Peptides Derived from Oats Improve Insulin Sensitivity and Lower Blood Glucose in Streptozotocin-Induced Diabetic Mice

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Abstract

Background: Oat, as a “third staple food” following rice and wheat in China, has been widely utilized due to the hypoglycemic activities of oat β-glucan. Oat also contains 15-20% protein, which consists of balanced amino acid profile devoid of anti-nutritional factors. Peptides from albumin, whey protein and other foods have been proposed to have hypoglycemic activities while the hypoglycemic activities of oat peptides have not been reported to our knowledge.

Results: The hypoglycemic activities and underlying mechanisms of alcalase2.4 L oat hydrolysates purified by ultrafiltration were investigated in streptozotocin-induced diabetic mice using metformin as the positive control. The hypoglycemic activities of oral administration of oat peptides (0.25, 0.5 and 1.0 g kg bw−1) were in a dose-dependent manner. In the highest does treatment group, mice had the significantly (P < 0.05) lower total food intake and fasting blood glucose (FBG) and the higher food efficiency, serum fasting insulin (Fins), insulin activity index (IAI), and hepatic glycogen content compared with the 0.25 and 0.5 g kg bw−1 treatment groups. Moreover, the FBG concentrations positively correlated with total food intake (r =0.994, p < 0.01) and negatively correlated with Fins concentrations (r = -0.979, P < 0.01), IAI (r = -0.942, P < 0.01), and hepatic glycogen contents (r = -0.952, P < 0.01).

Conclusion: The results indicated that the underlying mechanisms of the oat peptides’ antidiabetic action could be linked to more than one mechanism, such as controlling the food intake, stimulating the insulin secretion and insulin sensitizing, and elevating the glycogenesis.

Keywords: Oat peptides; Diabetic; Hypoglycemic activities; Insulin

Introduction

Type 2 diabetes mellitus (T2DM) affects 5-7% of the total world population [1,2]. Cardiovascular disease, T2DM, and other obesity related chronic diseases are major causes of morbidity and mortality. High fasting blood glucose (FBG) and insulin resistance typically precede T2DM. Dietary components such as polysaturated fats, viscous fibers, and polyphenolic compounds have been reported to reduce risk factors associated with T2DM.

Dietary protein is not only the source of essential amino acids required for growth or maintenance but also contains amino acid sequences for bioactive peptides that have antimicrobial, blood pressure-lowering (ACE inhibitory), anti-oxidative, cholesterol-lowering, and hypoglycemic properties [3,4]. The effects of different dietary protein and intake amount on hypoglycemic activities have received considerable attention. Gannon et al. showed that feeding T2DM patients with 50 g glucose in addition to 25 g various proteins led to increases in insulin secretion according to the protein type with the largest increases obtained with cottage cheese and least with egg protein [5]. Whey protein was demonstrated to have insulinotropic effects and reduced postprandial glycaemia by stimulating the glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) in C57BL/6J mice [6]. Power et al. also found that whey protein hydrolysates have a 45% greater insulintropic effect compared with the intact whey protein [7]. The protein from Thai bitter

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gourd (Momordica charantia) fruit pulp could decrease plasma glucose concentrations in both normal and streptozotocin (STZ)-induced diabetic rats in a dose-dependent manner [8]. One novel peptide (KLPGF) from albumin may have the anti-diabetic activity against amylase with an IC_{50} of 59.5 μmol L^{-1} and α-glucosidase with an IC_{50} of 120 μmol L^{-1} [4] and the peptide RVPSLM from egg white protein was also discovered as a potential α-glucosidase inhibitor with an IC_{50} of 23.1 mol L^{-1} [9].

Oat, as a “third staple food” following rice and wheat in China, has been widely utilized due to the need for high soluble fiber in consumer’s daily diet. Oat also contains 15-20% protein, which consists of balanced amino acid profile devoid of anti-nutritional factors [10,11] and has a chemical score of 72-74 [12]. Many studies have focused on the hypoglycemic activities of oat β-glucan [13-15]. However, the hypoglycemic activities of oat peptides have not been reported to our knowledge.

The aim of this study was to investigate the hypoglycemic activity of oat hydrolysates prepared by proteolysis by alcalase 2.4 L^{-1} on blood glucose concentration and insulin response in STZ-induced diabetic mice. STZ, a glucosamine–nitrosourea compound derived from Streptomyces achromogenes, specifically targets pancreatic β cells resulting in hypoinsulinemia and hyperglycemia and has been widely used to generate diabetic animal models [8-16,18].

Materials and Methods

Preparation of oat peptides with the α-glucosidase inhibitory activity

Naked oat (Bailang No.2) was provided by Baicheng Institute of Agricultural Products (Jilin, China) and ground in a hammer mill to pass through a 80-mesh screen. The powder was defatted by suspending in n-hexane, and drying the residue at ambient temperature. A 5% (w/w) suspension of the defatted oat meal was hydrolyzed by 4% Alcalase 2.4 L^{-1} (Novozyme Inc. Copenhagen, Denmark) at 55°C, pH 7.5 for 4 h with agitation by a SHZ-82A thermostatic oscillator (Guohua Electric Appliance Co., Ltd., Changzhou, China) at 200 w. After proteolysis, the suspension was cooled to the ambient temperature and the supernatant containing the protein hydrolysates separated from the insoluble residue by centrifugation at 8,000 × g for 30 min.

Peptides were separated from high molecular substances by ultrafiltration using a MSC300 ultrafiltration machine (Shanghai Mosu Science and Material Co., Ltd., Shanghai, China) according to the method of Hamada with slight modifications [19]. The crude hydrolysates were ultrafiltered through a Omega membrane with a molecular weight cut-off of 5,000 Da at 0.2 MPa, 20°C, and pH 7.0. Preliminary studies had shown that the fraction below 5 kDa had 18% higher α-glucosidase inhibitory activity than the fraction greater than 5 kDa. The fraction less than 5 kDa was collected, lyophilized, and stored at -20°C for the further study. The purity (65%) was determined by the Kjeldahl method using a conversion factor of 5.83 [20].

Experimental animals

Male ICR (Institute of Cancer Research) mice were obtained from SPF Animals Experiment Technology Co., Ltd. (Certification of Experimental Animal: No. SCXK (Jing) 2011-0004) (Beijing, China). Mice were housed at 20 ± 2°C with a 12 h alternating light-dark cycle. Food and water were available ad libitum. The experimental procedures were carried in conformity with international guidelines for care and use of laboratory animals. After the 3-day acclimation, ten mice were randomly allocated as the normal control (NC) group and intraperitoneally injected with 0.1 mol L^{-1}, pH 4.5 sodium citrate buffer solution at a dose of 150 mg/kg body weight after a 12 h fast. The remaining mice were intraperitoneally injected with 0.1 mol L^{-1}, STZ in pH 4.5 sodium citrate buffer solution (Sigma Chemicals, St. Louis, MO, USA) at a dose of 150 mg/kg body weight after a 12 h fast. After 2 weeks, mice with a fasting blood glucose (FBG) value higher than 11 mmol L^{-1} were considered diabetic and selected for the further pharmacological studies.

The diabetic mice were randomly divided into five groups of 10 mice. The groups included: a model control (MC), positive control (PC), low oat peptide (OPL), medium oat peptide (OPM), and high oat peptide (OPH). The MC group was fed a standard chow diet including wheat bran, corn, wheat flour, vegetable oil, vitamin, and mineral mix (Beijing Macao Cooperation Feed Co., Ltd., Beijing, China). The PC group, fed the standard chow diet, was orally administered 10 mg/ml metformin (0.6 g/kg body weight) (Tian’an Pharmaceutical Co., Ltd., Guizhou, China) daily. The OPL, OPM, and OPH groups were orally administered 0.25, 0.5 and 1.0 g/kg body weight in solutions containing 0.15, 0.3 and 0.6 g mL^{-1} oat peptides solution respectively.

The NC group was fed the standard chow diet and orally administered distilled water (1.0 g/kg body weight) daily. The experiment lasted for 4 weeks and food and water were available ad libitum during the period. Food intake and body weights were recorded weekly.

Blood glucose, insulin, and insulin activity index

During the feeding period, FBG values were determined from the tail vein using a One Touch Ultra blood glucose meter (Johnson & Johnson Medical Ltd., Shanghai, China) weekly for three times. After 4 weeks the mice were feed deprived for 12 h and sacrificed under mild isoflurane anesthesia. The blood samples were taken by orbital venous sinus puncture and separated by centrifuged at 3,000 × g for 15 min at 4°C. For the calculation of insulin activity index (IAI), the FBG values and serum fasting insulin (FINS) in serum were measured using a glucose kit (Leadman Group Co., Ltd., Beijing, China) and an insulin ELISA kit (Beckman Coulter Inc., Miami, FL, USA).

IAI was calculated using the following formula [15]:

\[ IAI = \frac{1}{\text{FINS} \times \text{FBG}} \]

FINS and FBG are the concentrations of serum insulin and blood glucose after a 12 h fast.

Hepatic glycogen

Livers were dissected out, washed in ice-cold saline solution to remove blood, frozen in liquid nitrogen, and kept at -80°C. The
hepatic glycogen content was determined using a commercially available enzyme assay kit (Nanjing Jiancheng Biocompany, Nanjing, China).

Identification of peptides by Nano-Ultra Performance Liquid Chromatography (UPLC)-MSE Tandem MS

The peptide sequences were indentified according to the method of Shen et al. [21].

After adjusting the pH to 8.5 with 1 M ammonium bicarbonate, oat peptides was chemically reduced for 45 min at 55°C by adding DL-Dithiothreitol (DTT) to 10 mM and carboxamidomethylated in 55 mM iodoacetamide for 30 min at room temperature in the dark. Then CaCl$_2$ was added to 20 mM, endoprotease Lys-C (Sigma Chemicals, St. Louis, MO, USA) was added to a final substrate/enzyme ratio of 100:1 (w/w), and the reaction was incubated at 37°C for 12 h. Nano-scale liquid chromatography (LC) separation of peptides digested by Lys-C and trypsin was performed with a nano ACQUITY system (Waters Corporation, Milford, MA, USA) equipped with a symmetry C18, 5 μm, 180 μm x 20 mm pre-column and an ethylene bridged hybrid (BEH) C18, 1.7 μm, 75 μm x 250 mm, analytical reversed-phase column (Waters Corporation, Milford, MA, USA). The column temperature was maintained at 35°C. The mobile phase A was deionized water with 0.1% formic acid and the mobile phase B was 0.1% formic acid in acetonitrile. The peptides were separated with a linear gradient of 0-40% mobile phase B over 80 min at 0.2 μL min$^{-1}$ followed by 10 min at 80% mobile phase B. The column was re-equilibrated at initial conditions for 20 min.

Analysis of oat peptides was performed using a SYNAPT high definition mass spectrometer (HDMS) (Waters Corporation, Milford, MA, USA). The source temperature was 90 °C. The mass range was from m/z 350 to 1600. The time-of-flight (TOF) analyzer of the mass spectrometer was calibrated with the MS/MS fragment ions of [Glu$_{5}$] fibrinopeptide B (Sigma Chemicals, St. Louis, MO, USA) from m/z 50 to 2000. Peak lists were generated using Protein Lynx Global Server version 2.3 (PLGS 2.3) software and automatically combined into a single pkf file for every LC-MS/MS run. Other search parameters were as follows: trypsin digest with one missed cleavage, no fixed modification and carboxamidomethyl of cysteine and oxidation of methionine.

Results

Changes in body weights and food intake

There were no significant differences in final body weights or weight gain among the different diabetic mice groups after 4 weeks (Table 1). The total food intake was significantly (P < 0.05) lower in the OPH and PC treatment groups compared with the NC group (Table 1). The total food intake was 19.4%, 10.9%, 5.3%, and 21.2% lower in OPH, OPM, OPL, and PC groups, respectively, compared with the NC group. Moreover, the total food intake was significantly (P < 0.05) inversely related to oat peptides’ dose and the OPH treatment group had the similar total food intake as the PC group. Because there were no differences in weight gain, the lower total food intake of the OPH, OPM, and PC groups translated into significantly (P < 0.05) higher food efficiencies. Compared with the MC group, the food efficiencies of OPH, OPM, and PC groups increased 25%, 19%, and 47%, respectively.

FBG and insulin responses

Prior to the treatment, the FBG concentrations showed no significant differences among all of the diabetic mice. However, the FBG concentrations of the diabetic mice were all significantly higher (P < 0.05) than that of the NC group (Figure 1). After the first week treatment, the FBG concentrations of mice in OPH, OPM, OPL, and PC groups significantly (P < 0.05) decreased 16.5%, 11.1%, 8.6%, and 19.9%, respectively. The FBG concentrations in OPH and PC were 30.2% and 38.9% lower after the 4-week administration. Compared with the MC treatment group, the FBG concentrations in OPH, OPM, and PC groups significantly (P < 0.05) decreased 31.6%, 14.4%, and 39.7%, respectively, at the end of the treatment.

Compared with the NC group, all the diabetic mice had significantly (P < 0.05) lower FINS concentrations (Table 2). However, the FINS concentrations in the OPH and PC treatment groups significantly (P < 0.05) increased 13.0% and 30.5% compared with the MC group. Mice in the OPH treatment group had the similar IAI to that of the PC group and the IAI in the OPH and PC treatment groups was 29.1% and 26.8% significantly (P < 0.05) higher than that of the MC group.

Hepatic glycogen content

Hepatic glycogen contents of all diabetic mice, except for the PC group, were significantly (P < 0.05) lower than that of the NC group (Figure 2). However, the OPH, OPM, and PC groups had significantly (P < 0.05) higher (16.8-82.4%) hepatic glycogen content compared with the MC group. Moreover, the increasing hepatic glycogen contents were observed in a dose-dependent manner in the OP treatment groups (R$^2$=0.9504).

Peptides sequences

The three most prominent ions had m/z ratios of 556.797, 663.32, and 711.87 were clarified. Continuum LC-MS data were processed and searched using PLGS 2.3 software. The peptides sequences of those ions were identified and the MS/MS spectra of Phe-Leu-Gln-Pro-Asn-Leu-Asp-Glu-His...
In preliminary studies we observed that low molecular weight oat protein hydrolysates prepared by nonspecific proteases decreased starch digestibility by inhibiting α-glucosidase activity. The purpose of this study was to determine if oat protein hydrolysates retained their bioactivity in vivo since digestion by stomach acidity and mammalian digestive enzymes could denature or further hydrolyze and eliminate their inhibitory activity. The STZ-induced diabetic mouse model was selected since it had been shown to have a hypoglycemic response to oat β-glucan treatment [14,15]. Metformin was used as a positive control because it is the first line treatment for T2DM. After a 4-week treatment we observed a dose dependent effect where the mice in the OPH group had the lowest total food intake (29.5 g/gm food intake), FINS, IAI (1.03), and hepatic glycogen content. There was a significant correlation between the total food intake and the FBG concentrations (r = 0.994, P < 0.01) and FINS concentrations (r = -0.965, P < 0.01). The results suggest that oat

### Table 1: Effects of different doses oat peptides on body weights in streptozotocin (STZ)-induced diabetic mice.

|          | 0         | 1 Week   | 2 Weeks  | 3 Weeks  | 4 Weeks  |
|----------|-----------|----------|----------|----------|----------|
|          | Body weight (g) |
| NC       | 29.40 ± 1.51a | 32.60 ± 1.96a | 34.80 ± 2.35a | 36.30 ± 2.54a | 38.10 ± 2.96a |
| MC       | 23.91 ± 1.22b | 25.92 ± 1.88b | 27.17 ± 2.21b | 28.67 ± 2.10b | 28.42 ± 2.64b |
| OPH      | 24.17 ± 1.80b | 24.83 ± 1.64b | 26.00 ± 1.95b | 27.33 ± 1.61b | 28.58 ± 1.31b |
| OPM      | 22.75 ± 1.60b | 24.45 ± 1.63b | 26.25 ± 1.96b | 27.08 ± 2.02b | 27.42 ± 2.27b |
| OPL      | 23.42 ± 1.88b | 25.08 ± 2.11b | 26.17 ± 2.17b | 27.25 ± 2.53b | 27.67 ± 2.53b |
| PC       | 23.00 ± 1.41b | 24.67 ± 1.37b | 25.92 ± 1.83b | 26.83 ± 1.59b | 28.08 ± 1.68b |

*Data presented as means ± standard deviations.

+ Different letters in the same column indicate significant differences at P < 0.05.

NC: normal control group; MC: model control group; OPH: high-dose oat peptides group (1.0 g/kg body weight); OPM: medium-dose oat peptides group (0.5 g/kg body weight); OPL: low-dose oat peptides group (0.25 g/kg body weight); PC: positive control group (0.6 g/kg body weight).

![Figure 1](image1.png)

**Figure 1** Effects of different doses oat peptides on fasting blood glucose (FBG) levels in streptozotocin (STZ)-induced diabetic mice during the treatment. Data presented as means ± standard deviations. NC: normal control group; MC: model control group; OPL: low-dose oat peptides group (0.25 g/kg body weight); OPM: medium-dose oat peptides group (0.5 g/kg body weight); OPH: high-dose oat peptides group (1.0 g/kg body weight); PC: positive control group (0.6 g/kg body weight).

![Figure 2](image2.png)

**Figure 2** Effects of different doses of oat peptides on the hepatic glycogen contents in streptozotocin (STZ)-induced diabetic mice after the 4 weeks treatment. Data are presented as means ± standard deviations. Different letters indicate significant difference at P < 0.05. NC: normal control group; MC: model control group; OPL: low-dose oat peptides group (0.25 g/kg body weight); OPM: medium-dose oat peptides group (0.5 g/kg body weight); OPH: high-dose oat peptides group (1.0 g/kg body weight); PC: positive control group (0.6 g/kg body weight metformin).

### Table 2: Effects of different doses oat peptides on fasting insulin (Fins) and insulin activity index (IAI) in streptozotocin (STZ)-induced diabetic mice.

|          | 0         | 1 Week   | 2 Weeks  | 3 Weeks  | 4 Weeks  |
|----------|-----------|----------|----------|----------|----------|
|          | Fins(µIU/L) |
| NC       | 24.50 ± 3.77a | 16.05 ± 2.83a | 18.65 ± 2.81a | 17.45 ± 3.84a | 16.15 ± 3.13a | 21.53 ± 2.55a |
| MC       | 18.83 ± 0.22c | 0.83 ± 0.17c | 1.03 ± 0.021c | 0.87 ± 0.23c | 0.86 ± 0.22c | 1.06 ± 0.15c |
| OPH      | 28.80 ± 0.5c | 36.60 ± 1.1c | 32.60 ± 0.08c | 22.30 ± 1.1c | 36.60 ± 1.1c | 22.30 ± 1.1c |
| OPM      | 27.67 ± 2.53c | 27.42 ± 2.27c | 28.58 ± 1.31c | 27.67 ± 2.53c | 27.67 ± 2.53c | 27.67 ± 2.53c |
| OPL      | 28.42 ± 2.64c | 28.58 ± 1.31c | 27.67 ± 2.53c | 27.67 ± 2.53c | 27.67 ± 2.53c | 27.67 ± 2.53c |
| PC       | 27.33 ± 1.61c | 26.83 ± 1.59c | 28.08 ± 1.68c | 27.67 ± 2.53c | 27.67 ± 2.53c | 27.67 ± 2.53c |

Data are presented as means ± standard deviations. Different letters indicate significant differences at P < 0.05.

NC: normal control group; MC: model control group; OPH: high-dose oat peptides group (1.0 g/kg body weight); OPM: medium-dose oat peptides group (0.5 g/kg body weight); OPL: low-dose oat peptides group (0.25 g/kg body weight); PC: positive control group (0.6 g/kg body weight metformin).

### Discussion and Conclusions

In preliminary *in vitro* studies we observed that low molecular weight oat protein hydrolysates prepared by nonspecific proteases...
Nano-UPLC-MS$^{5}$ Tandem MS spectra of fractions purified from oat hydrolysates. The mass spectrum of the fraction was acquired and the collision induced fragmentations of the fraction was illustrated. Following sequence interpretation and database searching, the MS/MS spectra of a single charged ion with m/z at 556.797, 663.32, and 711.87 were determined to be the peptides (A) Phe-Leu-Gln-Pro-Asn-Leu-Asp-Glu-His (FLQPNLEH), (B) Asp-Leu-Glu-Leu-Gln-Asn-Asn-Val-Phe-Pro-His (DLELQNNVFPH), and (C) Thr-Pro-Asn-Ala-Gly-Val-Ser-Gly-Ala-Ala-Gly-Ala-Gly-Ala-Gly-Lys-His (TPNVGSAGAGAGAGKH).

Figure 3
peptides reduce the FBG and increase FINS by regulating the food intake of diabetic mice. At a dose of 1200 and 2000 mg/kg body weight β-glucan from oat was also reported to reduce the food intake and FBG of STZ-induced diabetic mice [15]. The similarities in response to the β-glucan and peptide fractions were puzzling and may be due to a common highly bioactive constituent.

Insulin can both reduce endogenous glucose production and stimulate glucose utilization. Therefore, insulin-mediated suppression of endogenous glucose production and insulin-dependent stimulation are the primary alterations of glucose metabolism in T2DM [22]. In this study, the OPH and PC treatment groups had significantly (P < 0.05) higher FINS concentrations compared with the MC group. Meanwhile, a significant positive correlation between the FBG concentrations and FINS concentrations (r = 0.979, P < 0.01) was also reported to produce the significantly increasing hepatic glycogen content in alloxan-induced mice [25].

Although the underlying mechanisms of the proteins or peptides hypoglycemic activities are not yet fully understood, the differences in amino acid composition and protein digestibility are issues for consideration. Van Loon et al. showed that the insulin response to dietary amino acids, oligopeptides, and proteins (consumed with glucose) was related to the combination of leucine, phenylalanine, and arginine content [26]. In the present study, the peptides of FLQPNLDEH and DLELQNNVFPH both had two leucine residues and one phenylalanine residue, which might be related to the antidiabetic effects of oat hydrolysates. Whey protein stimulating insulin secretion to a greater extent might be due to its easy digestibility [27]. The true protein digestibility of oat was in the range of 90.3 to 94.2% [28], which might be in favor of the antidiabetic effects of oat peptides.

In summary, the present study showed that oat peptides at the higher doses had hypoglycemic activities on STZ-induced diabetic mice by reducing food intake, stimulating insulin secretion and insulin sensitivity, and elevating glycogenesis. The results indicate that the oat peptides’ antidiabetic action could be linked to more than one mechanism. The mechanism of increasing FINS by oat peptides is still not understood. We plan to conduct further studies that will focus on the induction of the surviving intrinsic pancreatic β-cells to secrete insulin or to prevent the disfunction of β-cells by oat peptides.

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