Effects of raw soybean-enriched diet on blood glucose in the KK-A\(^y\) mice, a type 2 diabetes model

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INTRODUCTION

Diabetes patients and those likely to be diabetic are estimated to total 7.4 million and 8.8 million, respectively, in Japan\(^1\). The improvement of dietary habits may be one of important means to prevent the onset of type 2 diabetes as well as other metabolic syndromes, and to retard the progression of such diseases.

Recent studies showed that some soybean constituents, e.g., isoflavones, have antioxidant and estrogenic activities\(^2\text{-}7\). Isoflavones also alleviated the symptoms of type 2 diabetes\(^6\text{-}7\), and enhanced lipid and glucose metabolisms through the peroxisome-proliferator activated receptor pathways\(^7\). Other constituents, like heat-labile protease inhibitors, i.e., the Bowman-Birk inhibitor, was reported to have an anticarcinogenic effect in animal experiments, although trypsin inhibitors have been considered as an antinutritional factor\(^3\text{-}8\text{-}9\).

In spite of these achievements, articles reporting the dietary significance of raw soybeans, which contain...
several heat-labile components, are relatively scarce. In this study, we investigated the effects of the long-term feeding of a raw soybean powder-enriched diet on blood glucose in type 2 diabetic KK-A^T mice, by measuring their body weight, diet/water intakes and blood/urinary glucose levels. Experiments were also done using a steamed soybean diet or diet without soybeans.

**EXPERIMENTAL METHODS**

1) **Diets**

Dried soybeans, a product of Hokkaido, Japan, and commercially available at a supermarket, were finely powdered using a blender, and added to chow MF (pellet type; Oriental Yeast, Tokyo, Japan) powdered as already mentioned. If necessary, the powdered soybeans were steamed by autoclaving at 120°C for 5 min.

2) **Animals and data measurements**

Female KK-A^T mice (type 2 diabetes model, Kyudo, Kumamoto, Japan, the 5th week of age), weighing about 24 g, were acclimated to the animal facility for 1 week, while fed the pelleted chow MF. The mice (now at the 6th week of age) were then randomized on the basis of their body weight and blood glucose level, and divided into 3 groups. Animals of the first group (‘raw’ soybean group) were fed the powdered MF diet containing 20% powdered raw soybeans for 5 weeks and then fed the same diet, but containing 30% raw soybean powder for another 8 weeks. The stepwise increase in the soybean ratio in this protocol stems from the observation that the augmented amount of soybeans during feeding reduced the increase in the blood glucose level in our preliminary experiments. The second group of animals (‘steamed’ soybean group) were treated according to the above mentioned schedule, but using the powdered steamed soybeans. Those of the third group of mice (‘MF’ group) were only fed the powdered MF diet for the 13 weeks. The compositions and energy contents of these experimental diets are summarized in Table 1. The mice were housed 3 to 4 each in mesh stainless steel cages in an air-conditioned room at 23 ± 1°C, and allowed ad libitum access to the diet in lid bowls as well as water supplied throughout the experimental periods.

We monitored the body weight, diet intake, water intake and blood glucose level of the mice once a week at 10:00 to 11:00 a.m. during the feeding period. Blood samples were obtained from the tail vein. This treatment was done with care so as not to stress the mice, but under non-anesthesia and non-fasting conditions in order to reduce changes in the blood glucose level as much as possible. The blood glucose concentration was determined by the glucose oxidase method using a commercial kit (Glutest Ace R, Sanwa Kagaku Kenkyusho, Nagoya, Japan). Urinary glucose was measured at the 3rd, 4th, 5th, 6th and 7th weeks of feeding using a commercial kit (Diastix, Bayer Health Care, U.K.). Data were analyzed using ANOVA4, a Web statistical analysis program. Differences in the data obtained between the three experimental groups were considered to be significant when the one-way analysis of variance (ANOVA) followed by Ryan’s method indicated the P level of less than 0.05. All the animals used in this study were treated in accordance with the guidelines issued by the Japanese Government and approved by the Animal Care and Use Committee of Kyushu Kyoritsu University.

**RESULTS**

When the body weights of the KK-A^T mice fed the different diets were measured (Figure 1A), no difference in the average value was observed between the ‘raw’ soybeans and ‘MF’ groups until the end of the feeding period. The body weight data tended to be high for the ‘steamed’ soybean group during the latter half of the feeding (the period of 30% soybean diet) compared to the other groups (final data: ‘steamed’ soybean group: 55.2 ± 1.2 g vs. ‘MF’ group: 52.3 ± 1.8 g). The diet intake (Figure 1B) of the ‘raw’ soybean group was significantly lower than that of other groups.

**Table 1** Composition of experimental diets (%)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Chow MF</th>
<th>With 20% soybean</th>
<th>With 30% soybean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>7.7</td>
<td>8.7</td>
<td>9.1</td>
</tr>
<tr>
<td>Protein</td>
<td>23.6</td>
<td>25.9</td>
<td>27.1</td>
</tr>
<tr>
<td>Fat</td>
<td>5.3</td>
<td>8.0</td>
<td>9.4</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>57.3</td>
<td>51.5</td>
<td>48.6</td>
</tr>
<tr>
<td>Ash</td>
<td>6.1</td>
<td>5.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Energy (kcal/g)</td>
<td>3.60</td>
<td>3.71</td>
<td>3.77</td>
</tr>
</tbody>
</table>

Data were from the product guide for MF and modified by calculation for soybean diets. The animals of the ‘raw’ and ‘steamed’ soybean groups (see text) received the diets with the same calculated composition and energy except that the soybean powders were steamed or not.
Fig. 1 Effects of raw soybeans on female type 2 diabetic model KK-Ay mice.
A, body weight; B, diet intake; C, water intake; D, blood glucose. ●, ‘raw’ soybean group; ■, ‘steamed’ soybean group; ○, ‘MF’ group. The soybean content in the diets was changed from 20% to 30% at the 5th week of feeding. The mice were housed 3 to 4 head each in mesh stainless steel cages. Each point ± bar in A and D represents the mean ± S.E. for 6 to 7 mice, and each point in B and C denotes the calculated amount of diet intake or water intake per mouse. Statistical significance vs. ‘MF’ group was shown by * or ** (P<0.05 and P<0.01, respectively), and that vs. ‘steamed’ soybean group was indicated by # (P<0.05). Diet/water intakes (B and C) were not individually measured and no statistical calculations were made.

The blood glucose levels are illustrated in Figure 1D. The ‘MF’ group showed an increase until the 3rd week of feeding and a trend toward a plateau thereafter. On the other hand, the level of the ‘raw’ soybean group did not increase during the first 6 weeks of feeding, exhibiting a significant difference vs. the ‘MF’ group at each of the 3rd, 4th, 5th, 6th and 7th weeks (P<0.01 or P<0.05) and vs. the ‘steamed’ soybean group at the 6th week (P<0.05). In the ‘raw’ soybean group, the blood glucose level seemingly increased at the 7th week. The levels of the ‘steamed’ soybean group were lower than those of the ‘MF’ group at the 3rd week (P<0.01), and became almost similar to those of the ‘MF’ group during the latter half of the experimental period.

Results of urinary glucose measured at the 3rd, 4th, 5th, 6th and 7th weeks of feeding are shown in Table 2. All data were the lowest for the ‘raw’ soybean group, but the differences vs. the ‘steamed’ soybean group was statistically insignificant. Differences in the ‘raw’ soy-
between the Lagerstroemia speciosa foods such as moment of hyperinsulinemia in KK-Ay mice by functional been focused on the restraint or delay in the develop-

soybeans or with the MF diet alone, using type 2 diabetic model animals. The increase in water consumption ob-

served in the animal groups fed the ’steamed’ and the ‘MF’ diets can be explained by the physiological response to suppress the elevation of osmotic pressure produced by a high blood glucose level in the KK-Ay mice. In our study, the KK-Ay mice that were fed the ’raw’ soybean diet tended to have a slower increases in their water intake and blood glucose level throughout the experimental period; the apparent differences between the ’raw’ and ’steamed’ soybean groups were consistently seen, with one of blood glucose results being statistically significant. We infer that the characteristics of the disease model mice were improved at least to some extent by feeding the raw soybean powder (note that the ’raw’ and ’steamed’ soybean diets were the same as for the indicated ingredients and energy, although possibly differed in digestibility of the proteins and inhibition to carbohydrate intake, which might be lessened by steaming of the raw soybeans). Such an e-

fect of the raw soybeans on the blood glucose level, however, seemed to gradually decrease until the end of the feeding period (Figure 1D). It was considered that some of the mice at this point were in an insulinopenic state (cf. ref. 12). Generally, the KK-Ay mice are recog-

nized as having hyperinsulinemia in connection with insulin resistance. In this decade, attention has been focused on the restraint or delay in the development of hyperinsulinemia in KK-Ay mice by functional foods such as Lagerstroemia speciosa L. (Banaba), Auricularia auricula-judae Quel. (Kikurage), Momordica charantia L. (Nigauri) and Tithonia diversifolia (Nitobegiku). Based on our results, this point remains uncertain without measuring the concentration of plasma insulin during the feeding periods.

In a previous study on streptozotocin-treated rats, the increasing effects of soybeans on the insulin mRNA ex-

pression in pancreatic β cells, as well as their decreasing effects for hyperglycemia, were significantly reduced when soybeans were heated, implies the possibility that soybeans contain some beneficial heat-labile components. Our preliminary study has indicated that the raw soybean powder prepared for the current experiments contained an antitryptic activity, but the steamed powder did not (unpublished data). Not only inhibitors, but also lectins and other proteins are contained in beans and may exert good or adverse effects. Further work is required for soybean components to be used to develop healthy foods.

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