Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation

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Abstract

In vivo effects of histidine and carnosine against diabetic deterioration in diabetic Balb/cA mice were studied. Histidine and carnosine at 0.5, 1 g/l were added into drinking water. After 4 weeks intake of these agents, the content of histidine and carnosine in plasma, heart and liver significantly elevated ($P<0.05$). The intake of these agents significantly decreased plasma glucose and fibronectin levels ($P<0.05$); however, only 1 g/l histidine and carnosine treatments significantly increased insulin level ($P<0.05$) in diabetic mice. Triglyceride level in heart and liver was dose-dependently reduced by histidine or carnosine treatments ($P<0.05$); however, only 1 g/l histidine and carnosine treatments significantly reduced cholesterol level in heart and liver ($P<0.05$). The administration of histidine or carnosine significantly enhanced catalase activity and decreased lipid oxidation levels in kidney and liver ($P<0.05$); however, only 1 g/l histidine and carnosine treatments significantly increased glutathione peroxidase activity ($P<0.05$). The increased interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha in diabetic mice were significantly suppressed by the intake of histidine or carnosine ($P<0.05$). In human low density lipoprotein, histidine or carnosine showed dose-dependently suppressive effect in glucose-induced oxidation and glycation ($P<0.05$). These data suggest that histidine and carnosine are potential multiple-protective agents for diabetic complications prevention or therapy.
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1. Introduction

Diabetic complications such as neuropathy, retinopathy, nephropathy, ageing and atherosclerosis contribute to the severity and mortality of diabetic patients. The clinical characteristics of these complications include hyperglycemia, hyperlipidemia, oxidative stress, and cytokine imbalance (Amaral et al., 2002; Strippoli et al., 2003; Goldberg, 2003; Duby et al., 2004). It has been documented that oxidative stress, hyperglycemia, inflammation and hyperlipidemia are closely interrelated in diabetic condition (Hunt and Wolff, 1991; Brownlee, 2001; Amaral et al., 2002), and involved in the development of diabetic complications. Furthermore, it is well known that the increased release of

Th1 type cytokines such as tumor necrosis factor (TNF)-alpha and Th2 type cytokines such as interleukin (IL)-6 are involved in the diabetic deterioration (Geerlings et al., 2000; Jialal et al., 2002; Hwang et al., 2003). Thus, it is very important to control these risk factors in order to improve diabetic complications.

Histidine and carnosine (beta-alanyl-L-histidne) are endogenously synthesized peptides present in brain, skeletal muscle and liver (Boldyrev and Severin, 1990). Several studies have reported that these compounds possessed marked antioxidant activities such as scavenging free radicals, binding divalent metal ions and anti-glycating action (Babizhayev et al., 1994; Lee et al., 1999; Ukeda et al., 2002). It has been reported that histidine and carnosine concentrations in rat tissues could be increased by dietary supplementation (Tamaki et al., 1985; Chan et al., 1994; Maynard et al., 2001), and then contributed to antioxidant protection in these tissues (Chan et al., 1994). However, it

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remains unclear that the tissue content of these peptides could be increased by dietary supplement in diabetic condition. The studies of Nagai et al. (2003) and Hwang et al. (2003) further indicated that carnosine and related compounds could affect glycemic control in diabetic animals. Also, it is unknown that the increased histidine and carnosine in animal tissues could improve diabetic complications such as oxidative stress, hyperlipidemia and cytokine imbalance. On the other hand, the protective effect of histidine and carnosine in human low density lipoprotein (LDL) against copper induced oxidative damage has been observed (Decker et al., 2001); however, it is unsure that these agents could protect LDL against glucose-induced oxidative and glycative damage.

The major purpose of this study was to evaluate the in vivo protection from histidine and carnosine in diabetic mice. The effects of these agents on the plasma level of glucose, insulin, fibronectin, TNF-alpha, IL-6, and tissue content of triglyceride and cholesterol were determined. Furthermore, the effects of these compounds in human LDL against glucose-induced oxidation and glycation were examined.

2. Materials and methods

2.1. Animal study

2.1.1. Animals and diabetic induction

Three- to four-week-old male Balb/cA mice were obtained from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Mice were housed on a 12-h light–12-h dark schedule, and fed with water ad libitum, and rat and mouse standard diet containing by weight (g/100 g): 64 starch, 23 protein, 3.5 fat, 5 fiber, 1 vitamin mixture and 3 salt mixture (PMI Nutrition International LLC, Brentwood, MO). Use of the mice was reviewed and approved by the Chungshan Medical University animal care committee. To induce diabetes, the mice were treated with streptozotocin (40 mg/kg bodyweight in 0.1 M citrate buffer, pH 4.5) i.p. for 5 consecutive days. Blood glucose level was monitored on days 2, 5 and 10 from the tail vein by using a one-touch blood glucose meter (Lifescan). Ten days after streptozotocin treatment, mice with fasting blood glucose levels over 13.8 mmol/l were defined as diabetic mice and used for this study.

2.1.2. Histidine and carnosine treatments

Histidine (99.5%) and carnosine (beta-alanyl-L-histidine, 99%) were purchased from Sigma Chemical (St. Louis, MO, USA). Each agent at 0.5 and 1 g/l was directly added into the drinking water. At the tenth day after streptozotocin treatment, diabetics has been induced; and then these diabetes mice were divided into five groups (15 mice in each group) for the intake of water, low histidine (0.5 g/l), high histidine (1 g/l), low carnosine (0.5 g/l) or high carnosine (1 g/l). All diabetic mice fed with regular diet and had free access at all times to diet and water. Nondiabetic mice treated with water were used for comparison. Daily-consumed water volume was recorded. Bodyweight was measured every week. Plasma glucose level was measured every week. After 4 weeks, mice were killed with carbon dioxide. Heart, liver and kidney from each mouse were collected and weighted. Blood was also collected, and plasma was separated from erythrocyte immediately. Each organ at 0.2 g was homogenized on ice in 2 ml phosphate buffer (pH 7.2) and the filtrate was collected. The protein concentration of plasma, heart filtrate, kidney filtrate, and liver filtrate was determined by the method of Lowry et al. (1951) using bovine serine albumin as a standard. In all experiments, sample was diluted to a final concentration of 1 g protein/l.

2.1.3. Histidine and carnosine analysis

The content of histidine and carnosine in plasma, heart and liver from mice was determined according to the method of Chan et al. (1994). Briefly, plasma at 0.1 ml or organ at 0.2 g was homogenized in 1 ml, 0.36 M perchloric acid and followed by centrifugation at $2000 \times g$ for 10 min. The supernatant was filtrated through a 0.45 μM filter membrane, and then 50 μl filtrate was derivatized with 200 μl fluoaldehyde *o*-phthalaldehyde reagent (Pierce, Rockford, IL, USA). Histidine and carnosine concentrations in the derivatized extract were determined by high performance liquid chromatography (HPLC) equipped with a 5 μm Hypersil ODS high-resolution column.

2.1.4. Plasma glucose, insulin and fibronectin determination

Plasma glucose level (mmol/l) was determined by glucose HK kit (Sigma Chemical, St. Louis, MO, USA). Fibronectin level (g/L) was determined by rabbit anti-rat fibronectin polyclonal antibody (Gibco-BRL, Grand Island, New York) and quantified by solid phase enzyme-linked immunosobent assay (ELISA) (Kanters et al., 2001). Insulin level (nmol/l) was measured in whole plasma by radio-immunoassay using the rat insulin kit (SRI-13K; Linco Research, St. Charles, Missouri, USA).

2.1.5. Triglyceride and cholesterol determination

Triglyceride and total cholesterol levels in heart and liver were determined by triglycerides/GB kit and cholesterol/HP kit (Boehringer Mannheim, Indianapolis, IN, USA), respectively. Total lipids were extracted from heart and liver, and then triglyceride concentration (mg/g wet heart or liver) was quantified by a colorimetric assay (Biggs et al., 1975). Total cholesterol (mg/g wet tissue) was measured using *o*-phthalaldehyde (Rudel and Morris, 1973).

2.1.6. Catalase and glutathione peroxidase assay

Catalase and glutathione peroxidase activities (U/mg protein) in kidney and liver were determined by catalase and

glutathione peroxidase assay kits (Calbiochem, EMD Biosciences San Diego, CA, USA).

2.1.7. Lipid oxidation determination

Lipid oxidation was determined by measuring the level of malondialdehyde (μmol/l) via an HPLC method (Jain and Palmer, 1997) in liver and kidney.

2.1.8. IL-6 and TNF-alpha measurement

Plasma levels of IL-6 and TNF-alpha were measured by ELISA using Cytoscreen Immunoassay Kits (BioSource International Camarillo, Camarillo, CA, USA). Samples were run in duplicates according to manufacturers instructions. The sensitivity of assay with the lower limit was at 5 nmol/l for IL-6, and 0.5 nmol/l for TNF-alpha.

2.2. Human LDL study

2.2.1. Subjects and blood sample collection

Informed consents for study participation were obtained from 15 male subjects with age range at 21–28 years old in Chungshan Medical University (Taichung City, Taiwan). These subjects were not affected by diabetic related inflammatory or macrovascular diseases. Peripheral blood sample, 15 ml, from each subject was drawn after an overnight fasting. LDL fractions with density 1.006–1.063 were isolated from plasma by sequential ultracentrifugation (Esterbauer et al., 1989). The isolated LDL was dialyzed against 1.5 mM phosphate buffer and sterilized with a 0.22 μM filter. The protein concentration of LDL was determined by the assay of Lowry et al. (1951) using bovine serum albumin as a standard. LDL fraction was diluted to a final concentration of 500 μg protein/ml using phosphate buffer.

2.2.2. LDL oxidation

The method of Jain and Palmer (1997) was used to measure malondialdehyde formation in LDL. LDL samples were treated with 1 or 2 μM histidine or carnosine agents, and further oxidation was initiated with 100 mM glucose at

37 °C, and oxidation level was determined after a 3-day incubation.

2.2.3. LDL glycation

The method of Duell et al. (1990) was used to measure the degree of LDL glycation. Briefly, 500 μl LDL solution was loaded on a Glycogel II column, and glycated LDL was eluted with 2 ml sorbitol buffer, pH 10.25. Two hundred microliters elute (glycated LDL solution) was mixed with 200 μl 4% $NaHCO_3$ and 200 μl 0.1% trinitrobenzoic acid. This mixture was flushed with N_2, sealed and incubated at 37 °C in the dark. After 2 h, the absorbance at 340 nm was measured spectrophotometrically. The blank was a mixture of LDL solution and $NaHCO_3$ in phosphate buffer. Further LDL glycation was performed according to the method described in Li et al. (1996). Glucose at 100 mM was directly added into LDL solution with 1 or 2 μM histidine or carnosine agents. This solution was sterile filtered, covered with N_2 a nd stored for 6 days at 37 °C in the dark. After a 6-day incubation, the glycation level was determined again. During glycation, samples were treated with EDTA (0.5 mM) to prevent the interference from LDL oxidation.

2.2.4. Statistical analyses

The effect of each treatment was analyzed from 15 mice or human blood samples ($n=15$). Data were subjected to analysis of variance (ANOVA) and computed using the SAS General Linear Model procedure (SAS, 1990). Differences with $P<0.05$ were considered to be significant.

3. Results

The content of histidine and carnosine in plasma, heart and liver from nondiabetic and diabetic mice after 4 weeks intake of these agents is presented in Table 1. The intake of these two compounds significantly increased the concentration of these agents in plasma and organs of diabetic mice ($P<0.05$). Glucose, insulin and fibronectin levels in plasma from mice are presented in Table 2. All diabetic mice have

Table 1

Histidine and carnosine concentrations in plasma (μg/ml) and in heart and liver (mg/100 g tissue) from nondiabetic mice (control) and diabetic mice treated with water (diabetes), histidine or carnosine[1]

	Histidine			Carnosine		
	Plasma	Heart	Liver	Plasma	Heart	Liver
Control	9.84±1.01[a]	20.12±2.06[a]	16.28±1.53[a]	n.d.[2]	11.16±1.05	2.09±0.43
Diabetes	8.05±0.86[b]	18.27±2.37[b]	14.46±2.01[b]	n.d.	10.81±0.89	1.97±0.57
Histidine, 0.5 g/l	11.35±0.75[a,b]	21.21±1.96[a,b]	17.49±2.11[a,b]	n.d.	12.27±1.14[a,b]	3.11±0.46[a,b]
Histidine, 1 g/l	12.39±1.06[a,b]	22.88±2.15[a,b]	20.32±2.31[a,b]	0.67±0.21[a,b]	13.15±1.21[a,b]	3.47±0.38[a,b]
Carnosine, 0.5 g/l	11.07±1.17[a,b]	23.20±2.47[a,b]	18.53±1.36[a,b]	n.d.	15.26±1.63[a,b]	4.85±0.62[a,b]
Carnosine, 1 g/l	12.21±0.88[a,b]	22.47±2.18[a,b]	20.32±1.84[a,b]	0.85±0.13[a,b]	16.17±1.32[a,b]	5.23±0.45[a,b]

[a] $P<0.05$ versus diabetes group.

[b] $P<0.05$ versus control group.

[1] Values are means±SD, $n=15$.

[2] n.d., not detectable.

Table 2
Glucose, insulin and fibronectin levels in plasma from nondiabetic mice (control) and diabetic mice treated with water (diabetes), histidine or carnosine[1]

	Glucose[2] (mmol/l)	Insulin (nmol/l)	Fibronectin (g/l)
Control	6.3±1.3[a]	11.4±1.5[a]	2.44±0.43[a]
Diabetes	20.6±2.8[b]	6.8±1.4[b]	7.12±0.55[b]
Histidine, 0.5 g/l	16.4±1.1[a,b]	7.0±1.8[b]	5.40±0.41[a,b]
Histidine, 1 g/l	14.3±1.3[a,b]	8.9±1.2[a,b]	4.02±0.35[a,b]
Carnosine, 0.5 g/l	16.2±1.2[a,b]	7.5±1.5[b]	5.16±0.21[a,b]
Carnosine, 1 g/l	13.8±1.0[a,b]	9.7±1.0[a,b]	3.89±0.34[a,b]

[a] $P<0.05$ versus diabetes group.
[b] $P<0.05$ versus control group.
[1] Values are means±SD, $n=15$.
[2] Plasma glucose levels in all diabetic mice were 13.4±2.3 mmol/l at the beginning of experiment.

significantly higher levels of glucose and fibronectin, and lower level of insulin than nondiabetic mice ($P<0.05$). The treatments from histidine and carnosine dose-dependently decreased glucose and fibronectin levels ($P<0.05$); however, histidine and carnosine administration only at 1 g/l significantly increased insulin level ($P<0.05$). As shown in Table 3, the treatments from histidine and carnosine dose-dependently decreased triglyceride level in heart and liver ($P<0.05$); however, these two agents only at 1 g/l significantly reduced cholesterol level in heart and liver ($P<0.05$).

In all diabetic mice, histidine and carnosine significantly increased catalase activity ($P<0.05$), and showed dose-dependent reducing effect in malondialdehyde level ($P<0.05$, Table 4). Glutathione peroxidase activity in kidney and liver was elevated only in 1 g/l histidine and carnosine treatments ($P<0.05$, Table 4). As shown in Table 5, the levels of both IL-6 and TNF-alpha were significantly increased in diabetic mice ($P<0.05$). The intake of these agents showed dose-dependent suppressive effect in the release of IL-6, but only 1 g/l histidine and carnosine treatments significantly reduced TNF-alpha level ($P<0.05$).

As shown in Table 6, 100 mM glucose significantly increased both oxidation and glycation levels ($P<0.05$) in human LDL; and the presence of histidine or carnosine showed dose-dependent effect in suppressing glucose-induced malondialdehyde formation and glycation ($P<0.05$).

4. Discussion

In our present study, carnosine or histidine was added into drinking water of diabetic mice, which resulted in the increased content of these peptides in plasma and organs. Although histidine and carnosine at low concentration could improve diabetic complications such as better glycemic control, lower triglyceride and proinflammatory cytokine levels, these agents only at high concentration could increase insulin secretion, reduce cholesterol accumulation and enhance glutathione peroxidase activity. These results indicated that these agents could alleviate diabetic deterio-

ration via both antioxidant activity and insulin restoration capability, and also suggested that the elevated insulin secretion consequently affected cholesterol metabolism and glutathione peroxidase activity. It is reported that orally administrated carnosine can be hydrolyzed to beta-alanine and histidine in the small intestine (Boldyrev and Severin, 1990); and carnosine can be synthesized in tissues from β-alanine and histidine by carnosine synthase (Tamaki et al., 1985). In our present work, we found histidine supplementation alone effectively increased carnosine content in organs, and supplementing carnosine alone also increased histidine content in organs. It seems that these two compounds maintain a balance in blood and tissues.

As reported by others (Hunt and Wolff, 1991; Goldberg, 2003; Limaye et al., 2003), the enhanced oxidative stress in diabetic condition may result from glucose toxicity and the activity loss of antioxidant enzymes. Thus, the use of proper antioxidative agents such as histidine or carnosine might spare catalase and glutathione peroxidase activities, which could consequently provide greater antioxidant protection for diabetic individuals against oxidation progression. Several studies have indicated that the antioxidative effect from histidine and carnosine is based on free radicals scavenge and divalent metal ions chelation (Babizhayev et al., 1994; Lee et al., 1999). However, the results of our present study further suggest that these agents might possess enzymatic antioxidant activity or sparing effect for antioxidant enzymes. Fibronectin is one of extracellular matrix proteins. It is known that both reactive oxygen species and protein kinase C activity increased under high glucose condition are responsible for the enhanced expression of this protein (Ha and Lee, 2000; Lee et al., 2003). Our current work found that histidine or carnosine supplementation could suppress fibronectin biosynthesis in diabetic condition. This anti-fibronectin effect from these agents might be partially due to their antioxidant activity, which reduced available reactive oxygen species for fibronectin biosynthesis stimulation. The influ-

Table 3
Triglyceride and cholesterol concentrations in heart and liver (mg/g wet tissue) from nondiabetic mice (control) and diabetic mice treated with water (diabetes), histidine or carnosine[1]

	Triglyceride (mg/g wet tissue)		Cholesterol (mg/g wet tissue)	
	Heart	Liver	Heart	Liver
Control	32.4±2.6[a]	29.2±2.4[a]	4.1±0.4[a]	3.4±0.3[a]
Diabetes	54.6±4.0[b]	40.6±3.2[b]	7.4±0.6[b]	6.7±0.5[b]
Histidine, 0.5 g/l	44.2±3.1[a,b]	36.1±2.0[a,b]	7.1±0.5[b]	6.3±0.4[b]
Histidine, 1 g/l	38.5±2.5[a,b]	32.6±1.3[a,b]	6.0±0.4[a,b]	5.4±0.3[a,b]
Carnosine, 0.5 g/l	43.1±3.7[a,b]	37.5±2.6[a,b]	6.8±0.3[b]	6.1±0.2[b]
Carnosine, 1 g/l	37.6±3.1[a,b]	34.2±1.9[a,b]	5.7±0.4[a,b]	5.0±0.5[a,b]

[a] $P<0.05$ versus diabetes group.
[b] $P<0.05$ versus control group.
[1] Values are means±SD, $n=15$.

Table 4

Malondialdehyde concentration, catalase activity and glutathione peroxidase activity in liver and kidney from nondiabetic mice (control) and diabetic mice treated with water (diabetes), histidine or carnosine[1]

	Malondialdehyde (μmol/l)		Catalase (U/mg protein)		Glutathione peroxidase (U/mg protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	1.18 ± 0.12^a	1.26 ± 0.11^a	24.7 ± 2.3^a	16.5 ± 1.9^a	28.2 ± 2.3^a	17.9 ± 0.7^a
Diabetes	3.43 ± 0.18^b	4.21 ± 0.21^b	15.8 ± 1.6^b	9.6 ± 1.3^b	19.5 ± 1.7^b	11.3 ± 1.4^b
Histidine, 0.5 g/l	$2.52\pm0.20^{a,b}$	$3.05\pm0.20^{a,b}$	$19.7\pm1.6^{a,b}$	$12.3\pm1.0^{a,b}$	20.5 ± 1.6^a	12.2 ± 1.2^b
Histidine, 1 g/l	$2.05\pm0.16^{a,b}$	$2.33\pm0.17^{a,b}$	$20.3\pm1.8^{a,b}$	$12.8\pm1.1^{a,b}$	$23.4\pm2.0^{a,b}$	$14.6\pm0.9^{a,b}$
Carnosine, 0.5 g/l	$2.37\pm0.10^{a,b}$	$2.78\pm0.21^{a,b}$	$21.5\pm1.4^{a,b}$	$11.6\pm1.3^{a,b}$	21.0 ± 1.2^a	11.8 ± 1.3^b
Carnosine, 1 g/l	$1.95\pm0.14^{a,b}$	$2.11\pm0.15^{a,b}$	$20.9\pm1.7^{a,b}$	$13.0\pm1.2^{a,b}$	$24.5\pm1.9^{a,b}$	$14.0\pm1.1^{a,b}$

[a] $P<0.05$ versus diabetes group.

[b] $P<0.05$ versus control group.

[1] Values are means $\pm$ SD, $n=15$.

ence of these agents on the activity of protein kinase C also needs further study. Our present work further found that histidine and carnosine at low concentration effectively reduced triglyceride accumulation in organs from diabetic mice; however, these agents showed marked cholesterol-lowering effect only at high concentration. It is highly possible that triglyceride-lowering effect from these agents at low concentration was simply due to the improved oxidative stress and glycemic control modify carbohydrate and lipid metabolisms. However, these agents at high concentration could partially restore insulin secretion and reduce both triglyceride and cholesterol accumulation. Apparently, the elevated insulin secretion improved lipid metabolism and provided more contribution toward cholesterol-lowering effect. These results seemingly imply that the influence of insulin to cholesterol regulation is more crucial than that to triglyceride regulation in diabetic condition.

Both IL-6 and TNF-alpha are proinflammatory cyto-kines, and the elevation of these two cytokines in diabetic patients has been reported (Geerlings et al., 2000; Jialal et al., 2002; Hwang et al., 2003; Aso et al., 2003). These authors indicated that the excessive production of TNF-alpha and/or IL-6 could impair insulin sensitivity and increase platelet sensitivity to thrombin activation, which elevates the risk of coagulation. The results of our present work agreed those previous studies because both IL-6 and TNF-alpha levels were markedly elevated in these diabetic mice; furthermore, we found that histidine and carnosine

supplementation could effectively downregulate IL-6 and TNF-alpha, this may consequently diminish inflammatory oriented endothelial dysfunction and coagulation risk. These results also partially explained why the diabetic mice with histidine or carnosine administration exhibited marked improvement in several diabetic clinical characteristics such as higher insulin level, lower blood glucose level and better lipid metabolism.

It is also known that poor glycemic control in diabetic patients caused LDL oxidation and glycation, which could further enhance the deterioration of diabetic complications and other vascular diseases (Picard, 1995; Moro et al., 1999). Our current work found that histidine and carnosine effectively protect LDL against glucose-induced oxidation and glycation. This protection may be simply due to these agents scavenge free radicals in LDL, and retard oxidation and glycation process. On the other hand, histidine and carnosine are amino acid-based hydrophilic compounds, and might possess greater affinity to water soluble molecules such as glucose, i.e. they may compete with protein part of LDL particle for glucose, which allowed them to interfere glycation development between glucose and LDL protein part. It should be pointed out that the used histidine and carnosine concentrations in human LDL experiments were 1 and 2 μM, these concentrations were less than the concentrations presented in the plasma and organs from histidine

Table 5

Interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) levels in plasma from nondiabetic mice (control) and diabetic mice treated with water (diabetes), histidine or carnosine[1]

	IL-6 (nmol/l)	TNF-α (nmol/l)
Control	24.6 ± 1.7^a	2.2 ± 0.2^a
Diabetes	78.4 ± 5.2^b	5.3 ± 0.3^b
Histidine, 0.5 g/l	$65.2\pm4.1^{a,b}$	5.4 ± 0.3^b
Histidine, 1 g/l	$50.8\pm3.5^{a,b}$	$4.2\pm0.2^{a,b}$
Carnosine, 0.5 g/l	$66.1\pm6.3^{a,b}$	4.9 ± 0.4^b
Carnosine, 1 g/l	$55.5\pm3.6^{a,b}$	$4.1\pm0.2^{a,b}$

[a] $P<0.05$ versus diabetes group.

[b] $P<0.05$ versus control group.

[1] Values are means $\pm$ SD, $n=15$.

Table 6

In vitro effect of 1 and 2 μM histidine or carnosine in human LDL against 100 mM glucose-induced oxidation and glycation[1]

	Oxidation[2] (nmol/mg LDL protein)	Glycation (%)
LDL	3.6 ± 1.2^a	3.2 ± 0.4^a
LDL+glucose	16.4 ± 1.1^b	9.3 ± 0.8^b
Histidine, 1 μM	$12.5\pm0.7^{a,b}$	$7.4\pm1.3^{a,b}$
Histidine, 2 μM	$10.6\pm0.5^{a,b}$	$5.3\pm1.0^{a,b}$
Carnosine, 1 μM	$13.2\pm1.0^{a,b}$	$7.2\pm0.9^{a,b}$
Carnosine, 2 μM	$9.4\pm0.8^{a,b}$	$5.9\pm1.2^{a,b}$

[a] $P<0.05$ versus diabetes group.

[b] $P<0.05$ versus control group.

[1] Values are means $\pm$ SD, $n=15$.

[2] Oxidation level of LDL at the beginning of incubation was 2.3 ± 1.1 nmol/mg LDL protein.

and carnosine supplemented mice. These results also suggest that these agents might provide effective protection for human against diabetic development or deterioration.

In conclusion, dietary supplementation of histidine and carnosine increased the content of these peptides in blood and organs, which consequently improved hyperglycemia, hyperlipidemia, oxidation and inflammation in diabetic mice. Furthermore, histidine and carnosine effectively inhibited glucose-induced oxidation and glycation in human LDL. These data support that these agents are potential multiple-protective agents for diabetic complications prevention or therapy.

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