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Original article

Influence of beef bowl (*gyudon*) materials on postprandial blood glucose.

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Abstract

***Objectives*:** This study was designed to examine the inhibition of postprandial blood glucose (PBG) levels in four materials of *gyudon*, a bowl of rice topped with beef. A previous report has already shown that beef-on-rice bowls, a fast food, are effective to alleviate hyperglycemia of PBG levels and to reduce glycative stress, compared with the cases of intaking steamed white rice alone. Based on the previous study, this study was intended to clarify how each material contributes to the reduction of PBG levels: 1. Raw sauce, *nama-tare*; 2. Cooked sauce, *ni-tare*; 3. Meat; 4. Onion. Moreover, analysis was added about the relation between PBG levels and physical information.

***Methods*:** A total of 19 healthy subjects (9 men and 10 women, mean age of 23.7 ± 5.1 years) were asked by written consent for participation in the experiment and were given anti-aging medical examinations. Fasting BS levels of subjects were evaluated on the day of the examination. Test food intake was lasted 10 minutes. 30, 45, 60, 90 and 120 minutes after starting to eat, BS levels and the area under the curve (AUC) were measured by OneTouch Ultra View: Jonson & Jonson. 10 subjects, consisting of 9 subjects from the 19 subjects and one more newly added subject, (4 men and 6 women) ingested the following types of test food: Steamed white rice + (One type of the test food material for one experiment) 1, 2, 3, 4 and 5 *gyudon*, a beef bowl. The results of these test foods were compared to steamed rice alone.

***Results*:** The case of one, *nama-tare*, raw sauce, showed no significant difference in PBG levels or AUC, compared with the case of steamed white rice alone. The PBG levels and AUC were significantly lowered in the following cases, compared with the case of steamed rice alone. 2. The PBG after 45 minutes in the cooked sauce, *ni-tare*, cases. 3. The PBG levels and AUC after 45 and 60 minutes in the meat cases. 4. The PBG after 45 minutes in the onion cases. 5. The PBG levels and AUC after 45 and 60 minutes in the beef bowl, *gyudon,* cases. Four material items were placed in descending order of strength for inhibitory effects on the elevation of glycemia; 3. meat > 2. *ni-tare*, cooked sauce = onion > *nama-tare*, raw sauce. Correlation between the PBG and physical information was recognized between BMI and AUC by using secondary regression expression; r = 0.400, p = 0.009. AUC was minimized in BMI 23.3 kg/m^2. Physical functions of age showed no significant correlation, mainly because the range of subject's age was narrow in this study.

***Conclusion*:** The component of beef had a great contribution, among ingredients of the beef bowl, to inhibit the PBG levels and to reduce glycative stress. Moreover, further increasing the amount of onion and using more kinds of vegetables would expand the potential of contribution to reduce glycative stress. This study also proved that the PBG levels were more effectively inhibited as the BMI was closer to 23.

***KEY WORDS*:** glycative stress, postprandial blood glucose, lipids, protein, body mass index (BMI)

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Introduction

Glycation is a reaction where reducing sugar, such as glucose, is nonenzymatically bound with protein and turns into glycoprotein. Stress to organisms by reducing sugar and aldehyde load is designated as *glycative stress* [1, 2]. Glycative stress is a risk factor for age-related deterioration and could be a progress factor in acceleration of skin aging and diabetic complications. Treatment methods for reducing glycative stress are categorized as follows: reduction of postprandial hyperglycemia, inhibition of advanced glycation end product (AGE) formation, and enhancement of AGE degeneration and excretion palpation. One of the methods for the inhibition of postprandial hyperglycemia is the dietary method, which is the method of choice. The diet of eating vegetable first has been commended because dietary fibers should be ingested before carbohydrates.

The previous research showed three groups that were compared in the examination of postprandial blood glucose (PBG). The groups were *gyudon* with ginger, *gyudon* without ginger and steamed rice. As a result, there was no significant difference in the area under curve (AUC). However, the changes in blood glucose values had a significant difference compared with steamed rice. They were measured 15 minutes and 60 minutes after the intake of *gyudon* with ginger, and the PBG levels were significantly low. Measured 60 minutes after the intake of *gyudon* without ginger, the PBG levels were significantly low [3]. The findings of the previous research suggested that *gyudon* is an effective diet to inhibit postprandial hyperglycemia. Following these previous findings, this study was designed to examine the food items contained in *gyudon* to elucidate how each item could function to inhibit postprandial hyperglycemia.

Methods

Subjects

Subjects were recruited from among persons with relevance to the Department of Anti-Aging Medicine, Doshisha University Faculty of Life and Medical Sciences as the previous experiment had been performed [3-5]. The subjects were comprised of healthy people ages 20 years or older who did not meet any of the following exclusion criteria: people with food or drug allergies, pregnant or lactating women, people under treatment with medication or follow-up, people who were diagnosed with diabetes mellitus, people with obvious cardiopulmonary functional disorder, people on therapeutic medication for hypertension, people with a past history of gastrointestinal surgery, or people with suspicion of infectious diseases. Other subjects judged by the investigators as being inappropriate for inclusion in the study were also excluded from the study.

A total of 19 healthy subjects (9 men and 10 women, mean age of 23.7 ± 5.1 years, height of 164.0 ± 6.4 cm, weight of 55.2 ± 7.7 kg, body mass index [BMI] of 20.4 ± 2.2 kg/m^2) were given anti-aging medical checkups [6-8] and examinations of blood glucose levels by the way of intaking the standard food of steamed rice. 10 subjects, consisting of nine subjects from the nineteen subjects and one more newly added subject, were given sufficient explanations of the study and agreed to it with prior written consent. Blood glucose tests were performed for each food item contained in *gyudon*.

Protocol for the blood glucose test

A blood glucose test was performed, as previously reported [3-5], according to the unified protocol established by the Japanese Association for the Study of Glycemic Index (JASGI) [9, 10]. On the day before the test the following was 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 postponed or terminated. On the day of the test, the reference diet and test food were taken over a period of 5 to 10 minutes with about 30 chews per mouthful required. Blood glucose was measured at 15 (the second time), 30 (the third time), 45 (the fourth time), 60 (the fifth time), 90 (the sixth time), and 120 minutes (the seventh time) after the start of taking each test food. AUC was calculated, using a PBG variation diagram. A glucose meter for self-monitoring (OneTouch Ultra View: Jonson & Jonson, Chiyoda-Ku, Tokyo, Japan) was used as test equipment.

Test foods

The test foods in this study were steamed white rice (200 g), raw sauce, *nama-tare*, of *gyudon* (35 g), cooked sauce, *ne-tare* of *gyudon* (35 g), beef of *gyudon* (65 g), onion of *gyudon* (25 g) and *gyudon* (325 g, the whole quantity). *Nama-tare* had the same ingredients and the same compound ratio of *ne-tare*, but was not heated. Raw sauce of *gyudon*, cooked sauce of *gyudon*, beef of *gyudon*, onion of *gyudon* and *gyudon* were provided by Yoshinoya Holdings Co., Ltd. (Nakano-ku, Tokyo, Japan). Steamed rice, as the standard food, were provided by Sato No Gohan (Sato Foods Co., Ltd. Niigata, Japan).

Anti-aging checkup

The anti-aging medical checkups provided the evaluations of the functional age including muscle age, bone age, hormone age, neurological age and blood vessel age. At the same time, height, weight, BMI and blood pressure were measured. To calculate the functional age of subjects, Age Management Check was used as previous article [6-8] (Ginga Kobo; Nagoya, Japan). Muscle age: Muscle mass and body tissue were evaluated with the bioelectric impedance method (a high-accuracy body composition meter, Physion MD, Nippon Shooter, Chiyoda-ku, Tokyo, Japan). Bone age: The stiffness of bones was evaluated with a supersonic method (A-1000: GE Yokokawa Medical System, Hino, Tokyo, Japan). Hormone age: Biochemical examination of blood was conducted. Fasting plasma glucose, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, hemoglobin A1c, immunoreactive insulin, insulin-like growth factor-I (IGF-I), dehydroepiandrosterone-sulfate (DHEA-s), cortisol, and triglyceride concentration levels in serum were measured. Neurological age: Executive brain function was evaluated with the Wisconsin Card Sorting Test (WCST). Blood vessel age: Estimated vascular age was evaluated by using Dyna Pulse SDP-100 Fukuda Denshi (Bunkyo-ku, Tokyo, Japan). The fingertip acceleration pulse wave was determined using the plethysmometer.

Statistical analysis

The amount of change, Δ of blood glucose value, was obtained by subtracting the zero-minute values from the blood glucose values, which were measured over time after starting to eat items of test food. AUC was evaluated from zero minutes to 120 minutes. Statistical analysis for comparison was based on one-way analysis of variance: unpaired Tukey Test, paired LSD Test (least significant difference), paired Holm Test, and paired t-test (IBM SPSS Statics23, IBM Japan, Minato ward, Tokyo, Japan).

Ethical Considerations

The examinations of this study were 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), Japan's Act on the Protection of Personal Information, 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, Labor and Welfare, and Ministry of Education, Culture, Sports, Science and Technology. Further, the Doshisha University Ethics Review Committee on Research with Human Subjects was held to deliberate the ethics and validity of this study. Under their approval, this clinical test was conducted with advanced registration (Application number: 15014, UMIN# 000018458).

Results

Comparison between five materials of *gyudon* and steamed rice were made. ***Fig. 1-6*** shows the transition in the blood glucose level from zero to 120 minutes. ***Table 1-2*** shows the comparison of each AUC and the comparison of PBG at 45 minutes. AUC comparison based on one-way analysis of variance recognized the significant differences ($p = 0.009$). Multiple comparisons by the LSD Test and Holm Test (three-pair comparison) presented the followings.

Significant differences were shown in the comparison between ③ meat + steamed rice and ⑥ steamed rice, and also the comparison between ⑤ *gyudon* and ⑥ steamed rice ($p < 0.05$). In the comparison, 45 minutes after eating, significant differences were also noted in the comparison between ③ meat + steamed rice and ⑥ steamed rice, and also in the comparison between ⑤ *gyudon* and ⑥ steamed rice ($p < 0.05$). P value in result reports showed the result of the paired t-test.

① *nama-tare* + steamed rice and ⑥ steamed rice, in comparison, showed no significant differences in each time on blood glucose transition and also no significant differences in AUC (***Fig. 1***).

② *Ni-tare* case: The PBG at 45 minutes in the case of ② *ni-tare* + steamed rice showed 67.4 ± 7.4 mg/dL and ⑥ steamed rice showed 75.3 ± 5.3 mg/dL; the former was significantly lower than the latter ($p = 0.048$). There were no significant differences in AUC in either two cases (***Fig. 2***).

③ Meat case: In the level of 45 minutes, ③ meat + steamed rice group showed (43.4 ± 3.4 mg/dL) and ⑥ steamed rice group showed (75.3 ± 5.3 mg/dL); the former was significantly lower than the latter ($p = 0.001$, ***Fig. 3***). In the level of 60 minutes, the former (41.1 ± 1.1 mg/dL) was significantly lower than the latter (63.4 ± 3.4 mg/dL, $p = 0.008$). In AUC, the former (3,793 ± 793 mg·min/dL) was significantly lower than the latter (5,333 ± 333 mg·min/dL, $p = 0.001$).

④ Onion case: The comparison between ④ onion + steamed rice and ⑥ steamed rice showed that the former (64.3 ± 4.0 mg/dL) was significantly lower than the latter (75.3 ± 5.3 mg/dL, $p = 0.044$, ***Fig. 4***). There was no significant difference in AUC between these two cases.

⑤ *Gyudon* case: The comparison between ⑤ *gyudon* and ⑥ steamed rice showed that the former (44.6 ± 4.6 mg/dL) was significantly lower than the latter (75.3 ± 5.3 mg/dL) in the evaluations 45 minutes after eating ($p = 0.001$, ***Fig. 5***). Also, in the comparison of 60 minutes after eating, the ⑤ *gyudon* case (33.6 ± 3.6 mg/dL) was significantly lower than the ⑥ steamed rice case (63.4 ± 3.4 mg/dL, $p = 0.008$). In comparison of AUC, the former (3,996 ± 996 mg·min/dL) was significantly lower than the latter (5,333 ± 333 mg·min/dL, $p = 0.011$).

These results, as described above, pointed out that meat greatly influenced blood glucose levels. Therefore the examination was conducted to examine the comparison between ③ meat + steamed rice and ⑤ *gyudon* to find which case inhibited the elevation of blood glucose levels more (***Fig. 6***). As a result, the former (39.8 ± 9.8 mg/dL) was significantly lower than the latter (60.8 ± 0.8 mg/dL) in the evaluation of 30 minutes ($p = 0.034$). There was no significant difference in the evaluation of AUC.

The evaluation data, which were obtained in the anti-aging medical checkups, were compared with the evaluation of AUC to examine the correlation. The functional age, including muscle age, bone age, hormone age, neurological age and blood vessel age, showed no significant correlation. However, there was a weak correlation recognized between AUC (y) and BMI (x) by the secondary regression expression ($r = 0.400$, $p = 0.009$, ***Fig. 7***) among the examinations which were conducted of physical information (height, weight, BMI, body composition, maximal blood pressure, diastolic pressure and fasting plasma glucose). The regression formula was $y = 63.804x^2 - 2,977.1x + 39,434$ and the regression line indicated that AUC showed the minimum value at BMI 23.3 kg/m^2.

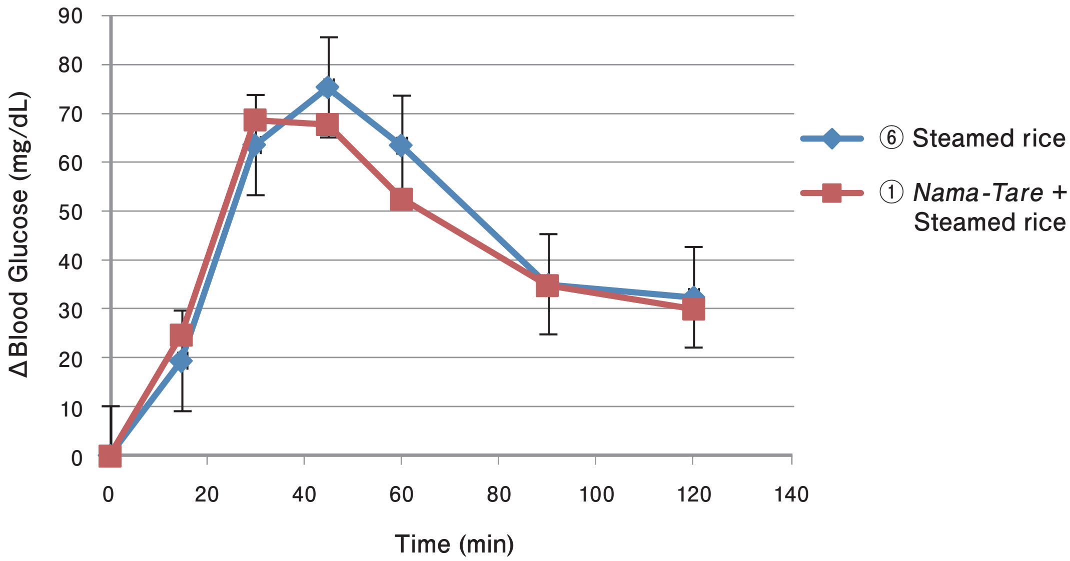

Fig. 1. ΔBlood glucose in ① *nama-tare* + steamed rice and ⑥ steamed rice. Results are expressed as mean ± standard error mean, n = 10. *Nama-tare* is a raw source without heating.

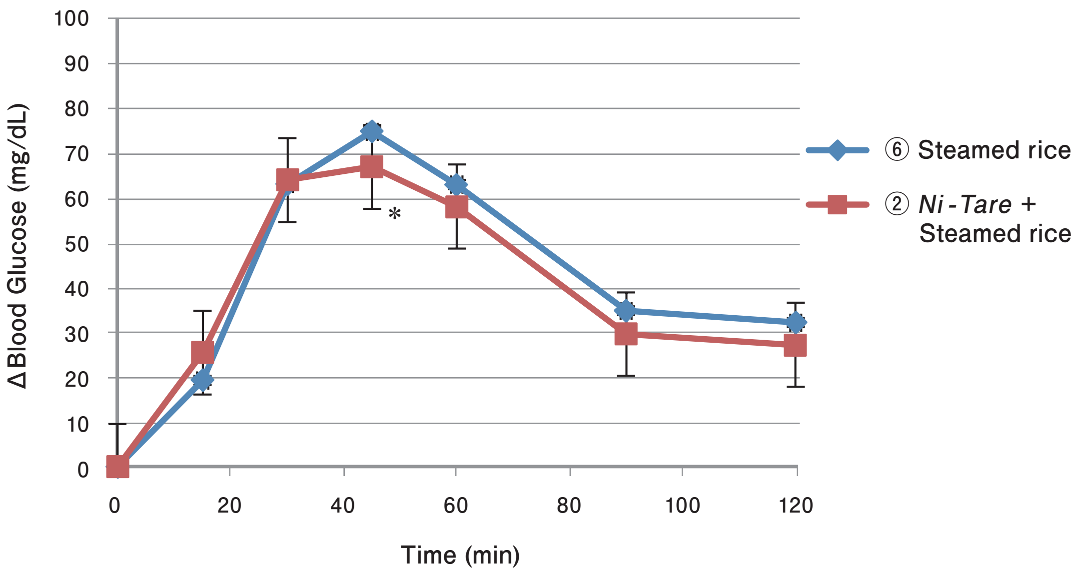

Fig. 2. ΔBlood glucose in ② *ni-tare* + steamed rice and ⑥ steamed rice. Results are expressed as mean ± standard error mean, * $p<0.05$ by paired t tests, n = 10. *Ni-tare* is made by boiling down both *nama-tare* (a raw source) and shaved pieces of dried bonito or other staffs together.

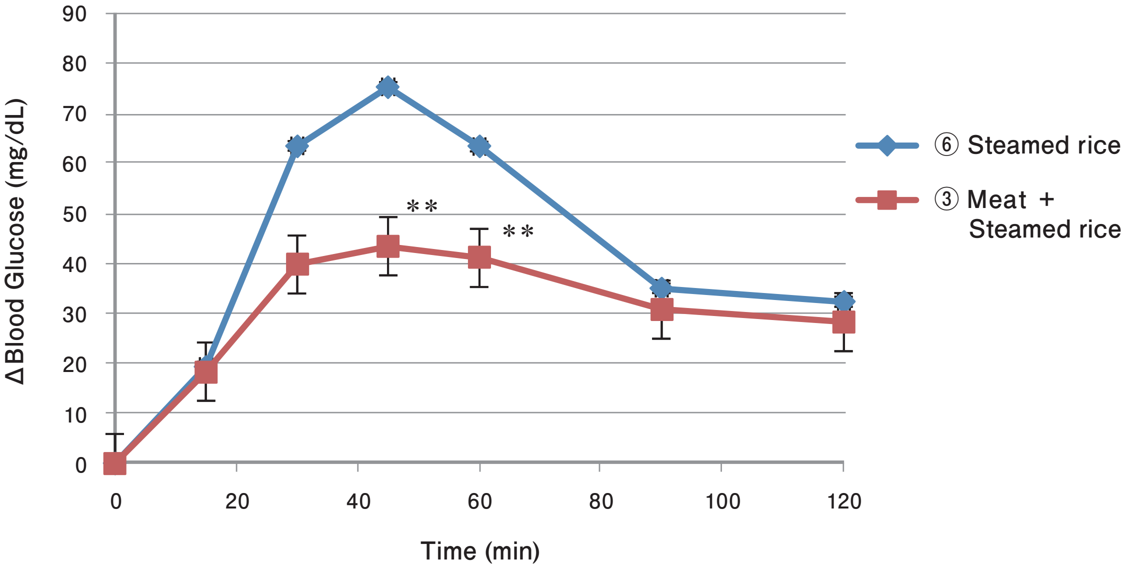

Fig. 3. ΔBlood glucose in ③ meat + steamed rice and ⑥ steamed rice. Results are expressed as mean ± standard error mean, ** $p<0.01$ by paired t tests, n = 10. AUC is significantly lower in ③ meat + steamed rice than that in ⑥ steamed rice ($p<0.05$). AUC, area under curve.

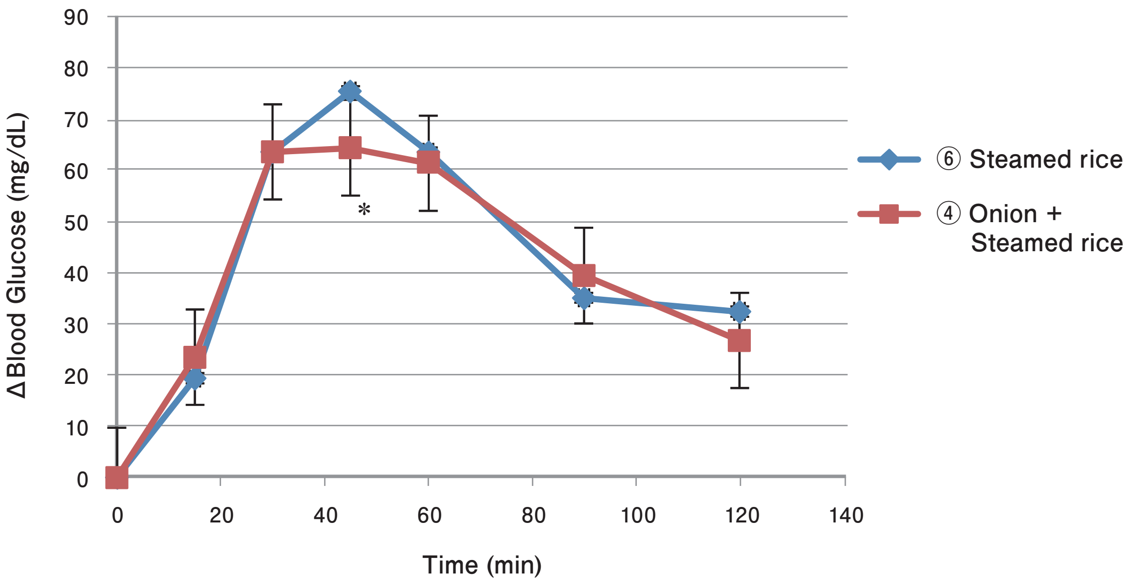

Fig. 4. ΔBlood glucose in ④ onion + steamed rice and ⑥ steamed rice. Results are expressed as mean ± standard error mean, * $p<0.05$ by paired t tests, n = 10.

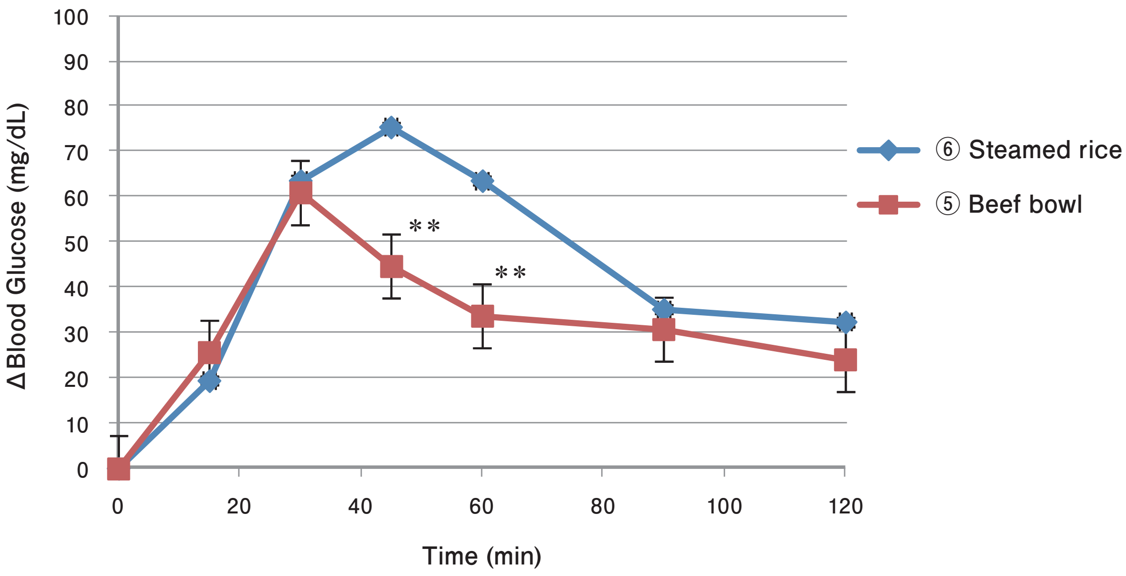

Fig. 5. ΔBlood glucose in ⑤ beef bowl (*gyudon*) and ⑥ steamed rice. Results are expressed as mean ± standard error mean, ** p<0.01 by paired t tests, n = 10. AUC is significantly lower in ⑤ beef bowl than that in ⑥ steamed rice (p<0.05). Beef bowl (*gyudon*) consists of steamed rice, meat, onion and *ni-tare*; AUC, area under curve.

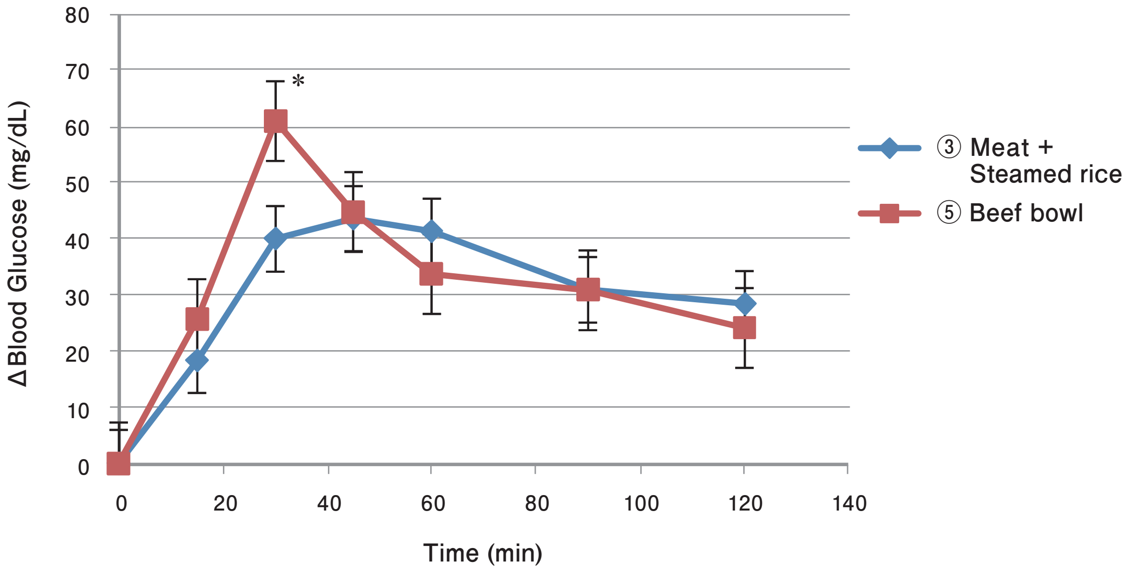

Fig. 6. ΔBlood glucose in ③ meat + steamed rice and ⑤ beef bowl (*gyudon*). Results are expressed as mean ± standard error mean, * p<0.05 by paired t tests, n = 10. Beef bowl (*gyudon*) consists of steamed rice, meat, onion and *Ni-tare*.

Table 1. Comparison of each AUC

	AUC (mg・min/dL)	p value vs. ⑥ steamed rice		
		Tukey	LSD *	Holm **
① *Nama-tare* + steamed riced	5097 ± 632	1.000	0.660	> 0.05
② *Ni-tare* + steamed rice	4979 ± 680	0.998	0.291	> 0.05
③ Meat + steamed rice	3793 ± 482	0.374	**0.024**	**0.024**
④ Onion + steamed rice	5226 ± 685	1.000	0.786	> 0.05
⑤ Beef bowl (*gyudon*)	3996 ± 444	0.533	**0.011**	**0.033**
⑥ Steamed rice	5333 ± 533			

Results are expressed as mean ± standard error mean, n = 10.* There is a significant difference in AUC in 6 groups with p = 0.009 by one-way ANOVA. ** Holm analysis is conducted in 3 pair cases, no significant difference when the number of cases is more than 3. AUC, area under curve; ANOVA, one way analysis of variance; LSD, least significant difference: *Nama-tare*, the raw source without heating; *Ni-tare* is made by boiling down both *nama-tare* and shaved pieces of dried bonito or other staffs together.

Table 2. Comparison of Δblood glucose at 45 minutes.

	ΔBlood glucose (mg/dL)	p value vs. ⑥ steamed rice		
		Tukey	LSD *	Holm **
① *Nama-tare* + steamed riced	67.8 ± 8.8	0.974	0.399	> 0.05
② *Ni-tare* + steamed rice	67.4 ± 5.5	0.968	**0.048**	> 0.05
③ Meat + steamed rice	43.4 ± 5.5	**0.028**	**0.002**	**0.018**
④ Onion + steamed rice	64.3 ± 8.6	0.878	**0.044**	> 0.05
⑤ Beef bowl (*gyudon*)	44.6 ± 6.8	**0.037**	**0.001**	**0.009**
⑥ Steamed rice	75.3 ± 7.3			

Results are expressed as mean ± standard error mean, n = 10. * There is a significant difference in 45-minute Δblood glucose in 6 groups with p < 0.001 by one-way ANOVA. ** Holm analysis is conducted in 10 pair cases. ANOVA, one way analysis of variance; LSD, least significant difference: *Nama-tare*, the raw source without heating; *Ni-tare* is made by boiling down both *nama-tare* and shaved pieces of dried bonito or other staffs together.

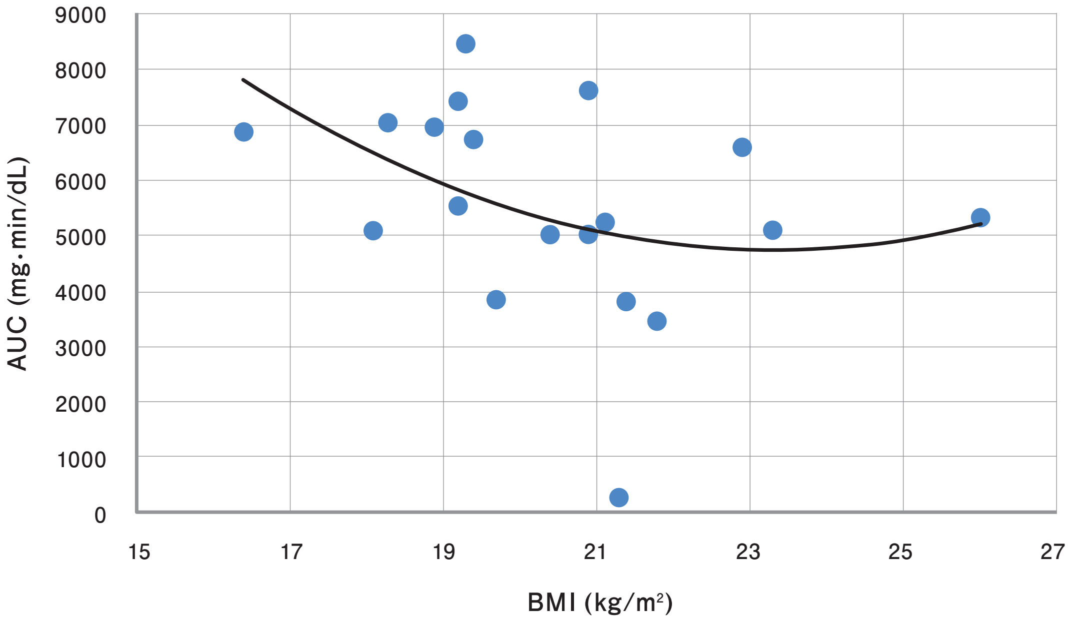

Fig. 7. **Correlationbetween BMI and AUC of postprandial blood glucose.**
$y = 63.804x^2 - 2976.1x + 39434$, $p = 0.009$, $r = 0.400$, $n = 19$. BMI, body mass index; AUC, area under curve in ⑥ Steamed rice.

Discussion

This study examined how the materials of *gyudon* influenced PBG levels. *Gyudon* is a rice bowl topped with beef. Moreover, analysis covered the influences caused by personal differences. This study clarified the PBG levels in the experiments where staple food and each food item were ingested at the same time. Although the order of eating food items was an important factor [11, 12], this study was rather focused on the individual evaluation for the inhibition of the elevation of PBG levels after simultaneously ingesting staple food (carbohydrate) and subsidiary food items, onion (dietary fiber), meat (protein and lipid), *tare*, and sauce (carbohydrates and amino acid). The results of this examination proved that meat most effectively functioned to inhibit the elevation of glycemia among materials comprising *gyudon*. Onion (dietary fiber) followed meat. Main nutrients are written in parentheses after the name of material. Concerning physical information, BMI had the greatest influence on change of blood glucose levels.

The results proved that the case of ③ meat + steamed rice and the case of ⑤ *gyudon*, both of which contained meat, remarkably inhibited the elevation of glycemia when samples one to five were compared with ⑥ steamed rice. ⑤ *gyudon* more effectively inhibited PBG levels than ⑥ steamed rice, while ⑤ *gyudon* had more nutrients than ⑥ steamed rice. The former had five grams more of carbohydrates, 13 g more of protein and 21 g more of lipids than the latter. The case of ③ meat + steamed rice had less amount of carbohydrate by only two to three grams than the case of ⑥ steamed rice. Considering these results, it could be proved that protein and lipids could function to inhibit the elevation of glycemia.

The statistical analyses of this study employed one-way analysis of variance. Unpaired Tukey Test, paired LSD Test (least significant difference), and a paired Holm Test were performed. Type I error (Even when there is no difference, it is misjudged and "there is difference" is shown) and type II error (Even when there is a difference, it is misjudged and "there is no difference" is shown) must be comprehensively reviewed [13]. Tukey Test showed that there was no significant difference in multiple comparison of AUC. LSD Test showed that there was significant difference in the comparisons between ③ and ⑥ and between ⑤ and ⑥ . The Tukey Test tends to be too strict in multiple comparisons like paired cases in this examination. There were possibilities of type II error. However, a paired t-test was performed in a manner of diversified combinations, and the t-test showed almost the same p value which was obtained by the LSD Test. The result of the statistical analysis tends to be lenient and Type I error must be considered. For AUC of the Holm Test there was a significant difference when three pairs were selected from ten pairs. However, the significant difference disappeared when four pairs were selected.

In the results of the analysis for the 45 minute evaluation, the Tukey Test, LSD Test and Holms Test all showed that there were significant differences in the comparisons between ③ and ⑥ and between ⑤ and ⑥. The Holm Test recognized a significant difference when ten pairs were selected. The results of the Holm Test could be found interesting as a statistical description to make a comprehensive judgment of Type I and Type II error.

There have been several studies which examined how the proteins intaken by eating meals influence the carbohydrate metabolism and insulin secretion. However, examinations of influences on the PBG levels were limited in number. Generally, proteins secrete a glucose-dependent insulinotropic polypeptide, which accelerates insulin secretion [14]. Lipids influence smooth movements through the gastro-entero-pancreatic and nervus vagus systems and also function to lower the gastric emptying rate [15]. Intaking protein plays a notable role in the secretion process of insulin and glucagon through incretin secretion [16]. Incretin and pancreatic hormones were examined over five hours after intaking lipids (oleic acid) and proteins (milk, egg proteins). It was pointed out that the secretion of GIP (glucose-dependent insulinotropic peptide) played a notable role in secreting pancreatic hormones in the initial stage of intaking protein, while lipids and proteins accelerated the secretion of incretin and pancreatic hormones [16]. Furthermore, some previous studies reported that intaking proteins could inhibit the elevation of blood glucose two to three times more effectively as a lipid and that there was no multiplier effect between them [17]. The reports also stated that the PBG levels were evaluated in comparison among standard food, food containing excessive carbohydrates, food containing excessive lipids, and food containing excessive proteins. The results of previous examinations proved that food containing excessive proteins slowed the elevation of blood glucose level the most drastically. The second best was food containing excessive lipids, the third was standard food, and food containing excessive carbohydrates had a remarkably high value of elevation of blood glucose [18]. Similarly, the experiments of this study showed that among the nutrients of beef, protein was the most likely to contribute to inhibit the elevation of blood glucose level.

④ Onion followed ③ meat in the inhibition of the blood glucose elevation. When the amount of onion nutrient was calculated, assuming that the onions used in this experiment had as much nutrient as ordinary onions, the onions of Test Food 3 contained more dietary fiber by 0.4 g/cooked onion 25 g than the Test Food ⑥ steamed rice alone.

Dietary fiber has the diversified mechanisms of gastrointestinal tract functions to influence the change in blood glucose levels [19-32]. Previous studies stated that gastrointestinal tract contents formed gel in the upper gastrointestinal tract, which boosted viscosity, lowered the moving speed in the gastrointestinal tract and the digestion absorption speed, and slowed carbohydrate absorption. Furthermore, mechanical stimulus promoted the secretion of glucagon peptide-1, which accelerated insulin secretion [33]. These two functions inhibited the elevation of PBG levels. It was recommended that the amount of dietary crude fiber a day should be one gram per 100 kcal intaken in a day [19].

Breakfast containing sufficient dietary fiber is, in particular, recommended. This reduces ghrelin (the hormone causing the feeling of hunger) from stomach, and promotes intestinum tenue, GIP (glucose dependent insulinotropic polypeptide), cholecystokinin and peptide YY (hormones causing the feeling of fullness) [34]. This also contributes to weight control. Dietary fiber inhibits the secretion of ghrelin [35].

This examination proved that the most effective food item of all the items was meat (protein and lipid). Onions (dietary fiber) followed meat. Lipids, as nutrition, could influence PBG levels. A previous report referred to the effects on the elevation of the PBG level by simultaneously intaking steamed rice (carbohydrate 50 g), as a staple food, and dietary fiber, vinegar, soybean curd, milk and cheese as the secondary foods. The report stated that AUC was reduced when steamed rice was intaken with 200 g of soybean curd and milk and cheese, which contained a great deal of lipids and proteins [22]. It was assumed that a certain amount of lipids and protein were required to reduce the elevation of the PBG level.

Free fatty acids plays a notable role of enhancing the secretion of insulin from pancreatic β cells, which could make a contribution to reducing the elevation of the PBG level [36]. However, high fat diets should not be necessarily encouraged, because high fat diets have high calorie counts and, what is worse, they harm the intestinal bacteria flora [37-41]. Linseed bread contains a great amount of lignin and α-linolenic acid and inhibits the elevation of the PBG level [42]. Active intake of α-linolenic is regarded as desirable.

The experiments of this study also suggested that the comparison between *nama-tare*, raw sauce, and *ni-tare*, cooked sauce, showed differences in the PBG curve. In the comparison between ① *nama-tare* + steamed rice and ② *ni-tare* + steamed rice, the case of ① *nama-tare* + steamed rice showed no significant difference in any evaluation, but the case of ② *ni-tare* + steamed rice was significantly lower in blood glucose levels than the case of ⑥ steamed rice at the evaluation of 45 minutes after intaking. It could be explained that proteins and lipids of meat could elute into *ni-tare*, and these components could function to inhibit the elevation of the PBG level. However, in this experiment, eluted substances were not detected.

② *ni-tare,* ③ meat and ④ onion, excluding ① *nama-tare*, showed the inhibition of blood sugar elevation. Therefore, it was expected that ⑤ *gyudon* was supposed to function the most effectively because of the fact that *gyudon* contains all the effective food items, ②, ③ and ④. However, the test results oppositely showed that ⑤ *gyudon* had higher blood sugar levels than ③ meat + steamed rice at the evaluation of 45 minutes after eating. The possible reason that ⑤ *gyudon*, which contained all the food items, was not the strongest inhibitor was that ② *ni-tare* contained carbohydrates, including sugar, as seasoning. However, judging from the experiments where ① *nama-tare* did not show high glucose levels, ① *nama-tare* + steamed rice and ② *ni-tare* + steamed rice did not show high levels compared with ⑥ steamed rice. The amount of carbohydrates which were contained in *tare*, sauce, were not regarded as a big factor influencing the fluctuation of blood glucose levels.

Considering these results above, it was clearly suggested that *gyudon* made a great contribution to the inhibition of the elevation in PBG levels, not thanks to dietary fiber, but thanks to protein (meat). Among the subject food items of this experiment, meat was larger in quantity by 40 g than onion. The effectiveness of the inhibition of it seemed to be remarkable.

This study reconfirmed that the intake of *gyudon* could inhibit the blood sugar elevation and reduce the glycative stress compared with the intake of steamed rice alone.

The further goal is to arrange the nutrition balance by analyzing the nutritional components among proteins, lipids, and carbohydrates (sugars, dietary fiber), although fine-tuning and replacement experiments of food items were not performed in this study. New menus would be created to enhance the effects on the inhibition of blood glucose levels compared with steamed rice alone. The misunderstanding that *gyudon* "fast foods could be undesirable for health" would be swept away and *gyudon* would be fairly evaluated as reducing glycative stress and a nutritionally well balanced diet.

This study examined the correlation between the results of physical measurements and the changes in PBG levels. Significant differences were recognized only in the evaluation of BMI. However, it was elucidated that the reduction of blood glucose levels was recognized when BMI was closer to 23, which is the standard value of BMI. It has been reported that the condition of a BMI of 23 enables life support and prevention of diseases, including diabetes, to be easily reached. This study confirmed the correlation between BMI and PBG levels. It was also suggested that people who have BMI values closer to the standard value of 22 have a tendency to have higher muscle mass than people with BMI values not close to 23. It is considered that, because glucose is consumed by the muscle, muscle mass could affect the inhibition of the blood glucose elevation. However, the results of this research require consideration of the ratio of subjects. The number of subjects with BMI values lower than 23 was greater than that of subjects with BMI values higher than 23. Moreover, it must be considered that the influences by the cases of low AUC could be sizable. These problems will require further examination.

It was assumed that there was no significant difference in any category, except for BMI, partly because there were no major differences recognized in any categories of anti-aging checkup where the subjects of the examination were young, age 25.2 on average. Furthermore, examination of subjects in a wider range of ages would have the possibility that categories other than BMI could have a relation with the fluctuation in PBG levels.

Research Limitations

Various factors are the cause for the fluctuation in the PBG levels of humans, such as calories of food, eating order [11, 12], nutrition balance among glucose, protein, lipids, dietary fiber, eating speed, the number of chewing times, gastrointestinal function, pancreas exocrine functions [43] and several others. Interdigestive migrating contractions (IMC), a strong contraction, occurred every two hours in digestive organs at the time of hunger [44]. When food is intaken, the movement drastically turns into a weak contraction compared with IMC. This movement occurs and is sustained which moves food through the gastrointestinal tracts. The movements of digestive organs concerning food intake are classified into three phases: preingestion phase, ingestion phase, and postingestion phase [44]. Many kinds of peptic juices are secreted and contribute to the digestion and the absorption of food. The secretion of insulin from the pancreas β cell is adjusted in a complicated mechanism by the stimulators, which are mainly composed of glucose and the amplifiers, including free fatty acids [36]. The examinations of this study did not completely cover all the factors. As all the studies have research limitations, the information which was obtained from the reactions of specific nutrients in the experiments could not explain all the phenomena in real life. Foods in daily life are usually intaken in a manner of a mixed diet.

Conclusion

This study concluded that meat, a food item of *gyudon*, had the greatest effect on the inhibition of glycative stress in these examinations. Increasing the amount of onion and using more kinds of vegetables would expand the potential to reduce glycative stress. This study also proved that BMI was determined as a physical finding which could affect the PBG level, which tended to be more effectively inhibited as the BIM was closer to 23.

Conflict of Interest Statement

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