Indonesia University

RESEACRH PROTOCOL

The Mediterranean Diet Based on Local Foods for Obese Patients: A Study of Anthropometric Parameters, Inflammatory Markers, and Gut Microbiota

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Table of Contents

Protocol Summary

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Protocol registration number
25-09-1322
Principal Investigator
dr. Diyah Eka Andayani, M.Gizi, Sp.GK (K)
Background and Rationale
Obesity is a global problem with incidents increasing every year and estimated to reach 21% by 2025. Based on data from the 2023 Indonesian Health Survey, the prevalence of obesity in Indonesia is 23.4%. This prevalence is highest in the Jakarta, at 31.8%. In addition, a report on health examinations at the Indonesia University found that 16.2% (50/321) of employees were obese. The modern diet-includes fast food, sweet, and fatty foods-is associated with metabolic diseases such as hypertension, glucose intolerance, and obesity. Weight loss in obesity can be achieved through behavioral modification, such as improving dietary patterns and increasing physical activity. Weight loss in the early stages can help predict the success of long-term weight loss management. The Mediterranean diet is one diet that can be used to reduce the risk of cardiovascular disease, but some studies show that the Mediterranean diet has a positive impact on weight loss in obese patients. The Mediterranean diet includes a plant-based foods, with olive oil as the main source of additional fat, as well as high intake of fish and seafood, moderate consumption of eggs, poultry, and dairy products (cheese and yogurt), and low consumption of red meat. In addition, the Mediterranean diet also includes activity management. Compared to other diets, the Mediterranean diet shows a decrease in BMI and weight, HbA1c levels, fasting blood sugar, fasting insulin, inflammatory markers, and can improve gut microbiota dysbiosis. The implementation of the Mediterranean diet often faces challenges related to cultural factors and the availability of local food ingredients. Considering the potential of the Mediterranean diet in modulating gut microbiota and metabolic status, as well as the challenges of its adaptation in Indonesia, this study aims to examine the effectiveness of a Mediterranean diet based on local Indonesian foods on changes in gut microbiota, body composition, and metabolic biomarkers in obese.
Study Objectives
To examine the efficacy of a Mediterranean diet adapted to local Indonesian foods on changes in gut microbiota, body composition, and metabolic biomarkers in obese individuals.

Study Design

This study will be conducted using a randomized, single-blind, clinical trial design. The intervention group consists of obese patients who receive dietary education and a Mediterranean diet adapted to local Indonesian foods. The control group is composed of obese patients who will receive low calorie diet and dietary education.

Participant Population

The inclusion criteria

1. Women who were obese, as defined by a BMI of at least 27.
2. Aged 18-50 years
3. Subjects with a non-shift work schedule
4. Willing to follow the research procedures and sign the informed consent form.

The exclusion criteria

1. Subjects with a history of type 2 diabetes, kidney disease, liver disease, or thyroid disorders, as indicated by medical history or medical records.
2. Contraindications for the MF-BIA examination include the use of pacemakers or implants and a history of amputation.
3. Pregnant or within 40 days of childbirth
4. Adults with edema
5. Taking medications with hyperglycemic effects, such as beta-blockers, thiazides, corticosteroids, calcineurin inhibitors, anti-dyslipidemic medications (e.g., statins, fibrates, niacin, and bile acid sequestrants), or antibiotics.
6. History of food allergies

Treatment of participants

The research subjects received diet around 1500 calories according to their randomized groups, consisting of breakfast, lunch, dinner, and snacks.

- Arm 1: The intervention group provide the composition was 50% carbohydrates, 30-34% fat (SFA ≤8%, MUFA 15-20%, PUFA 5-10%), and 17-20% protein.
- Arm 2: The control group receive 50-54% carbohydrates, 25-30% fat (SFA <12, MUFA 8-12%, PUFA 5-8%), 19-20% protein. The researchers developed the diet composition supplied to the catering service. Then, the catering service translated these compositions into meals.

Study Procedur

Screening, Randomisation and Follow-up

At screening: informed consent; Demographics (year of birth, race and gender at birth); allergies and medical history.

At washout period: participants were not permitted to consume foods or beverages containing probiotics or prebiotics (such as yogurt), weight-loss medications, or fiber supplements.

Follow up visit: Participants received meals according to their assigned group allocation. They were required to consume the meals within 15–20 minutes and reported to researcher every meal time.
At week 0 and week 4 examination: anthropometric measures (BMI, WHR), body composition parameters (fat mass, fat-free mass, visceral fat), IL-6 levels, I-FABP levels, the TyG index, and gut microbiota composition.

Statistical consideration

- Univariate Analysis: Ratio scales (age, BMI) and dependent variables (anthropometry, FFM, FM, visceral fat, TyG index, IL-6 levels, gut microbiota diversity, I-FABP levels) are presented with a normal distribution in the form of mean±SD, but if the data distribution is not normal, it is presented in the form of median (minimum–maximum values). Data normality was assessed using the coefficient of variation (CoV) and the Kruskal-Wallis test.
- Bivariate Analysis: If dependent variables were normally distributed, an unpaired t-test was performed. However, if the dependent variable data are not normally distributed (changes in all variables studied between the intervention group and the control group), the Mann-Whitney test will be performed. Analysis of the difference in the mean of the dependent variable data between baseline and week 4 of the study: if the data distribution is normal, a t-test will be performed, but if the data distribution is not normal, a Wilcoxon test will be performed.
- Multivariate Analysis: Linear regression test to control for confounding variables. Bivariate tests are required (if $p < 0.25$) and statistical tests are considered significant if the p-value is <0.05. Linear mixed model analysis is used to analyze the relationship between insulin resistance variables (TyG index) and changes in I-FABP or microbiota composition. Statistical tests are considered significant if the p-value is <0.001.

1. Background and rationale

Obesity is a global problem, with cases increasing each year and projected to reach 21% by 2025. Based on the 2023 Indonesian Health Survey, obesity prevalence in Indonesia is 23.4%, with the highest prevalence (31.8%) recorded in the Jakarta. Additionally, Indonesia University health examination report found that 16.2% (50 out of 321) of employees were obese. A study reported that obese female employees at Cipto Mangunkusumo Hospital (RSCM) had a mean BMI of 30.67 ± 4.91. Dietary patterns high in fast food, sweets and fatty foods are associated with increased risk factors for metabolic diseases, including hypertension, glucose intolerance, and obesity. Obesity can be addressed through behavioral modifications, including dietary improvement and increased physical activity. Early weight loss may serve as a predictor of long-term weight loss success.

Adherence to the Mediterranean diet has been associated with a reduced risk of cardiovascular disease and has demonstrated beneficial effects on weight loss among individuals with obesity. The Mediterranean diet is characterized by a high intake of plant-based foods, the use of olive oil as the main source of dietary fat, moderate consumption of fish, seafood, eggs, poultry, and dairy products, and limited intake of red meat. Furthermore, it integrates lifestyle behaviors such as regular physical activity. Compared with other dietary patterns, the Mediterranean diet is associated with reductions in BMI, body weight, HbA1c levels, fasting blood glucose, fasting insulin, and inflammatory markers, while also improving gut microbiota dysbiosis.

Gut microbiota is an important factor in obesity, as the diversity of its composition and metabolic activity is closely associated with nutritional intake, dietary patterns, and obesity. Gut microbiota dysbiosis is one of the underlying contributors to obesity-related susceptibility to chronic inflammation. Another condition is adipose tissue stimulates the release of proinflammatory adipokines—such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and resistin—which contribute to chronic inflammation.

Implementing the Mediterranean diet can be challenging due to cultural factors and the availability of local ingredients. Mediterranean diet can modulate gut microbiota and metabolic status and the challenges of adapting it in Indonesia, this study aims to evaluate the effectiveness of a Mediterranean diet based on local Indonesian foods in altering gut microbiota, body composition, and metabolic biomarkers in obese.

2. Hypothesis

The provision of a Mediterranean diet modification based on local food ingredients can result in greater weight loss, greater reductions in inflammatory markers, improve gut microbiota diversity compared to a balanced low-calorie diet in obese patients.

3. Study Objectives

General Objective

The implementation of a Mediterranean diet modified using local foods has the potential to improve anthropometric outcomes, inflammatory markers, and gut microbiota profiles in obese individuals, thereby providing a reference for dietary selection in obesity treatment.

Specific Objectives

- To determine the characteristics of the research subjects based on age, underlying disease, nutritional status, body composition, intake patterns, and physical activity.
- To determine the energy, protein, fat, and carbohydrate intake of research subjects before and after diet administration.
- To determine the effect of a Mediterranean diet based on local foods on the anthropometry of obese patients.
- To determine the anthropometric data (weight, height, and waist circumference), nutritional status, and body composition of research subjects before and after diet administration.
- To determine the differences in the mean anthropometry (weight, height, and waist circumference), nutritional status, and body composition of the research subjects in each group.

- To determine the effect of a modified Mediterranean diet based on local food ingredients on inflammatory marker values in obese patients.
- To determine changes in IL-6, I-FABP, and TyG index levels in research subjects before and after diet administration.
- To determine the differences in the mean levels of IL-6, I-FABP, and TyG index of the research subjects in each group.
- To determine the effect of a Mediterranean-based diet modification using local food ingredients on improving gut microbiota diversity and metabolomic profile in obese patients.
- To determine the gut microbiota diversity and metabolomic profile of the study subjects before and after the diet intervention.

4. Study Design

This study will be conducted using a single-blind randomized clinical trial to evaluate the effects of a locally adapted Mediterranean diet intervention on anthropometric measures (BMI, WHR), body composition parameters (fat mass, fat-free mass, visceral fat), IL-6 levels, I-FABP levels, the TyG index, and gut microbiota composition. The study will be conducted at the Nutrition Clinic of Cipto Mangunkusumo Hospital (RSCM) and the Faculty of Medicine, Indonesia University (FKUI), Jakarta. Analyses of gut microbiota diversity and metabolomics will be performed at the Department of Chemistry, FKUI, and at the Indonesian Medical Education and Research Institute (IMERI), FKUI Occupational and Bioinformatics Cluster. The study is scheduled to take place between 2025 and 2026, following approval from the Ethics Committee FKUI–RSCM.

5. Participant Population

Study population

a. Target Population

The target population of this study includes all obese patients living in Jakarta, Depok, and surrounding areas.

b. Accessible Population

The accessible population of this study consists of obese patients who are employees of FKUI and RSCM and are treated at the Nutrition Clinic.

c. Research Subjects

The research subjects are part of the accessible population selected based on the established inclusion and exclusion criteria.

a. The inclusion criteria
   - Women who were obese, as defined by a BMI of at least 27.
   - Aged 18-50 years
   - Subjects with a non-shift work schedule
   - Willing to follow the research procedures and sign the informed consent form.

b. The exclusion criteria
   - Subjects with a history of type 2 diabetes, kidney disease, liver disease, or thyroid disorders, as indicated by medical history or medical records.
   - Contraindications for the MF-BIA examination include the use of pacemakers or implants and a history of amputation.
   - Pregnant or within 40 days of childbirth
   - Adults with edema
   - Taking medications with hyperglycemic effects, such as beta-blockers, thiazides, corticosteroids, calcineurin inhibitors, anti-dyslipidemic medications (e.g., statins, fibrates, niacin, and bile acid sequestrants), or antibiotics.
   - History of food allergies

c. The DO criteria for this study are:

- If severe side effects occur
- The subject decides to withdraw from the study
- The subject does not comply with the established research protocol.

6. Treatment of Participant

Participants received a low-calorie diet of approximately 1.500 kcal/day according to their randomized group assignments, consisting of breakfast, lunch, dinner, and snacks. In the intervention group, the macronutrient distribution was 50% carbohydrates, 30–34% fat (SFA ≤ 8%, MUFA 15–20%, PUFA 5–10%), and 17–20% protein. In the control group, the diet provided 50–54% carbohydrates, 25–30% fat (SFA < 12%, MUFA 8–12%, PUFA 5–8%), and 19–20% protein.

Table 1. Diet formulation and composition in the intervention group and control group

Composition	**Intervention Group (%)**	**Control group (%)**
Protein	17-20	19-20
Carbohydrates	50	50-54
Fat	30-34	25-30
SFA	≤8	<12
MUFA	15-20	8-12
PUFA	5-10	5-8

The diets for both groups will be prepared by a certified diet catering service under the supervision of a clinical nutritionist. This catering service meets established certification standards and routinely prepares meals for individuals with special nutritional needs, including obesity. The dietary composition will be designed by the researchers and subsequently translated into meal plans by the catering service for both study groups. Details of the diet will be known only to the catering service and the research team to maintain blinding. Meals for both groups will be provided by the catering service from

Monday to Saturday, while on Sundays, dietary intake for both groups will be supervised directly by the researchers. In addition, participants will receive education on hunger management and will be permitted to consume additional foods that do not alter the core composition of the Mediterranean diet, such as antioxidant-rich foods, vegetables, and fruits.

Both groups will receive dietary education and physical activity guidance tailored to their weight loss needs. The dietary education includes instructions that participants are not permitted to consume foods, beverages, or supplements outside the provided catering menu without the researchers' knowledge. The prescribed physical activities consist of aerobic exercises, such as brisk walking, swimming, cycling, jogging, and light aerobic workouts, performed 3–5 times per week, with each session lasting 30–45 minutes.

7. Study Procedures

Initial screening period.

Data collection commenced after approval was obtained from the Ethics Committee FKUI–RSCM and research permission was granted by the RSCM Research Department. Prospective participants were interviewed to collect baseline characteristics using a screening questionnaire. Clinical assessments conducted during the screening phase included: informed consent; Demographics (year of birth, race and gender at birth); allergies and medical history.

Randomisation and wash out

To proceed to the randomization and washout phases, all participants were required to meet the eligibility criteria based on screening evaluation results. Clinical assessments conducted during the randomization and washout phases included anthropometric measurements, collection of 10 mL blood samples, and collection of 250 mg fecal samples. Analyses of gut microbiota diversity and metabolomics were performed at the Department of Chemistry FKUI, and at the Indonesian Medical Education and Research Institute (IMERI), FKUI Occupational and Bioinformatics Cluster.

A 7-day washout period was implemented. During this period, participants were not permitted to consume foods or beverages containing probiotics or prebiotics (such as

yogurt), weight-loss medications, or fiber supplements. Participants were instructed to perform light to moderate aerobic physical activity. Dietary intake during the washout period was assessed using food recall.

Follow Up Visits

Participants received meals according to their assigned group allocation. They were required to consume the meals within 15–20 minutes at the agreed mealtime. Participants were instructed to complete a food intake record and report their consumption to the researchers using intake forms and photographs of the food packaging. Weekly meetings were held via Zoom to evaluate dietary compliance, and participants were monitored daily through a WhatsApp group, with compliance verified using photographic evidence. Blood samples and fecal specimens were collected at baseline (week 0) and at week 4 of the study.

At week 0 and week 4 examination: anthropometric measures (BMI, WHR), body composition parameters (fat mass, fat-free mass, visceral fat), IL-6 levels, I-FABP levels, the TyG index, and gut microbiota composition.

8. Clinical and Toxicity Management Guideline

The Mediterranean diet has been widely consumed and recommended for individuals at risk of cardiovascular disease and obesity, with no reported adverse effects to date, provided that individuals are not allergic to the ingredients used. Potential risks associated with blood sample collection include pain, bruising, swelling, or infection at the venipuncture site. These risks will be minimized by using small-gauge needles, ensuring that blood is drawn by trained personnel, and implementing appropriate infection prevention measures prior to sample collection.

9. Adverse Event Recoding and Reporting

The researchers collaborated with food providers that met Hazard Analysis and Critical Control Points (HACCP) standards and operated under the supervision of a clinical nutritionist. In the event that any adverse conditions arose as a result of the provided food,

participants were instructed to immediately contact the researchers, who would facilitate access to appropriate medical care.

Timely and complete reporting of all adverse events (AEs) helps to identify any adverse medical occurrences, thereby enabling:

- Protection of the safety of study participants
- A better understanding of the overall safety profile of the study intervention
- Recognising food-related allergies or toxicities
- Appropriate modification of study protocols
- Improvements in study design or procedures
- Adherence to regulatory requirements

10. Biological samples

Blood and Feces Sample Collection Procedure

When blood is drawn, the subject must sit for at least 5 minutes before sampling. Blood sampling is performed on the anterior cubital vein, sterilizing the area with an alcohol swab. The tourniquet should not be applied for more than 2 minutes. 10 ml of blood is drawn and placed in a red vacutainer. Collected blood must be labeled with the patient's identity, date of collection (format DD/MMM/YYYY), and time of collection (24-hour format). Samples must arrive at the laboratory within 3 hours of blood collection (the time required for centrifugation of storage samples and biochemical and lipid testing within 4 hours of blood collection). All samples must be stored and transported from the local site at room temperature (20°C-25°C) to the responsible laboratory.

Fecal samples were collected by subjects at home in the morning using sterile containers provided. Instructions for collection and storage were provided in writing. After collection, samples were immediately stored in a coolbox with dry ice, then frozen at -80°C in the laboratory to prevent microbial DNA degradation. Collected feces must be labeled with the patient's identity, date of collection (DD/MMM/YYYY format), and time of collection (24-hour format). Collected stool samples must be immediately stored at a low temperature,

usually -80°C, to prevent metabolite degradation. In addition, stool can be stored at 4°C in an anaerobic atmosphere for less than 24 hours, and can be stored at -20°C for several days (anaerobic atmosphere or ambient environment).

Processing of Samples

The TyG index was calculated based on fasting blood glucose and triglyceride levels obtained from venous blood serum samples. Fasting glucose and triglyceride concentrations were measured using the glycerol phosphate oxidase–phenol aminophenazone (GPO–PAP) enzymatic method with a clinical spectrophotometer, in accordance with standard laboratory protocols.

Interleukin-6 (IL-6) levels were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) method with a Human IL-6 ELISA Kit. Absorbance was read at a wavelength of 450 nm using a microplate reader.

Intestinal fatty acid–binding protein (I-FABP) levels were determined using a Human I-FABP ELISA Kit based on the sandwich ELISA method, following the manufacturer's protocol.

Metabolomic profile analysis was performed using the Shimadzu GCMS-QP2020 NX system.

Gut microbiota diversity was assessed using the QIAGEN QIAamp PowerFecal Pro DNA Kit. All procedures were conducted according to the manufacturer's instructions. DNA concentration and purity were evaluated using a NanoDrop spectrophotometer and agarose gel electrophoresis.

11. Analysis Plan

Univariate Analysis: Ratio scales (age, BMI) and dependent variables (anthropometry, FFM, FM, visceral fat, TyG index, IL-6 levels, gut microbiota diversity, I-FABP levels) are presented with a normal distribution in the form of mean±SD, but if the data distribution is not normal, it is presented in the form of median (minimum–maximum values). Data normality was assessed using the coefficient of variation (CoV) and the Kruskal-Wallis test for normality.

Bivariate Analysis: If dependent variables were normally distributed, an unpaired t-test was performed. However, if the dependent variable data are not normally distributed (changes

in all variables studied between the intervention group and the control group), the Mann-Whitney test will be performed. Analysis of the difference in the mean of the dependent variable data between baseline and week 4 of the study: if the data distribution is normal, a t-test will be performed, but if the data distribution is not normal, a Wilcoxon test will be performed.

Multivariate Analysis: Linear regression test to control for confounding variables. Bivariate tests are required (if $p < 0.25$) and statistical tests are considered significant if the p-value is <0.05. Linear mixed model analysis is used to analyze the relationship between insulin resistance variables (TyG index) and changes in I-FABP or microbiota composition. Statistical tests are considered significant if the p-value is <0.001.

12. Data Collection, Source Documents, Record Retention, and Confidentiality of Data

The Principal Investigator or designee is responsible for preparing and maintaining adequate and accurate data pertinent to the investigation on each individual in the study. All data collected in this study will be kept confidential. Data processing and storage will only record identities in coded form. Furthermore, the presentation of research results at scientific meetings/conferences and publications in scientific journals will not include the names of research subjects. Soft files will be stored in password-protected folders, while hard files will be stored in locked research cabinets. Only the research team will have access to research documents.

13. Ethics Committee/Regulatory Approval and Informed Consent

This study will be conducted in compliance with the ethical principles outlined in the National Statement on Ethical Conduct in Research Involving Humans and the Declaration of Helsinki, and in accordance with Good Clinical Practice (GCP) guidelines and all applicable regulatory requirements. The study will be reviewed by the relevant local ethics committees, in accordance with current local guidelines.

Informed consent and procedures

The principal investigator will prepare the informed consent which must: include all elements required by ICH-GCP; all applicable regulatory requirements; and adhere to the ethical principles that have their origin in the Declaration of Helsinki.

The Principal Investigator is responsible for ensuring that participants, or their legally authorized representatives (LARs), receive comprehensive and clearly communicated information regarding the study objectives, potential risks, and all other relevant aspects of the clinical trial.

14. Financing and Insurance

Research funding is fully covered by the Researcher.

15. Quality Control and Quality Assurane

For implementing and maintaining quality control and quality assurance systems with written standard operating procedures to ensure the study is conducted and data are generated, documented and reported in compliance with the protocol, Good Clinical Practice standards and all applicable local laws and regulations relating to the conduct of a clinical study.

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Abbervation List

BMI : Body Mass Index

DNA : Deoxyribonucleic Acid

ELISA : Enzyme-Linked Immunosorbent Assay

FFM : Fat-Free Mass

FM : Fat Mass

I-FABP : Intestinal Fatty Acid Binding Protein

IL-6 : Interleukin-6

TyG : Triglyceride-Glucose Index

MCP-1 : Monocyte Chemoattractant Protein-1

MF-BIA: Multi-frequency Bioelectrical Impedance Analysis

MUFA : Monounsaturated Fatty Acids

PUFA : Polyunsaturated Fatty Acid

RSCM : Cipto Mangunkusumo Hospital

SFA : Saturated Fatty Acid

TNF-α : Tumor Necrosis Factor Alpha

WHO : World Health Organization

WHR : Waist-to-Hip Ratio

PARTICIPANT INFORMATION AND CONSENT FORM

I am dr. Diyah Eka Andayani, M.Gizi, SpGK(K), from the Department of Nutrition, Faculty of Medicine, Indonesia University–Cipto Mangunkusumo Hospital. Under the supervision of Dr. dr. Diana Sunardi, M.Gizi, SpGK(K), I will conduct a study entitled The Mediterranean Diet Based on Local Foods for Obese Patients: A Study of Anthropometric Parameters, Inflammatory Markers, and Gut Microbiota.

I will first provide you with detailed information about this study and invite you to participate. Individuals who agree to participate will be asked to sign an informed consent form in the presence of a witness. You may withdraw from the study at any time without penalty. You also have the right to receive updated information from the research team regarding the intervention being tested, if applicable. Choosing not to participate will not affect the care or services you receive at this hospital, nor will it affect your relationship with the researchers.

If you do not understand any statement in this form, you may ask me about it.

1. Research objectives

The objective of this study is to determine whether a modified Mediterranean diet based on local foods can improve body weight, waist circumference, body composition (including body fat mass), inflammatory markers, and gut microbiota composition in obese patients, thereby providing a reference for dietary selection in obese management.

The Mediterranean diet is a diet rich in fiber sources such as vegetables and fruits with olive oil as the main source of additional fat, as well as high intake of fish and seafood. Moderate consumption of eggs, poultry, and dairy products (cheese and yogurt), and low consumption of red meat. In addition, the Mediterranean diet also includes physical activity such as regular exercise.

2. Participation in the study

This study will last approximately 28 days and will be preceded by a 7-day pre-study period. During the assessment phase, measurements of body weight, height, waist circumference, hip circumference, and body composition will be taken, which will require approximately 20 minutes. Blood sampling will take approximately 5–10 minutes. You will also be asked to collect stool samples according to procedures that will be explained to you.

Following these assessments, participants will receive meals prepared by the research team from Monday to Saturday, consisting of three main meals and two snacks per day. The Sunday meal

menu will be supervised by the researcher. On the 29th day, the baseline examinations will be repeated.

The diet will be arranged and provided by a catering service from Monday through Saturday, while on Sundays the meal menu will be supervised by the researchers. Participants are expected to consume only the provided meals and follow the dietary recommendations, as well as engage in physical activity as advised. By agreeing to participate in this study, you will be asked to adhere to the study schedule and confirm your ability to comply with all study requirements.

3. Reasons you have been invited to participate in this study
   a. Women who are obese based on a nutritional status of ≥ 27
   b. Aged 18-50 years
   c. Willing to follow the research procedures and sign the informed consent form.
   d. No history of type 2 diabetes mellitus, kidney, liver, or thyroid disorders based on medical history or medical records
   e. No contraindications for body composition measurement: use of pacemakers, implants, history of amputation
   f. Not pregnant or in the postpartum period (up to 40 days after giving birth)
   g. Not experiencing edema
   h. Not taking medications that increase blood sugar (e.g., beta blockers, thiazides, corticosteroids, and calcineurin inhibitors) or lower blood lipids (e.g., statins, fibrates, niacin, bile acid sequestrants)

4. Research procedures

You will undergo the following procedures:

   a. Interview and identification of dietary history using a food record sheet completed by a nutritionist covering food consumed during the previous 24 hours. This interview will last 30 minutes
   b. A physical examination will be conducted by a trained measurement officer to assess body weight, height, and body composition, including fat mass, muscle mass, and body fluids. The examination will take approximately 15 minutes.
   c. Blood sampling of 2 tablespoons.
      Blood sampling will be performed by a trained and experienced officer. Prior to blood collection, the participant will be asked to sit for at least 5 minutes. Blood will be drawn from the antecubital vein (located in the crease of the arm at the elbow) after the area has been cleaned with alcohol. A tourniquet will be applied to the upper arm to facilitate venous access. A total of 10 mL of blood (approximately 2 tablespoons) will be collected and placed into appropriate blood collection tubes.

d. Stool sample collection: 1 teaspoon
Stool samples will be collected by the participant at home in the morning using a sterile container provided by the study team. Participants are advised to urinate before stool collection. The stool should first be collected in a large plastic container provided, after which approximately 250 mg (about 1 teaspoon) of stool should be transferred using a spoon into the sterile container. The sample should then be submitted to the study staff within 2 hours of collection.

All of these tests will be conducted at the beginning and end of the research day.

5. Risks, side effects, and management

The Mediterranean diet has been widely consumed and promoted among individuals at risk of cardiovascular disease and obesity. To date, no adverse effects have been reported, provided that individuals are not allergic to the ingredients used. Potential risks associated with blood collection include pain, bruising, swelling, or infection at the puncture site. These risks can be minimized through the use of small-gauge needles, procedures performed by trained personnel, and adherence to infection prevention measures prior to blood collection.

6. Benefits

The benefits to you include measurements of body weight, waist circumference, and body composition (such as body fat mass), laboratory tests for inflammatory markers and intestinal bacterial composition, as well as provision of balanced meals. All tests are provided free of charge, and all related costs are covered by the researchers.

7. Compensation

You will receive transportation compensation of Rp 50,000 for each visit to the research site and compensation of Rp 100,000 for time spent participating in the study. These compensations will be provided at the beginning and at the end of the research period. If you experience any injury or illness as a result of participating in this research, please contact the study physician immediately, who will assist you in obtaining the necessary medical care.

8. Funding

Research funding is fully covered by the Researcher.

9. Confidentiality

All data collected in this study will be kept confidential. Data processing and storage will not include your name and will use coded identifiers only. The presentation of research results at scientific meetings or conferences and publication in scientific journals will not include any information that could identify you.

10. Obligations of research subjects

As a research participant, you are required to follow the study rules and instructions outlined above. If anything is unclear, you may ask the research team for further clarification. During the study period, you are not permitted to take any supplements or herbal medicines other than those provided by the researchers.

11. Right to refuse and withdraw

As a research participant, you are required to follow the study rules and instructions outlined above. Even if you agree to participate, you have the right to withdraw from the study at any time. This decision will not affect your relationship with the investigator or the standard of care provided at this hospital. At the end of this explanation, you will be given time to consider the decision you wish to make.

12. Post-trial access

At the end of the study, all participants will receive follow-up monitoring of body weight, waist circumference, and body composition (including body fat mass), as well as one month of dietary education, provided that the study results demonstrate benefits for obese patients.

13. Additional Information

You will be given the opportunity to ask any questions you may have regarding this study. If at any time you experience side effects or require further explanation, you may contact dr. Diyah Eka Andayani, M.Gizi, SpGK(K), at 0813-1063-7429, Department of Nutrition, Faculty of Medicine, University of Indonesia / Dr. Cipto Mangunkusumo Hospital, Jakarta.

CONSENT FORM

All of these explanations have been conveyed to me and all of my questions have been answered by Dr. Diyah Eka Andayani, M.Gizi, SpGK(K). I understand that if I need further clarification, I can ask dr. Diyah Eka Andayani, M.Gizi, SpGK(K).

Consent	
I have read all the explanations about this study. I have been given the opportunity to ask questions and all my questions have been answered clearly. I am willing to participate in this research study voluntarily. _____ Name of subject _____ Sign Date_____ day/month/year	I confirm that participants were given the opportunity to ask questions about this study, and all questions were answered correctly. I confirm that consent was given voluntarily. _____ Name of researcher/approval requester _____ Sign Date_____ day/month/year

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