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A "PAKU-PAKU KOUBO-KUN" containing yeast and mulberry extract postprandial glycemic control in healthy Japanese men and women: a randomized, placebo-controlled, crossover study

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ABSTRACT

Objective: This study's purpose was to verify the PAKU-PAKU KOUBO-KUN (PPKK) containing yeast and mulberry extract concentrate's effects on suppressing elevated postprandial blood glucose (PBG).

Methods: Two randomized, placebo-controlled, crossover studies (TRIAL-1 and TRIAL-2) were conducted. Both studies included healthy Japanese adults with a maximum PBG concentration (Cmax) in the range of 140–199 mg/dL. Study subjects were randomly assigned to take PPKK or placebo food. Then subjects consumed 200 g of cooked rice within 10 minutes of test food consumption. Blood glucose (BG) levels were evaluated before intervention, and 30 min, 60 min, 90 min, and 120 min after consumption. The main endpoint was the incremental area under the curve (IAUC) of PBG.

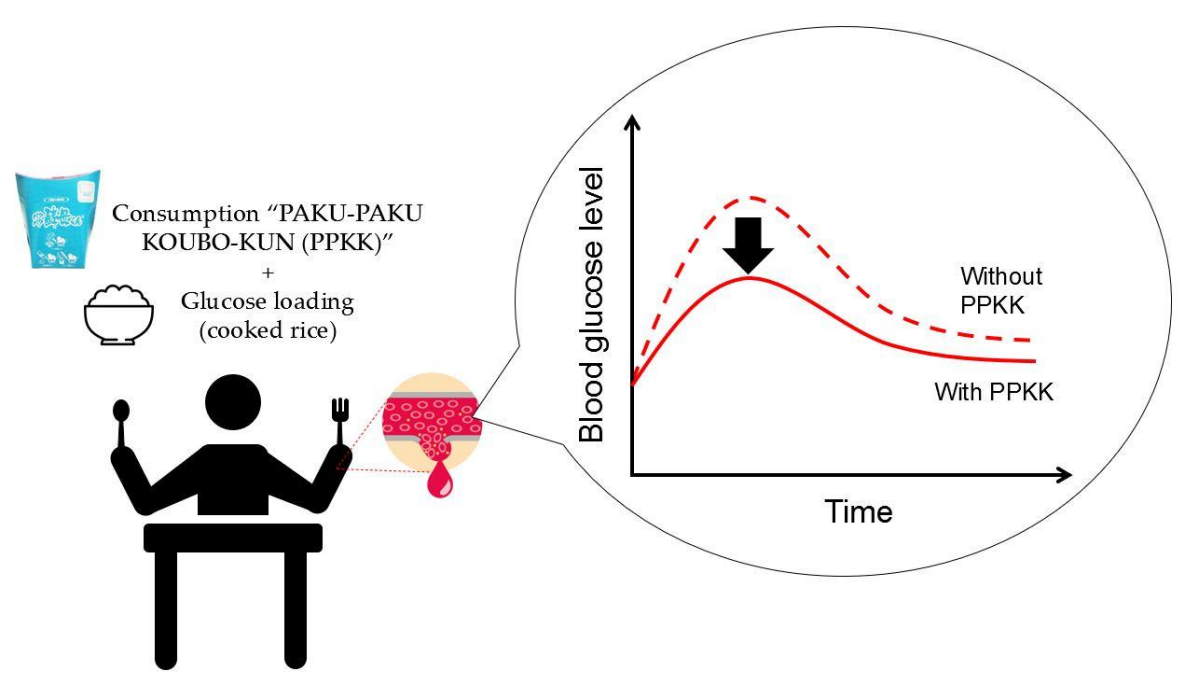
Results: The analysis included 36 subjects in TRIAL-1 and 41 subjects in TRIAL-2. A combined analysis (n = 71) was conducted. Both individual studies and combined analysis showed that PPKK significantly reduced the IAUC of PBG. In particular, BG levels were significantly lower at 30 min, 60 min, and 90 min after intervention. No adverse effects were identified.

Conclusions: These results indicated that PPKK moderated the increase in PBG and enhanced glucose metabolism.

Trial registration: UMIN-CTR: UMIN000042445 and UMIN000045341

Foundation: Mainichiegao Co., Ltd.

Keywords: postprandial blood glucose, yeast, mulberry, prediabetes, glycemic control



INTRODUCTION

Type 2 diabetes mellitus (T2D) is considered one of the most severe chronic diseases, affecting the nerves, eyes, and kidneys [1]. T2D decreases life expectancy and increases patients' healthcare costs [1]. T2D's global prevalence has become substantial. The International Diabetes Federation (IDF) Diabetes Atlas reported T2D prevalence to be 10.5% (537 million adults) in 2021, and this number is expected to increase to 783 million by 2045 [2].

According to the 2019 National Health and Nutrition Survey in Japan, the percentage of people with strongly suspected diabetes was 19.7% for men and 10.8% for women [3]. The percentage of people for whom the possibility of diabetes cannot be ruled out (prediabetes) was 12.4% for men and 12.9% for women [3]. We multiplied the population projection estimate by the 2019 national population data [4], and we discovered that the total number of people with strongly suspected diabetes was approximately 18 million and the total number of people with prediabetes was approximately 16 million. The Healthy Japan 21 (the second phase) set a goal of "preventing an increase in the number of diabetic persons" with the target number of 10 million people [5]. The current prevalence of both the strongly suspected diabetic group and the prediabetic group far exceeds this standard.

T2D should be prevented in the prediabetic stage. Diet and lifestyle improvements are effective [6,7] and particularly important in controlling postprandial blood glucose (PBG) levels [8,9]. Postprandial hyperglycemia

is considered a more independent risk factor for cardiovascular disease than fasting blood glucose (BG), affecting metabolic organs, worsening insulin secretion, and causing vascular endothelial damage and chronic abnormal metabolic function [8,10]. Therefore, PBG level control is important for health maintenance.

PAKU-PAKU KOUBO-KUN (PPKK, manufactured by Mainichiegao Co., Ltd.) contains mulberry extract, mulberry leaf powder, and three types of yeast (baker's yeast, wine yeast, and sake yeast). Mulberry leaves contain 1-deoxynojirimycin (DNJ) [11], which has α-glucosidase inhibitory activity [12], and mulberry extract has been shown to inhibit sucrase, maltase, and isomaltase activities in rat small intestinal mucosa [13,14].

Chromium, one of the components contained in yeast, is suspected to have an inhibitory effect on PBG levels [15,16]. Furthermore, insulin is known to promote glucose uptake via glucose transporter type 4 (GLUT4) and to promote glucose metabolism by acting on the glycolytic system in skeletal muscle and adipocytes. *In vitro* chromium has been shown to promote insulin's sugar transport via GLUT4 [17]. In addition, yeast cell walls contain β-glucan [18], which has been shown to suppress increased PBG levels [18]. This effect is thought to be related to β-glucan's ability to form an unstirred water layer and resist the convective effect of intestinal contractions, thereby reducing glucose absorption by the small intestine [19]. Therefore, PPKK, which contains mulberry leaf components (mulberry extracts and leaf powders) and yeast, may have a hypoglycemic effect, but it has not been clarified whether such an effect can be obtained in a product that combines both components.

In this study, we examined the PPKKs' antiglycemic effect on PBG levels based on the two studies' results (UMIN000042445 and UMIN000045341).

METHODS

Study Design: Two randomized controlled crossover trials (TRIAL-1 and TRIAL-2) were conducted. In each clinical trial, subjects were allocated in a 1:1 ratio in sequences A and B. TRIAL-1 protocol (approval number: 2011-04463-0001-09-TC; approval date: November 11, 2020) and TRIAL-2 protocol (approval number: 2108-04463-0010-09-TC; approval date: August 25, 2021) were approved by the Ethics Committee of Medical Corporation Seishinkai, Takara Clinic. The University Hospital Medical Information Network Clinical Trials Registry (TRIAL-1, UMIN000042445; TRIAL-2, UMIN000045341) was the protocol registration site. These study protocols followed the Declaration of Helsinki (2013) and the Ethical Guidelines for Medical and Biological Research Involving Human Subjects of the Ministry of Education, Culture, Sports, Science, and Technology; the Ministry of Health, Labor, and Welfare; and the Ministry of Economy, Trade, and Industry of Japan principles.

Subjects: Table 1 shows the inclusion and exclusion criteria (TRIAL-1 and TRIAL-2). The inclusion and exclusion criteria were slightly modified based on the reports of Hashizume *et al*. [20] and Baba *et al*. [21]. The enrolled subjects were recruited using the website "GO-TOROKU" (https://www.go106.jp/) operated by ORTHOMEDICO Inc. (Tokyo, Japan). The protocol was comprehensively explained to all subjects at the Takara clinic or the office of ORTHOMEDICO, Inc. Written informed consent was provided by all subjects in the trial. No subjects were associated with sponsors or members of the funding companies in the trial. TRIAL-1 was conducted in Minami-machi Clinic (Tokyo, Japan) and TRIAL-2 was conducted in Medical Corporation Seishinkai (Tokyo, Japan).

Table 1. Inclusion and exclusion criteria.

	Criteria
Inclusion criteria (TRIAL-1)	1. Healthy Japanese adults. 2. The maximum concentration (Cmax) of PBG is 140–199 mg/dL during screening. 3. Cmax of PBG during screening was relatively high. 4. Subjects qualified by the physician to participate in the study.
Inclusion criteria (TRIAL-2)	1. Healthy Japanese adults. 2. Cmax of PBG is 140–199 mg/dL during screening. 3. The incremental area under the curve (IAUC) of PBG is relatively large during screening. 4. Subjects qualified by the physician to participate in the study.
Exclusion criteria (TRIAL-1 and TRIAL-2)	1. At least one malignant tumor, myocardial infarction, or heart failure in previous medical history 2. With an implantable cardioverter defibrillator or pacemaker 3. Under treatment for either disease as follows: cardiac arrhythmia, hepatic disorder, renal disorder, cerebrovascular disorder, rheumatism, diabetes mellitus, dyslipidemia, hypertension, or other chronic diseases 4. "Foods for Specified Health Uses," "Foods with Functional Claims," or other functional food/beverage are used or consumed on a daily basis 5. Taking medicines (including herbal medicines) and supplements 6. Allergic to medicines and/or test food in this study 7. Pregnant, breastfeeding, or planning to become pregnant subjects 8. Enrolled in other clinical trials within the last 28 days before agreeing to participate in this trial or plans to participate in another trial simultaneously 9. Subjects who the physician judged to be inappropriate to participate in the study

Determination of Sample Size: Sample size determinations for TRIAL-1 and TRIAL-2 are described below. To date, there are no studies evaluating the effect of single ingestion of this food on the PBG elevation suppression in humans. Therefore, the IAUC difference between test food consumption and placebo consumption was hypothetically large in this study. We defined an effect size (d) of 0.80 [22], a significance level (α) of 0.05, statistical power (1−β) of 0.80, and an individual difference index (θ) of 1.5. Using these definitions, 34 subjects in a study (17 in each sequence) were calculated. We set a statistical power of 0.86 to maximize the statistical power as much as possible on a budget and considered dropouts and noncompliance with the protocol during the study

period; thus, the number of subjects was finally set at 44 (22 in each sequence).

Selection, Randomization, and Blinding: In TRIAL-1 and TRIAL-2, the number of subjects who provided informed consent was 247 and 120, respectively. Of these subjects, 44 per trial were enrolled by the physician in accordance with the eligibility criteria in Table 1. The sponsor provided test foods to the contract research organization (CRO). After a representative of the CRO (the test food administrator) confirmed the test foods that were indistinguishable, the test food administrator provided the code of the test foods to an allocation controller who was not involved in this study. The stratified random assignment factors were age, gender, and IAUC of PBG at a screening in TRIAL-1 and age, gender, IAUC, and Cmax of PBG at a screening in TRIAL-2. The allocation controller randomly allocated study participants based on a computer-generated list. Then, the test foods administrator, who was provided the allocation table with the coded sequences by the allocation controller, sent the test foods to each subject according to the table. The allocation controller concealed the allocation table until the key-opening day. The sequence assignments and subjects of allocation were unknown to all participants, sponsor staff, CRO, and clinic staff involved the study. The randomization and blinding procedures were based on the reports of Hashizume *et al*. [20] and Sugimoto *et al*. [23].

Intervention: Each TRIAL subjects were assigned to sequence A, which received placebo after consuming PPKK and sequence B, which received PPKK after consuming placebo. There were two periods (Period I and Period II) of intervention, with at least a 1-week washout period between periods. Subjects took two packs of PPKK or placebo with approximately 200 mL of water after taking blood samples prior to intervention. The carbohydrate load was Sato No Gohan (retort cooked rice, 200 g; Sato Foods Industries Co., Ltd., Niigata, Japan), and each subject ate rice within 10 min.

PPKK contained yeast, mulberry extract, sweet potato extract powder, rooibos tea extract powder, crystalline cellulose, and Tomat-O-Red 10%CWD (2.13 g/package as test food), with mulberry leaf as the main ingredient. Meanwhile, the placebo did not contain mulberry leaves and instead included shell calcium, reduced maltose, maltose, and red cabbage dye (2.13 g/package as placebo). Both packages were confirmed to be non-identical in odor, flavor, and color by the ethics committee.

Primary Outcome: TRIAL-1 was the Cmax of PBG. TRIAL-2 was the IAUC of PBG. Venous blood collection points from subjects were set before test food consumption and 30 min, 60 min, 90 min, and 120 min after a glucose load. LSI Medience Corporation (Tokyo, Japan) was used for BG analysis. The measurement method and blood collection timing were based on the report of Hashizume *et al*. [20]

Secondary Evaluation Items: TRIAL-1 was the PBG IAUC and PBG at each measurement point. TRIAL-2 was the PBG Cmax and PBG at each measurement point.

Safety Evaluation: Safety evaluation was assessed based on physical examination, urinalysis parameters, blood analysis parameters, a medical questionnaire,

and a dietary survey by a Calorie and Nutrition Diary (CAND) [24]. These items were the same as those reported by Sugimoto *et al*. [23].

Statistical Analysis: Statistical analyses were performed with two-sided tests with a 5% significance level. SPSS ver. 23.0 (IBM Japan, Ltd., Tokyo, Japan) for Windows was used. The analysis subjects for both TRIAL-1 and TRIAL-2 were per protocol set (PPS) and were analyzed with an emphasis on the primary outcome. The multiplicity problem caused by the multiple secondary outcomes was not considered.

The background data were demographically aggregated according to the analyzed subjects. Gender was compared using chi-square test. Age, height, weight, BMI, and body fat percentage were compared using the Welch's *t* test.

A general linear mixed model analysis of variance (ANOVA) was used to analyze the primary and secondary outcomes of TRIAL-1 and TRIAL-2 with sequence factors (Sequence A or B), subjects nested in the sequence, study period (the periods I and II), and test foods (PPKK or placebo) to calculate the period effect, carryover effect, and treatment effect. IAUC were presented with the estimated marginal means (EMM), standard error (SE) based on EMM, and the 95% confidence intervals (CI) for each food and the difference between foods. BG levels at each measurement point were indicated as mean and standard deviation (SD).

A merged analysis was conducted to integrate the IAUC of TRIAL-1 and TRIAL-2 and to improve their accuracy as posteriori analysis. Merged analysis was analyzed using a general linear mixed model ANOVA with sequence factors, subjects nested in the sequence, study period, test food, and study (TRIAL-1 and TRIAL-2) to compare between groups. The statistics to be calculated were the same as in TRIAL-1 and TRIAL-2.

The side effects incidence and adverse events were tabulated by group for safety evaluation items, and the 95% CI for the incidence in each group as well as the difference in incidence between groups were calculated.

RESULTS

Analysis Set: The recruitment and study periods of TRIAL-1 were from November 17, 2020, to February 17, 2021, and from November 17, 2020, to May 1, 2021, respectively. In TRIAL-2, the recruitment and study periods were from September 3 to November 28, 2021, and from September 3, 2021, to February 14, 2022, respectively. TRIAL-1 has eight subjects and TRIAL-2 has three subjects who had poor compliance as a result of the examination logs and hearings, and were excluded from the analysis. Therefore, TRIAL-1 has 36 subjects (19 men and 17 women), TRIAL-2 has 41 subjects (20 men and 21 women) who were included in the statistical analysis on PPS (Figure 1). The subjects' background information is shown in Table 2.

BG Levels After Glucose Loading: TRIAL-1 was 84.7 ± 3.7 mg/dL·h for PPKK and 95.9 ± 3.7 mg/dL·h for the placebo and TRIAL-2 was 59.7 ± 2.5 mg/dL·h for PPKK and 68.3 ± 2.5 mg/dL·h for the placebo; the IAUC was significantly lower when PPKK was ingested (P = 0.039, P = 0.021, respectively) for the mean and SE of the IAUC. The merged analysis additionally confirmed that the IAUC for PBG was −9.8 mg/dL·h lower when the PPKK was consumed than when the placebo was consumed, 72.2 ± 3.7 mg/dL·h and 82.0 ± 3.7 mg/dL·h, respectively (P = 0.002, Table 2).

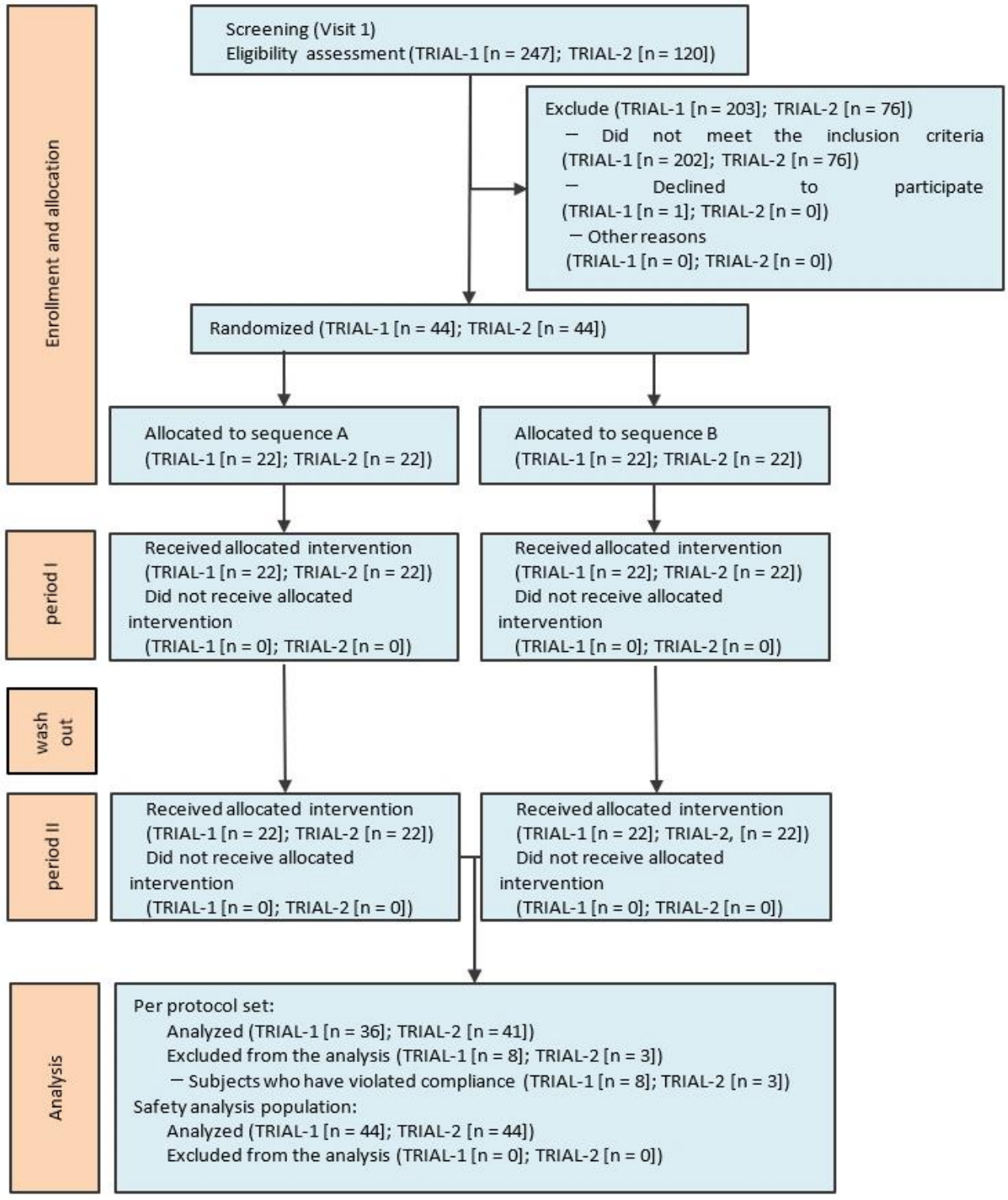


Figure 1. Flowchart of study subjects

The IAUC results showed that PPKK consumption lowered PBG levels, but there was no significant difference in PBG of Cmax between the test foods (data not shown). However, assessing BG levels at each measurement point, TRIAL-1 showed that PPKK significantly affected intervention 30 min after the loading ($P < 0.001$), while there was a carryover effect ($P = 0.004$; Figure 2 A). TRIAL-2 showed that PPKK significantly affected intervention 30 min, 60 min and 90 min after the loading ($P < 0.001$, $P < 0.001$, $P < 0.001$,

respectively; Figure 2B). Merged analysis showed that PPKK significantly affected intervention 30 min, 60 min and 90 min after the loading (P = 0.007, P = 0.008, P = 0.015, respectively), while there was a carryover effect at 60 min after a load (P = 0.038; Figure, 2C). These results indicate that PPKK suppressed the elevation in BG levels after glucose loading.

Safety Assessment: No side effects were observed during the study period in both TRIAL-1 and TRIAL-2. One adverse event was observed in TRIAL-1, in which a subject experienced dry eye. However, the investigator determined there was no causal relationship with the test food based on the criteria established at the time of study design.

DISCUSSION

There were two studies (UMIN000042445 and UMIN000045341) which confirmed the PPKK effect on PBG levels, both with a total of about 40 subjects, which confirmed a significant decrease in the IAUC of PBG levels. The two studies were combined to examine the PPKK effect on suppressing PBG level elevation, and the combined analysis showed a reduction in the IAUC of PBG level since the subjects in both studies had similar attributes.

PPKK also contains baker's yeast, sake yeast, and wine yeast. Yeast cell walls contain β-glucan [18], which is known to suppress the increase in PBG levels [18]. This effect is thought to be related to β-glucan's ability to form an unstirred water layer and resist the convective intestinal contractions effect, thereby reducing glucose absorption by the small intestine [19]. The β-glucan amount contained in PPKK is uncertain because there is a report showing that β-glucan suppressed elevated BG levels and that baker's yeast, sake yeast, and wine yeast in PPKK might affect the decreasing IAUC of PKG.

Table 2. Subjects' background information.

Item	TRIAL-1		*P* value	TRIAL-2		*P* value
	Sequence A	Sequence B		Sequence A	Sequence B	
Subjects (Men/Women)	18 (10/8)	18 (9/9)	1.000	20 (9/11)	21 (11/10)	0.758
Age (years)	45.1 ± 14.4	45.7 ± 13.7	0.887	46.7 ± 14.3	45.1 ± 12.2	0.703
Height (cm)	166.3 ± 8.6	164.0 ± 9.0	0.429	163.3 ± 7.7	169.1 ± 8.8	0.030
Weight (kg)	60.0 ± 15.3	64.2 ± 15.7	0.417	57.6 ± 9.2	60.7 ± 10.0	0.319
BMI (kg/m^2)	21.5 ± 4.3	23.6 ± 4.3	0.151	21.5 ± 2.3	21.1 ± 2.3	0.589
Body fat percentage (%)	23.7 ± 7.1	26.8 ± 8.6	0.257	23.0 ± 6.6	21.3 ± 6.3	0.425

[1] The data are described as the mean and standard deviation (SD).
[2] BMI, Body mass index.
[3] The intervention sequence was PPKK to placebo in Sequence A, placebo to PPKK in Sequence B.

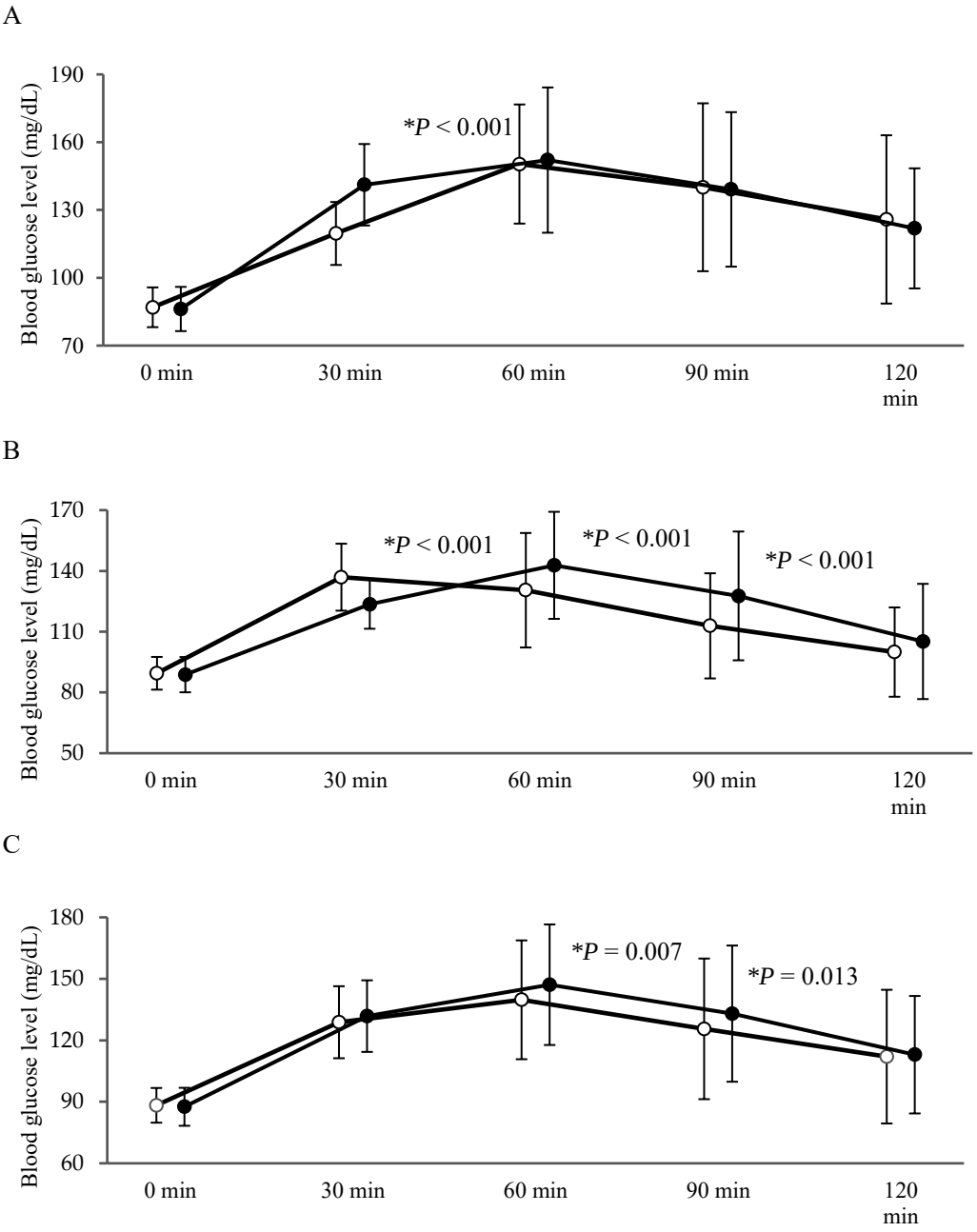


Figure 2. BG levels at intake of test food and placebo TRIAL-1 (n = 33, A), TRIAL-2 (n = 41, B), TRIAL-1 and TRIAL-2 combination results (n = 71, C) were shown. A 200 g cooked rice with approximately 200 mL of water was consumed for glucose loading 10 min after two PPKK packets or placebo were consumed. The open circle was PPKK and the closed circle was placebo.

The elevated BG level suppression caused by PPKK intake confirmed in this study is speculated to be primarily due to the effects of 1-DNJ and β-glucan. Suppression of increasing BG levels is important for diabetes prevention [8,9]. It is also desirable to improve glucose metabolism because prediabetes is caused by metabolic abnormalities [28,29]. Several studies have shown that continuous intake of yeast has a hypoglycemic effect. Continuous intake of 10-g/day brewer's yeast for 12 weeks significantly reduced BG levels at 0 min, 60 min, and 90 min after an oral 75-g glucose-tolerance test in adult men and women (aged 40–76 years; average age, 51 years) who were not taking oral hypoglycemic agents [30].

A study conducted in Iran in which T2D patients (aged 35–55 years) were additionally given 1800

mg/day of beer yeast for 12 weeks reported significantly reduced fasting BG levels and HbA1c after the intervention [31].

In vivo and *in vitro Saccharomyces pastorianus* has additionally been found to exhibit hypoglycemic effects; the mechanism is thought to be an increase in GLUT4, phosphotyrosine phosphatase, and insulin receptor localization [32]. 1-DNJ has also been suggested to modulate glucose metabolism via GLUT4 [33,34]. These studies suggest that continuous PPKK intake, which contains yeast, may improve glucose metabolism, and the effect of suppressing elevated BG levels may be enhanced as a result of improved glucose metabolism. In fact, the degree of reduction in postprandial glycemic inhibition after glucose loading was greater after the 4-week continuous intake of 1-DNJ, in comparison to a single intake of 1-DNJ in a study by Kim et al. [27]. Therefore, the hypothesis will be tested in future studies because the PPKK effect in suppressing PBG elevation may increase with continuous intake.

CONCLUSIONS

In this study, we sought to examine the results of two clinical trials that verified the PPKK effect in suppressing PBG levels. PPKK showed the ability to decrease PBG IAUC in response to a glucose load of 200 g of rice, and the results of the two integrated clinical trials also showed the suppressive effect in each clinical trial. Therefore, we believe that PPKK is a useful in preventing diabetes by moderating the increase in PBG levels by consuming it before a meal.

List of Abbreviations: PPKK: PAKU-PAKU KOUBO-KUN, PBG: postprandial blood glucose, BG: blood glucose, Cmax: maximum blood concentration, IAUC: incremental area under the curve, T2D: Type 2 diabetes mellitus, IDF: International Diabetes Federation, DNJ: 1-deoxynojirimycin, GLUT4: glucose transporter type, PPS: per protocol set, ANOVA: analysis of variance, EMM: estimated marginal means, SE: standard error, CI: confidence intervals, SD: standard deviation

Competing Interests: Junyoo Takizawa is the CEO of MAINICHIEGAO. CO., LTD., and Asami Baba is part of ORTHOMEDICO Inc. MAINICHIEGAO. CO., LTD. was a sponsor of this study and provided expenses and fees for the experiment and subsequent drafting of the manuscript. The study was jointly conducted by MAINICHIEGAO. CO., LTD. and ORTHOMEDICO Inc. Tsuyoshi Takara (MD) is the director of the Medical Corporation Seishinkai, Takara Clinic, and he was the principal investigator and managed the physical condition of all subjects at his clinic.

Authors' Contributions: Conceptualization, Junyoo Takizawa; methodology, Asami Baba; formal analysis, Asami Baba; investigation, Asami Baba and Tsuyoshi Takara; resources, Junyoo Takizawa; data curation, Asami Baba; writing—original draft preparation, Junyoo Takizawa; writing—review and editing, Asami Baba and Tsuyoshi Takara; visualization, Asami Baba; supervision, Junyoo Takizawa and Tsuyoshi Takara; project administration, Asami Baba and Tsuyoshi Takara. All authors have read and agreed to the published version of the manuscript.

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