MR scanner (Magnetom, Siemens-Healthcare, Germany). Measurements were performed in a supine position with the widest part of the right calf placed in the middle of RF coil in the magnet iso-center using single-voxel MR spectroscopy positioned in gastrocnemius and soleus muscle. Dynamic 7T ^{31}P-MRS was used for non-invasive evaluation of changes in oxidative metabolism in gastrocnemius muscle during submaximal exercise performed *in situ*, using MR-compatible ergometer (Ergospect, Austria) and a surface ^{1}H/^{31}P coil (Rapid Biomedical, Germany) as previously described [25,26]. Using this technique, dynamic changes of phosphocreatine content and recovery, maximal oxidative flux and muscle pH were assessed during an acute bout of 6 min exercise and within a 6 min lasting recovery period.

Statistical analysis

Pearson linear correlation analysis was performed, and paired *t*-test was applied to assess the carnosine-supplementation effects together with the two-way RM-ANOVA to evaluate group effects (differences between individuals stratified according to baseline muscle carnosine content), time effects (effects of carnosine supplementation, irrespective of baseline muscle carnosine) and group/time interactions. Normally distributed data were analysed using a parametric test (Welch´s *t*-test) and the data which were not normally distributed with a non-parametric test (Mann-Whitney U test). Supplementation-induced fold-change is a ratio of post- to pre-supplementation values. Data are presented as mean ± STD, $p<0.05$. JMP ver.4 (SAS, USA) was used.

Results

The supplementation-induced change of muscle carnosine content (in both gastrocnemius and soleus muscle) correlated negatively with the concurrent change of the time needed for post-exercise recovery of muscle phosphocreatine (τ_{PCr}) (Fig. 2A). The importance of carnosine for muscle energy metabolism could also be indicated by a positive correlation between muscle carnosine and resting phosphocreatine content (gastrocnemius muscle; R=0.663, p=0.026). In addition, increase in muscle carnosine was paralleled by increased urinary carnosine propanal (Fig 2B), and by decreased serum content of glycated albumin (Fig. 2C). Change in muscle carnosine correlated positively with the concurrent change of serum carnosinase-1 (CN1) protein content (R=0.693, p=0.0003).

The observed complex interrelations at the baseline non-exercise state could be explained by the supplementation-induced decrease in serum CN1 activity, a tendency to increase muscle carnosine content (in both muscles), increase in resting level of muscle phosphocreatine, and elevated urinary carnosine excretion (Table 1). However, it could be speculated that the variability in muscle carnosine loading could perhaps prevents the supplementation-induced change in the post-exercise phosphocreatine recovery (τ_{PCr}, time constant of PCr recovery) and exercise-related ATP flux (Qmax) i.e. the functional parameters active in m. gastrocnemius during or immediately after in-magnet exercise (Table 1).

We therefore explored the variability in the supplementation-induced increase of muscle carnosine. Concordant response to carnosine supplementation in both examined muscles is indicated by correlation between supplementation-induced change of carnosine in m. soleus and m. gastrocnemius (R=0.881, p=0.014). Individual differences in muscle mass or dietary preference for proteins did not explain variability in muscle carnosine loading capacity but could be related to serum CN1 content (Table 1). Moreover, a negative correlation between baseline/pre-intervention muscle carnosine content and its supplementation-induced increase (gastrocnemius muscle; R=-0.789, p=0.031) indicated that individuals with lower baseline muscle carnosine are more likely to accumulate carnosine during supplementation (Table 1). The median value of baseline muscle carnosine was therefore used to stratify the population into low- and high-carnosine containing groups. Individuals in low carnosine group were characterized by lower BMI and waist circumference, and higher habitual ambulatory activity, while resting pH of their gastrocnemius muscle was lower (Fig. 2D-F, Table 1). Intramuscular carnosine content tended to correlate positively with resting intramuscular pH (soleus muscle; r=0.409, p=0.059).

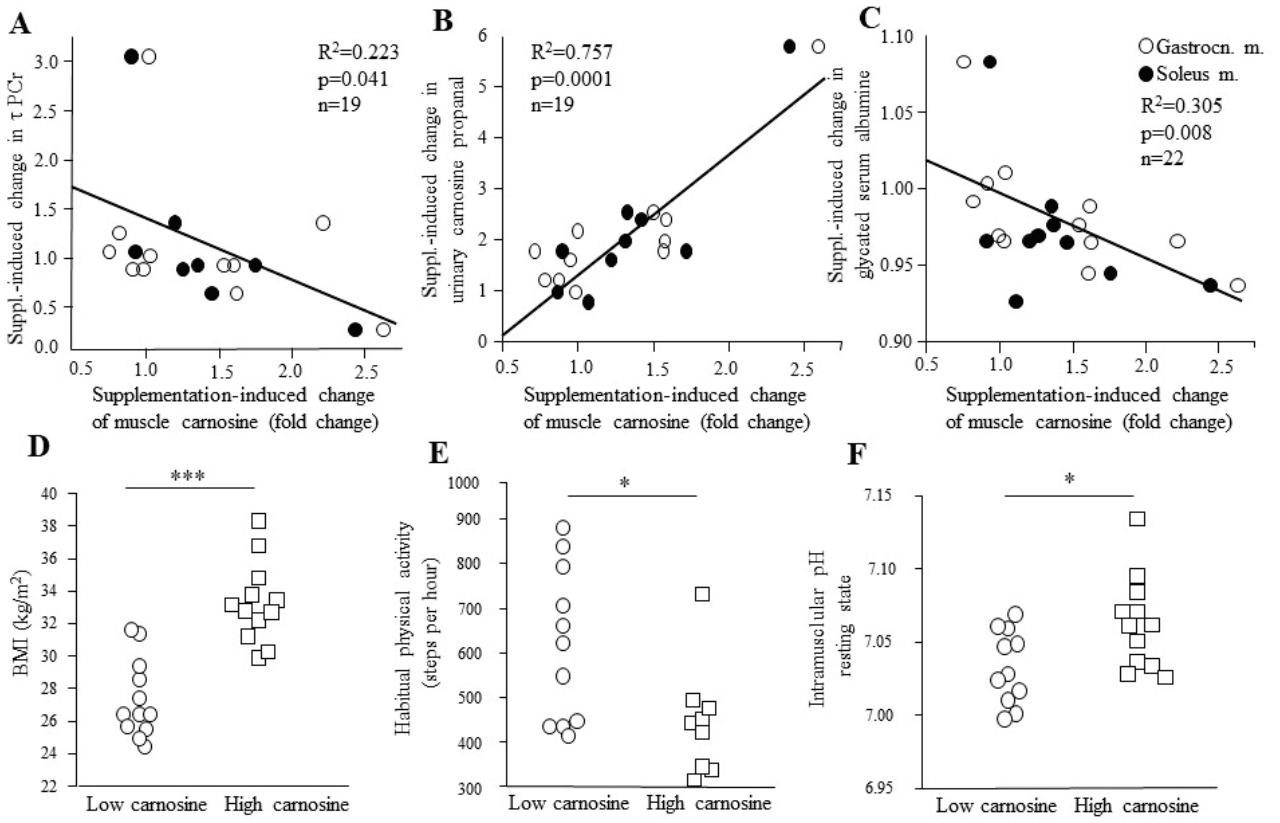

Fig. 2. Interrelations between supplementation-induced change of skeletal muscle carnosine content and parallel changes (**A**) in time constant of phosphocreatine recovery (τ_{PCr}), (**B**) of urinary carnosine propanal content, (**C**) of glycated serum albumin (gHSA). Low and high carnosine groups differed in (**D**) body mass index (BMI), (**E**) habitual ambulatory activity and (**F**) resting intramuscular pH, * $p<0.015$, *** $p<0.001$.

Discussion

Here we described the link between changes in muscle carnosine content induced by carnosine supplementation and changes in the whole-body and muscle energy metabolism, enhanced reactive carbonyl species sequestering capacity and reduced protein glycation. Supplementation with carnosine or its precursor β-alanine has been shown to improve dynamic knee extension torque in sprint-trained athletes [27] as well as performance capacity during high intensity cycling (110 % Wmax) [28] and anaerobic exercise [29]. Recently we showed that muscle carnosine concentration correlates positively with the dynamics of intramuscular phosphocreatine post-exercise recovery in a cohort of 15 young and 19 elderly individuals. This indirectly indicates that carnosine content might relate to specific aspects of muscle energy metabolism [30]. Here, we extend this observation by showing that supplementation-induced change in muscle carnosine is paralleled by change of post-exercise phosphocreatine recovery dynamics (τ_{PCr}), and that muscle carnosine correlates with resting muscle phosphocreatine content. One recent study measured both carnosine and phosphocreatine in young healthy humans with the aim to assess the relationship between post-exercise phosphocreatine recovery to O_2 supply and muscle fiber type, however, the authors used carnosine as a surrogate measure for muscle fiber type only and did not report on a biochemical or physiological relationship to phosphocreatine [31]. It is plausible to speculate that relationship between muscle carnosine and phosphocreatine could be physiologically relevant. Perhaps the pH buffering and antioxidant properties of carnosine, together with its capacity to reduce lactic acid accumulation [32], and to enhance oxidative capacity of skeletal muscle, could be translated to improved dynamics of the phosphocreatine post-exercise recovery.

Importantly, supplementation-induced increase in muscle carnosine was paralleled by a concurrent decrease of serum protein glycation and a correspondent increase of toxic carbonyl species urinary excretion. These observations are in line with the finding that improvements in glucose metabolism and diabetic

Table 1. Effects of carnosine supplementation on parameters of whole-body and muscle-specific energy metabolism.

Clinical phenotypes	**Entire population** (n=12)		**Lower baseline muscle carnosine** (n=6)		**Higher baseline muscle carnosine** (n=6)		**Repeated measures 2-way ANOVA**					
	Baseline	**Carnosine suppl.**	**Baseline**	**Carnosine suppl.**	**Baseline**	**Carnosine suppl.**	Group		Time		Group × time	
Sex (F/M)	2/10	2/10	2/4	2/4	0/6	0/6						
	mean ± STD	mean ± STD	mean ± STD	mean ± STD	mean ± STD	mean ± STD	F-value	p-value	F-value	p-value	F-value	p-value
Age (years)	40.1±6.7		40.5±6.1		39.7±7.9							
Carnosine *m. gastr.* (mM)	4.75±1.74	5.95±2.41	**3.42±1.35**	**6.43±3.35***	**6.09±0.74**[#]	5.47±1.00	0.729	0.413	**6.46**	**0.029**	**14.8**	**0.003**
[♪]**Carnosine** *m. soleus* (mM)	**3.72±1.45**	**4.81±1.22***	**3.03±0.68**	**4.64±1.09***	4.75±0.78	5.01±1.46	% -	-	-	-	-	-
Carnosine urine (µmol/l)	**0.80±0.60**	**1.33±0.81**[§]	**0.63±0.85**	1.10±0.99	**0.96±0.35**	**1.56±0.62***	0.06	0.819	3.68	0.084	0.03	0.865
Carnosine propanal urine (nmol/ml)	0.68±0.59	0.82±0.66	0.73±0.33	0.85±0.27	0.63±0.16	0.85±0.27	0.032	0.861	0.48	0.504	0.04	0.854
Glycated HSA (%)	17.4±0.8	17.2±0.9	17.6±0.3	17.1±0.03	17.2±0.3	17.3±0.3	0.3	0.865	1.62	0.232	4.61	0.057
CN1 content (µg/ml)	58.4±19.7	54.4±17.2	64.8±26.0	62.1±19.5	52.1±9.2	46.7±11.2	2.08	0.180	1.68	0.224	0.19	0.675
CN1 act. (µmol/ml/h)	**2.09±0.59**	**1.57±0.64***	2.24±0.77	1.68±0.68	1.93±0.34	1.46±0.64	0.77	0.400	**6.75**	**0.027**	0.06	0.816
Body weight (kg)	96.0±18.2	97.0±16.8	**83±5.7**	85.7±5.7	**109±4.6**[#]	108.3±4.6	**11.2**	**0.007**	1.32	0.277	3.79	0.080
BMI (kg/m^2)	30.0±4.2	30.3±3.6	**26.8±2.6**	27.7±1.0	**33.2±2.7**[#]	33.0±1.1	**16.6**	**0.002**	1.29	0.283	4.12	0.070
Body fat (%)	30.4±6.6	31.1±6.1	30.0±1.1	30.9±3.6	30.8±1.5	31.3±1.4	0.02	0.886	3.46	0.092	0.40	0.540
Muscle mass (%)	32.0±3.8	31.8±3.9	31.8±5.4	32.0±2.2	32.2±1.6	31.6±0.8	0.01	0.989	0.53	0.484	1.80	0.210
Waist circumference (cm)	99.3±11.8	99.8±11.5	**91.3±4.1**	91.3±4.0	**107.3±3.3**[#]	107.0±3.2	**8.74**	**0.014**	0.45	0.518	1.25	0.29
Handgrip strength – right (kg)	43.1±10.9	44.2±10.6	41.9±4.6	43.2±4.5	44.2±4.6	45.2±4.3	0.12	0.741	1.85	0.204	0.05	0.838
Handgrip strength – left (kg)	38.4±8.6	37.4±6.4	35.7±3.1	35.3±3.1	41.1±3.0	39.5±3.1	1.35	0.273	0.65	0.438	0.24	0.638
Habitual activity (steps/active hour[@])	639±229	728±229	675±165	740±190	495±55	600±171	**5.96**	**0.025**	0.03	0.855	2.43	0.103
Protein preference (score 0-9)[&]	6.3±1.1	6.3±1.2	6.4±0.4	6.9±0.4	5.9±0.5	5.4±0.5	0.26	0.976	0.01	0.987	0.95	0.354
REE (kcal/kg/24 h)	31.8±6.2	28.6±2.6	32.6±2.3	28.9±2.2	31.0±2.0	28.3±1.8	0.22	0.646	3.34	0.097	0.07	0.792

RQ (VCO_2/VO_2)	0.80±0.09	0.83±0.08	0.84±0.09	0.83±0.04	0.76±0.08	0.83±0.03	0.71	0.421	1.17	0.305	1.86	0.202
pH baseline *m. gastr.*	7.04±0.04	7.05±0.04	7.03±0.01	7.03±0.01	7.05±0.01	7.07±70.01	**6.34**	**0.033**	0.74	0.412	0.21	0.654
pH post-exercise *m. gastr.*	6.96±0.12	6.94±0.13	6.95±0.05	6.95±0.05	7.01±0.06	6.93±0.06	0.08	0.79	0.84	0.383	0.83	0.387
PCr baseline *m. gastr.* (mM)	**32.7±2.8**	**35.2±4.9**[§]	31.5±1.9	33.6±1.7	33.7±1.5	36.9±1.5	1.58	0.241	4.23	0.07	0.06	0.806
τ_{PCr} *m. gastr.* (s)	51.2±21.2	50.8±18.2	57.3±9.3	44.5±8.5	46.1±7.6	57.2±7.6	0.01	0.969	0.01	0.949	2.43	0.154
Qmax *m. gastr.* (mM/s)	0.45±0.20	0.51±0.17	0.47±0.09	0.52±0.08	0.43±0.08	0.51±0.08	0.07	0.798	2.65	0.138	0.01	0.948

Carnosine content in skeletal muscle was assessed by ^{1}H-MRS (Magnetic Resonance Spectroscopy). Data are presented as mean ± STD. * denotes supplementation-induced change (paired *t*-test, $p<0.05$). [§] denotes a trend to supplementation-induced change (paired *t*-test, $p<0.1$). [#] marks the difference between individuals with high and low baseline muscle carnosine (unpaired *t*-test, $p<0.05$). RM two-way ANOVA was used. [@] active hour: at least 50 steps per hour. BMI, Body Mass Index; BP, blood pressure; REE, Resting Energy Expenditure; RQ, respiratory quotient; FFA, Free Fatty Acids; HSA, Human Serum Albumin; CN1, carnosinase-1; A.U., Arbitrary Units; PCr, phosphocreatine; τ_{PCr}, time constant of phosphocreatine/PCr recovery; Qmax, maximal oxidative flux. [♪] measurement was successfully performed in 9 individuals. [&] Food preference questionnaire [24].

nephropathy related to 18-weeks of carnosine supplementation were linked to enhanced carnosine adducts urinary excretion in mice model of type 2 diabetes [9] as well as with our previous work showing that carnosine treatment is accompanied by increased urinary content of carnosine and carnosine-acrolein adducts [23]. Carnosine supplementation decreased serum carnosinase-1 activity, and supplementation-induced changes of CN1 protein content correlated with changes in muscle carnosine, indicating the putative importance of the dynamic regulation of CN1.

Carnosine supplementation increased muscle carnosine content specifically in individuals with lower baseline carnosine content, who had lower BMI and abdominal adiposity, were more active in their everyday life, but their muscle mass, preference for dietary protein or compliance to the supplementation, was not different from those who accumulated less muscle carnosine. Our previous report showed that muscle carnosine content increased with the progression of obesity-related metabolic disease and the highest levels were found in patients with newly diagnosed yet untreated type-2-diabetes [20]. Variability of muscle capacity to accumulate carnosine following carnosine/β-alanine supplementation was reported in young and/or physically active individuals [33-36], and in healthy elderly [29]. Individuals in low carnosine group were characterized by higher habitual ambulatory activity and more acidic resting pH of their gastrocnemius muscle. Carnosine content in soleus muscle tended to correlate positively with resting intramuscular pH. Carnosine is well-known for its pH buffering capacity [37]. We have previously shown that baseline intramuscular pH of less carnosine accumulating in soleus muscle containing more oxidative muscle fibers was slightly lower (pH 7.01±0.03), than that in gastrocnemius muscle (pH 7.05±0.02) [18], which is in line with our current observations. Limitations of this pilot-study is a small number of participants and missing MRI/MRS examination in the placebo control group. The outcomes of this report have thus rather exploratory and observational character. However, this study has the capacity to evaluate interrelations between supplementation-induced changes in muscle carnosine and changes in dynamic parameters of exercise-related muscle energy metabolism, carnosine adducts excretion and albumin glycation, as well as to evaluate carnosine-supplementation related effects in patients with low and high baseline muscle carnosine. The changes in muscle carnosine content induced by 12-week carnosine supplementation are linked to the accelerated muscle post-exercise phosphocreatine recovery, decreased protein glycation and enhanced reactive carbonyl species sequestering capacity. These observations suggest that carnosine accumulation in skeletal muscle following the supplementation is greater in leaner, more active individuals, and is paralleled by more pronounced reduction in protein glycation, increased reactive carbonyl species removal and accelerated muscle post-exercise PCr recovery. These effects might contribute to enhanced skeletal muscle metabolism and performance, and subsequently lower the risk of metabolic disease development.

Conflict of Interest

There is no conflict of interest.

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References

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet 2017;390:2627-2642. https://doi.org/10.1016/S0140-6736(17)32129-3
2. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-1053. https://doi.org/10.2337/diacare.27.5.1047
3. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393-403. https://doi.org/10.1056/NEJMoa012512
4. Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, ET AL. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343-1350. https://doi.org/10.1056/NEJM200105033441801
5. Baye E, Ukropcova B, Ukropec J, Hipkiss A, Aldini G, de Courten B. Physiological and therapeutic effects of carnosine on cardiometabolic risk and disease. Amino Acids 2016;48:1131-1149. https://doi.org/10.1007/s00726-016-2208-1
6. Schön M, Mousa A, Berk M, Chia WL, Ukropec J, Majid A, Ukropcová B, de Courten B. The potential of carnosine in brain-related disorders: a comprehensive review of current evidence. Nutrients 2019;11:1196. https://doi.org/10.3390/nu11061196
7. Boldyrev A, Aldini G, Derave W. Physiology and pathophysiology of carnosine. Physiol Rev 2013;93:1803-1845. https://doi.org/10.1152/physrev.00039.2012
8. Sauerhöfer S, Yuan G, Braun GS, Deinzer M, Neumaier M, Gretz N, Floege J, ET AL. L-carnosine, a substrate of carnosinase-1, influences glucose metabolism. Diabetes 2007;56:2425-2432. https://doi.org/10.2337/db07-0177
9. Albrecht T, Schilperoort M, Zhang S, Braun JD, Qiu J, Rodriguez A, Pastene DO, ET AL. Carnosine attenuates the development of both type 2 diabetes and diabetic nephropathy in BTBR ob/ob mice. Sci Rep 2017;7:44492. https://doi.org/10.1038/srep44492
10. Aldini G, Orioli M, Rossoni G, Savi F, Braidotti P, Vistoli G, Yeum K-J, ET AL. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. J Cell Mol Med 2011;15:1339-1354. https://doi.org/10.1111/j.1582-4934.2010.01101.x
11. Cripps MJ, Hanna K, Lavilla C JR, Sayers SR, Caton PW, Sims C, De Girolamo L, ET AL. Carnosine scavenging of glucolipotoxic free radicals enhances insulin secretion and glucose uptake. Sci Rep 2017;7:13313. https://doi.org/10.1038/s41598-017-13649-w
12. Holeček M, Vodeničarovová M. Effects of histidine supplementation on amino acid metabolism in rats. Physiol Res 2020;69:99-111. https://doi.org/10.33549/physiolres.934296
13. Menon K, Marquina C, Liew D, Mousa A, de Courten B. Histidine-containing dipeptides reduce central obesity and improve glycaemic outcomes: A systematic review and meta-analysis of randomized controlled trials. Obes Rev 2020;21:e12975. https://doi.org/10.1111/obr.12975
14. Nagai K, Tanida M, Niijima A, Tsuruoka N, Kiso Y, Horii Y, Shen J, Okumura N. Role of L-carnosine in the control of blood glucose, blood pressure, thermogenesis, and lipolysis by autonomic nerves in rats: involvement of the circadian clock and histamine. Amino Acids 2012;43:97-109. https://doi.org/10.1007/s00726-012-1251-9
15. De Courten B, Jakubova M, de Courten MPJ, Kukurova IJ, Vallova S, Krumpolec P, Valkovic L, ET AL. Effects of carnosine supplementation on glucose metabolism: Pilot clinical trial. Obesity 2016;24:1027-1034. https://doi.org/10.1002/oby.21434
16. Baye E, Ukropec J, de Courten MP, Vallova S, Krumpolec P, Kurdiova T, Aldini G, Ukropcova B, de Courten B. Effect of carnosine supplementation on the plasma lipidome in overweight and obese adults: A pilot randomised controlled trial. Sci Rep 2017;7:17458. https://doi.org/10.1038/s41598-017-17577-7
17. Baye E, Ukropec J, de Courten MPJ, Mousa A, Kurdiova T, Johnson J, Wilson K, ET AL. Carnosine supplementation improves serum resistin concentrations in overweight or obese otherwise healthy adults: a pilot randomized trial. Nutrients 2018;10:1258. https://doi.org/10.3390/nu10091258

18. Just Kukurová I, Valkovič L, Ukropec J, de Courten B, Chmelík M, Ukropcová B, Trattnig S, Kršňák M. Improved spectral resolution and high reliability of in vivo 1H MRS at 7 T allow the characterization of the effect of acute exercise on carnosine in skeletal muscle. NMR Biomed 2016;29:24-32. https://doi.org/10.1002/nbm.3447
19. Gualano B, Everaert I, Stegen S, Artioli GG, Taes Y, Roschel H, Achten E, ET AL. Reduced muscle carnosine content in type 2, but not in type 1 diabetic patients. Amino Acids 2012;43:21-24. https://doi.org/10.1007/s00726-011-1165-y
20. De Courten B, Kurdiova T, De Courten MPJ, Belan V, Everaert I, Vician M, Teede H, ET AL. Muscle carnosine is associated with cardiometabolic risk factors in humans. PLoS One 2015;10:e0138707. https://doi.org/10.1371/journal.pone.0138707
21. Matthews JJ, Dolan E, Swinton PA, Santos L, Artioli GG, Turner MD, Elliott-Sale KJ, Sale C. The effect of carnosine or beta-alanine supplementation on markers of glycaemic control and insulin resistance in human and animal studies: a protocol for a systematic review and meta-analysis. Syst Rev 2020;9:282. https://doi.org/10.1186/s13643-020-01539-8
22. Holeček M. Side effects of amino acids supplements. Physiol Res 2022;71:29-45. https://doi.org/10.33549/physiolres.934790
23. Regazzoni L, de Courten B, Garzon D, Altomare A, Marinello C, Jakubova M, Vallova S, ET AL. A carnosine intervention study in overweight human volunteers: Bioavailability and reactive carbonyl species sequestering effect. Sci Rep 2016;6:27224. https://doi.org/10.1038/srep27224
24. Geiselman PJ, Anderson AM, Dowdy ML, West DB, Redmann SM, Smith SR. Reliability and validity of a macronutrient self-selection paradigm and a food preference questionnaire. Physiol Behav 1998;63:919-928. https://doi.org/10.1016/S0031-9384(97)00542-8
25. Valkovič L, Ukropcová B, Chmelík M, Baláž M, Bogner W, Schmid AI, Frollo I, ET AL. Interrelation of 31P-MRS metabolism measurements in resting and exercised quadriceps muscle of overweight-to-obese sedentary individuals. NMR Biomed 2013;26:1714-1722. https://doi.org/10.1002/nbm.3008
26. Valkovič L, Chmelík M, Just Kukurová I, Jakubová M, Kipfelsberger MC, Krumpolec MP, Tušek Jelenc M, ET AL. Depth-resolved surface coil MRS (DRESS)-localized dynamic 31P-MRS of the exercising human gastrocnemius muscle at 7 T. NMR Biomed 2014;27:1346-1352. https://doi.org/10.1002/nbm.3196
27. Derave W, Özdemir MS, Harris RC, Pottier A, Reyngoudt H, Koppo K, Wise JA, Achten E. β-Alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. J Appl Physiol 2007;103:1736-1743. https://doi.org/10.1152/japplphysiol.00397.2007
28. Hill CA, Harris RC, Kim HJ, Harris BD, Sale C, Boobis LH, Kim CK, Wise JA. Influence of β-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. Amino Acids 2007;32:225-233. https://doi.org/10.1007/s00726-006-0364-4
29. Del Favero S, Roschel H, Solis MY, Hayashi AP, Artioli GG, Otaduy MC, Benatti FB, ET AL. Beta-alanine (CarnosynTM) supplementation in elderly subjects (60-80 years): Effects on muscle carnosine content and physical capacity. Amino Acids 2012;43:49-56. https://doi.org/10.1007/s00726-011-1190-x
30. Krumpolec P, Klepochová R, Just I, Tušek Jelenc M, Frollo I, Ukropec J, Ukropcová B, ET AL. Multinuclear MRS at 7T uncovers exercise driven differences in skeletal muscle energy metabolism between young and seniors. Front Physiol 2020;11:644. https://doi.org/10.3389/fphys.2020.00644
31. Heskamp L, Lebbink F, van Uden MJ, Maas MC, Claassen JAHR, Froeling M, Kemp GJ, Boss A, Heerschap A. Post-exercise intramuscular O2 supply is tightly coupled with a higher proximal-to-distal ATP synthesis rate in human tibialis anterior. J Physiol 2021;599:1533-1550. https://doi.org/10.1113/JP280771
32. Derave W, Ozdemir MS, Harris RC, Pottier A, Reyngoudt H, Koppo K, Wise JA, Achten E. beta-Alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. J Appl Physiol 2007;103:1736-1743. https://doi.org/10.1152/japplphysiol.00397.2007
33. Baguet A, Reyngoudt H, Pottier A, Everaert I, Callens S, Achten E, Derave W. Carnosine loading and washout in human skeletal muscles. J Appl Physiol 2009;106:837-842. https://doi.org/10.1152/japplphysiol.91357.2008
34. Stegen S, Bex T, Vervaet C, Vanhee L, Achten E, Derave W. ß-alanine dose for maintaining moderately elevated muscle carnosine levels. Med Sci Sport Exerc 2014;46:1426-1432. https://doi.org/10.1249/MSS.0000000000000248
35. Bex T, Chung W, Baguet A, Stegen S, Stautemas J, Achten E, Derave W. Muscle carnosine loading by beta-alanine supplementation is more pronounced in trained vs. untrained muscles. J Appl Physiol 2014;116:204-209. https://doi.org/10.1152/japplphysiol.01033.2013

36. Blancquaert L, Everaert I, Missinne M, Baguet A, Stegen S, Volkaert A, Petrovic M, ET AL. Effects of histidine and β-alanine supplementation on human muscle carnosine storage. Med Sci Sport Exerc 2017;49:602-609. https://doi.org/10.1249/MSS.0000000000001213
37. Culbertson JY, Kreider RB, Greenwood M, Cooke M. Effects of beta-alanine on muscle carnosine and exercise performance: a review of the current literature. Nutrients 2010;2:75-98. https://doi.org/10.3390/nu2010075