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ORIGINAL ARTICLE

Effects of marine collagen peptides on glucose metabolism and insulin resistance in type 2 diabetic rats

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Abstract The present study was conducted to investigate the effects of marine collagen peptides (MCPs) on glucose metabolism and insulin resistance using a rat model of type 2 diabetes mellitus (T2DM). Forty T2DM obese Wistar rats were randomly assigned to receive varying doses of MCPs or a vehicle control for 4 weeks. Blood glucose and insulin levels, as well as oxidative stress and inflammation were measured. The expression of glucose transporter type 4 (GLUT4) in skeletal muscles and peroxisome proliferator-activated receptor-α (PPAR-α) in livers of T2DM rats was also measured. It was found that in the group of 9.0 g/kg/day MCPs significantly improved glucose, insulin, and homeostatic model assessment-insulin resistance, and increased the insulin sensitivity index (ISI). In addition, the groups of 4.5 and 2.25 g/kg/day MCPs significantly improved liver steatosis. It was also found that MCPs decreased expression of oxidative stress biomarkers and inflammatory cytokines and adipocytokines in T2DM rats. In conclusion, medium and high doses of MCPs (≥4.5 g/kg/day) improved glucose metabolism and insulin sensitivity in T2DM rats. These beneficial effects of MCPs may be mediated by decreasing oxidative stress and inflammation and by up-regulating GLUT4, and PPAR-α activity.

Keywords Marine collagen peptide · Insulin resistance · Oxidative stress · Inflammatory cytokines · Adipocytokine

Introduction

The global prevalence (age-standardized) of type 2 diabetes mellitus (T2DM) has nearly doubled since 1980, rising from 4.7% (108 million) to 8.5% (422 million) in the adult

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population (Jornayvaz 2016). Patients with type 2 diabetes have an increased risk of cardiovascular disease and microvascular disease (WHO, Globa lreport on diabetes 2016, http://www.who.int/diabetes/global-report/en/). Treatment options for type 2 diabetes have greatly expanded, and available medications reduce glucose levels through a variety of mechanisms, including increased insulin sensitivity and insulin secretion (Tahrani et al. 2011). Interestingly, dietary supplements, including marine bioactive peptides, have potential to prevent and treat type 2 diabetes.

Marine collagen peptides (MCPs), which are enzymatically hydrolyzed from the skin of chum salmon (Oncorhynchus keta), are oligopeptide compounds with a molecular mass ranging from 100 to 860 Da (Wang et al. 2015). MCPs have been shown to exhibit beneficial effects in metabolic syndrome, which is a cluster of the most dangerous risk factors for cardiovascular disease, including type 2 diabetes, abdominal obesity, hyperlipidemia, and hypertension (Alberti et al. 2005). MCPs can inhibit angiotensin I-converting enzyme (ACE) (Kim et al. 2012), and therefore have potential to reduce hypertension and hyperlipidemia (Wang et al. 2010). Moreover, MCPs have been shown to improve insulin sensitivity in insulin-resistant individuals and reduce the incidence of associated metabolic disorders (Zhu et al. 2010c). Recently, we demonstrated that MCPs improve glucose and lipid metabolism and blood pressure in patients with type 2 diabetes (Liu et al. 2008; Zhu et al. 2009, 2010a, b, 2013; Cui-Feng et al. 2010). However, the underlying mechanisms of MCPs effects are not fully understood.

Insulin resistance is a primary factor in the pathophysiology of type 2 diabetes. Oxidative stress, increased inflammation, and increased activity of the glucose transporter type 4 (GLUT4) and peroxisome proliferator-activated receptor-α (PPAR-α), have all been implicated in the pathogenesis of insulin resistance (Tangvarasittichai et al. 2016; Lindsay et al. 2002; Duncan et al. 2004; Shepherd and Kahn 1999; Olefsky and Saltiel 2000). MCPs can reduce insulin resistance in patients with type 2 diabetes. We therefore hypothesized that MCPs have beneficial effects on reducing oxidative stress, inflammation, and GLUT4 and PPAR-α expression. To test this hypothesis, we investigated of effects of MCPs on oxidative stress, inflammation, and expression of GLUT4 and PPAR-α in a rat model of T2DM.

Materials and methods

Preparation and identification of MCPs

MCPs (GLPGPLGPAGPK) were derived from the skin of wild-caught chum salmons (average body weight 1.47 kg), which were donated by CF Haishi Biotechnology Ltd. Co. (Beijing, China). Briefly, fish were cleaned and the meat was minced, followed by defatting (Liang et al. 2014). The materials were homogenized in distilled water and digested with mixed proteases (3000 U/g protein) at 40 °C for 3 h. The resultant hydrolysate was centrifuged at 26,000 × *g* and the supernatants were subsequently filtered through a ceramic membrane (200 mm) for purification. MCP powder was generated by ultrafiltration.

The isolated MCPs were analyzed by high-performance liquid chromatography (with a cutoff of 10,000 Da) to remove undigested proteins, followed by desalination, cryoconcentration under vacuum at 70 °C, decolorization with medicinal.

charcoal (Hangmu Corporation, Hangzhou, China), and lyophilization (Waters Corp., Milford, MA, USA) using a Phenomenex C_{18} column (10 × 250 mm), and rinse with Acetonitrile 0.05 mol/L phosphate buffer (pH 3.2, 10: 90) at a flow rate of 2.0 mL/min. We analyzed the sample at 260 nm using a Water 486 tunable UV detector.

Subsequently, the MCPs were characterized by 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 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 high-performance liquid chromatography. The MCPs contained oligopeptides with molecular weights of 130–3000 Da (S1), accounting for >95% of total proteins with a purity ≥90.6% (Pei et al. 2010).

Rat model of T2DM

Sixty adult male Wistar rats (160–180 g, aged 6 weeks) were obtained from the Department of Animal Service of Shandong University Health Science Center, Shandong, China, and were used for the experiments. The protocol followed the institutional and national guidelines for the care and use of animals. The Animal Ethics Review Committee of Southern Medical University Health Science Center, Shenzhen, China, approved all experimental procedures involving animals for this study. The rats were housed individually in rooms with controlled environmental conditions (12-h light/dark cycle, temperature approximately 25 ± 2 °C, 50% humidity) in an approved animal facility, after a 1-week adaptation period with free access to tap water and standard chow diet (SCD). Rats were assigned randomly to two groups: the normal control group (Group N, $n = 10$) and the high-cholesterol, high-fat diet (HCHFD)-induced diabetes model group ($n = 50$).

Experimental design and T2DM rat model

T2DM was induced according to methods described in our previous study (Srinivasan et al. 2005). After 2 weeks of

HCHFD feeding, freshly prepared streptozotocin [STZ, 30 mg/kg body weight (BW)] in citrate buffer (0.1 M, pH 4.5) was administered intraperitoneally three times a week (Liang et al. 2012). At approximately 2 weeks following STZ, fasting blood glucose (FBG) levels were measured using a blood glucose meter (Oxidase methods, OneTouch Ultra; Lifescan, Wayne, PA, USA). Of the 50 rats, 45 rats were considered to have T2DM with an FBG level ≥11.1 mmol/l. Forty of these diabetic rats were then randomly assigned to four groups: vehicle (group D, $n = 10$), low dose MCPs (2.25 g/kg/day; group L, $n = 10$), medium dose MCPs (4.5 g/kg/day, group M, $n = 10$), or high dose MCPs (9.0 g/kg/day, group H, $n = 10$) (Liang et al. 2012).

MCPs or vehicle were administered intragastrically every morning for 4 weeks. Rats were then tube fed for 4 weeks, with or without different doses of MCPs (via oral gavage) or vehicle (1 mL H_2O via oral gavage). The same amount of drinking water served as the blank control. Food and water intake were measured daily, and BW was measured weekly throughout the study.

The composition of the HCHFD (g/100 g food) was: 31% beef tallow, 25% cornstarch, 15.4% casein (78% protein), 8.3% maltodextrin, 6.3% sucrose, 5.0% cellulose, 4% corn oil, 3.5% mineral premix, 1% vitamin premix, 0.3% L-cysteine, and 0.2% choline bitartrate. Sucrose solution (30%) was provided as drinking fluid to HCHFD-fed rats. The SCD contained 47% cornstarch, 14.2% maltodextrin, 7% corn oil, no beef tallow, and the same percentages of other ingredients as the HCHFD. SCD-fed rats were provided normal drinking water. Mineral and vitamin mixtures purchased from Harlan Teklad (Madison, WI, USA) were fed to all rats (Wang et al. 2011). All control and STZ treated rats continued on their original diets for the duration of the study. The vehicle control group received normal saline.

At days 0 and 28 of treatment, blood samples were collected by retro-orbital sinus puncture under chloroform anesthesia. FBG levels were determined using a glucometer (OneTouch Ultra; Lifescan, Wayne, PA USA).

Biochemical analysis

After 28 days of treatment and before sacrifice for organ collection, rats were fasted overnight and weighed, and blood samples were then collected by heart puncture using a needle under chloroform anesthesia. Two tubes were used to obtain serum (with heparin) and plasma (without heparin) for each rat. Then, serum and plasma were prepared by centrifuging samples at 4000 rpm for 10 min. Plasma glucose, free fat acid (FFA), blood triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) were measured enzymatically using standard kits (Accurex Biomedical Pvt. Ltd., Thane, India) and a semi-automated analyzer (Photometer 5010 V5+; Berlin, Germany).

Enzyme-linked immunosorbent assays (ELISAs)

Fasting blood insulin (FBI), biomarkers of oxidative stress and inflammation, including malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), nitric oxide (NO), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), C-reactive protein (CRP), and adipocytokines, including leptin, resistin, adiponectin and PPAR-γ, were measured using commercially available ELISA kits according to the manufacturers' instructions (Millipore Co., Billerica, MA, USA). All samples and standards were determined in triplicate. Homeostatic model assessment-insulin resistance (HOMA-IR) and insulin sensitivity index (ISI) in diabetic rats were calculated as follows: HOMA-IR = (FBG × FBI)/22.5; ISI = 1/(FBG × FBI) (Bhatnagar et al. 2011).

Histological analysis

Four weeks after HCHFD treatment and MCP administration, all rats were sacrificed. Livers were fixed in 4% phosphate-buffered saline-buffered formalin for at least 24 h, followed by processing for conventional paraffin embedding. Sections (5 um thickness) were mounted on glass slides, dewaxed, rehydrated with distilled water, and stained with hematoxylin and eosin (H&E). The livers were examined to identify pathological changes.

Immunohistochemistry (PPAR-α and GLUT4)

Paraffin-embedded tissues of liver and skeleton muscle were sectioned (5 um), and antigen retrieval was performed using 10 mM sodium citrate buffer. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide in methanol for 15 min. Tissues were treated with polyclonal rabbit anti-PPAR-α or anti- GLUT4 (Cell Signaling Technology, Inc., Danvers, MA USA; 5% free-fat milk dilution 1:400) overnight at 4 °C. Specific labeling was detected with a peroxidase-conjugated goat anti-rabbit IgG and avidin–biotin peroxidase complex. Slides were then mounted with coverslips and analyzed by two pathologists who were unaware of the origin of the slides. PPAR-α positive cells in the livers were counted randomly in ten fields from each sample. The cumulative optical density value [IOD, IOD = area (area) × average density (density scheme)] was used to evaluate the signal of GLUT4 in the skeletal muscle, with a higher IOD value indicating a stronger immunohistochemical signal.

Statistical analysis

All data are expressed as means and standard error. The significance of differences between experimental and control values were examined using one-way analysis of variance. SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used to perform analyses. A p value <0.05 was considered significant.

Results and discussion

MCPs improve glucose metabolism and insulin resistance and prevent body weight loss in type 2 diabetic rats

The high dose of MCPs significantly prevented body weight loss ($p < 0.0001$; H vs D) during T2DM development in rats (Fig. S1A). To determine whether MCPs improve glucose metabolism and insulin resistance in rat model, we measured blood glucose and insulin levels in diabetic rats, diabetic rats treated with MCPs, and normal controls. The glucose and insulin levels were significantly higher in the diabetic rats compared to the normal controls ($p < 0.001$). The high dose of MCPs significantly decreased glucose ($p = 0.026$; H vs D) and insulin ($p < 0.001$; H vs D) levels in the T2DM rats (Fig S1B). These results suggested that a high dose of MCPs decreased glucose and insulin levels in diabetic rats.

To further evaluate the insulin-resistant state, we measured HOMA-IR and ISI in diabetic rats, diabetic rats treated with MCPs, and normal controls. We found that HOMA-IR was significantly increased in the diabetic rats compared to the normal controls ($p < 0.001$). The high dose of MCPs significantly decreased HOMA-IR ($p = 0.003$). Consistently, ISI was significantly lowered in the diabetic rats compared to the normal controls ($p < 0.001$), and ISI was significantly higher in the diabetic rats treated with high dose MCPs compared to the diabetic controls ($p = 0.003$). These results indicate that MCPs improve insulin resistance in diabetic rats (Fig. S1B).

MCPs improve blood lipid metabolism in type 2 diabetic rats

Tissue damage from blood lipid metabolism disorder is a manifestation of insulin resistance during T2DM. To investigate the beneficial effects of MCPs on blood lipid metabolism, we assayed biomarkers of blood lipids (FFA, TG, TC, HDL cholesterol, and LDL cholesterol) levels. After 4 weeks, the medium and high doses of MCPs significantly improved blood lipid levels (FFA, $p = 0.026$; H vs D; TG, $p = 0.001$, 0.0005; M and H vs D) (Fig. S2).

MCPs decrease oxidative stress and inflammatory cytokines in type 2 diabetic rats

Oxidative stress has been implicated in insulin resistance (Muoio and Newgard 2008; Matsuda and Shimomura 2013). To investigate whether MCPs decreased oxidative stress, we measured blood levels of SOD, GSH, NO, and MDA in diabetic rats, diabetic rats treated with MCPs, and normal. The levels of GSH and NO were significantly lower in the diabetic controls compared to the normal controls ($p = 0.005$ and $p = 0.002$, respectively), whereas the levels of MDA were significantly higher in the diabetic controls compared to the normal controls ($p < 0.001$). This suggested that oxidative stress increased in diabetic rats. After treatment with MCPs, the levels of SOD, GSH and NO were significantly increased in the diabetic rats treated with the high dose of MCPs compared to the diabetic controls, whereas the levels of MDA were significantly decreased in the diabetic rats treated with MCPs compared to the diabetic controls. We further found that MCPs decreased oxidative stress and inflammation in T2DM rats, as indicated by increased levels of GSH ($p = 0.044$; H vs D), SOD ($p < 0.001$; M and H vs D), NO ($p < 0.05$; M and H vs D), and decreased expression of MDA ($p < 0.001$; L, M and H vs D). These results indicate that MCPs decrease oxidative stress in diabetic rats (Fig. 1A).

It is well recognized that inflammatory cytokines are manifestation of oxidative stress (Ceriello and Motz 2004). To test whether MCPs decreased inflammation in diabetic rats, we measured blood levels of TNF-α, IFN-γ and CRP in diabetic rats, diabetic rats treated with MCPs, and normal controls. The TNF-α, IFN-γ and CRP levels were significantly higher in the diabetic controls compared to the normal controls ($p = 0.006$, $p < 0.001$, and $p < 0.001$, respectively), indicating that inflammation increased in diabetic rats. After treatment with MCPs, the TNF-α levels were significantly decreased in the diabetic rats treated with the high dose of MCPs compared to the diabetic controls ($p = 0.026$; H vs D), whereas the IFN-γ levels were significantly decreased in the diabetic rats treated with MCPs compared to the diabetic controls ($p < 0.001$; L, M and H vs D). Interestingly, the CRP levels were significantly lower in all three MCP treatment groups compared to the diabetic control group ($p < 0.001$; L, M and H vs D) (Fig. 1B). These results suggest that MCPs decrease inflammatory cytokines in diabetic rats.

MCPs regulate levels of adipocytokines in type 2 diabetic rats

Adipocytokines have been associated with the development of diabetes (Lindsay et al. 2002; Duncan et al. 2004; Abate et al. 2014; Silha et al. 2003). To test if MCPs alter

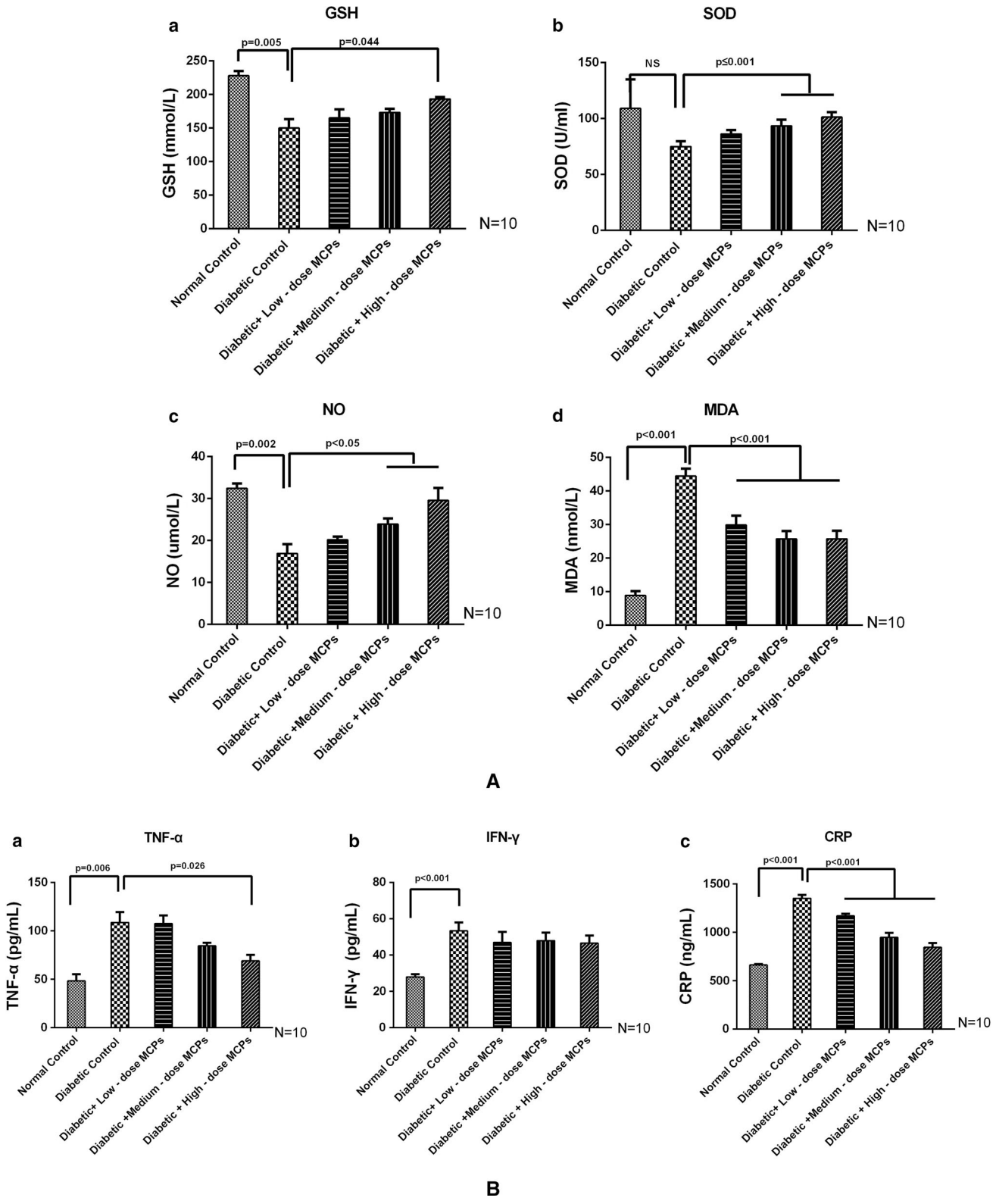

Fig. 1 Effects of MCPs on the expression of oxidative stress biomarkers in type 2 diabetic rats. Normal rats received a vehicle control and obese Wistar rats with type 2 diabetes received a vehicle control or different doses of MCPs. Each group included 10 rats. After 4 weeks, blood levels of SOD, GSH, NO, and MDA (**A**), and TNF-α, IFN-γ, and CRP (**B**) were measured

adipocytokine levels in diabetic rats, we measured blood levels of adiponectin, leptin and resistin in diabetic rats diabetic rats treated with MCPs and normal controls. We found that adiponectin levels were significantly lower in the diabetic rats compared to the normal controls ($p < 0.001$). MCP treatment significantly increased adiponectin in all three treatment groups of diabetic rats compared to the diabetic control group ($p < 0.001$). Leptin and resitin were significantly higher in the diabetic rats compared to the normal controls ($p = 0.027$ and $p = 0.011$, respectively). Moreover, in rats with HCHFD-induced T2DM, we found that MCPs increased adiponectin ($p < 0.001$; L, M and H vs D) and decreased leptin ($p = 0.034$; H vs D) and resistin ($p = 0.014$, 0.006; M and H vs D) levels (Fig. 2). These results suggest that MCPs enhance insulin sensitivity through regulating adipocytokine release in diabetic rats.

MCPs up-regulate GLUT4 expression in the skeletal muscle of diabetic rats

GLUT4, the insulin-responsive glucose transporter expressed in adipose tissue and skeletal muscle, plays an important role in glucose uptake and insulin sensitivity (Liu et al. 2008). To test whether MCPs impact expression of GLU4, we measured GLUT4 levels in the skeletal muscle via immunohistochemistry. We found that the expression of GLUT4 in the skeletal muscle was significantly lower in the diabetic rats compared to the normal controls ($p < 0.001$), and that the high dose of MCPs significantly increased GLUT4 expression in the diabetic rats ($p = 0.005$; Fig. 3). These results suggest that a high dose of MCPs can enhance insulin sensitivity through up-regulating GLUT4 expression in diabetic rats.

MCPs improve liver steatosis in type 2 diabetic rats

Liver steatosis is a manifestation of insulin resistance. To investigate the effects of MCPs on liver steatosis, we measured liver steatosis in diabetic rats, diabetic rats treated with MCPs, and normal controls by H&E staining. We found that MCP treatment significantly improved liver steatosis in the diabetic rats (Fig. 4). These results suggest that MCPs enhance insulin sensitivity and improve liver steatosis.

MCPs up-regulate PPAR-α expression in the livers of diabetic rats

PPAR-α is a nuclear hormone receptor and plays an important role in the modulation of insulin sensitivity (Grygiel-Gorniak 2014; Kusminski et al. 2016). To test whether MCPs increase the expression of PPAR-α, we measured the levels of PPAR-α in the liver via immunohistochemistry. The expression of PPAR-α in the liver was significantly decreased in the diabetic rats compared to the normal controls ($p = 0.005$), and MCP treatment significantly increased PPAR-α expression in livers of diabetic rats ($p < 0.05$; M and H vs D) (Fig. 4). These results suggest that MCPs enhance insulin sensitivity through increasing expression of PPAR-α in the liver.

It has long been understood that proper diet, exercise and drugs are the cornerstones in the prevention and treatment of insulin resistance. However, a number of medications are associated with severe side effects and high costs. MCPs have been widely used as functional foods or dietary supplements due to their homology with human collagen in terms of structure, safety profile, stability, biocompatibility (Ennaas et al. 2016), high bioavailability through gastrointestinal barrier, and potent

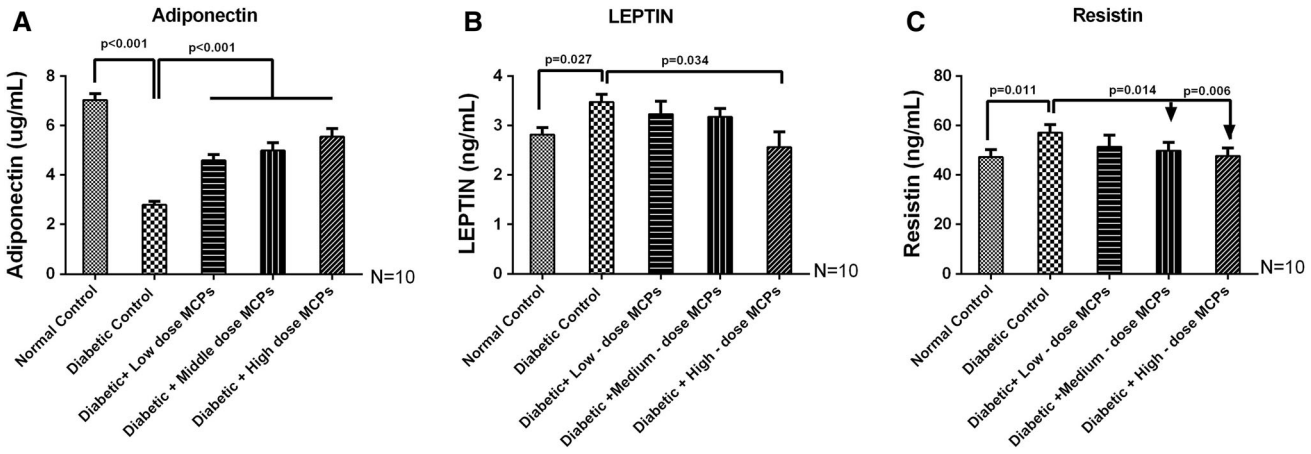

Fig. 2 MCPs regulate expression of inflammation biomarkers in type 2 diabetic rats. Normal rats received a vehicle control and obese Wistar rats with type 2 diabetes received a vehicle control or different doses of MCPs. Each group included 10 rats. After 4 weeks, blood levels of adiponectin (**a**), leptin (**b**) and resistin (**c**) were measured

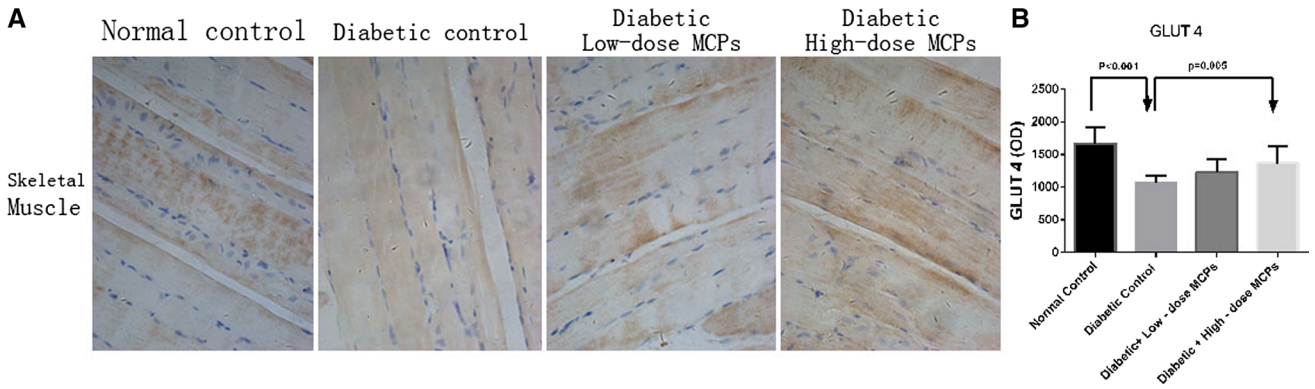

Fig. 3 MCPs up-regulate GLUT4 expression in the skeletal muscle of type 2 diabetic rats. Normal rats received a vehicle control and obese Wistar rats with type 2 diabetes received a vehicle control or different doses of MCPs. Each group included 10 rats. After 4 weeks, rats were sacrificed, and skeletal muscle was observed using immunohistochemistry. The cumulative optical density value (IOD) was used to evaluate the signal of GLUT4 in the skeletal muscle, with a higher IOD value indicating a stronger immunohistochemical signal

bioactivities (De Luca et al. 2016). However, there is very little evidence describing the mechanistic benefits of MCPs for T2DM. In this study, we showed that MCPs improve glucose metabolism and insulin resistance in a rat model of type 2 diabetes. This improvement is probably due to the beneficial effects of MCPs on oxidative stress, inflammation, and GLUT4 and PPAR-α expression. To our knowledge, this is the first report revealing the mechanisms by which MCPs improve insulin resistance in type 2 diabetes.

Insulin resistance is a key feature of the progression of type 2 diabetes. Our study showed that MCPs significantly improved glucose metabolism and insulin resistance in type 2 diabetic rats, as indicated by decreased levels of glucose and insulin and HOMA-IR, as well as increased ISI. These observations in the type 2 diabetic rats are consistent with our previous findings in patients with type 2 diabetes (Zhu et al. 2010a, b). Moreover, we found that MCPs improve liver steatosis and blood lipids in type 2 diabetic rats, providing additional evidence that MCPs can improve insulin resistance (Kang et al. 2015). Oxidative stress plays important roles in insulin resistance (Tangvarasittichai 2015). Since inflammatory mediators such as adhesion molecules and interleukins induce oxidative stress, inflammation is now recognized as one manifestation of oxidative stress (Roebuck 1999) and can be involved in the pathogenesis of insulin resistance and type 2 diabetes (Hu and Stampfer 2003; Schmidt et al. 1995; Kaul et al. 2010).

We subsequently investigated and identified possible mechanisms that could contribute to the effects of MCPs, including oxidative stress and inflammation. The major concern regarding safety and clinical feasibility of regular intake of MCPs has been raised from the well established fact that induction of collagen synthesis, mainly assessed by increased hydroxyproline levels, is often associated with oxidative stress (De Luca et al. 2016). Our current observations demonstrate that MCPs significantly reduced biomarkers of oxidative stress and inflammation (Imperatore et al. 2014), suggesting that MCPs improve insulin resistance through regulating oxidative stress and inflammation during diabetes induced by HCHFD. Adipocytokines have been implicated in the development of insulin resistance and diabetes (Abate et al. 2014; Lindsay et al. 2002; Duncan et al. 2004). Adiponectin is decreased and leptin and resistin are increased in obese and insulin resistant patients (Silha et al. 2003). Our rat model of type 2 diabetes had decreased adiponectin and increased leptin and resistin levels, which is consistent with the findings in patients (Shepherd and Kahn 1999). Interestingly, our study shows that even a low dose of MCPs can increase the levels of adiponectin and decrease the levels of leptin and resitin in type 2 diabetic rats.

PPAR-α is primarily expressed in tissues with a high capacity for fatty acid oxidation, such as the liver, heart, and skeletal muscle. PPAR-α also plays a role in glucose homeostasis and insulin resistance development (Grygiel-Gorniak 2014) If the concentration of fatty acids increases, PPAR-α is activated and takes up oxidized forms of these acids. Oxidation of fatty acids is mainly present in the liver where it prevents steatosis in the case of starvation and fasting. During the influx of fatty acids, transcription of PPAR-α regulated genes is stimulated and the oxidation systems (microsomal omega-oxidation system, and mitochondrial and peroxisomal beta-oxidation) are activated. This activation and increased PPAR-α sensing in the liver result in increased energy burning and reduced fat storage. PPAR-α functions as a lipid sensor such that it controls energy combustion. It also plays a prominent role in the pathogenesis of fatty liver disease (FLD), and ligands of this receptor might be effective at reducing hepatic staetosis and insulin resistance by increasing energy utilization (Grygiel-Gorniak 2014).

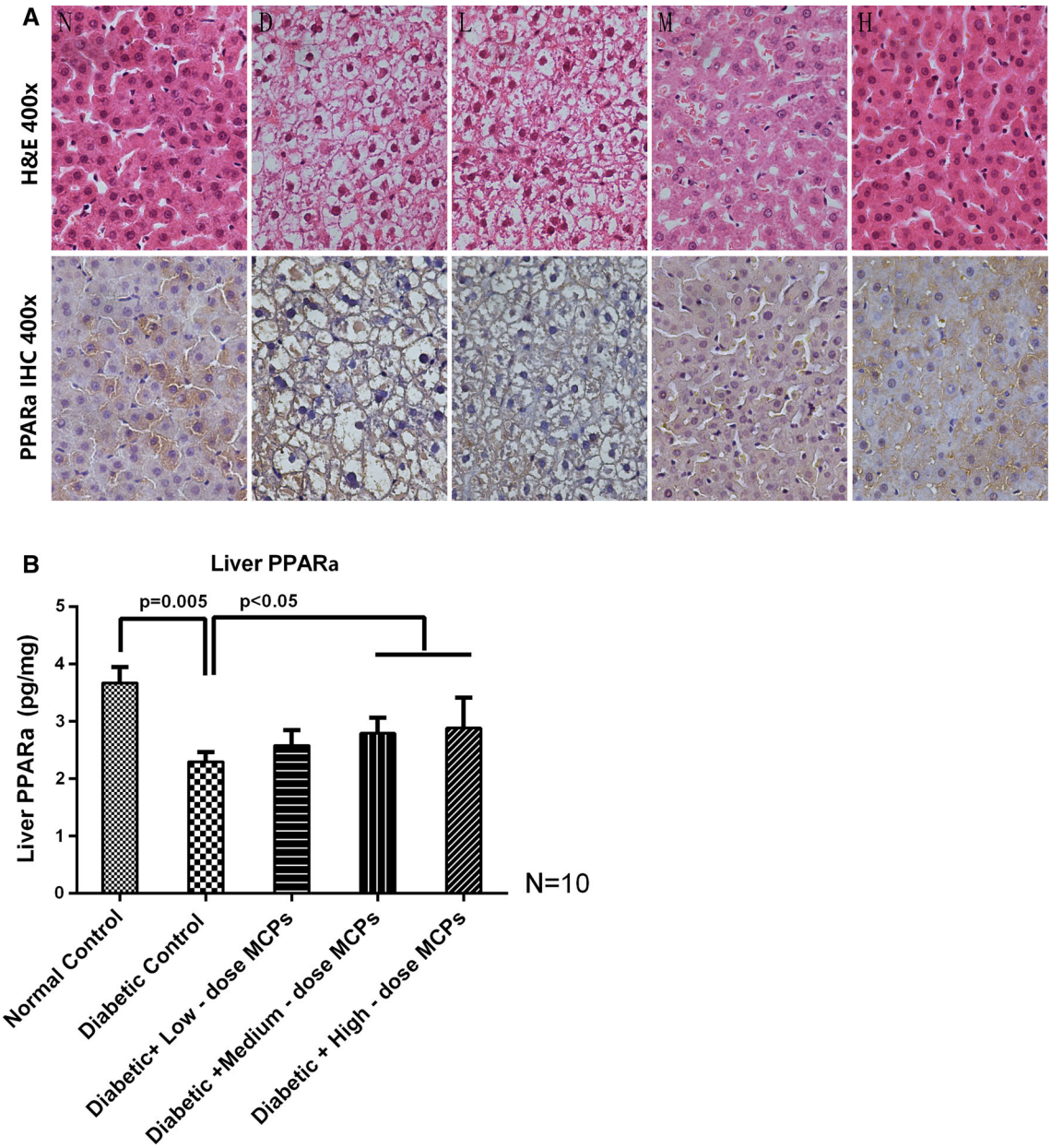

Fig. 4 MCPs improve liver steatosis and up-regulate PPAR-α expression in livers from type 2 diabetic rats. Normal rats received a vehicle control and obese Wistar rats with type 2 diabetes received a vehicle control or different doses of MCPs. Each group included 10 rats. After 4 weeks, rats were sacrificed, and livers were stained by H&E and immnuohistochemistry. PPAR-α positive cells were counted randomly in ten fields from each sample

Three pathways regulate blood glucose levels: the AMP-activated protein kinase (AMPK), phosphatidylinositol 3-kinase (PI3 K), and mitogen-activated protein kinase (MAPK) pathways. The AMPK pathway is mainly regulated by exercise, whereas the PI3 K and MAPK pathways are active when insulin binds to its receptor in the peripheral tissues (adipose, liver, and skeletal muscle) to regulate blood glucose. Among these three pathways, the PI3 K/AKT pathway is of vital significance and has been extensively studied (Lee et al. 2016). Active PI3 K stimulates the phosphorylation and activation of its downstream molecule AKT, thus promoting the transmembrane activity of GLUT4 and consequently increasing glucose uptake (Moraes-Vieira et al. 2016).

Skeletal muscle cells consume nearly 80% of ingested glucose; therefore, they have the highest level of insulin-stimulated glucose uptake and are the main site of insulin resistance (Wu et al. 2016). GLUT4 translocation to the cell surface is the final step of the insulin-stimulated glucose uptake process, and the quantity of GLUT4 at the

muscle cell surface is the rate-limiting step of glucose disposal (Wu et al. 2016). Therefore, we examined the expressions of GLUT4 in muscle. Our immunohistochemistry experiments highlighted the effects of MCPs on the expression of GLUT4 and PPAR-α, both of which play important roles in insulin sensitivity (Shepherd and Kahn 1999; Olefsky and Saltiel 2000). Decreased expression and impaired cell membrane translocation of GLUT4 are involved in the pathogenesis of type 2 diabetes (Yan et al. 2014). Our study showed that expression of GLUT4 in skeletal muscle was significantly decreased in type 2 diabetic rats, and that the high dose MCP treatment significantly increased GLUT4 expression. Furthermore, we observed that MCPs significantly increased PPAR-α expression in the livers of diabetic rats.

Conclusion

Our study shows that certain doses of MCPs (≥4.5 g/kg BW/d) improve glucose metabolism and insulin resistance in a rat model of type 2 diabetes. This improvement might be mediated by the beneficial effects of MCPs on oxidative stress, inflammation, and GLUT4 and PPAR-α expression (S Fig. S3). Further investigation is needed to confirm these conclusions.

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Compliance with ethical standards

Conflict of interest The authors have no potential conflicts of interest relevant to this article.

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