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Hypoglycemic and hypolipidemic properties of polysaccharides from *Enterobacter cloacae* Z0206 in KKAy mice

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ABSTRACT

The water-soluble polysaccharides (EPS) were isolated from culture broth of *Enterobacter cloacae* Z0206, and the hypoglycemic and hypolipidemic effects of EPS were investigated. In this study, KKAy mice were gavaged once daily with either distilled water or EPS (200 mg/kg body weight) for 6 weeks. Results showed that EPS possessed significant hypoglycemic effects. Improved oral glucose tolerance, reduced serum insulin levels as well as decreased serum triglycerides (TG), cholesterol (TC) and low density lipoprotein cholesterol (LDL-c) were observed after treatment with EPS. Furthermore, EPS upregulated the expression of glucokinase (GK), HSL (Hormone Sensitive Lipase), adipose triglyceride lipase (ATGL), carnitine palmitoyl transferase 1-α (CPT1-α), glucose transporter 2 (Glut2), adenosine monophosphate activated protein kinase (AMPK) and silent information regulator 1 (Sirt1), but downregulated the gene expression of glucose-6-phosphatase (G6P) and fatty acid synthase (FAS) in the liver. These results suggest that EPS exhibits hypoglycemic and hypolipidemic effects possibly through regulating AMPK- and SirT1-mediated effects on carbohydrate and lipid metabolism.

1. Introduction

Diabetes mellitus is the most common chronic metabolic disease in modern society (Dey et al., 2003). More than 90% of diabetic populations are diagnosed with non-insulin-dependent diabetes (type 2 diabetes), which is characterized by hyperglycemia and dyslipidemia resulting from defects in both insulin secretion and action (Defronzo, Bondonna, & Ferranini, 1992). Insulin resistance has been evidenced to be a key factor in the onset and progress of type 2 diabetes which results in the impairment in insulin-regulated metabolic actions, including glucose transport, glycogen synthesis and gene expression characteristics (Defronzo & Ferrannnini, 1991). Ameliorating insulin resistance is an important strategy in the development of new pharmacological treatment for type 2 diabetes. There are many oral anti-diabetic agents for the treatment of type-2 diabetes currently, such as biguanides and sulfonylureas, but these synthetic agents are associated with certain adverse side effects and even toxicity (Grover, Yadav, & Vats, 2002). Therefore, it is necessary to explore and discover other alternative drugs and natural therapies which are safer and more effective.

Polysaccharides are considered an important class of bioactive natural products, which have been widely studied in the biochemical and medical areas due to specific bioactivities, such as immunostimulatory, antioxidant, antiviral (Jin et al., 2010), antitumor (Tong, Xia, Feng, Sun, & Gao, 2009) and anti-diabetic (Li et al., 2006) activities. For the past decades, polysaccharides isolated from *Astragalus* (Mao et al., 2007), *Ganoderma* (Zhu et al., 2013) and *Ophiopogon* japonicas (Xu et al., 2011) have been proved to exert hypoglycemic and hypolipidemic effects. Studies have shown that tissue antioxidant status may play an important role in the etiology of diabetes (Valko et al., 2007). Thus, oxidative stress may constitute a focal point for multiple therapeutic interventions, and for therapeutic synergy. Ma, Liu, Yu, Chen, and Zhang (2009) found that *Lycium barbarum* polysaccharides noticeably inhibited against lipid oxidation in high fat fed mice on the basis of their antioxidant activities. Xue also reported that sulfated *achyranthes bidentata* polysaccharides exert protective effects in streptozotocin-induced diabetic rats, possibly by reducing oxidative stress and hence protects organism from oxidative damage (Xue, Chen, Lu, & Jin, 2009).

In our previous study, a fucose-containing EPS was purified from the crude exopolysaccharides produced by *Enterobacter cloacae* Z0206. EPS with the molecular weight of 1.1×10^6 was composed of L-fucose, D-glucose, D-galactose, D-glucuronic acid and pyruvic

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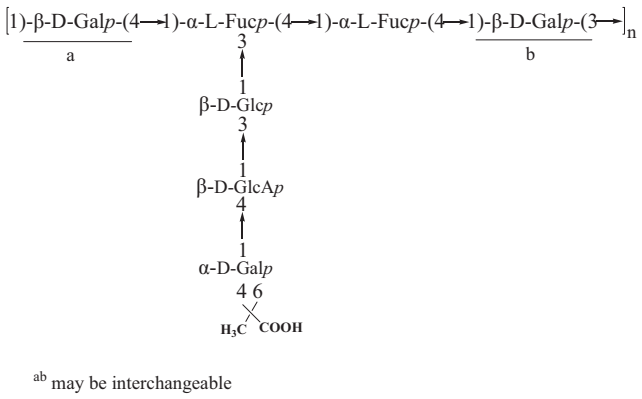

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Fig. 1. The repeating unit structure of the EPS from *Enterobacter cloacae* Z0206.

acid in the approximate molar ratio of 2:1:3:1:1. The structure of the EPS was elucidated by a combination of chemical and ESI-MS as well as NMR (Fig. 1) (Wang, Yang, & Wang, 2013). EPS has been shown to improve the immune responses and antioxidant activity in immunosuppressed mice induced by cyclophosphamide (Jin et al., 2010). However, little is known about the anti-diabetic effects of EPS. In this study, the hypoglycemic and hypolipidemic effects of EPS on diabetic KKAy mice was carried out, which would allow a better understanding of the functional effects about those macromolecules, and be beneficial to explore new more bioresources.

2. Materials and methods

2.1. Materials

Enterobacter cloacae, an exopolysaccharide producing bacterial strain, was identified and kept in our laboratory, and it has been collected by China General Microbiological Culture Collection Center (CGMCC). Assay kits for glycogen, hexokinase (HK) and free fatty acids (FFA) were the products of NanJing Biotechnology Co. Ltd. (NanJing, China).

2.2. Preparation of EPS

Preparation and purification of EPS were carried out according to the previous report of our research team with little modification (Wang et al., 2013). Briefly, the exopolysaccharide production was performed in a 10 dm^3 bioreactor (Shanghai Biotech Ltd., China) with 7 dm^3 cultivation medium (dextrose, 2.5%; peptone, 0.5%; yeast extract, 0.5%; K_2HPO_4, 0.2%; KH_2PO_4, 0.1% and $MgSO_4{\cdot}7H_2O$, 0.05%) at 30 °C for 48 h. The initial pH was 7.5 and the inoculation volume was 5.0% (v/v).

After cultivation, the broth was centrifuged (11,000 rpm) for 20 min to remove the mycelia. Supernatant was evaporated under reduced pressure at 50 °C, and then 3 vol. of cold 95% EtOH was added. The precipitates were collected by centrifugation at 11,000 rpm for 15 min and freeze-dried to yield a yellow powder. The collected yellow powder was redissolved in distilled water and deproteinized by a combination of trypsin and papain enzymolysis and Sevag method (Staub, 1965). The supernatant were dialyzed against deionized water (8000–14,000, molecular weight cut-off) and then lyophilized to obtain EPS, which was subjected to subsequent.

2.3. Animals

Male KKAy and C57BL/6J mice at the age of 6 weeks were obtained from the animal experimental center of Zhejiang University (Hangzhou, China). The mice were maintained under controlled conditions of 12 h light/12 h dark cycle and 60 ± 10% relative humidity at 22 ± 2 °C with free access to food and water. The mice were acclimatized to diet and general conditions of laboratory for 1 week. All experimental procedures involving the use of animals were approved by the experimental Animal Care Review Committee, Zhejiang University.

2.4. Experimental design

The KKAy mice were a cross between glucose-intolerant black KK female mice and male, yellow, obese Ay mice and are known to serve as excellent models of type 2 diabetes, while C57BL/6J mice with normal diets are generally used as non-diabetic controls. In the experiments, KKAy mice were assigned to groups based on the fasting blood glucose and body weight and were divided into two groups with similar initial blood glucose levels and initial weight (n = 8 per group). From 8 weeks of age, the KKAy mice fed a high-fat diet were gavaged once daily with either distilled water or EPS (200 mg/kg body weight) for 6 weeks. The lean mice (C57BL/6J) received the same volume of distilled water by oral administration during the same time. Blood glucose levels were tested (after 12 h fasting) using a One-Touch Basic Glucose Monitor (Johnson, USA) once every 7 days. Body weight was also recorded once a week. At the end of the experiment, the animals were fasted overnight and sacrificed by cervical dislocation. And blood samples were taken to determine plasma biomarkers. The liver was removed after the blood was collected, rinsed with a physiological saline solution, and immediately stored at −80 °C.

2.5. Oral glucose tolerance test (OGTT)

After 6 weeks treatment with EPS, the KKAy mice were made to fast for 12 h and then orally administered with glucose (2.0 g/kg body weight). Blood glucose level was measured at 0, 30, 60, 120 and 180 min respectively after glucose administration with a One-Touch Glucose Monitor (Lifescan, USA) via blood drops obtained by clipping the tail of the mice.

2.6. Blood biochemical measurement

At the end of the 6 weeks experimental period, mice were fasted for 12 h, blood samples were then collected under anaesthetized conditions and placed into prechilled tubes. The samples were immediately centrifuged at 3000 rpm for 5 min and the serum was separated for further analyses. Serum insulin was assayed by mouse insulin enzyme-linked immunosorbent assay (ELISA) kit. Levels of serum lipids including TC, TG, LDL-c and high-density lipoprotein cholesterol (HDL-c) were measured by a automatic biochemical analyzer. Free fatty acid (FFA) content in the serum was determined by an assay kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) as per the manufacturer's instructions.

2.7. Liver glycogen and hexokinase measurement

At the end of 42 days, all the mice were sacrificed, livers were weighed and washed with cold physiological saline followed by removal of visible fat and connective tissue in ice bath. Glycogen levels were measured using the anthracenone method. Activity of HK was measured using the glucose 6 phosphate dehydrogenase coupling colorimetric method. The measurements of such indexes were performed using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

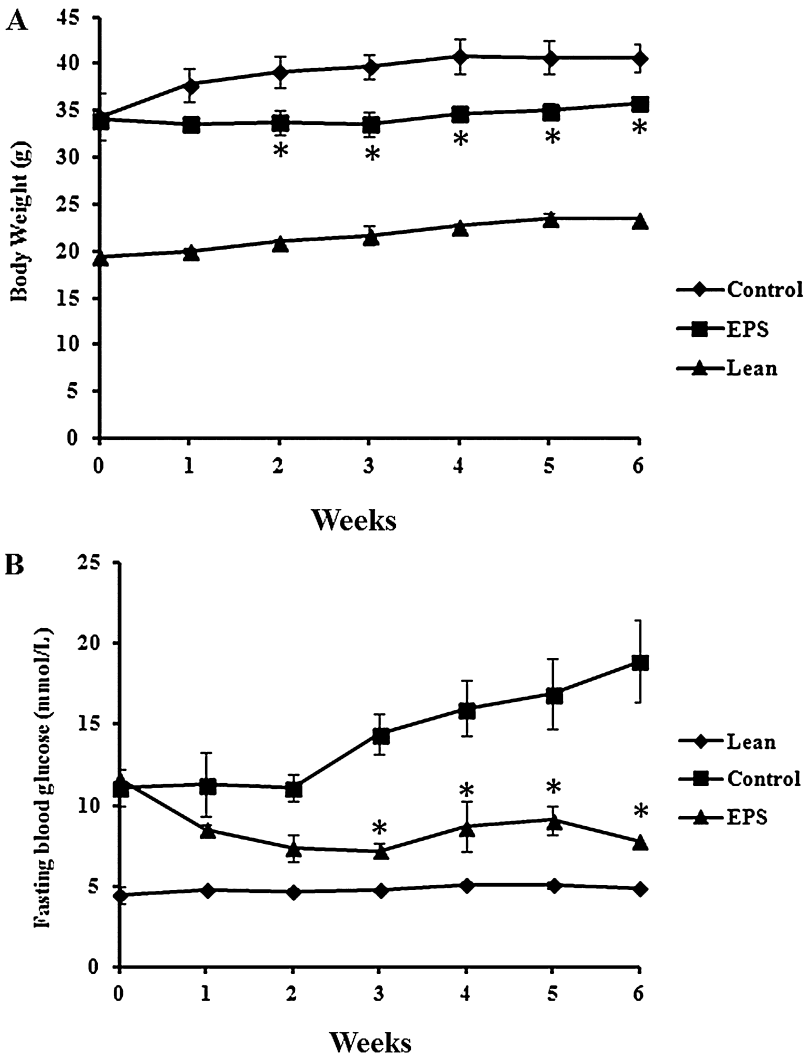

Fig. 2. Effect of EPS on body weight (A) and fasting blood glucose values (B) in KKAy mice (data are expressed as means ± SD ($n = 8$)).

2.8. Real-time PCR

Total RNA was extracted from the liver tissue using the TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) and RNA concentration was quantified by the NanoDrop ND-1000 spectrophotometer. PCR analysis was performed using SYBR Green normalized to 18S (StepOnePlus™ System; Applied Biosystems). G6P, GK, HSL, ATGL, FAS and CPT1-α transcripts were quantitated using RT-PCR technology on the LightCycle1.5 (MasterCycler EP gradient realplex4 Eppendor, Germany). Quantitative RT-PCR for each gene was performed using the method (95 °C for 30 s, and 40 cycles of 95 °C for 5 s and 60 C for 30 s). PCR primers used were: G6P: 5′-AAT CTC CTC TGG GTG GC-3′ (forward); 5′-TGC TGT AGT AGT CGG TGT CC-3′ (reverse); GK: 5′-TCC CTG TAA GGC ACG AAG A-3′ (forward); GK: 5′-GAG AAG TCC CAC GAT GTT GTT-3′ (reverse); ATGL: 5′-GAG CCC CGG GGT GGA ACA AGA T-3′ (forward); ATGL: 5′-AAA AGG TGG TGG GCA GGA GTA AGG-3′ (reverse); HSL: 5′-GCC GGT GAC GCT GAA AGT GGT-3′ (forward); HSL: 5′-CGC GCA GAT GGG AGC AAG AGG T-3′ (reverse); FAS: 5′-ATC CTG GAA CGA GAA CAC GAT CT-3′ (forward); FAS: 5′-AGA GAC GTG TCA CTC CTG GAC TT-3′ (reverse); CPT-1α: 5′-GTG TCC AAG TAT CTG GCA GTC-3′ (forward); CPT-1α: 5′-TCA GGG TAT TTC TCA AAG TCA A-3′ (reverse); 18S: 5′-CGG ACA CGG ACA GGA TTG ACA-3′ (forward); 18S: 5′-CCA GAC AAA TCG CTC CAC CAA CTA-3′ (reverse). Relative expression was assessed by the Ct formula. All samples were run in triplicate and the average values were calculated.

2.9. Western blotting assay

100 mg of liver tissue was crushed in lysis buffer, the homogenate was centrifuged at 14,000 × g for 15 min at 4 °C and the supernatant was collected and stored at −80 °C. The protein concentrations were measured with BCA protein assay reagent. The protein supernatants were subjected to SDS-PAGE and blotted onto apolyvinylidene difluoride membrane followed by incubation with the primary antibodies anti-AMPK, anti-*p*AMPK, anti-Glut2 and anti-Sirt1. The proteins were detected with an ECL system.

3. Results

3.1. Effect of EPS on body weight and fasting blood glucose levels

The changes of body weight in different experimental groups are shown in Fig. 2. During the treatment, the untreated KKAy mice had more body weight gain than the EPS-treated animals. After treatment with EPS, the body weight was significantly lower ($p < 0.05$) than that of the untreated KKAy mice group, which suggested that EPS attenuated the body weight gain at the doses used in this study.

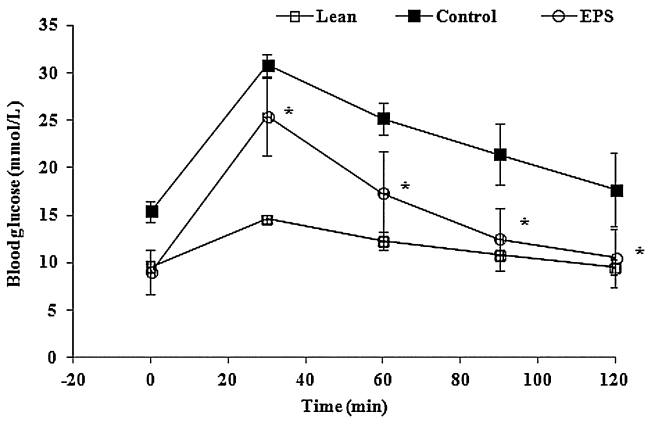

Fig. 3. Effect of EPS on OGTT in KKAy mice (Data are expressed as means ± SD ($n = 5$). $^{*}p < 0.05$ compared with control group of KKAy mice).

As shown in Fig. 2, blood glucose levels in the diabetic KKAy mice were significantly higher than those of the lean mice. The KKAy mice administered EPS for 42 days exhibited a significant decrease ($p < 0.05$) in fasting blood glucose level compared with the diabetic KKAy mice.

3.2. Effect of EPS on the oral glucose tolerance test

After 42 days of EPS treatments, glucose tolerance was examined for the three experimental groups using the OGTT methodology. Fig. 3 shows the changes in the levels of blood glucose during OGTT (2 g glucose/kg body weight). The diabetic KKAy mice in the control group showed a significantly stronger hyperglycemic response to oral glucose administration compared to C57BJ/6L mice. The blood glucose level of EPS-treated group was significantly lower ($p < 0.05$) than that of the untreated diabetic KKAy mice at each time point, which indicated that EPS showed a remarkable improvement in overall glucose response.

3.3. Effect of EPS on serum insulin and lipids levels in KKAy mice

KKAy mice are characterized by advanced hyperinsulinemia, so the effect of EPS administration on serum insulin levels was investigated. As shown in Fig. 4, the untreated diabetic KKAy mice exhibited a much higher ($p < 0.05$) serum insulin concentration than that of the lean mice. Administration of EPS significantly decreased ($p < 0.05$) serum insulin levels of the KKAy mice.

The effect of EPS on serum lipid concentration was also studied after oral administration for 42 days. As shown in Fig. 4, there was a significant increase in the levels of serum TG, TC and LDL-c of the untreated diabetic mice in comparison with lean mice. The serum HDL-c level in untreated diabetic KKAy mice decreased numerically without a significant difference compared with lean mice. Administration of EPS resulted in a significant diminution ($p < 0.05$) of elevated serum TC, TG, and LDL-c levels. No significant effects on HDL-c were observed following EPS treatment compared with lean mice.

At the same time, the KKAy mice showed increased serum FFA levels when compared with the lean mice. After 42 days treatment with EPS, the serum FFA levels in the fasted KKAy mice were reduced ($p < 0.05$) compared to untreated diabetic KKAy mice.

3.4. Effect of EPS on hexokinase and glycogen content in the liver of KKAy mice

Fig. 5 shows the changes in the activity of hexokinase and glycogen content in the liver of normal and experimental mice. The activity of hepatic hexokinase and the glycogen content were significantly decreased ($p < 0.05$) in the untreated diabetic mice when compared with the lean mice. Administration of EPS to diabetic KKAy mice resulted in a significant ($p < 0.05$) recovery of the activity of the hexokinase, along with restoration of the glycogen content.

3.5. Expressions of glucose and lipid metabolism genes and proteins

To figure out the underlying molecular mechanism of EPS on blood glucose and serum lipid levels, we sought to evaluate its effect on several important genes and proteins in the glucose and lipid metabolism in the liver tissue. Firstly, we examined the expressions of GK, G6P, HSL, ATGL, CPT-1α, FAS using quantitative RT-PCR. As depicted in Fig. 6A, the expression of GK was significantly upregulated ($p < 0.05$) after treatment with EPS, while the expression of G6P was reduced ($p < 0.05$) compared with untreated KKAy mice. As shown in Fig. 6B, there was a significant increase ($p < 0.05$) in ATGL, HSL and CPT1-α gene expression in the liver tissue of KKAy mice supplemented with EPS compared with untreated KKAy mice, while FAS mRNA expression was downregulated by 30% ($p < 0.05$) in EPS treated groups. In an attempt to understand the ameliorating effect of EPS on glucose and lipid metabolism in the liver tissue, we further examined the protein expression of Glut2, Sril1, AMPK and *p*AMPK in untreated and treated KKAy mice. Western blot analysis showed that EPS treatment significantly enhanced Glut2, Sirtl1, AMPK and *p*AMPK protein levels in KKAy mice (Fig. 6C).

4. Discussion

Polysaccharides are a kind of macromolecules exist widely in nature, which are considered as an important class of bioactive natural products with specific bioactivities, such as immunostimulating, antioxidant, antiviral and anti-diabetic activities (Huynh, Chaubet, & Jozefonvicz, 2001; Wang, Zhang, Yu, & Cheung, 2009). Nowadays, lots of literatures have reported that the polysaccharides from *Ganoderma atrum* (Gao, Lan, Dai, Ye, & Zhou, 2004), *Lycium barbarum* (Luo, Cai, Yan, Sun, & Corke, 2004), *Astragalus* (Chen, Li, & Yu, 2008) and so on, have anti-hyperglycemic or anti-hyperlipidemic activities.

Type 2 diabetes are a group of prevalent metabolic disease in the world, which is characterized by hyperglycemia and hyperlipidemia due to an imbalance between endocrine pancreatic function and peripheral insulin sensitivity including skeletal muscle, liver and adipose tissue (Bavenholm, Pigon, Ostenson, & Efendic, 2001). KKAy mice are a cross between glucose-intolerant black KK female mice and yellow obese Ay male mice. They are characterized by obesity and the development of insulin resistance, hyperinsulinemia and dyslipidemia. Therefore, KKAy mice are excellent model of type 2 diabetes diseases (Srinivasan & Ramarao, 2012).

In the present study, we adopted KKAy mice as a model of type 2 diabetes to investigate the effects of EPS on glucose and lipid metabolism in vivo and found that the KKAy diabetic mice exhibited a significant increase of body weight and fasting blood glucose levels compared with lean mice. The treatment with EPS caused a significant decrease in blood glucose levels ($p < 0.05$) compared with untreated diabetic KKAy mice. We hypothesize that regulating intestinal microflora, reducing food absorption not consumption may play a role in the hypoglycemic activity of EPS (Xu et al., 2011). The much lower body weight observed in treated diabetic mice might be the result of reduction of the fat storage or enhancement of lipolysis.

Healthy individuals maintain stable blood glucose levels by basal insulin secretion (Raghavan & Garber, 2008), but type 2 diabetes is characterized by hyperglycemia resulting from insulin

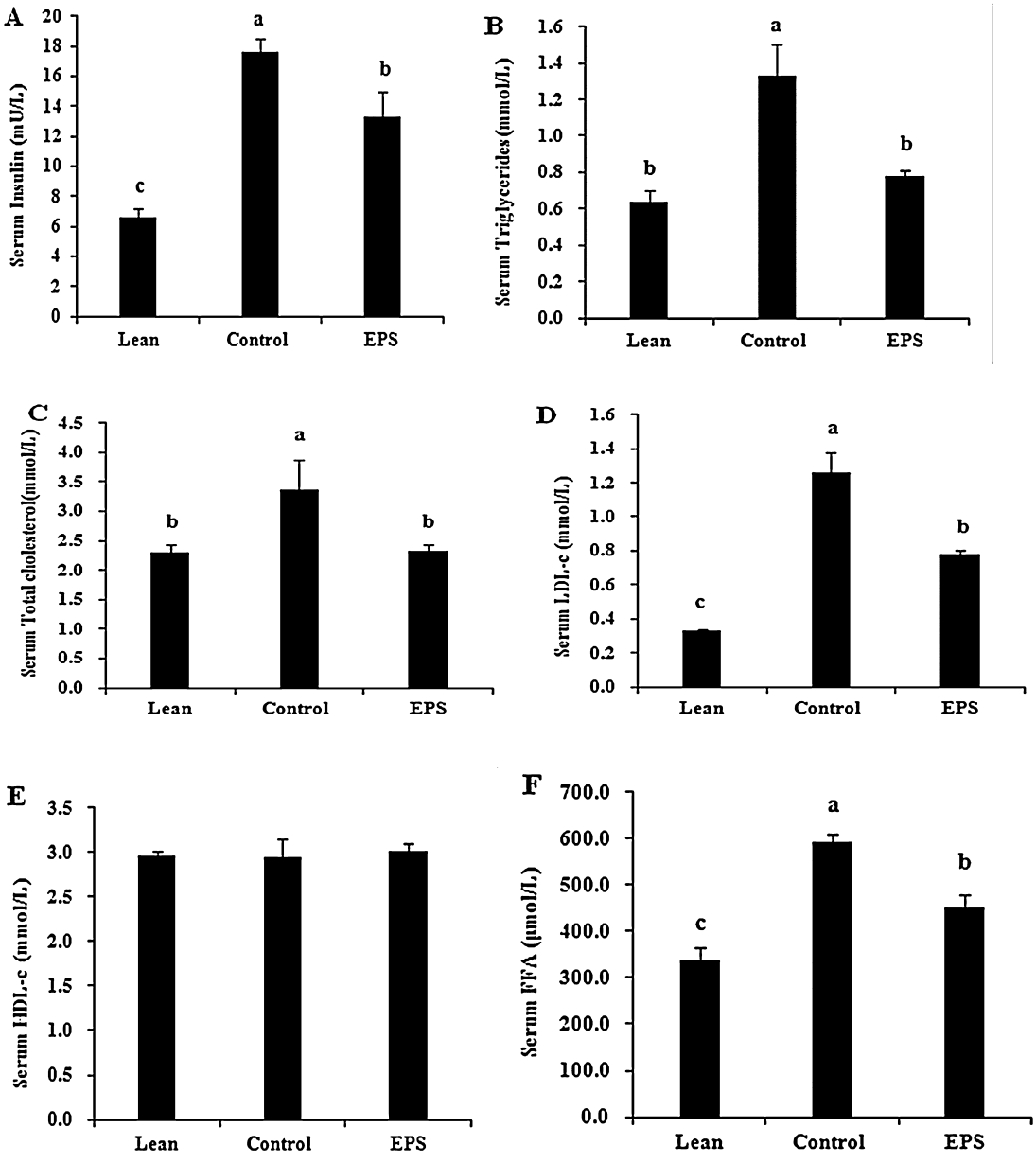

Fig. 4. Effect of EPS on serum insulin (A), TG (B), TC (C), LDL-c (D), HDL-c (E) and FFA (F) levels in KKAy mice (data are expressed as means ± SD ($n = 6$); $^{a, b, c}$ means within the same column lacking a common superscript differ ($p < 0.05$)).

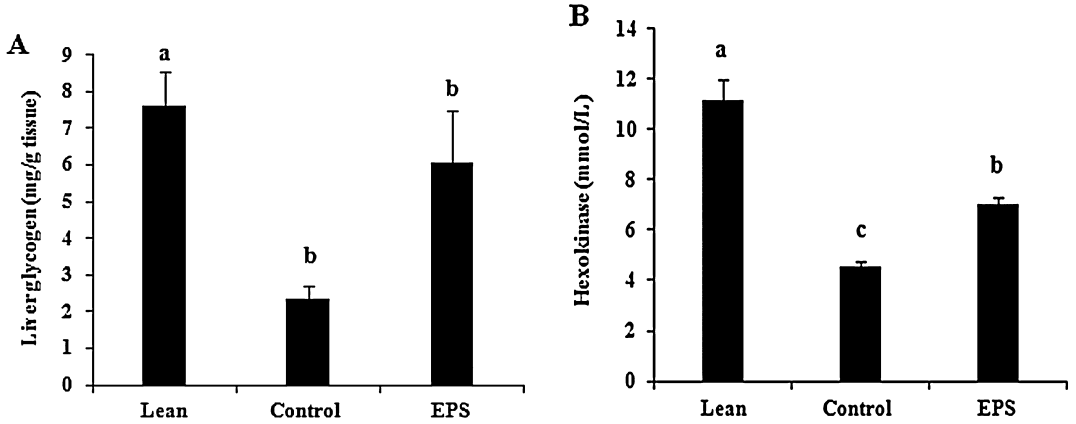

Fig. 5. Effect of EPS on hepatic glycogen content (A) and activity of hexokinase (B) in the liver of KKAy mice (Data are expressed as means ± SD. (n = 6); $^{a, b, c}$ means within the same column lacking a common superscript differ ($p < 0.05$)).

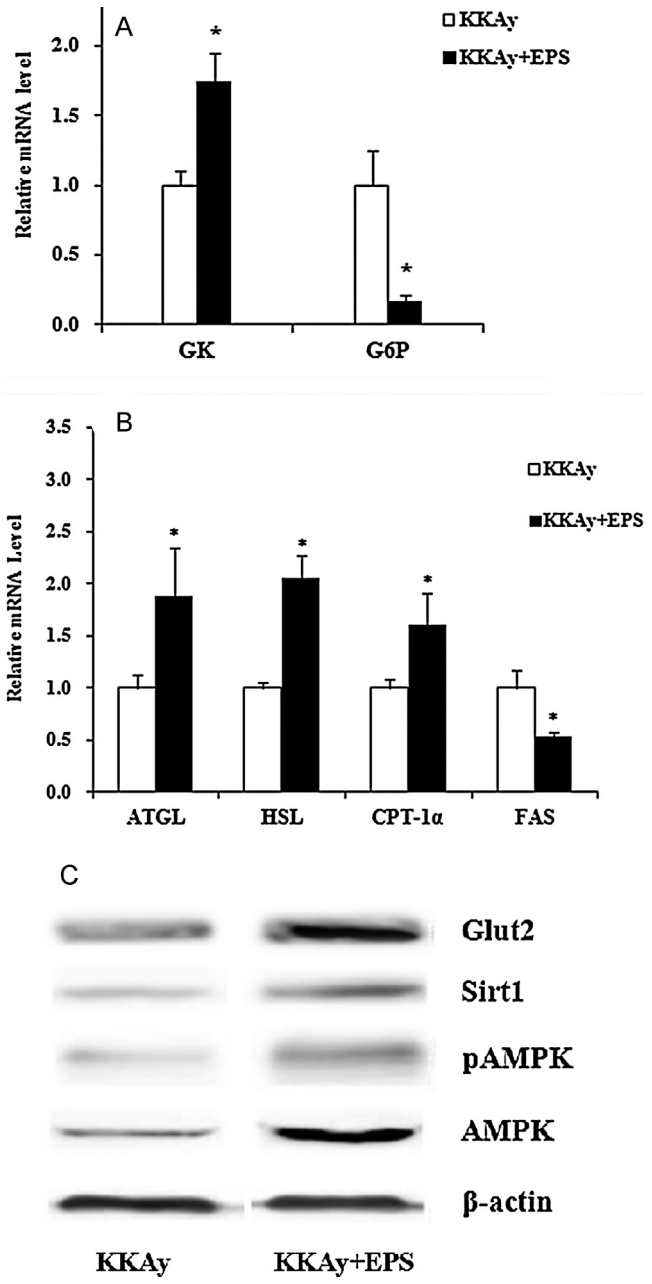

Fig. 6. Effect of EPS on the glucose and lipid metabolism in the liver of KKAy mice. (A) the gene expression of GK and G6P; (B) the gene expression of ATGL, HSL, CPT1-α and FAS ($n = 3$, means ± SD; $^*p < 0.05$, vs. control group of KKAy mice); (C) expression of Glut 2, AMPK, *p*AMPK and Sirt1.

resistance and inadequate insulin (Ke, Delerue, Gladbach, Götz, & Ittner, 2009). Such compensatory hyperinsulinemia is probably the cause of many other disorders such as dyslipidemia and hypertension (Li, 2007). The result of OGTT in the present experiment showed that the severely impaired glucose tolerance confirmed the insulin resistant state of the KKAy mice. After EPS treatments, there was a significantly increased glucose disposal, presumably due to an increase in insulin sensitivity. At the same time, the serum insulin level of KKAy mice was significantly reduced when supplemented with EPS, indicating insulin-sensitizing activity of EPS. Our results suggested that EPS had the hypoglycemic effect by increasing whole-body insulin sensitivity.

Besides hyperglycemia and insulin resistance, the evolution of type 2 diabetes frequently combined with dyslipidemia (Cha et al., 2005), which is often important determinant of the course and status of the diabetes mellitus (Fumelli, Romagnoli, Carlino, Fumelii, & Boemi, 1996). In the present study, marked increase in TC, TG and LDL-c were observed in untreated diabetic KKAy mice. These results are in agreement with other studies that the control KKAy mice showed significant elevation in the levels of plasma TC, TC and LDL-c (Wang et al., 2012). It is reported that some polysaccharides such as chitin and K-carrageenan oligosaccharides have overall positive effect on lipid metabolism and cholesterol (Muzzarelli, 1997). We similarly found that administration of EPS resulted in an obvious decrease in serum TG, TC, and LDL-c, indicating that EPS attenuated the disorder of lipid metabolism.

FFA plays important roles in many tissues and FFA levels are commonly elevated in obese individuals and patients with type 2 diabetics (Boden & Shulman, 2002), resulting from lipolysis in the peripheral adipose tissue in an insulin-resistant condition (Defronzo & Ferrannnini, 1991). In our study, serum FFA levels were elevated 1.75-fold in untreated diabetic KKAy mice compared with non-diabetic controls. Treatment with EPS significantly reduced the serum FFA levels. Zhu et al. (2013) also found that administration of *Ganoderma atrum* (PSG-1) at 200 and 400 mg/kg BW caused a significant reduction in the serum FFA levels compared with untreated diabetic rats (Zhu et al., 2013). The reduction of serum FFA levels possibly contributes to the ameliorating effect of EPS in insulin resistance in KKAy mice.

Glycogen is the primary intracellular storable form of glucose and it directly reflects the insulin activity since it regulates glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Muthulakshmi & Saravanan, 2013). In the present study, a significant decrease in the glycogen content of liver was observed in untreated diabetic KKAy mice and treatment with EPS increased liver glycogen content to a significant level. Hexokinase is the key enzyme catalyzing glucose into glucose 6 phosphate in the first step in glycolysis (Laakso, Malkki, & Deeb, 1995). HK deficiency in diabetic mice could result in decreased glycolysis and decreased utilization of glucose for energy production (Gancedo & Gancedo, 1971). Chen et al. (2013) reported that HK activity in liver was significantly reduced in diabetic KKAy mice (Chen et al., 2013). In agreement with this author, we observed a significant decrease in hepatic HK activity in untreated KKAy mice, which was probably due to the insensitivity of cells to insulin. Administration of EPS mice resulted in significant increase in the activity of hexokinase. The increased hepatic HK activity may contribute to the restoration of serum glucose and insulin levels in EPS treated KKAy mice.

Gluts are a wide group of membrane proteins, which facilitate the transport of glucoses over the plasma membrane. Glut2 is the principal transporter for the transfer of glucose between the liver and blood and renal glucose reabsorption. In this experiment, we revealed that the EPS could significantly improve the expression of Glut2 in liver tissue of KKAy mice, suggesting an increased absorption of glucose from blood to the liver. At the same time, the gene expression of GK, the key enzyme in the phosphorylation of glucose, was upregulated whereas the gene expression of G6P, the regulatory enzyme catalyzes the final step in gluconeogenesis, was downregulated in KKAy mice supplemented with EPS. Overall these investigational studies indicated that the role of EPS in glucose metabolism as increasing glycolysis, while decreasing gluconeogenesis.

ATGL is a kind of enzyme catalyses the initial step in triglyceride hydrolysis in mammalian cells (Smirnova et al., 2006). HSL is an intracellular neutral lipase that hydrolyzes triacylglycerols, diacylglycerols and monoacylglycerols (Kraemer & Shen, 2002). ATGL and HSL are both involved in intracellular degradation of triacylglycerols (Morak et al., 2012). In the present study, we found that ATGL and HSL gene expression in liver were significantly increased in mice supplemented with EPS. It suggested that decomposition of

triglycerides in the liver was enhanced in KKAy mice treated with EPS.

FAS, an enzyme which catalyses the final step in the fatty acid synthesis, plays an important role in fatty acid biosynthesis (Kuhajda et al., 1994). CPT 1 is an enzyme responsible for the entry of long-chain fatty acid into the mitochondria matrix and their subsequent β-oxidation (McGarry & Brown, 1997). The increase in CPT-1α and decrease in FAS in the liver of KKAy mice indicated that EPS had the effect on stimulating the degradation of fatty acid, while weakening the fatty acid synthesis.

The AMPK is a serine/threonine kinase that functions as an intracellular energy sensor (Hardie, 2004). The activation of AMPK switches on catabolic pathways, such as fatty acid oxidation and glycolysis (Steinberg & Kemp, 2009). Studies have demonstrated that impairment of AMPK activity resulted in elevated esterification and reduced ability to oxidize fatty acid (Gaidhu, Anthony, Patel, Hawke, & Ceddia, 2010), but a short-term overexpression of AMPK led to an increase in hepatic fatty acid oxidation in mice (Foretz et al., 2005). Besides, AMPK could suppress the fatty acid synthesis by inhibiting FAS (Ruderman & Prentki, 2004). Sirt1, another metabolic sensor, is also a crucial link in a regulatory network for nutrient metabolic homeostasis (Canto & Auwerx, 2009). Emerging evidence supports the notion that Sirt1 works in tandem with AMPKα to regulate energy metabolism (Hou et al., 2008). Hwang et al. (2013) found that sapoinis prevents lipid accumulation through Sirt1 and CaMKKb/AMPK activation in high-glucose-induced HepG2 cells. Our results showed that EPS induced the expression of AMPK, *p*AMPK and Sirt1. This indicated that EPS may potentially help not only block the process in fatty acid synthesis but also augment β-oxidation and glycolysis through stimulating AMPK and Sirt1, so we conjectured that EPS possibly mediated lipid-lowering effects through the Sirt1/AMPK signaling pathway in the liver of KKAy mice. Therefore, treatment with EPS could activate energy sensing molecules, including AMPK and SIRT1, which in turn induce catabolic pathways such as fatty acid oxidation and glycolysis and inhibit anabolic pathways such as fatty acid synthesis.

In summary, our current studies demonstrated EPS produced by *Enterobacter cloacae* Z0206 has significant hypoglycemic activity and insulin-sensitizing effects in diabetic KKAy mice and pointed out that EPS may serve as a potential hypoglycemic and hypolipidemic agent in treating diabetic patients. In addition, the deeper mechanism of anti-diabetic effect of EPS is necessary to be further studied in both vivo and vitro.

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