



## Histidine-Dipeptides in Relation to Diabetes and Obesity

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### ABSTRACT

Diabetes is a serious public health concern, and innovative, low-cost therapies to prevent or slow disease development are needed. Obesity is a globally epidemic disease that is threatening the public health concern and also increasing the incidence of other diseases as diabetes, heart disease and cancer. Diabetes and obesity are related mostly to each other as they share common pathophysiological processes and lead to the same sequences, so changing the lifestyle is mandatory. The oxidation of polyunsaturated fatty acids and carbohydrates leads to the formation of reactive carbonyl molecules and also advanced glycoxidation/lipoxidation end products (AGEs/ALEs) are formed which ultimately leading to cellular dysfunction. As a result, agents able to detoxify these harmful molecules by creating unharmed molecules, and reverse the glycated protein are needed. Histidine-containing dipeptides (HDPs) are proven through different studies to be one of these agents. HDPs are found in mammals, amphibians and fish, with a wide range of ratios and concentrations. Anserine, L-carnosine and homocarnosine are related dipeptides produced by vertebrate muscles. Carnosine and anserine can form unharmed molecules, which may assist to ameliorate some of the metabolic dysregulation seen in diabetes and obesity. Homocarnosine has shown anti-inflammatory characters, DNA damage and AGEs inhibition, this review gives attention to the protective effects of histidine-dipeptides against diabetes and obesity.

**Key words:** Diabetes, Obesity, Histidine- Containing dipeptides.

### INTRODUCTION

Diabetes is a series of metabolic disorder grouped together by a single diagnostic criterion: hyperglycemia, caused by absolute or relative insulin insufficiency, usually in the setting of  $\beta$ -cell malfunction, insulin resistance, or both (Care 2006). Diabetes is traditionally divided into two types: type 1 diabetes; T1D, (insulin-dependent diabetes) and T2D; the most common diabetes subtype, accounting for 90-95 percent of cases, formerly non-insulin-dependent diabetes mellitus, as well as other subtypes as monogenic diabetes, gestational diabetes and a late-onset autoimmune form (American Diabetes Association 1997; Udler et al. 2018).

Diabetes can cause damage to several organs for example heart, kidneys, nerves and so on, raising the risk of cardiovascular illness. The nerve damage occurring in the feet as one of the diabetic complications leads to decrease in nerve supply and also increasing foot ulcers incidence may finally end with amputation (Fowler 2008; Khanolkar et al. 2008; Snelson et al. 2021). Obesity, particularly central obesity, is a primary risk point for type 2 diabetes, and other illnesses, Obesity is caused mostly by a rise in the bulk of adipose tissue and also due to producing of pathogenetic compounds by larger fat cells (Bray 2004; Menon et al. 2020).

Both obesity and Type 2 diabetes can be treated and controlled via lifestyle interruption programs that are exercise and a nutritious diet. These programs have significant impacts on whole-body metabolism, including enhanced insulin sensitivity (Gaesser et al. 2011). The weight-loss drugs that were first produced are sympathomimetics, amphetamines, and chemical uncouplers, that act by raising metabolic rate so lowering body weight, but the unfavorable cardiovascular consequences of these medicines were so severe (Mills et al. 2004). Other drugs aimed at reducing food consumption, as serotonergics, dopaminergics, and endocannabinoid antagonists, it's safe but less effective than the above-mentioned medications. Also, they have cardiovascular and psychological side effects (Kirilly et al. 2012). Leptin also has the ability to enhance satiety and boost energy expenditure in obesity treatment (Heymsfield et al. 1999).

Inhibitors of sodium-glucose co-transporter 2 (SGLT2) and analogues of the incretin hormone glucagon-like peptide 1 (GLP-1) (Tuch 2016) are two medications used to treat type 2 diabetes. Surgery is the most significant treatment in weight loss and preceded by improvements in glucose metabolism (Stefater et al. 2012).

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As a result of newly identified peptide effects on various physiological systems, there has been a peptide revolution all over the world. Small peptides are classified as quasi-hormones and pharmacological agents that can alter the physiological functioning of cells. The PepT1 H<sup>+</sup>/peptide co-transporter allows bioactive short peptides to be taken directly into the bloodstream from the intestine (Wang et al. 2019). The pancreas is one of the most significant organs after enteric absorption because it is exposed to large amounts of absorbed bioactive peptides. The pancreas' beta cells are one of the most important biological targets for bioactive peptides (Udenigwe et al. 2021). In this review, we focus on the possible mechanisms of HDPs in the treatment of obesity and diabetes and reducing their complications. We also, suggesting using HDPs nano-formulation to improve its solubility and for better targeting into organs, studies should be done in controlled human and animal research. More studies should be done in the future for N-acetylcarnosine and Ophidine/balenine for discovering more functions of both as they have limited studies.

### Histidine-Containing Dipeptides

HDPs are found in humans, birds and fish with a wide range of ratios and concentrations (Wu 2009; Wu 2020). Table 1 represents different types of HDPs and their structure (Abe 1995; Peters et al. 2020). Carnosine is internally formed by carnosine synthase 1. Anserine and ophidine are formed through carnosine methylation, N-Acetyl-L-carnosine made through carnosine acetylation. Dipeptidase carnosinase (EC 3.4.13.20, CN1) degraded the dipeptides carnosine, anserine, and homocarnosine (O'Dowd et al. 1988; de Souza Gonçalves et al. 2021). Carnosine scavenges carbonyls, inhibits glycation, and functions as an angiotensin-converting enzyme (ACE) inhibitor. Carnosine can suppress the development of N-carboxyethyl lysine (CEL) and methylglyoxal (MG)-induced advanced glycation end-products (AGE) (Hipkiss et al. 1994; Vistoli et al. 2017).

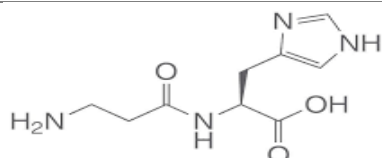
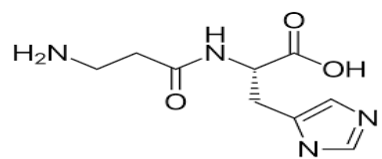
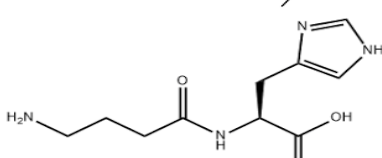
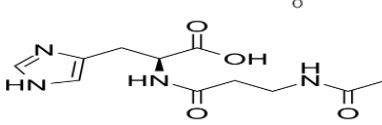
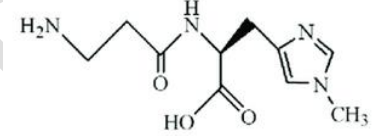
### Carnosine (β-alanyl-L-histidine)

Carnosine is largely found in skeletal muscles, and lesser amounts in the brain and kidneys of humans (Guiotto et al. 2005; de Souza Gonçalves et al. 2021). Carnosine is a cytoplasmic dipeptide made up of β-alanine and L-histidine that is water-soluble. L-biological histidine's action is conferred by the dipeptide's imidazole ring, whereas β-alanine regulates its rate of synthesis. It has the chemical formula C<sub>9</sub>H<sub>14</sub>N<sub>4</sub>O<sub>3</sub>, a molar mass of 226.236 g.mol<sup>-1</sup> and a melting point of 253°C (Hipkiss 2009; Vraneš et al. 2021). L-carnosine has a short half-life in humans, just a few minutes, as it is inhibited by carnosinases, whereas it has a half-life of 78 minutes in mice serum (Peters et al. 2020).

### Metabolism of Carnosine

Five major mechanisms in the metabolism of carnosine can be formulated in greater detail. First from the diet when significant amounts of carnosine are consumed, carnosine is partially intact transferred across intestinal cells and emerged in the blood. At the intestinal epithelium, carnosine is transported from the lumen into the enterocytes via the proton-coupled peptide transporter 1

**Table 1:** different type of HDPs and their structure

Carnosine (β-alanyl-L-histidine)	
Anserine (β-alanyl-Np-methyl-L-histidine)	
Homocarnosine (γ-aminobutyric acid-L-histidine)	
N-acetylcarnosine (NAC; N-acetyl-β-alanyl-L-histidine)	
Ophidine/balenine (β-alanyl-Nt-methyl-histidine (HDPs))	

(PEPT 1) in an active, electrogenic way by H<sup>+</sup> symport (Yeum et al. 2010). Low concentrations of carnosine, on the other hand, are thought to be primarily digested by carnosinase 2 in enterocytes then delivered through the bloodstream by other transporters. Mechanisms of carnosine uptake by enterocytes are represented in Fig. 1 (Perim et al. 2019). Second, carnosine is rapidly destroyed by carnosinase 1 in the circulation if it reaches the bloodstream (and is not digested by enterocytes). Carnosinase 1 is quite active in the circulation of humans, while it is not found in the circulation of rodents (Everaert et al. 2012).

Third, as previously stated, no or very little carnosine is moved through the muscles after consumption. The amino acid transporters move some amino acids and histidine through the skeletal muscle, while β-alanine is transported via taurine transporters (Perim et al. 2019). Fourth, brain tissues are likewise equipped with taurine transporters; they have relatively high amounts of carnosine and a metabolism that is remarkably comparable to that of skeletal muscle. Peptide transporter 2 is important for maintaining carnosine (and another peptide) homeostasis in the CSF (Boldyrev et al. 2013). Finally, carnosine can occur in urine if substantial dosages of carnosine are administered, and carnosine is not degraded by carnosinase 2 or 1 in circulation. Carnosine, on the other hand, is primarily reabsorbed from in the renal cells via carnosinase 2 (Everaert et al. 2019). So the above mentioned studies concluded the different patterns for carnosine absorption and metabolism.

### Health Benefits of Carnosine

Carnosine's antioxidant capabilities originate from direct action with O<sub>2</sub> and scavenge peroxy and superoxide particles, avoiding their toxic impact. Carnosine's direct interaction with these particles suppresses the formation of

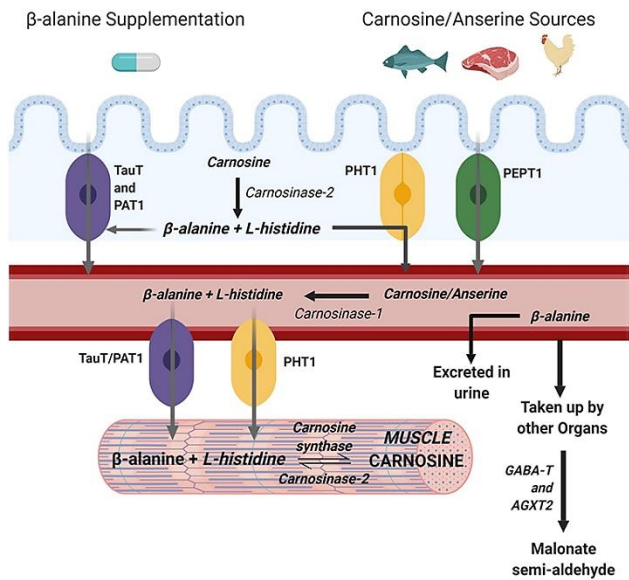


Fig. 1: Metabolism of carnosine (Perim et al. 2019).

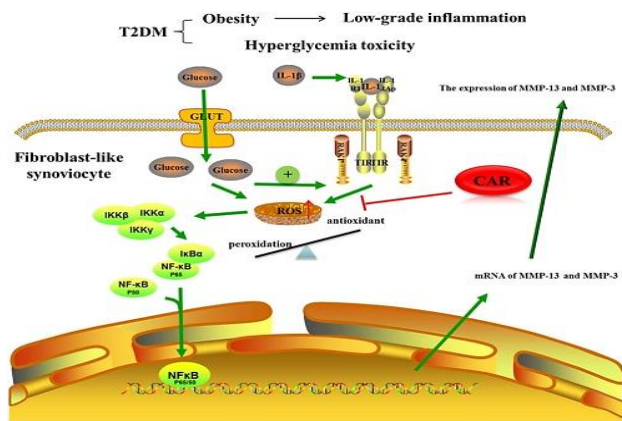


Fig. 2: The mechanism of carnosine on IL-1 $\beta$ -induced FLSs in a high glucose environment. Carnosine alleviated MMP-3 and MMP-13 expression by decreasing ROS content and suppressing nuclear translocation of NF- $\kappa$ B p65 in IL-1 $\beta$ -induced FLSs under a high glucose environment (Yang et al. 2018).

lipid peroxidation end substances, which are harmful to the body (Guiotto et al. 2005). Carnosine's capacity to chelate metals, particularly transition metals, which would naturally produce free radicals via the Fenton action, is another component of its antioxidant activity (Ma et al. 2020).

Carnosine's capacity to suppress copper and zinc-related neurotoxicity by intact with these metals has also been reported (Trombley et al. 2000). Carnosine administration in rats resulted in decreased LDH and AST in plasma, indicating its antioxidant impact, and improved histopathological structure in cardiac tissues (Derave et al. 2019).

### Mechanisms of Action of Carnosine in Insulin Resistance

Through TGF- $\beta$ /Smad3 signaling pathway, pleiotropic effects on various systems such as glucose and lipid metabolism, insulin resistance, and inhibits reactive oxygen species (ROS) formation (Saadat et al. 2021). The pancreatic islet cell is a prime target of disease in diabetes because of its specific function of insulin production and glucose-stimulated insulin secretion. Insulin has been identified as a TGF- $\beta$  target gene in several investigations,

and the TGF- $\beta$  signaling effector Smad3 was revealed to involve the insulin gene promoter and suppress insulin gene transcription. Smad3 small interfering RNAs, on the other hand, relax insulin transcriptional regulation and increase insulin levels. Smad3-deficient animals have moderate hyperinsulinemia and mild hypoglycemia, which is consistent with this. Furthermore, *in vivo*, Smad3 absence helps in the improvement of glucose tolerance and glucose-stimulated insulin secretion. Carnosine diminishes TGF- $\beta$  production, which helps in preventing diabetic nephropathy (Siriwattanasit et al. 2021).

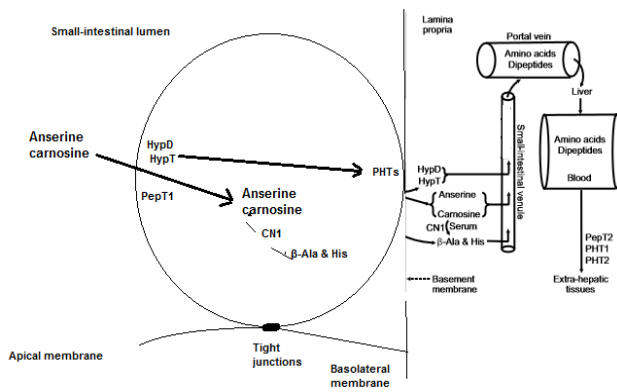
Obesity and hyperglycemia cause the development of AGEs and the overproduction of ROS. Increased levels of ROS and AGEs increase the nuclear factor- $\kappa$ B (NF- $\kappa$ B) resulting in the release of cytokines, TGF- $\beta$ , chemokines, TNF- $\alpha$ , IL-1, IL-6 and vesicular cell adhesion molecules (VCAMs), all of which contribute to diabetic complications. Carnosine supplementation lowered oxidative stress in mice by interacting with glycated proteins and preventing the development of AGE and ROS and alleviated MMP-3 and MMP-13 expression. It also reduced the expression of NF- $\kappa$ B through acting with singlet O $_2$  and also scavenge peroxy and superoxide particles, to avoid ROS generation (Fig. 2) (Yang et al. 2018).

The PI3K/AKT signaling pathway is needed for normal metabolism, and any error leads to obesity and type 2 diabetes. AKT is activated by PI3K and regulates glycolysis, Glucose 6-phosphate and glycogen synthase kinase 3 (GSK3), to provide cellular energy and inhibit glycogen synthesis (Rains and Jain 2011). AKT targets FoxO proteins that induce gluconeogenesis while inhibiting mTOR complex 1 (mTORC1) to decrease fat and protein production, lowering glucose levels. Eventually, GSK3 prevents glycogen production by inhibiting glycogen synthase (GS). GSK3 is phosphorylated by AKT, which has an inhibitory effect on it. Lipid metabolism is regulated through sterol regulatory element-binding proteins (SREBP), which promote cholesterol and fatty acid buildup. As a result, PI3K/AKT controls glucose metabolism via FoxO1 and GSK-3, as well as lipid metabolism via mTORC1 and SREBP (Xue et al. 2018; Xu et al. 2021). Carnosine has also been shown to stop the Akt/mTOR/p70S6K pathway in a human cell line (Zhang and Liu 2014).

Diabetic problems are exacerbated by reactive dicarbonyl chemicals like methylglyoxal. Carnosine and anserine, which have been found to reduce diabetic nephropathy had their methylglyoxal -scavenging capacities tested *in vitro* and *in vivo* (Yilmaz et al. 2017).

Carnosine supplementation for 12 weeks on type 2 diabetes patients leads to decrease in fat mass and fat-free mass, fasting blood glucose, glycated haemoglobin, carboxymethyl lysine, serum triglyceride, and TNF- $\alpha$  levels and thus improving their weight and glucose level (Houjehani et al. 2018).

The effect of carnosine medication on diabetic complications was studied using various rat models of type 1 and 2 diabetes. They repeatedly show that exogenous carnosine had renoprotective effects. Carnosine and carnosine-derivate treatment significantly reduced albuminuria and proteinuria. Diabetic type 2 mice models were given an early therapy that reduced proteinuria and improved vasculopathy (Peters et al. 2018).



**Fig. 3:** Absorption of carnosine and anserine are taken up by the enterocyte across its apical membrane via specific transporters. Inside the cell anserine is not degraded, and carnosine undergoes limited catabolism. Anserine and the products of carnosine hydrolysis ( $\beta$ -alanine and histidine) exit the enterocyte across its basolateral membrane into the lamina propria of the intestinal mucosa via specific transporters (Wu 2016).

Carnosine reduced albuminuria carnosinases by one-third and half in ApoE null mice fed a high-fat diet (Menini et al. 2012) and obese rats (Aldini et al. 2011). In db/db and BTBR ob/ob mice, carnosine therapy reduced glomerular hypertrophy while maintaining podocyte number (Albrecht et al. 2017).

Overall, carnosine supplementation in diabetic mice with low serum CN1 activity decreased damage caused by experimental DN. Carnosine-treated rodents with type 2 diabetes, type 1 diabetes, enhanced glucose homeostasis via decreasing blood glucose and/or HbA1c level (Nagai et al. 2012).

### Side Effects of Carnosine

When circulating  $\beta$ -alanine concentrations reach 100M, the only negative impact revealed called paresthesia, that is a benign sense of tingling and numbness (Derave et al. 2019). So, patients should be supplied with a slow-release  $\beta$ -alanine compound or not exceed a dose of 10 mg/kg of body weight to avoid paresthesia (Boldyrev et al. 2013).

### Anserine ( $\beta$ -alanyl-N<sup>p</sup>-methyl-L-histidine),

Anserine chemical formula is  $C_{10}H_{16}N_4O_3$  which is represented in Table 1. Carnosine N-methyltransferase (EC 2.1.1.22) methylates carnosine into anserine (Derave et al. 2019). Anserine is primarily present in birds' skeletal muscles and some fish (Boldyrev et al. 2013), and cattle (Wu 2016), but not in human tissues.

### Absorption of Anserine by the Small Intestine and Transport in Blood

In humans, anserine obtained from the diet is absorbed via the small intestine, moved in the blood, and taken via extra-intestinal tissues as in carnosine absorption mentioned before, except the degradation of anserine into  $\beta$ -alanine and 1-methyl-histidine by CN1 in plasma and by CN2 in tissues (Wu 2016). Mechanisms of anserine uptake by enterocytes is represented in Fig. 3.

### Metabolism of Anserine

Carnosinase degrades anserine at a lower rate than carnosine (Boldyrev et al. 2013), so, diet containing

anserine as beef can increase anserine levels in human plasma (Everaert et al. 2019).

### Health Benefits of Anserine

Anserine's biochemical characteristics differ from those of carnosine in a few ways as it does not chelate copper and does not have an impact on nitric oxide in cells (Boldyrev et al. 2013). Anserine (1 mM) induces the expression of heat shock protein-70 in renal tubular cells supplied with 25 mM glucose or 20-100M hydrogen peroxide, other than carnosine although both have an antioxidative impact (Peters et al. 2018). It inhibits carnosinase activity (Derave et al. 2019). As a result, anserine may enhance the impacts of carnosine inside the body.

In addition to animal studies, clinical trials with people have shown that anserine has favorable benefits on metabolic, neurological, immunological, cardiovascular, and renal functions. In several models characterized by oxidative stress, nutritional supplementation with anserine has been demonstrated to be helpful. Intraperitoneal injection of anserine to hyperglycemic rats suppressed sympathetic nerve activity and lowered hyperglycemia and plasma glucagon concentrations (Kubomura et al. 2010).

### Mechanisms of Action of Anserine in Insulin Resistance

Anserine acts with the reactive carbonyl particles forming unharmed one. Anserine can change the insulin effect in muscle cells via oxidative damage to proteins (Yeum et al. 2020). *In vitro*, anserine can reduce MG-induced AGE and CEL production. AGE levels in the blood and tissues are higher in both type 1 and 2 diabetes. Anserine works to prevent this by inhibiting RAGE expression and act with AGEs, as well as lowering serum glucose levels and/or trapping MG (Brings et al. 2017). Heat shock proteins (HSPs) help to reduce insulin resistance and hyperglycemia in T2D patients. Furthermore, enhanced HSP70 expression has been shown to improve insulin and glycemic control. Furthermore, anserine can effectively inhibit protein carbonylation, the most severe alteration caused by ROS in proteins (Peters et al. 2018). In diabetic db/db mice, intravenous injection of anserine (every two days for six days) decreased blood glucose level by 20%, vascular permeability, and proteinuria by 50% (Peters et al. 2018).

In 2014 a study was made on people felled with fatigue and stress and some neurological disorders, they supplemented with anserine for weeks after while the stress, fatigue, hyperglycemia and hypertension decreased, physical capacity and exercise performance improved, and neurological disorders were controlled and inhibited (Szczeński et al. 2014). Anserine supplementation decreased glucose, proteinuria, and vascular permeability in diabetic mice (Peters et al. 2018).

### Homocarnosine ( $\gamma$ -aminobutyric acid-L-histidine)

Homocarnosine–carnosine synthase produces a dipeptide made up of histidine and GABA. In comparison to concentrations in other mammals, it is present abundantly in CSF (up to 4 IM), especially children, than blood (about 100 nM) (Menon et al. 2020).

In skeletal muscle and the brain, homocarnosine may act as an endogenous antioxidant. Homocarnosine appears

to be a neuromodulator that acts as an inhibitor. Homocarnosine effects on carnosine pathway via monitoring of CN1 activity. Because the enzyme involved in carnosine biosynthesis (carnosine synthase and CARNS) regulates homocarnosine biosynthesis. Some studies hypothesized that skeletal muscle homocarnosine biosynthesis is inhibited in the absence of the substrate GABA (Perry et al. 1979; Peters et al. 2010). Homocarnosine has shown anti-inflammatory characters (Huang et al. 2018), DNA damage and AGEs inhibition, furthermore, it resists serum carnosinase hydrolysis more than carnosine (Pavlin et al. 2016). It has antioxidant, a free radical scavenger, and metal chelating properties as other HDPs. As a result, they may operate as neuroprotective agent in copper-dependent toxic situations and as neuroprotective agent in general in physiological and pathological circumstances (Grasso et al. 2014).

Homocarnosinosis is a hereditary condition caused by accumulation of L-homocarnosine in the brain tissue and fluid. L-homocarnosine and carnosine have also been shown to have neuroprotective effects in ischemia injury in PC12 cell, a clonal cell line derived from a pheochromocytoma of the rat adrenal medulla. Human glial tumors had lower L-homocarnosine levels than normal brain tissue, whereas the brain tissues of experimental traumatic brain injury animal models had higher L-homocarnosine levels. L-homocarnosine concentrations in the brain and CSF have been reported to be in the range of 2-50 M in several investigations (Menon et al. 2020).

### **Mechanisms of Action of Homocarnosine in Insulin Resistance**

Reactive dicarbonyl chemicals induce diabetic problems in part by covalently modifying proteins, culminating in AGEs. The synthesis of the MG-derived AGE methylglyoxalhydroimidazolone1 has been linked to the development of diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy in several investigations. Histidine peptides interact with AGEs while also reducing serum glucose levels and/or trapping MG in the body (Brings et al. 2017).

Furthermore, homocarnosine as a potential therapy against diabetic nephropathy patients should be approached with caution. Also, homocarnosine therapy has the potential to minimize carnosine breakdown in the blood. High levels of homocarnosine, on the other hand, may result in high levels of its breakdown products,  $\gamma$ -aminobutyric acid (GABA) and histidine. Histidine is rapidly turned into histamine and leading to enhanced both renal insufficiency and nerve activity (Kumrungsee et al. 2020).

### **N-acetylcarnosine (NAC; N-acetyl- $\beta$ -alanyl-L-histidine)**

NAC, the acetylated form of carnosine is found in the CNS of vertebrates. It also has an antioxidative impact and insulates the eye lens against lipid oxidation. NAC enters the cornea and is turned into carnosine, making it a therapeutic alternative for cataract sufferers (Dubois and Bastawrous 2017).  $\beta$ -alanine may aid in the scavenging of lipid peroxidation products as well as metal-ion chelation. Due to powerful protection against oxidative stress in

membranes and aqueous environment, NAC holds potential as an *in vivo* powerful antioxidant.

### **Mechanisms of Action of N-acetylcarnosine in Insulin Resistance**

Diabetics' complications can lead to cataracts. Surgical removal of a cataract, in diabetics leads to suction on the retinas in some cases (Wang et al. 2018). Cataract could lead to vision loss. In beginning of cataract. products named primary lipid peroxidation (LPO), lipid hydroperoxides, cetodienes, and dialdehydes characterized could be found. NAC has been shown to be effective in treating cataracts via regulating of ROS and aldehyde in ocular tissues and fluids. The dipeptide compounds disclosed here, as well as their ophthalmic preparations, can be used to treat diabetes-related problems that result from normal ageing glycosylation processes (Babizhayev 2017; Jeevanandam et al. 2021).

### **Ophidine/balenine ( $\beta$ -alanyl-N<sup>t</sup>-methyl-histidine (HDPs)**

Using comparative chromatography and amino acid sequencing techniques, balenine was found in muscle samples from 9 mammalian species, including man and chicken. Balenine, like carnosine and anserine, has also been proposed as a natural component of muscle (Wada et al. 2016). It is worth noting, though, that methylation of the imidazole ring (as opposed to acetylation of the  $\beta$ -amine group) preserves the molecule's anti-radical function. In basic chemical models, this derivative also displays antioxidant, proton buffering, and heavy metal chelating properties, although their biological activity has not been investigated (Guiotto et al. 2005). Sugino et al. (2013) discovered that balenine had an anti-fatigue effect. Furthermore, Wada et al. (2016) discovered that balenine possesses dementia-relieving properties (Valdersnes et al. 2017). All HDPs, including carnosine, anserine, and balenine, could react with deadly aldehydes, according to studies. With carbohydrate-derived aldehydes such as MG and glyceraldehyde, on the other hand, balenine showed notably high reactivity, equivalent to or higher than carnosine; however, the decrease in balenine concentration could not be substantiated in the case of unsaturated aldehydes (Mori et al. 2019). More studies concentrating on new balenine functions are expected to be published in the future. The metabolic function of methylhistidines is unknown, as are the components of ophidine, and validation data for balenine in published methods is scarce or nonexistent.

### **Conclusions and Future Prospective**

This review summarized several studies about the protective effects of histidine-dipeptides against diabetes and obesity. The focus of this review is about the possible mechanisms of HDPs in the treatment of obesity and diabetes and reducing their complications. HDPs nano-formulation suggested to enhance the solubility of HDPs and increase the targeting toward the organs, more studies should be done in human and animal models in comparison with other treatments and also the crude HDPs without nano-formulation. Also, more studies should be done in the future for N-acetylcarnosine and Ophidine/balenine for discovering more functions and mechanisms of both as they have limited studies.

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