Effect of whey protein on insulin sensitivity in mouse models for diabetes induced by streptozotocin

ABSTRACT

Some studies demonstrated that dairy whey protein (WP) is of benefit to diabetes patients. In the present study, mouse models for type 2 diabetes mellitus (T2DM) and type 1 DM (T1DM) were induced by single injection of 100 and 160 mg/kg streptozotocin (STZ). WP solution was intragastrically administered every other day for 4 weeks. Body weight, food and water intake were recorded once a week. Fasting blood glucose, fasting insulin and HOMA-IR, as an index of insulin resistance, were evaluated. As a result, mice drank more in both STZ groups compared with the control group. Body weight in 100 mg/kg STZ group was similar to that of the control group after a transient decrease when STZ injection was administered. Insulin secretion significantly decreased after the injection of 100 mg/kg STZ and became ineffective after 160 mg/kg STZ. 40% WP administration decreased insulin level by 33.2 and 45.3% in the control group and 100 mg/kg STZ group, respectively. HOMA-IR was significantly decreased by 20 and 40% WP administration in the control group and by 40% WP administration in 100 mg/kg STZ group. In conclusion, WP administration improves insulin resistance in normal mice and non-obese T2DM mice induced by STZ.

Key words: Whey protein, diabetes, insulin, insulin resistance.

INTRODUCTION

In general, diabetes mellitus (DM) is classified into two types: type 1 DM (T1DM) and type 2 DM (T2DM). T1DM is characterized by complete destruction of pancreatic beta cells resulting in absolute insulin deficiency, and T2DM by a combination of resistance to insulin action and inadequate compensatory insulin secretory response. T1DM is the most common type of diabetes in children and adolescents, and T2DM often occurs in adulthood despite the increasing patients in youth (Maahs et al, 2010). DM is an important cause of mortality and morbidity worldwide, through both direct clinical sequelae and increase mortality from complications (Maahs et al, 2010; Danaei et al, 2011). Thus, strategies of DM prevention and treatment are urgently needed to reduce the huge burden of DM.

A large body of evidence has demonstrated that DM incidence is related to dietary patterns and effective management for DM patients should be achieved with an appropriate diet. Milk is a naturally nutrient-rich food, including high quality proteins: casein and whey protein (WP). WP accounts for 20% of total protein in bovine milk and is usually a by-product of cheese manufactured from cow’s milk. An increasing number of studies have demonstrated the beneficial effect of WP intake on obese individuals, T2DM patients and insulin-resistant rats by multimechanisms, including weight loss, satiety increase and regulation of insulin sensitivity (Pal et al, 2010; Frid et al, 2005; Belobrajdic et al, 2004; Shertzer et al, 2011). Badr (2012) and Badr et al (2012) also found that WP improved the healing and closure of diabetic wounds in T1DM mice thus, WP is a potential supplementation as an adjuvant.
therapy for DM patients. Common methods for diabetes model in rodents include high-fat diet feeding and streptozocin (STZ) injection. High-fat diet induces obesity and T2DM after a long-term feeding. STZ is a pancreatic poison and can result in both T1DM and T2DM with a single injection of different amounts (Ito et al., 2001; Giarratana et al., 2007). In this preliminary study, we established mouse models for T1DM and T2DM using STZ and observed the effect of WP on mice growth, food and water intake, and insulin sensitivity.

MATERIALS AND METHODS

A total of 90 male ICR mice were purchased at 8 week of age from Shanghai laboratory animal center (Shanghai, China). The mice were housed in air-conditioned animal room at 23°C±2°C with a 12-h light-dark cycle. All experiments involving mice were approved by the Animal Ethics Committee of Soochow University. After one week of acclimation, mice were randomly divided into 3 groups. Two of the groups received a single i.p. injection of 100 and 160 mg/kg STZ (sigma, St. Louis, MO) respectively. STZ freshly dissolved in 0.1 mol/L citrate buffer (pH 4.2) to indicated concentrations. According to Ito et al. (2001) and Giarratana et al. (2007) reports, a single injection of 100 or 160 mg/kg STZ induced the model of T2DM and T1DM, respectively. The remaining 30 mice were injected citrate buffer in equivalent volume as the control group.

Three days later, mice in 3 groups were further assigned into 4 administrations (7, 7, 8 and 8 mice, respectively), each intragastrically administering with 0, 10, 20 and 40% of WP every other day. WP concentrate (80%) was purchased from Warrnambool Cheese & Butter Factory Co Ltd (Alansford, south-west Victoria, Australia) and dissolved in distilled water. The experiment lasted 4 weeks.

Before administration and at the end of experiment, blood was taken from veins of the eyes after overnight food deprivation and fasting blood glucose was immediately determined using Roche blood glucose meter (E Hoffmann-La Roche Ltd, Basel, Switzerland). The remaining blood was immediately centrifuged and plasma was stored at -80°C for the determination of fasting insulin using rat/mouse insulin ELISA kit (Millipore, Billerica, MA). Insulin resistance was estimated by homeostasis model assessment-insulin resistance (HOMA-IR) using the following formula:

\[
\text{HOMA-IR} = \frac{\text{fasting blood glucose (mmol/L)}}{22.5} \times \text{fasting insulin (pmol/L)}
\]

During the experiment, the general status of mice was observed every day. Body weight, food and water intake were recorded once a week. At autopsy, the head of pancreas was stored in 10% neutral buffered formalin and then embedded in paraffin. Sections were cut at 3 μm and stained with hematoxylin and eosin for morphological examination.

All data are described as mean±SD. Differences were analyzed by one-way analysis of variance using SPSS programs (SPSS Inc., Chicago, IL). P-values less than 0.05 were considered significant.

RESULTS

All mice were alive during the experiment. Appetite and activity of mice decreased in 100 mg/kg STZ group. Besides poor appetite and less activity, the typical diabetic symptoms, such as obvious loss of weight, polyuria, polydipsia, urine foul smell like the odor of rotten apples, were observed in 160 mg/kg STZ group. Morphological examination showed clumped or funicular distribution of pancreas islet in the control group. In 100 mg/kg STZ group, pancreas islet was infiltrated by lymphocyte with unclear cell boundary. In 160 mg/kg STZ group, the number and size of islet further decreased with focal fibrosis, karyolysis and karyopyknosis occurred in islet cells (Figure 1). During the experiment, the body weight of mice in the control group gradually increased. Food and water intake kept constant with slight fluctuation. In 100 mg/kg STZ group, the body weight decreased after STZ injection and showed catch-up growth during the experiment. Mice drank more in 40% WP administration than others. In the 160 mg/kg STZ group, the body weight of mice markedly decreased after STZ injection and did not reach the basal
level until the end of experiment. Food intake increased to a high platform level and water intake continued to increase during the experiment (Figure 2).

The differences at the end were statistically analyzed among the 3 groups and among 4 WP administrations. Compared with the control group, body weight was significantly decreased (31.1±0.5 g vs. 41.3±0.3 g, P<0.01) and food intake was significantly increased (12.3±0.7 vs. 7.6±0.2, P<0.01) in 160 mg/kg STZ group. Mice drank more in 100 and 160 mg/kg STZ groups than in the control group (13.7±4.4 g, 59.5±2.2 g vs. 6.0±0.3 g, both P<0.01). The amount of drink increased by almost 10 times in 160 mg/kg STZ group. In 100 mg/kg STZ group, mice drank 9.7±2.1 g, 13.3±2.0 g, 11.9±1.8 g and 19.9±2.1 g of water in 0, 10, 20 and 40% WP administrations, respectively (compared with 0%: P<0.01 for 10 and 40% WP, P<0.05 for 20% WP). There were no other significant differences observed.

After modeling, the insulin levels were 149.9±25.4 pmol/L, 107.6±27.1 pmol/L and 3.3±1.0 pmol/L, and glucose 4.0±0.6 mmol/L, 6.5±0.9 mmol/L, 21.8±2.6 mmol/L, in normal, 100 and 160 mg/kg STZ groups, respectively. Thus, injection of 100 mg/kg STZ significantly decreased insulin levels and 160 mg/kg STZ completely destroyed the islet function without effective insulin secretion. In normal group and 100 mg/kg STZ group, 40% WP administration significantly decreased insulin level by 33.2 and 45.3%, respectively. In 100 and 160 mg/kg STZ groups, levels of blood glucose reached 20 mmol/L with a slightly decrease in 40% WP administrations (Table 1). HOMA-IR was used to estimate insulin resistance. In the control group, 20 and 40% WP administration significantly decreased HOMA-IR compared with no administration (34.5±14.0, 28.2±14.8 vs. 60.3±22.8 P<0.01). In 100 mg/kg STZ group, 40% WP administration had significantly lower value than the 3 others (47.6±12.7 vs. 145.7±46.3, 109.9±39.0 and 126.8±51.2, P<0.01).

**DISCUSSION**

In the present study, we established mouse models for T1DM and T2DM using single injection of STZ. Typical diabetes...
symptoms appeared in both models. In the T1DM model (160 mg/kg STZ group), there was no effective insulin secretion due to the destruction of pancreatic beta cells. In this case, WP administration cannot improve insulin sensitivity. It suggests that whey or its metabolites is dependent on the normal, at least in part, pancreatic islet to play a physiological function. In normal mice and T2DM model (100 mg/kg STZ group) mice, high WP administration decreased the insulin levels and insulin resistance, indicating the rise of insulin sensitivity.

There is plausible that mice drank more after STZ injection. Furthermore, WP administration increased water intake in T2DM model. Amino acids hydrolyzed from protein cannot be stored and the resulting excessive amino nitrogen has to be excreted via urine. For the urinary excretion and filtration of urea, water is needed. The increased drink after increased whey intake was also observed by Freudenberg et al (2013). Thus, the harmful effect of high protein on renal function should be considered in diabetes patients.

Animal studies prefer to use obese model or insulin-resistant model induced by obesity. Although so-called obesity epidemic accounts for the increased incidence of T2DM across the world, not all of patients with T2DM occur in obese population. In the present study, high WP administration decreased plasma insulin levels and improved insulin sensitivity in normal mice and non-obese T2DM model mice. There is consistent evidence in animal study and human study. Belobrjadic et al (2004) found whey protein concentration administrated for 8 weeks decreased plasma insulin by 40% and increased insulin sensitivity in insulin-resistant rats. These changes were explained by the reduction in visceral fat. However, fasting insulin levels and insulin resistance scores were significantly decreased without the change of body fat after 12-week whey intake in overweight/obese individuals (Pal et al., 2010). Although we did not determine the body composition of mice, body weight is similar among WP administrations each model, which is supported by Noatsch study (Noatsch et al., 2011). However, postprandial studies have demonstrated that whey protein has a stimulating effect on insulin secretion in healthy subject and in diabetes (Frid et al., 2005; Nilsson et al., 2007). The key mechanism is not known and further studies are required to resolve why whey protein has an insulinotrophic effect in the short term, but can decrease plasma insulin level and improve insulin sensitivity in the long term.

### Table 1. Effect of WP on fasting blood glucose and fasting insulin before and after WP intervention in 3 groups (n=10).

<table>
<thead>
<tr>
<th>Group</th>
<th>Insulin (pmol/L)</th>
<th>Glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 4</td>
</tr>
<tr>
<td></td>
<td>Control group</td>
<td></td>
</tr>
<tr>
<td>WP 0%</td>
<td>149.3±21.0</td>
<td>159.7±52.1</td>
</tr>
<tr>
<td>10%</td>
<td>144.8±19.5</td>
<td>148.5±57.5</td>
</tr>
<tr>
<td>20%</td>
<td>158.7±25.5</td>
<td>129.4±35.7</td>
</tr>
<tr>
<td>40%</td>
<td>153.3±20.8</td>
<td>106.6±63 b</td>
</tr>
<tr>
<td></td>
<td>105.6±25.2 c</td>
<td>159.9±24.4</td>
</tr>
<tr>
<td>10%</td>
<td>101.9±29.1 c</td>
<td>116.4±38.5 b</td>
</tr>
<tr>
<td>20%</td>
<td>108.8±33.4 c</td>
<td>131.3±33.8 a</td>
</tr>
<tr>
<td>40%</td>
<td>113.9±25.9 c</td>
<td>57.5±10.5 ac</td>
</tr>
<tr>
<td></td>
<td>3.44±1.13 c</td>
<td>1.98±0.81 c</td>
</tr>
<tr>
<td>10%</td>
<td>3.13±1.19 c</td>
<td>2.60±0.71 c</td>
</tr>
<tr>
<td>20%</td>
<td>3.38±0.45 c</td>
<td>2.54±0.74 c</td>
</tr>
<tr>
<td>40%</td>
<td>3.44±1.51 c</td>
<td>3.44±1.38 bc</td>
</tr>
</tbody>
</table>

*P <0.01*: significantly different from the control group in the same whey treatment; *P <0.05*: significantly different from the 0% WP in the same group.

### Conclusion

WP administration for 4 weeks increased insulin sensitivity, as indicated by the decrease of HOMA-IR, in normal mice and non-obese T2DM mice induced by STZ. On the other hand, no effect of WP on T1DM model was observed because of the complete destruction in pancreatic beta cells.

### Competing interests

The authors have no conflict of interests to disclose.
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