Study Protocol and Statistical Analysis Plan

Development of a Novel Asian Mediterranean Diet and Its Acceptability in NAFLD

02 Feb 2024

Associate Professor Verena Tan Ming Hui

SINGAPORE INSTITUTE OF TECHNOLOGY 10 Dover Drive, Singapore 138683

Overview

This will be the first study that aims to develop an Asian version of the clinically beneficial Mediterranean diet using ingredients commonly consumed in the Asian diet. With the developed AMD, we then aim to evaluate its acceptability among subjects with NAFLD. Despite numerous studies conducted using the Mediterranean diet in NAFLD, there is a paucity of information of the acceptability of Mediterranean diet among Asian patients with NAFLD. This can be attributed to the cultural differences in dietary habits between the western Mediterranean diet and the Asian cuisine. This study aims to reconstruct the traditional Mediterranean diet using Asian recipes and food preparation techniques and evaluate its acceptability in Asian subjects with NAFLD.

Study Protocol

There will be 2 phases to this study:

Phase 1 – Asian Mediterranean Diet Development

This phase of the study involves desktop research and recipe trials in SIT, and does not require IRB review as no human subjects are involved. We will ascertain the nutritional composition of the traditional Mediterranean diet by conducting a comprehensive literature review. This review will help us map out the portion sizes of foods commonly consumed in Mediterranean countries that adhere closely to this diet. Following this, we will identify substitute ingredients that are frequently consumed in the Asian diet. Subsequently, we will review the polyphenol studies and match the polyphenol content of both Mediterranean to Asian ingredients. The serving sizes of these ingredients will be determined based on this mapping. Using this information, we will develop a 4-week cycle menu for an Asian Mediterranean diet.

Phase 2 – Sensory Evaluation Study

We will collaborate with a food catering company with SFA license to produce the recipes for 1 of the weeks in the 4-week cycle menu. Recipe trials will be conducted by the food company. Finalized recipes will be packaged as ready-to-eat meals for use in Phase 2 of our study.

Study Population

We plan to recruit 30 adults diagnosed with NAFLD from the Department of Hepatology in Singapore General Hospital.

Inclusion criteria include:

- Diagnosis of NAFLD, as determined clinically by imaging evidence of hepatic steatosis on ultrasound/CT/MRI.
- Aged from 21 to 60 years old.

Exclusion criteria include:

- Secondary causes of NAFLD (e.g. medication-induced), unstable body weight (variation >5% within the preceding 3 months),
- Current use of weight loss medications,
- Other liver diseases (viral hepatitis, auto-immune or cholestatic liver disease, Wilson's disease, hemochromatosis, alpha-1 anti trypsin deficiency),
- Unstable diabetes (HbA1c >8.5%),
- Renal failure,
- Inability to provide informed consent,
- Pregnancy and lactation,
- Problems with tasting and smelling,
- Food allergy/intolerances/restrictions.

Study Visits Procedure

All eligible subjects will be given a participant information sheet and a consent form if they wish to participate in the study. The PI will also be available for any clarifications either through phone call or via email. With permission from the subjects, the SIT research team will message them to remind subjects of the requirements before coming for their study visits. Anthropometric data (weight, height, body mass index, waist circumference) and body composition using bioelectrical impedance will be assessed.

Recruited subjects will be invited to SIT@Dover campus tentatively in the months of Mar 2024 to Dec 2024 for two sessions of sensory evaluation test on the acceptability of the Asian Mediterranean diet (AMD) sample meals. All the ready meals will be heated up to

the required temperature before consumption. Subjects will taste a total of 14 different food items, over 2 study visits. This is to minimize taste fatigue if more than 5-7 samples are tasted at each study visit. After tasting the food items, subjects will be required to evaluate the food items tasted. A sensory evaluation form will be provided to the subjects. Subjects are required to complete the form before leaving the food tasting lab. The duration of each session is expected to take 60 minutes. Reimbursement will be given upon the completion of both study visits.

Sensory evaluation food tasting preparatory steps will involve:

1. All ready meals will be reheated to the appropriate consumption temperature when the subject arrives at SIT's food-tasting lab.
2. All trays, crockeries and cutleries will be provided. Environment settings will be standardised throughout all sessions.
3. Subject verification and registration will be carried out before subjects enter the food-tasting lab.
4. Before the commencement of the food-tasting session, subjects will be briefed on the food-tasting procedure and instructions.
5. A total of 7 food items will be served to participants and 50g of each food product will be portioned out. A pictorial illustration of the actual ready meal will be provided to the subject.
6. Subjects will be required to fill up the online food-tasting evaluation forms.
7. Throughout the tasting session, subjects will not be allowed to communicate with other subjects in the food-tasting lab.
8. Subjects will leave the food-tasting lab when they have finished evaluating all the food items.
9. Trays, crockeries, and cutleries will be collected back.

Statistical Analysis Plan

As we are piloting the novel Asian Mediterranean diet, the primary objective is to obtain comments and feedback on the acceptability of the ready meals from NAFLD patients. The sensory data from this study were analysed using a Kruskal–Wallis test, and a one-

way analysis of variance (ANOVA) was used with significant differences detected for $p < 0.05$.

Information about liver enzymes (ALT, AST, Albumin, bilirubin, alkaline phosphatase, and GGT), platelet count, and lipid profile (total cholesterol, LDL, HDL, TG) will be obtained from the subjects' medical records using the most recent laboratory data. Subjects will be required to complete a semi-quantitative food frequency questionnaire (FFQ) that includes foods and beverages commonly consumed in Singapore. The aim is to assess subjects' usual dietary intake over the past 6 months and evaluate their baseline dietary intake and adherence to the Mediterranean Diet.

Data collected by collaborators will be anonymized. Recruited participants will be de-identified through a subject ID to ensure the responses are kept anonymous. Participants' identifiable information like names, contact numbers, emails, gender, race, and age will be collected and stored securely in an encrypted password-protected file. Identity of participants will be kept in a separate file from the research data. Only the study investigators will have access to this file. The files containing all research data will be password-protected and stored in project PDDS folder. Access to data will be restricted to only authorised people who are involved in this study. The data will be retained in SIT for 10 years in line with SIT Research Data Management Policy Version 1.1.