Antidiabetic Effect and Mechanism of Chitooligosaccharides

Chuanxia Ju, a Wang Yue, a Zhihong Yang, a Quanfang Zhang, b Xue Yang, a Zhantao Liu, a and Fang Zhang a

a Pharmaceutical Department, Medical College of Qingdao University; Qingdao 266021, Shandong, China; and b Cardiovascular Department, The Affiliated Hospital of Medical College of Qingdao University; Qingdao 266003, Shandong, China. Received March 5, 2010; accepted May 10, 2010; published online June 15, 2010

The aim of this study was to observe the antidiabetic effect and mechanism of chitooligosaccharides (COS). Type 2 diabetic rats were fed a high-energy diet together with an injection of streptozotocin (STZ). After 8 weeks of COS treatment, the changes in glycometabolism, insulin sensitivity, serum hepatic marker enzyme levels, liver glycogen content, expressions of glucose transporter GLUT-4, malonaldehyde content, superoxide dismutase activity and morphology of the pancreases were observed. The results showed that COS significantly reduced fasting blood glucose (FBG), fasting insulin (FINS), increased the insulin sensitivity index (ISI) and improved oral glucose tolerance. COS increased liver glucokinase activity and glycogen content and upregulated the expressions of GLUT-4 mRNA in adipose and soleus muscle. They also raised the superoxide dismutase activity and reduced the malonaldehyde content in pancreas homogenate. Pancreas hematoxylin/eosin (HE) staining of the diabetic rats showed ruptured islet, but changes of pancreatic islet in the animals were minimized by administration of COS. The effect of COS on pancreatic β cell (INS-1) in vitro was also examined. It was found that COS played important roles in INS-1 cells by promoting proliferation, increasing glucose stimulated insulin release, upregulating the expressions of GLUT-2 mRNA and protecting against STZ-induced apoptosis. The results from the present study indicate COS have protective effect for type 2 diabetes by ameliorating insulin resistance, promoting the proliferation of β cells, increasing insulin secretion and protecting β cells.

Key words chitooligosaccharide; type 2 diabetes; insulin resistance; β cell

Chitooligosaccharides (COS) are partially hydrolyzed products of chitosan which is obtained by deacetylation of chitin, a major component of the exoskeleton in crustaceans. COS are of great interest in pharmaceutical and medicinal applications due to their high solubility and nontoxicity. Previous studies have shown that COS have various properties such as free radical scavenging activity, protective effects against infections and antitumor enhancing properties. COS also show a very promising antidiabetic effect in non-obese diabetic streptozotocin (STZ)-induced rats. However, the antidiabetic effect has not been duplicated in insulin-resist rats, and little information on the exact mechanism of action has been available to date. The aim of this study was to determine the relationship between the antidiabetic effects of COS and improvement insulin resistance and β cell dysfunction.

MATERIALS AND METHODS

Treatment of Animals The experiments were conducted on male Sprague-Dawley rats (Experimental Animal Center of Hebei Province, China) weighing 160—180 g. Rats were randomized into two groups. The control group was fed a basal diet whereas the others consumed a high-energy diet (HD) of 20% sucrose and 10% lard. All animal care and surgery protocols were designed in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals published by the NIH, U.S.A. Five weeks later, a diabetic model was induced from epididymal fat pads of rats as described in a previous report. The diabetic rats were randomized into 4 groups, namely: model group; COS L (COS, 500 mg/kg·d, average molecular: 1.5 kDa, deacetylated degree: 86.5%, Jinan Haidebei Marine Bioengineering Co., Ltd., China); COS H (COS, 1000 mg/kg·d) group and Met group (metformin, 200 mg/kg·d, Sinways Pharmaceutical Co., Ltd., China). All rats were administered by gavage. The body weight and feed intake were recorded, and 8 weeks later, an oral glucose tolerance test was performed. After administration of 20% glucose solution (2 g/kg), blood samples were drawn from the tail vein at 0, 30, 60 and 120 min. Blood glucose was checked by a portable glucometer. Blood samples of COS treatment group were centrifuged at 3000 rpm for 15 min and the separated serum was collected. Soleus muscle from rat hindlimb was removed and trimmed of connective tissue, fat and nerves. Adipose was isolated from epididymal fat pads of rats as described in a previous report. Soleus muscle and adipose were frozen rapidly in liquid nitrogen and stored until further analysis. The liver and pancreases were carefully removed, and pancreas to body weight ratio was evaluated. Part of the pancreas was fixed with 4% paraformaldehyde, embedded in paraffin and sequentially sectioned. After hematoxylin/eosin (HE) staining, a section of each block was observed at a magnification of 400×. Part of the pancreas was homogenized in physiological saline with a homogenizer at 4°C.

Analysis of Blood Samples Fasting blood glucose (FBG) was determined by enzymatic methods using an automatic blood chemical analyzer (Hitachi 747 auto-analyzer, Tokyo, Japan). Fasting insulin (FINS) was measured with a radioimmunoassay kit (Beijing Northern Institute of Biotechnology, China). Insulin sensitivity index (ISI=ln(1/FBG×diabetic rats). The diabetic rats were randomized into 4 groups, namely: model group; COS L (COS, 500 mg/kg·d, average molecular: 1.5 kDa, deacetylated degree: 86.5%, Jinan Haidebei Marine Bioengineering Co., Ltd., China); COS H (COS, 1000 mg/kg·d) group and Met group (metformin, 200 mg/kg·d, Sinways Pharmaceutical Co., Ltd., China). All rats were administered by gavage. The body weight and feed intake were recorded, and 8 weeks later, an oral glucose tolerance test was performed. After administration of 20% glucose solution (2 g/kg), blood samples were drawn from the tail vein at 0, 30, 60 and 120 min. Blood glucose was checked by a portable glucometer. Blood samples of COS treatment group were centrifuged at 3000 rpm for 15 min and the separated serum was collected. Soleus muscle from rat hindlimb was removed and trimmed of connective tissue, fat and nerves. Adipose was isolated from epididymal fat pads of rats as described in a previous report. Soleus muscle and adipose were frozen rapidly in liquid nitrogen and stored until further analysis. The liver and pancreases were carefully removed, and pancreas to body weight ratio was evaluated. Part of the pancreas was fixed with 4% paraformaldehyde, embedded in paraffin and sequentially sectioned. After hematoxylin/eosin (HE) staining, a section of each block was observed at a magnification of 400×. Part of the pancreas was homogenized in physiological saline with a homogenizer at 4°C.
FINS) was calculated according to the method of Li and Pan. Analysis of Glucose Transporter-4 (GLUT-4) mRNA in Soleus Muscle and Adipose The expression of GLUT-4 mRNA was determined using reverse transcription-polymerase chain reaction (RT-PCR) with total RNA. Total RNA was isolated from the soleus muscle and adipose using Trizol reagent (Sigma, U.S.A.). CDNA was synthesized from 2 μg of each RNA sample using oligo(dT)_{12-18} (0.5 μg) and reverse transcriptase (15 U) at 42 °C for 60 min. For PCR, 12 μl of 1×PCR buffer containing 0.42 U of Taq polymerase (Roche Diagnostics GmbH, Germany) and 0.24 μM of primer was added. Primers for rat GLUT-4 were, sense: 5'-AGA GTG CCT GAA ACC-3', anti-sense: 5'-CCC TAA GTA TTC AAG TTC TG-3'. Primers for rat β-actin were, sense: 5'-TAC AAC CTC TTT GCA GCT CC-3', anti-sense: 5'-GGG TCT TCA TGA GTG AGT CAG TC-3'. The PCR conditions were as follows: 1 cycle of 94 °C for 5 min; 25 cycles of 94 °C for 30 s, 60 °C for 1 min, and 70 °C for 1 min; and 1 cycle of 72 °C for 5 min. The RT-PCR products were separated and visualized on an ethidium bromide-stained 1.0% agarose gel. The semi-quantitative analysis of each product was performed by densitometry using Image J and calculated relative to β-actin.

Measurement of Superoxide Dismutase Activity and Malonaldehyde Content in Pancreas Homogenate Superoxide dismutase activity was assayed by the method of Kakkar et al. with a superoxide dismutase kit (Nanjing Jiancheng Bioengineering Institute, China). Malonaldehyde content was estimated by the method of Draper and Hadley with a malonaldehyde kit (Nanjing Jiancheng Bioengineering Institute). Protein contents in the tissue homogenate were measured by the Bradford method.

Measurement of Serum Hepatic Marker Enzymes and Glycogen Content in Liver Homogenate Two hundred milligrams of liver was homogenized in physiological saline at 4 °C, and glycogen content was measured by the anthrone-sulfuric colorimetric method. Liver glucokinase activity was tested using commercial kits (Nanjing Jiancheng Bioengineering Institute). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using a chemistry analyzer (Hitachi 747 auto-analyzer, Tokyo).

Cell Culture The INS-1 cell (China Center for Type Culture Collection, China) was maintained in RPMI 1640 (Hy Clone, U.S.A.) supplemented with 10% fetal calf serum (PAA, Austria), 2 mmol/l l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mmol/l sodium pyruvate, and 50 μmol/l 1-mercaptoethanol.

Effects of COS on Proliferation of INS-1 Cells After incubation with COS at different concentrations, the INS-1 cell viability was estimated with CCK-8 kits (Dojindo, Japan), and the absorbance was determined at 450 nm using an enzyme-linked immunosorbent assay (ELISA) reader.

Measurement of Glucose-Stimulated Insulin Release After 48 h of treatment with COS, INS-1 cells were washed with Krebs-Ringer bicarbonate-Hepes buffer (KRBB) and incubated for an additional 60 min in 1 ml of KRBB buffer containing 1.67 and 16.7 mmol/l of glucose, respectively. Media were stored at −20 °C until insulin measured with a radioimmunoassay kit (Beijing Northern Institute of Biotechnology, China).

Analysis of GLUT-2 mRNA in INS-1 Cells After incubation with COS for 48 h, the expressions of GLUT-2 mRNA in INS-1 cells were determined using RT-PCR. Primers for rat GLUT-2 were, sense: 5'-CAA TTT CAT CAT CGC CCT CT-3', anti-sense: 5'-GGA TCT TCA TGA GTG AGT CAG TC-3'. The PCR conditions were as follows: 1 cycle of 94 °C for 5 min; 25 cycles of 94 °C for 30 s, 60 °C for 1 min, and 70 °C for 1 min; and 1 cycle of 72 °C for 5 min. The RT-PCR analysis was done as described above.

Flow Cytometry Analysis of Proinflammatory Cytokine-Induced Apoptosis Interleukin (IL)-1β (10 μg/l), interferon (IFN)-γ (50 μg/l) and tumor necrosis factor (TNF)-α (50 μg/l) (Pepro Tech Inc., U.S.A.) were used to induce the apoptosis of INS-1 cells. COS was added to the medium 1.5 h before cytokines, and then cultured with cytokines for 12 h, the treated cells were then harvested by centrifugation and washed with phosphate buffered saline (PBS) three times. The cells were fixed with ice-cold PBS, stained with annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (Beijing Biosea Biotechnology Co., Ltd., China), and then measured by flow cytometry to detect apoptosis. A minimum of 10000 events was analyzed in each experiment.

Flow Cytometry Analysis of STZ-Induced Apoptosis Two millimolar per liter STZ was used to induce the apoptosis of INS-1 cells. COS was added to the medium 1.5 h before STZ. After being cultured with STZ for 12 h, the treated cells were harvested and then measured by flow cytometry to detect apoptosis. The procedure was done as described above.

Statistical Analysis All data were expressed as the mean±S.D. Statistical significance was determined using t-test or analysis of variance with Bonferroni post-hoc comparison. A level of p<0.05 was accepted as indicating statistical significance.

RESULTS

Effects of COS on the Feed Intake, Body Weight Gain, FBG, FINS and ISI As shown in Table 1, feed intake and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Model group</th>
<th>COS L group</th>
<th>COS H group</th>
<th>Met group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake/rat/d (g)</td>
<td>26.9±2.1</td>
<td>30.6±4.2</td>
<td>28.4±4.1</td>
<td>29.5±2.9</td>
<td>28.2±3.4</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>364.4±39.7</td>
<td>339.7±93.9</td>
<td>377.8±84.8</td>
<td>369.7±91.6</td>
<td>397.5±74.6</td>
</tr>
<tr>
<td>FBG (mmol/l)</td>
<td>4.47±0.25</td>
<td>14.72±2.15*</td>
<td>16.46±2.08*</td>
<td>9.73±2.47</td>
<td>4.92±0.84*</td>
</tr>
<tr>
<td>FINS (mIU/l)</td>
<td>28.04±3.26</td>
<td>45.47±5.07*</td>
<td>37.83±3.56*</td>
<td>32.32±4.96*</td>
<td>39.94±3.26</td>
</tr>
<tr>
<td>ISI</td>
<td>-4.86±0.23</td>
<td>-6.51±0.43*</td>
<td>-5.98±0.36*</td>
<td>-5.75±0.38*</td>
<td>-5.82±0.23*</td>
</tr>
</tbody>
</table>

*p<0.05 vs. Control group; *p<0.05 vs. Model group; **p<0.05 vs. Met group.
body weight gain in 13 weeks were not different. Rats of the model group showed significant increase in FBG, FINS and decrease in ISI when compared with control rats \((p<0.05)\). Relatively lower FBG and FINS concentrations were observed in the COS H and COS L groups. COS or metformin treatment induced an increase in ISI \((p<0.05)\), and there were marked differences in FBG and FINS between the COS H and metformin (Met) group \((p<0.05)\). These results suggest that COS are effective to reduce diabetes-related symptoms in an HD diet-fed STZ-induced type 2 diabetes rat model.

**Effects of COS on Glucose Tolerance** Table 2 showed that model rats had significantly higher levels of blood glucose than non-diabetic rats after glucose administration, indicating the presence of abnormal glucose tolerance in the model group. In diabetic rats, the increase in glucose level at 30, 60 and 120 min was significantly attenuated by the treatment with COS or metformin \((p<0.05)\). On the other hand, there were marked differences in blood glucose levels after glucose loading between COS and Met treated animals \((p<0.05)\). This showed that COS improved oral glucose tolerance, but did not normalize it.

**Effect of COS on Liver Glycogen, Liver Glucokinase Activity and Serum Hepatic Marker Enzymes** Compared to control rats, rats of the model group had significantly lower liver glycogen level and liver glucokinase activity \((p<0.05)\). Administration of COS or metformin to diabetic rats could increase liver glycogen level and liver glucokinase activity \((p<0.05)\), but there were marked differences between COS and Met treated animals \((p<0.05)\). STZ induced significant elevations in ALT and AST levels when compared to control values \((p<0.05)\). ALT and AST values of the COS-treated group were relatively lower than the model group, but the data were not significantly different (Table 3).

**Effect of COS on GLUT-4 mRNA in Soleus Muscle and Adipose** We found decreases of GLUT-4 mRNA in the soleus muscle and adipose of model group compared to the control group. With the COS or Met treatment for 8 weeks, the GLUT-4 mRNA expressions were elevated in soleus muscle and adipose \((p<0.05, \text{Fig. 1})\).

**Effect of COS on Superoxide Dismutase Activity and Malonaldehyde Content in Pancreas Homogenates** As

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>Control group</th>
<th>Model group</th>
<th>COS L group</th>
<th>COS H group</th>
<th>Met group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min</td>
<td>4.97±0.35</td>
<td>15.53±2.36*</td>
<td>10.44±1.23</td>
<td>9.68±2.11</td>
<td>5.14±0.76</td>
</tr>
<tr>
<td>30 min</td>
<td>7.53±1.12</td>
<td>20.20±3.46*</td>
<td>14.42±3.74*</td>
<td>13.44±2.13*</td>
<td>7.74±0.99</td>
</tr>
<tr>
<td>60 min</td>
<td>6.54±1.56</td>
<td>29.48±4.72*</td>
<td>16.63±3.26*</td>
<td>15.49±3.08*</td>
<td>7.81±1.02</td>
</tr>
<tr>
<td>120 min</td>
<td>6.39±0.79</td>
<td>28.42±3.69*</td>
<td>15.76±2.87*</td>
<td>14.62±2.81*</td>
<td>7.64±0.95</td>
</tr>
</tbody>
</table>

\(* p<0.05 \text{ vs. Control group}; \# p<0.05 \text{ vs. Model group}\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Model group</th>
<th>COS L group</th>
<th>COS H group</th>
<th>Met group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver glycogen (mg/100 g tissue)</td>
<td>727.22±67.54</td>
<td>314.09±34.26*</td>
<td>460.47±83.67*</td>
<td>518.21±69.46*</td>
<td>646.21±75.36*</td>
</tr>
<tr>
<td>Liver glucokinase (U/mg prot)</td>
<td>1.23±0.24</td>
<td>0.49±0.09*</td>
<td>0.62±0.14*</td>
<td>0.69±0.18*</td>
<td>0.83±0.21*</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>36.45±6.17</td>
<td>67.24±24.29*</td>
<td>54.16±27.34</td>
<td>52.96±19.76</td>
<td>60.35±28.64</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>143.79±27.56</td>
<td>196.72±63.49*</td>
<td>184.33±47.68</td>
<td>176.11±34.92</td>
<td>204.72±42.54</td>
</tr>
</tbody>
</table>

\(* p<0.05 \text{ vs. Control group}; \# p<0.05 \text{ vs. Model group}\)

<table>
<thead>
<tr>
<th>Variable</th>
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<th>COS H group</th>
<th>Met group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (U/mg prot)</td>
<td>50.12±3.75</td>
<td>31.56±7.28*</td>
<td>42.37±8.45*</td>
<td>45.16±9.84*</td>
<td>35.04±8.97</td>
</tr>
<tr>
<td>Malonaldehyde (nmol/mg prot)</td>
<td>2.06±0.69</td>
<td>4.65±1.34*</td>
<td>2.24±0.81*</td>
<td>2.49±1.07*</td>
<td>3.86±1.52</td>
</tr>
<tr>
<td>Pancreas to body ratio (%)</td>
<td>0.24±0.08</td>
<td>0.15±0.06*</td>
<td>0.19±0.05</td>
<td>0.20±0.04*</td>
<td>0.17±0.07</td>
</tr>
</tbody>
</table>

\(* p<0.05 \text{ vs. Control group}; \# p<0.05 \text{ vs. Model group}\)
shown in Table 4, significantly decreased superoxide dismutase activity and elevated malonaldehyde content were observed in diabetic rats of the model group \((p<0.05)\). Administration of COS to diabetic rats caused a rise in the reduced superoxide dismutase activity and reduced the increased malonaldehyde content \((p<0.05)\). Administration of metformin to diabetic rats could not bring these values back to those of the control rats, and there were marked differences in superoxide dismutase between COS H and Met groups \((p<0.05)\).

**Effects of COS on Pancreas to Body Weight Ratio**

The change of pancreas to body weight ratio is shown in Table 4. In diabetic rats of the model group this ratio was significantly decreased \((p<0.05)\). Administration of high-dose COS restored pancreas to body weight ratio to near the control level \((p<0.05)\), while treatment with metformin had no effect on this ratio in diabetic rats.

**Morphology of the Rat Pancreas**

Pancreatic histology of the control rats was normal. On the contrary, the diabetic islet significantly atrophied. The injury of pancreatic tissues was repaired in the COS-treated group, but metformin had no protective effect on pancreatic islet of the damaged diabetic pancreas (Fig. 2).

**Effect of COS on Proliferation of INS-1 Cells**

As shown in Table 5, after incubation with 100 and 500 mg/l COS for 72 h, optical density (O.D.) values were increased \((p<0.05)\), suggesting that COS could promote the growth of INS-1 cells.

**Effect of COS on Glucose Stimulated Insulin Release in INS-1 Cells**

After treatment of COS for 48 h, glucose-stimulated insulin release was increased in 100, 500 mg/l concentrations of COS in 1.67 mmol/l glucose \((p<0.05)\) and significantly increased in 100, 500 mg/l concentrations of COS in 16.7 mmol/l glucose \((p<0.05\), Table 6).

**Effect of COS on GLUT-2 mRNA Relative Expression in INS-1 Cells**

RT-PCR analysis indicated the GLUT-2 mRNA levels in INS-1 cells were significantly increased by 100 and 500 mg/l COS \(\text{sec.}\) Fig. 3.

**Effect of COS on Apoptosis in INS-1 Cells**

The STZ-induced apoptotic rate of INS-1 cells was significantly higher than that in control cells \((41.36\pm4.83\% \text{versus} 7.74\pm0.70\%, p<0.05\).

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**Table 5. Effect of COS on Proliferation of INS-1 Cells \((n=6)\)**

<table>
<thead>
<tr>
<th>Group</th>
<th>0 h</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.246±0.019</td>
<td>0.363±0.043</td>
<td>0.433±0.020</td>
<td>0.529±0.056</td>
</tr>
<tr>
<td>COS 100 mg/l</td>
<td>0.251±0.025</td>
<td>0.381±0.034*</td>
<td>0.594±0.061*</td>
<td>0.643±0.062*</td>
</tr>
<tr>
<td>500 mg/l</td>
<td>0.257±0.031</td>
<td>0.395±0.046*</td>
<td>0.575±0.032*</td>
<td>0.617±0.047*</td>
</tr>
</tbody>
</table>

\(\text{*} p<0.05 \text{versus Control group.}\)

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**Table 6. Effect of 48 h Treatment with COS on Glucose Stimulated Insulin Release in INS-1 Cells \((n=6)\)**

<table>
<thead>
<tr>
<th>Insulin (mIU/l)</th>
<th>1.67 mmol/l glucose</th>
<th>16.7 mmol/l glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.20±0.21</td>
<td>3.35±0.24</td>
</tr>
<tr>
<td>COS 100 mg/l</td>
<td>1.35±0.23*</td>
<td>4.15±0.22*</td>
</tr>
<tr>
<td>500 mg/l</td>
<td>1.54±0.18*</td>
<td>4.70±0.15*</td>
</tr>
</tbody>
</table>

\(\text{*} p<0.05 \text{versus Control group.}\)
0.05). When treated with COS (100, 500 mg/l) for 12 h, apoptotic rates were decreased to 36.04±3.42% and 28.80±2.95%, respectively (p<0.05) (Fig. 4).

The cytokines-induced apoptotic rate of INS-1 cells was also significantly higher than that in control cells (45.36±3.70% vs. 7.27±0.80%, p<0.05). COS (100, 500 mg/l), however, did not decrease the apoptotic rates (46.07±4.30%, 46.40±4.20%).

DISCUSSION

Type 2 diabetes is one of the most common syndromes prevalent in society. It is characterized by a triad of resistance to insulin action on glucose uptake in peripheral tissues, especially skeletal muscle and adipocytes, impaired insulin action to inhibit hepatic glucose production, and dysregulated insulin secretion.13) The type 2 diabetes model induced by high energy intake with STZ is characterized by both insulin resistance and obvious β cell dysfunction.7) In the present study, rats of the model group showed metabolic disorders including hyperglycemia, hyperlipidemia (data not shown), insulin resistance and ruptured islet. COS have antidiabetic effects in vivo by reducing FBG and FINS, increasing ISI, improving oral glucose tolerance, reducing insulin resistance and protecting islet.

Insulin resistance due to the increased production and decreased utilization of glucose is the key primary defect underlying the development of type 2 diabetes.13) The liver, which accounts for approximately 80% of endogenous glucose production, is primarily responsible for increased FBG.14) In this study, a diminished liver glycogen synthesis was found in diabetic rats. A significant elevation in liver glycogen accompanied by a significant increase in liver glucokinase by COS was also observed. This implied that COS transformed more blood glucose into liver glycogen. The failure of peripheral tissues in the body to properly utilize glucose results in chronic hyperglycemia. The adipose tissue and skeletal muscle are the primary targets of insulin-stimulated glucose uptake. GLUT-4 is a major glucose transporter present in skeletal muscle and adipocytes. GLUT-4 is now not only considered to be a transporter but also an important regulatory factor of overall glucose metabolism.15,16) In this study, GLUT-4 expressions in adipose tissue and soleus muscle of diabetic animals were decreased. However, with treatment of COS the GLUT-4 levels were increased, suggesting that COS upregulated GLUT-4 mRNA and improved insulin resistance directly. This is the first report that establishes a direct effect of COS on the regulation of GLUT-4 expression in diabetic animals in vivo, and may at least partially explain the antidiabetic effects of COS.

Type 2 diabetes mellitus manifests itself in individuals who lose the ability to produce sufficient quantities of insulin to maintain normoglycemia in the face of insulin resistance.17) The ability to secrete adequate amounts of insulin depends on β-cell function and mass. In the present study, we found that COS minimized ruptured changes of pancreas in diabetic rats, and restored the pancreas to body weight ratio in vivo. COS also protected against the apoptosis induced by STZ, promoted the proliferation of INS-1 cells and stimulated insulin release through the upregulation of GLUT-2 gene expression in vitro. These data suggest that the preservation of β cell mass and function was attributable to the efficacy of COS.

The increased β-cell apoptosis plays a central role in the deficit of β-cell mass in the pathophysiology of type 2 diabetes. It has been suggested that β-cell damage is due to the combined consequence of increased circulating glucose, saturated fatty acids, inflammatory mediators and oxid-
tive stress.\textsuperscript{21–23} In this study, STZ and proinflammatory cytokines, respectively, were used to induce apoptosis of INS-1 cells. STZ is a glucosamine-nitrosourea compound that shows selective cytotoxicity to pancreatic cells and is widely used to generate diabetic animal models. STZ generates reactive oxygen species, which contribute to DNA fragmentation and evokes other deleterious changes in $\beta$ cells.\textsuperscript{24,25} We found COS protected INS-1 cells against the apoptosis induced by STZ, raised the reduced superoxide dismutase activity and reduced the increased malonaldehyde content in pancreas homogenate. This is consistent with the previous study which showed COS can improve the activity of superoxide dismutase and decrease the content of malonaldehyde in serum.\textsuperscript{12} These results indicated that the mechanism of COS' protective effects may be due to scavenging of free radicals to protect $\beta$ cell against oxidative damage induced by STZ. Proinflammatory cytokines, particularly IL-1$\beta$ in combination with IFN-$\gamma$ and/or TNF-$\alpha$, have been implicated in the elimination of $\beta$-cells in diabetes.\textsuperscript{26,27} Proinflammatory cytokines can induce $\beta$ cell apoptosis through the intrinsic mitochondrial apoptotic pathway.\textsuperscript{28} But the result showed that COS could not protect INS-1 cells against damage induced by proinflammatory cytokines. The different apoptotic pathway induced by STZ and proinflammatory cytokines may account for the discrepancy.

The therapeutic effects of COS on type 2 diabetes might be related to its ability of increasing liver glycogen synthesis and GLUT-4 gene expression in soleus muscle and adipose tissue to improve insulin resistance. On the other hand, COS has an antidiabetic effect by playing important roles in $\beta$ cells through promoting proliferation, increasing insulin release and GLUT-2 mRNA levels and protecting against STZ-induced apoptosis. The antioxidant activity of COS may be partially responsible for its protective properties. This research offers a limited experimental basis for elucidating the antidiabetic mechanism of COS.

Acknowledgement The research was funded by Qingdao Municipal Science and Technology Commission, China.

REFERENCES