European Journal of Sport Science

ISSN: 1746-1391 (Print) 1536-7290 (Online) Journal homepage: http://www.tandfonline.com/loi/tejs20

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Guilherme Giannini Artioli, Craig Sale & Rebecca Louise Jones

To cite this article: Guilherme Giannini Artioli, Craig Sale & Rebecca Louise Jones (2018): Carnosine in health and disease, European Journal of Sport Science, DOI: 10.1080/17461391.2018.1444096

To link to this article: https://doi.org/10.1080/17461391.2018.1444096

Published online: 04 Mar 2018.

European Journal of Sport Science, 2018
https://doi.org/10.1080/17461391.2018.1444096

ORIGINAL ARTICLE

Carnosine in health and disease

GUILHERME GIANNINI ARTIOLI[1], CRAIG SALE[1], & REBECCA LOUISE JONES [2]

[1]*Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement (SHAPE) Research Centre, School of Science and Technology, Nottingham Trent University, Nottingham, UK &* [2]*School of Sport Science and Physical Activity, Institute for Sport and Physical Activity Research, University of Bedfordshire, Bedford, UK*

Abstract
Carnosine was originally discovered in skeletal muscle, where it exists in larger amounts than in other tissues. The majority of research into the physiological roles of carnosine have been conducted on skeletal muscle. Given this and the potential for muscle carnosine content to be increased with supplementation, there is now a large body of research examining the ergogenic effects (or otherwise) of carnosine. More recent research, however, points towards a potential for carnosine to exert a wider range of physiological effects in other tissues, including the brain, heart, pancreas, kidney and cancer cells. Taken together, this is suggestive of a potential for carnosine to have therapeutic benefits in health and disease, although this is by no means without complication. Herein, we will provide a review of the current literature relating to the potential therapeutic effects of carnosine in health and disease.

Keywords: *Health, metabolism, nutrition, physiology*

Highlights
- Carnosine and its analogues (anserine and balenine) are naturally-occurring histidine-containing dipeptides (HCDs) expressed in different tissues, such as skeletal muscle, cardiac muscle, kidneys, and some regions of the brain. Roles of HCDs include: neutralisation of reactive species, detoxification and acid-base regulation.
- HCDs are thought to have several potential applications to health and disease, such as healthy ageing, improved cognitive function, prevention of diabetes complications, protective effects during acute kidney failure, and anti-neoplastic effects.
- Evidence for the health related and disease prevention effects are still emerging and the clinical efficacy of HCDs merit further confirmation by clinical trials. Since HCDs content in some tissues increase with dietary ingestion of HCDs, nutritional strategies may be easily developed to explore the therapeutic potential of HCDs.

Introduction

Carnosine, a histidine-containing dipeptide (HCD), is synthesised by bonding of the amino acids β-alanine and L-histidine, a reaction catalysed by carnosine synthase (Figure 1). Carnosine has been identified in vertebrates, including horses, greyhounds, camels and humans (see review by Boldyrev, Aldini, & Derave, 2013). Several animal species express other HCDs (anserine and ophidine/balenine), which are methylated forms of carnosine. Carnosine was, until recently, the only known HCD in human tissues, largely reported in skeletal and cardiac muscle, liver tissue and regions of the central nervous system. Peters et al. (2015) showed, however, that anserine is also expressed in the renal cortex of human kidney, in concentrations approximately two times higher than those of carnosine.

Carnosine is considered an intracellular pH buffer, but several other physiological roles relevant to the function and homeostasis of different tissues have been proposed, including the regulation of Ca^{2+} transients in sarcoplasm and the sensitivity of skeletal muscle contractile apparatus to Ca^{2+}, protection against reactive species, protection against glycation and inhibition of the formation of glycation-end products (Boldyrev et al., 2013). These properties are attracting attention relating to its potential ability to improve normal function, prevent ageing and help

Correspondence: Guilherme G. Artioli, Musculoskeletal Physiology Research Group, Sport, Health and Performance Enhancement (SHAPE) Research Centre, School of Science and Technology, Nottingham Trent University, Clifton Campus, Erasmus Darwin Building, Clifton Lane, Clifton, Nottingham NG11 8NS, UK. E-mail: guilherme.artioli@ntu.ac.uk

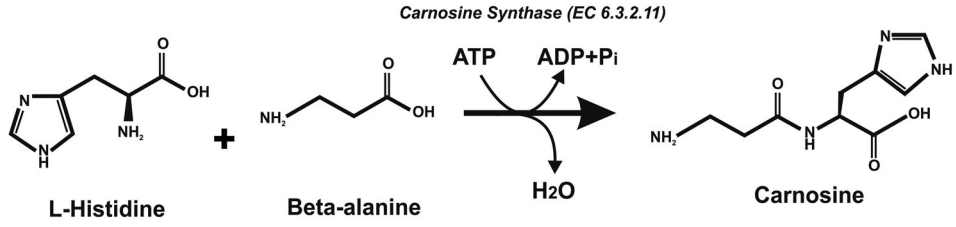

Figure 1. Endogenous synthesis of carnosine by the enzyme carnosine synthase. The molecular masses of the three compounds (carnosine: 226 g mol^{-1}; L-histidine: 155 g mol^{-1}; β-alanine: 89 g mol^{-1}) indicates a loss of 18 g mol^{-1} during carnosine formation, which is consistent with the loss of one water molecule.

treat conditions in which glycation and oxidative stress play critical roles (e.g. diabetes and neurodegenerative diseases). Due to the physiological properties of carnosine associated with anti-proliferation (Shen et al., 2014), and its ability to act as a neurotransmitter (Tiedje, Stevens, Barnes, & Weaver, 2010), the potential for carnosine to act as an anti-cancer agent and to improve cognitive function has also been examined. Furthermore, it has been suggested that carnosine may not display the same functions in different tissues, and may even fulfil several functions within one tissue (Boldyrev et al., 2013).

In humans, increasing the availability of free β-alanine, either via dietary (e.g. beef, chicken, pork, turkey and fish) or supplemental sources (either β-alanine or carnosine), results in increased skeletal muscle carnosine content (Harris et al., 2006). The relationship between β-alanine intake and skeletal muscle carnosine content occurs in a dose-dependent manner (Stellingwerff et al., 2012). Nonetheless, it remains uncertain how carnosine concentrations are regulated in other tissues. Although β-alanine and carnosine supplementation have been gaining attention due to a robust body of evidence to support their ergogenic effects (Saunders et al., 2017), there is also great potential for application in health and disease. In this review, we will use current knowledge on carnosine and other HCDs to explore the potential impact of carnosine on tissue function, specifically regarding health and disease. We discussed the importance of HCDs to healthy ageing as well as potential clinical applications in conditions such as neurodegenerative diseases, diabetes, kidney failure and cancer. However, the multiple roles played by HCDs in multiple organs and tissues imply that their applications extend to a number of other conditions that cannot be thoroughly discussed in a brief review. We advise the reader that there are other potential applications that will not be covered in this review, such as autism, schizophrenia, stroke, heart failure, circulatory disorders, wound healing, sepsis and cataracts.

Carnosine in health

Ageing

Ageing is a multifactorial process, resulting from several persistent deleterious effects that negatively alter cellular and organism homeostasis. McFarland and Holliday (1994, 1999) suggested that carnosine could act as an anti-ageing agent, with improvements to the Hayflick limit (the maximum number of times cells can divide), and the apparent rejuvenation of senescent cells. Numerous possible anti-ageing outcomes of carnosine have been proposed, including effects on reactive species (Kohen, Yamamoto, Cundy, & Ames, 1988), inhibitory effects on glycolysis in tumour cells (Iovine et al., 2012), stimulatory effects on mitochondrial activity (Renner, Asperger, et al., 2010), reduced toxic metabolite and methylglyoxal formation (Hipkiss, Michaelis, & Syrris, 1995), reduction of translation initiation factor phosphorylation, slowing translation, thus reducing error-protein generation (Son, Satsu, Kiso, Totsuka, & Shimizu, 2008), and slowed telomere shortening (Shao, Li, & Tan, 2004). Although these findings are promising, current evidence is mostly limited to *in vitro* and cultured cell models, with little being known about the physiological relevance of these properties. It also remains unclear exactly how carnosine can affect cellular lifespan and the onset of age-related changes in tissue function.

Studies in humans (Tallon, Harris, Maffulli, & Tarnopolsky, 2007) and animals (Johnson & Hammer, 1992) suggest that skeletal muscle carnosine content declines with ageing. Emerging evidence from human studies shows improvements in exercise performance following β-alanine supplementation in older adults (del Favero et al., 2012; Stout et al., 2008). β-Alanine supplementation in older adults increases skeletal muscle carnosine content (+85%; del Favero et al., 2012), with responsiveness to supplementation occurring in a similar manner to younger individuals (Stellingwerff et al., 2012). In older adults, increased skeletal muscle carnosine was accompanied by improved exercise tolerance in constant-load submaximal and incremental tests

(del Favero et al., 2012). β-Alanine supplementation has also been shown to delay the onset of neuromuscular fatigue in older adults (Stout et al., 2008), with performance improvements being twofold greater than those achieved by younger individuals (Stout et al., 2006). Although increased exercise tolerance and delayed fatigue may suggest that muscle function and quality of life could be improved, measures associated with skeletal muscle function (e.g. timed-stands, timed-up-and-go and hand-grip strength) and quality of life have remained unaffected by increased skeletal muscle carnosine concentrations (del Favero et al., 2012). This might be due to the low sensitivity of these tests, limited by a "ceiling effect" in healthy populations. Thus, alterations to functional and health-related measures may be more apparent in frail individuals, or those with more substantial impairments in physical function, which was not the case in the assessed population (del Favero et al., 2012). It remains plausible, therefore, that increased muscle carnosine content may be able to improve skeletal muscle function and improve the mobility of older individuals; in turn reducing functional decline and risk of disability. Although more evidence is needed, these data imply that increasing skeletal muscle carnosine concentrations in older adults may result in improved muscle performance, alongside reducing the negative impact of ageing, albeit these effects may occur indirectly.

Cognitive function

Carnosine synthase, an enzyme present in the human skeletal muscle, has been reported in different areas of the mammalian brain (Murakami & Furuse, 2010). As well as carnosine, this ATP-dependent enzyme can synthesise homocarnosine, although this occurs at a lower efficiency. Carnosine is likely to be taken up into brain regions following its release from glial cells, since neurons, mainly those of the olfactory bulb (where carnosine is in greater concentrations), are unable to synthesise carnosine (Hoffmann, Bakardjiev & Bauer, 1996). β-Alanine can be rapidly transported into the brain, with research in rodents demonstrating increased carnosine content in the cerebral cortex and hypothalamus following β-alanine supplementation (Murakami & Furuse, 2010). *In vitro* studies with isolated human retina showed that β-alanine can be transported into and can accumulate in neuronal cells (Bruun & Ehinger, 1974). It is unclear, however, whether brain carnosine can be increased with β-alanine supplementation and, if so, in what regions this would occur. An *in vivo* study assessing the effects of β-alanine supplementation on brain carnosine in humans has shown no increase in carnosine in the posterior cingulate cortex following four weeks of β-alanine supplementation (Solis et al., 2015). Notably, the posterior cingulate cortex is highly active and fulfils various functions, such as memory, focus of attention, processing and learning. It must be noted, however, that there are limitations in non-invasive quantification of carnosine in the human brain, such as the inability to distinguish carnosine from homocarnosine signals. It remains unknown whether other areas can benefit from β-alanine supplementation.

Since the identification of HCDs in the brain, it has been proposed that increasing their availability could alter cognitive function and/or neuropsychological measures. In rodents experiencing stressful conditions, increased brain carnosine content achieved via β-alanine supplementation resulted in improvements to time in the maze (Murakami & Furuse, 2010), an indicator of learning and memory. In humans, examination of functional MRI and neuropsychological assessments was undertaken to examine the effect of daily doses of carnosine and anserine over a three-month supplementation period in middle-aged and older individuals (Rokicki et al., 2015). Improvements in verbal episodic memory performance and decreased connectivity in the default mode network were reported following supplementation. These enhancements in cognitive function are in line with earlier research in a similar elderly population, during which dietary anserine and carnosine supplementation significantly increased mean values of the Short Test of Mental Status; specifically, subscores of construction/copying, abstraction and recall (Szczesniak, Budzen, Kopec, & Rymaszewska, 2014).

The effects of β-alanine supplementation and the purported increased levels of HCDs in the brain on cognitive function in healthy individuals have been investigated; the results, however, are conflicting. A two-part investigation was the first to examine the effects of β-alanine supplementation on brain homocarnosine/carnosine signal in humans, and the mediating effects of β-alanine supplementation on the acute effect of exercise on cognitive function (Solis et al., 2015). Following four weeks of β-alanine supplementation, there were no reported changes in the brain homocarnosine/carnosine signal in either vegetarian or omnivorous individuals, nor were there any effects of β-alanine supplementation on cognitive function at rest or following exercise. Similar responses were reported following four weeks of β-alanine supplementation in military personnel, with enhanced vertical jump relative peak power, marksmanship and target engagement speed compared to placebo, but no impact on cognitive function (Hoffman et al., 2014). It should be noted

that muscle and brain carnosine concentrations were not determined following supplementation. The experience of both physiological and psychological decline is not uncommon in military personnel undertaking prolonged, high-intensity military training, simulated combat exercise or military duties. As such, Hoffman, Landau, et al. (2015) examined the effect of β-alanine supplementation on gastrocnemius muscle and brain carnosine content, physiological (2.5-km run, a 1-min sprint, 50-m casualty carry, repeated 30-m sprints with target shooting) and cognitive function (2-min serial subtraction test) measures in military personnel experiencing stressful conditions (active firing was taking place). Thirty days of β-alanine supplementation significantly elevated muscle carnosine content and improved both physiological performance (simulating a 50-m casualty carry) and cognitive function (2-min serial subtraction test) compared to placebo (Hoffman, Landau, et al., 2015). There was no detectable change in brain carnosine content following β-alanine supplementation, meaning that the underlying mechanism for improved cognitive function remains unclear.

The manner in which the subtraction test (cognitive performance) was conducted could explain differences in these data, since active firing was taking place, which, when coupled with the stress of the test, may have contributed to participants experiencing high levels of anxiety. Later research in a murine model investigated the effects of 100 mg kg^{-1} β-alanine supplementation for 30 days on brain carnosine concentrations and behavioural and neuroendocrine responses in a model of post-traumatic stress disorder (PTSD; Hoffman, Ostfeld, et al., 2015). Animals supplemented with β-alanine and exposed to predator-scent stress had significantly greater carnosine concentrations in the hippocampus, cortex, hypothalamus, amygdala and thalamus sections of the brain than animals that were provided with a normal diet, before exposure to the stressor. A 19% lower startle response in the acoustic startle response test and 15% lower freezing behaviour upon cue-exposure was reported. These data suggest that β-alanine supplementation is able to attenuate some of the behaviours associated with experiencing high levels of stress and/or anxiety. To explore the potential mechanisms of these data, brain-derived neurotrophic factor (BDNF), a key factor in the neuronal remodelling and potential modulator of synaptic plasticity and neurotransmitter release was examined. The animals experiencing predator-scent stress and β-alanine supplementation expressed greater BDNF compared to those exposed to stress and fed the control diet (Hoffman, Ostfeld, et al., 2015). The authors speculated that the reduction in PTSD behaviours may be mediated by the maintenance of BDNF levels in the hippocampus during stress and/or anxiety. Overall, the current body of research highlights the potential ability of β-alanine supplementation to alter cognitive function and/or psycho-neurological measures; although the stressfulness of the environment and anxiety levels of the individual may be critical in mediating this interaction.

Carnosine in disease

Neurodegenerative diseases

Alzheimer's and Parkinson's diseases are two of the most common neurodegenerative diseases, both characterised by mitochondrial energy dysfunction that ultimately leads to increased oxidative stress, chronic inflammation, and exacerbation of the ubiquitin–proteasome system; progressive loss of cellular function and neuronal cell death is a common endpoint of these diseases.

In Alzheimer's disease, carnosine inhibits the toxic effects of β-amyloid accumulation in cultured neuronal cells (Preston, Hipkiss, Himsworth, Romero, & Abbott, 1998); furthermore, carnosine can inhibit protein crosslinking and aggregation – two of the most extensive molecular features of Alzheimer's pathogeny – mediated by glycation-end products (AGEs; Corona et al., 2011). Using transgenic mouse models of Alzheimer's disease, which expresses both β-amyloid and tau-dependent pathologies, Corona et al. (2011) showed that carnosine supplementation reduced the accumulation of β-amyloid and suppressed mitochondrial dysfunction. Using a similar murine model, oral carnosine supplementation was effective in preventing cognitive decline (i.e. contextual memory assessed in a fear conditioning experiment), which was attributed to the ability of carnosine to inhibit the polymerisation of β-amyloid protein (Herculano et al., 2013).

Inadequate blood flow to the brain occurs in Alzheimer's disease; mediated by microvascular defects in brain blood vessels and accompanied by impaired clearance of neurotoxins. In healthy elderly humans, Hisatsune et al. (2016) showed that supplementation with a formula containing anserine:carnosine (3:1, 1 g/day for 3 months) counteracted the decline in blood flow in the posterior cingulate cortex associated with ageing, providing confirmatory evidence of the ability of HCDs to improve cerebral blood flow. Importantly, better preservation of memory in healthy elderly individuals was also reported. Similar results were shown in a murine model of Alzheimer's disease (Kaneko, Enya, Enomoto, Ding, & Hisatsune, 2017). Eight weeks of anserine

supplementation improved memory impairment in the Morris water maze and contextual fear conditioning tests. Moreover, anserine resulted in suppressed glial inflammatory reactions in the hippocampus and increased pericytes coverage in brain capillaries – an indicator of neurovascular-unit function (Kaneko et al., 2017). No effects of anserine were, however, shown on β-amyloid plaques. In light of this evidence, carnosine has been postulated to control the progression of Alzheimer's disease; clinical studies, however, are yet to be conducted to assess the relevance of carnosine-based therapies on Alzheimer's disease progression.

In Parkinson's disease, Boldyrev et al. (2008) enrolled 36 patients in an open-label clinical trial to receive DOPA (dihydroxyphenylalanine) or DOPA + carnosine (1.5 g/day) for 30 days. The patients treated with DOPA + carnosine presented with a significantly better (+36%) clinical improvement, as assessed by the Unified Parkinson's Disease Rating Scale, in comparison with the conventional treatment alone (+16%). Larger improvements in physical symptoms, such as rigidity and tremors in the upper body extremities, were also reported, accompanied by the increased activity of Cu/Zn-superoxide dismutase in red blood cells and decreased protein carbonyls and lipid hydroperoxides in plasma. Although these promising results suggest that carnosine could potentiate the effects of conventional treatments, no further evidence is available relating to the therapeutic effects of carnosine in Parkinson's disease. Future studies should confirm the clinical relevance of carnosine as an adjuvant treatment for Parkinson's disease, as well the underpinning mechanisms.

Type II diabetes

Type II diabetes and its complications are amongst the most common causes of morbidity and mortality in Western societies. Diabetes complications are multifactorial, but the long-term accumulated cellular damage caused by the formation of AGEs likely play a central role. AGEs are generated during chronic hyperglycaemia and are related to the increased formation of free radicals and protein crosslinks, thus inducing oxidative stress, morpho-functional alterations of cells and extracellular matrices and systemic low-grade inflammation.

Animal models of diabetes have shown diminished carnosine in plasma, retina, liver and kidneys in both type I and type II diabetes (Sauerhofer et al., 2007). In humans, studies have been limited to the analysis of carnosine in the skeletal muscle and results are equivocal. A cross-sectional study has shown that patients with type II, but not type I, diabetes have 45% less muscle carnosine in comparison with healthy controls (Gualano et al., 2012). This reduction was attributed to carnosine being consumed by AGEs and free radicals, which are both hallmarks of insulin resistance, although such assertions need confirmation. Cripps et al. (2017) reported that carnosine is an effective scavenger of reactive oxygen and nitrogen species, resulting in a doubling of insulin secretion from isolated mouse islets and INS-1 β-cells. In addition, carnosine also reversed glucolipotoxic inhibition of insulin secretion and enhanced glucose uptake into C2C12 skeletal muscle cells.

The recent identification of adducts of carnosine with by-products of aldehyde detoxification (e.g. acrolein) suggests that carnosine may be consumed by sub-products linked with the pathogenesis of diabetes complications, such as exacerbated lipoxidation (Bispo, de Arruda Campos, Di Mascio, & Medeirosa, 2016). Contrary to evidence that carnosine may be reduced in type II diabetes due to its ability to entrap toxic sub-products, Stegen et al. (2015) reported higher intramuscular carnosine concentrations in individuals classified as pre-diabetic (+30%) and type II diabetic (+39%) in comparison with lean controls. This finding was possibly not related to insulin resistance per se, but perhaps to a higher distribution of type II fibres in these individuals. This, however, was not directly determined in the study. Alternatively, the authors speculated that increased carnosine could be a result of a compensatory mechanism that would take place when carnosine is consumed at high rates by elevated oxidative stress, carbonyl stress, excessive glycation and aldehyde detoxification.

Due to these properties, carnosine has been considered to play a clinically significant role in diabetes treatment, especially in preventing diabetic complications. Although evidence is still emerging, both animal (Lee, Hsu, Lin, & Yin, 2005) and human (Janssen et al., 2005) studies have indicated the potential of carnosine to delay diabetes progression. Carnosine supplementation is capable of improving glycaemic control and reducing glycated haemoglobin in a murine model of type II diabetes (Sauerhofer et al., 2007). Similar results were shown in obese mice that developed diabetes; 18 weeks of carnosine supplementation reduced glycaemia, improved glycaemic control and preserved the integrity of the kidney (Albrecht et al., 2017). In line with these findings, in obese Zucker rats, 24 weeks of carnosine supplementation blunted the development of dyslipidaemia and hypertension, whilst preventing damage to the kidney (Aldini et al., 2011). Furthermore, carnosine supplementation counteracted the deterioration of kidney function, blood lipid profiles,

insulin secretion and insulin resistance. More evidence from animal studies suggests that the mechanisms of carnosine protection may also involve decreased apoptosis of glomerular cells (Peters et al., 2014) or normalisation of vascular permeability (Peters et al., 2012).

In humans, early evidence of the protective effects of carnosine on diabetes progression and complications came from association studies with patients carrying polymorphisms on the genes encoding for serum and tissue carnosinases (i.e. CNDP1 and CNDP2). These genes are adjacently located in the 18q chromosome, a locus that has been previously linked to diabetic nephropathy (Vardarli et al., 2002). One particular polymorphism on exon 2 of the CNDP1 gene seems to be the one most associated with diabetic complications (Janssen et al., 2005); this polymorphism consists of different repeats of trinucleotides resulting in a different number of leucine repeats that vary from five to seven. In fact, several different polymorphisms leading to a different number of leucine repeats have been described, with shorter repeats being associated with lower plasma carnosinase activity and longer repeats with higher plasma carnosinase activities (Janssen et al., 2005). Accordingly, patients with the short repeat alleles tended to be more protected against diabetic nephropathy, suggesting that carnosine may play a protective role in diabetes (Janssen et al., 2005). Similar results were confirmed in other (Freedman et al., 2007), but not all (Wanic et al., 2008) studies.

Clinical studies with carnosine supplementation are scarce, but emerging evidence appears to confirm some of the theoretical mechanisms of action, particularly those related to carbonyl species quenching (Bispo et al., 2016). A pilot clinical trial provided evidence of the ability of carnosine supplementation to protect against diabetes development in non-diabetic obese individuals (De Courten et al., 2016). Overweight and obese adults were randomly allocated to ingest carnosine (2 g/day) or a placebo for 12 weeks. An increase in fasting insulin and insulin resistance was reported with placebo, but not carnosine. The group receiving carnosine also had improved responses to an oral glucose test (i.e. lower glucose and insulin), which was only shown in a subgroup of individuals with impaired glucose tolerance. Such findings, although promising, require further confirmation.

Acute kidney failure

Acute kidney failure is defined as a sudden, sustained impairment of kidney function, typically for periods of 1–7 days, resulting in reduced glomerular filtration rate, urinary volume, electrolyte imbalance and impaired pH regulation. Kidney damage induced by ischemia/reperfusion is a key factor involved in the pathogenesis of acute kidney failure and it is commonly observed in various clinical conditions, such as recovery from cardiac arrest, kidney transplantation and partial nephrectomy (Thadhani, Pascual, & Bonventre, 1996). Acute kidney failure may also result in acute tubular necrosis and increased renal vascular resistance (Thadhani et al., 1996), thereby being typically accompanied by impaired renal blood flow (Basile, Anderson, & Sutton, 2012). Although the molecular mechanisms underpinning these responses are not fully elucidated, ATP depletion, oxidative damage, phospholipase activation, neutrophil infiltration and exacerbation of adrenergic activation have been shown to play a central role in the pathogenesis of acute kidney failure (Basile et al., 2012). Any substance capable of suppressing or attenuating any of the processes involved in its pathogenesis may, therefore, be protective during episodes of acute failure (Fujii et al., 2003), thus reducing the area of the kidney where cells suffer lethal injuries. Reducing lethal injuries to the point of non-lethality is critical for recovery and regeneration (Basile et al., 2012).

In this respect, carnosine may have a protective effect, as suggested by Kurata et al. (2006) using an animal model of acute kidney failure induced by ischaemia/reperfusion. Intra-cerebroventricular injection of carnosine before acute kidney failure suppressed the increase in adrenergic activity in the renal sympathetic nerves, which was paralleled by attenuation in kidney dysfunction. Interestingly, no effect of *N*-α-acetyl-L-carnosine (a carnosine analogue resistant to carnosinases) was observed, whereas the injection of L-histidine resulted in similar protective effects to those of carnosine. Thus, the mechanism of protection may involve the activation of histamine H_3 receptors in the central nervous system, which was confirmed by the infusion of thioperamide (a histamine receptor antagonist) that eliminated the protective effects of carnosine (Kurata et al., 2006). Similar findings were shown in a rat model of acute kidney failure (Fujii et al., 2003), which further expanded the evidence that carnosine ameliorates adrenergic activation by reducing epinephrine release from the kidneys in animals treated with carnosine. The results by Kurata et al. (2006) and Fujii et al. (2003) indicate a protective role of carnosine that is independent of antioxidant activity, although oxidative stress is critically involved in the pathogenesis of acute kidney damage. There remains, therefore, an unexplored potential for carnosine to protect the kidneys and preserve function in acute episodes. Evidence from studies in humans, however, are still absent.

Cancer

The potential for carnosine to exert anti-neoplastic effects was first proposed by Nagai and Suda (1986) who implanted sarcoma-180 tumour cells under the skin of mice. Carnosine, β-alanine and a saline control were administered subcutaneously 24 h later in close proximity to the tumour implantation site, with administration continuing on every other day. Administration with both β-alanine and carnosine reduced tumour growth and reduced mortality, compared to those treated with carnosine alone.

Despite these encouraging findings, work in this area did not gain much momentum until 10 years later when Holliday and McFarland (1996) showed that 20 mM of carnosine was able to selectively inhibit the growth of transformed and neoplastic cells. Subsequently, much of the work in this area has stemmed from the laboratory of Frank Gaunitz (University of Leipzig), whose group has performed a series of experiments showing positive effects of carnosine on reducing the growth of human glioblastoma cells (Renner, Zemitzsch, et al., 2008) and in reducing the growth and size of tumours implanted subcutaneously in nude mice (a strain with no or poor functioning thymus that results in reduced T-cell production and an impaired immune system, Renner, Zemitzsch, et al., 2010). In the latter study, Renner, Zemitzsch, et al. (2010) subcutaneously implanted NIH3T3 fibroblasts in mice, which continually express human epidermal growth factor receptor 2 in two independent experiments. The mice in both studies then received daily intraperitoneal injections of 500 μL of a 1-M carnosine solution, with treated mice being compared to control mice receiving intraperitoneal injections of sodium chloride. Although carnosine was not able to completely reverse tumour growth and development in this study, the aggressive growth of the tumour was delayed in comparison with controls in both experiments. Interestingly, microscopic examination of the tumours indicated that there were a lower number of mitoses per high-powered field when compared with untreated mice, suggesting a direct anti-proliferative action of carnosine *in vivo*. These encouraging studies demonstrating the anti-proliferative effects of carnosine on models of glioblastoma (a highly aggressive form of brain cancer that can be very difficult to treat) have been extended to other cancer cell lines, including those from the colon (Iovine et al., 2012), gut (Shen et al., 2014) and ovaries (Mikula-Pietrasik & Ksiazek, 2016), showing that evidence is gathering in support of direct anti-tumour actions of carnosine.

The effects of carnosine on tumour cells seem in direct contrast to the effects on other cell types, where carnosine has been shown to increase cell viability. It has been suggested that these apparent differences can be reconciled, however, by considering the different metabolic differences between cancer cells and other cells where carnosine is seemingly pro-proliferative (Gaunitz & Hipkiss, 2012). Most cells derive their energy from oxidative phosphorylation under normoxic conditions, whereas cancer cells have a high dependence on glycolysis (known as the Warburg effect) for their ATP production, which results in the production of lactate even in the presence of sufficient oxygen (Oppermann, Schanbel, Meixensberger, & Gaunitz, 2016). It has been suggested that the primary mechanism for the anti-neoplastic effects of carnosine relates to its ability to inhibit glycolysis (Renner, Asperger, et al., 2010). Hipkiss and Gaunitz (2014) recently suggested that there are several possibilities to explain carnosine's effects on glycolytic activity that, in turn, underpin the anti-neoplastic effects shown in cancer cells. These were reported to include effects on (a) glycolytic enzymes, (b) metabolic regulatory activities, (c) redox biology, (d) protein glycation, (e) glyoxalase activity, (f) apoptosis, (g) gene expression and (h) metastasis. Oppermann et al. (2016) provided some evidence in support of this, but also suggested that the anti-neoplastic effects of carnosine on glioblastoma cells are attenuated in the presence of higher pyruvate, which is independent of oxidative phosphorylation.

Perspectives

Although identified ~100 years ago, only in the last 30 years has research identified carnosine as a factor that might exert an influence on health and disease. Evidence is indicative of the ability of the carnosine to influence health and disease status, most clearly in the skeletal muscle and brain at this stage. Many of these developments are, however, recent and numerous important questions remain unanswered, particularly with regard to the precise mechanisms by which carnosine acts and the real impact of carnosine on both health and disease status. More mechanistic approaches, such as knocking-out genes that control carnosine content in tissues (e.g. carnosine synthase and tissue carnosinase), could be used, as well super-expressing them. Alternative approaches to gather further knowledge on the physiological roles of carnosine may comprise comparative physiology studies. Further studies should explore whether these properties are physiologically relevant to cellular and tissue functions and whether they can be translated into therapeutic benefits, especially in diseases where small-scale clinical studies have

shown promising results, such as diabetes. Large-scale clinical studies are indeed necessary to provide stronger evidence on the actual clinical benefits of interventions to increase carnosine levels on primary and secondary disease prevention, as well as treatment. Future studies should also look at improving previous clinical research by using large sample sizes, long follow-up periods and measures that link the biological actions of carnosine to their clinical effects. Effective ways to apply the therapeutic effects of increased carnosine levels in different human tissues must also be more clearly established, since the lower bioavailability of carnosine may impose significant limitations to supplementation strategies involving the intact dipeptide. Alternative strategies, such as the use of carnosine-analogues or anserine, both resistant to carnosinases, should also be explored in the future. Whether or not β-alanine supplementation can increase carnosine concentrations in tissues other than skeletal muscle remains unknown and should, therefore, be explored. Finally, increased carnosine in muscle in response to exercise may be a novel long-term adaptation to exercise training that might explain some of the benefits of exercise to health – this novel possibility could also be explored in future studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by FAPESP [grant number 2014/11948-8].

ORCID

Rebecca Louise Jones http://orcid.org/0000-0001-9657-9448

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