

Original Article

Effects of mulberry leaves & water chestnut husk tea on postprandial and second meal interstitial glucose in healthy adults: A randomised, double-blind, placebo-controlled crossover study

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Background and Objectives: Postprandial hyperglycaemia is a modifiable risk factor, even in healthy adults, and dietary strategies to attenuate postprandial glycaemic excursions are of clinical interest. This study investigated whether mulberry leaf and water chestnut husk tea (MW tea) attenuates postprandial and second-meal glycaemic responses in adults. **Methods and Study Design:** This randomised, double-blind, placebo-controlled crossover study with a washout period of at least one week examined the effects of MW tea consumption on postprandial and second-meal glycaemic responses. Twenty healthy adults consumed MW or placebo tea in a randomised crossover sequence before breakfast. Interstitial glucose levels were continuously monitored using flash glucose monitoring (FreeStyle Libre Pro). The primary outcome was the incremental area under the curve (Δ IAUC) of interstitial glucose after breakfast, and the secondary outcome was the Δ IAUC after lunch (second meal). **Results:** Significant intervention-by-time interactions were observed after both breakfast and lunch (second meal). The IAUC of interstitial glucose after breakfast (primary outcome) and lunch (secondary outcome) differed between the MW and placebo tea conditions. Compared with placebo tea, MW tea was associated with attenuated postprandial interstitial glucose responses after both breakfast and lunch, as quantified by Δ IAUC, with effect sizes ranging from moderate to large and achieved statistical power ($1-\beta$) generally in the moderate-to-high range. **Conclusions:** These findings suggest that a single intake of MW tea may attenuate postprandial and second-meal glycaemic responses in healthy adults. These findings should be interpreted with caution and require further confirmation.

Key Words: functional tea, glycaemic response, second-meal effect, continuous glucose monitoring, healthy adults

INTRODUCTION

Postprandial hyperglycaemia and impaired glucose tolerance are recognised risk factors for type 2 diabetes mellitus and cardiovascular disease.¹⁻³ Accordingly, dietary approaches that attenuate postprandial glycaemic excursions have attracted increasing attention in preventive clinical nutrition. Functional foods that suppress postprandial hyperglycaemia and oxidative stress,^{4,5} and those that induce second-meal effects,^{6,7} have been investigated as practical strategies to improve glycaemic control.⁸⁻¹⁰

Mulberry leaf extracts have been shown to suppress postprandial hyperglycaemia in both healthy individuals and patients with type 2 diabetes.¹¹⁻¹³ These effects are largely attributed to 1-deoxynojirimycin (DNJ), an α -glucosidase inhibitor that delays carbohydrate digestion.^{14,15}

Water chestnut husk contains hydrolysed polyphenols such as eugenin, 1,2,3,6-tetra-O-galloyl-D-glucopyranose, and trapain,¹⁶ which exhibit antioxidant activity and suppress postprandial blood glucose elevation in mice and humans through α -glucosidase inhibition.^{16,17}

Based on these complementary bioactive properties, we developed a tea composed of mulberry leaves and water chestnut husks (MW tea) mixed at a ratio of 95:5 (%). This ratio was selected to reflect favourable in vitro bioactivity, including α -glucosidase inhibition and antioxidant capacity, while maintaining practical feasibility for preparation and consumption as a tea beverage, given limitations in the amount of water chestnut husk that could be incorporated.¹⁸ In a previous randomised controlled trial, MW tea significantly reduced the incremental area under the curve (IAUC) of postprandial blood

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glucose and insulin levels in Japanese adults with borderline diabetes,¹⁸ and its glucose-lowering effect was greater than that reported for mulberry leaves^{11, 19} or water chestnut husk¹⁷ alone. However, it remains unclear whether MW tea exerts similar effects in young adults without impaired glucose tolerance and whether a single ingestion can influence glycaemic responses not only after the first meal but also after a subsequent meal. In particular, the presence of a second-meal effect after a single intake of a functional beverage in healthy young adults has not been well characterised.

Therefore, this randomised, double-blind, placebo-controlled crossover study investigated the effects of a single dose of MW tea on postprandial and second-meal interstitial glucose responses after breakfast and lunch in healthy adults using flash glucose monitoring.

We hypothesised that the ingestion of a single dose of MW tea would attenuate both postprandial and second-meal glycaemic responses in healthy adults.

This study is expected to contribute to the field of preventive clinical nutrition by identifying a practical dietary strategy for glycaemic control in individuals without diabetes.

METHODS

Study design, setting, and ethical approvals

This was a randomised, double-blind, placebo-controlled, crossover study using a test food (MW tea) or placebo food (placebo tea), with a washout period of at least one week between test days. Participants were recruited between March and August 2024. The experiments were conducted in a controlled laboratory setting at Nakamura Gakuen University in 2024. No significant changes were made to the methods after the trial commenced. This study was conducted according to the guidelines of the Declaration of Helsinki. All procedures involving human patients were approved by the Ethics Committee of Nakamura Gakuen University (approval number: rinri-21-008) in 2021, and the most recent amendment was approved on 18 January 2023. The study plan was registered with the University Hospital Medical Information Network Center (registration no. UMIN000053152, https://center6.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000057645) before study initiation. All the participants provided written informed consent. Participation was voluntary, participants could withdraw at any time without penalty, and participation or non-participation had no effect on their coursework or academic evaluation. Recruitment, enrolment, and allocation were conducted by research staff members, independent of the participants' instructors, to avoid potential coercion.

Sample size

Based on a previous study¹⁷ with a similar design using MW tea, the required sample size was calculated to be 13 cases, with a detectable difference in Δ I_{AUC} of interstitial glucose between MW tea and placebo tea set at 2000 mg·min/dl (standard deviation 2200 mg·min/dl), significance level set at 5%, and statistical power at 90%. Considering that the participants were healthy young adults without impaired glucose tolerance, were neither over-

weight nor extremely underweight, and accounting for potential dropouts, the target sample size for this study was set at 20, which was approximately 1.5 times the calculated sample size. This increase in the target sample size was introduced to account for (i) possible data loss specific to flash glucose monitoring (e.g. sensor detachment and calibration errors), (ii) potential protocol deviations or dropouts between periods, and (iii) the need for greater precision in secondary analyses (e.g. second-meal effects) and mixed-effects modelling.

Participants

The inclusion criteria for this study were as follows: university students aged 18–29 years; body mass index (BMI) between 16.0 and 23.0 kg/m², as defined with reference to previous studies in Japanese young adults;²⁰ no food allergies; no regular use of medications and supplements; no history of diabetes or impaired glucose tolerance; and no pregnancy or breastfeeding. A lower limit of 16.0 kg/m² was pre-specified in the protocol approved by the Ethics Committee of Nakamura Gakuen University because the overall project initially considered an exploratory comparison between the underweight and normal-weight subgroups.

Allocation

The participants were assigned IDs in the order of enrolment. After enrolment, an independent researcher who was not involved in the data collection, intervention delivery, or statistical analysis generated a random allocation sequence using a computer-generated random number list. The participants were stratified according to age, sex, BMI, and appendicular skeletal muscle mass index (ASMI) to ensure balance across intervention sequences. Within each stratum, the participants were randomly assigned to one of two crossover sequences: MW tea followed by placebo tea or placebo tea followed by MW tea.

The allocation sequence was managed exclusively by an independent researcher and was not accessible to the investigators responsible for participant enrolment, intervention administration, or outcome assessment.

Participants were enrolled by the first author, and assignment to the intervention sequence was implemented by another research team member according to a pre-generated allocation list.

Thus, allocation concealment was maintained until the point of intervention assignment.

Blinding

This was a double-blinded study. Participants, care providers, and outcome assessors were blinded to the group assignments. To ensure blinding, MW and placebo teas were packaged in identical stick-type containers. Both beverages were designed to resemble typical tea infusions, and their appearance, taste, and aroma matched as closely as possible. A pre-test conducted by the research staff confirmed that participants could not distinguish between the MW and placebo teas based on sensory characteristics. The test beverages were labelled with a code, and only one researcher, who was not involved in participant interaction or data collection, had access to the key.

Blinding was maintained until data collection and initial statistical analyses were completed.

Diet

Mulberry leaves were dried and ground into a powder using Sato Tea Manufacturing (Kumamoto, Japan). Water chestnut husks were roasted and ground into powder using Kenkochaen (Fukuoka, Japan). These were blended at a ratio of 95:5 (mulberry leaf: water chestnut husk, w/w %) and individually packaged as 3 g of powder in stick-like bags by Kyushu Pharmaceutical Industry (Saga, Japan). MW tea (Kanzaki M&M, Saga, Japan) was used as the test food. Placebo tea was prepared based on previous studies¹⁸ and was designed to match the appearance and smell of the test food, with due consideration given to hygiene. It consisted of a mixture of corn flour (2.5 g) (Pioneer Planning Corporation, Tokyo, Japan), barley tea powder (Yanagiya, Aichi, Japan), and food colouring (Tamaphilin; Tama Biochemical, Tokyo, Japan). Barley tea and corn flour were selected because they are caffeine-free, are commonly consumed in Japan, and do not contain polyphenols, which are the major bioactive components of MW tea. Therefore, they were considered appropriate placebo ingredients as they were unlikely to influence postprandial glucose responses.

Both the MW tea and placebo tea were mixed with 200 mL of hot water and served to the participants. Breakfast (loaded food A) consisted of 200 g vacuum-packed rice (Sato Foods Industries, Aichi, Japan), which provided 294 kcal and 67.8 g carbohydrates.¹⁸ Lunch (loaded food B) for the second-meal effect consisted of 200 g of vacuum-packed rice (Sato Shokuhin Kogyo, Aichi, Japan) and 180 g of retort curry (House curry house curry house curry <sweet>), providing 154 kcal and 18.6 g carbohydrates. Each loaded food sample was heated in a microwave oven and served to participants. The nutritional characteristics of the test, placebo, and loaded foods are summarised in Table 1.

Interstitial and blood glucose measurements

Subcutaneous interstitial fluid glucose levels were continuously monitored using flash glucose monitoring (FGM) with FreeStyle Libre Pro (Abbott Diabetes Care, Alameda, CA, USA). Although this system measures interstitial glucose rather than blood glucose, its values are highly correlated with capillary glucose and are widely accepted as surrogates for blood glucose in clinical

nutrition studies. Because interstitial glucose reflects blood glucose with a short physiological delay, particularly during periods of rapid glycaemic change, the present study focused on integrated measures (Δ IAC) and time-matched comparisons rather than isolated peak values. Capillary self-monitoring blood glucose (SMBG) measurements, performed using the FreeStyle Precision Neo device (Abbott Diabetes Care, Alameda, CA, USA), at predefined time points were used to confirm the validity of the interstitial glucose readings. The sensors were placed on the upper arm for at least 48 h before testing, to ensure proper calibration. To validate interstitial readings, capillary blood samples were obtained via fingertip before breakfast and at 30, 60, and 120 minutes postprandially, using the same SMBG meter as described above, instead of a Libre electrode. The total observation period lasted approximately 300 min and included assessments after both breakfast and lunch. Glucose levels were measured 16 times, incorporating baseline values prior to each meal and at 15-minute intervals at 120 min post-breakfast and 90 min post-lunch. This setup allowed a detailed, minimally invasive assessment of postprandial and second-meal glycaemic responses. The accuracy and validity of the FreeStyle Libre Pro system have been established in previous studies using capillary and venous blood as reference standards.²¹

Body measurements

Participants who agreed to participate in the study were measured for height and weight while lightly dressed, without shoes or socks, after an overnight fast of at least 12 h, and their bladders were emptied. Height and body weight were recorded to 0.1 cm and 0.1 kg, respectively, and 1 kg was subtracted from the recorded body weight to adjust for the weight of the clothes. Body composition was measured using an InBody 770 Body Component Analyzer (InBody) based on the bioelectrical impedance method.

Experimental protocol

The participants were instructed not to eat or drink anything except water and designated loaded foods from 9:00 pm on the day before the experiment until the end of the test session. The experimental protocol is illustrated in Figure 1. Before consuming the loaded food, baseline interstitial and blood glucose values were collected from each participant using FGM with a FreeStyle Libre Pro,

Table 1. Nutrition facts for placebo, test and loaded foods[†]

Nutrition facts	MW tea	Placebo tea	Breakfast [‡] (loading food A)	Lunch [§] (loading food B)
Weight (g)	3.0	2.5	200	380
Energy (kcal)	8.5	9.3	294	571
Protein (g)	0.6	0.1	4.6	11.8
Fat (g)	0.1	0.0	0.0	12.8
Carbohydrates (g)	1.8	2.2	67.8	101.3
Fibre (g)	1.14	0.08	3.0	5.3

MW tea: mixed tea of mulberry leaves and water chestnut husk

[†]The nutritional values of loaded foods A and B are based on information provided by the manufacturers

[‡]Breakfast (loaded food A): 200 g of vacuum-packed rice

[§]Lunch (loaded food B): 200 g of vacuum-packed rice and 180 g of vacuum-packed instant curry

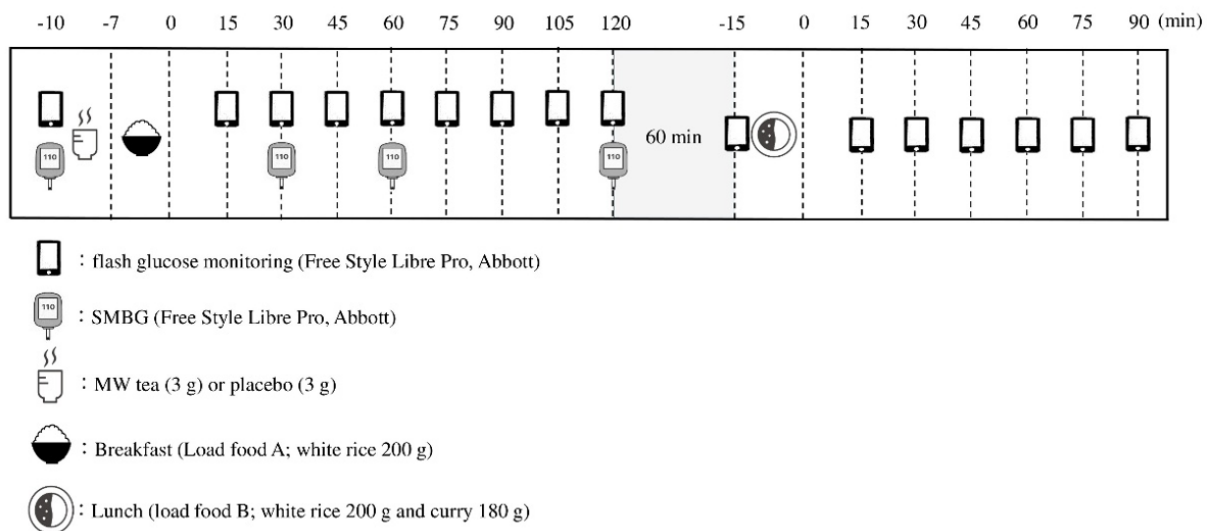


Figure 1. Experimental protocol. MW, mulberry leaves and water chestnut husk; SMBG, self-monitoring of blood glucose

and SMBG using the FreeStyle Precision Neo® device. The participants were instructed to consume either MW tea or placebo tea within 3 min, followed by breakfast (loaded with food A) within 7 min. Interstitial glucose levels were measured every 15 min for 120 min after breakfast using the FreeStyle Libre Pro. Simultaneously, the SMBG was performed for 30, 60, and 120 min using the same SMBG meter as described above.

An additional experiment was conducted 180 min after breakfast to verify the effects of the second meal. Each participant was then asked to consume lunch (loaded with food B) within 15 min. Thereafter, interstitial glucose levels were measured every 15 min using the FreeStyle Libre Pro for 90 min. Participants were instructed to remain seated throughout the experiment.

The participants were asked not to make any major changes in their lifestyle, including diet and exercise, until all food-loading tests were completed, including a one-week washout period.¹⁸

Outcomes

The primary outcome was the difference in the area under the curve of elevated interstitial glucose (Δ IAUC of total glucose area), measured using the FreeStyle Libre Pro. Measurements were taken every 15 min from 0 to 120 min after the consumption of MW or placebo tea and breakfast (loaded food A). In addition, SMBG was performed at 0, 30, 60, and 120 min, using the same SMBG meter as described above.

The secondary outcome was the IAUC of the total interstitial glucose area measured using the FreeStyle Libre Pro from 0 to 90 min after consuming lunch (loaded food B), with measurements taken every 15 min. No changes in the pre-specified primary or secondary outcomes were observed after the trial commenced.

Statistical analyses

Normality tests (Shapiro–Wilk test) were performed, and the subsequent test method was selected based on the distribution of the data. Data are presented as mean \pm standard deviation or median [25–75%]. When required

to meet the model assumptions, the outcome or explanatory variables were log-transformed before parametric analyses. To appropriately account for within-subject correlations in repeated measurements, a linear mixed-effects model was applied with fixed effects for intervention (MW vs. placebo), time, and their interaction, with sex and BMI included as covariates, and a random intercept for participants. Additional models were fitted, with further adjustments for fasting interstitial glucose levels. The results from the linear mixed-effects models are presented in Figure 2 as estimated marginal means with 95% confidence intervals (CIs). The intervention-by-time interaction was evaluated within the model, and the differences between the intervention conditions at each time point were interpreted descriptively based on the estimated marginal means. The primary and secondary outcomes, as well as the correlation analyses between interstitial glucose and capillary glucose (SMBG), were predefined prior to the study.

For comparative and multiple regression analyses, the outcome variable was defined as the relative difference in total IAUC between the MW tea and placebo tea conditions, calculated as $(\text{IAUC}_{\text{MW}} - \text{IAUC}_{\text{placebo}}) / \text{IAUC}_{\text{placebo}} \times 100$.

The relative difference in total IAUC between the two conditions was compared using a paired t-test or Wilcoxon signed-rank test, as appropriate. Effect sizes were calculated for paired comparisons: Cohen's *d*_z was used for normally distributed paired data, and the effect size *r* was derived from the Wilcoxon signed-rank test for non-normally distributed data ($r = |Z| / \sqrt{N}$). The 95% CIs for the mean differences were calculated to aid in the interpretation of the effect magnitude. Achieved statistical power ($1 - \beta$) was estimated post hoc based on the observed effect sizes and sample size. Effect sizes were interpreted according to conventional criteria, with Cohen's *d*_z values of approximately 0.2, 0.5, and 0.8, indicating small, medium, and large effects, respectively, and Wilcoxon *r* values of approximately 0.1, 0.3, and 0.5 indicating small, medium, and large effects, respectively.

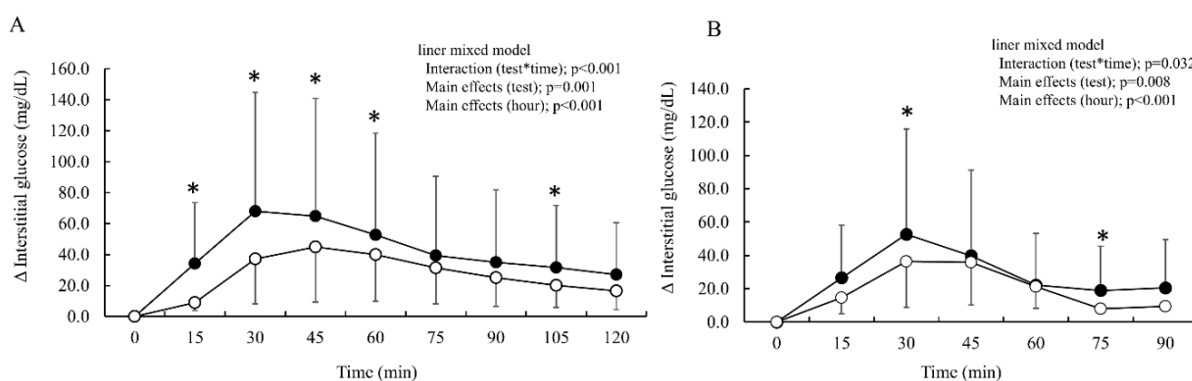


Figure 2. Changes (Δ) in glucose levels in interstitial fluid after breakfast (loaded food A) and lunch (loaded food B): comparison between MW tea and placebo tea. Values are presented as estimated marginal means (EMMs) \pm 95% confidence intervals (CIs) from the linear mixed-effects model adjusted for sex and BMI. (a) Breakfast (loaded food A): A significant intervention-time interaction was observed. Asterisks indicate the time points at which Bonferroni-adjusted post-hoc tests showed significant differences between MW tea and placebo tea. (b) Lunch (loaded food B): A significant intervention-time interaction was observed. Asterisks indicate time points with significant Bonferroni-adjusted differences between interventions. In additional models, with further adjustments for fasting interstitial glucose, the results remained unchanged for both breakfast and lunch.

White circles represent MW tea, and black circles represent placebo tea.

* $p < 0.05$, MW tea vs. placebo tea at the corresponding time points (post-hoc test with multiplicity adjustment).

LMM, linear mixed-effects model; MW, mulberry leaf and water chestnut husk

Pearson's correlation coefficient was used to analyse the correlations between bivariate quantitative variables, and multiple regression analyses were performed on an exploratory basis to assess the factors associated with the relative difference in the total IAUC of the interstitial glucose curve, with age, sex, BMI, %Fat, ASMI, and fasting interstitial glucose included as explanatory variables.

All statistical analyses were performed using SPSS version 27 (IBM, Armonk, NY, USA), with post hoc power calculations conducted using G*Power 3.1.9.7; statistical significance was set at $p < 0.05$ (two-tailed) unless otherwise stated. No interim analyses were performed and no stopping guidelines were established for this study.

RESULTS

Twenty individuals consented to participate and met the inclusion criteria. Their characteristics are presented in Table 2; none dropped out during the study period. No participants were excluded after randomisation and there were no losses to follow-up. The trial proceeded as planned with no early termination. All 20 participants completed both intervention conditions (MW and placebo teas) and were included in the final analysis. As this was a crossover trial, each participant served as a control and all analyses were conducted based on the assigned intervention sequences.

First, to confirm the validity of the interstitial glucose concentrations measured using the FreeStyle Libre Pro at 30, 60, and 120 min after breakfast (loaded food A) following the consumption of MW or placebo tea, we analysed the relationship between the interstitial glucose concentration and capillary blood glucose levels measured using SMBG. Strong-to-moderate positive correlations were observed across all time points (Supplementary Figure 1).

Next, we investigated the effects of MW tea on post-prandial interstitial glucose levels. A significant intervention-by-time interaction was observed after breakfast (loaded food A) ($p < 0.001$, adjusted for sex and BMI) (Figure 2a). The results remained unchanged after additional adjustments for fasting interstitial glucose levels. Consistent with this interaction, Δ interstitial glucose levels tended to be lower in the MW tea condition than in the placebo tea condition at 15, 30, 45, 60, and 105 min after consumption of breakfast (Figure 2a).

In addition, the Δ IAUC of total glucose area from 0 to 120 min was significantly lower for MW tea than for placebo tea ($p < 0.001$). Similar results were obtained at 30, 60, and 90 min (Table 3). Effect size estimates for these comparisons ranged from moderate to large, with achieved statistical power ($1-\beta$) generally in the moderate-to-high range (Table 3).

Table 2. Characteristics of the participants[†]

	All	Men	Women
N	20	10	10
Age (years)	21.0 [21.0–21.8]	21.0 [20.0–21.5]	21.0 [21.0–22.0]
BMI (kg/m ²)	20.3 (1.7)	20.8 (1.9)	19.9 (1.4)
FFM (kg)	40.7 (6.7)	47 (2.7)	34.4 (1.7)
Fat mass (%)	22.3 (6.9)	17.3 (4.9)	27.4 (4.5)
ASMI (kg/m ²)	6.4 [5.7–7.3]	7.3 [7.1–7.4]	5.8 [5.6–5.9]
Glucose (mg/dL)	89.3 (6.4)	90.9 (6.3)	87.8 (6.2)

BMI, body mass index; FFM, fat-free mass; ASMI, appendicular skeletal muscle mass index; Glucose, fasting interstitial glucose.

[†]Data are expressed as number of participants, mean (standard deviation), or median [25th–75th percentile].

Table 3. Incremental area under the curve of change (Δ) in interstitial glucose levels after breakfast (loaded food A) and lunch (loaded food B): Comparison between MW tea and placebo tea^{†‡§}

Time points	Placebo	MW tea	Mean difference
After breakfast (loaded food A)			
30 min (mg x 30min/dL)	1023 (261)	420 (225)	-603
60 min (mg x 30min/dL)	1736 [1284–2518]	1181 [846–1378]	-624
90 min (mg x 30min/dL)	1249 (749)	961 (514)	-288
120 min (mg x 30min/dL)	645 [504–1464]	540 [407–900]	-323
Total area (mg x 120min/dL)	4800 [3966–6023]	3266 [2102–3825]	-1837
After lunch (loaded food B) (second-meal test)			
30 min (mg x 30min/dL)	765 (358)	540 (342)	-225
60 min (mg x 30min/dL)	1155 (604)	929 (413)	-225
90 min (mg x 30min/dL)	484 [240–662]	304 [238–512]	-232
Total area (mg x 90min/dL)	2522 (1173)	1840 (715)	-682

Time points	95% CI (lower to upper)	<i>p</i>	Effect size (Cohen's dz / Wilcoxon r)	1- β
After breakfast (loaded food A)				
30 min (mg x 30min/dL)	-730.9 to -474.3	<0.001	2.14 [¶]	1.00
60 min (mg x 30min/dL)	-943.1 to -304.2	<0.001	0.67 ^{††}	
90 min (mg x 30min/dL)	-564.0 to -12.0	0.047	0.48 [¶]	0.66
120 min (mg x 30min/dL)	-587.9 to -57.8	0.059	0.40 ^{††}	
Total area (mg x 120min/dL)	-2596.0 to -1078.2	<0.001	0.74 ^{††}	
After lunch (loaded food B) (second-meal test)				
30 min (mg x 30min/dL)	-451.8 to 1.8	0.057	0.45 [¶]	0.48
60 min (mg x 30min/dL)	-482.4 to 31.6	0.090	0.40 [¶]	0.40
90 min (mg x 30min/dL)	-442.0 to -21.5	0.057	0.30 ^{††}	
Total area (mg x 90min/dL)	-1215.5 to -148.7	0.017	0.58 [¶]	0.69

MW, mulberry leaves and water chestnut husk.

[†]Data are expressed as number of participants, mean (standard deviation), or median [25th–75th percentile].

[‡]The upper section of the table presents results after breakfast (first meal), and the lower section presents results after lunch (second meal). Within each section, results are organised by time point to facilitate comparison between MW tea and placebo.

[§]For non-normally distributed variables, 95% confidence intervals are provided for descriptive purposes only; effect size interpretation is based on Wilco

[¶]Cohen's dz values of 0.2, 0.5, and 0.8 indicate small, medium, and large effects, respectively.

^{††}Wilcoxon r values of 0.1, 0.3, and 0.5 indicate small, medium, and large effects, respectively.

As to whether there was a second-meal effect, a significant intervention-by-time interaction was observed after lunch (loaded food B) ($p = 0.032$, adjusted for sex and BMI) (Figure 2b). The results remained unchanged after additional adjustments for fasting interstitial glucose levels. Consistent with this interaction, Δ interstitial glucose levels tended to be lower in the MW tea condition than in the placebo tea condition at 30 and 75 min after lunch (Figure 2b). The Δ IAUC of total glucose area from 0 to 90 min was lower for MW tea than for placebo tea, indicating a moderate effect size ($p = 0.017$, Cohen's dz = 0.58, $1 - \beta = 0.69$) (Table 3).

Finally, exploratory stepwise multiple regression analyses were performed to examine the factors associated with the relative difference in total glucose IAUC between MW tea and placebo tea, accounting for potential confounding factors including age, sex, BMI, %Fat, ASMI, and fasting interstitial glucose (Table 4). BMI was negatively associated with the relative difference in the total glucose IAUC at breakfast (first meal), independent of age, sex, fat mass, ASMI, and fasting interstitial glucose. At lunch (second meal), the relative difference in total glucose IAUC was also negatively associated with both BMI and fasting interstitial glucose.

No adverse events or unintended effects were reported with either the MW or placebo tea during the study.

DISCUSSION

The results of this study support our hypothesis that a single intake of MW tea attenuates both postprandial and second-meal glycaemic responses in healthy young adults. This is, to our knowledge, the first study to suggest that MW tea, made from a mixture of mulberry leaves and water chestnut husk, may reduce the Δ IAUC of glucose after both the first meal (breakfast) and the subsequent second meal (lunch) in healthy, non-obese, non-diabetic young adults.

First, MW tea significantly reduced interstitial glucose levels and Δ IAUC after breakfast (loaded food A). This finding supports the results of our previous study,¹⁸ which showed that MW tea reduced postprandial hyperglycaemia in middle-aged and older Japanese adults with borderline diabetes. This study confirmed the interstitial glucose-lowering effect of MW tea in healthy young adults, supporting its potential as a functional dietary component. The hypoglycaemic effect is thought to result from the inhibition of α -glucosidase by 1-DNJ contained in mulberry leaves^{14, 15} and polyphenols in water chestnut husk,¹⁶ which delay the absorption of glucose in the intestinal tract.¹⁸ In addition, trace amounts of dietary fibre in MW tea may play a role in lowering blood glucose levels.^{9, 10} However, the relative contribution of each component and precise underlying mechanisms remain unclear and require further investigation.

Table 4. Multiple stepwise linear regression analysis of factors associated with the relative difference in total glucose IAUC between the mulberry leaves and water chestnut husk tea and placebo tea

	Partial regression coefficient (β)	Standardized partial regression coefficient (β)	95% CI (lower to upper)	<i>p</i>
After breakfast (loaded food A)				
BMI (kg/ m ²)	-6.264	-0.475	-12.011 to -0.517	0.034
Age (years)				0.497
Sex				0.793
Fat mass (%)				0.925
ASMI (kg/m ²)				0.826
Glucose (mg/dL)				0.180
<i>R</i> ²	0.226			
Adjusted <i>R</i> ²	0.183			0.034
After lunch (load food B) (second-meal test)				
BMI (kg/m ²)	-0.078	-0.473	-0.148 to 0.000	0.030
Age (years)				0.608
Sex				0.460
Fat mass (%)				0.809
ASMI (kg/m ²)				0.470
Glucose (mg/dL)	-0.011	-0.459	-0.021 to 0.009	0.042
<i>R</i> ²	0.210			
Adjusted <i>R</i> ²	0.166			0.045

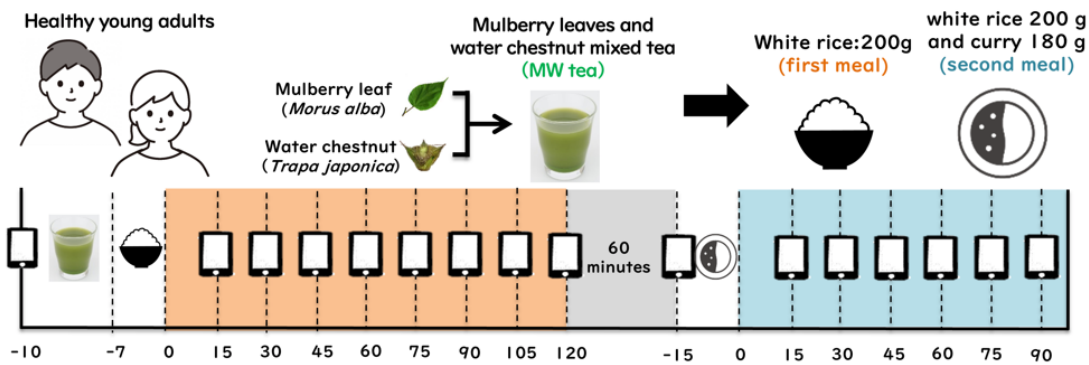
BMI, body mass index; ASMI, appendicular skeletal muscle mass index; Glucose, fasting interstitial glucose; *R*², coefficient of determination; adjusted *R*², adjusted coefficient of determination.

Objective variable: Relative difference in total IAUC between MW tea and placebo tea, calculated as [Total IAUC of MW tea - Total IAUC of placebo tea] / Total IAUC of placebo tea * 100

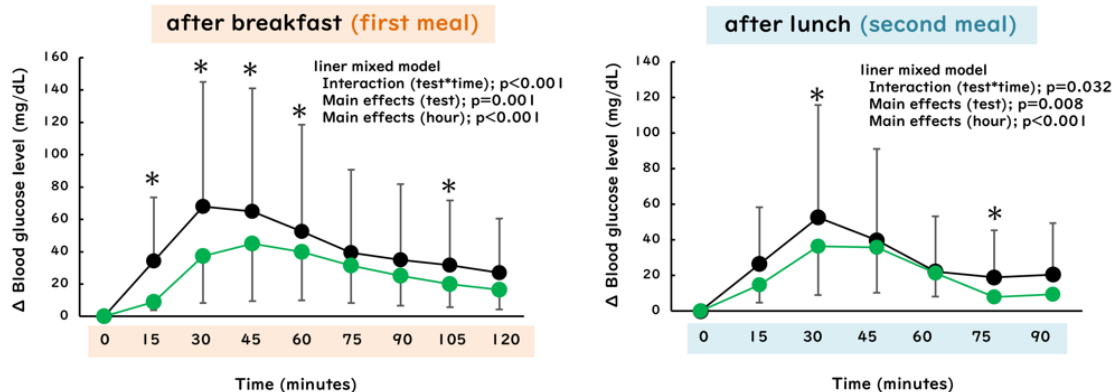
Explanatory variables: age, sex, BMI, ASMI, %Fat, Glucose

The upper section of the table presents results after breakfast (first meal), and the lower section presents results after lunch (second meal). For each meal, partial regression coefficients (β), standardised partial regression coefficients, and their 95% confidence intervals are shown for each explanatory variable.

Values represent the relative difference in total IAUC between the MW tea and placebo tea conditions; more negative values indicate a greater relative glucose-lowering effect of MW tea compared with placebo



Inhibition of postprandial blood glucose elevation



Graphical abstract.

Jenkins et al.⁶ and Wolever et al.⁷ found that the glycaemic index of a meal before a second meal was a determinant of the improvement in glucose tolerance observed with subsequent food intake, a phenomenon termed the second-meal effect. The second-meal effect has been observed in high-fibre foods,^{9, 10, 21, 22} foods low in carbohydrates, and foods high in proteins,⁸ although its underlying mechanism remains unclear. In this study, drinking MW tea before breakfast influenced the Δ IAC of glucose from 0 to 90 min after lunch (second meal), suggesting the presence of a second-meal effect. Although loaded food B (lunch) contained more energy and carbohydrates than loaded food A (breakfast), the Δ IAC for interstitial glucose was lower, possibly due to delayed gastric excretion of carbohydrates caused by the protein and lipids in the instant curry in loaded food B.²³ However, the mean difference, effect size, and achieved statistical power for the second-meal effect were all smaller than those observed after loaded food A (breakfast). In addition, this effect was identified as a statistically significant intervention-by-time interaction in the linear mixed-effects model after adjusting for covariates. However, the magnitude of the effect, as assessed by the between-condition difference in Δ IAC, was modest and should be interpreted cautiously.

Previous studies have suggested dietary fibre as a primary factor underlying the second-meal effect.^{9, 10, 21, 24} However, the dietary fibre content of the MW tea used in the present study was only 1.14 g, and the observed second-meal effect occurred after a single intake rather than continuous consumption. This finding suggests that, although even a small amount of dietary fibre may contribute to the observed response, it is difficult to explain the effect solely by dietary fibre. In addition, the α -glucosidase inhibitory activity of 1-DNJ and polyphenols derived from water chestnut husk contained in MW tea may contribute to the second-meal effect. Indeed, previous *in vitro* studies have reported that a complex of 1-DNJ and the natural flavonoid chrysin reversibly and competitively inhibits α -glucosidase inhibitory activity and alters enzyme structure.²⁵ However, previous studies have also shown that the glucose-lowering effects of 1-DNJ and polyphenols are generally short-lived and largely confined to the immediate postprandial period.^{11, 16, 17, 19} Taken together, these findings suggest that the second-meal effect observed several hours after a single intake of MW tea may reflect the combined effects of dietary fibre and other bioactive components rather than a single dominant mechanism. Nevertheless, because gastric emptying and baseline metabolic variability were not directly assessed in the present study, these mechanistic interpretations remain speculative and warrant further investigation.

In a multiple regression analysis to examine the factors associated with the relative difference in the total IAC between MW tea and placebo tea, BMI was identified as a common influential factor for both breakfast (first meal) and lunch (second meal) independent of sex and age. Fasting interstitial glucose levels were also identified as a significant factor during the second meal. Similar inter-individual variability in dietary intervention responses has been reported in previous nutritional studies.²⁶⁻²⁸ Howev-

er, these regression analyses were exploratory in nature, and the results should be interpreted cautiously given the limited sample size. Accordingly, the present findings should be interpreted as preliminary and cannot be directly extrapolated beyond healthy young adults or long-term clinical outcomes.

This study has several limitations. First, the sample size in this study was relatively small, and some analyses were exploratory; therefore, the findings should be interpreted with caution when considering their clinical implications. Second, the loaded foods used for breakfast and lunch consisted of single foods and did not include vegetable dishes or soups, meaning that they might not necessarily reflect the general diet of Japanese people. However, approximately 70% of young Japanese adults (aged 20–29 years) do not consume meals consisting of staple foods, main dishes, or side dishes more than twice a day.²⁹ In future studies, it will be necessary to examine whether similar effects can be obtained when a meal includes a staple meal, main dish, or side dish. Third, although FGM enables minimally invasive and frequent glucose assessment over a 300-minute observation period, it measures interstitial rather than blood glucose and may be less accurate than capillary or venous sampling during rapid glycaemic changes. Nevertheless, interstitial glucose values obtained using FreeStyle Libre Pro are highly correlated with capillary and venous glucose levels and are widely accepted as surrogates for blood glucose responses in clinical studies. The accuracy and validity of this system have been demonstrated previously,²⁴ and in the present study, interstitial glucose values also showed a strong to moderate positive correlation with capillary SMBG. Therefore, although potential physiological lag effects should be considered, the consistency between interstitial and capillary glucose measurements supports the validity of the observed interventional effects.

Fourth, this study did not capture postprandial insulin trends. However, in a previous study on middle-aged and older Japanese individuals with borderline diabetes, we found that insulin levels during the consumption of MW tea were lower than those during the consumption of placebo tea.¹⁸ Therefore, it is reasonable to assume that the hypoglycaemic effect of MW tea in the present study was independent of insulin. Fifth, dinner on the day before testing was not standardised. As carbohydrate intake on the preceding day is known to influence the next-day postprandial glucose responses,³⁰ this could have introduced variability into our results. However, because the study employed a randomised crossover design, each participant served as their own control, which likely minimised the influence of daily dietary variations. Furthermore, although some participants had relatively low BMI values, the crossover design minimised between-subject variability, and BMI was explicitly adjusted as a covariate in the linear mixed-effects models and included in the regression analyses, thereby reducing the potential influence of body composition-related heterogeneity. Nevertheless, the heterogeneity related to body composition cannot be entirely excluded. Sixth, the mechanisms of interstitial glucose reduction after breakfast and lunch and the contributions of the individual components are unknown and require further study. In addition, we did not

investigate the effects of the antioxidant activity of MW tea on the interstitial glucose levels or vascular endothelial function, which are important topics for future research. Furthermore, the contents of active substances in MW tea, such as 1-DNJ and polyphenols, were not directly measured in the present study. Future studies should include detailed compositional analyses to clarify the relationship between these bioactive components and the observed glycaemic responses. These limitations should be addressed in future studies to validate our findings. Despite these limitations, the present study provides preliminary evidence suggesting that MW tea, a natural blend rich in polyphenols and 1-DNJ, can modulate postprandial glycaemic responses and may contribute to a second-meal effect even after a single intake in healthy young adults. Given the growing recognition of postprandial hyperglycaemia as a modifiable risk factor for type 2 diabetes and cardiovascular disease, MW tea may represent a potentially safe dietary component with preliminary relevance to glycaemic modulation under controlled experimental conditions.

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DISCLOSURE ON THE USE OF AI AND AI-ASSISTED TECHNOLOGIES

No AI or AI-assisted technologies were used in the data collection, analysis, or creation of images or graphical elements for this manuscript. The authors reviewed and edited the content and take full responsibility for the content of the publication.

CONFLICT OF INTEREST AND FUNDING DISCLOSURES

The authors declare no conflict of interest.

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