Short-Term Effect of Non-Nutritive Sweeteners (Sucralose and Saccharin) Consumption on Glycaemic Control and Gut Microbiota in Type 2 Diabetes Patients: Study Protocol for a Double-Blind, Randomised, Placebo-Controlled, Crossover Trial

Huey Shin Tan, Harvinder Kaur Gilcharan Singh, Snigdha Misra, Vanitha Mariappan, Nor Aini Jamil @ A. Wahab, Suhaili Naim Mustapa

Submitted to: JMIR Research Protocols
on: August 20, 2025

Table of Contents

Short-Term Effect of Non-Nutritive Sweeteners (Sucralose and Saccharin) Consumption on Glycaemic Control and Gut Microbiota in Type 2 Diabetes Patients: Study Protocol for a Double-Blind, Randomised, Placebo-Controlled, Crossover Trial

Huey Shin Tan[1*]; Harvinder Kaur Gilcharan Singh[1*]; Snigdha Misra[2*]; Vanitha Mariappan[3*]; Nor Aini Jamil @ A. Wahab[1*]; Suhaili Naim Mustapa[4*]

[1]Centre for Community Health Studies (ReaCH) Faculty of Health Sciences Universiti Kebangsaan Malaysia Kuala Lumpur MY
[2] Jeffrey Cheah School of Medicine and Health Sciences Monash University Malaysia Kuala Lumpur MY
[3]Center for Toxicology and Health Risk Studies (CORE) Faculty of Health Sciences Universiti Kebangsaan Malaysia Kuala Lumpur MY
[4] Klinik Kesihatan Setapak Kuala Lumpur MY
[*]these authors contributed equally

Corresponding Author:
Huey Shin Tan
Centre for Community Health Studies (ReaCH)
Faculty of Health Sciences
Universiti Kebangsaan Malaysia
Jalan Raja Muda Abdul Aziz, Wilayah Persekutuan Kuala Lumpur
Kuala Lumpur
MY

Abstract

Background: Non-nutritive sweeteners (NNS) are widely used as sugar substitutes to help individuals with diabetes manage glycaemic control. However, emerging evidence suggests that even low doses of NNS, such as saccharin and sucralose, may adversely affect metabolic health by impairing glycaemic regulation. These effects appear to be mediated, in part, by changes in the gut microbiome. In Malaysia, where gut microbiome research is still limited, particularly among individuals with Type 2 Diabetes Mellitus (T2DM) further investigation is needed to inform guidelines for NNS use.

Methods: This is a double-blind, randomised, placebo-controlled, crossover trial designed to evaluate the short-term effects of saccharin and sucralose on glycaemic control and the gut microbiota. A total of 33 adults with T2DM will consume sucralose (5 mg/kg body weight), saccharin (2 mg/kg body weight), or a placebo (calcium carbonate) in capsule form daily for seven days in each intervention arm with a month wash-out period. Data collection will include anthropometric measurements, biochemical assessment for glycaemic control, dietary food records, physical activity levels, and stool samples for 16S rRNA gene sequencing to assess gut microbiota composition. This trial is registered at ClinicalTrials.gov (NCT07124585).
Discussion: This trial will contribute novel insights into the effects of short-term NNS consumption on glycaemic control and gut microbiota composition in individuals with T2DM. This finding may support evidence-based recommendations for NNS use in diabetes management and enhance understanding of microbiome-diet interactions in an ethnically diverse Asian population.

(JMIR Preprints 20/08/2025:82695)
DOI: https://doi.org/10.2196/preprints.82695

Preprint Settings

1) Would you like to publish your submitted manuscript as preprint?

✓ **Please make my preprint PDF available to anyone at any time (recommended).**
Please make my preprint PDF available only to logged-in users; I understand that my title and abstract will remain visible to all users.
Only make the preprint title and abstract visible.
No, I do not wish to publish my submitted manuscript as a preprint.

2) If accepted for publication in a JMIR journal, would you like the PDF to be visible to the public?

✓ **Yes, please make my accepted manuscript PDF available to anyone at any time (Recommended).**
Yes, but please make my accepted manuscript PDF available only to logged-in users; I understand that the title and abstract will remain v
Yes, but only make the title and abstract visible (see Important note, above). I understand that if I later pay to participate in <a href="http
No. Please do not make my accepted manuscript PDF available to anyone. I understand that if I later pay to participate in <a href="https:/

Original Manuscript

Title

Short-Term Effect of Non-Nutritive Sweeteners (Sucralose and Saccharin) Consumption on Glycaemic Control and Gut Microbiota in Type 2 Diabetes Patients: Study Protocol for a Double-Blind, Randomised, Placebo-Controlled, Crossover Trial

Abstract

Background: Non-nutritive sweeteners (NNS) are widely used as sugar substitutes to help individuals with diabetes manage glycaemic control. However, emerging evidence suggests that even low doses of NNS, such as saccharin and sucralose, may adversely affect metabolic health by impairing glycaemic regulation. These effects appear to be mediated, in part, by changes in the gut microbiome. In Malaysia, where gut microbiome research is still limited, particularly among individuals with Type 2 Diabetes Mellitus (T2DM) further investigation is needed to inform guidelines for NNS use.

Methods: This is a double-blind, randomised, placebo-controlled, crossover trial designed to evaluate the short-term effects of saccharin and sucralose on glycaemic control and the gut microbiota. A total of 33 adults with T2DM will consume sucralose (5 mg/kg body weight), saccharin (2 mg/kg body weight), or a placebo (calcium carbonate) in capsule form daily for seven days in each intervention arm with a month wash-out period. Data collection will include anthropometric measurements, biochemical assessment for glycaemic control, dietary food records, physical activity levels, and stool samples for 16S rRNA gene sequencing to assess gut microbiota composition. This trial is registered at ClinicalTrials.gov (NCT07124585).

Discussion: This trial will contribute novel insights into the effects of short-term NNS consumption on glycaemic control and gut microbiota composition in individuals with T2DM. This finding may support evidence-based recommendations for NNS use in diabetes management and enhance understanding of microbiome-diet interactions in an ethnically diverse Asian population.

Keywords: non-nutritive sweetener; artificial sweetener; glycaemic control; gut microbiota; saccharin; sucralose; type 2 diabetes

Protocol Version

Version 1, dated 05 August 2025

Names and Affiliations of Protocol Contributors

Tan Huey Shin[1], Harvinder Kaur Gilcharan Singh[1], Snigdha Misra[2], Vanitha Mariappan[3], Suhaili Naim Mustapa[4], Nor Aini Jamil @ A.Wahab[1]

[1]Centre for Community Health Studies (ReaCH), Faculty of Health Sciences, Universiti Kebangsaan Malaysia. Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur.

[2]Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia. Jalan Lagoon Selatan, Bandar Sunway, 47500 Petaling Jaya, Selangor.

[3]Center for Toxicology and Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia. Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur.

[4]Klinik Kesihatan Setapak. Tingkat 2, Wisma Rampai, Blok Pasifik, Jln. 34/26, Taman Sri Rampai, 53300, Wilayah Persekutuan Kuala Lumpur.

Roles of Protocol Contributors

Conceptualization: HKG, SM, THS, VM, NAJ, SN

Methodology/Study design: HKG, SM, THS, VM, NAJ, SN

Funding acquisition: HKG, SM, VM, NAJ, SN

Investigation (study conduct): THS, SN

Project administration (clinical oversight): THS, SN

Writing – original draft: THS

Writing – review & editing: THS, HKG

Supervision: HKG, SM, VM, NAJ, SN

Name and Contact Information for the Trial Sponsor

Bahagian kecemerlangan penyelidikan IPT

Jabatan Pendidikan Tinggi

Kementerian Pendidikan Tinggi

Aras 7, no. 2, menara 2

Jalan p5/6, presint 5

62200 w.p. putrajaya

No. Tel. : +603-8870 6974/6975

Role of Trial Sponsor

The sponsor does not play any role in the study design, data collection, analysis, interpretation, or

publication of results.

Composition of the Coordinating Centre and Oversight Responsibilities

This study is coordinated by the principal investigator with support from the co-investigators. Day-to-day trial activities, including recruitment, consent, data and sample collection, will be conducted by the student researcher under close supervision. The site investigators, who are also physicians, will oversee clinical procedures and protocol adherence, assisted by clinic nurses. No formal steering or independent monitoring committee is established due to the small scale and minimal-risk nature of the trial; oversight will be maintained through regular supervisory meetings.

Trial Registration

This trial is registered at ClinicalTrials.gov (NCT07124585) on 8/8/2025.

Access to Protocol, Statistical Code, and Participant Data

The full study protocol and statistical analysis code will be made publicly accessible upon publication of the trial results. De-identified participant-level data may be shared upon reasonable request, subject to approval by the Universiti Kebangsaan Malaysia Research Ethics Committee and in accordance with data governance and participant privacy policies.

Data Sharing

The full study protocol and statistical analysis code will be made publicly available upon publication of the trial results. Individual participant-level data will be de-identified, and a data dictionary will be included to facilitate reuse. These materials will be deposited in an institutional or journal-affiliated open-access repository linked to the trial publication. Additional requests for access to de-identified participant data not included in the repository may be considered on a case-by-case basis, subject to approval by the Universiti Kebangsaan Malaysia Research Ethics Committee and in accordance with data governance and participant privacy regulations.

Funding and Conflicts of Interest

The Fundamental Research Grant Scheme (FRGS) is a research funding initiative managed by the Ministry of Higher Education Malaysia (MOHE), administered through the Department of Higher Education (JPT). Grant number: FRGS/1/2024/SKK06/UKM/03/4. The investigators declare no financial or personal conflicts of interest related to this study. No industry funding or involvement is

associated with the design, conduct, analysis, or reporting of this trial.

Dissemination Policy

The findings of this trial will be disseminated through peer-reviewed publications, national and international scientific conferences, and public reporting in ClinicalTrials.gov. Results will be prioritised for publication in open-access journals to maximise accessibility. As stated in the Participant Information Sheet, individuals who express interest in the study outcomes will be provided with a plain-language summary once the trial has been completed and analysed.

Background

Non-nutritive sweeteners (NNS) are widely used as sugar substitutes to reduce caloric intake and manage blood glucose levels. Although previously considered metabolically inert, emerging evidence suggests that NNS may influence host metabolism through effects on the gut microbiota (1). A landmark study in 2014 demonstrated that NNS-induced glucose intolerance in mice was mediated by gut microbiota alterations and transferable to germ-free mice via fecal microbiota transplantation (2). However, subsequent studies reported inconsistent findings, some show NNS alter microbial composition and glycaemic control (3-6), while others find no significant effects (7-9).Variability in results likely reflects differences in NNS type, study design, host characteristics, and baseline microbiota profiles. Although all types of NNS are categorized as sugar substitutes, they differ in their chemical structures, metabolism, and gut accessibility. Therefore, findings from one NNS cannot be generalized to all types of sweeteners. A most recent findings by Suez et al. in 2022 showed that while all tested NNS altered the gut microbiome, only sucralose and saccharin significantly impaired glycaemic responses (3). These effects were reproduced in germ-free mice colonised with microbiota from exposed individuals, strengthening the causal link between NNS, microbiota, and metabolic outcomes. Therefore, our study will focus specifically on sucralose and saccharin.

Some observational studies have raised concerns about the long-term consumption of NNS, such as sucralose and saccharin, potentially leading to glycaemic dysregulation, increased insulin resistance, or metabolic disturbances (2, 3, 10-13). However, these effects were generally observed at higher doses and with prolonged exposure. In contrast, this study adopts a short-term, controlled intervention using doses well within the acceptable daily intake recommended by major health authorities, including the U.S. Food and Drug Administration (FDA), European Food Safety Authority (EFSA), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (14-17).

Both sweeteners have been extensively evaluated and are considered safe for human consumption within their established Acceptable Daily Intakes (ADI).

To date, most NNS studies have focused on healthy populations, overlooking the fact that T2DM itself is associated with gut dysbiosis and altered metabolic signaling (18, 19). In 2024, the International Diabetes Federation (IDF) estimated that nearly 4.75 million adults in Malaysia are living with diabetes (20). The use of NNS in Malaysia is expected to rise due to growing public awareness of sugar's adverse effects; and the implementation of a sugar tax on sugar-sweetened beverages. Thus, prompting the food industry to favour NNS in product reformulation. This study aims to evaluate the effects of sucralose and saccharin on glycaemic control and gut microbiota composition in Malaysian adults with T2DM. Findings will help clarify the potential risks or benefits of NNS use in this high-risk group and inform public health guidance in the context of rising NNS consumption.

Objectives

The primary objective of this study is to assess the change in glycaemic control following NNS consumption, whereas the secondary objective is to evaluate changes in gut microbiota composition and diversity.

Patient and Public Involvement

Patients or the public will not involve in the design, or conduct, or reporting, or dissemination plans of this research.

Trial Design

This is a double-blind, randomised, placebo-controlled crossover trial. A crossover design was chosen to account for interindividual variability in the gut microbiota, as each participant will serve as their own control across three interventions in random order. A washout period is included to avoid any carryover effects.

Trial Setting

This study will be conducted at Klinik Kesihatan Setapak, a government-funded primary care clinic in Setapak, Kuala Lumpur, and Hospital Canselor Tuanku Muhriz (HCTM), the teaching hospital of Universiti Kebangsaan Malaysia. As part of the national public healthcare system, these institutions provide subsidised services and represent typical settings for diabetes care in the Malaysian public

sector.

Eligibility Criteria

This study aims to recruit a cohort of patients whose conditions are stable that can best represent the Malaysian with early-phase diagnosed T2DM phenotypes. The exclusion criteria will primarily focus on lifestyle behaviours or dietary practices that deviate from typical Malaysian lifestyles and while also considering behaviours that could significantly alter the gut microbiota.

Inclusion Criteria

1. Malay Male citizens of Malaysia.
2. Aged between 30 and 50 years.
3. BMI between 23-27.4 kg/m² (Overweight).
4. Diagnosed with diabetes for a duration between 1-5 years.
5. Currently on oral antidiabetic medications and not on insulin.
6. Initial HbA1c less than 10%.
7. Patients with other stable, non-severe chronic conditions (e.g., hypertension, dyslipidemia) may be included if they have been on a stable prescribed oral treatment for at least 6 months.
8. Patients who have undergone dietary counselling for diabetes and are willing to maintain their habitual diabetes-friendly diet and usual physical activity patterns throughout the study period.
9. Willing to refrain from alcohol throughout the study period.
10. Must be literate in English or Bahasa Malaysia.

Exclusion Criteria

1. Current smoking or alcohol use.
2. Experienced more than a 5% change in body weight within the past 3 months.
3. Acute illness or significant cardiovascular, psychological, neurological, renal, or endocrine diseases, apart from diabetes.
4. Intolerance or allergy to test products.
5. Special dietary practices (e.g., intermittent fasting, vegetarian, ketogenic diet) that deviate from typical Malaysian dietary patterns.
6. On treatment, medications and food supplements (e.g. probiotics) that can significantly alter intestinal function and gut microbiome.

7. Involvement in clinical trials within the last 3 months.

Intervention

Participants will receive one of three interventions sucralose, saccharin, or placebo (calcium carbonate) administered once daily for seven days during each intervention phase with a four week of washout period between intervention phases. Dosages will be based on body weight, following national clinical guidelines: 5 mg/kg for sucralose and 2 mg/kg for saccharin (21). Participants will consume the capsule each morning after breakfast with plain water to standardise timing and reduce missed doses.

Placebo

Calcium carbonate at a low dose of 500 mg is generally recognised as safe and is commonly used in clinical studies as a placebo. Specifically, a previous study on sucralose using calcium carbonate as placebo, with 750 mg dosage per day for one week, observed no significant changes in gut and glycaemic parameters in healthy individuals (9). At commonly used low doses of calcium carbonate, serious adverse effects are not expected; minor gastrointestinal symptoms (e.g., constipation, bloating, dyspepsia) rare and occur more often with higher or prolonged dosing of calcium salts (22).

Subject Withdrawal

Participants may withdraw voluntarily at any time. They may also be withdrawn if they develop conditions requiring medications that affect the gut microbiota (e.g., antibiotics) or if continued participation compromises safety or study validity. Participant who misses two or more doses in any intervention phase will be considered non-compliance and will result in withdrawal. Withdrawn subjects will not be replaced unless resources allow. Data collected prior to withdrawal will be included in the intention-to-treat analysis. All withdrawals will be documented, and necessary medical care will be ensured. No modifications to the allocated interventions will be permitted, as this is a blinded crossover study.

Adherence Promotion and Monitoring Strategies

To promote adherence to the intervention protocols, participants will be briefed during enrolment on the study purpose, product safety, and the importance of compliance. Daily log sheets will be provided for participants to record sachet intake, and daily reminders will be sent throughout each 7-day intervention phase. Participants will be required to return any unconsumed capsules at the end of

each phase, and adherence will be assessed based on the number of capsules returned and the completeness of log entries. Regular contact will be maintained to address any concerns and reinforce adherence throughout the trial.

Relevant concomitant care permitted or prohibited during the trial

The intervention is not expected to interfere with ongoing treatments.

Outcomes

The primary outcome of this study is to assess the change in glycaemic control, measured by insulin sensitivity using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), which will be calculated from fasting glucose and insulin levels following NNS consumption (23). The secondary outcome is to evaluate changes in gut microbiota composition and diversity following NNS consumption. Microbiota outcomes will be assessed via 16S rRNA gene sequencing. Alpha diversity and beta diversity metrics will be used to evaluate within- and between-group microbiota differences. Differential abundance analysis will be conducted to identify specific taxanomy associated with each intervention.

Adverse Event Reporting and Harms

Harms will be defined and assessed in accordance with institutional and international clinical research standards. An adverse event (AE) is defined as any untoward medical occurrence in a participant, regardless of its suspected relation to the intervention. Serious adverse events (SAEs) are defined as events that result in death, are life-threatening, require hospitalization or prolongation of existing hospitalization, or result in significant disability.

All AEs, whether non-serious or serious, will be systematically documented throughout the study. This includes both solicited events (e.g., through structured questioning during follow-up) and spontaneously reported events from participants. For each AE, the following details will be recorded: description, classification (e.g., gastrointestinal, dermatological, respiratory, or other), onset and resolution dates, intensity, frequency, action taken (including discontinuation of the study product), and any concomitant medications used.

Assessment of each AE will be conducted by the study physician, who will determine its potential relationship to the investigational products (sucralose or saccharin). Causality will be categorised as unrelated, unlikely, probable, or certain. For events assessed as probable or certain, additional follow-up will be conducted; where feasible, a de-challenge or re-challenge strategy may

be applied.

Any SAE will be reported to the study physician and principal investigator within 24–48 hours of recognition, via telephone or electronic communication, irrespective of its suspected relation to the intervention. Ongoing SAEs at study completion will be followed until resolution or stabilization. All SAEs and relevant safety concerns will be reported to the institutional ethics committee in compliance with local regulatory requirements.

Given that this is a short-term, low-risk dietary intervention, the above approach provides proportionate and systematic monitoring for participant safety.

Participant timeline

Each participant will be enrolled for 12 weeks, comprising a 1-week run-in period, three 7-day intervention phases (sucralose, placebo, saccharin), and two 4-week washout periods between the intervention phases. A total of six study visits will be conducted: three pre-intervention and three post-intervention. During the run-in and washout phases, participants will maintain their habitual lifestyle and avoid all non-nutritive sweeteners. The full study timeline, including assessments and interventions, is outlined in Figure 1. Recruitment is expected to begin in November 2025 and continue until May 2026, or until the target sample size is achieved.

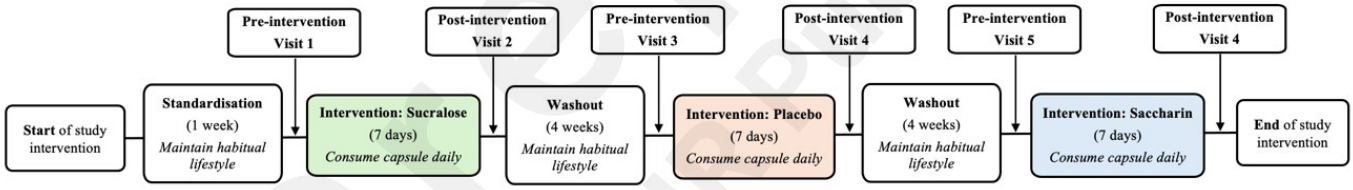


Figure 1. Overview of the Study Visits

Sample Size

The sample size for this crossover trial was estimated based on detecting a medium effect size (Cohen's f = 0.25), consistent with findings from a recent meta-analysis of soluble fiber supplementation in T2DM, which reported moderate standardised mean differences for HOMA-IR (SMD = –0.58; 95% CI: –0.86 to –0.29) (24). Using G*Power 3.1.9.6, we computed the required sample size for a within-subject repeated-measures ANOVA with three conditions (sucralose, saccharin, placebo), assuming $\alpha = 0.05$, 80% power, and a moderate correlation ($r = 0.5$) among repeated measurements (25). The analysis indicated that a minimum of 28 participants would be required. To account for potential dropout or non-compliance, we will recruit 33 participants.

Recruitment

Recruitment will be conducted in person at study sites through clinic listings and referrals. Face-to-face recruitment enhances participant engagement and understanding of study procedures.

Randomisation and Blinding

Randomisation and allocation concealment will be managed by external personnel not involved in recruitment, data collection, analysis, or interpretation. Only this individual will have access to the randomisation key linking participant codes to their assigned test products.

1. Randomisation List Generation: A computer-generated block randomisation list will be created by the external personnel using software such as Randomizer.org (26). Each participant will be assigned to one of the possible intervention sequences (e.g., A-B-C, B-C-A, C-A-B), where A = sucralose, B = saccharin, and C = placebo. This ensures balance across study arms while maintaining unpredictability of allocation. No stratification factors will be used.

2. Product Preparation and Initial Labelling: The project coordinator will prepare the three investigational products (IPs) for each participant, dosed according to body weight. Each type of NNS (sucralose, saccharin, or placebo) will be encapsulated and packaged in identical opaque capsules, placed into individual plastic bags labelled with the participant's ID and the general product name (e.g., "Sucralose", "Saccharin", or "Placebo"), without indicating the randomisation order. These packages will then be handed over to the external personnel for relabelling.

3. Relabelling by External Personnel: The external personnel will relabel the three intervention packages for each participant as "First Intervention IP," "Second Intervention IP," and "Third Intervention IP" based on the assigned randomisation sequence. This relabelling process will be video-recorded to ensure accuracy, transparency, and traceability of the blinding procedure.

4. Product Return and Dispensing: After relabelling, the test products will be returned to the project coordinator, who will dispense them to participants according to the scheduled intervention timeline.

5. Blinding Maintenance and Unblinding: Both participants and research staff involved in data collection will remain blinded to the intervention allocation throughout the study. The randomisation code will be securely stored by the external personnel and only accessed in the event of a medical

emergency or after all interventions and data collection are completed, prior to data analysis. All investigational products (placebo, sucralose, and saccharin) will be encapsulated in identical opaque capsules to maintain blinding of participants and study personnel. Unblinding will only occur in medical emergencies where knowledge of the intervention is required for patient safety, upon request by the principal investigator. All unblinding events will be documented and reported to the ethics committee.

Data Collection Methods

Data and biospecimens will be collected at baseline and after each intervention phase as outlined in Table 1. Trained personnel will conduct all assessments using standardised procedures to ensure data quality, including duplicate measurements where applicable.

Table 1. Summary of Participant Visits

**Due to formatting, Table 1 is at the end of the page*

Anthropometric Data

Weight, height, BMI and waist circumference will be collected using calibrated digital scales and stadiometers, following standard protocols (27-29). Body composition will be assessed via bioelectrical impedance analysis (BIA) machine, with pre-measurement guidelines provided to ensure accuracy (30).

Blood Sample

Approximately 15 mL blood will be collected by a certified nurse or phlebotomist following a 30-minute rest. Analyses will include fasting glucose, insulin, HbA1c, lipid profile, kidney function, and insulin resistance (HOMA-IR). Samples will be handled and transported under controlled conditions, with archiving at –80°C for future research.

Blood Pressure

Blood pressure will be measured after a 5-minute rest, with two readings averaged per standard clinical guidelines.

Questionnaire

Sociodemographic and medical history will be collected using a structured questionnaire and cross-verified with medical records. Dietary intake will be assessed using a 3-day food records (two weekdays, one weekend), supported by photographic aids. Food records will be analysed based on Malaysian food composition data (31). Physical activity will be measured using the validated International Physical Activity Questionnaire–Short Form (IPAQ-SF), including the Malay version (IPAQ-M) where applicable (32, 33).

Stool Sample

Stool samples (~1 g) will be self-collected by participants using DNA/RNA Shield Fecal Collection Tubes from Zymo Research with detailed instructions and video guidance according to the product protocol (34). Samples will be collected pre- and post-intervention and stored at –20°C until analysis.

Gut Microbiota Analysis

Gut microbiota analysis will be conducted via 16S rRNA sequencing (V3–V4 region) using the Illumina MiSeq platform. DNA extraction will follow Promega's Maxwell® RSC protocol. Bioinformatics will be performed using QIIME 2 with DADA2 for denoising and SILVA 138 for taxonomic assignment (35).

Microbiome Analysis

Stool samples will be subjected to 16S rRNA gene sequencing targeting the V3–V4 region, following Illumina's 16S Metagenomic Sequencing protocol (36). Amplicons will be barcoded and pooled equimolarly before sequencing on the Illumina MiSeq platform (2 × 250 bp). Raw reads will be demultiplexed, quality filtered, and denoised using DADA2 in QIIME 2, generating amplicon sequence variants (ASVs) and filtering out chimeras(35, 37). Taxonomic assignment will be performed using a pre-trained classifier on the SILVA 16S rRNA database (35). Diversity analyses will be conducted to evaluate within-sample (alpha diversity: Shannon, Faith's PD) and between-sample (beta diversity: Bray–Curtis, UniFrac distances) variation (38-41). Differential abundance testing will be performed using appropriate statistical models (e.g., ANCOM-BC or MaAsLin2), with corrections for multiple comparisons (42). All downstream analyses will be guided by metadata and study endpoints, focusing on identifying microbial shifts following the intervention.

Archived Biospecimens for Future Research

Leftover blood and stool samples from each visit will be stored for future research. These biospecimens will be stored under appropriate biobanking conditions at –80°C in the designated institutional laboratory facilities. Potential analyses may include investigations into disease biomarkers, gene expression, or microbial functionality, contingent on funding and scientific relevance. If no approved study is initiated within seven years, samples will be disposed of in accordance with institutional biobank protocols and ethical standards.

Plans to Promote Participant Retention and Complete Follow-up

To enhance retention, the study coordinator will maintain regular contact with participants via phone calls or messaging applications (e.g., WhatsApp) to address concerns, provide reminders, and offer continuous support. Participants will be allowed flexible scheduling for their study visits, especially during washout periods, provided that a minimum 4-week washout interval is maintained. Clear instructions and culturally appropriate materials will be provided to encourage adherence and address potential barriers, such as hesitation toward NNS intake or stool sample collection. Reasons for withdrawal or non-compliance will be documented to aid in interpretation and transparency of findings.

Data Management

All data will be managed in accordance with the Malaysian Personal Data Protection Act (PDPA) 2010 and the Ministry of Health (MOH) Good Clinical Practice (GCP) Guidelines (43, 44). Data from physical forms will be entered into an electronic database by trained research personnel, with double data entry and cross-verification performed where feasible to promote accuracy. Range checks and logical validation rules will be applied during entry to identify out-of-range or inconsistent values. Each participant will be assigned a unique identification code to ensure that all data are de-identified prior to analysis.

Physical documents, such as consent forms and questionnaires, will be stored in locked filing cabinets at the study site, with access restricted to authorised personnel. Electronic datasets and scanned files will be kept on password-protected, encrypted institutional servers or secure cloud storage systems in compliance with institutional data governance policies, with regular backups performed to prevent data loss. Access to all data, whether physical or digital, will be limited to authorised members of the research team.

Upon study completion, data will be archived at the principal investigator’s institutional office for a minimum of seven years, in accordance with MOH requirements. After this period, all

identifiable data will be permanently destroyed using secure disposal methods. Archived biospecimens will be anonymised, and all handling will comply with institutional ethical guidelines. No data or samples will be shared without prior ethics approval and appropriate legal safeguards. Any future use of biospecimens will require additional ethics committee approval, and re-consent from participants will be obtained as necessary.

Statistical Methods

Clinical data will be expressed as mean ± standard deviation unless stated otherwise. Data will be analysed using IBM SPSS Statistics version 28.0 (45). Normality of continuous variables will be assessed using the Shapiro Wilk test. Participants' baseline characteristics will be summarised using descriptive statistics. Differences in glycaemic responses across the three study arms will be assessed using either one-way ANOVA or the Kruskal Wallis test, depending on data distribution. Changes in clinical variables before and after the intervention will be analysed using repeated-measures ANOVA, with time (pre vs post), group (intervention arm), and the group × time interaction as factors. Sociodemographic characteristics, dietary intake, and physical activity level will be considered as covariates where appropriate. A p-value of <0.05 will be considered statistically significant.

Analysis Populations and Handling of Missing Data

All randomised participants will be included in the primary analysis according to the intention-to-treat (ITT) principle, with participants analysed in the group to which they were originally assigned, regardless of subsequent adherence. For participants who withdraw or discontinue the intervention, data collected up to the point of withdrawal will be retained and included in analyses. A per-protocol (PP) analysis may also be conducted as a secondary approach, limited to participants who complete the study without major protocol deviations.

Missing data will be evaluated for both extent and mechanism (e.g., missing completely at random, missing at random, or not missing at random). For data assumed to be missing at random, simple imputation methods (e.g., mean substitution or last observation carried forward [LOCF]) may be applied for minimal missingness. In cases of more extensive or systematic missingness, multiple imputation will be considered, provided assumptions are met.

Sensitivity analyses will be performed to evaluate the impact of different missing data handling approaches on the robustness of study findings. All methods used for handling missing data will be reported transparently in the final analysis.

Methods for additional analyses

N/a. No additional analyses are currently planned beyond the primary and secondary objectives of the study. However, if exploratory analyses become relevant based on the data collected, such as potential subgroup trends or adjustments for covariates like age, sex, BMI, dietary intake, or physical activity, they may be conducted at the analysis stage. Any such analyses will be clearly identified as exploratory and interpreted with appropriate caution.

Data Monitoring Committee

A formal Data Monitoring Committee (DMC) will not be established, as this trial involves a short-term dietary intervention of seven days, using non-nutritive sweeteners with minimal known risk and non-invasive procedures. Oversight of participant safety, data integrity, and protocol adherence will instead be carried out by the research team, with the site investigator playing a key role in monitoring clinical procedures. Any adverse events or protocol deviations will be documented and reported according to institutional ethics requirements and standard operating procedures. Should unforeseen safety concerns arise, the study team will consult the ethics committee and relevant institutional authorities. No interim analyses or stopping guidelines are planned, as the study intervention is of short duration and low risk. All data will be analysed only after the intervention and final data collection are complete.

Trial Monitoring

Given the small scale and minimal risk of the trial, no formal external monitoring procedures will be implemented. Trial conduct will be monitored internally by the research team through routine supervisory meetings and periodic checks by the site investigator to ensure participant safety, accurate data collection, and adherence to the protocol.

Research Ethics Approval

Ethics approval was obtained from the Universiti Kebangsaan Malaysia Research Ethics Committee (UKM/PPI/111/8/JEP-2025-322) on 15/7/2025.

Plans for communicating important protocol modifications to relevant parties

This protocol is reported in accordance with SPIRIT guidelines (46). Any significant modifications to the study protocol will be promptly submitted for review and approval by the relevant Research

Ethics Committee. If approved, updates will also be made to trial registries as applicable. Participants will be informed of relevant changes that may affect their continued participation, and updated consent will be obtained when necessary. All protocol amendments will be documented and communicated to the study team to ensure consistency in implementation.

Informed consent

The study coordinator will obtain written informed consent from all participants prior to enrolment. Potential participants will first receive a verbal overview, followed by a private session where the Participant Information Sheet is explained in English or Malay. Only competent adults will be recruited; therefore, no surrogate consent or assent procedures are required. An additional optional consent form will be provided for the collection and future use of de-identified biological specimens in ancillary studies. Signed consent forms will be kept by the principal investigator and stored securely for seven years in accordance with institutional policy.

Confidentiality

Personal information (e.g., name, contact details, medical history) will be collected only as required for study purposes and stored separately from research data using unique identifiers. All research data will be de-identified before analysis. Access to identifiable information will be limited to authorised study personnel. Only de-identified, aggregated data will be used in publications or presentations.

Signed documents and personal records will be retained securely by the principal investigator in accordance with Malaysian PDPA 2010 and institutional policy (44). After the retention period, all identifiable records will be permanently destroyed.

Provisions for post-trial care

No formal post-trial follow-up is planned for participants who complete the study without adverse effects. However, any participant who reports an adverse event related to the investigational product will receive appropriate medical attention from the attending physician affiliated with the research team. All adverse events, including any occurring after the trial period, will be documented and managed in accordance with the study’s safety protocol and institutional ethical guidelines. Compensation for harm is not anticipated, as the interventions involve substances generally recognised as safe at the tested dosages.

Discussion

This study protocol addresses important and underexplored questions regarding the metabolic and microbiome effects of NNS in Malaysian adults with T2DM. While ethical and safety considerations have been outlined, several operational challenges require further discussion.

A key challenge is participant adherence, particularly given the use of NNS at the upper limits of the acceptable daily intake (ADI). Individual attitudes toward artificial sweeteners may vary, and behaviour in a free-living population is inherently unpredictable. To address this, participants will receive clear instructions, culturally appropriate educational materials, and regular reminders to promote accurate self-reporting and protocol compliance. Transparent communication will help build trust and encourage honest reporting of intake and adverse experiences.

Another limitation is the inability to fully control lifestyle behaviours, namely, diet and physical activity, which may confound glycaemic and microbiome outcomes. While participants will be asked to maintain their usual lifestyle, changes may occur over the study period. To mitigate this, dietary intake and physical activity will be assessed at each intervention phase using standardised recall methods. This allows for the tracking of major deviations that may influence outcome interpretation. At the same time, participant autonomy and voluntary participation is respected, and a pragmatic balance between study rigour and real-world feasibility is maintained.

Lastly, given the limited existing gut microbiome data from Malaysian T2DM populations, interpretation of microbial shifts must consider the influence of heterogeneous diets, genetics, and lifestyle patterns. Robust baseline data collection is therefore critical, both to support internal validity and to enable future comparisons with regional and international cohorts. By anticipating and addressing these context-specific challenges, this study aims to generate relevant insights into NNS effects in a Southeast Asian setting and inform the design of future human trials investigating metabolic microbiome interactions.

Trial status

This protocol is Version 1.0, dated 05 August 2025. Participant recruitment will commence on November 2025, and is expected to be completed by approximately May 2025.

Abbreviations

Type 2 Diabetes Mellitus (T2DM)

Non-nutritive sweeteners (NNS)

Body mass index (BMI)

Investigated product (IP)

References

1. Rother KI, Conway EM, Sylvetsky AC. How Non-nutritive Sweeteners Influence Hormones and Health. Trends Endocrinol Metab. 2018;29(7):455-67.
2. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 2014;514(7521):181-6.
3. Suez J, Cohen Y, Valdés-Mas R, Mor U, Dori-Bachash M, Federici S, et al. Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. Cell. 2022;185(18):3307-28.e19.
4. Méndez-García LA, Bueno-Hernández N, Cid-Soto MA, De León KL, Mendoza-Martínez VM, Espinosa-Flores AJ, et al. Ten-Week Sucralose Consumption Induces Gut Dysbiosis and Altered Glucose and Insulin Levels in Healthy Young Adults. Microorganisms. 2022;10(2).
5. Laforest-Lapointe I, Becker AB, Mandhane PJ, Turvey SE, Moraes TJ, Sears MR, et al. Maternal consumption of artificially sweetened beverages during pregnancy is associated with infant gut microbiota and metabolic modifications and increased infant body mass index. Gut Microbes. 2021;13(1):1-15.
6. Frankenfeld CL, Sikaroodi M, Lamb E, Shoemaker S, Gillevet PM. High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States. Ann Epidemiol. 2015;25(10):736-42.e4.
7. Ahmad SY, Friel J, Mackay D. The Effects of Non-Nutritive Artificial Sweeteners, Aspartame and Sucralose, on the Gut Microbiome in Healthy Adults: Secondary Outcomes of a Randomized Double-Blinded Crossover Clinical Trial. Nutrients. 2020;12(11).
8. Serrano J, Smith KR, Crouch AL, Sharma V, Yi F, Vargova V, et al. High-dose saccharin supplementation does not induce gut microbiota changes or glucose intolerance in healthy humans and mice. Microbiome. 2021;9(1):11.
9. Thomson P, Santibañez R, Aguirre C, Galgani JE, Garrido D. Short-term impact of sucralose consumption on the metabolic response and gut microbiome of healthy adults. Br J Nutr. 2019;122(8):856-62.
10. Bueno-Hernández N, Esquivel-Velázquez M, Alcántara-Suárez R, Gómez-Arauz AY, Espinosa-Flores AJ, de León-Barrera KL, et al. Chronic sucralose consumption induces elevation of serum insulin in young healthy adults: a randomized, double blind, controlled trial. Nutr J. 2020;19(1):32.
11. Dalenberg JR, Patel BP, Denis R, Veldhuizen MG, Nakamura Y, Vinke PC, et al. Short-Term Consumption of Sucralose with, but Not without, Carbohydrate Impairs Neural and Metabolic Sensitivity to Sugar in Humans. Cell Metab. 2020;31(3):493-502.e7.
12. Lertrit A, Srimachai S, Saetung S, Chanprasertyothin S, Chailurkit LO, Areevut C, et al. Effects of sucralose on insulin and glucagon-like peptide-1 secretion in healthy subjects: a randomized, double-blind, placebo-controlled trial. Nutrition. 2018;55-56:125-30.
13. Romo-Romo A, Aguilar-Salinas CA, Brito-Córdova GX, Gómez-Díaz RA, Almeda-Valdes P. Sucralose decreases insulin sensitivity in healthy subjects: a randomized controlled trial. Am J Clin Nutr. 2018;108(3):485-91.
14. (EFSA) TEFSA. Sweeteners 2025 July 4 [cited 2025 Aug 18]. Available from: https://www.efsa.europa.eu/en/topics/topic/sweeteners.
15. (FDA) USFaDA. Aspartame and Other Sweeteners in Food 2025 [cited 2025 Aug 18]. Available from: https://www.fda.gov/food/food-additives-petitions/aspartame-and-other-sweeteners-food.
16. (JECFA) EotJFWECoFA. SUCRALOSE: World Health Organization (WHO); [cited 2025 Aug 18]. Available from: https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/2340.
17. (JECFA) EotJFWECoFA. SACCHARIN [cited 2025 Aug 18]. Available from: https://apps.who.int/food-additives-contaminants-jecfa-database/Home/Chemical/3164.
18. Chong S, Lin M, Chong D, Jensen S, Lau NS. A systematic review on gut microbiota in type 2 diabetes mellitus. Front Endocrinol (Lausanne). 2024;15:1486793.
19. Wen X, Qi LM, Zhao K. Influence of gut bacteria on type 2 diabetes: Mechanisms and therapeutic strategy. World J Diabetes. 2025;16(1):100376.
20. Federation. ID. Malaysia Brussels, Belgium: International Diabetes Federation; [cited 2025 Aug 12]. Available from: https://idf.org/our-network/regions-and-members/western-pacific/members/malaysia/.

21. (MDA) MDA. Medical Nutrition Therapy Guidelines for Type 2 Diabetes Mellitus.; 2013.
22. Fritz K TK, Parmar M. Calcium Carbonate. Treasure Island (FL): StatPearls Publishing; 2023 [cited 2025 2025 Aug 4]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK562303/.
23. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.
24. Xie Y, Gou L, Peng M, Zheng J, Chen L. Effects of soluble fiber supplementation on glycemic control in adults with type 2 diabetes mellitus: A systematic review and meta-analysis of randomized controlled trials. Clin Nutr. 2021;40(4):1800-10.
25. Faul F, Erdfelder E, Buchner A, Lang A-G. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. Behavior Research Methods. 2009;41(4):1149-60.
26. Urbaniak GC PS. Research Randomizer. Version 4.0 ed2013.
27. Centre NIoHRNSBR. Procedure for measuring adult height Southampton: NIHR Southampton Biomedical Research Centre; [Available from: https://www.uhs.nhs.uk/Media/Southampton-Clinical-Research/Procedures/BRCProcedures/Procedure-for-adult-height.pdf.
28. Centre NIoHRNSBR. Procedure for measuring adult weight Southampton: NIHR Southampton Biomedical Research Centre; [Available from: https://www.southamptonbrc.nihr.ac.uk/_files/ugd/178f68_ca76432d55fa4024a769e845860b2d21.pdf.
29. Organization WH. Waist circumference and waist–hip ratio: report of a WHO expert consultation. Geneva: World Health Organization; 2011.
30. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Manuel Gómez J, et al. Bioelectrical impedance analysis-part II: utilization in clinical practice. Clin Nutr. 2004;23(6):1430-53.
31. Malaysian Food Composition Database (MyFCD) [Internet]. 2015. Available from: https://myfcd.moh.gov.my/index.html.
32. Chu AH, Moy FM. Reliability and validity of the Malay International Physical Activity Questionnaire (IPAQ-M) among a Malay population in Malaysia. Asia Pac J Public Health. 2015;27(2):Np2381-9.
33. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95.
34. Corporation ZR. DNA/RNA Shield Fecal Collection Tube Irvine, CA: Zymo Research; [Available from: https://www.zymoresearch.com/products/dna-rna-shield-fecal-collection-tube.
35. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology. 2019;37(8):852-7.
36. Illumina. 16S Metagenomic Sequencing Library Preparation San Diego (CA): Illumina; 2013 [cited 2025 Aug 4]. Available from: https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf.
37. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581-3.
38. Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. Ecological monographs. 1957;27(4):326-49.
39. Faith DP. Conservation evaluation and phylogenetic diversity. Biological conservation. 1992;61(1):1-10.
40. Lozupone C, Lladser ME, Knights D, Stombaugh J, Knight R. UniFrac: an effective distance metric for microbial community comparison. Isme j. 2011;5(2):169-72.
41. Shannon CE. A mathematical theory of communication. ACM SIGMOBILE mobile computing and communications review. 2001;5(1):3-55.
42. Mallick H, Rahnavard A, McIver LJ, Ma S, Zhang Y, Nguyen LH, et al. Multivariable association discovery in population-scale meta-omics studies. PLoS computational biology. 2021;17(11):e1009442.
43. (NCCR) NCfCR. Malaysian Guideline for Good Clinical Practice. ed. t, editor. Petaling Jaya, Selangor, Malaysia: National Pharmaceutical Regulatory Agency (NPRA), Ministry of Health Malaysia; 2020.
44. Malaysia TCoPDP. Personal Data Protection Act 2010 (Act 709). Kuala Lumpur2010.
45. Corp I. IBM SPSS Statistics for Macintosh Version 28.0. Armonk, NY: IBM Corp; 2021.
46. Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013

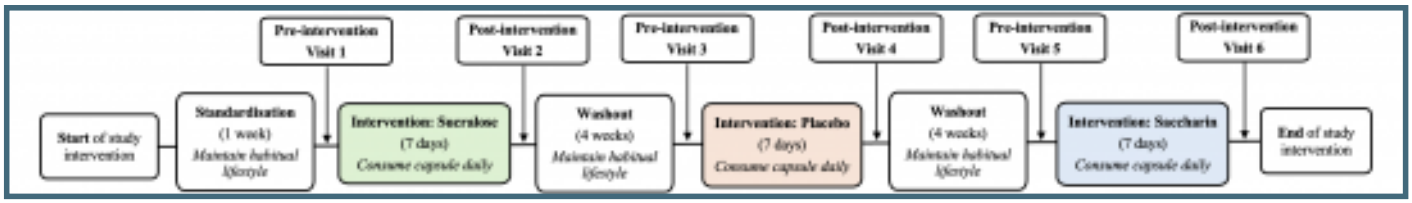
explanation and elaboration: guidance for protocols of clinical trials. Bmj. 2013;346.

Table 1. Summary of Participant Visits

	Screening	Intervention 1		Intervention 2		Intervention 3	
Visit		1	2	3	4	5	6
	Anthropometry						
Weight	X	X		X		X	
Height	X						
Waist Circumference		X		X		X	
Body Composition		X		X		X	
Blood pressure		X		X		X	
	Biological Specimens						
Blood		X	X	X	X	X	X
Stool		X	X	X	X	X	X
	Questionnaire						
Sociodemographic & medical history	X						
Medication log	X	X		X		X	
Dietary record	X	X		X		X	
Physical Activity log	X	X		X		X	
Test product compliance record			X		X		X
Adverse Effect Reports			X		X		X

Supplementary Files

Figure 1. Overview of the Study Visits.



Multimedia Appendixes

Peer-Review Reports.
URL: http://asset.jmir.pub/assets/f9a6ea7338d47a4261c1085be4256bc8.pdf

CONSORT (or other) checklists

SPIRIT 2025 checklist for protocol paper.
URL: http://asset.jmir.pub/assets/1e86f602863d2342a2f3e26336a3ca5a.pdf

Related publication(s) - for reviewers eyes onlies

UKM ethic approval.
URL: http://asset.jmir.pub/assets/3d8e7f3c7595612e581d7989c0723b70.pdf