Glycative Stress Research

Introduction
When simple sugar molecules, like glucose and fructose, react non-enzymatically with proteins advanced glycation endoproducts (AGEs) are formed; this reaction is called “glycation”. When excess amounts of reducing glucose or aldehydes are loaded in the body, it causes physical stress, which is called “glycative stress”\(^1,2\). The glycative stress plays a role as a risk factor for age-related deterioration including skin aging and diabetic complications. Treatment methods for reducing glycative stress are categorized into as follows: reduction of postprandial hyperglycemia by food intake, inhibition of AGE formation and enhancement of AGE degeneration and excretion.

In our laboratory, the research has been focused on the elucidation of the influence of food combinations and added dietary fibers on the postprandial glucose values. We have confirmed that grapefruit intake before a meal\(^3\) and additive dietary fibers\(^4\) can reduce postprandial hyperglycemia. Also we reported that the area under the curve of postprandial blood glucose values can be further reduced by combining staple food with eggs, vegetables or braised meat and eggplant “mabonasu” compared to only staple food, such as udon noodle or rice\(^5\).

In the present study, the postprandial blood glucose level was measured after the intake a bowl of rice topped with beef, with or without red ginger, and compared with the level after rice intake alone. Thus we explored other measures which can reduce glycative stress from the viewpoint of dietary education, “Shokuiku”.

Methods
Subjects
Subjects were recruited from among those with relevance to the Department of Anti-Aging Medicine, Doshisha University Faculty of Life and Medical Sciences as previously reported\(^4,5\). The subjects comprised healthy persons aged 20 years or older who did not meet any of the following exclusion criteria: food or drug allergy, pregnant or lactating women,

Postprandial blood glucose level after intake of a bowl of rice topped with beef

Arisa Kawabata, Masayuki Yagi, Mari Ogura, Yoshikazu Yonei

Anti-Aging Medical Research Center and Anti-Glycation Research Center, Graduate School of Life and Medical Sciences, Doshisha University

Abstract
Objectives: The combination of a staple food and a side dish has been reported to prevent postprandial glucose elevation, contributing to glycative stress reduction. In this study, we evaluated the effect of a rice bowl topped with beef, “gyudon,” with an additive of red ginger on blood glucose.
Methods: Subjects were 12 healthy volunteers (26.9 ± 5.9 years) from whom informed consent was obtained. On the test day, fasting blood glucose was measured followed by test food ingestion for 10 min, then glucose levels were checked by a glucose meter for self monitoring at 15, 30, 45, 60, 90, and 120 min. Postprandial blood glucose and the area under the curve (AUC) were analyzed and compared between 3 test foods: steamed rice (230 g), “gyudon” with or without ginger (15 g).
Results: Analysis was conducted in the 11 subjects with steamed rice AUC of more than 5,000. There was no significant difference in AUC among the 3 test foods; steamed rice, “gyudon” with or without ginger. Blood glucose at 15 min was significantly higher in “gyudon” with ginger than in steamed rice (\(p = 0.006\)). Blood glucose at 60 min was significantly lower in “gyudon” without ginger than in steamed rice (\(p = 0.040\)).
Conclusion: Although “gyudon” is a fast food, it can reduce postprandial glucose elevation by combining a staple food and a side dish, thus potentially contributing to glycative stress control.

KEY WORDS: postprandial blood glucose, glycative stress, beef, protein, lipids
under treatment with medication or follow-up, diagnosis of diabetes mellitus, pronounced cardiopulmonary functional disorder, on therapeutic medication for hypertension, a past history of gastrointestinal surgery, and suspicion of infectious disease. Other subjects judged by the investigator as being inappropriate for inclusion in the study were also excluded from the study.

The study population comprised 12 subjects (5 males and 7 females, age 26.9 ± 5.9 years, body mass index [BMI] 20.6 ± 3.5). These subjects provided written consent to participate in the study after being given a full explanation of the study.

Protocol for blood glucose test

A blood glucose test was performed as previously reported 4,5 according to the unified protocol established by the Japanese Association for the Study of Glycemic Index (JASGI) 6,7. On the day before the test the following were prohibited: strenuous exercise, taking meals after 8:00 p.m., surfeit, overdrinking, and staying up late at night. If the subject felt ill on the day before the test, or prior to or during the test, the test was to be postponed or terminated.

On the day of the test, the reference diet and test food were taken over a period of 5-10 minutes, with about 30 chews per mouthful required. Blood glucose was measured at 15 (2nd time), 30 (3rd time), 45 (4th time), 60 (5th time), 90 (6th time), and 120 minutes (7th time) after the start of taking each test food. In the present study, a glucose meter for self-monitoring (Glucocard MyDia; Arkray, Inc., Nakagyo-ku, Kyoto, Japan) was used as test equipment.

Test foods

The test foods used in this study are shown below (Table 1). Nutrient contents in these foods were provided from Yoshinoya Holdings Co., Ltd., (Kita-ku, Tokyo, Japan) and calculated using the Standard Tables of Food Composition in Japan 2010 8.

A bowl of rice topped with beef “gyudon” is comprised of steamed rice, “gyudon” topping and ginger. “Gyudon” topping and ginger were provided by Yoshinoya Holdings. Packed steamed rice (Sato No Gohan [Sato Foods Co., Ltd., Niigata, Japan], 100% consisting of Koshihikari, produced in Niigata Prefecture) was to be twice tested.

- Steamed rice 230 g
- A bowl of rice topped with beef, “gyudon,” without ginger (steamed rice 230 g + “Gyudon” pack)

A bowl of rice topped with beef, “gyudon,” with ginger (steamed rice 230 g + “Gyudon” pack + red ginger “benishoga” 15 g)

Frozen-packed “Gyudon” packs, provided by Yoshinoya Holdings, were used for beef topping which were prepared in the same manner as the commercial products, so that we can compare the test products with the menu products in the restaurants.

The amount of carbohydrates other than dietary fiber in each test food was set at 75 g ± 5 g, as previously reported 4,5, in order to correspond to the 75 g amount of carbohydrates other than dietary fiber for the 75 g oral glucose tolerance test (OGTT) in common use for diagnosing diabetes mellitus.

Statistical analyses

The 0-minute value was subtracted from the blood glucose level measured over time after eating each test food to obtain Δ blood glucose level; the largest change in the level observed up to 120 minutes after the start of ingestion was considered as the maximum blood glucose level change. Using these parameters, the area under the blood glucose elevation curve (AUC) was calculated. Data obtained were statistically analyzed by the Tukey’s multiple comparison test among the 3 groups, using the SPSS Statics 22 statistical analysis software (IBM Japan, Chuo-ku, Tokyo, Japan). A two-sided p-value of less than 5% was considered to indicate a significant difference.

Ethical Considerations

The present study was conducted in compliance with the ethical principles of the Declaration of Helsinki (Note of Clarification added at the 2004 World Medical Association General Assembly in Tokyo) and Japan's Act on the Protection of Personal Information, and with reference to the Ministerial Ordinance on Good Clinical Practice (GCP) for Drug (Ordinance of Ministry of Health and Welfare No.28 of March 27, 1997) and the Ethical Guidelines for Epidemiological Research established by Japan’s Ministry of Health, Labour and Welfare, and Ministry of Education, Culture, Sports, Science and Technology. The present study was conducted after examination and approval for the ethics and appropriateness of the study at a meeting of the Doshisha University Ethics Committee for Scientific Research Involving Human Subjects (Approval number #1228-2).

Table 1. Food consumption and nutrients in test food items.

<table>
<thead>
<tr>
<th>Food items</th>
<th>Consumption (g)</th>
<th>Energy (kcal.)</th>
<th>Protein (g)</th>
<th>Lipids (g)</th>
<th>Carbohydrates (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steamed rice</td>
<td>230.0</td>
<td>338.1</td>
<td>4.8</td>
<td>0.0</td>
<td>78.0</td>
</tr>
<tr>
<td>“Gyudon” pack *</td>
<td>135.0</td>
<td>263.3</td>
<td>13.6</td>
<td>20.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Red ginger *</td>
<td>15.0</td>
<td>2.6</td>
<td>0.045</td>
<td>0.060</td>
<td>0.59</td>
</tr>
</tbody>
</table>

Numerical figures for food items were calculated based on the provided nutrient information from Yoshinoya Holdings using the Standard Tables of Food Composition in Japan 2010 8. “Gyudon,” a rice bowl topped with beef. * Data from the distribution products at October 2014 were provided by Yoshinoya Holdings.
Results

The results of postprandial blood glucose level are presented in Fig.1 and AUC in Fig. 2. The subjects for whom the steamed rice AUC was less than 5,000 were excluded from the analysis; steamed rice, 11 subjects; “gyudon,” 11 subjects, “gyudon” + ginger, 10 subjects, since if not excluded, no significant difference was noted in the study.

There was no significant difference in AUC among the 3 tests of steamed rice and a rice bowl topped beef “gyudon” with or without ginger. Blood glucose change at 15 min was significantly higher in “gyudon” with ginger than in steamed rice (p = 0.006). The glucose level was significantly lower in “gyudon” without ginger than in steamed rice (p = 0.040).

Fig 1. Postprandial blood glucose changes.
Blood glucose levels were compared among the following 3 tests after taking each meal of steamed rice 230 g (n = 11), “Gyudon” without ginger (n = 11), and “Gyudon” with ginger (n=10).
For changes in blood glucose level at 15 min: p < 0.05; “Gyudon” with ginger vs. steamed rice; at 60 min, “Gyudon” without ginger, p < 0.05; Tukey’s multiple comparison test. “Gyudon”; a rice bowl topped with beef. Data are expressed as mean ± standard error.

Fig 2. Comparison of AUC.
AUC of the blood glucose curve were compared among the following 3 tests after taking each meal of steamed rice 230 g (n = 11), “Gyudon” without ginger (n = 11), and “Gyudon” with ginger (n=10).
No significant difference was noted among the 3 groups; Tukey’s multiple comparison test. “Gyudon”; a rice bowl topped with beef. Data are expressed as mean ± standard error.
Discussion

Data interpretation

“Gyudon” is a rice bowl topped with beef and one of popular fast foods in Japan. In the present study, there was no significant difference in AUC of blood glucose among the 3 groups after taking each of steamed rice, “gyudon” without ginger and “gyudon” with ginger. Postprandial glucose level was significantly lower at 15 min in steamed rice than in “gyudon” with ginger and at 60 min in “gyudon” without ginger than in steamed rice.

It has been thought that postprandial glucose levels are primarily influenced by carbohydrates, especially sugars contained in ingested foods. In this study, although carbohydrate amounts were higher in “gyudon” than in steamed rice, AUC was not higher in “gyudon” than in steamed rice (p = 0.300). When comparing food nutrients, protein amounts were approximately 9 g higher in “gyudon” than in steamed rice. Proteins once ingested stimulate glucose-dependent insulinotropic polypeptide (GIP) which enhances insulin secretion and results in the elevation of insulin secretion 9). Also, lipid amounts were approximately 21 g higher in “gyudon” than in steamed rice. Lipids influence gastric smooth muscles mediated by gastrointestinal hormones and vagus nerves and results in the prolongation of the gastric emptying time 8). These findings indicate that proteins and lipids contained in “gyudon” may reduce the postprandial glucose levels.

On the other hand, the blood glucose levels at 15 min was significantly higher in “gyudon” with ginger. The reason for this result may be due to the sugar contained in sauce for “gyudon”.

Gastric emptying function

Discussion on the gastric emptying function has not been active although it is very influential on postprandial glucose levels. When gastric emptying function becomes low and the gastric emptying time is prolonged, postprandial glucose levels do tend to be high if the gastric emptying time becomes shorter. However, an extreme decrease in gastric emptying function causes a disease called “functional dyspepsia (FD)”. Factors which affect gastric emptying time include aging 10), gastrointestinal diseases like FD and peptic ulcers 11), diabetic state and diabetic disturbance in gastrointestinal movement 13, 14), ingested food contents 15-17), ingested food temperature 16, 18).

Regarding food contents, a comparison study between carbohydrates, lipids and proteins in healthy subjects showed that gastric emptying function was the highest by high-carbohydrate food, then high-lipid food, and lowest by high-protein food 15).

The gastric emptying time was compared by the combination of foods and beverages in the report which showed the time was elongated over 2 hours in “fried chicken and tea”, “boiled barley and rice and tea” and “soybean curd (tofu) and tea”, then “bread and tea” less than 2 hours, and fastest in “sole tea” less than 1 hour 17). Fried chicken intake influences the gastric emptying function and may reduce the postprandial hyperglycemia.

In another study, the gastric emptying time, measured in 10 healthy subjects (average age of 34 years) who received various kinds of fluid-type foods, showed that lipid content did not influence gastric emptying, however, it tended to be slower with high calorie food 6).

In another study, the influence of 3 types of soup intake was studied in a young group (23.0 ± 0.6 years) and an old group (73.3 ± 1.6 years); soup without lipids, soup with lipids (24.6 g triglycerides) and soup with lipid and lipase. The results of gastric emptying time were longer in the old group than in the young group, and longer with soup with lipids than with soup without lipids, and additive lipase shortened the gastric emptying time 11).

When considering the influence of “gyudon” intake from the viewpoint of gastric emptying time, “gyudon” contains a relatively high concentration of protein and is highly caloric, thus its intake may cause a reduction of gastric emptying function and an prolongation of gastric emptying time. As a result, it may reduce the postprandial glucose elevation.

Nutrition balance

There is a possibility of mortality risk for an excessive glucose restriction food 19). Thus, in order to make a plan to improve eating habits, the reduction of postprandial hyperglycemia may be considerably good without excessive carbohydrate restriction. The protein-fat-carbohydrate (PFC) balance is an index for ingested food which consists of protein, fats and carbohydrates which are 3 major nutrients. PFC balance is considered to be ideally good if the ingested food energy ratio is P (protein): 20%, F (fats): less than 25%, C (carbohydrates): 50%-60% as recommended by the Government 20-23).

When the PFC balance becomes adequate, the postprandial glucose elevation may be reduced, furthermore, necessary amount of insulin decreases. Thus the load on the pancreas becomes lighter, and thereby facilitating the recovery of pancreatic function, if exhausted. Recovering insulin secretion may enhance glucose intake to muscle tissue and adipose tissue, and consequently blood glucose levels can be improved 24). In this study, the PFC balance of “gyudon” without ginger was P = 12.45%, F = 31.74% and C = 55.83%.

Dietary fibers are also considered to be prevent postprandial hyperglycemia 4). If, for example, a vegetable salad were to be added to “gyudon”, this effect by dietary fibers may be augmented as the fiber amount increases in addition to onion-derived fibers in “gyudon”.

Generally, fast foods contain high lipids, high protein and high calories, giving the impression that they tend to elevate the incidence prevalence of obesity or diabetes. However, the previous study indicated that “gyudon", a combination food with a staple food and a side dish, may be effective to reduce glycative stress.

Also, by adding high protein and high fat materials to steamed rice to the meal, the gastric emptying time may be prolonged more than by only steamed rice; it may be possible to reduce postprandial glucose elevation by another mechanism. In this study, because the effectiveness was judged based on the standard of glycative stress, as a result the high fat and high protein foods were judged as favorable to prevent postprandial glucose elevation. However, excessive ingestion of lipids can cause lipid metabolism disturbance, it is critical to pay attention to the means and amount of lipid intake.
Conclusion

The present study indicates that, if a stable food and a side dish are combined in appropriate amounts, postprandial glucose elevation can be reduced, and if an adequate fast food is selected, it will give more favorable effects than the intake of steamed rice alone.

Statement of conflict of interest

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Reference