Effect of Marine Collagen Peptides on Markers of Metabolic Nuclear Receptors in Type 2 Diabetic Patients with/without Hypertension[1]

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Objective   To explore Effects of marine collagen peptides (MCPs) on markers of metablic nuclear receptors, i.e peroxisome proliferator-activated receptor (PPARs), liver X receptor (LXRs) and farnesoid X receptor (FXRs) in type 2 diabetic patients with/without hypertension. **Method**   Study population consisted of 200 type 2 diabetic patients with/without hypertension and 50 healthy subjects, all of whom were randomly assigned to MCPs-treated diabetics ($n=50$), placebo-treated diabetics ($n=50$), MCPs-treated diabetics with hypertension ($n=50$), placebo-treated diabetics with hypertension ($n=50$), and healthy controls ($n=50$). MCPs or placebo (water-soluble starch) were given daily before breakfast and bedtime over three months. Levels of free fatty acid, cytochrome P450, leptin, resistin, adiponectin, bradykinin, NO, and Prostacyclin were determined before intervention, and 1.5 months, and 3 months after intervention. Hypoglycemia and the endpoint events during the study were recorded and compared among the study groups. **Result**   At the end of the study period, MCPs-treated patients showed marked improvement compared with patients receiving placebo. The protection exerted by MCPs seemed more profound in diabetics than in diabetics with hypertension. In particular, after MCPs intervention, levels of free fatty acid, hs-CRP, resistin, Prostacyclin decreased significantly in diabetics and tended to decrease in diabetic and hypertensive patients whereas levels of cytochrome P450, leptin, NO tended to decrease in diabetics with/without hypertension. Meanwhile, levels of adiponectin and bradykinin rose markedly in diabetics following MCPs administration. **Conclusion**   MCPs could offer protection against diabetes and hypertension by affecting levels of molecules involved in diabetic and hypertensive pathogenesis. Regulation on metabolic nuclear receptors by MCPs may be the possible underlying mechanism for its observed effects in the study. Further study into its action may shed light on development of new drugs based on bioactive peptides from marine sources.

Key words: Marine collagen peptide; Peroxisome proliferator-activated receptor (PPAR); Liver X receptor; Farnesoid X receptor; Metabolic nuclear receptor

INTRODUCTION

Hypertension is a severe and common morbid *condition in patients with diabetes, acting* together with diabetes in elevating the risk of diabetic complications. Hypertensive patients with diabetes are much more likely predisposed to cardiovascular events as compared with the general population. Previous studies provided compelling evidence that intensive blood pressure control brought substantial benefits to diabetic patients with hypertension[1-3]. A recent study also suggested that combination of two or more antihypertensive agents, angiotensin-converting enzyme (ACE) inhibitors in particular, may offer additional renal and cardiovascular protection in diabetic patients with hypertension[4].

Owing to the well-established correlation of the two chronic conditions with metabolism syndrome, there has been an upsurge of interest in molecular mechanisms underlying these metabolic diseases. As a class of ligand-activated transcriptional factors including PPARs, LXRs, and FXRs, metabolic nuclear receptors were attracting increasing attention following discoveries of their roles in regulating lipid and glucose homeostasis. They were at the intersection of key pathways involved in the pathogenesis of diabetes and cardiovascular disease. Synthetic PPAR agonists were already used to treat dyslipidaemia and diabetes. PPAR-α agonist fibrates could lower plasma triglycerides and increased HDL

cholesterol[5] while PPAR-γ agonist thiazolidinediones could reduce insulin resistance and blood glucose levels[6]. LXRs and FXRs also played a role in regulation of lipid and glucose metabolism. A synthetic LXR agonist improved glucose tolerance in a model of diet-induced obesity and insulin resistance[7]. Moreover, a study demonstrated that FXR was decreased in animal models of diabetes[8]. A synthetic FXR agonist GW4064 resulted in a dose-dependent decrease in plasma triglyceride levels[9]. Therefore, therapeutic agents designed to affect pathways involving metabolic nuclear receptors may be beneficial for diabetics and hypertensives.

Although search for biologically active compounds from marine sources remains a relatively new field, marine organisms represent a vast source of novel active substances and hold a promising future for new drug discoveries because of their biodiversity of marine environment. A growing number of literatures demonstrated that peptides from marine organisms had a wide spectrum of effects, such as tumor suppression, antihypertension, and cholesterol reduction[10]. A previous study showed that treatment with dietary cod (morrhua) proteins improved insulin sensitivity in insulin-resistant individuals and reduced insulin-resistance related metabolic disorders, both contributing to the prevention of type 2 diabetes[11]. Researchers have also found that small collagen peptides in fish hydrolysate inhibited the activity of angiotensin I converting enzyme, suggesting collagen peptides as promising candidates of ACE inhibitors[12]. On that basis, we hypothesize that treatment with MCPs may offer protection for patients with diabetes and/or hypertension by modulating participants in the pathways involving metabolic nuclear receptors.

In the present prospective study, we attempted to explore effects of MCPs, small bioactive peptides from deep-sea fish, on markers of metabolic nuclear receptors such as PPARs, LXRs, and FXRs in typeII diabetic patients with/without hypertension. Findings from this study may be of help for potential therapeutic use of marine-derived bioactive peptides in individuals with diabetes and hypertension.

MATERIAL AND METHODS

Subjects

Two hundred patients with type 2 diabetes alone, type 2 diabetes and hypertension, and 50 age-matched healthy volunteers were recruited from two local communities (Lianhuabei and Jingtian) in Shenzhen and Shenzhen Hospital of Beijing University during the period from January 2007 to October 2008. The study population consisted of MCPs-treated diabetics (n=50, group A), placebo-treated diabetics (n=50, group B), MCPs-treated diabetics with hypertension (n=50, group C), placebo-treated diabetics with hypertension (n=50, group D), and placebo-treated healthy controls (n=50). All subjects participated in the study on a voluntary and informed basis. Written consent was obtained from all subjects. The study was approved by the Ethnical Committee of Shenzhen Hospital of Beijing University.

Diagnostic Criteria

Diabetes   In accordance with recommendations from American Diabetes Association (1999), diagnosis of diabetes was made when the level of fasting blood glucose (FBG) is 7.0 mmol/L or above. diabetic patients with secondary hypertension, coronary heart disease, liver or kidney diseases, cerebrovascular diseases, and their sequelae, malignancies or on insulin treatment were excluded from this study.

Hypertension   Based on hypertension guidelines published by WHO/ISH in 1999, diagnosis of hypertension was established when systolic blood pressure is 140 mmHg or above and/or diastolic blood pressure is 90 mmHg above. Patients with secondary hypertension, cardiomyopathy, severe abnormalities of liver and kidney function, and history of coronary bypass surgery were excluded from this study.

Healthy controls were those who did not have hypertension, diabetes, coronary heart disease, or hyperlipemia.

Preparation and Identification of MCPs

MCPs used in this study were prepared from wild marine fish caught from the East China Sea, and provided by the CF Haishi Biotechnology (Beijing, China). Briefly, fish were cleaned and the meat was minced. The materials were homogenized in distilled water and digested with mixed proteases (3 000 U/g protein) at 40 ℃ for 3 h. The resultant hydrolysate was centrifuged at 26 000 g and subsequently filtered through ceramic membranes (200 μm) for purification. MCPs powder was generated by ultra-filtration, desalination, cryoconcentration under vacuum at 70 ℃, decolorization, and lyophilization.

MCPs prepared as above mentioned were analyzed by high-performance liquid chromatography (HPLC, Waters Corp., Milford, MA, USA) and matrix-assisted laser desorption

ionization time-of-flight mass spectrometry (MALDI-TOF-MS, LDI-1700, Linear Scientific Inc., Reno, NV, USA). The amino acid composition of the MCPs was further analyzed using an H835-50 automatic amino acid analyzer (Hitachi, Tokyo, Japan), and the amount of free amino acids was measured by the HPLC. These assays showed that MCPs contained over 95% of oligopeptides with molecular weights of 130-3000 dalton (Da). Analysis by MALDI-TOF-MS further revealed that the molecular weight of the main components in the peptide mixture ranged from 100 to 860 Da, and 85.86% of the peptide mixtures has a molecular weight between 300 and 800 Da, of which fractions with 300-500 Da accounted for 28.50% and fractions with 700-860 Da 48.09%. Based on the above data, we postulated that the main component in the peptide mixture may have two to six amino acid residues in length.

Treatment and Measurement

All participants received education about the diet and exercise programs. Patients with T2DM and hypertension were prescribed with anti-diabetic medicines, tolbutamide (500-3000 mg/d), and/or metformin (500-1500 mg/d), and anti-hypertensive drugs, metoprolol (50-100 mg/d), verapamil (80-240 mg/d), or losartan (25-100 mg/d) for control hyperglycemia and hypertension, but without insulin, insulin secretion stimulators, inhibitor for angiotensin-converting enzyme and anti-hyperlipidemic medicines.

Guidance on diet and lifestyle was prescribed to all subjects. MCPs or placebo (water-soluble starch) were taken daily before breakfast and bedtime at a dose of 6.5 g in parallel with routine clinic drugs for three months. After an overnight fast for 12 h, blood serum samples were collected at intermedian cubital vein on early morning for determining levels of hs-CRP, free fatty acid, cytochrome P450, leptin, resistin, adiponectin, bradykinin, NO, and Prostacyclin before intervention, 1.5 months, and 3 months after intervention. Markers of metabolic nuclear receptors including free fatty acid, cytochrome P450, leptin, resistin, adiponectin, bradykinin, NO, and Prostacyclin were assayed by enzyme-linked immunoassay using reagents from Pennsylvania State University Animal Diagnostics Lab (ADL, USA).

Statistical Analysis

All quantitative data were expressed as mean±standard deviation (S). A paired t test was used to compare data from the same group at different time periods whereas multiple t test was utilized to determine the differences among different groups. A P value less than 0.05 was considered to be statistically significant. All data were analyzed with the SPSS 13.0 software.

RESULTS

Baseline Characteristics of the Study Population

No significant differences were observed with respect to sex ratio, age, and body weight index (BMI) among the study groups. Disease duration in diabetic patients with hypertension was significantly longer than that in diabetic patients. But no marked difference in disease duration was noted between paired groups (Table 1).

TABLE 1

Baseline Characteristics of The Study Population ($\bar{x} \pm s$)

Group	Number (Male/Female)	Age (Year)	Disease Duration (Month)	BMI（kg/m^2）
Healthy Control (Group N)	50(24/26)	65.78±12.47	-	24.19±3.23
MCPs-treated Diabetics (Group A)	50(26/24)	64.20±8.66	67.00±35.71	24.28±3.24
Placebo-treated Diabetics (Group B)	50(25/25)	61.79±13.34	65.53±65.01	23.35±3.39
MCPs-treated Diabetics with Hypertension (Group C)	50(28/22)	67.64±8.27	102.58±108.04	25.17±2.45
Placebo-treated Diabetics with Hypertension (Group D)	50(27/23)	63.66±9.83	106.23±99.86	24.82±3.25
Independent Samples T Test	**F=0.236** **P=0.870**	**F=3.465** **P=0.053**	**F=6.387** **P=0.055**	**F=1.458** **P=0.252**

Note. One-Way ANOVE was used for groups.

Changes in Serum Free Fatty Acid, Cytochrome P450, and hs-CRP Levels before and after Intervention

Throughout the whole study, the level of free fatty acid was significantly higher in diabetic patients with/without hypertension than in healthy controls. After intervention with MCPs, levels of free fatty acid decreased significantly in diabetics and tended to decrease in diabetic and hypertensive patients. Following placebo administration, however, levels of free fatty acid increased significantly in controls and tended to increase in both diabetics and diabetics with hypertension. As for the level of cytochrome P450, it was markedly higher in diabetics with hypertension than in diabetics and healthy controls throughout the study. Nevertheless, after MCPs intervention, levels of cytochrome P450 tended to decrease in diabetics and diabetics with hypertension whereas levels of cytochrome P450 tended to increase in healthy controls and increased precipitously in diabetics and diabetics with hypertension after taking placebo.

Throughout the study, levels of hs-CRP in diabetics and diabetics with hypertension were significantly higher than those in healthy controls whereas levels of hs-CRP in diabetics with hypertension were in turn significantly higher than those in diabetics. After MCPs intervention, levels of hs-CRP tended to decrease in diabetics with hypertension and fell markedly in diabetics. In contrast, after placebo administration, levels of hs-CRP rose significantly in diabetics three months later, but showed no significant response to placebo in diabetics with hypertension.

TABLE 2

Changes in Free Fatty Acid, Cytochrome P450, and hs-CRP Levels before and after Intervention $(\bar{x} \pm s)$

Group	Months of Intervention	Free Fatty Acid (ug/mL)	Cytochrome P450 (ng/mL)	hs-CRP (ug/mL)
Healthy Control (Group N)	0	101.53±43.10	5.98±4.58	0.21±0.12
	1.5	115.76±54.47[*]	6.37±3.91	0.23±0.13
	3.0	125.24±57.35[**▲▲]	6.91±4.17	0.23±0.13
MCPs-treated Diabetics (Group A)	0	279.97±136.15[NN]	7.21±4.18	0.75±0.54[NN]
	1.5	250.98±114.40[*NN]	7.17±4.55	0.68±0.51[**NN]
	3.0	247.41±114.25[**NN]	8.30±3.57	0.65±0.47[**NN]
Placebo-treated Diabetics (Group B)	0	285.51±83.20[NN]	8.08±4.08	0.74±0.45[NN]
	1.5	281.34±83.70[NN]	8.76±4.90[*]	0.77±0.48[NN]
	3.0	287.65±96.54[NN]	9.74±4.94[**▲▲]	0.79±0.49[*NN]
MCPs-treated Diabetics with Hypertension (Group C)	0	343.17±222.67[NN]	11.54±10.25[NNAB]	2.97±6.42[NNAB]
	1.5	312.34±203.00[NN]	10.62±10.55[N]	2.78±5.85[NNAB]
	3.0	327.73±208.51[NNA]	8.68±8.65	2.72±5.75[NNAB]
Placebo-treated Diabetics with Hypertension (Group D)	0	347.18±100.78[NN]	11.93±5.91[NNAAB]	2.86±5.54[NNAB]
	1.5	346.20±109.97[NNAA]	13.39±7.19[*NNAAB]	2.90±5.75[NNAB]
	3.0	349.93±113.55[NNAA]	14.00±7.55[** NNAABBCC]	2.90±5.65[NNAB]

Note. One-Way ANOVE was used for group. Paired-Samples t Test was used for different time. Values are $(\bar{x} \pm s)$. *$P<0.05$, **$P<0.01$ compared with the levels before intervention; ▲$P<0.05$, ▲▲$P<0.01$ compared with levels 1.5 months after intervention; N$P<0.05$, NN$P<0.01$ compared with levels of group N; A$P<0.05$, AA$P<0.01$ compared with levels of group A; B$P<0.05$, BB$P<0.01$ compared with levels of group B; C$P<0.05$, CC$P<0.01$ compared with levels of group C; D$P<0.05$, DD$P<0.01$ compared with levels of group D.

Changes in Leptin, Resistin, and Adiponectin Levels before and after Treatment

Throughout the study, levels of leptin and resistin in diabetics with/without hypertension were all significantly higher than that in healthy controls whereas no major difference of adiponectin levels was noted between all groups. However, following MCPs intervention, leptin levels tended to decrease in diabetics and diabetics with hypertension whereas

after placebo administration, leptin levels increased significantly in diabetics, diabetics with hypertension, and healthy controls. Meanwhile, after MCPs intervention, levels of resistin tended to decrease in diabetics with hypertension and decreased dramatically in diabetics. In contrast, after placebo administration, levels of resistin tended to increase in healthy controls and diabetics, and increased significantly in diabetics with hypertension. As for the level of adiponectin, it tended to increase in diabetics with hypertension and rose markedly in diabetics three months after MCPs administration. Nevertheless, levels of adiponectin tended to decrease in healthy controls, diabetics, and diabetics with hypertension after taking placebo, yet not to a significant level (Table 3).

TABLE 3

Changes in Leptin, Resistin, and Adiponectin Levels before and after Intervention ($\bar{x} \pm s$)

Group	Months of Intervention	Leptin(ng/mL)	Resistin(ng/mL)	Adiponectin(ng/mL)
Healthy Control (Group N)	0	1.21 ± 1.89	0.76 ± 1.86	38.59 ± 8.15
	1.5	$1.56 \pm 2.11^{*}$	0.83 ± 1.99	40.41 ± 7.47
	3.0	$1.79 \pm 2.52^{*}$	0.83 ± 1.98	40.42 ± 7.98
MCPs-treated diabetics (Group A)	0	5.52 ± 7.02^{NN}	4.44 ± 2.21^{NN}	37.18 ± 10.22
	1.5	5.04 ± 6.55^{NN}	$4.75 \pm 2.43^{*NN}$	38.18 ± 11.38
	3.0	5.10 ± 6.88^{N}	$4.70 \pm 2.56^{*NN}$	$41.98 \pm 10.83^{*▲▲}$
Placebo-treated Diabetics (Group B)	0	5.51 ± 5.03^{NN}	4.54 ± 3.60^{NN}	37.09 ± 8.64
	1.5	$5.93 \pm 5.08^{*NN}$	4.40 ± 3.56^{NN}	37.05 ± 9.41
	3.0	$5.93 \pm 4.79^{*NN}$	4.42 ± 3.56^{NN}	37.85 ± 10.05
MCPs-treated Diabetics with Hypertension (Group C)	0	4.39 ± 5.47^{N}	4.35 ± 2.62^{NN}	35.69 ± 11.04
	1.5	4.07 ± 5.42	4.36 ± 2.90^{NN}	36.30 ± 14.88
	3.0	4.44 ± 5.58	4.36 ± 2.76^{NN}	39.28 ± 17.06
Placebo-treated Diabetics with Hypertension (Group D)	0	4.24 ± 4.65^{N}	4.45 ± 3.71^{NN}	34.67 ± 9.87
	1.5	4.33 ± 4.81^{N}	$4.29 \pm 3.56^{*NN}$	33.92 ± 7.93
	3.0	$4.47 \pm 5.06^{▲N}$	$4.38 \pm 3.62^{*NN}$	34.15 ± 9.44^{NAA}

Note: One-Way ANOVE was used for group. Paired-Samples t Test was used for different time. Values are $\bar{x} \pm s$. $^{*}P<0.05$, $^{**}P<0.01$ compared with the levels before intervention; $^{▲}P<0.05$, $^{▲▲}P<0.01$ compared with levels 1.5 months after intervention; $^{N}P<0.05$, $^{NN}P<0.01$ compared with levels of group N; $^{A}P<0.05$, $^{AA}P<0.01$ compared with levels of group A; $^{B}P<0.05$, $^{BB}P<0.01$ compared with levels of group B; $^{C}P<0.05$, $^{CC}P<0.01$ compared with levels of group C; $^{D}P<0.05$, $^{DD}P<0.01$ compared with levels of group D.

Changes in Bradykinin, NO, and Prostacyclin Levels before and after Treatment

Throughout the study, levels of bradykinin were significantly higher in diabetics with/without hypertension than in healthy controls; in addition, levels of NO were markedly higher in diabetics than in diabetics with hypertension and healthy controls. Although levels of NO were higher in diabetics with hypertension than in healthy controls, but not to a significant level. Moreover, levels of Prostacyclin in diabetics with hypertension were significantly higher than those in healthy controls and diabetics throughout the study.

After MCPs administration, levels of bradykinin in healthy controls tended to increase and rose markedly in diabetics and diabetics with hypertension. In contrast, bradykinin levels fell remarkably in diabetics and diabetics with hypertension three months after taking the placebo. Meanwhile, after MCPs administration, NO levels tended to decrease in diabetics and diabetics with hypertension, but not to a significant level. As for the level of Prostacyclin, three months following placebo administration it increased markedly in diabetics, diabetics with hypertension, and healthy controls. However, levels of Prostacyclin tended to decrease in diabetics with hypertension and fell dramatically in diabetics after taking MCPs (Table 4).

TABLE 4

Changes in Bradykinin, NO, and Prostacyclin Levels before and after Intervention ($\bar{x} \pm s$)

Group	Months of Intervention	Bradykinin (ng/mL)	NO (μmol/L)	Prostacyclin (PIG2) pg/mL
Healthy Control (Group N)	0	24.37 ±14.48	12.94 ±7.35	114.58 ±167.71
	1.5	29.19 ±16.39	12.78 ±7.90	117.19 ±167.30
	3.0	28.78 ±15.64	13.41 ±6.92	118.04 ±172.28
MCPs-treated Diabetics (Group A)	0	55.19 ±13.73[NN]	27.53 ±15.19[NN]	181.08 ±93.96
	1.5	51.65 ±15.32[NN]	24.61 ±15.87[NN]	165.65 ±86.87
	3.0	59.60 ±15.99[*▲▲NN]	24.59 ±12.80[NN]	154.93 ±87.52[*]
Placebo-treated Diabetics (Group B)	0	55.76 ±17.70[NN]	27.13 ±10.49[NN]	197.51 ±108.59
	1.5	53.51 ±17.00[*NN]	29.01 ±9.90[NN]	205.92 ±110.32
	3.0	53.89 ±16.64[*NN]	29.34 ±9.62[NN]	208.02 ±112.06[*]
MCPs-treated Diabetics with Hypertension (Group C)	0	63.44 ±21.38[NN]	17.71 ±17.29[AABB]	257.98 ±236.46[NN]
	1.5	64.99 ±21.17[NNAB]	17.90 ±11.86[ABB]	236.74 ±195.32[N]
	3.0	66.64 ±20.80[*NNB]	14.59 ±11.27[AABB]	239.31 ±183.62[N]
Placebo-treated Diabetics with Hypertension (Group D)	0	69.00 ±27.10[NNAB]	17.27 ±7.28[AABB]	251.74 ±166.05[NN]
	1.5	62.86 ±24.13[**NNA]	18.75 ±5.24[ABB]	257.89 ±168.49[NNA]
	3.0	61.84 ±22.93[**NN]	18.99 ±5.28[ABB]	262.42 ±174.76[*NNA]

Note. One-Way ANOVE was used for group. Paired-Samples *t* Test was used for different time. Values are ($\bar{x} \pm s$). [*]$P<0.05$, [**]$P<0.01$ compared with the levels before intervention; [▲]$P<0.05$, [▲▲]$P<0.01$ compared with levels 1.5 months after intervention; [N]$P<0.05$, [NN]$P<0.01$ compared with levels of group N; [A]$P<0.05$, [AA]$P<0.01$ compared with levels of group A; [B]$P<0.05$, [BB]$P<0.01$ compared with levels of group B; [C]$P<0.05$, [CC]$P<0.01$ compared with levels of group C; [D]$P<0.05$, [DD]$P<0.01$ compared with levels of group D.

DISCUSSION

Diabetes is a prevalent chronic condition causing substantial morbidity and mortality, and affecting currently 40 million people in China[13]. As an extremely common morbidity in diabetic patients, hypertension leads to increased risk of cardiovascular disease[14]. According to an epidemiological study in 2006, 26.7% of hypertensive patients in China also suffered from diabetes[15]. Impaired lipid and glucose metabolism underlied the pathogenesis of both diabetes and hypertension. Therefore, as a class of ligand-activated nuclear receptors closely involved in metabolism, PPARs, LXRs, and FXRs has been attracting increasing attention in the study of pathogenesis and treatment of metabolic diseases. A growing body of evidence demonstrated that they played critical roles in controlling lipid and glucose homeostasis[5-9].

In the present study, we examined effects of MCPs on markers of PPARs, LXRs, and FXRs in type 2 diabetic patients with/without hypertension. Results from our study showed that MCPs could offer protection against diabetes and hypertension by affecting levels of molecules involved in diabetic and hypertensive pathogenesis.

Collagen is the main component of connective tissue in animals and the most abundant and widely distributed protein in mammals. Traditionally, collagen has been isolated from the skins of land-based animals, such as cow and pig. However, the outbreak of mad cow disease and foot-and-mouth disease has given rise to mounting concern over the safety of collagen products from livestock. In addition, use of livestock as the source of collagen may be limited due to religious or customary practices in some parts of the world. Therefore, the need to look for new sources of collagen becomes increasingly important. Aquatic animals, especially their skin, bones, scales, are rich in collagen. In light of the high yield of collagen from fish, fish skin provides an attractive source for collagen.

Recent studies showned that peptides derived from enzymatic hydrolysis of collagen possess a variety of physiological activities[16-17]. Many efforts are currently underway to assess the potential of marine-based collagen peptides as ACE inhibitors, antioxidants, and anti-tumor agents. For instance,

researchers found that small collagen peptides in the hydrolysate of livestock and fish inhibited the activity of angiotensin I converting enzyme, suggesting collagen peptides as promising candidates of ACE inhibitors[18]. Consistent with their studies, a recent study proved that peptides derived from catfish protein isolate significantly promoted the reduction of blood pressure via ACE inhibition[19]. Owing to their antihypertensive and antioxidative effects, collagen peptides hold enormous potential for development of health products against hypertension and diabetes.

Previous studies have established the correlation of free fatty acids, cytochrome P450, and hs-CRP with diabetes[20-22]. Consistent with these findings, we demonstrated in the present study that levels of free fatty acids, cytochrome P450, and hs-CRP were significantly higher in diabetics with hypertension than in healthy controls, confirming their involvement in diabetics. We also found that levels of hs-CRP in diabetics with hypertension were markedly higher than those in patients with diabetes alone, suggesting hypertension exacerbates inflammation in diabetics. However, after MCPs administration, levels of free fatty acid and hs-CRP in diabetics decreased dramatically and tended to decrease in diabetic and hypertensive patients. In contrast, their levels increased in patients receiving placebo. These data suggest that MCPs could lower the levels of free fatty acid, cytochrome P450, and hs-CRP in diabetic patients with/without hypertension. It suggests that MCPs could provide protection for diabetic patients with hypertension, and that this protection is more evident for patients with diabetes alone.

To further assess effects of MCPs on diabetic patients with/without hypertension, we performed assays on changes of three adipocyte-secreted hormones: leptin, resistin, and adiponectin, which are tightly implicated in diabetes[23]. Havel et al.[24] showed that adiponectin levels in patients with type 2 diabetes were lower than in non-diabetic patients. A study demonstrated that adiponectin could increase insulin sensitivity and worked with leptin to normalize insulin action in severely insulin-resistant animals. In another study, Steppan et al.[25] proved that administration of anti-resistin antibody improved blood glucose and insulin action in mice with diet-induced obesity and suggested that resistin potentially linked obesity to diabetes. As shown in this report, increase of leptin and resistin levels together with decrease of adiponectin level might be implicated in the pathogenesis of diabetes. Leptin might exert its effect during early stages of diabetes, but attenuated its action in later development of diabetes. Following MCPs intervention, levels of leptin and resistin tended to decrease in diabetics and diabetics with hypertension, whereas levels of

adiponectin tended to increase in diabetics with hypertension and rose markedly in diabetics three months after MCPs administration. By comparison, changes of these hormones showed a reverse trend in subjects receiving placebo. These data lend further support to the presumption that increase of leptin and resistin levels together with decrease of adiponectin level are implicated in the pathogenesis of diabetes. MCPs could exert protective effects for diabetics.

Furthermore, previous study has indicated that bradykinin increases insulin sensitivity and stimulates glucose uptake both in vivo and in vitro[26]. In addition, a study showed that chronic in vivo treatment with bradykinin could significantly improve whole body glucose tolerance[27]. Another study also suggested that inappropriate high levels of Prostacyclin might be involved in the development of diabetes[28]. Results from our study showed that decreased or increased levels of bradykinin and Prostacyclin might underlie the development of diabetes and its associated vascular complications. Abnormal increase of NO levels might also contribute to the onset of diabetes. After MCPs administration, levels of bradykinin rose markedly in diabetics and diabetics with hypertension. In contrast, bradykinin levels fell remarkably in diabetics and diabetics with hypertension three months after taking the placebo. Meanwhile, levels of Prostacyclin fell dramatically in diabetics after taking MCPs. These results indicated that MCPs could elevate the bradykinin level and lower the Prostacyclin level. It suggested that MCPs might exert its protective effects partly by elevating the bradykinin level and reducing the Prostacyclin level.

Taken together, results from this study showed the beneficial effects of MCPs against diabetes and hypertension by affecting levels of molecules involved in diabetic and hypertensive pathogenesis. Regulation on metabolic nuclear receptors by MCPs was the possible underlying mechanism for its observed effects in the study. This study also provideed information on the use of bioactive peptides from marine sources in the prevention and treatment of type 2 diabetes and hypertension.

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