Anti-cancer actions of carnosine and the restoration of normal cellular homeostasis

Mark D. Turner [a, *], Craig Sale [b], A. Christopher Garner [c], Alan R. Hipkiss [d]

[a] Centre for Diabetes, Chronic Diseases and Ageing, School of Science and Technology, Nottingham Trent University, Clifton, Nottingham NG11 8NS, UK
[b] Sport, Health and Performance Enhancement Research Centre, School of Science and Technology, Nottingham Trent University, Clifton, Nottingham NG11 8NS, UK [c] Department of Chemistry and Forensics, School of Science and Technology, Nottingham Trent University, Clifton, Nottingham NG11 8NS, UK
[d] Aston Research Centre for Healthy Ageing, Aston University, Birmingham B4 7ET, UK.

A B S T R A C T

Carnosine is a naturally occurring dipeptide found in meat. Alternatively it can be formed through synthesis from the amino acids, β-alanine and L-histidine. Carnosine has long been advocated for use as an anti-oxidant and anti-glycating agent to facilitate healthy ageing, and there have also been reports of it having anti-proliferative effects that have beneficial actions against the development of a number of different cancers. Carnosine is able to undertake multiple molecular processes, and it's mechanism of action therefore remains controversial - both in healthy tissues and those associated with cancer or metabolic diseases. Here we review current understanding of its mechanistic role in different physiological contexts, and how this relates to cancer. Carnosine turns over rapidly in the body due to the presence of both serum and tissue carnosinase enzymes however, so its use as a di-etary supplement would require ingestion of multiple daily doses. Strategies are therefore being developed that are based upon either resistance of carnosine analogs to enzymatic turnover, or else β-alanine supplementation, and the development of these potential therapeutic agents is discussed.

A R T I C L E I N F O

Keywords:
Histidine-containing dipeptide Carnosine analog
β-Alanine
Glycation
Reactive species scavenging

1. Introduction

Carnosine is a histidine-containing dipeptide that is synthesised by the bonding of β-alanine and L-histidine, which is catalysed by the enzyme carnosine synthase. The tissues of several animal species also contain other common histidine-containing dipeptide variants (anserine and ophidine/balenine), which are different methylated analogues of carnosine. Whilst it has long been considered that carnosine is the only histidine-containing dipeptide expressed in human tissues [1], this was relatively recently challenged by Peters et al. [2] who also reported the expression of anserine in the renal cortex of humans. Carnosine was first discovered and reported in 1900 by Russian chemists Gulewitsch and Amiradzibi [3], who were interested in identifying nitrogen containing non-protein compounds in meat extract [4].

Subsequently, histidine containing dipeptides have been identified across a number of mammalian species and fish, with carnosine, in particular, being identified in vertebrates that include horses, greyhounds, camels and humans (for a comprehensive review on this topic, please see [1]). In mammals, carnosine has only been reported to be present in high (i.e., millimolar) concentrations in the skeletal muscle and olfactory bulb, with the skeletal muscle having the highest carnosine content given its significant mass. Whilst carnosine is present in a number of other tissues (including other regions of the brain, the kidney, the spleen and the blood), it tends to only be present within the micromolar range. In skeletal muscle, and possibly these other tissues, species is the main determinant of the carnosine content [5], with intramuscular content being about ten times higher in hunting or evading animals than in humans.

The rate limiting factor for tissue carnosine synthesis in humans is thought to be the availability of β-alanine from the diet [6]. β-alanine is endogenously produced in relatively small amounts from uracil degradation in the liver [7], but dietary intake accounts for the majority of its availability, although this assumes an omnivorous rather than vegetarian diet. The omnivorous diet provides around ~300–550 $mg \cdot d^{-1}$ of β-

Abbreviations: 4E-BP, elongation initiation factor 4E binding protein; AGE, advanced glycation end-product; CNDP1, serum carnosinase; CNDP2, tissue carnosinase; eiF, elongation initiation factor; GS, glutamine synthetase; HO-1, heme oxygenase-1; MG, methylglyoxal; mTOR, mechanistic target of rapamycin; NRF2, nuclear factor erythroid 2–related factor 2; RAGE, AGE receptor; RNS, reactive nitrogen species; ROS, reactive oxygen species

alanine, but the vegetarian diet includes almost no β-alanine. Whilst carnosine can also be ingested in the diet, and can enter the enterocytes via the peptide transporter 1 (PEPT1) [8], it is then rapidly hydrolysed into its constituent amino acids [9] by the tissue carnosinase (CNDP2) present in the jejunal mucosa [10], although basolateral peptide transporters [11] might also support the entry of a small amount of carnosine into the circulation. Although small amounts of carnosine have been measured in the urine several hours after ingestion [12], the majority of the carnosine entering the circulation is rapidly hydrolysed by the highly active serum carnosinase (CNDP1) [9].

In humans, increasing the availability of free β-alanine, either through the ingestion of high β-alanine containing foods, such as fish, fowl, beef and pork, or through supplementation with either β-alanine or carnosine, results in increased skeletal muscle carnosine content [6], although it remains uncertain how carnosine concentrations are regulated in other tissues.

2. Physiological role of carnosine in healthy tissues

Perhaps the most widely researched role for carnosine, at least in humans, is its ability to act as an intracellular pH buffer. The pKa of the imidazole ring on the histidine residue of carnosine is 6.83 [13], meaning that it is well placed to act as a pH buffer across the physiological pH transit range of tissues. The majority of the research in this regard has focussed upon the skeletal muscle, where the pH transit range exists between approximately 7.0 at rest to between 6.5 [14] or 6.1 [15] (depending upon how this is measured) following high-intensity exercise. Given this, the focus of much of the research on carnosine in humans has related to implications for improving exercise capacity during high-intensity exercise, where supplementation with β-alanine has been shown to result in an ergogenic effect (for a summary see the systematic review and meta-analysis of Saunders et al. [16]).

pH buffering is by no means the only purported physiological role for carnosine however, as other roles relevant to the function and/or maintenance of cellular homeostasis have also been proposed. These include the regulation of Ca^{2+} transients in the sarcoplasm, influencing the sensitivity of the skeletal muscle contractile apparatus to Ca^{2+}, metal ion chelation and reactive species scavenging and protecting the cell against glycation and glycation end products. It is, of course, quite plausible that carnosine could have different functions in different tissues or, indeed, the same functions in different tissues or it could even have multiple functions within one tissue. Much of this, however, remains to be elucidated.

2.1. Regulation of Ca^{2+} transients and influencing sensitivity of the contractile apparatus to Ca^{2+}

It has been suggested that carnosine might help to improve contractile function in striated muscles as a result of improved Ca^{2+} handling and heightened sensitivity of the contractile apparatus to Ca^{2+}, which was initially demonstrated in several studies using chemically skinned skeletal muscle fibres [17,18] and cardiac muscle [19]. Using a mechanically skinned skeletal muscle fibre model, which overcomes some of the reported criticisms of the chemical skinning approach and maintains normal excitation-contraction coupling mechanisms, Dutka & Lamb [20] confirmed the finding that carnosine increased Ca^{2+} sensitivity of the contractile apparatus in a concentration dependent manner. They did not, however, report any effect of carnosine on Ca^{2+} release from the sarcoplasmic reticulum [20]. In a follow-up study, the same group [21] examined the effects of carnosine provided to mechanically skinned type I and type II fibres dissected from the *m. vastus lateralis* of humans using needle biopsy. They showed that increasing muscle carnosine content increased Ca^{2+} sensitivity of the contractile apparatus in both fibre types and likely increased Ca^{2+} release from the sarcoplasmic reticulum in type I fibres [21]. Taken together the above results suggest that increasing intramuscular carnosine content might help to offset the decline in muscle performance during fatigue, in addition to the potential effects of carnosine on intramuscular pH buffering mentioned above, although there is potential cross-over between the two effects (for a more detailed explanation see Matthews et al. [22]).

Whilst the data from these isolated muscle preparations seem encouraging, the data from in vivo human investigations do not seem to support such an important effect of carnosine on Ca^{2+} mediated muscle function. Both Hannah et al. [23] and Jones et al. [24] showed no effect of β-alanine supplementation (with a view to increasing intramuscular carnosine content, although this was not directly determined) on peak force, time to peak tension, or on maximum and explosive force generation in voluntary and electrically evoked muscle contractions. Similarly, there was no effect of β-alanine supplementation on force-electromyography or force-frequency relationship, which would have been expected with a carnosine mediated improvement in calcium sensitivity and handling in the skeletal muscle. That said, both studies [23,24] did show an effect of β-alanine supplementation on half-relaxation time of the knee extensors, which might be explained by an enhanced reuptake of calcium into the sarcoplasmic reticulum that is influenced by a carnosine effect on sarcoendoplasmic reticulum calcium transport ATPase pump activity, although this remains a working hypothesis that requires further investigation to confirm. Reducing muscle relaxation rates are likely important for the development of muscle power output, which could likewise be important for exercise performances involving fast, repetitive contractions with limited recovery time. Whilst the findings described above are largely confined to skeletal muscle, it would also be of interest to examine the potential actions of carnosine on calcium handling and sensitivity in the heart, where should it exert a positive effect, carnosine could potentially play a role in heart health. In line with this, and linking the two previously mentioned physiological roles of carnosine together, Swietach et al. [25] suggested that carnosine can act as a diffusible cytoplasmic buffer, exchanging Ca^{2+} for H^+ that can move Ca^{2+} within the cytoplasm in response to H^+ gradients.

2.2. Metal ion chelation and reactive species scavenging

A potentially important role for carnosine in cellular protection against damage, is its purported role as an antioxidant given that oxidative stress is recognised as a causative factor in ageing and several pathologies (including cancer, neurodegenerative disorders and diabetes). The potential for carnosine to act as an antioxidant was initially studied in the late 1980s, with these studies being well reviewed by the same researcher some years later [1]. The specific mechanism underpinning any potential antioxidant effect of carnosine remains unclear, although several possibilities have been suggested, including metal ion chelation and scavenging of reactive species and peroxyl radicals [1].

Several in vitro investigations have suggested that carnosine could be an effective chelating agent against metal ions, including copper, zinc, iron, cobalt, nickel and cadmium, meaning that it can form stable bonds with these transitional metal ions and prevent them from completing undesirable downstream reactions complicit in cellular damage [26]. That said, only two of these complexes have received a reasonable amount of attention, with the most investigated links being those formed between carnosine and both zinc [27–31] and copper [26,31–35], albeit primarily in vitro and not under physiologically relevant conditions. The zinc-carnosine complex has received perhaps the most attention in recent years due to its potential therapeutic application as a drug [36] provided against gastric ulcers [29] and *Helicobacter pylori* infection [27,28].

2.3. Protecting the cell against glycation and glycation end products

Carnosine has also been suggested as an anti-glycating agent, in that it can 'trap' advanced glycation end products that are generated as a result of Maillard reactions of protein amino groups with reactive carbonyl species [26]. These reactive carbonyl species are thought to be an important cause of macromolecular damage to proteins, nucleic acids, and lipids, which increases with ageing and in ageing specific diseases [37]. Hipkiss and colleagues [38] demonstrated that carnosine is glycated by reactive carbonyl species and can thus act as a sacrificial amino store, sparing proteins and lipids from damage. Further evidence in support of this comes from studies that have, for example, reported the presence of glycated forms of carnosine, such as carnosine-4-hydroxynonenal in the urine of obese rats [39] or carnosine-acrolein in human urine [40]. F urther studies have suggested similar effects of carnosine, or the methylated variants of carnosine, on damaging lipid peroxidation products in the skeletal muscle [41–43].

To summarise, there are a number of purported biological actions of carnosine that could underpin improvements in normal cellular function or indeed to offset challenges to the homeostasis of the cell. These actions suggest interesting therapeutic possibilities that might help in the fight against ageing and age associated conditions, such as diabetes or neurodegenerative diseases, in which glycation and oxidative stress are implicated. In addition, due to the physiological properties of carnosine associated with anti-proliferation [44,45], the potential for carnosine to be used in the prevention and/or treatment of cancer is worthy of further exploration.

3. Anti-cancer actions of carnosine

Many years have elapsed since it was discovered that carnosine could exert an inhibitory effect on growth of transformed cells, whilst seemingly having little or no effect on proliferation of normal (untransformed) cells [46]. Various suggestions can be made to explain the dipeptide's selectivity; these include effects on components of energy metabolism and its regulation, as well as changes in protein synthesis and catabolism. It is suggested that none of the possible mechanisms, discussed below, are by necessity mutually exclusive.

3.1. Carnosine and energy metabolism

A characteristic of many tumour cells is enhanced glycolytic activity [47]. A number of studies have shown that carnosine seems to suppress not only glycolytic ATP synthesis [44,48] but also mitochondrial activity [49,50]. The mechanisms responsible could include direct interference with relevant enzymes and/or metabolic intermediates. For example, it has been shown that carnosine can react non-enzymically with glyceraldehyde-3-phosphate which would decrease glycolytic ATP generation [51]. Another possibility is that carnosine could interfere with cell signalling, such as the mechanistic target of rapamycin (mTOR) regulatory complex, in a manner similar to rapamycin [51,52]. Indeed it has been suggested that carnosine may possess rapamycin-like activity [53,54], although it has also been shown that carnosine inhibits growth of rapamycin insensitive glioblastoma cells [55]. In addition, it is possible that glycolytic regulation could be mediated via increased expression of pyruvate dehydrogenase kinase by means of epigenetic effects [56], resulting in inhibition of glycolysis in general [52,57,58].

One by-product of enhanced glycolytic activity is the accumulation of methylglyoxal (MG) following the spontaneous decomposition of certain glycolytic intermediates, the triose-phosphates dihydroxyacetone phosphate and glyceraldehyde-3-phosphate [59]. MG is a highly reactive bicarbonyl which is usually regarded as deleterious. MG is capable of inflicting much macromolecular damage, such as formation of advanced glycation end-products (AGEs) following its reaction with histidine, arginine, lysine and cysteine residues in proteins, and guanidine in nucleic acids. In most normal cells MG is detoxified by the action of the glyoxalase system in which the bicarbonyl is converted to (*R*)-lactate. Many years ago carnosine was shown (in a model system) to protect proteins against MG-mediated modification, by presumably reacting directly with MG [60]. However, the expected carnosine-MG adduct has not been detected in vivo, whereas adducts between carnosine and propanal, propanol and acrolein have been found in human tissues [40,61], suggesting (speculatively) that the putative carnosine-MG adduct might be their precursor via the activity of unidentified aldo-keto-reductases.

In tumour cells, however, MG is not necessarily deleterious; there are examples, when the bicarbonyl is present at low doses, in which MG enhances growth of certain tumour cells, possibly by way of MG-induced hormetic effects. Furthermore, MG can glycate (and consequently inactivate) defensive stress/chaperone proteins [62,63], and thus decrease binding to the large tumour suppressor protein-1 (LATS1) [63]. It has been shown that putative carbonyl scavengers, such as carnosine, can counter the effects of MG in tumour cells [62–65], which reinforces the notion that carnosine's putative reaction towards MG may have physiological relevance.

3.2. Carnosine and gene expression

It has frequently been suggested that carnosine can induce the expression of the nuclear factor erythroid 2–related factor 2 (NRF2) transcription factor which regulates expression of a number of functions, via expression of up to 500 genes (in humans), which combat oxidative stress and inflammation via anti-oxidant and autophagic activities [66,67]. It is likely that many of the beneficial effects exerted by carnosine towards health and lifespan (including cancer prevention) could derive from increased expression of NRF2 [68]. This includes action through the heat shock protein family member, heme oxygenase-1 (HO-1), in a model system employing LPS-stimulated RAW 264.7 macrophages [66]. However, it should be pointed out that whilst evidence for carnosine-enhanced NRF2 expression was also observed in a system using MG-treated human umbilical vein cells, expression of HO-1 was suppressed by carnosine.in this case [69]. Whilst it is not clear why carnosine seems to have opposite effects on HO-1 expression in these two systems, it is possible that macrophages and umbilical vein cells differ in response to carnosine, and/or the dipeptide's ability to react directly with MG.

In 2008, Son et al. [70] showed that carnosine could inhibit interleukin-8 production via effects on mRNA translation. They showed that carnosine inhibited phosphorylation of translation initiation factor (eiF4E) binding protein (4E-BP). Generation of the complete and functional translation initiation complex eiF4G1 is essential for translation of m6A-containing (i.e., capped) mRNA species; absence of the phosphorylated 4E-BP prevents translation of capped mRNAs whilst permitting translation of cap-independent mRNAs. Subsequently it was indeed shown that hypo-phosphorylation of 4E-BP suppresses cap-dependent mRNA translation but permitted cap-independent mRNA translation. Furthermore, inhibition of translation of capped-mRNA has emerged as a strategy for suppressing growth of cancer cells [71–74]. It is interesting to note that cap-independent mRNA translation is upregulated in certain long-lived mice [[74]], possibly resulting from decreased mTOR function, an observation consistent with carnosine's putative anti-ageing activity [37].

3.3. Carnosine and proteolysis

There is evidence showing that carnosine can influence protein breakdown as well as synthesis. A recent study by Fang et al. [75] has shown that carnosine stimulates the intra-cellular proteolysis of glutamine synthetase (GS) in human glioma cell lines; GS half-life was reduced from between 8 and 10 h to between 5 and 1 h [75], mediated

mostly by upregulation of the ubiquitin-proteasome mechanism. Many tumour cells synthesise large amounts of glutamine, which acts as a metabolic source of precursors for synthesis of amino acids and purine nucleotides. Thus, the enhanced proteolysis of GS would limit supply of macromolecular precursors and thereby suppress growth of tumour cells.

There are few studies on the effects of carnosine on proteolysis in tumour cells. It was shown [76] that in cultured human fibroblasts grown in the presence of the dipeptide, proteolysis of long-lived proteins (radiolabelled for 72 h) was increasingly enhanced as the carnosine-treated cells approached replicative senescence [76], although there were no effects of carnosine on the proteolysis of short-lived proteins (i.e., radiolabelled for 5–30 min) [76]. It is relevant to note that the anti-ageing agent rapamycin has also been shown to stimulate proteolysis of long-lived proteins in cultured human fibroblasts [77], an observation consistent with the idea that carnosine has properties analogous to those of rapamycin.

3.4. Carnosine and clearance of senescent cells

Age is an acknowledged risk factor for cancer, and it is highly likely that cellular senescence contributes to tumour development and metastasis. It is interesting to note that carnosine has recently been shown to stimulate macrophage-mediated clearance of senescent skin cells via a mechanism that involved autophagy and upregulation of AGE receptors (RAGEs) and scavenger receptor CD36 [78], most likely mediated via the AKT signalling pathway and the mTOR metabolic regulator.

4. Alternative therapeutic strategies based upon carnosine

As detailed above, carnosine could provide a therapeutic, anticancer action by multiple mechanisms. One possible issue with this strategy, however, is that carnosine is enzymatically hydrolysed in the body by carnosinase, and consequently when administered orally in humans there is low bioavailability, with circulating concentrations only reaching micromolar levels. Carnosinase was originally identified in the kidney of a hog in 1951 [79]. Subsequently, a human serum carnosinase was identified as separate and more highly expressed than the tissue carnosinase [80]. Consequently, taking carnosine as a supplement is likely to require sustained administration of high doses to achieve even the modest beneficial effects reported for diseases such as diabetes [81], but even this has significant issues. The identification of human tissues that can synthesise carnosine from its precursors is therefore required, such that an understanding of the potential for tissue 'loading' might be gained. Of course, this might not be possible either, and so other strategies might be required to realise any of the purported therapeutic benefits of carnosine. Such strategies include the design of molecules that retain the beneficial biological actions of carnosine, whilst at the same time being resistant to carnosinase degradation, or else extend the half-life of endogenous carnosine through inhibition of carnosinase enzyme activity.

4.1. Carnosine analogs and carnosinase inhibitors

A number of approaches have been taken to utilise carnosine-like molecules to treat conditions where reactive species contribute to disease pathology. By so doing, it is possible to generate new classes of therapeutic agents that offer significantly enhanced efficacy over carnosine itself. Some of the leading candidates are illustrated in Fig. 1, with modifications from the structure of carnosine shown in red.

N-acetyl (*S*)-carnosine, a derivative of the naturally occurring isomer, is licenced for use in eye drops with reportedly beneficial effects against cataracts [82,83]. The reactive carbonyl quenching activity of (*R*)-carnosine, the enantiomer (mirror image) of the naturally occurring dipeptide has also been examined [84]. Whilst the enantiomer was not active in comparison to the naturally occurring carnosine, testing of a number of (*R*)-carnosine derivatives, designed to improve the bioavailability of the compound, led to the identification of an active pro-drug in animal studies. The authors suggested that a combination of the known stability of the (*R*)-enantiomer to enzymic hydrolysis, and improved absorption of the pro-drug, were responsible for the observed results. Three further functional analogs of carnosine have also been developed as potential therapeutic agents; carnosinol, a carnosinase resistant, reactive carbonyl scavenger, in which the carboxylic acid group of carnosine is reduced to an alcohol [85,86]; carnostatine, a carnosinase inhibitor structurally similar to carnosine with potential therapeutic modality for renal disease [87]; and 3-alanyl-β-2-thienyl-*DL*-alanine, a thiophene analog of carnosine that has been shown to inhibit the growth of *E. coli* [88].

4.2. β-Alanine

In addition to developing pharmacological agents based on slowly hydrolysable carnosine analogs, an alternative approach is to control cellular homeostasis through dietary supplementation with the rate-limiting constituent component of carnosine, namely, β-alanine. It has previously been reported that β-alanine supplementation could increase intramuscular carnosine content in humans where it was shown to have

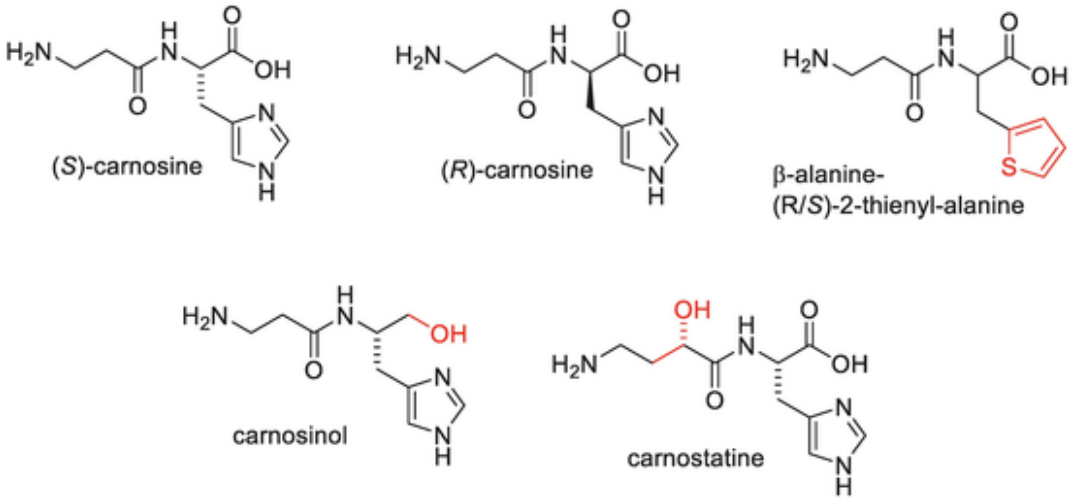

Fig. 1. (*S*)-carnosine and potential therapeutic analogs. Biologically active (*S*)-carnosine is shown top left. Analog modifications from the structure of (*S*)-carnosine are shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the online version of this chapter.)

a positive effect on cellular performance [6,89]. Provided that cells express carnosine synthase, it is therefore possible that supplementation with β-alanine could increase the intracellular pool of carnosine (much the same as it does in the skeletal muscle), thereby resulting in similar downstream positive effects as supplementation with carnosine itself.

It is worth noting however, that long-term β-alanine supplementation could potentially reduce plasma histidine content [90], although this possibility currently requires further investigation with a greater temporal pattern of sampling. Nevertheless, given that the imidazole group of histidine might exert anti-neoplastic effects in itself, theoretically at least this might reduce the therapeutic effectiveness of β-alanine supplementation against cancer specifically. There is also a further cautionary note to the use of β-alanine supplementation as a therapeutic strategy, in that the ingestion of high doses (generally above 20 $mg{\cdot}kgbm^{-1}$) has the potential to induce paresthesia in humans [91], as indeed does supplementation with carnosine where doses are high enough to increase the plasma β-alanine level to a similar extent following hydrolysis of the intact dipeptide by plasma carnosinase. Although, as yet, there is little evidence to suggest that this influences the efficacy of the supplement, it is however possible that this might reduce an individual's willingness to take it. This aside though, the approach remains a viable alternative to dietary supplementation with carnosine, or associated analogs.

5. Conclusions

Research shows that carnosine is pluripotent affecting metabolism, especially glycolysis, thereby decreasing the generation of deleterious metabolites, such as triose-phosphates and their decomposition product MG. These metabolic effects may result from carnosine-induced changes in gene mRNA transcription and translation, as well as enhanced protein degradation of selected proteins, possibly originating via changes in phosphorylation of regulatory/signalling proteins, most likely mediated via increased expression of the NRF2 transcription factor. It remains uncertain whether the intact unmodified carnosine molecule is responsible or whether the dipeptide behaves as a sentinel by becoming modified by metabolic toxins, such as MG or ROS, given carnosine's ready reactivity to many deleterious aldehydes, as well as ROS and RNS. It is concluded, therefore, that the putative protective activity of carnosine (and related analogs) towards tumour development likely derives from effects on a combination of targets, all of which contribute, in smaller or larger degrees, to suppress growth of tumour cells, primarily by suppressing glycolysis and thus the supply of metabolic precursors and their toxic metabolite MG, as exemplified by the metabolically-selective effects of the dipeptide on the growth of yeast cells [92]. Given the potential for carnosine to exert physiological effects in a number of cells and tissues, including skeletal muscle, brain, heart, pancreas, kidney, and cancer cells, it is therefore likely that carnosine and associated analogs will be increasingly utilized as therapeutic agents to treat a number of different types of cancer. Each needs to be taken on a case by case basis however, and the effectiveness of these molecules should form the focus for numerous future clinical investigations.

CRediT authorship contribution statement

Mark D. Turner: Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Craig Sale:** Writing – original draft, Writing – review & editing. **A. Christopher Garner:** Writing – original draft, Writing – review & editing, Visualization. **Alan R. Hipkiss:** Writing – original draft, Writing – review & editing.

Declaration of competing interest

MDT received a British Council grant award to support a PhD studentship focused on research into carnosine (grant number: s209524711). CS is the recipient of funding to support a PhD programme of work from Natural Alternatives International (NAI) and has received *β*-alanine supplements free of charge from NAI for use in experimental investigations. NAI have also supported open access page charges for some CS manuscripts.

References

[1] A.A. Boldyrev, G. Aldini, W. Derave, Physiology and pathophysiology of carnosine, Physiol. Rev. 93 (2013) 1803–1845, https://doi.org/10.1152/physrev.00039.2012.

[2] V. Peters, C.Q.F. Klessens, H.J. Baelde HJ, B. Singler, K.A. Veraar, A. Zutinic, J. Drozak, J. Zschocke, C.P. Schmitt, E. de Heer. Intrinsic carnosine metabolism in the human kidney. Amino Acids 47 (2015) 2541–2550. doi:https://doi.org/10.1007/s00726-015-2045-7.

[3] W.I. Gulewitsch, S. Amiradžibi, Ueber das Carnosin, eine neue organische Base des Fleischextractes, Berichte Dtsch. Chem. Ges. 33 (1900) 1902–1903, https://doi.org/10.1002/cber.19000330275.

[4] A.A. Boldyrev, Carnosine: new concept for the function of an old molecule, Biochem. Mosc. 77 (2012) 313–326, https://doi.org/10.1134/S0006297912040013.

[5] R.C. Harris, J.A. Wise, K.A. Price, H.J. Kim, C.K. Kim, C. Sale, Determinants of muscle carnosine content, Amino Acids 43 (2012) 5–12, https://doi.org/10.1007/s00726-012-1233-y.

[6] R.C. Harris, M.J. Tallon, M. Dunnett, L. Boobis, J. Coakley, H.J. Kim, J.L. Fallowfield, C.A. Hill, C. Sale, J.A. Wise, The absorption of orally supplied β-alanine and its effect on muscle carnosine synthesis in human vastus lateralis, Amino Acids 30 (2006) 279–289, https://doi.org/10.1007/s00726-006-0299-9.

[7] M.M. Matthews, T.W. Traut. Regulation of N-carbamoyl beta-alanine amidohydrolase, the terminal enzyme in pyrimidine catabolism, by ligand-induced change in polymerization. J. Biol. Chem. 262 (1987) 7232–7237.

[8] D.O. Son, H. Satsu, Y. Kiso, M. Shimizu, Characterization of carnosine uptake and its physiological function in human intestinal epithelial Caco-2 cells, Biofactors 21 (2004) 395–398, https://doi.org/10.1002/biof.552210177.

[9] T.L. Perry, S. Hansen, D.L. Love, Serum-carnosinase deficiency in carnosinaemia, Lancet 291 (1968) 1229–1230, https://doi.org/10.1016/s0140-6736(68)91924-7.

[10] F. Sadikali, R. Darwish, W.C. Watson, Carnosinase activity of human gastrointestinal mucosa, Gut 16 (1975) 585–589, https://doi.org/10.1136/gut.16.8.585.

[11] T. Terada, K. Sawada, H. Saito, Y. Hashimoto, K. Inui. Functional characteristics of basolateral peptide transporter in the human intestinal cell line Caco-2. Am. J. Phys. 276 (1999) G1435–1441. doi: https://doi.org/10.1152/ajpgi.1999.276.6.G1435.

[12] M.L. Gardner, K.M. Illingworth, J. Kelleher, D. Wood, Intestinal absorption of the intact peptide carnosine in man, and comparison with intestinal permeability to lactulose, J. Physiol. 439 (1991) 411–422, https://doi.org/10.1113/jphysiol.1991.sp018673.

[13] E.C.B. Smith, The buffering of muscle in rigor; protein, phosphate and carnosine, J. Physiol. 92 (1938) 336–343, https://doi.org/10.1113/jphysiol.1938.sp003605.

[14] K. Sahlin, R.C. Harris, B. Nylind, E. Hultman, Lactate content and pH in muscle samples obtained after dynamic exercise, Pflugers Arch. 367 (1976) 143–149, https://doi.org/10.1007/BF00585150.

[15] J.W. Pan, J.R. Hamm, H.P. Hetherington, D.L. Rothman, R.G. Shulman, Correlation of lactate and pH in human skeletal muscle after exercise by1H NMR, Magn. Reason. Med. 20 (1991) 57–65, https://doi.org/10.1002/mrm.1910200107.

[16] B. Saunders, K. Elliott-Sale, G.G. Artioli, et al., β-Alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis, Br. J. Sports Med. 51 (8) (2017) 658–669, https://doi.org/10.1136/bjsports-2016-096396.

[17] M.A. Batrukova, A.M. Rubtsov. Histidine-containing dipeptides as endogenous regulators of the activity of sarcoplasmic reticulum Ca-release channels. Biochim. Biophys. Acta 1324 (1997) 142–150. doi:https://doi.org/10.1016/S0005-2736(96)00216-7.

[18] C. Lamont, D.J. Miller. Calcium sensitizing action of carnosine and other endogenous imidazoles in chemically skinned striated muscle. J. Physiol. 454 (1992) 421–434. doi:https://doi.org/10.1113/jphysiol.1992.sp019271.

[19] G.P. Zaloga, P.R. Roberts, K.W. Black, M. Lin, G. Zapata-Sudo, R.T. Sudo, T.E. Nelson, Carnosine is a novel peptide modulator of intracellular calcium and contractility in cardiac cells, Am. J. Phys. 272 (1997) H462–H468, https://doi.org/10.1152/ajpheart.1997.272.1.H462.

[20] T.L. Dutka, G.D. Lamb, Effect of carnosine on excitation–contraction coupling in mechanically-kkinned rat skeletal muscle, J. Muscle Res. Cell Motil. 25 (2004) 203–213, https://doi.org/10.1023/B:JURE.0000038265.37022.c5.

[21] T.L. Dutka, C.R. Lamboley, M.J. McKenna, R.M. Murphy, G.D. Lamb, Effects of carnosine on contractile apparatus Ca^{2+} sensitivity and sarcoplasmic reticulum Ca^{2+} release in human skeletal muscle fibers, J. Appl. Physiol. 112 (2012) 728–736, https://doi.org/10.1152/japplphysiol.01331.2011.

[22] J.J. Matthews, G.G. Artioli, M.D. Turner, C. Sale, The physiological roles of carnosine and β-alanine in exercising human skeletal muscle, Med. Sci. Sports Exerc. 51 (2019) 2098–2108, https://doi.org/10.1249/MSS.0000000000002033.

[23] R. Hannah, R.L. Stannard, C. Minshull, G.G. Artioli, R.C. Harris, C. Sale. β-Alanine supplementation enhances human skeletal muscle relaxation speed but not force production capacity. J. Appl Physiol. 118 (2015) 604–612. doi:https://doi.org/10.1152/japplphysiol.00991.2014.

[24] R.L. Jones, C.T. Barnett, J. Davidson, B. Maritza, W.D. Fraser, R. Harris, C. Sale, β-

Alanine supplementation improves in-vivo fresh and fatigued skeletal muscle relaxation speed, Eur. J. Appl. Physiol. 117 (2017) 867–879, https://doi.org/10.1007/s00421-017-3569-1.
[25] P. Swietach, J.-B. Youm, N. Saegusa, C.-H. Leem, K.W. Spitzer, R.D. Vaughan-Jones, Coupled Ca2+/H+ transport by cytoplasmic buffers regulates local Ca2+ and H+ ion signaling, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) E2064–E2073, https://doi.org/10.1073/pnas.1222433110.
[26] S. Velez, N.G. Nair, V.P. Reddy, Transition metal ion binding studies of carnosine and histidine: biologically relevant antioxidants, Colloids Surf. B. Biointerfaces. 66 (2008) 291–294, https://doi.org/10.1016/j.colsurfb.2008.06.012.
[27] O. Handa, N. Yoshida, Y. Tanaka, M. Ueda, T. Ishikawa, T. Takagi, N. Matsumoto, Y. Naito, T. Yoshikawa, Inhibitory effect of polaprezinc on the inflammatory response to Helicobacter pylori, Can. J. Gastroenterol. 16 (2002) 785–789, https://doi.org/10.1155/2002/631070.
[28] R. Ishihara, H. Iishi, N. Sakai, H. Yano, N. Uedo, H. Narahara, K. Iseki, T. Mikuni, S. Ishiguro, M. Tatsuta, Polaprezinc attenuates Helicobacter pylori-associated gastritis in Mongolian gerbils, Helicobacter. 7 (2002) 384–389, https://doi.org/10.1046/j.1523-5378.2002.00114.x.
[29] S. Furuta, S. Toyama, M. Miwa, T. Itabashi, H. Sano, T. Yoneta, Residence time of polaprezinc (zinc L-carnosine complex) in the rat stomach and adhesiveness to ulcerous sites, Jpn. J. Pharmacol. 67 (1995) 271–278, https://doi.org/10.1254/jjp.67.271.
[30] A. Torreggiani, S. Bonora, G. Fini, Raman and IR spectroscopic investigation of zinc (II)–carnosine complexes, Biopolymers. 57 (2000) 352–364, https://doi.org/10.1002/1097-0282(2000)57:6<352::AID-BIP50>3.0.CO;2-Y.
[31] P. Mineo, D. Vitalini, D.L. Mendola, E. Rizzarelli, E. Scamporrino, G. Vecchio, Electrospray mass spectrometric studies of L-carnosine (β-alanyl-L-histidine) complexes with copper(II) or zinc ions in aqueous solution, Rapid Commun. Mass Spectrom. 16 (2002) 722–729, https://doi.org/10.1002/rcm.633.
[32] H. Dobbie, W.O. Kermack, Complex-formation between polypeptides and metals: 2. The reaction between cupric ions and some dipeptides, Biochem. J. 59 (1955) 246–257, https://doi.org/10.1042/bj0590246.
[33] E.J. Baran, Metal complexes of carnosine, Biochemistry (Mosc) 65 (2000) 789–797.
[34] A. Torreggiani, M. Tamba, G. Fini, Binding of copper(II) to carnosine: Raman and IR spectroscopic study, Biopolymers 57 (2000) 149–159, https://doi.org/10.1002/(SICI)1097-0282(2000)57:3<149::AID-BIP3>3.0.CO;2-G.
[35] L. Schröder, C.H.J. Schmitz, P. Bachert. Carnosine as molecular probe for sensitive detection of cu(II) ions using localized 1H NMR spectroscopy. J. Inorg. Biochem. 102 (2008) 174–183. doi:https://doi.org/10.1016/j.jinorgbio.2007.07.033.
[36] T. Matsukura, H. Tanaka, Applicability of zinc complex of L-carnosine for medical use, Biochemistry (Mosc) 65 (2000) 817–823.
[37] A.R. Hipkiss, E. Baye, B.de Courten, Carnosine and the processes of ageing, Maturitas 93 (2016) 28–33, https://doi.org/10.1016/j.maturitas.2016.06.002.
[38] A.R. Hipkiss, J. Michaelis, P. Syrris, Non-enzymatic glycosylation of the dipeptide L-carnosine, a potential anti-protein-cross-linking agent, FEBS Lett. 371 (1995) 81–85, https://doi.org/10.1016/0014-5793(95)00849-5.
[39] G. Aldini, M. Orioli, G. Rossoni, F. Savi, P. Braidotti, G. Vistoli, K.J. Yeum, G. Negrisoli, M. Carini. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. J. Cell. Mol. Med. 15 (2011) 1339–1354. doi:https://doi.org/10.1111/j.1582-4934.2010.01101.x.
[40] V.S. Bispo, I.P. de Arruda Campos, P. Di Mascio, M.H.G. Medeiros. Structural elucidation of a carnosine-acrolein adduct and its quantification in human urine samples. Sci. Rep. 6 (2016) 19348. doi:https://doi.org/10.1038/srep19348.
[41] G. Aldini, M. Carini, G. Beretta, S. Bradamante, R.M. Facino, Carnosine is a quencher of 4-hydroxy-nonenal: through what mechanism of reaction?, Biochem. Biophys. Res. Commun. 298 (2002) 699–706, https://doi.org/10.1016/S0006-291X(02)02545-7.
[42] M.J. Cripps, K. Hanna, C. Lavilla Jr., S.R. Sayers, P.W. Caton, C. Sims, L.D. Girolamo, C. Sale, M.D. Turner, Carnosine scavenging of glucolipotoxic free radicals enhances insulin secretion and glucose uptake, Sci. Rep. 7 (2017) 13313, https://doi.org/10.1038/s41598-017-13649-w.
[43] T. Nagasawa, T. Yonekura, N. Nishizawa, D.D. Kitts, In vitro and in vivo inhibition of muscle lipid and protein oxidation by carnosine, Mol. Cell. Biochem. 225 (2001) 29–34, https://doi.org/10.1023/a:1012256521840.
[44] Y. Shen, J. Yang, J. Li, X. Shi, L. Ouyang, Y. Tian, J. Lu, Carnosine inhibits the proliferation of human gastric cancer SGC-7901 cells through both of the mitochondrial respiration and glycolysis pathways, PLoS One 9 (2014) e104632, https://doi.org/10.1371/journal.pone.0104632.
[45] Y. Bao, S. Ding, J. Cheng, Y. Liu, B. Wang, H. Xu, Y. Shen, J. Lyu, Carnosine inhibits the proliferation of human cervical gland carcinoma cells through inhibiting both mitochondrial bioenergetics and glycolysis pathways and retarding cell cycle progression, Integr. Cancer Ther. 17 (2018) 80–91, https://doi.org/10.1177/1534735416684551.
[46] R. Holliday, G.A. McFarland, Inhibition of the growth of transformed and neoplastic cells by the dipeptide carnosine, Br. J. Cancer 73 (1996) 966–971, https://doi.org/10.1038/bjc.1996.189.
[47] H. Oppermann, L. Schnabel, J. Meixensberger, F. Gaunitz, Pyruvate attenuates the anti-neoplastic effect of carnosine independently from oxidative phosphorylation, Oncotarget 7 (2016) 85848–85860, https://doi.org/10.18632/oncotarget.13039.
[48] C. Renner, A. Asperger, A. Seyffarth, J. Meixensberger, R. Gebhardt, F. Gaunitz, Carnosine inhibits ATP production in cells from malignant glioma, Neurol. Res. 32 (2010) 101–105, https://doi.org/10.1179/016164109X12518779082237.
[49] J.-Y. Cheng, J.-B. Yang, Y. Liu, M. Xu, Y.-Y. Huang, J.-J. Zhang, P. Cao, J.-X. Lyu, Y. Shen, Profiling and targeting of cellular mitochondrial bioenergetics: inhibition of human gastric cancer cell growth by carnosine, Acta Pharmacol. Sin. 40 (2019) 938–948, https://doi.org/10.1038/s41401-018-0182-8.
[50] H. Oppermann, C. Birkemeyer, J. Meixensberger, F. Gaunitz, Non-enzymatic reaction of carnosine and glyceraldehyde-3-phosphate accompanies metabolic changes of the pentose phosphate pathway, Cell Prolif. 53 (2020) e12702, https://doi.org/10.1111/cpr.12702.
[51] H. Fan, Y. Wu, S. Yu, X. Li, A. Wang, S. Wang, W. Chen, Y. Lu, Critical role of mTOR in regulating aerobic glycolysis in carcinogenesis, Int. J. Oncol. 58 (2021) 9–19, https://doi.org/10.3892/ijo.2020.5152.
[52] H. Mirzaei, M.R.J. Hamblin. Regulation of glycolysis by non-coding RNAs in cancer: switching on the Warburg effect. Mol. Ther. Oncolytics.19 (2020) 218–239. doi: https://doi.org/10.1016/j.omto.2020.10.003.
[53] Z. Zhang, L. Miao, X. Wu, G. Liu, Y. Peng, X. Xin, B. Jiao, X. Kong. Carnosine inhibits the proliferation of human gastric carcinoma cells by retarding Akt/mTOR/p70S6K signaling. J. Cancer 5 (2014) 382–389. doi: https://doi.org/10.7150/jca.8024.
[54] Y. Huang, J. Wang, M. Luo, D. Yan, C. Zhang, Carnosine attenuates vascular smooth muscle cells calcification through mTOR signaling pathway, Aging Med. (Milton) 3 (2020) 153–158, https://doi.org/10.1002/agm2.12125.
[55] H. Oppermann, H. Faust, U. Yamanishi, J. Meixensberger, F. Gaunitz, Carnosine inhibits glioblastoma growth independent from PI3K/Akt/mTOR signalling, PLoS One 14 (2019) e0218972, https://doi.org/10.1371/journal.pone.0218972.
[56] H. Oppermann, A. Alvanos, C. Seidel, J. Meixensberger, F. Gaunitz, Carnosine influences transcription via epigenetic regulation as demonstrated by enhanced histone acetylation of the pyruvate dehydrogenase kinase 4 promoter in glioblastoma cells, Amino Acids 51 (2019) 61–71, https://doi.org/10.1007/s00726-018-2619-2.
[57] D. Wu, A. Dasgupta, A.D. Read, R.E.T. Bentley, M. Motamed, K.H. Chen, R. Al-Qazazi, J.D. Mewburn, K.J. Dunham-Snary, E. Alizadeh, L. Tian, S.L. Archer. Oxygen sensing, mitochondrial biology and experimental therapeutics for pulmonary hypertension and cancer. Free Radic. Biol. Med. (2021) S0891-5849(21)00001-0. doi: https://doi.org/10.1016/j.freeradbiomed.2020.12.452.
[58] J. Moreno-Felici, P. Hyroššová, M. Aragó, S. Rodríguez-Arévalo, P.M. García-Rovés, C. Escolano, J.C. Perales, Phosphoenolpyruvate from glycolysis and PEPCK regulate cancer cell fate by altering cytosolic Ca^{2+}, Cells 9 (2019) 18, https://doi.org/10.3390/cells9010018.
[59] C. Nigro, A. Leone, F. Fiory, I. Prevenzano, A. Nicolò, P. Mirra, F. Beguinot, C. Miele, Dicarbonyl stress at the crossroads of healthy and unhealthy aging, Cells 8 (2019) 749, https://doi.org/10.3390/cells8070749.
[60] A.R. Hipkiss, H. Chana, Carnosine protects proteins against methylglyoxal mediated protein modifications, Biochem. Biophys. Res. Commun. 248 (1998) 28–32, https://doi.org/10.1006/bbrc.1998.8806.
[61] T.E. O'Toole, X. Li, D.W. Riggs, D.J. Hoetker, S.P. Baba, A. Bhatnagar, Urinary levels of the acrolein conjugates of carnosine are associated with cardiovascular disease risk, Int. J. Mol. Sci. 22 (2021) 1383, https://doi.org/10.3390/ijms22031383.
[62] J. Bellier, M.J. Nokin, M. Caprasse, A. Tiamiou, A. Blomme, J.L. Scheijen, B. Koopmansch, G.M. MacKay, B. Chiavarina, B. Costanza, G. Rademaker, F. Durieux, F. Agirman, N. Maloujahmoum, P.G. Cusumano, P. Lovinfosse, H.Y. Leung, F. Lambert, V. Bours, C.G. Schalkwijk, R. Hustinx, O. Peulen, V. Castronovo, A. Bellahcène, Methylglyoxal scavengers resensitize KRAS-mutated colorectal tumors to cetuximab, Cell Rep.30 (2020) 1400–1416, https://doi.org/10.1016/j.celrep.2020.01.012.
[63] M.-J. Nokin, F. Durieux, P. Peixoto, B. Chiavarina, O. Peulen, A. Blomme, A. Turtoi, B. Costanza, N. Smargiasso, D. Baiwir, J.L. Scheijen, C.G. Schalkwijk, P.D. Tullio, E. Bianchi, M. Thiry, K. Uchida, D.A. Spiegel, J.R. Cochrane, C.A. Hutton, E.D. Pauw, P. Delvenne, D. Belpomme, V. Castronovo, A. Bellahcèn, Methylglyoxal, a glycolysis side-product, induces Hsp90 glycation and YAP-mediated tumor growth and metastasis, Elife 5 (2016) e19375, https://doi.org/10.7554/eLife.19375.
[64] M.-J. Nokin, J. Bellier, F. Durieux, O. Peulen, G. Rademaker, M. Gabriel, C. Monseur, B. Charloteaux, L. Verbeke, S.van Laere, P. Roncarati, M. Herfs, C. Lambert, J. Scheijen, C. Schalkwijk, A. Colige, J. Caers, P. Delvenne, A. Turtoi, V. Castronovo, A. Bellahcène, Methylglyoxal, a glycolysis metabolite, triggers metastasis through MEK/ERK/SMAD1 pathway activation in breast cancer, Breast Cancer Res. 21 (2019) 11, https://doi.org/10.1186/s13058-018-1095-7.
[65] S. Menini, C. Iacobini, M. Vitale, C. Pesce, G. Pugliese. Diabetes and pancreatic cancer - a dangerous liaison relying on carbonyl stress. Cancers (Basel).13 (2021) 313. doi: https://doi.org/10.3390/cancers13020313.PMID: 33467038.
[66] C.G. Fresta, A. Fidilio, G. Lazzarino, N. Musso, M. Grasso, S. Merlo, A.M. Amorini, C. Bucolo, B. Tavazzi, G. Lazzarino, S.M. Lunte, F. Caraci, G. Caruso, Modulation of pro-oxidant and pro-inflammatory activities of M1 macrophages by the natural dipeptide carnosine, Int. J. Mol. Sci. 21 (2020) 776, https://doi.org/10.3390/ijms21030776.
[67] K. Zhao, Y. Li, Z. Wang, N. Han, Y. Wang, Carnosine protects mouse podocytes from high glucose induced apoptosis through PI3K/AKT and Nrf2 pathways, Biomed. Res. Int. 28 (2019) 4348973, https://doi.org/10.1155/2019/4348973.
[68] M.L. Pall, S. Levine, Nrf2, a master regulator of detoxification and also antioxidant, anti-inflammatory and other cytoprotective mechanisms, is raised by health promoting factors, Sheng Li Xue Bao 67 (2015) 1–18.
[69] J.D. Braun, D.O. Pastene, A. Breedijk, A. Rodriguez, B.B. Hofmann, C. Sticht, E. von Ochsenstein, H. Allgayer, J. van den Born, S. Bakker, S.J Hauske, B. K. Krämer, B.A. Yard, T. Albrecht. Methylglyoxal down-regulates the expression of cell cycle associated genes and activates the p53 pathway in human umbilical vein endothelial cells Sci. Rep. 9 (2019) 1152. doi: https://doi.org/10.1038/s41598-018-37937-1.
[70] D.O. Son, H. Satsu, Y. Kiso, M. Totsuka, M. Shimizu, Inhibitory effect of carnosine on interleukin-8 production in intestinal epithelial cells through translational regulation, Cytokine 42 (2008) 265–276, https://doi.org/10.1016/j.cyto.2008.02.011.
[71] B.R.E. Harris, D. Wang, Y. Zhang, M. Ferrari, A. Okon, M.P. Cleary, C.R.Wagner, D.-

Q. Yang. Induction of the p53 tumor suppressor in cancer cells through inhibition of cap-dependent translation. Mol. Cell. Biol. 38 (2018) e00367–17. doi: https://doi.org/10.1128/MCB.00367-17.

*M.*R.K. Srivastava, J. Khan, A. Arumugam, S. Muzaffar, P. Guroji, M.S. Gorbatyuk, C.A. Elmets, A.T. Slominski, M.S. Mukhtar, M.J. Athar, 5'-Cap-dependent translation as a potent therapeutic target for lethal human squamous cell carcinoma, Invest. Dermatol. 141 (2021) 742–753, https://doi.org/10.1016/j.jid.2020.08.021.

*MI.*S. Jo, A. Lockridge, R. Mohan, N. Esch, R. Schlichting, N. Panigrahy, A. Essawy, E. Gustafson, E.U. Alejandro, Translational factor eIF4G1 regulates glucose homeostasis and pancreatic beta-cell function, Diabetes. 70 (2021) 155–170, https://doi.org/10.2337/db20-0057.

*MII.*U. Ozkurede, R. Kala, C. Johnson, Z. Shen, R.A. Miller, G.G. Garcia, Cap-independent mRNA translation is upregulated in long-lived endocrine mutant.Mice, Mol. Endocrinol. 63 (2019) 123–138, https://doi.org/10.1530/JME-19-0021.

*MIII.*Y.-J. Fang, M. Wu, H.-N. Chen, T.-T. Wen, J.-X. Lyu, Y. Shen, Carnosine suppresses human glioma cells under normoxic and hypoxic conditions partly via inhibiting glutamine metabolism, Acta Pharmacol. Sin. (2020), https://doi.org/10.1038/s41401-020-0488-1.

*MIV.*A.R. Hipkiss, C. Brownson, M.F. Bertani, E. Ruiz, A. Ferro, Reaction of carnosine with aged proteins: another protective process? Ann, NY Acad. Sci. 959 (2002) 285–294, https://doi.org/10.1111/j.1749-6632.2002.tb02100.x.

*MV.*J. Zhao, B. Zhai, S.P. Gygi, A.L. Goldberg, mTOR inhibition activates overall protein degradation by the ubiquitin proteasome system as well as by autophagy, Proc. Natl. Acad. Sci. U. S. A. 2 (2015) 15790–15797, https://doi.org/10.1073/pnas.1521919112.

*MVI.*X. Li, K. Yang, S. Gao, J. Zhao, G. Liu, Y. Chen, H. Lin, W. Zhao, Z. Hu, N. Xu, Carnosine stimulates macrophage-mediated clearance of senescent skin cells through activation of the AKT2 signaling pathway by CD36 and RAGE, Front. Pharmacol. 11 (2020) 593832, https://doi.org/10.3389/fphar.2020.593832.

*MVII.*E.B. Smith, The specificity of certain peptidases, Advances Enzymol. 12 (1951) 191–257, https://doi.org/10.1002/9780470122570.ch4.

*MVIII.*J.F. Lenney, R.P. George, A.M. Weiss, C.M. Kucera, P.W. Chan, G.S. Rinzler. Human serum carnosinase: characterization, distinction from cellular carnosinase, and activation by cadmium. Clin.. Chim. Acta. 123 (1982) 221–231. doi: https://doi.org/10.1016/0009-8981(82)90166-8.

*MIX.*Y. Liu, A. Cotillard, C. Vatier, J.-P. Bastard, S. Fellahi, M. Stévant, O. Allatif, C. Langlois, S. Bieuvelet, A. Brochot, A. Guilbot, K. Clément, S.W. Rizkalla, A dietary supplement containing cinnamon, chromium and carnosine decreases fasting plasma glucose and increases lean mass in overweight or obese pre-diabetic subjects: a randomized, placebo-controlled trial, PLoS One 10 (2015) e0138646, https://doi.org/10.1371/journal.pone.0138646.

*MX.*D.L. Williams, P. Munday, The effect of a topical antioxidant formulation including N-acetyl carnosine on canine cataract: a preliminary study, Vet. Ophthalmol. 9 (2006) 311–316, https://doi.org/10.1111/j.1463-5224.2006.00492.x.

[83] M.A. Babizhayev, Biomarkers and special features of oxidative stress in the anterior segment of the eye linked to lens cataract and the trabecular meshwork injury in primary open-angle glaucoma: challenges of dual combination therapy with N-acetylcarnosine lubricant eye drops and oral formulation of nonhydrolyzed carnosine, Fundam. Clin. Pharmacol. 26 (2012) 86–117, https://doi.org/10.1111/j.1472-8206.2011.00969.x.

[84] M. Orioli, G. Vistoli, L. Regazzoni, A. Pedretti, A. Lapolla, G. Rossoni, R. Canevotti, L. Gamberoni, M. Previtali, M. Carini, G. Aldini, Design, synthesis, ADME properties, and pharmacological activities of β-alanyl-D-histidine (D-carnosine) prodrugs with improved bioavailability, ChemMedChem 6 (2011) 1269–1282, https://doi.org/10.1002/cmdc.201100042.

[85] E.J. Anderson, G. Vistoli, L.A. Katunga, K. Funai, L. Regazzoni, T.B. Monroe, E. Gilardoni, L. Cannizzaro, M. Colzani, D. De Maddis, G. Rossoni, R. Canevotti, S. Gagliardi, M. Carini, G. Aldini. A carnosine analog mitigates metabolic disorders of obesity by reducing carbonyl stress. J. Clin. Invest. 128 (2018) 5280–5293. doi: https://doi.org/10.1172/JCI94307.

[86] R. Rezzani, G. Favero, M. Ferroni, C. Lonati, M.H. Moghadasian, A carnosine analog with therapeutic potentials in the treatment of disorders related to oxidative stress, PLoS One 14 (2019) e0215170, https://doi.org/10.1371/journal.pone.0215170.

[87] J. Qiu, S.J. Hauske, S. Zhang, A. Rodriguez-Niño, T. Albrecht, D.O. Pastene, J. van den Born, H.van Goor, S. Ruf, M. Kohlmann, M. Teufel, B.K. Krämer, H.P. Hammes, V. Peters, B.A. Yard, A. Kannt, Identification and characterisation of carnostatine (SAN9812), a potent and selective carnosinase (CN1) inhibitor with in vivo activity, Amino Acids 51 (2019) 7–16, https://doi.org/10.1007/s00726-018-2601-z.

[88] F.W. Dunn. Synthesis and microbiological properties of a thiophene analogue of carnosine. J. Biol. Chem. 227 (1957) 575–581.

[89] C.A. Hill, R.C. Harris, H.J. Kim, B.D. Harris, C. Sale, L.H. Boobis, C.K. Kim, J.A. Wise, Influence of beta-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity, Amino Acids 32 (2007) 225–233, https://doi.org/10.1007/s00726-006-0364-4.

[90] L. Blancquaert, I. Everaert, M. Missinne, A. Baguet, S. Stegen, A. Volkaert, M. Petrovic, C. Vervaet, E. Achten, M.D. Maeyer, S.D. Henauw, W. Derave, Effects of histidine and β-alanine supplementation on human muscle carnosine storage, Med. Sci. Sports Exerc. 49 (2017) 602–609, https://doi.org/10.1249/MSS.0000000000001213.

[91] G.G. Artolli, B. Gualano, A. Smith, J. Stout, A.H. Lancha Jr., Role of beta-alanine supplementation on muscle carnosine and exercise performance, Med. Sci. Sports Exerc. 42 (2010) 1162–1173, https://doi.org/10.1249/MSS.0b013e3181c74e38.

[92] S.P. Cartwright, R.M. Bill, A.R. Hipkiss. L-carnosine affects the growth of Saccharomyces cerevisiae in a metabolism-dependent manner. PLoS One 7 (2012) e45006. doi: https://doi.org/10.1371/journal.pone.0045006.