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Original paper

Physical effects of a 12-week protocol of daily *gyudon* (beef bowl) ingredients consumption: Implications for fast food research

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Abstract

***Objectives*:** *Gyudon*, a bowl of rice topped with beef and onions, is a fast food item. We evaluated the physical effect and safety of a 12-week protocol of once daily *gyudon* ingredients consumption.

***Methods*:** A total of 24 adult volunteers (12 men and 12 women, mean age 44.8 ± 8.5 years) were included and asked to consume "Frozen *Gyudon* Ingredients" (heated according to the printed instructions), the test food, once daily for 12 consecutive weeks. The test food, provided by Yoshinoya Holdings Ltd. (Tokyo, Japan), was identical to the regular-size *gyudon* provided at Yoshinoya stores. The subjects underwent physical and hematological/biochemical examinations before and at 4, 8 and 12 weeks after the start of test food consumption and at 4 weeks after the completion of test food consumption. This study was designed to compare the test food with other food items under development in a double-blind, parallel-group study and was conducted with approval by an ethics committee. The current report however, only presents the results of the group of subjects who consumed the test food.

***Results*:** The compliance rate for test food consumption during the observation period was more than 94%, with no subject withdrawn. No significant change was observed in body weight (63.1 ± 13.9 kg at baseline) or body fat percentage (23.2 ± 6.4% at baseline) during the test food consumption period. As for blood pressure, no significant change was observed in systolic blood pressure while a 4.7% increase in diastolic blood pressure from the baseline value (70.4 ± 9.5 mmHg) was noted at week 4 ($p < 0.05$), with no significant change from baseline at week 8 or 12. Among lipid metabolism parameters, the total cholesterol level showed a 4.4% increase from the baseline value (197.3 ± 27.8 mg/dL) at week 8 ($p < 0.05$), with no significant change from baseline at week 12. The LDL and HDL cholesterol levels showed no significant change during the consumption period. Although the triglyceride level showed a 30.3% significant increase from the baseline value (76.3 ± 33.2 mg/dL) at week 8 ($p < 0.05$), there was no significant increase at week 12. The fasting blood glucose level increased from baseline (85.4 ± 9.2 mg/dL) by 3.5% at week 4 ($p < 0.05$), with no significant change from baseline at week 8 or 12. No adverse event related to the test food was observed during the observation period.

***Conclusion*:** Under the current study conditions, no adverse event and no significant change in glycolipid metabolism parameters was observed during or after the 12-week protocol of daily test food (*gyudon* ingredients) consumption. Further well-designed studies are needed to verify the safety of the test food.

***KEY WORDS*:** *Gyudon*, continuous intake, fast food, trans fatty acids, glycative stress

Introduction

Gyudon is a bowl of rice topped with thin-sliced (cut or chopped) beef and onion simmered with sugar and soy sauce. Its sweet soy sauce taste is preferred by the majority of Japanese people and its attractive flavor of *umami*-rich beef tallow renders it a famous national dish of Japan.

Japan has recently seen an increasing prevalence of metabolic syndrome and an associated rapid increase in lifestyle-related diseases. The major causes of these trends are decreased physical activity and increased energy intake due to the Westernization of diet. However, there is a strong trend among people to link the image of a Western diet with meat consumption. This trend is related to *gyudon*, a cuisine that proposes to be a Japanese traditional way of consuming

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beef, thereby alluding that it is not an unhealthy food.

We have measured changes in postprandial glucose levels after consumption of various food ingredients [1, 2] and found that consuming rice with *gyudon* resulted in reduced postprandial hyperglycemia compared to consuming white rice alone [3]. Meanwhile, no study has examined the physical effect of the continuous consumption of *gyudon* ingredients. The objective of this study was to evaluate the health effect of the current commercial product by analyzing the physical effect of a 12-week protocol of daily *gyudon* consumption, in particular focusing on changes in lipid metabolism parameters known to be associated with glycative stress. Although this study was designed to compare the test food with other products under development in a double-blind, parallel-group study, the current report only presents the results of the group of subjects who consumed the test food (*gyudon* ingredients).

Methods

Subjects

The subjects consisted of 24 adult volunteers aged ≥20 and <65 years (12 men and 12 women, mean age 44.8 ± 8.5 years). Subjects were excluded from the study if they: 1) were regularly taking a medicine, health food or food for specified health use considered to possibly affect study results; 2) had a previous or current history of a serious disorder of heart, liver, kidney or gastrointestinal tract; 3) were pregnant, suspected of being pregnant, or breastfeeding; 4) were heavy alcohol drinkers, defined as those with an average daily alcohol consumption of >60 g, according to References on Promotion of Healthy Japan 21 (Second edition); or 5) had extremely irregular dietary habits or irregular rhythm of life due to shift-based or night shift work.

When the subjects were examined for baseline glucose metabolism parameters, 5 of 24 subjects had high normal to borderline levels of fasting plasma glucose (FPG) during the pre-study screening or a 75-g oral glucose tolerance test (OGTT) 2-hour value of 140-199 mg/dL. With regard to dyslipidemia, 8 and 4 of 24 subjects had borderline (123-139 mg/dL) and abnormal (140-187 mg/dL) levels of low density lipoprotein cholesterol (LDL-C), respectively, while no subject had a high density lipoprotein cholesterol (HDL-C) level of <40 mg/dL or a triglyceride (TG) level of ≥150 mg/dL. As for lifestyle-related variables, 15 of 24 subjects were consuming alcohol (equivalent to ≥2 gou *sake*/day), 4 were smokers (≥20 cigarettes/day) and 2 were former smokers. Exercise habit (≥twice/week) was reported by 7 of 24 subjects.

Subjects were asked to consume one serving of test food "Frozen *Gyudon* Ingredients" (heated according to the printed instructions) daily by adding it to any one of the three daily meals for 12 consecutive weeks. The test food, provided by Yoshinoya Holdings Ltd. (Tokyo, Japan), was identical to the regular-size *gyudon* (135 g) provided at Yoshinoya stores.

The study was conducted at Chiyoda Paramedical Care Clinic (Tokyo, Japan) between April 2015 and October 2015. Subjects were given a full explanation of the study and provided a written informed consent.

Test food

Each bag of Yoshinoya Frozen *Gyudon* Ingredients (content: 135 g) was defined as 1 serving. The component composition and nutritional analysis results of the test food are shown in ***Tables 1*** and ***2***, respectively.

Table 1. Component of the test sample in 1 package for 1 day.

Item	Unit	Amount
Beef	g	65
Onion	g	30
Sauce (Tare)	g	40

Table 2. Nutrition analysis of the test sample.

Item	Unit	Amount per 100 g
Energy	kcal	249
Protein	g	9.6
Lipid	g	20.8
Carbohydrate	g	5.8
Sodium	mg	696

※135 g per package

Test parameters

Baseline characteristics, anthropometry and physical examination

The following subject's baseline characteristics were evaluated: gender, date of birth, age, alcohol habits, dietary habits, medical history, comorbidities (subjective and objective symptoms), allergy (food and drug), bowel movement history, names of drugs taken, names of medicines, health food and food for specified health use taken regularly, and volume of blood samples collected within 12 months. Subjects were also interviewed regarding their physical condition. Anthropometric parameters included body height and weight as measured with InBody 3.2 (InBody Japan, Tokyo, Japan), body fat percentage as measured by the bioelectrical impedance method on InBody 3.2 (InBody Japan), and body mass index (BMI). On physical examination, systolic and diastolic blood pressures and pulse rate were measured.

Subjects were asked to record the following information in a diary during the study period: the details of meals (frequency and amount), alcohol consumption, amount of exercise, frequency of bowel movement, sleeping time, subjective symptoms, presentation to a medical institution and details of treatment received, and use of medicines and health food.

Hematology and biochemistry

The following hematological and biochemical parameters were measured to assess the safety of the test food. The hematology parameters included white blood cell count (WBC; /μL), red blood cell count (RBC; $x10^4$/μL), hemoglobin (Hb; g/dL), hematocrit (Hct; %) and platelet count (Plt; $x10^4$/μL). The biochemical parameters included total protein (g/dL), albumin (g/dL), AST (GOT; IU/L), ALT (GPT; IU/L), LDH (IU/L), ALP (IU/L), γ-GTP (IU/L), CPK (IU/L), blood urea nitrogen (BUN; mg/dL), creatinine (mg/dL), uric acid (mg/dL), serum electrolytes (sodium, potassium and chloride; mEq/L) and calcium (mg/dL). The glycolipid metabolism parameters included FPG (mg/dL), total cholesterol (TC; mg/dL), HDL-C (mg/dL), LDL-C (mg/dL) and TG (mg/dL). The hematological and biochemical examinations were performed at LSI Medience Corporation (Tokyo, Japan). Urinalysis included a semi-quantitative measurement of protein, sugar, urobilinogen and bilirubin.

Subjects were instructed to fast from 21:00 on the day before testing until the completion of testing, during which no beverage other than water was allowed. On the day of testing, subjects were prohibited from smoking from rising until the completion of testing. Subjects were asked to come to the clinic in the morning and undergo a series of tests and a medical interview, followed by blood collection. Subjects were kept in a resting state in the clinic and asked to stay in the clinic until the completion of testing.

Subjects with normal high to borderline FPG levels at the pre-study screening were subjected to OGTT, in which only the 2-hour data were collected.

Study schedule

This study consisted of the pre-study screening and the main study. For the pre-study screening, the consenting subjects were asked to come to the clinic within 4 weeks before the start of the main study and undergo the necessary tests. During the main study, each subject made 5 visits to the clinic (before and at 4, 8 and 12 weeks after the start of test food consumption, and at 4 weeks after the completion of test food consumption) to undergo the necessary tests.

Statistical Analysis

For each parameter, the number of subjects, mean and standard deviation (SD) were calculated and tested for statistical significance as described below. All statistical tests were performed with a significance level of 5%. For the results of anthropometry, physical examination, hematological and biochemical examinations, the Dunnett's test was performed to compare the measured values at each measurement point (at weeks 4, 8 and 12 and 4-week follow-up) with those at baseline. The Wilcoxon's one-sample test was used to compare urinalysis parameters (protein, sugar, urobilinogen and bilirubin) at each measurement point with those at baseline.

Ethical considerations

Subjects were examined by physicians for any change in their physical condition caused by test food consumption. This study was conducted in compliance with the Declaration of Helsinki and the Ethical Guidelines for Medical Research in Humans and in accordance with the Good Clinical Practice (GCP), with ongoing consideration to the protection of subjects' human rights. This double-blind, parallel-group study was conducted in accordance with the protocol which had been reviewed for ethical adequacy and appropriateness of the study plan and approved by Research Ethics Committee at Chiyoda Paramedical Care Clinic. This report only presents the results of the group of subjects who consumed the test food (*gyudon* ingredients).

Results

Compliance to test food consumption

The mean compliance rate for test food consumption by the subjects was 99.6 ± 1.1%, ranging from 94.0% to 100%. Thus, all subjects achieved more than 90% of compliance to test food consumption. There was no significant difference in the compliance rate between men and women. With no subject withdrawn prematurely, all 24 subjects were included in the analysis.

Results of anthropometry and physical examination (Table 3)

No significant change in body weight was observed at week 4, 8 or 12 compared to baseline (63.1 ± 13.9 kg), whereas a significant decrease to 62.3 ± 13.5 kg (-1.3%) was observed at the 4-week follow-up ($p < 0.01$). The changes in body weight in individual subjects ranged from -4.5 to 1.6 kg.

For BMI, no significant change was observed at weeks 4, 8 or 12 compared to baseline (22.6 ± 3.6 kg/m^2), whereas a significant decrease to 22.3 ± 3.6 kg/m^2 (-1.3%) was observed at the 4-week follow-up ($p < 0.01$). The changes in BMI in individual subjects ranged from -1.5 to 0.6 kg/m^2.

Table 3. Physical information.

Item	Unit	Before	4 weeks	8 weeks	12 weeks	4 weeks after the test
Weight	kg	63.1 ± 13.9	62.8 ± 13.7	62.9 ± 14.0	63.0 ± 13.5	62.3 ± 13.5 **
BMI	kg/m²	22.6 ± 3.6	22.5 ± 3.6	22.5 ± 3.7	22.6 ± 3.6	22.3 ± 3.6 **
Body fat	%	23.2 ± 6.4	22.7 ± 6.5	22.9 ± 6.6	22.8 ± 7.0	22.5 ± 7.1 *
Blood pressure (systolic)	mmHg	115.2 ± 13.7	117.0 ± 14.1	116.9 ± 13.9	115.4 ± 15.7	115.9 ± 13.7
(diastolic)	mmHg	70.4 ± 9.5	73.7 ± 9.7 *	72.2 ± 10.4	73.3 ± 12.0	72.5 ± 9.7
Pulse rate	Bpm	73.6 ± 10.3	75.4 ± 9.2	73.3 ± 9.7	73.5 ± 9.5	74.5 ± 9.6

Data are expressed as mean ± standard deviation. *p<0.05, **p<0.01 vs Pre values by Dunnett' s test, n = 24. BMI, body mass index.

As for body fat percentage, no significant change was observed at week 4, 8 or 12 compared to baseline (23.2 ± 6.4%), whereas a significant decrease to 22.5 ± 7.1% (-2.2%) was observed at the 4-week follow-up ($p < 0.05$). The changes in body fat percentage in individual subjects ranged from -4.4 to 2.3%.

In terms of blood pressure, the diastolic blood pressure significantly increased from 70.4 ± 9.5 mmHg at baseline to 73.7 ± 9.7 mmHg (+4.7%) at week 4 ($p < 0.05$), whereas no significant difference compared to baseline was observed at week 8 or 12. No significant change was observed in systolic blood pressure. The changes in systolic and diastolic blood pressures in individual subjects ranged from -13 to 16 mmHg and -13 to 17 mmHg, respectively.

No significant change was observed in pulse rate.

While there were significant differences between men and women in the baseline values of body weight and BMI, no significant sex difference was observed in percent changes in the post-baseline values of these variables. No significant sex difference was observed in any other variable.

*Hematological and biochemical examinations (**Tables 4** and **5**)*

When the measured values of biochemical and hematological parameters at each measurement point were compared to the baseline values, significant changes were observed in the following parameters. Since significant sex differences were observed in the baseline values of RBC, Hb, Ht, γ-GTP, CPK and Cre, the values of these variables are presented separately for men and women. No significant sex difference was observed in any other variable.

Among hematological parameters, Ht in women significantly decreased from 40.7 ± 2.9% at baseline to 39.1 ± 2.4% (-3.9%) at week 4 ($p < 0.05$), whereas no significant difference compared to baseline was observed at week 8. No significant change was observed in WBC, RBC, Hb or Plt.

Among biochemical parameters, TP showed no significant change from baseline (7.08 ± 0.35 g/dL) at week 4, 8 or 12, but showed a significant increase to 7.21 ± 0.30 g/dL (+1.8%) at the 4-week follow-up ($p < 0.05$). LDH significantly increased from 154.5 ± 21.5 U/L at baseline to 164.1 ± 28.2 U/L (+6.2%) at week 12 ($p < 0.01$), whereas no significant difference compared to baseline was observed at the 4-week follow-up. γ-GTP in women showed no significant change from baseline (14.3 ± 3.5 U/L) at week 4, 8 or 12, but showed a significant increase to 17.3 ± 7.2 U/L (+21.0%) at the 4-week follow-up ($p < 0.05$).

In electrolyte analysis, the potassium level showed a significant increase from 4.13 ± 0.25 mEq/L at baseline to 4.30 ± 0.41 mEq/L (+4.1%) at week 8 ($p < 0.05$), whereas no significant difference compared to baseline was observed at week 12.

Among glycolipid metabolism-related parameters known to be associated with glycative stress, the TC level showed a significant increase from 197.3 ± 27.8 mg/dL at baseline to 206.0 ± 31.3 mg/dL (+4.4%) at week 8 ($p < 0.05$), whereas no significant difference compared to baseline was observed at week 12. TC also significantly increased to 205.2 ± 28.4 mg/dL (+4.0%) at the 4-week follow-up ($p < 0.05$). No significant change was observed in LDL-C or HDL-C. TG significantly increased from 76.3 ± 33.2 mg/dL at baseline to 99.4 ± 71.1 mg/dL (+30.3%) at week 8 ($p < 0.05$), whereas no significant difference compared to baseline was observed at week 12. FPG significantly increased from 85.4 ± 9.2 mg/dL at baseline to 88.4 ± 8.2 mg/dL (+3.5%) at week 4 ($p < 0.05$), whereas no significant difference compared to baseline was observed at or after week 8.

*Qualitative urinalysis (**Table 6**)*

Qualitative urinalysis showed a change to (±) (from (-) at baseline) in 3 subjects at week 8, changes to (±) in 3 subjects and to (+) (from (-) at baseline) in 1 subject at week 12, and changes to (±) in 4 subjects and to (+) (from (-) at baseline) in 1 subject at the 4-week follow-up. When the semi-quantitsative values of urinalysis parameters at each measurement point were compared to the baseline values, no significant change was observed in any of the parameters tested. No abnormal changes in qualitative sugar, urobilinogen or bilirubin levels were observed in any of the subjects.

Adverse events

The number of adverse events observed during the study period is shown in **Tables 7** and **8**. The incidence of adverse events was 16.7% (4/24). No serious adverse event was observed in this study. The subjective symptoms reported as adverse events included acute pharyngitis in 1 man and cold symptoms (cough, malaise and anorexia) in 1 man and 1 woman. These events were considered unrelated to the test food consumption. Abnormal laboratory changes reported as adverse events included a change in urine protein in a woman and an increase in γ-GTP in a man. These events were

Table 4. Peripheral blood examination.

Item	Unit	Sex	Before	4 weeks	8 weeks	12 weeks	4 weeks after the test
WBC	/μL	–	5741.7 ± 1657.1	5729.2 ± 1503.5	5208.3 ± 1468.8	5358.3 ± 1472.6	5391.7 ± 1410.5
RBC	10^4/μL	Male	493.7 ± 27.0	499.3 ± 34.6	498.5 ± 27.1	489.7 ± 25.7	492.8 ± 28.7
		Female	428.2 ± 38.7	428.2 ± 35.3	430.3 ± 40.7	421.0 ± 40.8	435.8 ± 27.9
Hb	g/dL	Male	15.1 ± 1.0	15.1 ± 1.0	15.2 ± 0.9	15.0 ± 0.8	14.9 ± 1.0
		Female	12.6 ± 0.9	12.6 ± 0.9	12.7 ± 1.1	12.5 ± 1.1	12.7 ± 0.9
Ht	%	Male	47.3 ± 2.2	46.3 ± 2.8	46.5 ± 2.0	47.0 ± 2.2	46.4 ± 2.7
		Female	40.7 ± 2.9	39.1 ± 2.4 **	39.8 ± 3.1	39.8 ± 3.0	40.6 ± 2.6
Plt	10^4/μL	–	25.1 ± 3.4	25.8 ± 3.8	25.4 ± 3.4	24.6 ± 3.4	26.1 ± 4.3

Data are expressed as mean ± standard deviation. **p<0.01 vs Pre values by Dunnett's test, n = 24.

Table 5. Peripheral blood examination.

Item	Unit	Sex	Before	4 weeks	8 weeks	12 weeks	4 weeks after the test
TP	g/dL	–	7.08 ± 0.35	7.06 ± 0.31	7.18 ± 0.36	7.18 ± 0.41	7.21 ± 0.30 *
ALB	g/dL	–	4.36 ± 0.24	4.38 ± 0.18	4.40 ± 0.23	4.42 ± 0.24	4.43 ± 0.20
AST (GOT)	U/L	–	17.5 ± 3.6	17.3 ± 4.1	18.0 ± 4.4	17.9 ± 4.3	18.4 ± 5.2
ALT (GPT)	U/L	–	16.7 ± 8.7	16.5 ± 8.5	17.5 ± 8.9	16.3 ± 8.3	18.1 ± 9.3
LDH (LD)	U/L	–	154.5 ± 21.5	155.5 ± 24.5	155.3 ± 24.5	164. ± 28.2 **	159.0 ± 24.0
T-BIL	mg/dL	–	0.76 ± 0.25	0.78 ± 0.24	0.80 ± 0.29	10.78 ± 0.28	0.83 ± 0.26
ALP	U/L	–	166.8 ± 45.2	171.4 ± 46.8	173.7 ± 49.6	173.4 ± 54.4	173.9 ± 49.9
γ-GTP	U/L	Male	31.7 ± 18.2	32.6 ± 19.8	34.5 ± 22.4	36.6 ± 32.2	37.2 ± 25.8
		Female	14.3 ± 3.5	14.9 ± 5.3	15.4 ± 5.4	15.0 ± 6.0	17.3 ± 7.2 *
CPK	U/L	Male	138.3 ± 53.1	123.3 ± 33.3	110.0 ± 32.0	180.0 ± 144.9	151.6 ± 67.2
		Female	74.2 ± 30.6	76.8 ± 30.6	93.5 ± 56.0	82.6 ± 42.2	74.2 ± 31.1
BUN	mg/dL	–	11.4 ± 3.1	11.6 ± 2.8	11.1 ± 2.8	11.6 ± 3.0	12.2 ± 4.4
Cre	mg/dL	Male	0.862 ± 0.103	0.833 ± 0.104	0.859 ± 0.128	0.858 ± 0.127	0.848 ± 0.142
		Female	0.592 ± 0.068	0.566 ± 0.049	0.600 ± 0.062	0.601 ± 0.075	0.614 ± 0.055
Na	mEq/L	–	140.3 ± 1.8	139.8 ± 1.5	140.5 ± 1.6	140.3 ± 1.7	140.3 ± 1.3
Cl	mEq/L	–	104.4 ± 1.8	104.0 ± 1.8	104.5 ± 1.9	103.7 ± 2.0	104.0 ± 1.4
K	mEq/L	–	4.13 ± 0.25	4.27 ± 0.37	4.30 ± 0.41 *	4.30 ± 0.34	4.24 ± 0.32
Ca	mg/dL	–	9.42 ± 0.38	9.45 ± 0.38	9.48 ± 0.35	9.47 ± 0.37	9.53 ± 0.30
TC	mg/dL	–	197.3 ± 27.8	202.5 ± 34.4	206.0 ± 31.3 *	199.0 ± 29.9	205.2 ± 28.4 *
LDL-C	mg/dL	–	116.8 ± 26.9	120.8 ± 28.7	120.8 ± 30.2	117.1 ± 27.2	121.0 ± 22.5
HDL-C	mg/dL	Male	59.8 ± 8.7	59.1 ± 10.8	60.0 ± 8.9	61.3 ± 7.4	59.7 ± 8.3
		Female	67.0 ± 19.5	67.1 ± 18.5	70.4 ± 21.8	64.7 ± 20.0	65.8 ± 23.3
TG	mg/dL		76.3 ± 33.2	77.9 ± 38.1	99.4 ± 71.1 *	84.5 ± 37.6	92.5 ± 56.0
FPG	mg/dL		85.4 ± 9.2	88.4 ± 8.2 *	86.1 ± 7.5	86.6 ± 8.1	85.0 ± 9.8

Data are expressed as mean ± standard deviation. *p<0.05, **p<0.01 vs Pre values by Dunnett's test, n = 24.

Table 6. Physical information.

Item	Judgement	Before	4 weeks	8 weeks	12 weeks	4 weeks after the test
Protein	(–)	21	22	19	17	17
	(+–)	1	2	4	6	6
	(1+)	2	0	0	1	1
Sugar	(–)	24	24	24	24	24
	(+–)	0	0	0	0	0
	(1+)	0	0	0	0	0
Urobilinogen	(–)	0	0	0	0	0
	(+–)	24	24	24	24	24
	(1+)	0	0	0	0	0
Bilirubin	(–)	24	24	24	24	24
	(+–)	0	0	0	0	0
	(1+)	0	0	0	0	0

No significant difference from pre-values "Before" is analyzed by Wilcoxon test.

Table 7. Adverse event in subjective symptoms.

Sex	Intake start date	Manifestation date to disappearance date	Event	Treatment	Treatment content	Degree	Follow-up	Outcome	Seriousness	Test continuation	Causal relationship
Female	May 13	July 31 to August 7	Acute pharyngitis	Doctor consultation and medication	PL 3 tablet/day Flomox 3 tablet/day Transamine 3 tablet /day	Moderate	No	Recovered	Not serious	Yes	No
Male	May 14	July 14 to 22	Cold-like symptoms (cough, fatigue and appetite loss)	Medication	Pabron Gold A 3 tablet/day	Mild	No	Recovered	Not serious	Yes	No

Degree is divided into "mild", "moderate" and "severe", and seriousness into "not serious" and "serious".

Table 8. Adverse event in examination data.

Sex	Event	Time course					Treatment	Degree	Outcome	Follow-up	Serious-ness	Test continuation	Causal relationship
		Before	4 weeks	8 weeks	12 weeks	4 weeks after the test							
Female	Proteinuria (+)	(–)	(–)	(±)	(±)	(+)	No	Mild	Unknown	No	Not serious	Yes	No
Female	γ-GTP elevation	72	75	83	125	101	No	Mild	Unknown	No	Not serious	Yes	No

Degree is divided into "mild", "moderate" and "severe", and seriousness into "not serious" and "serious".

considered unrelated to the test food consumption.

In the first case, urine protein (+) was observed at the 4-week follow-up and reported as an adverse event. Because this change was observed at the 4-week follow-up in a subject during her menstrual period, the event was considered to be unrelated to the test food consumption. Since this was a menstruation-related change, no further follow-up was considered necessary.

In the second case, the γ-GTP value, with a baseline value of 72 U/L, started to gradually increase from week 4 and reached 125 U/L at week 12, the highest value during the study period, followed by a slight decrease at the 4-week follow-up. Thus, this change was originally considered as an adverse event. A detailed interview with this subject revealed that the subject tends to drink more alcohol at an increased frequency during the summer season. Thus, the observed increase in γ-GTP was considered to be due to changes in the subject's drinking habit and was considered unrelated to the test food consumption. Since a decrease in the value was observed at the 4-week follow-up, no further follow-up was considered necessary for this variable.

Discussion

The accumulation of AGEs and carbonyl stress have beenThe objective of this study was to evaluate the health effect of *gyudon*, a representative national dish of Japan, using the current commercial product as the first step to further refine the dish as a healthier food choice. Since it is difficult to conduct a study using *gyudon* bowls served at stores due to subject management issues, we conducted the study using frozen *gyudon* ingredients, an equivalent product in terms of nutritional composition. We evaluated the safety and health effect of *gyudon* ingredients by measuring anthropometric, hematological/biochemical and urine parameters and abnormal changes in these parameters exceeding the range of physiological variations before, after and at 4 weeks after the completion of a 12-week protocol of daily *gyudon* consumption. The results showed no significant change in body weight or body fat percentage and only slight changes in some physical parameters, including a 4.7% increase in diastolic blood pressure. With regard to glycolipid metabolism parameters, no significant change was observed in LDL-C and, with the exception of TG which exhibited a 30.3% increase, only transient, slight changes were observed in TC (+4.4%) and FPG (+3.5%). No serious adverse event related to the test food was observed during the observation period.

Fast foods and health

There has been a gradual increase in fat intake among Japanese since around 1990. The mean total cholesterol level in Japanese has become comparable to that in Americans, with recent data showing a higher mean total cholesterol level in Japanese women than in American men and women [4]. Factors contributing to this trend include the westernization of diet and popularization of fast food chains, family restaurants and convenience stores.

Geographically easy access to supermarkets, fast food restaurants, food outlets or convenience stores is known to influence people's dietary habits [5]. The availability of supermarkets has been positively correlated with vegetable/fruit intake while the availability of fast food restaurants and convenience stores has been positively correlated with the intake of ice cream, salty snacks, meat, confectionery and sugar-sweetened beverages and negatively correlated with the intake of vegetables/fruits and low-fat food. The availability of fast food restaurants also affects vegetable intake. A study on the correlation between the number of fast food restaurants and vegetable intake in each prefecture revealed a significant negative correlation between the two variables [6].

How often do typical Japanese people use fast food restaurants? Although information is limited regarding the frequency of use of fast food restaurants, data have suggested that "junk food" intake accounts for about one-third of the standard calorie intake and that this percentage is higher in younger age groups. A particularly high frequency of the use of fast food restaurants and other dine-out restaurants was reported by children, being about 8 times a month [7]. The frequency of fast food intake among junior high school students was distributed as follows: "almost every day" accounting for 1.0%, "3-4 times a week" for 2.3%, "once or twice a week" for 17.3%, "a few times a month" for 70.0% and "none" for 9.3% [8]. Another survey, conducted with 45 non-dialysis patients with chronic kidney disease (CKD), showed that 26.7% of the patients used fast food restaurants once a month and 8.9% used them once a week [9]. People who have started living apart from their family for work are known to become more dependent on fast food restaurants or other dine-out places and take-out foods. In a study comparing those living apart from their family for work and those living with their family, no significant difference was observed in the prevalence of obesity, dyslipidemia or diabetes. However, the prevalence of hypertension symptoms in each group was 0% and 1.6% in those in their 20s, 14.9% and 4.1% in those in their 30s, 21.4% and 13.8% in those in their 40s, and 27.6% and 23.2% in those in their 50s, respectively, with significant differences observed between groups in those in their 30s and 40s [10].

Several reports are also available regarding the association between the frequency of use of fast food restaurants and lifestyle-related diseases. A study examining the effect of fast foods on pregnancy showed no significant correlation between the frequency of use of fast food restaurants during before-pregnancy and children's birthweight, but it did show a significant correlation in the preference for fast foods between before-pregnancy and children's birthweight [11]. More specifically, those mothers who delivered children with a birthweight of less than 2500 g were less likely to answer that they liked fast foods and tended to be aware of the high-calorie content of fast foods and thus avoid them.

Regarding the effect of fast foods on younger children, there has been a report on the association between fast food intake and prosocial behavior of children. A logistic regression analysis of the association between factors known to affect children's prosocial behavior and fast food intake was conducted in a total of 246 kindergarten pupils (age 5-6 years) in a Japanese kindergarten in Shanghai, China, and in kindergartens in Osaka and Wakayama Prefectures in Japan. A significant correlation was observed between the factor "getting anxious or reluctant in interacting with children when they are defiant" and fast food intake only in Shanghai [12]. In Wakayama, a significant correlation was found between TV viewing time and fast food intake. In Osaka, the amount of

outdoor play time was identified as a significant factor. Fast food is only one of the many factors that constitute the living environment. Therefore, the association between people's health status or lifestyle-related disease and fast food intake should be analyzed from a broader point of view.

The percentage of obese children in Japan reached a peak in 2006 and slightly declined thereafter. However, the percentage remained at a high level of about 8%, which is suggested to be due to junk food/fast food intake. Nowadays, children are able to buy anything they want at a reasonable price, anytime and anywhere [7]. Since children's lifestyle is largely influenced by their parents' and other family members' lifestyle, it is necessary to provide these children with appropriate advice and support taking into account the situation of each family.

A survey has been conducted in junior high schools to collect information about their desire for slenderness, access to information on diet, dietary habits, bone mass, exercise and lifestyle. The survey revealed a stronger desire for slenderness among girls than boys and the fact that more than half of the girls of standard weight have a desire to be slender. In addition, a comparison of the girls of standard weight with and without a desire for slenderness identified fast food intake, as well as information on diet, daily exercise hours and having breakfast or not, as a factor closely related to the desire for slenderness [13]. These results suggest the importance of the proper understanding of health information for health promotion and maintenance.

A correlation between oral hygienic status and fast food intake has been suggested in a survey of 997 female junior-high and high school students. In this survey, a questionnaire was administered to assess associations of subjective oral symptoms, including "dryness of the mouth and throat", "sticky feeling in the mouth" and "a sense of swallowing difficulty in the absence of sufficient moisture", with risk factors related to these symptoms, including 1) intake of coffee, tea or green tea, 2) skipping a meal, 3) eating fast foods, 4) having a meal while drinking water or tea, 5) finishing a meal within 10 minutes, 6) nasal congestion, 7) mouth breathing and 8) stress [14]. A logistic regression analysis identified "nasal congestion", "mouth breathing" and "stress" as significant factors associated with "sticky feeling in the mouth", while "eating fast foods", as well as "junior-high or high school", "skipping a meal", "having a meal while drinking water or tea", "nasal congestion" and "stress", were identified as factors significantly associated with "a sense of swallowing difficulty in the absence of sufficient moisture". No reference was made to the frequency of use of fast food restaurants.

With the changing social environment, the dietary habits of pupils and students have also changed, generating problems such as eating alone and skipping meals. Psychophysiological investigations have also been conducted to assess the association between fast food intake and lifestyle of children. Dietary habits such as eating fast foods and high saturated fat diets have been shown to increase the risk of depression [15].

A survey on the association between stress responses (psychological/physical stress) and lifestyle factors in 114 children of grades 4 to 12 showed that children consuming instant foods at least once a week had significantly higher scores for physical stress response, bad mood/anger, lack of motivation and depression/anxiety, as compared to those consuming instant foods less than once a week [16]. In addition, children consuming fast foods at least once a week had significantly higher scores for lack of motivation and depression/anxiety, as compared to those consuming fast foods less than once a week.

In a survey to assess the physical and mental health status of 244 male high school students (89 grade-10, 81 grade-11 and 74 grade-12 students) using the Todai Health Index [17], the frequent users of convenience stores or fast food restaurants had significantly higher scores for "irregularity of life" among grade-10, "multi-complaints" among grade-11, and "mouth and anus" and "irregularity of life" among grade-12 students. This result suggests that the frequency of use of fast food restaurants affects the mental status of male high school students.

A logistic regression analysis of the association between dietary habits and depression symptoms in 331 hospital nurses (108 males and 223 females) showed an increasing age-adjusted odds ratio with an increasing frequency of eating instant foods and late evening meals [18]. In contrast, an increasing frequency of consuming dark-colored vegetables, seaweed and mushrooms was associated with a decrease in age-adjusted odds ratio.

Other reported associations between fast food intake and diseases include an association between lower fast food intake and reduced prostate cancer risk [19] and an association between higher fast food intake and higher likelihood of increased prostate specific antigen (PSA) levels above the reference range [20].

There are some positive aspects of fast food restaurants. In Aichi Prefecture, there is a polarization of attitude to passive smoking prevention at eating places depending on the type of business; smoking bans have been actively implemented at fast food and curry restaurants, but seldom implemented at bars, barbecue restaurants, pubs and *okonomiyaki* restaurants [21]. Efforts should be made to find better ways to use fast food restaurants by improving our understanding of the nutritional value of foods provided at these restaurants [22].

The "fast food restaurants" that have been discussed in this report actually include various types of restaurants that provide various types of foods, including hamburgers, fried chicken, *kaitenzushi* (conveyer belt sushi), *udon*, *soba*, curry rice and *gyudon*. Therefore, future studies should be designed to address the safety and health management aspects of individual food ingredients.

Interpretation of data

In the present study, in blood testing, a transient decrease in Ht (-3.9% at week 4 in women) was observed. Based on the data from the 14 clinical studies we have conducted [23-36], 2-4% decreases in RBC, Hb or Ht were observed at week 4 in 3 [23, 27, 30] of the 6 studies [23, 25, 27, 30, 32, 33] in which blood collection was done at week 4. In one study, although not at week 4, a significant decrease in Ht was observed at week 8 [33]. In terms of sex difference, all 4 studies included only women, with 1 study involving post-menopausal women [30] and 3 studies involving both pre- and post-menopausal women [23, 27, 33]. The 2 studies showing no significant difference at week 4 included both men and women [25, 32].

Of the 8 studies in which blood collection was done at week 8 and not at week 4 [24, 26, 28, 29, 31, 34-36], only 1 study (involving only women) showed a significant decrease in Ht at week 8 [31], whereas no significant difference was observed in the remaining 7 studies [24, 26, 28, 29, 34-36]. Of these 7 studies,

6 included both men and women [24, 26, 28, 29, 34, 35] and 1 included only women (both pre- and post-menopausal) [36].

Mild variations due to a small blood sampling volume of 10-20 mL may be interpreted as significant differences by a paired t-test if the variations are in the same direction. These observations are common in studies involving only women and are probably influenced by such factors as age, presence/absence and volume of menstruation, and potential iron deficiency. In the present study, a mild decrease in Ht was observed only at week 4. The reason for the absence of significant differences at weeks 8 and 12 could not be identified.

In biochemistry, an increased LDH (+6.2% at week 12) was observed. This change was within the range of physiological variation, although its cause could not be identified. Increases in TP and γ-GTP (women) were observed only after the completion of test food consumption and thus were considered unrelated to the test food.

As for electrolytes, a transient increase in the potassium level (+4.1% at week 8) was observed. The transient and mild increase in the potassium level was within the range of physiological variation and was considered negligible. The potassium source in the test food was onion (containing 150 mg of potassium per 100 g edible part), with each serving of the test food containing about 30 mg of potassium. The test food contains about 940 mg of sodium; therefore, *gyudon* has a relatively low sodium/potassium ratio compared to other Japanese dishes. Typical Japanese people are taking too much sodium, with a mean daily sodium intake of about 10.0 g (Ministry of Health, Labour and Welfare) [37], which is way over the levels recommended by the Japanese Society of Hypertension (<6 g/day) and the WHO (<5 g/day). Given this situation, foods with a low sodium/potassium ratio should be preferred.

The PFC balance is a measure of dietary nutritional balance in terms of the three major nutrients found in food, i.e. protein, fat and carbohydrate. In our previous analysis, the PFC balance of *gyudon* was P = 12.45%, F = 31.74% and C=55.82% [3]. The ideal PFC balance is said to be 20% protein (P), <25% fat (F) and 50-60% carbohydrate (C), relative to the total energy intake from a meal [38-40]. The PFC balance should be used as a measure for assessing the nutritional balance of a meal as a whole and may not be pertinent in the assessment of the nutritional balance of individual food items. The nutritional balance of a meal can be regarded as the sum of individual food ingredients. Therefore, maintaining the PFC balance of individual food ingredients within a reasonable range may lead to a better PFC balance of a meal as a whole. The PFC balance of gyudon, although with slightly lower protein and higher fat ratios compared to the ideal balance, is within the reasonable range compared to that of the staple food alone.

Glycolipid parameters are strongly influenced by dietary habits. In this study, abnormal changes in glycolipid parameters included transient increases in TC (+4.4%), TG (+30.3% at week 8) and FPG (+3.5% at week 4). Based on the PFC balance of *gyudon*, a slightly higher fat ratio than the ideal balance might have contributed to the observed increases in TC and TG. The change in TC was transient and mild in nature and was within the range of physiological variation. In addition, no significant change was observed in LDL-C. Therefore, given that the usual frequency of *gyudon* consumption is estimated to be 2-8 times a month, which is less than the frequency of test food consumption in the present study, the impact of these changes is considered to be limited.

The observed change in TG, although appearing to be somewhat large, was transient in nature and within the range of physiological variation. A large standard deviation also suggests that the change was likely due to other foods than the test food consumed by some subjects. In daily dietary life, *gyudon* is often consumed with various other types of foods. Balanced dietary habits should therefore be encouraged. The observed increase in FPG was also transient and mild in nature and within the range of physiological variation.

The results of these glycolipid parameters suggest that 12 weeks of daily *gyudon* consumption does not increase the levels of glycative stress-related factors. Rather, *gyudon* has been associated with a lower postprandial peak plasma glucose level compared to white rice alone, which is composed mainly of carbohydrates, and thus *gyudon* is considered to cause less glycative stress than meals containing a disproportionately high percentage of the carbohydrate-rich staple food, such as white rice alone [3].

Issues to be addressed in future studies: Trans fatty acids

Trans fatty acids (TFAs) are generated as a result of the partial hydrogenation of double bonds in vegetable fats (unsaturated fatty acids). Unlike natural polyunsaturated fatty acids, which are liquid at room temperature, these artificially synthesized polyunsaturated fatty acids, i.e. TFAs, are semi-solid, keep for a long time and improve the flavor and taste of foods, especially fried foods [41]. Most TFAs are generated as a result of hydrogenation during the manufacturing process of hardened oil. An excessive intake of TFAs has been shown to increase the risk of heart diseases. The World Health Organization (WHO) recommends the intake of TFAs to be less than 1% of total energy intake.

In 2015, the previous three legal provisions relating to food labeling were integrated into the Food Labeling Act. This new food labeling system still has several issues to be addressed, such as the handling of cholesterol, TFAs and potassium, and the appropriateness of mandatory nutrition labeling for liquor products. Since the labeling of TFA content is not mandatory in Japan, many Japanese, both children and adults, may be consuming TFAs without knowing it [42, 43]. The mean daily intake of TFAs from industrial products is estimated to be 0.82 g. The mean daily intake of TFAs estimated from the data from a 6-day daily diet survey in 118 Japanese college students in the Kanto region and Okinawa prefecture (57 males and 61 females) was 0.43 g for males in Kanto, and 0.30 g for males in Okinawa, 0.49 g for females in Kanto and 0.73 g for females in Okinawa [44].

The intake of TFAs has been shown to correlate positively with the intake of non-essential food items, such as cookies, cakes and pastries. As most Japanese foods contain a small amount of TFAs, the daily intake of TFAs by Japanese does not substantially exceed the US regulation level of 500 mg per serving [45]. However, care should be taken as some types of foods, such as fast foods, may contain an amount of TFAs exceeding the acceptable level. In the younger population, high serum TFA levels have also been reported in patients with non-alcoholic steatohepatitis (NASH) [46] and coronary artery disease [47].

A field study of TFA intake in 467 young Japanese subjects ranging from infants to high school students, where the mean daily TFA intake was estimated to be 0.63 ± 0.37 g, suggested that infants have a greater intake (relative to body

size) of TFAs from tasty food items, as compared to children in other age groups [48]. The estimated source of TFAs was fast foods. When the frequency of use of fast food restaurants was analyzed, while Mos Burger, Lotteria, Mister Donuts and Kentucky Fried Chicken were used at an almost comparable frequency, McDonald's was more frequently used than the other 4 fast food chains.

TFA-rich fast food items include donuts, *karaage*, French fries, pies, fried chicken, pizza, pork cutlet, mince cutlet, shrimp cutlet, tempura and deep-fried food in general. By comparison, the fat component of *gyudon* is solely derived from beef, with about half of the fatty acid content accounted for by saturated fatty acids and the remaining half by oleic acid (an omega-9 fatty acid abundant in olive oil), and a negligible part by omega-6 fatty acids (about 240 mg per 65 g beef in a regular-size *gyudon*; in-house data). It contains no TFAs derived from harmful additives. Thus, *gyudon* can be said to be a fast food item with a low TFA content. This should be further clarified in future studies.

Limitations of study

The current study did not cover all glycolipid metabolism-related parameters (e.g. HbA1c). Oxidized and glycated forms of LDL, as well as advanced glycation end products (AGEs) as a measure of glycative stress, should have also been analyzed.

The subjects were not adequately managed or evaluated for dietary intake and exercise amount during the intervention period. Dietary details were recorded in a diary by the subjects, but this could not be analyzed quantitatively. The same applies to the evaluation of exercise amount. Future studies should be designed to allow for the proper evaluation of dietary intake and exercise amount.

In this study, the subjects were asked to consume the test food, which was identical to *gyudon* served at stores, once daily and achieved a 99% compliance rate for test food consumption, where even the least compliant subject achieved 94%. However, we did not specifically instruct them to eat *gyudon* for breakfast, lunch or dinner, as it has a relatively high fat content. It was also impossible to collect all the details of meals consumed by the subjects. Therefore, we could not perform chrono-nutritional analysis.

Conclusion

In this study, a 12-week protocol of daily test food (*gyudon* ingredients) consumption resulted in no significant change in parameters related to metabolic syndrome or other lifestyle-related diseases, such as body weight, body fat percentage, blood pressure, TG, LDL-C and FPG, or the development of adverse events. While the present study has a significance in the pursuit of food safety, further investigations are needed to verify the safety of *gyudon*.

Conflict of Interest Statement

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