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Title: Comparative Evaluation of Low-Carbohydrate and Balanced Diets on Anthropometric and Cardiovascular Risk Markers in Healthy Volunteers: A Randomized Controlled Trial

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INTRODUCTION

Cardiovascular disease (CVD) is one of the diseases whose prevalence is currently increasing, with the age of sufferers getting younger. In addition, CVD is the leading cause of death in the world, with an estimated 20.5 million people dying, which is 32% of all deaths in the world. [1] Based on Basic Health Research (Riskesdas 2018), the prevalence of heart disease in Indonesia is 1.5%, with a prevalence in DKI Jakarta of 1.9%. [2] Cardiovascular disease is associated with lipid profile disorders, [3] nutritional status obesity,[4] and hypertension.[5]

Carbohydrates are the primary energy-producing macronutrient, but carbohydrate consumption is currently suspected to be a contributing factor in various diseases. A meta-analysis found that individuals with high carbohydrate intake had a 1.15-fold higher risk of CVD compared to individuals with low carbohydrate intake (Hazard Ratio (RH) = 1.15; 95% confidence interval (CI) = 1.07–1.23).[6] Carbohydrate intake stimulates insulin secretion, which promotes fat storage and inhibits adipose tissue lipolysis and fatty acid oxidation. Carbohydrate restriction can correct abnormal fatty acid composition. A cross-sectional study conducted by Kalumpiu et al. [5] showed a significant association between blood pressure, total cholesterol, and triglycerides with cardiovascular risk scores. Another study showed that normal HDL-C concentrations were 0.18 times less likely to be associated with poor Health-Related Quality of Life (HRQoL) (Adjusted Odds Ratio (aOR) = 0.18; 95%CI = 0.03–0.91; p = 0.038).[3]

Chronic inflammation is a major factor in the development of atherosclerosis. High concentrations of inflammatory markers such as C-reactive protein (CRP) and high-sensitivity C-reactive protein (hsCRP) are strong predictors of CVD risk and are associated with insulin resistance and metabolic syndrome. C-reactive protein (CRP/hsCRP) is a key indicator of systemic inflammation and a predictor of cardiovascular events, and is closely associated with visceral adiposity, which is induced by adipocyte-mediated cytokines.[7,8] Furthermore, there is strong evidence that apolipoprotein (apo) B is a more accurate indicator of CVD risk than total cholesterol and LDL. [9]

The interaction between carbohydrate intake and inflammatory markers and their relationship to predictors of atherosclerotic CVD remains understudied, particularly in Indonesia. Lifestyle changes, including dietary changes, are the primary approach to managing cardiometabolic risk factors.[10] The main cardiometabolic risk factors are diabetes, hypertension, dyslipidemia, and excess abdominal fat, all of which are affected by dietary changes. [11]

Several studies have demonstrated the contribution of carbohydrate intake to inflammation. [12-14] Studies have shown that low-carbohydrate diets are associated with a greater risk reduction compared with low-fat diets. [15] A systematic review and meta-analysis of cohort studies published in 2024 showed that high carbohydrate intake is associated with an increased risk of CVD death. [16] Another study, a systematic

review and meta-analysis of prospective studies published in 2024, showed that individuals with high carbohydrate intakes had a 1.15-fold increased risk of CVD compared with individuals with low carbohydrate intakes (RH = 1.15; 95% CI = 1.07–1.23). [17]

While Western [18] and Mediterranean [19] diets have been well studied, other dietary patterns, particularly in Eastern populations, such as Indonesia, require further investigation to understand their impact on cardiovascular parameters. To date, published dietary intervention studies examining the impact on atherosclerotic cardiovascular disease parameters in the adult population in Indonesia are still limited, primarily focusing on dietary knowledge. This study will fill this gap by providing robust empirical evidence to support the development of more effective dietary policies and guidelines.

The novelty of this study is that, to the researchers' knowledge, it is the first intervention study in Indonesia to directly compare the effects of two types of diet composition (low-carbohydrate versus different balanced diets) on anthropometric parameters, hsCRP, and ApoB serum concentration, markers of cardiovascular inflammation in healthy adult volunteers, with a control group. Most previous studies in Indonesia analyzing the relationship between diet and cardiovascular disease risk used a cross-sectional design, which only provides a single point in time. This study employed an intervention design with a control, enabling direct observation of changes in cardiovascular parameters in response to dietary interventions. This is expected to result in dietary composition recommendations tailored to the characteristics and needs of Indonesian adults.

Research objectives:

Primary outcome:

Change from baseline in Body Mass Index (BMI) at 4 weeks

Secondary outcome:

1. Change from baseline in Mean Seated Systolic Blood Pressure at 4 weeks
2. Change from baseline in Mean Seated Diastolic Blood Pressure at 4 weeks
3. Change from baseline in Serum Apolipoprotein B (ApoB) concentration at 4 weeks
4. Change from baseline in serum High-Sensitivity C-Reactive Protein (hsCRP) concentration at 4 weeks

METODE

Design:

This study is a randomized, controlled, open-label clinical trial.

Settings & Time:

Faculty of Medicine, Trisakti University, Jakarta, Indonesia.

The study was conducted for 1 year, May 2025–May 2026.

Study Population

This study recruited from residents living near or employees working at Trisakti University. The study subjects will be randomly assigned to three intervention groups: one group following a low-carbohydrate diet (45 participants), one group following a balanced diet (45 participants), and one group following a control group (45 participants). The study will be conducted in a 1:1:1 ratio by personnel blinded to the intervention.

Before participation, all individuals provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Research Ethics Committee, Faculty of Medicine Universitas Trisakti, under ethics approval No. 002/KER/FK/1/2025.

Subject recruitment criteria:

Inclusion criteria for this study are age 18–59, willingness to participate for the duration of the study (one month), ability to follow the prescribed meal plan and examination schedule, and informed consent to participate in the study, which included diet tracking, biometric testing, and adherence to the intervention program.

Exclusion criteria included a history of type 1 or type 2 diabetes, autoimmune disease, thyroid disease that can affect carbohydrate metabolism, major surgery in the past six months, as this can affect carbohydrate metabolism and the body's response to dietary changes, eating disorders, allergies or intolerances to ingredients used in the study meal plan, participation in other clinical trials that could affect the results of this study, impaired kidney function with serum creatinine ≥1.5 mg/dL, and abnormal liver function with alanine aminotransferase and aspartate aminotransferase concentrations exceeding threefold.

Subject Size Estimation:

The sample size was calculated using a one-way ANOVA test with a statistical significance concentration of $\alpha = 0.05$ and a power of 80%, using the effect size approach (Cohen's $f = 0.44$). Therefore, the planned sample size was 41 subjects in each group, plus 45 dropouts per group.

Participant Selection Process and Criteria

The selection process includes:

1. Recruitment: Participants will be recruited from Trisakti University employees, and recruitment will be disseminated through various channels, including social media, health forums, and interfaculty collaboration to ensure participant diversity.
2. Selection: Potential participants in the control or case groups will undergo a screening process to ensure they meet inclusion criteria, which may include age range (e.g., 18–59 years), stable health status, and ability to comply with the study requirements. Exclusion criteria include individuals who do not meet the study participant criteria.

3. Research Consent: All participants must provide informed consent, stating their understanding of the study's purpose, procedures, risks, and benefits.

Research Procedures

1. Biometric testing is essential for establishing baseline research parameters and monitoring changes throughout the study. The procedures include:

a. Baseline Assessment: At the beginning of the study, participants will undergo comprehensive biometric testing in the laboratory, and other assessments will include anthropometric measurements (weight, height, waist circumference), blood pressure, lipid profile, and inflammatory biomarkers (hsCRP, ApoB).

b. Follow-up Testing: After the intervention, participants will undergo follow-up testing to track changes in research parameters, allowing for assessment of the effects of the dietary intervention.

2. Diet Monitoring and Intervention Strategies

Diet Recording: Participants will record their food choices and meal times and respond to surveys related to their eating habits. This data will provide insight into dietary patterns and their relationship to the study parameters.

3. Ethical Considerations

This study will be conducted in accordance with the ethical principles of the Declaration of Helsinki. All participants will provide written informed consent before data collection.

Dietary Intervention:

Participants will be randomly divided into three study groups, with two groups (a balanced diet group and a low-carbohydrate diet group) receiving three meals and two snacks for 20 days (on weekdays for four weeks). The **balanced diet group** will receive a dietary intervention consisting of 45-65% of total calories from carbohydrates, 20-25% from fat, and 10% from protein. The **low-carbohydrate diet group** will receive a dietary intervention focused on a diet with <45% of total energy from carbohydrates. Total calories will be calculated using the Brocca equation (height - 100 cm) for normal weight. The third group, as a **control** (without dietary intervention), will maintain their usual diet.

Study dropout criteria: consuming 80% or less of the intake (subjects in the balanced diet and low-carbohydrate diet groups), and if subjects (all groups) submitted food records for 80% or less of the required submissions.

Measurements

Questionnaires will be used to measure characteristics (age, gender, education concentration, employee status), habit history (smoking, alcohol consumption, physical activity), and dietary assessment.

Age was categorized into <40 years and $\geq$ 40 years, sex into male and female, concentration of education into high school, bachelor's degree, master's degree/doctoral degree, jobs into employees, professionals/health workers, and not working, smoking into yes and no, physical activity in to yes and

physical activity into <150 minutes/weeks and ≥ 150 minutes/weeks.
Dietary intake measured by a 3 x 24 hr record (2 weekdays and one weekend). During the study period, all participants will submit food records three times a week (two days on weekdays and one day on weekends). At the end of each week, the food record from the participant will be assessed by the nutritionist for compliance, and the participant's intake will be analyzed.

Anthropometric measurements included (weight, height, and BMI), and blood pressure (systolic and diastolic). Body weight measurements were taken in the morning between 8:00 and 10:00 am. Subjects were instructed not to eat breakfast before body weight measurements.
Height in meters will be measured using Seca 213 scales (Hamburg, Germany) at an accuracy of 0.1 cm. Weight in kg was determined using Seca 876 scales(Hamburg, Germany) at 0.1 kg accuracy. Subjects were asked to remove their footwear, hat, hair accessories, or any high hairdos, take off belts, and empty their pockets to remove cell phones, wallets, or coins. In measuring height, the subject was asked to stand with the feet together, heels against the wall, knees straight, and eyes on the same concentration with the ears. The measuring arm was slid gently down onto the head, and the subject was asked to breathe in, with the results being recorded at an accuracy of 0.1 cm. In determining the body weight, the portable scale was placed on a firm and flat surface. The scale was then switched on until the digits 0.0 appeared, then the subject was asked to step onto the scale, face forward, arms at the sides, while standing still, then the weight was recorded at an accuracy of 0.1 cm. Body mass index was calculated by dividing the weight in kg by the square of the height in meters, and was classified into non-obese (BMI <25 kg/m^2) and obese (≥ 25 kg/m^2) in accordance with the WHO Asia-Pacific BMI categories.

The blood pressure in mmHg was measured using an Omron HEM7120 (Vietnam) between 8:00 and 10:00 am. Subjects were instructed not to exercise, not to consume coffee for at least 4 hours prior to the blood pressure test, to get sufficient sleep (7–8 hours), and not to use antihypertensive medication. Blood pressure was measured while sitting, and the average of 2 measurements was calculated. Blood pressure checks were performed by doctors who were not members of the research team to ensure the objectivity of the findings.

Laboratory measurement
Approximately 5 mL of venous blood was collected from each participant after an overnight fast using serum separator tubes. Following centrifugation, serum samples were either analyzed immediately or stored at 2–8 °C until analysis. A portion of the collected blood was used for screening the eligibility of study participants. The assay required 6 µL of serum per test to determine apolipoprotein B (Apo B) and high-sensitivity C-reactive protein (hsCRP) serum concentrations.

Apo B measurement

Serum apolipoprotein B (ApoB) was measured using an automated immunoturbidimetric assay (Tina-quant® Apolipoprotein B ver.2, Roche Diagnostics, Mannheim, Germany) on a cobas® c analyzer. The method is based on the formation of antigen–antibody complexes between ApoB in the sample and specific anti-human ApoB antibodies, which are quantified.

Calibration was performed using Roche Calibrator f.a.s. Lipids, with traceability to the IFCC/WHO reference material SP3-07 for apolipoprotein B. Internal quality control materials at normal and pathological concentrations were analyzed routinely. The analytical measuring range was 0.2–4.0 g/L, and assay precision met acceptable clinical laboratory performance criteria. turbidimetrically.

Apo B serum concentration will be presented in mg/dL

hsCRP measurement

High-sensitivity C-reactive protein (hsCRP) concentrations were measured using a particle-enhanced immunoturbidimetric assay (Cardiac C-Reactive Protein [Latex] High Sensitive, Roche Diagnostics, Mannheim, Germany) on a cobas® c analyzer. The method is based on the agglutination of latex particles coated with monoclonal anti-CRP antibodies in the presence of CRP, which is quantified turbidimetrically. Calibration was performed using Roche Calibrator f.a.s. Proteins, with traceability to the IFCC/BCR reference material (CRM 470). Internal quality control materials were analyzed routinely. The analytical measuring range was 0.15–20.0 mg/L, and assay precision fulfilled acceptable clinical laboratory performance criteria for hsCRP serum concentration measurement.

hsCRP serum concentration will be presented in mg/dL

Statistical Analysis

Normality testing was performed on numerical scale data using the Kolmogorov–Smirnov test. Normally distributed data are presented as mean ± SD, while non-normally distributed data are presented as median (25–75%). Categorical data are presented as proportions.

Differences between the three groups' data categories will be assessed using the chi-square test. If the chi-square test did not meet the requirements, as in the case of the smoking status variable, a chi-square test with Bonferroni correction was used to assess the difference between the low-carb or balanced diet group and the control group, with a significance concentration of $p<0.025$. The delta % variable was used to assess the change in variable values at the end of treatment compared to the start of treatment. The formula used was delta% = [(A-B)/A] x 100, where A = initial-treatment value and B = post-treatment value. A repeated measures ANOVA test was used to assess differences between baseline and after 4 weeks, and differences between the three groups before and after the intervention. The significance concentration considered was $p<0.05$. The analysis was performed using SPSS v31.

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Document: Informed Consent Form

Title: Comparative Evaluation of Low-Carbohydrate and Balanced Diets on Anthropometric and Cardiovascular Risk Markers in Healthy Volunteers: A Randomized Controlled Trial

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CONSENT FORM

Title of the study: Comparative Evaluation of Low-Carbohydrate and Balanced Diets on Anthropometric and Cardiovascular Risk Markers in Healthy Volunteers: A Randomized Controlled Trial

Name: _____

Age: _____

Contact number: _____

- I confirm that I have read and understood the information about the project as provided in the Participant Information Sheet dated [Insert Date].
- I confirm that I have had the opportunity to ask questions and the researcher has answered any questions about the study to my satisfaction.
- I understand that my participation is voluntary and that I am free to withdraw from the project at any time, without having to give a reason and without any consequences.
- I understand that I can withdraw my data from the study at any time.
- I understand that any information recorded in the investigation will remain confidential and no information that identifies me will be made publicly available.
- I consent use of this data in research, publications, sharing and archiving as explained in the Participant Information Sheet.
- I agree / do not agree (delete as appropriate) to take part in the above study.

Name of Participant Date :

Doctor: Yenny

Date / Signature

……..

……

Interview Signature Date: