Jicama (Pachyrhizus erosus) extract increases insulin sensitivity and regulates hepatic glucose in C57BL/Ksj-db/db mice

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This study investigated the effect of jicama extract on hyperglycemia and insulin sensitivity in an animal model of type 2 diabetes. Male C57BL/Ksj-db/db mice were divided into groups subsequently fed a regular diet (controls), or diet supplemented with jicama extract, and rosiglitazone. After 6 weeks, blood levels of glucose and glycosylated hemoglobin were significantly lower in animals administered the jicama extract than the control group. Additionally, glucose and insulin tolerance tests showed that jicama extract increased insulin sensitivity. The homeostatic index of insulin resistance was lower in the jicama extract-treated group than in the diabetic control group. Administration of jicama extract significantly enhanced the expressions of the phosphorylated AMP-activated protein kinase and Akt substrate of 160 kDa, and plasma membrane glucose transporter type 4 in skeletal muscle. Jicama extract administration also decreased the expressions of glucose 6-phosphatase and phosphoenolpyruvate carboxykinase in the liver. Jicama extract may increase insulin sensitivity and inhibits the glucose genesis in the liver.

Key Words: jicama extract, insulin sensitivity, postprandial hyperglycemia, inulin, db/db mice, hepatic glycogen

Diabetes is the most serious health concern worldwide. Type 2 diabetes mellitus is characterized by high blood glucose levels resulting from a combination of peripheral insulin resistance, decreasing pancreatic β-cell function, and impaired regulation of hepatic glucose production. Although current management of diabetes involves exercise and diet modification, medications are typically required as the disease progresses. Sometimes, these medications can induce undesirable side-effects such as hypoglycemia, poor control of postprandial blood glucose level, and weight gain. Therefore, there has been a growth in research focused on natural products with therapeutic potential for diabetes.

Insulin helps to maintain glucose homeostasis and promote glucose utilization in the body. Insulin increases glucose utilization in peripheral organs such as skeletal muscle and adipose tissue and suppresses hepatic glucose production. AMPK is an important enhancer of insulin sensitivity. Activation of AMPK enhances insulin-stimulated glucose transport. These changes result in improved hyperglycemia and insulin sensitivity in type 2 diabetes. Zang et al. reported that a number of natural components of human diet strongly activate AMPK in the muscle tissue, accounting for the anti-diabetic effect of these natural derivatives.

Jicama (Pachyrhizus erosus), also called yam bean, is an edible tuberous root grown in many parts of America, Southeast Asia, and Western Africa. Jicama is characterized by a crispy texture and sweet and starchy taste. It is rich in fructooligosaccharides and inulin, a soluble fiber. Jicama is consumed worldwide. Recently, jicama was placed in the spotlight as a healthy food ingredient. Jicama is used as a health-promoting food in a number of countries. Several studies have described the chemical constituents of jicama. Additionally, further studies have reported the many biological benefits of jicama, including its immunomodulatory activity and ability to reduce the risk of colon cancer. However, the effect of jicama extract on type 2 diabetes has not been determined to date, particularly with regard to potential improvements in hyperglycemia and insulin sensitivity. Therefore, our current study was designed to evaluate the effect of jicama extract on hyperglycemia and insulin sensitivity in C57BL/Ksj-db/db mice that exhibit a number of characteristics of type 2 diabetes.

Materials and Methods

Preparation of jicama extract. Jicama was purchased from Hadong (Kyongsang-nam-do, Korea). Jicama was washed in distilled water, and then cut into portions 0.1–1 cm in size. Sliced jicama was dried at 60°C and ground into a powder. For extraction, dried jicama powder was soaked with water at room temperature overnight. After soaking, the extract was sonicated three times for 5 h at 60°C and filtered through filter paper (Whatman No.1, Fairfield, NY). Suspensions were centrifuged at 450 × g for 30 min (MICRO 17R, Hanil, Inc). Jicama extract was stored in a freezer until used.

Animals and diets. Male C57BL/KsJ-db/db mice were purchased from Japan SLC (Hamamatsu, Japan). Five-week-old db/db mice were fed a pelleted commercial chow diet for 2 weeks after arrival. They were subsequently randomly divided into 3 groups (n = 7 per group): db/db mice in the control group (DMC) were fed a standard semi-synthetic diet (AIN-93G) for 6 weeks, while animals in the other 2 groups were fed the AIN-93G diet supplemented with either rosiglitazone (0.005% w/w) or Jicama extract (0.5%, w/w) (Table 1). All mice were kept individually caged in light-12 h on/12 h off and temperature-controlled room with food and water available ad libitum. At the end of the experimental period, mice were anesthetized with ether after withholding food for 12 h, and blood samples were collected from the inferior vena cava to determine levels of plasma biomarkers. After collection of the blood sample, the liver was dissected out, rinsed with physiological saline solution. All procedures were approved by the animal ethics committee of our university.

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Blood glucose and glycosylated hemoglobin levels. The glucose concentration in the venous blood drawn from the tail vein was measured using a glucometer (Roche Diagnostics GmbH, Mannheim, Germany) every week after a 12-h fast. Anticoagulated whole-blood samples were hemolyzed and the concentration of glycosylated hemoglobin (HbA1c) was measured. HbA1c levels were determined by immuno-turbidimetry.

Plasma insulin level. Blood samples from the inferior vena cava were collected into heparin-coated tubes. After centrifugation at 1,000 × g for 15 min at 4°C, plasma was carefully removed from the sample. Levels of plasma insulin were determined by radioimmunoassay with enzyme-linked immunosorbent assay (ELISA) kit (Millipore Corporation, Billerica, MA).

Homeostatic index of insulin resistance and quantitative insulin sensitivity check index. Homeostatic index of insulin resistance (HOMA-IR) and quantitative insulin sensitivity check index (QUICKI) were determined using the homeostasis model assessment with the following equation [Eq. (1)]:

\[
\text{HOMA-IR} = \frac{\text{Fasting glucose (mmol/L)} \times \text{fasting insulin (IU/ml)}}{22.51}
\]

QUICKI was calculated using the inverse of the sum of the logarithms of fasting insulin and fasting glucose by using the following equation [Eq. (2)]:

\[
\text{QUICKI} = \frac{1}{\log (\text{fasting glucose mg/dl}) + \log (\text{fasting insulin IU/ml})}
\]

Intraperitoneal glucose tolerance test and Intraperitoneal insulin tolerance test. An intraperitoneal glucose tolerance test (IPGTT) was performed at 5 weeks after the start of the experimental diets. Following an overnight fast, mice were injected intraperitoneally with glucose [0.5 g/kg of body weight (BW)], and their blood glucose levels were determined in tail blood samples 0, 30, 60, and 120 min after glucose administration. Additionally, an intraperitoneal insulin tolerance test (IPITT) was performed during the last week of the experimental period. After an overnight fast, human insulin (0.33 U/kg of BW) was administered by intraperitoneal injection to the mice, and blood samples were collected for determination of glucose levels 0, 30, 60, and 120 min later.

Hepatic glycogen assay. Glycogen concentration was determined using the approach previously presented by Seifert and Dayton(14) with some modifications. Liver tissue was homogenized in 5 volumes of and 30% (w/v) ice-cold KOH solution and dissolved in a boiling water bath (100°C) for 30 min. Glycogen was precipitated with ethanol, and then pelleted, washed, and resolubilized in distilled water. Resulting solution was treated with an anthrone reagent [2 g anthrone/1 L of 95% (v/v) H₂SO₄], and its absorbance was measured at 620 nm.

Plasma membrane fraction of skeletal muscle. Muscle tissue was placed in a buffer (5 mM sodium azide, 0.25 M sucrose, 0.1 mM phenylmethylsulfonyl fluoride, 10 mM NaHCO₃, pH 7.0) at 4°C. Sub-fractionation of muscle membrane was performed as Baron et al.(15) using procedures modified from those described by Klip et al.(16,17) Dissected skeletal muscle was homogenized and centrifuged at 1,000 × g for 10 min, and the supernatant collected and stored. The resulting pellet was resuspended in the buffer and re-homogenized in a glass homogenization tube. The supernatant was collected and combined with the first supernatant, and the combination centrifuged at 9,000 × g for 10 min. The resulting supernatant was then centrifuged at 190,000 × g for 60 min. Obtained membranes were subsequently applied to a discontinuous sucrose gradient containing 25, 30 and 35% (wt/vol) solutions and were centrifuged at 190,000 × g for 16 h. Membranes were collected from the top of each sucrose gradient, resuspended in the buffer, pelleted by centrifugation at 190,000 × g for 60 min, and resuspended in buffer.

Western blot. Western blot analysis was performed on tissue extract of the liver and skeletal muscle tissue. Liver and skeletal tissue homogenated in ice-cold lysis buffer were centrifuged at 14,000 rpm/min at 4°C, for 15 min and the supernatant were collected. Protein concentrations in the supernatants were measured using the protein kit (Bio-Rad, Hercules, CA). Then, 30 μg protein samples were separated on 12% resolving Tris-HCl gels. Separated proteins were transferred to nitrocellulose membranes. The membranes were blocked in 5% skim milk in Tris-buffered saline, 0.1% Tween-20 for 1 h at room temperature. Blocked membranes were incubated with antibodies overnight at 4°C. Antibodies against phospho AMPK (pAMPK), AMPK phospho-AS160 (Thr172), AS160, plasma membrane GLUT4 (PM-GLUT4), GLUT4, G6Pase and phosphoenolpyruvate carboxylase (PEPCK) were purchased from Cell Signaling Technology (Beverly, MA). The membranes were then washed and probed with a secondary antibody for 1 h at room temperature. Each antigen-antibody
complex was visualized using ECL western Blotting Detection Reagents and detected by chemiluminescence with LAS-1000 plus (FUJIFILM, Tokyo, Japan). Band densities were determined by an image analyzer (Multi Gauge ver. 3.1, FUJI FLIM Corporation, Valhalla, NY) and normalized to β-actin for total protein.

**Statistical analyses.** Data are represented as means ± SD. Statistical analyses were performed using SAS software. Differences between groups were evaluated for significance using one-way analysis of variance (ANOVA) followed by post-hoc Duncan’s multiple range tests.

**Results**

**Body weight, food intake and water intake.** Throughout the experiment, body weights of all mice were monitored weekly. Fig. 1 showed the weekly changes in body weight during 6 weeks. At the start of the study, body weights of mice in db/db-control, db/db-RG, and db/db-JCE groups did not differ significantly. Body weight increased gradually during the 6-week period. At the end of the study, mice in the db/db-RG group exhibited significantly higher body weights than animals in the db/db-control and db/db-JCE groups. Body weight increased gradually during the 6-week period. At the end of the study, mice in the db/db-RG group exhibited significantly higher body weights than animals in the db/db-control and db/db-JCE groups. Daily food intakes of mice in the db/db-control, db/db-RG, and db/db-JCE groups were 5.51 ± 0.77, 4.81 ± 1.55, and 5.03 ± 0.96 g/day, respectively. Especially, water intake was observed to be significantly higher in db/db-JCE group mice than animals in the db/db-JCE group. Daily water intake was 28.57 ± 3.02 and 15.52 ± 3.39 ml/day in the db/db-control and db/db-JCE groups, respectively.

**Blood glucose and HbA1c levels.** Fasting blood glucose levels in db/db-JCE group mice are shown in Fig. 2. At the start of the study, blood glucose levels were not significantly different between groups; However, fasting blood glucose levels in db/db-JCE group mice after 2 weeks were significantly lower than those in the db/db-RG control group mice. Blood glucose levels measured in db/db-control group mice were elevated throughout the experiment, likely reflecting the progress of diabetic mellitus. As shown in Table 3, HbA1c values were 12.92 ± 0.31 and 9.11 ± 0.38 in db/db-control and db/db-JCE groups, respectively. HbA1c values in mice in the db/db-JCE group were significantly lower than those measured in the db/db-control group mice ($p<0.05$).

**Plasma insulin levels, HOMA-IR and QUICKI.** Plasma insulin levels measured in experimental animals are shown in Table 3. Plasma insulin levels were markedly lowered in the mice of the db/db-RG group compared to those in the db/db-control group. Similarly, db/db-JCE group mice exhibited significantly lowered plasma insulin levels than mice in the db/db-control group. HOMA-IR values were significantly lower in the db/db-JCE group than in the db/db-control group ($p<0.05$). Additionally, QUICKI values were significantly higher in the db/db-JCE group than in the db/db-control group.

**Intraperitoneal glucose tolerance test and Intraperitoneal insulin tolerance test.** We performed IPGTT in db/db mice that were administered the jicama extract to evaluate its ability to improves glucose tolerance. The results are presented as percentages of the measurement performed at glucose injection ($t = 0$). As shown in Fig. 3A, blood glucose levels in the db/db-JCE group peaked at 60 min after injection and recovered to levels close to the basal value at 120 min. Similar pattern was observed in mice in

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**Table 2.** Effects of jicama extract dietary supplement on water and food intakes of C57BL/KsJ-db/db mice

<table>
<thead>
<tr>
<th></th>
<th>db/db (diabetes mellitus control)</th>
<th>db/db-RG</th>
<th>db/db-JCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water intake (ml/day)</td>
<td>28.57 ± 3.02*</td>
<td>15.52 ± 3.39a</td>
<td>4.74 ± 1.74*</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>5.51 ± 0.77m</td>
<td>5.03 ± 0.96b</td>
<td>4.81 ± 1.55</td>
</tr>
</tbody>
</table>

*db/db (diabetes mellitus control), C57BL/KsJ-db/db mice supplemented with AIN-93G diet; db/db-RG: C57BL/KsJ-db/db mice fed AIN-93G diet supplemented with rosiglitazone (0.005 g/100 g diet); db/db-JCE: C57BL/KsJ-db/db mice fed AIN-93G diet supplemented with jicama extract (0.5 g/100 g diet). Values are presented as means ± SD, $n = 7$ per group. **Mean values designed by different letters are significantly different between groups ($p<0.05$).
the db/db-RG group. However, no significant changes in blood glucose levels were observed in db/db-control group. IPITT was also performed to determine the effects of jicama supplementation on insulin tolerance. The results are presented as percentages of the measurement at insulin injection (t = 0). As shown in Fig. 3B, db/db-JCE and db/db-RG groups exhibited a rapid reduction in blood glucose levels compared with the db/db-control group, with blood glucose levels decreasing within 120 min of the insulin injection.

Expression of PM-GLUT4, GLUT4, pAMPK, AMPK, pAS160 and AS160 in the skeletal muscle. As shown in Fig. 4A, we showed the gene expression of PM-GLUT4, GLUT4, pAMPK, AMPK, pAS160 and AS160 protein in skeletal muscle. db/db-JCE group significantly increased PM-GLUT4 and total GLUT4, compared with those in the db/db control group (Fig. 4B). AMPK gene expression was no significance relation between db/db-JCE and db/db group. However, phosphorylation of AMPK and AS160 in db/db-JCE group was significantly enhanced, compared to db/db control group. Therefore, the phosphorylation levels of AMPK and AS160, two upstream regulators of plasma membrane GLUT4, were significantly enhanced by supplementation of jicama, suggesting that jicama extract may stimulate glucose uptake in skeletal muscles through activation of AMPK-AS160-GLUT4 pathway.

Expression of gluconeogenic enzyme G6Pase and PEPCK and hepatic glycogen level in liver. As shown in Fig. 5A and B, the expression of PEPCK in the liver was significantly decreased in db/db-JCE group, with 2-fold lower levels than those in the db/db-control group. Similarly, the expression of G6pase in db/db-JCE group was significantly decreased compared to that in db/db-control group. Therefore, the expression levels of gluconeogenic enzymes G6Pase and PEPCK in the liver were significantly lower in the db/db-JCE group than the db/db-control group. Hepatic glycogen concentrations in the db/db-JCE group was significantly higher than concentrations in the db/db control group, with values of 116.49 ± 21.65 mg/g liver measured in db/db-JCE and db/db-control groups, respectively (Fig. 5C).

Discussion

Major features of type 2 diabetes are low insulin sensitivity and pancreatic β-cell dysfunction. The fundamental treatment of diabetes, therefore, aims to increase insulin sensitivity and alleviate hyperglycemia. The aim of this study was to investigate the effect of jicama extract on improving hyperglycemia and insulin sensitivity using db/db mice, an animal model of type 2 diabetes. Mice in db/db-JCE group exhibited an increased body weight. However, this increase was significantly lower than that observed in the db/db-RG group. Elevations in body weight in the db/db-RG group mice were attributable to increased adipose tissue mass. Such increase in adipose tissue mass has also been observed in patients undergoing rosiglitazone treatment. Czonski-Murray et al. reported that rosiglitazone intake is associated with weight gain. In light of these findings, weight gain can be a side-effect of rosiglitazone use.

Table 3. Effects of jicama extract dietary supplementation on blood glycosylated hemoglobin levels and markers of insulin resistance in C57BL/KsJ-db/db mice

<table>
<thead>
<tr>
<th></th>
<th>db/db</th>
<th>db/db-JCE</th>
<th>db/db-RG</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c</td>
<td>12.92 ± 0.31*</td>
<td>9.11 ± 1.06*</td>
<td>7.45 ± 0.4*</td>
</tr>
<tr>
<td>Plasma insulin (pmol/L)</td>
<td>251.04 ± 25.24a</td>
<td>191.85 ± 4.65a</td>
<td>139.06 ± 21.96c</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>40.43 ± 4.03*</td>
<td>22.26 ± 0.53a</td>
<td>5.30 ± 0.84a</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.22 ± 0.01*</td>
<td>0.24 ± 0.01a</td>
<td>0.29 ± 0.01a</td>
</tr>
</tbody>
</table>

Mean values designated by different letters are significantly different between groups (p<0.05). HbA1c, blood glycosylated hemoglobin; HOMA-IR, homeostatic index of insulin resistance; QUICKI, quantitative insulin sensitivity check index.
Water intake was notably higher in db/db-control group than the db/db-JCE group. Marked increase in water intake likely reflects polydipsia, one of the major diabetes symptoms of diabetes. Supplementation with jicama reduced the symptoms of diabetes, especially polydipsia, suggesting that it can reduce the water intake.

The increase in fasting blood glucose levels was significantly attenuated in db/db-JCE group mice from the 2nd week of the experimental diet. Lowering blood glucose levels can prevent diabetic complications. Additionally, mice in the db/db-JCE group significantly lower HbA1c levels. HbA1c levels are indicative of the average blood glucose levels over the preceding 2–3 months. Lowering HbA1c level can reduce the risk of macrovascular complications of type 2 diabetes. These findings suggest that supplementation with jicama extract can alleviate hyperglycemia in type 2 diabetes. One possible factor by which jicama extract can alleviate hyperglycemia could be inulin, which is founded in jicama. Jicama is rich in fructooligosaccharides and inulin, a soluble fiber.

The present study demonstrated that supplementation with the jicama extract can improve plasma insulin levels. The results of this study show the levels of plasma insulin in db/db-control group mice to be higher than those in db/db-JCE group mice, suggesting that db/db-control group mice still showed hyperinsulinemia. In general, db/db mice exhibit an initial phase of hyperinsulinemia, while insulinopenia can progressively develop with age. It is a features commonly observed in the late stage of type 2 diabetes. In current study, plasma insulin levels in db/db-JCE group mice were significantly lower than those measured in db/db-control group mice, while the levels were significantly higher than those measured in db/db-RG group mice. Therefore, mice in the db/db-control group still displayed hyperinsulinemia. On the other hand, db/db-RG and db/db-JCE group mice exhibited reduced hyperinsulinemia. Kaur and Gupta reported that inulin and oligofructose modulate levels of insulin and glucagon hormones, thereby regulating carbohydrate metabolism by lowering blood glucose levels.

Chilelli et al. reported that HOMA-IR and QUICKI calculated from fasting insulin and glucose levels are the commonly used measures of insulin sensitivity. HOMA-IR was significantly lowered in the db/db-JCE group mice than the values measured in db/db-control group mice. Our results suggest that supplementation with jicama extract may enhance insulin sensitivity.

Consistent with these findings, Kim and Egan reported that dietary fiber might increase insulin sensitivity. The result of IPITT
revealed improved glucose levels in db/db-JCE and db/db-RG group mice, compared to the levels measured in db/db-control group mice. Moreover, IPGTT outcomes were improved in db/db-JCE group mice compared to db/db-control group mice. These results indicate that jicama extract may have potential as insulin sensitizer, comparing glucose tolerance to values measured in db/db-control group mice. Accordingly, blood glucose-lowering response in db/db-JCE group mice appear to reflect improved insulin sensitivity, which is required for the reduction of blood glucose levels, while db/db-control group mice did not show any alterations in the postprandial glucose level.

Changes in the expression of genes related to insulin sensitivity were examined using western blotting. Insulin binding to the insulin receptor regulates glucose uptake into cells via GLUT4, suggesting a key role for GLUT4 in glucose uptake and metabolism. Fryer et al. reported that increased expression of AMPK stimulates glucose uptake in to the cells, in association with increased translocation of GLUT4 to the plasma membrane. In this study, supplementation with jicama extract significantly increased the expression of GLUT4 in plasma membrane. Additionally, it increased the expression of phosphorylated AMPK and AS160 in db/db-JCE group mice, suggesting that jicama extract may stimulate glucose uptake in skeletal muscles through activation of the AMPK-AS160-GLUT4 pathway. Activation of AMPK resulted in increased expression of GLUT4, which enhances glucose uptake into these tissues. This change can contribute to the amelioration of hyperglycemia. AS160 is a substrate that links insulin signaling with GLUT4 trafficking. Phosphorylation of AS160 was shown to increased GLUT4 vesicle movement to the plasma membrane. Therefore, AS160 plays an important role in the regulation of insulin-induced GLUT4 translocation. Yun et al. reported that inulin and soluble fiber contained in jicama extract may activate AMPK.

In the liver, the expression of key gluconeogenic enzymes, G6Pase and PEPCK, was significantly decreased by supplementation with the jicama extract. These results suggest that jicama extract may reduce blood glucose levels by stimulating hepatic glycogen synthesis and inhibiting gluconeogenesis. In the postprandial state, the liver is stimulated by insulin to take up more glucose from the blood and synthesize glycogen, consequently...
attenuating the postprandial hyperglycemia. Conversely, in the starved state, liver generates more glucose to maintain adequate blood glucose levels. However, in the diabetes condition, glycogen synthesis is inhibited while gluconeogenesis is abnormally enhanced because of the inefficient utilization of glucose. Pushparaj et al. proposed that reduced activities of PEPCK and G6Pase enzymes correspond to decreased blood glucose levels, since less glucose is being produced and released into the blood stream. In our current study, the hepatic glycogen concentration in db/db-JCE group mice was significantly higher than in the db/db control group mice. Luo et al. reported that inulin decreased basal hepatic glucose production in a process separated from insulin-stimulated glucose metabolism. Several drug targets in the liver offer new ways of attenuating excessive hepatic glucose production.

In conclusion, this study demonstrates that supplementation with jicama extract enhances insulin sensitivity and regulates hepatic glucose in db/db mice. Jicama extract enhanced the expression of GLUT4 in plasma membrane of skeletal muscle through activation of pAMPK and pAS160 and suppressed hepatic gluconeogenesis, potentially ameliorating hyperglycemia. Supplementation with jicama extract therefore elicits beneficial effects in type 2 diabetes animals, suggesting that it may have potential for use as an anti-diabetes supplement.

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Author Contributions
Ji-Sook Han conceived and designed the experiments; Hyun-Ah Lee and Chan Joo Park performed the experiments; Chan Joo Park wrote the paper.

Conflicts of Interest
No potential conflicts of interest were disclosed.

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