Full Paper

Carnosine Ameliorates Stress-Induced Glucose Metabolism Disorder in Restrained Mice

Bun Tsoi^{1,2,†}, Rong-Rong He^{1,2,†}, Dong-Hui Yang¹, Yi-Fang Li¹, Xiao-Di Li¹, Wei-Xi Li¹, Keiichi Abe³, and Hiroshi Kurihara^{1,2,*}¹*Institute of Traditional Chinese Medicine and Natural Products,*²*BRAND'S Health Foundation Health Science Research Centre of Jinan University, Jinan University, Guangzhou 510632, China*³*BRAND'S Centre for Health and Nutritional Sciences, China Square Central, Singapore 048423, Singapore*

Received July 25, 2011; Accepted September 26, 2011

Abstract. Carnosine is a natural dipeptide that has shown multiple benefits in the treatment of various diseases. This study investigated the ameliorative effects of carnosine on glucose metabolism in restraint-stressed mice. Our results showed that restraint stress could significantly influence glucose metabolism, as reflected by lowered glucose tolerance, hepatic and muscle glycogen content, and increased plasma corticosterone concentration in mice. Oral administration of carnosine (150 and 300 mg/kg) not only reverted stress-induced decline in glucose tolerance and glycogen content in liver and muscle, but also reduced plasma corticosterone level. Carnosine has also significantly suppressed mRNA expression of glucose-6-phosphatase, while elevating glycogen synthase 2, glucokinase and glucose transporter 2 expressions in the liver. The obtained results demonstrated the harmful effects induced by restraint stress, while proving that carnosine could ameliorate stress-induced glucose metabolism disturbance. It is presumable that carnosine exerts its anti-stress effects by indirectly affecting the histaminergic neuron system, modulating the stress-activated hypothalamic–pituitary–adrenal axis and improving glucose metabolism through regulation of the enzymes in the glucose metabolic pathways.

Keywords: carnosine, restraint stress, glucose metabolism disorder, corticosterone, gene expression

Introduction

In most developed and developing countries, people have to face different kinds of pressures and stresses in their lives. Research has proved that immune functions were commonly lower in organisms under stressed conditions (1, 2). It has been shown that stress is strongly associated with the development of other diseases such as diabetes mellitus (3). As one of the leading causes of mortality in the world (4), the association between stress and development of diabetes mellitus has been extensively explored (5). Several lines of evidence showed that diabetes mellitus is usually accompanied by abnor-

mal blood glucose level and increased production of free radicals caused by oxidative stress (6, 7). Carnosine (β -alanyl-L-histidine), a naturally occurring dipeptide in the human body, was found to be a potent antioxidant (8) and is highly abundant in excitable tissues such as skeletal muscles and brain tissues (9, 10). Various reports showed that carnosine could scavenge reactive oxygen species (11), inhibit lipid oxidation (12), prevent glycation (13, 14), and modulate immune functions (15, 16) and has anti-tumor (17) and anti-aging (18) activities, and so on. There were also studies suggesting the use of carnosine for treatment of cataracts (19) and autism in children (20). However, only few studies focused on the benefits of using carnosine to treat glucose metabolism disorder. The aim of the present study was to discover the effect of carnosine on mice loaded with restraint stress. The restraint-stress mouse model is one of the animal models frequently used to study the harmful effects

[†]These authors contributed equally to this study.

*Corresponding author. hiroshi_kurihara@163.com

Published online in J-STAGE on November 26, 2011 (in advance)

doi: 10.1254/jphs.11131FP

of stress (21). Some of the known effects of restraint stress include irregular neuroendocrine hormones secretion (22), tissue injuries (23), fluctuation of blood glucose (24), and so on. The current study analyzed the malignant effect of the stress response on glucose metabolism of restrained mice. Further, the mechanisms of carnosine on restraint stress were studied.

Materials and Methods

Animals

Six-week-old male Kunming mice, weighing from 18 to 22 g, were purchased from the Guangdong Medicinal Laboratory Animal Centre, Guangzhou, China. The animals were kept in a specific pathogen-free animal room at $23 \pm 2^\circ\text{C}$ with a 12-h dark-light cycle and fed with standard laboratory diet and tap water. The animals were allowed to acclimatize to the environment for 1 week before the experiment. All the animal experiments were conducted in accordance with the *Guide for Care and Use of the Laboratory Animals* published by the U.S. NIH (publication No. 85-23, revised 1996).

Glucose tolerance test

After 1 week of acclimatization, a small group of normal control mice ($n = 6$) was used to perform a preliminary experiment. A dose of 2 g/kg glucose was orally administrated to the mice to confirm the time needed for peak glucose concentration. A second group of animal was randomly divided into four groups with 8 mice in each group. Carnosine (Sigma, St. Louis, MO, USA) was dissolved in distilled water and orally administered to the mice for 7 days consecutively. The dosages of carnosine were 150 and 300 mg/kg per day for 2 groups of mice, respectively. Normal control and restraint-stressed mice were only administrated distilled water once daily. Restraint stress procedures were performed according to the protocol established by Yamaoka et al. (25) with slight modifications and was started 30 min after the last drug administration. All mice except the normal control group were fixed in an oval restraint cage for 20 h (26). Normal control mice were fasted during the restraint procedure. All animals were allowed to rest for 30 min before the glucose tolerance test. A dose of 2 g/kg glucose solution was orally administrated to all mice after the resting period. Blood was drawn from tail intravenously at 20-min intervals immediately after glucose administration and mixed with heparin sodium. All samples were centrifuged at 5000 rpm for 5 min to obtain plasma for testing glucose concentrations by commercial kits (Shanghai Rongsheng Biotech Co., Ltd., Shanghai, China).

Corticosterone determination by HPLC

Mice were anesthetized by ether and sacrificed 30 min after the restraint stress procedure. Blood samples were transferred to centrifuge tubes with heparin and centrifuged for 5 min at 5000 rpm. The obtained plasma was mixed with 0.075 mg/ml cortisol (Sigma) and extracted by ethyl acetate twice. Organic layers were combined, washed by NaOH and distilled water and dried under liquid nitrogen. Mobile phase (acetonitrile-water, 38:72, v/v, 100 μl) was added into the organic layer to acquire corticosterone (CORT) content by HPLC analysis (Hitachi, Tokyo). The chromatography protocol was designed according to Woodward and Emery (27) with the following conditions: 254-nm UV detector; 5C18 column (4.6×150 mm, 5 μm ; Waters, Milford, MA, USA); acetonitrile-water mobile phase (38:72, v/v); flow rate at 1 ml/min.

Measurement of hepatic and muscle glycogen content

Liver and hind leg tissues were obtained from anesthetized mice and washed with normal saline. All tissues were dried, weighed, and put into test tubes with lye solution in a ratio of 1:3 (mg: μl). The test tubes were placed in a boiling water bath for 20 min and then cooled by running water. Hydrolyzed hepatic (1%) and muscle glycogen solutions (5%) were mixed with distilled water in a ratio of 1:96 and 1:16 of tissue weight, respectively, to obtain sample solutions. The prepared sample solutions were then combined with chromogenic reagent and placed in the boiling water bath for 5 min further. All samples were cooled down by running water and absorbance was determined at 620 nm (Thermo Scientific, Waltham, MA, USA).

Determination of mRNA expressions of glucose metabolism related enzymes and GLUT2 in liver

The gene expression protocol was performed according to Yao et al. with slight modification (28). Total RNA of liver tissues were extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed with mouse Moloney leukemia virus reverse transcriptase and oligo (dT) primers (Tiangen Biotech Co., Ltd., Beijing, China), followed by PCR amplification. Thereafter, cDNA was amplified together with Taq polymerase (Tiangen) using specific primers. The sequence of primers (Invitrogen) for mouse glucose-6-phosphatase (G6PC), glycogen synthase 2 (GYS2), glucokinase (GK), and glucose transporter (GLUT2) is listed in Table 1. The PCR products were fractionated on a 1% agarose gel (Invitrogen) and visualized by ethidium bromide staining. Band intensity was measured by the BIO-RAD Image Analysis system (BIO-RAD, Hercules, CA, USA), then quantified by Quantity One (BIO-RAD), and ex-

Table 1. Sequence of primers used for RT-PCR

Gene name	Sequence	Product size (bp)
<i>G6PC</i>	(F) 5'- CGCTATCTCCAAGTGAAT -3' (R) 5'- TGTAGTAGTCGGTGTCCAG -3'	235
<i>GYS2</i>	(F) 5'- ATGGCGATGAAAGCGGTGTG -3' (R) 5'- TTACTGCGCAATCCCAATCAC -3'	465
<i>GK</i>	(F) 5'- AAGATGTTGCCACCTAC -3' (R) 5'- GGATGCCCTTGTCTATGT -3'	325
<i>GLUT2</i>	(F) 5'- CCTTCCAGTTCGGCTAT -3' (R) 5'- CCTTCCAAGTTGTCCC -3'	308
<i>18S</i>	(F) 5'- GGGAGAGCGGGTAAGAGA -3' (R) 5'- ACAGGACTAGGCGGAACA -3'	241

pressed as the ratios to *18S*.

Statistical analyses

All data are represented as the mean $\pm$ standard error of the mean (S.E.M.). The data obtained were analyzed by ANOVA followed by Dunnett's significant post-hoc test using the SPSS 18.0 statistical software (IBM, Endicott, NY, USA). A *P* value of < 0.05 was considered statistically significant.

Results

Effect of carnosine on glucose tolerance in restraint stressed mice

As illustrated in Fig. 1A, glucose concentrations of normal control mice peaked around 20 min after glucose injection. Glucose tolerance was determined and we found that the plasma glucose elimination was significantly lower in the restraint-stressed mice when compared to the normal control ($P < 0.01$) after the peak concentration, which was compared in Fig. 1B (29). Oral administration of carnosine improved glucose elimination by approximately 10% ($P < 0.05$), while a high dose of carnosine significantly accelerated the elimination of plasma glucose by around 17% ($P < 0.01$).

Effect of carnosine on plasma CORT concentration in restraint stressed mice

Results showed that plasma CORT content in mice loaded with restraint stress was remarkably elevated by 175.76% when compared to the normal control ($P < 0.01$). Both doses of arnosine decreased CORT level, by 56.24% and 60.64%, respectively ($P < 0.01$) (Fig. 2).

Effect of carnosine on hepatic and muscle glycogen content in restraint-stressed mice

Hepatic and muscle glycogen content measured in restrained mice was obviously lower than those of the

Fig. 1. Influence of carnosine on glucose tolerance. A) Blood glucose concentration in normal mice. The results were reported as the mean $\pm$ S.E.M. ($n = 6$). B) Percentage glucose level after peak concentration. The results were reported as the mean $\pm$ S.E.M. ($n = 8$). Data were regarded statistically significant at $*P < 0.05$ and $**P < 0.01$ vs. normal control; $^{\Delta}P < 0.05$ and $^{\Delta\Delta}P < 0.01$ vs. restraint stress.

normal control ($P < 0.01$). Administration of carnosine improved hepatic glycogen content to 64.95% ($P < 0.05$)

Fig. 2. Effect of carnosine on plasma CORT concentration in restraint-stressed mice. The results are reported as the mean $\pm$ S.E.M. ($n = 8$). Data were regarded statistically significant at ** $P < 0.01$ vs. normal control and $\Delta\Delta P < 0.01$ vs. restraint stress.

and 98.63% ($P < 0.01$) of the normal level. At the same time, muscle glycogen content was also increased to 21.10% ($P < 0.05$) and 48.62% ($P < 0.01$) of the normal level (Fig. 3).

Effect of carnosine on mRNA expressions of glucose metabolism related enzymes and GLUT2 in liver of restraint-stressed mice

The mRNA expressions obtained were compared with *18S* which served as an internal standard. Restraint stress significantly up-regulated the mRNA expression of *G6PC* ($P < 0.01$) when compared with the control. On the contrary, *GYS2*, *GK*, and *GLUT-2* expressions were remarkably suppressed in restrained mice ($P < 0.01$). With the administration of a low dose of carnosine, stress-induced changes in *G6PC*, *GYS2*, *GK*, and *GLUT2* expressions were reversed when compared with restraint-stressed mice ($P < 0.05$), while a high dose significantly recovered these gene expressions to different extents as shown in Fig. 4 ($P < 0.01$).

Fig. 3. Effect of carnosine on hepatic and muscle glycogen content in restraint-stressed mice. The results are reported as the mean $\pm$ S.E.M. ($n = 8$). Data were regarded statistically significant at ** $P < 0.01$ vs. normal control; $\Delta P < 0.05$ and $\Delta\Delta P < 0.01$ vs. restraint stress.

Fig. 4. Effect of carnosine on mRNA expressions of glucose metabolism-related enzymes and *GLUT2* in liver of restraint-stressed mice. The results are reported as the mean $\pm$ S.E.M. ($n = 8$). Data were regarded statistically significant at ** $P < 0.01$ vs. normal control; $\Delta P < 0.05$ and $\Delta\Delta P < 0.01$ vs. restraint stress.

Discussion

Diabetes mellitus is one of the leading causes of death in recent centuries (30). While the pathological mechanism of diabetes mellitus is still ill-defined, environmental factors seem to be important in the development of the disease. In the 19th century, people noticed an elevation of blood glucose after acute trauma (31). Since then people realized the potential relationships between stress and glucose metabolism disorder (32, 33). In research, different types of stress have been applied on animals to study their influences on glucose metabolisms, including endoplasmic reticulum stress, oxidative stress, restraint stress, and so on (34–36). The present study employed a 20-h restraint stress to study its effects on glucose metabolism in mice (26). Results showed that restraint stress lead to glucose metabolism disorder in mice, which was reflected by abnormal glucose tolerance level, lowered glucose elimination rate, and decreased hepatic and muscle glycogen contents. Carnosine administration was found to attenuate such detrimental effects of stress on glucose metabolism. At the same time, glycogen content was also recovered in both carnosine groups. These revealed that carnosine could improve glucose disorder caused by restraint stress.

A large body of evidence shows that stress could alter glucose metabolism (24, 35–38). It is generally established that enzyme activities are changed by stress response (39). Therefore, expressions of the key enzymes in the glucose metabolic pathway were examined using RT-PCR. As illustrated in the results, mRNA expression of the important gluconeogenesis controlling enzyme *G6PC* was significantly up-regulated by restraint stress. This result is in accordance to previous research suggesting that the stress reaction elevated *G6PC* activity in mice (40, 41). On the other hand, *GYS2*, *GK*, and *GLUT2* expressions were lowered in restraint stressed mice. In accordance with previous research, glycogen content and glycogen synthase activity were significantly lowered by the stress reaction (37, 42). Hyperglycemia could also reduce glycogen synthase (43) and *GK* activities (44, 45). It had also been proved that *GLUT* expressions were affected by stress (38, 46). The data from previous studies supported that restraint stress influenced glucose metabolic enzyme activities in mice, which in turn altered its blood glucose level. In our experiment, carnosine was given to restraint-stressed mice and was shown to be beneficial on improving activities of glucose metabolic enzymes. Both dosages of carnosine lowered the expression of *G6PC* in restrained mice. Moreover, the expressions of *GYS2*, *GK*, and *GLUT2* were also increased by carnosine. These results proved that carnosine might improve glucose metabolism partially by inhibiting glu-

coneogenesis, promoting the synthesis of glycogen, enhancing glycolysis, and stepping up the transportation of glucose from blood to liver through regulating glucose metabolic enzymes.

In our experiments, we also observed an elevation of plasma CORT concentration in restraint-stressed mice. As the release of CORT is an indicator for the activation of the hypothalamic–pituitary–adrenal axis (HPA axis), it is believed that the hyper-activation of HPA axis was caused by restraint stress (47, 48). At the same time, blood glucose was also found to be increased after the restraint stress reaction (49, 50). When carnosine was orally administered to restrained mice, plasma CORT was significantly lowered. It showed that carnosine could relieve the hyper-activated HPA axis. It had been proved that carnosine could easily pass through the blood–brain barrier (51). Recent studies by Zhu et al. have also demonstrated that carnosine could activate histamine neurons in histidine decarboxylase knock-out mice (52). Besides, it was found that carnosine could be transformed into histidine, which is important for the synthesis of histamine in the brain, and act on histaminergic neurons system to relieve stress-induced damages (48, 53, 54). Carnosine presumably exerted its anti-stress effect by passing through the BBB (55). On the other hand, it was found that glucocorticoid could depress the expression of histidine decarboxylase (56). Therefore, the anti-stress carnosine calmed the excited HPA axis and most importantly promoted the reaction of the carnosine–histidine–histamine metabolic pathway to achieve its ameliorating effects on glucose metabolism.

In the current study, restraint stress was found to be affecting glucose metabolism. Through the examination of glucose tolerance, CORT, and glycogen contents, it is confirmed that blood glucose level was remarkably influenced. Experimental results proved that CORT, an end-product of the HPA axis, was significantly increased by restraint stress. Restraint stress also altered the activities of several glucose metabolism enzymes, including *G6PC*, *GYS2*, *GK*, and so on. Glucose transportation was also found to be slowed down by the stress reaction. Administration of carnosine improved glucose metabolism in restraint-stressed mice, which include better glucose tolerance, lowered CORT concentration, and increased glycogen content. Gene expressions of various enzymes, receptor proteins, and *GLUT2* were also improved. It is believed that carnosine exerted its anti-stress effects by calming the hyper-excited HPA axis and at the same time regulated hepatic glucose output, activated glycogen synthase kinase, and accelerated glucose transport to balance the fluctuation of blood glucose level.

Acknowledgments

This work was supported by the Fundamental Research Funds for the Central Universities (no. 21611396) and BRAND'S Centre for Health and Nutritional Sciences in Singapore.

References

- Yang EV, Glaser R. Stress-induced immunomodulation and the implications for health. *Int Immunopharmacol*. 2002;2:315–324.
- He RR, Yao XS, Li HY, Dai Y, Duan YH, Li YF, et al. The anti-stress effects of *Sarcandra glabra* extract on restraint-evoked immunocompromise. *Biol Pharm Bull*. 2009;32:247–252.
- Surwit R, Schneider M, Feinglos M. Stress and diabetes mellitus. *Diabetes Care*. 1992;15:1413.
- Murray C, Lopez A. Mortality by cause for eight regions of the world: global burden of disease study. *Lancet*. 1997;349:1269–1276.
- Mathers C, Loncar D. Updated projections of global mortality and burden of disease, 2002–2030: data sources, methods and results. World Health Organization. 2005.
- Maritim A, Sanders R, Watkins J III. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol*. 2003;17:24–38.
- Rains J, Jain S. Oxidative stress, insulin signaling and diabetes. *Free Radic Biol Med*. 2010;50:567–575.
- Guiotto A, Calderan A, Ruzza P, Borin G. Carnosine and carnosine-related antioxidants: a review. *Curr Med Chem*. 2005;12:2293–2315.
- Reddy WJ, Hegsted DM. Measurement and distribution of carnosine in rat. *J Biol Chem*. 1962;237:705–706.
- Zapp J, Wilson D. Quantitative studies of carnosine and anserine in mammalian muscle. *J Biol Chem*. 1938;126:19.
- Kurihara H, Shibata H, Tsuruoka N, Kiso Y, Abe K, Nagai H, et al. Carnosine modulates stress-attenuated plasma antioxidative capacity. *Food Sci Technol Res*. 2009;15:179–184.
- Decker E, Faraji H. Inhibition of lipid oxidation by carnosine. *J Am Oil Chem Soc*. 1990;67:650–652.
- Reddy VP, Garrett MR, Perry G, Smith MA. Carnosine: a versatile antioxidant and antiglycating agent. *Sci Aging Knowledge Environ*. 2005;2005:pe12.
- Lee YT, Hsu CC, Lin MH, Liu KS, Yin MC. Histidine and carnosine delay diabetic, deterioration in mice and protect human low density lipoprotein against oxidation and glycation. *Eur J Pharmacol*. 2005;513:145–150.
- Tan KM, Candlish JK. Carnosine and anserine as modulators of neutrophil function. *Clin Lab Haematol*. 1998;20:239–244.
- Soliman K, Abou-El Dobal S, Marei N. Effect of carnosine administration on the immune response of rabbit to *Schistosoma mansoni* antigens. *J Egypt Soc Parasitol*. 2003;33:663–678.
- Holliday R, McFarland GA. Inhibition of the growth of transformed and neoplastic cells by the dipeptide carnosine. *Br J Cancer*. 1996;73:966–971.
- Boldyrev AA, Gallant SC, Sukhich GT. Carnosine, the protective, anti-aging peptide. *Biosci Rep*. 1999;19:581–587.
- Boldyrev AA, Dupin AM, Bunin A, Babizhaev MA, Severin SE. The antioxidative properties of carnosine, a natural histidine containing dipeptide. *Biochem Int*. 1987;15:1105–1113.
- Chez MG, Buchanan CP, Aimonovitch MC, Becker M, Schaefer K, Black C, et al. Double-blind, placebo-controlled study of L-carnosine supplementation in children with autistic spectrum disorders. *J Child Neurol*. 2002;17:833–837.
- Buynitsky T, Mostofsky DI. Restraint stress in biobehavioral research: recent developments. *Neurosci Biobehav Rev*. 2009;33:1089–1098.
- Van de Kar LD, Blair ML. Forebrain pathways mediating stress-induced hormone secretion. *Front Neuroendocrinol*. 1999;20:1–48.
- Jaeschke H. Mechanisms of oxidant stress-induced acute tissue-injury. *Proc Soc Exp Biol Med*. 1995;209:104–111.
- McCowen KC, Malhotra A, Bistran BR. Stress-induced hyperglycemia. *Crit Care Clin*. 2001;17:107–124.
- Yamaoka Y, Kawakita T, Nomoto K. Protective effect of a traditional Japanese medicine, Bu-zhong-yi-qi-tang (Japanese name: Hochu-ekki-to), on the restraint stress-induced susceptibility against *Listeria monocytogenes*. *Immunopharmacology*. 2000;48:35–42.
- Kurihara H, Yao X, Nagai H, Tsuruoka N, Shibata H, Kiso Y, et al. Anti-stress effect of BRAND's essence of chicken (BEC) on plasma glucose levels in mice loaded with restraint stress. *J Health Sci*. 2006;52:252–258.
- Woodward CJ, Emery PW. Determination of plasma corticosterone using high-performance liquid chromatography. *J Chromatogr*. 1987;419:280–284.
- Yao N, Lan F, He RR, Kurihara H. Protective effects of bilberry (*Vaccinium myrtillus* L.) extract against endotoxin-induced uveitis in mice. *J Agric Food Chem*. 2010;58:4731–4736.
- Konrad D, Bilan PJ, Nawaz Z, Sweeney G, Niu WY, Liu Z, et al. Need for GLUT4 activation to reach maximum effect of insulin-mediated glucose uptake in brown adipocytes isolated from GLUT4myc-expressing mice. *Diabetes*. 2002;51:2719–2726.
- Xu J, Kochanek K, Murphy S, Tejada-Vera B. Deaths: final data for 2007. In: Hyattsville MD editor. National vital statistics reports, NVSR Volume 58, Number 19. 73. Hyattsville, MD: National Center for Health Statistics; 2010. pp. (PHS) 2010-1120.
- Cryer P. Glucose homeostasis and hypoglycemia. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, editors. *Williams textbook of endocrinology*. 10th ed. Philadelphia: Saunders; 2003. p. 1585–1618.
- Laugero KD. Reinterpretation of basal glucocorticoid feedback: implications to behavioral and metabolic disease. *Vitam Horm*. 2004;69:1–29.
- Maccari S, Morley-Fletcher S. Effects of prenatal restraint stress on the hypothalamus-pituitary-adrenal axis and related behavioural and neurobiological alterations. *Psychoneuroendocrinology*. 2007;32:S10–S15.
- Oezcan U, Yilmaz E, Oezcan L, Furuhashi M, Vaillancourt E, Smith RO, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science*. 2006;313:1137–1140.
- Ishizuka T, Hinata T, Watanabe Y. Superoxide induced by a high-glucose concentration attenuates production of angiogenic growth factors in hypoxic mouse mesenchymal stem cells. *J Endocrinol*. 2011;208:147–159.
- Nirupama R, Devaki M, Yajurvedi HN. Repeated stress exposure results in hyperglycemia for a prolonged period in rat. *J Expl Zool India*. 2010;13:537–540.
- Matte C, Stefanello FM, Mackedanz V, Pederzoli CD, Lamers ML, Dutra CS, et al. Homocysteine induces oxidative stress, inflammatory infiltration, fibrosis and reduces glycogen/glycopro-

- tein content in liver of rats. *Int J Dev Neurosci.* 2009;27:337–344.
- 38 Cohen H, Kozlovsky N, Savion N, Matar MA, Loewenthal U, Loewenthal N, et al. An association between stress-induced disruption of the hypothalamic-pituitary-adrenal axis and disordered glucose metabolism in an animal model of post-traumatic stress disorder. *J Neuroendocrinol.* 2009;21:898–909.
 - 39 Losser MR, Damoiseil C, Payen D. Bench-to-bedside review: glucose and stress conditions in the intensive care unit. *Crit Care.* 2010;14:231.
 - 40 Momoda TS, Schwindt AR, Feist GW, Gerwick L, Bayne CJ, Schreck CB. Gene expression in the liver of rainbow trout, *Oncorhynchus mykiss*, during the stress response. *Comp Biochem Physiol Part D Genomics Proteomics.* 2007;2:303–315.
 - 41 Wang D, Wei YR, Schmoll D, Maclean KN, Pagliassotti MJ. Endoplasmic reticulum stress increases glucose-6-phosphatase and glucose cycling in liver cells. *Endocrinology.* 2006;147:350–358.
 - 42 Palsamy P, Subramanian S. Modulatory effects of resveratrol on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. *Chem Biol Interact.* 2009;179:356–362.
 - 43 Noyman I, Marikovsky M, Sasson S, Stark AH, Bernath K, Seger R, et al. Hyperglycemia reduces nitric oxide synthase and glycogen synthase activity in endothelial cells. *Nitric Oxide.* 2002;7:187–193.
 - 44 Torres TP, Catlin RL, Chan R, Fujimoto Y, Sasaki N, Printz RL, et al. Restoration of hepatic glucokinase expression corrects hepatic glucose flux and normalizes plasma glucose in Zucker diabetic fatty rats. *Diabetes.* 2009;58:78–86.
 - 45 Pari L, Sathesh MA. Antidiabetic activity of *Boerhaavia diffusa* L.: effect on hepatic key enzymes in experimental diabetes. *J Ethnopharmacol.* 2004;91:109–113.
 - 46 Wiseman S, Osachoff H, Bassett E, Malhotra J, Bruno J, VanAggelen G, et al. Gene expression pattern in the liver during recovery from an acute stressor in rainbow trout. *Comp Biochem Physiol Part D Genomics Proteomics.* 2007;2:234–244.
 - 47 Parker KJ, Schatzberg AF, Lyons DM. Neuroendocrine aspects of hyperportisolism in major depression. *Horm Behav.* 2003;43:60–66.
 - 48 Wu XH, Ding MP, Zhu-Ge ZB, Zhu YY, Jin CL, Chen Z. Carnosine, a precursor of histidine, ameliorates pentylenetetrazole-induced kindled seizures in rat. *Neurosci Lett.* 2006;400:146–149.
 - 49 Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev.* 2000;21:55–89.
 - 50 Kainuma E, Watanabe M, Tomiyama-Miyaji C, Inoue M, Kuwano Y, Ren HW, et al. Association of glucocorticoid with stress-induced modulation of body temperature, blood glucose and innate immunity. *Psychoneuroendocrinology.* 2009;34:1459–1468.
 - 51 Lu YQ, He RR, Watanabe H, Abe K, Sakurai E, Yanai K, et al. Effects of a chicken extract on food-deprived activity stress in rats. *Biosci Biotechnol Biochem.* 2010;74:1276–1278.
 - 52 Zhu Y-Y, Zhu-Ge Z-B, Wu D-C, Wang S, Liu L-Y, Ohtsu H, et al. Carnosine inhibits pentylenetetrazol-induced seizures by histaminergic mechanisms in histidine decarboxylase knock-out mice. *Neurosci Lett.* 2007;416:211–216.
 - 53 Otani H, Okumura A, Nagai K, Okumura N. Colocalization of a carnosine-splitting enzyme, tissue carnosinase (CN2)/cytosolic non-specific dipeptidase 2 (CNDP2), with histidine decarboxylase in the tuberomammillary nucleus of the hypothalamus. *Neurosci Lett.* 2008;445:166–169.
 - 54 He RR, Yao N, Wang M, Yang XS, Yau CC, Abe K, et al. Effects of histamine on lipid metabolic disorder in mice loaded with restraint stress. *J Pharmacol Sci.* 2009;111:117–123.
 - 55 Kozan R, Sefil F, Baglrlcl F. Anticonvulsant effect of carnosine on penicillin-induced epileptiform activity in rats. *Brain Res.* 2008;1239:249–255.
 - 56 Zahnow CA, Panula P, Yamatodani A, Millhorn DE. Glucocorticoid hormones downregulate histidine decarboxylase mRNA and enzyme activity in rat lung. *Am J Physiol.* 1998;275:L407–L413.