Chapter 24

Carnosine as a Putative Antioxidant in Usage Against Liver Disease

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Summary Points

- This chapter focuses on the putative role of carnosine on acute and chronic liver diseases.
- Oxidative stress is believed to be a major factor in most of the liver pathologies.
- The protective effect of carnosine has been shown in several animal models that exhibit liver injury.
- Besides antioxidation, the protective potential of carnosine against liver injuries can be attributed to its antiinflammatory, antiapoptotic, antifibrotic, and antiglycating actions.

ABBREVIATIONS

AGE	advanced glycation end product
ALD	alcoholic fatty liver disease
ALE	advanced lipoxidation end product
ASH	alcoholic steatohepatitis
CCl_4	carbon tetrachloride
CN	carnosinase
CYP2E1	cytochrome P450 2E1
DC	diene conjugate
DEN	*N*-diethylnitrosamine
DOX	doxorubicin
GCs	glucocorticoids
GPx	glutathione peroxidase
GSH	glutathione
HFD	high fat diet
HFr	high fructose containing diet
4-HNE	4-hydroxynonenal
HPA	hypothalamic-pituitary-adrenal
IL	interleukin
MDA	malondialdehyde
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
Nrf-2	nuclear factor E2-related factor 2
PC	protein carbonyl
RNS	reactive nitrogen species
ROS	reactive oxygen species
SOD	superoxide dismutase

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SREBR sterol regulatory element binding protein
TAA thioacetamide
TNF-α tumor necrosis factor-α

KEY FACTS

- Carnosine is a water-soluble compound, composed of β-alanine and L-histidine.
- Carnosine has three ionizable groups: the amino group of the β-alanine residue, the carboxylic group, and the nitrogens of the imidazole ring.
- Since the p*K*a value of imidazole ring is close to intracellular pH, carnosine acts as a potent buffer in physiological pH range.
- Carnosine acts as an antioxidant by scavenging free radicals and aldehydes, chelating transition metals, thereby reducing oxidative stress.

Definition of words and terms:

- *Bcl-2:* It is encoded by Bcl-2 gene and is important as an antiapoptotic protein**.**
- *Bax:* Apoptosis regulator protein, which is a member of Bcl-2 gene family. It promotes apoptosis by binding to Bcl-2 protein.
- *Ki-67:* A nuclear protein that is associated with cellular proliferation.
- *Reactive oxygen species (ROS):* They are chemically reactive molecules, mostly being free radicals in nature. They contain oxygen atom and form during normal oxygen metabolism. Although they are helpful in cellular reactions, elevated levels cause damage in the cell.
- *Oxidative stress:* The cumulative damage caused by cellular oxidants when the balance between oxidant production and antioxidant defense is disturbed for the favor of oxidants.
- *Lipid peroxidation:* The oxidation chain reactions of lipids containing carbon—carbon double bonds. It is triggered by reactive oxygen species and lead to degradation of the molecules.
- *Advanced glycation endproducts (AGEs):* These molecules form when carbohydrate residues bind with proteins by the reaction called glycation. They may be formed in the cells or in food by heating. Presence of these products leads to premature aging and they take role in age-related diseases.
- *Advanced lipoxidation end products (ALEs):* They are a variety of adducts and crosslinks which are generated by the nonenzymatic reaction of reactive carbonyl species produced by lipid peroxidation and the lipid metabolism, with the nucleophilic sites of macromolecules.
- *Cytokine:* They are a group of molecules taking part in hormonal regulation and cell signaling. They are mostly protein or glycoprotein in nature. They are mostly referred as agents responsible for immune reactions. Those, which start an inflammatory response are grouped as proinflammatory and those that reduce inflammation are grouped as antiinflammatory.
- *Transforming growth factor β1 (TGF-β1):* A multipotential cytokine that regulates cell growth, its differentiation, processes of apoptosis, extracellular matrix production as well as inducing fibrosis in a variety of tissues.
- *High fat diet (HFD):* It is a type of experimental diet in which 35%–60% of total calories are obtained from fat.
- *High fructose containing diet (HFr):* It is a type of diet containing 60% fructose for generating insulin resistance in experimental animals.
- *Nuclear factor erythroid 2-related factor 2 (Nrf2):* It is a redox-regulated transcription factor and the master regulator of inducible antioxidant responses of cell to excess ROS.

INTRODUCTION

Carnosine (β-alanyl L-histidine) is a naturally occurring dipeptide found especially in animals (Fig. 24.1). It was first isolated from skeletal muscle extracts in 1900 by a Russian scientist, Vladimir S. Gulewitch. In fact, skeletal muscle and olfactory bulb are the main tissues that possess carnosine in millimolar range.[1] Male gender, young age, and diet based on meat eating are factors that positively affect carnosine concentration in human body.[2] It is shown that white muscle with ongoing glycolitic process has more carnosine than aerobic red muscle.[3]

The cytosolic carnosine synthase is known to synthesize carnosine from β-alanine and L-histidine using ATP.[3] It mainly exists in skeletal and heart muscles and certain brain sections. While the imidazole ring of L-histidine functions as the reactive part of carnosine, β-alanine appears to regulate the synthesis (Fig. 24.2).[1] Carnosine is hydrolyzed by the enzyme called carnosinase. The two identified forms are serum carnosinase (CN1) and cytosolic tissue carnosinase, otherwise called nonspecific dipeptidase (CN2). CN1 is found in humans but is not present in nonprimate mammals other than the Syrian

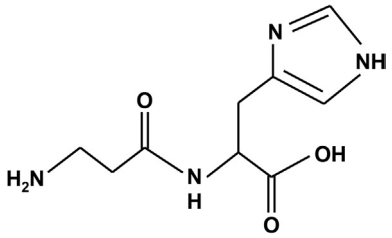

FIGURE 24.1 **Structural formula of carnosine.**

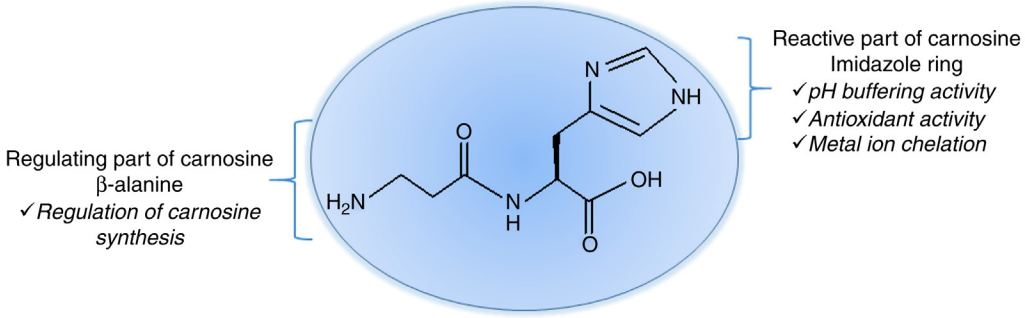

FIGURE 24.2 **The relationship between structure and activity of carnosine.**

golden hamster. CN1 which is secreted into circulation, is expressed in human liver and brain; whereas CN2 is expressed everywhere except serum and cerebrospinal fluid.[1]

Although cooking style may affect the availability, fish and meat are the main carnosine rich dietary food. Dietary carnosine can be transported across the brush-border membrane through an intestinal peptide transporter (human proton/peptide cotransporter 1; PEPT1; Fig. 24.3).[1] CN2 in enterocytes hydrolyzes carnosine that is absorbed by the small intestine. Upon the enzyme's saturation, the unhydrolyzed carnosine enters the blood to be the substrate of CN1. Only after chronic carnosine supplementation, a rather constant serum level can be achieved due to CN1 saturation.[4,5]

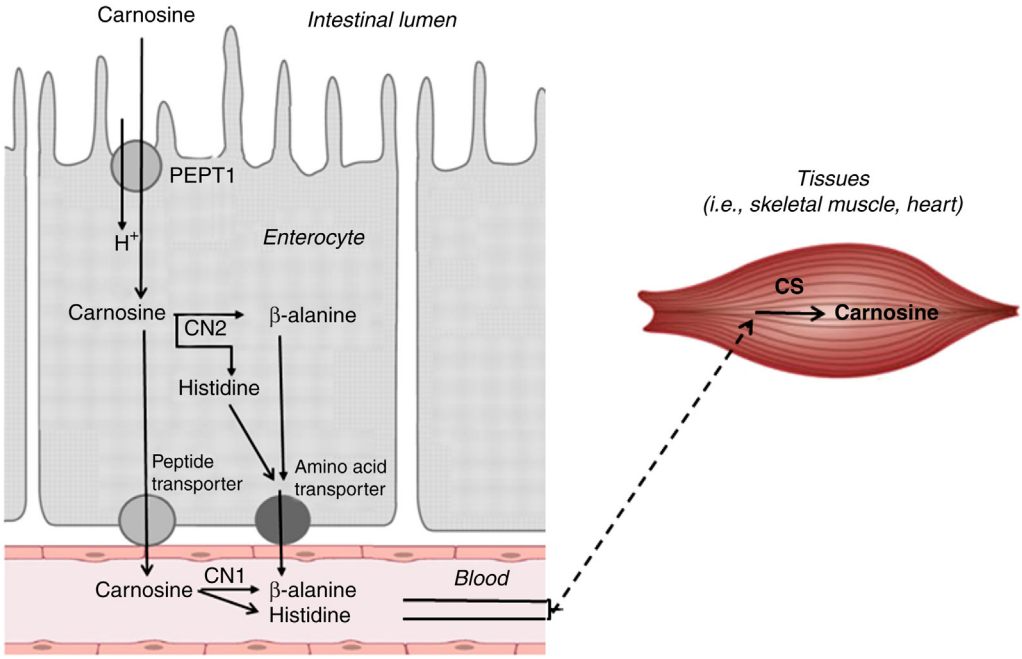

FIGURE 24.3 **Possible metabolic fate of dietary carnosine in humans.** *CN1*, Carnosinase 1; *CN2*, carnosinase 2; *PEPT1*, proton/peptide cotransporter 1; *CS*, carnosine synthase.

The water-soluble carnosine is established as a powerful physiological buffer. The nitrogen atoms of its imidazole ring having a pK_a ($pK_a = 6.72$) value close to the physiological pH, play the main role in the buffering activity. This buffering property is assumed to be the reason why white muscles that undergo intense glycolysis ending up with high lactic acid, have more carnosine. It is also known to activate carbonic anhydrase so to enlarge bicarbonate buffer capacity.[1,3]

Carnosine is demonstrated as a direct acting antioxidant reacting with reactive oxygen (ROS) and nitrogen (RNS) species in various studies.[1,4] It is shown to quench superoxide and hydroxyl radicals and prevent nitration of tyrosine. Hypochlorite anion formed by the reaction of superoxide and chlorine ions, and peroxynitrite by the reaction of superoxide with nitric oxide can powerfully be scavenged by carnosine. It inhibits lipid peroxidation and reacts with the harmful aldehydes like 4-hydroxynonenal (4-HNE), malondialdehyde (MDA), and acetaldehyde.[3,4]

Metal ion chelating activity of carnosine is one of its important properties that contribute to its protective role in oxidative stress. It is known to form complexes with metal ions, such as Cu^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, and Zn^{2+}. Thus, hazardous actions of such ions, which may be in contact with cellular macromolecules, may be avoided.[1]

Various carbonyl compounds, such as formaldehyde, methylglyoxal, acetaldehyde, MDA participate in processes of lipid oxidation, protein glycation, formation of crosslinks, and end up with production of advanced lipoxidation end products (ALEs) and advanced glycoxidation end products (AGEs).[3] Carnosine is shown to inhibit protein glycation and also be capable of reversing the reaction by transglycation. Its action of inhibiting AGE formation is referred to its metal ion chelating activity since oxidations through metals may lead to AGEs and ALEs. It is also proposed that carnosine could act upon preformed AGEs/ALEs.[1,4]

Carnosine is known to affect many age-related dysfunctions.[5] This action is generally assumed as an antiaging property though sometimes it is attributed to its antioxidant and antiglycating activities. It is also considered to take part in gene silencing and protection against telomere shortening.

These functions enable carnosine to be an efficient antioxidant and cell protector. Therefore, it has been employed in many oxidative stress-induced pathologies like atherosclerosis, diabetes, aging, and ischemia-reperfusion.[1,3,5] Indeed, its actions not only as antioxidative but also as antiinflammatory, antiapoptotic, antifibrotic, and antiglycating may prove helpful in the treatment of liver diseases of various origins in rodents. L-Carnosine has a long half-life in rodents, which lack serum carnosinase, and carnosine administration elevate their plasma and tissue carnosine levels.[6] However, L-carnosine has a limited use in humans due to its breakdown to L-histidine and β-alanine by serum and tissue carnosinases. To overcome this unfortunate issue, carnosinase-resistant enantiomer D-carnosine and L-carnosine derivatives like *N*-acetyl carnosine or trolox-L-carnosine are being used instead of L-carnosine.[3,7]

CARNOSINE AGAINST OXIDATIVE LIVER DAMAGE

There are not many studies about the use of carnosine against hepatic oxidative stress generated by various means. Most of them concern animal models. There exists a limited number dealing with humans. Table 24.1 summarizes doses, delivery route, and duration time of carnosine in various experimental liver injury models.

Ischemia-Reperfusion-Induced Liver Injury and Carnosine

Hepatic ischemia-reperfusion injury is one of the mechanisms that induce oxidative stress leading to damage and inflammation. Restorations of blood flow and oxygen supply lead inflammatory factors to the injured area and intensify ROS production. Benefits of antioxidants have been suggested for hepatic ischemia-reperfusion injury.[8] Two studies are present describing the beneficial role of carnosine against liver ischemia-reperfusion injury.

Carnosine ameliorates the histopathological damage induced by ischemia in rats when given beforehand. The decreases in serum transaminase activities and hepatic MDA levels and caspase-3 activity along with elevated hepatic nitrite levels and catalase and glutathione peroxidase (GPx) activities were observed.[9]

Application of carnosine following ischemia generation and before reperfusion improved liver histopathology, with decreased serum transaminase and hepatic myeloperoxidase activities and increased hepatic gluthathione (GSH) content.[10] The ameliorating action of carnosine was more distinctive when used together with melatonin.

Acetaminophen-Induced Liver Injury and Carnosine

Use of acetaminophen (paracetamol; *N*-acetyl-*p*-aminophenol) which has analgesic and antipyretic effects is usually safe at therapeutic doses. *N*-acetyl-*p*-benzoquinonemine is produced through its metabolic activation by cytochrome P450 2E1 (CYP2E1). Upon binding covalently to hepatic GSH and proteins during drug overdose, this reactive metabolite causes

TABLE 24.1 Doses, Delivery Route, and Duration Time of Carnosine in Various Experimental Liver Injury Models

Liver Injury Model	Species	Doses	Delivery Route	Duration Time	References
Ischemia-reperfusion	Rats	250 mg/kg	i.p.	Single dose	[9]
Ischemia-reperfusion	Rats	250 mg/kg	i.p.	Single dose	[10]
Acetaminophen-induced liver injury	Mice	Various 0.5, 1, and 2 g/L	In drinking water	4 weeks	[13]
TAA-induced liver injury	Rats	2 g/L	In drinking water	3 months	[14]
TAA-induced liver injury	Rats	250 mg/kg	i.p.	Single dose	[15]
Carbon tetrachloride–induced liver injury	Rats	250 mg/kg/day	i.p.	6 weeks	[16]
Acute cadmium toxicity	Mice	10 mg/kg/day	i.p.	3 days	[17]
Lead toxicity	Rats	10 mg/kg/day	Oral gavage	8 weeks	[18]
DEN-induced hepatic injury	Rats	2 g/L	In drinking water	6 weeks	[19]
DOX-induced hepatic injury	Rats	250 mg/kg/day	i.p.	12 days	[20]
Formaldehyde toxicity	Rats	100 mg/kg/day	p.o.	4 weeks	[21]
Titanium dioxide exposure	Mice	200 mg/kg/day	p.o.	2 weeks	[22]
Sepsis	Rats	250 mg/kg	i.p.	Single dose	[24]
Stress induced liver injury	Rats	250 mg/kg/day	i.p.	5 days a week for 21 days	[25]
Stress induced liver injury	Mice	150 and 350 mg/kg/day	p.o.	7 days	[26]
Age-induced liver injury	Rats	250 mg/kg/day	i.p.	1 month	[27]
Age-induced liver injury	Rats	250 mg/kg/day	i.p.	5 days per week for two months	[28]
D-Galactose induced aging model	Rats	250 mg/kg/day	i.p.	5 days per week for two months	[29]
Alcoholic liver injury	Rats	2 g/L	In drinking water	4 weeks	[30]
Alcoholic liver injury	Rats	100 mg/kg	Gavage	Twice a day	[31]
Alcoholic liver injury	Mice	0.5, 1, and 2 g/L	In drinking water	3 week	[6]
ASH	Rats	250 mg/kg	i.p.	2 doses	[32]
NAFLD	Rats	1 g/L	In drinking water	8 week	[33]
NAFLD	Rats	2 g/L	In drinking water	8 week	[34]
NAFLD	Mice	1 g/L	In drinking water	4 week	[35]

Abbreviations: ASH, Alcoholic steatohepatitis; DEN, *N*-diethylnitrosamine; DOX, doxorubicin; NAFLD, nonalcoholic fatty liver disease; TAA, thioacetamide.

GSH depletion Thus stimulated oxidative stress leads to hepatic mitochondrial dysfunction along with increased ROS and peroxynitrite productions and release of proinflammatory cytokines. This chain of reactions ends up as liver failure.[11,12]

There is one study in literature about the effect of carnosine on acetaminophen–induced liver injury.[13] Application of various doses of carnosine or histidine for 4 weeks, before acetaminophen treatment, increased reduced GSH and α-tocopherol, reserving hepatic GPx, catalase and superoxide dismutase (SOD) activities in mice. Ascorbic acid levels were elevated by high dose of carnosine. Carnosine appears to spare hepatic GSH, α-tocopherol, and ascorbic acid. Its antioxidant action is evident by reducing hepatic interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) levels, lipid peroxidation, and CYP2E1 activity. It is described to be effective at transcriptional level by suppressing the mRNA expressions of TNF-α and monocyte chemoattractant protein and enhancing GPx mRNA expression. Histidine is also found to exhibit protective actions similar to carnosine.

Thioacetamide-Induced Liver Injury and Carnosine

Propagation of liver injury is actualized by the use of the fungicide thioacetamide (TAA) in various experimental models. Its biotransformation is mediated mainly by CYP2E1. TAA is converted to thioacetamide sulfoxide, which undergoes fur-

ther conversion to thioacetamide sulfdioxide. During its bioactivation, ROS like superoxide anion and hydrogen peroxide are also produced. Covalent binding of TAA metabolites and ROS to hepatocellular molecules generate oxidative stress, which trigger the steps that proceed to liver injury. While a single dose of TAA administration has been shown to cause acute liver injury characterized by centrilobular necrosis, chronic applications result in hepatic cirrhosis.[14]

There are not many studies about the use of carnosine in prevention or attenuation of oxidative stress in TAA-induced liver injury. The study by Mehmetçik and coworkers is the only study implicating the effective antioxidant role of carnosine in acute TAA-induced liver necrosis.[15] Carnosine efficiently prevented TAA-induced necrosis and decreased oxidative stress parameters like hepatic MDA and diene conjugate (DC) levels in rats, when coadministered with TAA. However, it was ineffective on improving the TAA-induced depression in the antioxidant system except for the increase in vitamin E levels.

Chronic application of TAA resulted in elevated hydroxyproline and lipid peroxide levels as indications of hepatic fibrosis along with depressed antioxidant system in rats.[14] Carnosine when given together with TAA for 3 months was not effective on the regression of fibrosis. It was found to decrease the hepatic lipid peroxidation without any change in hepatic GSH levels, SOD, and GPx activities and their protein expressions.

Carbon Tetrachloride-Induced Liver Injury and Carnosine

Carbon tetrachloride (CCl_4) is one of the most powerful hepatotoxins. Acute and chronic experimental liver injury induced by CCl_4 administration is widely investigated in rodents. Conversion of CCl_4 to trichloromethyl radical by microsomal CYP2E1 leads to liver damage. Carnosine was found to ameliorate histopathological changes caused by hepatic fibrosis due to multiple CCl_4 injections. Its antioxidant and antiinflammatory actions were exhibited by reductions of hepatic MDA, nitric oxide and TNF-α levels, and elevations in GSH and nuclear factor E2-related factor 2 (Nrf-2) levels and SOD activity. Carnosine also prevented hepatic stellate cell activation shown by reduced α-smooth muscle actin.[16]

Cadmium and Lead-Induced Liver Injury and Carnosine

Cadmium being a very toxic heavy metal has an extensive industrial use. Electroplating, production of industrial paints and nickel-cadmium batteries are among its various sources. There may be extreme danger for human health upon exposure to cadmium-contaminated food, water, or air, especially near industrial areas.

Acute cadmium exposure is an important experimental heavy metal toxicity model. Cadmium plays an eminent role in the activation of inflammatory cells that inevitably leads to the generation of ROS and subsequently to lipid peroxidation. It has also a depleting effect on cellular antioxidants. Acutely exposed cadmium accumulates in the liver and cause hepatotoxicity.[12]

The only study evaluating the hepatoprotective effect of carnosine in acute cadmium toxicity belongs to Fouad and coworkers.[17] Carnosine, which was given to mice during cadmium exposure, depressed hepatic lipid peroxidation and restored hepatic antioxidant system by increasing GSH levels, and SOD and catalase activities. The recovery of liver injury was detected by light and electron microscopy. Carnosine lowered hepatic cadmium ion concentration both by acting as a metal chelator itself and by increasing levels of GSH which is also an important cadmium chelator. The antiinflammatory and antiapoptotic actions of carnosine were exposed through attenuation of myeloperoxidase and caspase-3 activities. Carnosine promises to be an explicit remedy in ameliorating the hepatotoxicity of acute cadmium exposure.

Lead being also a very toxic metal, targets mainly the liver to exhibit its toxic effects. Lead can be encountered in water carrying pipes, lead-acid car batteries, paints, television and computer screen glasses, cables, solders, and pesticides. Lead in gasoline produces lead salts when it is burned in car engines. These salts pollute the atmosphere, soil, and water through car exhausts. Thus, lead is introduced to human body by means of various contaminated food, water, and cigarette smoke.

Exposure to lead is assumed to produce ROS and depress antioxidant activity in the liver. Hepatic lead-induced lipid peroxidation was reported to be inhibited, along with the recovery of the antioxidant system by carnosine treatment in rats.[18]

Liver Injury Induced by Several Toxic Agents and Carnosine

N-Diethylnitrosamine (DEN) is known as both a hepatotoxin and hepatocarcinogen. It is a substrate of CYP2E1 and plays an active role in ROS generation. Treatment with carnosine prior to application of DEN reduced hepatic oxidative stress in rats.[19]

Doxorubicin (DOX) which is an effective anticancer antibiotic is proposed to generate oxidative stress. Carnosine was found to restore the prooxidant state and ameliorated the histopathologic changes in liver, kidney, and heart tissues following administration of DOX in rats.[20]

Although formaldehyde occurs naturally in mammals contributing to purine synthesis, its intake is known to be toxic. Ingesting formaldehyde can produce severe injury to upper gastrointestinal tract. It is known to generate oxidative stress and inflammation in animals. Altered sensitivity of the immune system by formaldehyde also influences the present oxidative stress. When increasing doses of formaldehyde were given to rats, total oxidant levels, and apoptosis are increased and antioxidant capacity is found decreased in serum, liver, and lung. Carnosine supplementation reduces oxidative stress and apoptosis.[21]

Titanium dioxide nanoparticles are used in the manufacture of dental implants, spinal fixation devices, and cardiovascular stents. These devices may produce unwanted nanoparticle debris in the systems they are implanted due to changed conditions. Nanoparticles entering nuclei affect DNA and generate ROS leading to DNA damage and apoptosis. Besides the debris in liver, heart, and many other organs is assumed to cause inflammation. Titanium dioxide nanoparticles application to mice increased serum liver function enzymes activities, hepatic TNF-α, IL-6, and MDA levels and reduced GSH content. This treatment was reported to increase hepatic apoptosis and DNA damage. Carnosine was found to alleviate the produced toxic effects.[22]

Sepsis-Induced Liver Injury and Carnosine Response

An infection triggering an exaggerated immune response may lead to the damage of body organs generating the condition called sepsis. Coagulation together with the suppression of fibrinolysis and production of cytokines and chemokines which amplifies the inflammatory response result in tissue hypoxia and ischemia proceeding to the ultimate end as organ failure and death.[23]

Hepatic dysfunction is the manifestation of sepsis in the liver. Kupffer cells remove endotoxins and produce the mediators, such as TNF-α, interleukin-1 (IL-1), superoxide radical, and nitric oxide. The prooxidant chaos created in the liver may lead to apoptosis and necrosis. In the only study investigating the effect of carnosine on sepsis, septic shock in rats was induced by laparotomy and cecal ligation.[24] Carnosine was applied when the first signs of septic shock emerged, which were determined using blood pressure measurements. It is found to return serum transaminase activities back to normal, and also lower both liver and serum MDA levels. The antioxidant action of carnosine along with its efficient improvement of the liver histopathology is overtly described by the study.

Stress-Induced Liver Injury and Carnosine

Stress is a condition encountered in everyday life, affecting the adaptation of an organism to the environment and impairing the quality of life. Exposure to stress conditions may lead to an organ's dysfunction by causing the secretion of neuroendocrine hormones and inhibiting the immune system. Stress activates the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the enhanced release of glucocorticoids (GCs). The liver, having a high metabolic capacity becomes the primary target of elevated GCs in stress conditions. Prolonged exposure to high concentrations of GCs causes increased ROS production and depressed antioxidant system in tissues including liver.[25]

Carnosine is reported to ameliorate the detrimental effects that emerge in several stress conditions. It relieves the hyperactivated HPA axis and decreases GCs levels. There are few studies about the hepatic stress and carnosine in literature. In the study of Tsoi and coworkers, the disturbed hepatic glucose metabolism was improved by carnosine in restraint-stressed mice, while GC levels decreased.[26] In the other study,[25] carnosine efficaciously amended the disrupted prooxidant–antioxidant balance in the brains and livers of rats exposed to chronic (cold and immobilization) stress. Although its effects were more significant in the brain, it was capable of depressing hepatic oxidative stress. Carnosine treatment elevated hepatic ascorbic acid levels but it was not influential on the other elements of the antioxidant system.

Age-Induced Liver Injury and Carnosine

Liver is affected by aging as its metabolic and detoxifying functions reduce and it becomes open to harmful attacks. Mitochondrial dysfunction, ROS production, and oxidative stress are well known to contribute to liver aging. The triggered apoptosis, which is explicit during aging, may attribute to the enhancement of the process thus leading to diseases related to increased age.

When carnosine was supplied to aged rats for 1 month, the hepatic oxidative stress was suppressed as indicated by reduced MDA, DC and protein carbonyl (PC) levels. Hepatic GSH and vitamin E content were elevated but the antioxidant

enzyme activities remained unchanged. Carnosine was observed to be selectively effective on liver but not on heart and brain.[27]

The efficiency of carnosine increases when used in combination with lipophilic antioxidants like vitamin E or melatonin.[10,28] Indeed supplementation of carnosine in combination with vitamin E was influential on depressing the oxidative stress in the liver as well as the heart and the brain of aged rats.[28]

D-galactose overload is an experimental model that mimics the normal aging process in experimental animals. While accumulated galactitol leads to osmotic and oxidative stresses, the reaction of galactose with amine groups of proteins produces AGEs. In galactose-treated rats, hepatic oxidative stress was induced as indicated by increased MDA and PC levels and decreased GSH content and antioxidant enzyme activities.[29] Elevated hepatic Bax expression was the indicator of induced apoptosis, while decreased Ki-67 expression showed reduced proliferation. Carnosine supplementation that was simultaneously applied with galactose, decreased serum transaminase activities, and improved the disrupted liver histopathology. As an effective antioxidant, it was able to depress the oxidative stress and increase the GSH content and SOD and GPx activities without changing the mRNA expressions. Carnosine-induced decrease in Bax expression and increase in Ki-67 expression exhibited the antiapoptotic and proliferative actions of the compound, respectively.

Alcoholic Liver Injury and Carnosine

Ethanol metabolism, which takes place mainly in the liver, is based on oxidations. The three hepatic enzyme systems that catalyze the oxidation of ethanol to acetaldehyde are cytosolic alcohol dehydrogenase, microsomal ethanol oxidizing system of the smooth endoplasmic reticulum, and catalase in peroxisomes. Acetaldehyde, in return, is oxidized to acetate by aldehyde dehydrogenase. These oxidation reactions which enhance NADH production and thus change the ratio of NAD^+/NADH, cause the inhibition of gluconeogenesis and fatty acid oxidation leading to the generation of fatty liver. Aldehyde oxidase and xanthine oxidase can also oxidize acetaldehyde to acetate. CYP2E1, the main enzyme of the microsomal ethanol oxidizing system, is especially upregulated in chronic ethanol drinking. ROS are produced by CYP2E1, and the free radical generation accompanies the oxidation of NADPH to $NADP^+$ in chronic alcohol consumption. In addition, activated hepatic macrophages generate proinflammatory cytokines, such as TNF-α and IL-6. These factors induce inflammation and fibrosis through the increased production of ROS. On the other hand, acetaldehyde may form antigenic adducts when it is bound to cellular proteins, to promote inflammation (Fig. 24.4).[8]

Many researchers investigate the hepatic prooxidant–antioxidant balance during ethanol metabolism and several substances are used to recover the balance, which is disturbed in favor of prooxidation, and the consequent liver injury. Few studies are present that investigate the role of carnosine in the oxidative stress caused by ethanol.

In the study of Artun and coworkers carnosine pretreatment was followed by binge ethanol administration (5 g/kg as three doses in every 12 h) to rats.[30] Carnosine was shown to decrease the elevated plasma transaminase activities and hepatic lipid peroxidation. It was unable to recover the depressed antioxidant system and hepatic steatosis. However, hepatic

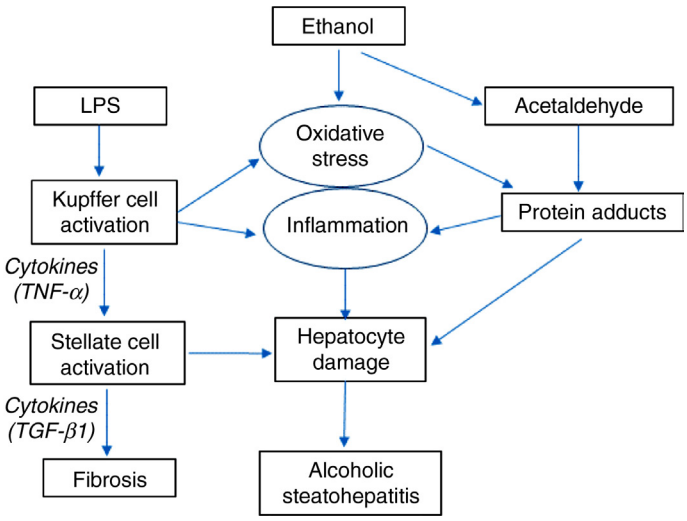

FIGURE 24.4 **Schematic presentation of ethanol plus lipopolysaccharide (LPS)-induced liver injury.**

GSH levels were increased due to carnosine treatment. In another study of binge ethanol administration,[31] carnosine was applied 1 h before every ethanol treatment (5 g/kg; oral in three doses) in rats. Carnosine also lowered plasma transaminase activities, hepatic lipid peroxidation, and myeloperoxidase activity and increased GSH content. Binge ethanol administration-induced apoptosis was suppressed as demonstrated by the declines in Bax and caspase-3 expressions and the increase in antiapoptotic Bcl-2 expression using immunohistochemical evaluations. Carnosine treatment also ameliorated the alcohol-induced morphological changes in hepatocytes as observed using electron microscopy.

There is one study regarding the role of carnosine in chronic ethanol treatment in mice.[6] The chronic ethanol application of 4 weeks was followed by carnosine usage in three different doses (0.5, 1, 2 g/L in drinking water for 3 weeks). Carnosine posttreatment proved to abate alcoholic hepatotoxicity in all doses. Similar to the findings of the two above-mentioned studies, plasma transaminase levels and hepatic lipid peroxidation were lower and hepatic GSH levels were increased with carnosine. The antioxidant and antiinflammatory actions of carnosine were clearly manifested in this study. Elevated hepatic activities of GPx and catalase and mRNA expression of catalase and decreased CYP2E1 activity are reflections of its protective properties. The depressions of mRNA expressions and hepatic levels of IL-6 and TNF-α and the decreased C-reactive protein levels by carnosine prohibited the generation of inflammation due to chronic ethanol metabolism. In this study, histidine exhibited similar antioxidative and antiinflammatory effects.

Alcoholic fatty liver disease (ALD) exhibits a wide range of liver injury from simple steatosis to alcoholic steatohepatitis (ASH), advanced fibrosis, and cirrhosis. ASH being the second stage of the disease is the rat-limiting step and is characterized by steatosis accompanied by neutrophil infiltration and hepatic necrosis. Indeed in experimental ASH model induced by applying ethanol and lipopolysaccharide to rats, carnosine treatment reduced hepatic ROS, and MDA levels and myeloperoxidase activity together with transforming growth factor β1 and collagen 1α1 expressions. Amelioration of hepatic injury due to ASH was observed with histopathological and biochemical findings.[32]

Nonalcoholic Fatty Liver Disease and Carnosine

Nonalcoholic fatty liver disease (NAFLD) shows a spectrum similar to ALD. Liver injury from simple steatosis to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and cirrhosis are also observed. Insulin resistance, obesity, dyslipidemia, hypertension, and diabetes may all lead to the development of NAFLD by stimulation of hepatocyte fat accumulation. The subsequent generation of oxidative stress followed by lipid peroxidation and inflammation are also accepted to play the leading roles in the manifestation of hepatic injury related to NAFLD.[8] There are few reports on the effects of carnosine in NAFLD.

High fat diet (HFD) is known to induce obesity, insulin resistance, dyslipidemia, and hepatic steatosis, together with the production of ROS and lipid peroxidation. In the study of Mong and coworkers, carnosine was given to rats fed on HFD (containing 60% of calories as fat) for 8 weeks.[33] Carnosine effectively reduced the body weights, improved insulin resistance, and dyslipidemia. Acting as an antilipogenic agent, it diminished the activities and mRNA expressions of some lipogenic enzymes, such as malic enzyme, fatty acid synthase, and 3-hydroxy-3-methylglutaryl coenzyme A reductase in the liver and adipose tissue. Sterol regulatory element binding proteins (SREBRs) are transcription factors responsible for fatty acid and cholesterol synthesis. SREBR-1c modulates the mRNA expressions of enzymes that catalyze fatty acid synthesis, and SREBR-2 modulates the mRNA expressions of those that catalyze cholesterol synthesis. Carnosine was capable of suppressing expressions of both transcription factors in liver and adipose tissue, thus attenuating hepatic steatosis through an antisteatotic action.

High fructose containing diet (HFr) is an experimental model of generating insulin resistance. Disrupted glucose tolerance, hypertriglyceridemia, and fatty liver are other pathologies observed beyond insulin resistance. Oxidative stress is assumed to play an important role in this model, thus usage of several antioxidants became an issue in various researches. In the study of Giriş and coworkers,[34] rats fed with HFr containing 60% fructose, were given carnosine alone or with α-tocopherol for 8 weeks. Insulin resistance and hypertriglyceridemia were not affected by carnosine alone, although hepatic steatosis and lipid peroxidation were diminished. Carnosine in combination with α-tocopherol, on the other hand, was found effective in decreasing inflammation, insulin resistance, hepatic steatosis and lipid peroxidation, and elevating GPx activity and expression. The results of this recent study also support the increased efficiency of carnosine when used in combination with α-tocopherol.

A poignant relationship between diabetes and NAFLD appears to exist. Although it is obscure whether diabetes foretells the development of NAFLD or vice versa; both play an eminent role for the progression of one another. The effectiveness of carnosine on liver has also been reported in streptozotocin-induced diabetic mice.[35] Carnosine treatment following the induction of diabetes, improved the diabetic complications by enhancing hepatic antioxidant activity, and restoring insulin secretion. It suppressed lipid peroxidation and increased catalase and GPx activities. Reduction in the levels of the proinflammatory cytokines of IL-6 and TNF-α also supported the antiinflammatory action of carnosine.

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