Postprandial Effects of Macronutrient Composition Meals on the Metabolic Responses and Arterial Stiffness Indices of Lean and Obese Male Adults: A Protocol of pilot Study

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Study Protocol

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Abstract

Background & Objective: Prior studies have shown that meal composition may affect the metabolic responses and arterial stiffness indices. Due to the lack of a comprehensive study that concurrently compares metabolic responses and vascular stiffness indices after receiving three different meals in lean and obese men, this pilot study has been conducted with a three-phase parallel design, aiming to investigate the effects of meal composition on the metabolic parameters and arterial stiffness indices of lean and obese adults. Materials and

Methods: This pilot, parallel clinical trial has been performed on 20 male adults aged 18-35 years who are disease-free and selected based on the inclusion and exclusion criteria at Mashhad University of Medical Sciences, Iran. The subjects have completed three interventions at a one-week interval, including high carbohydrate (70% carbohydrates, 10% protein, 20% fat), high protein (30% protein, 50% carbohydrates, 20% fat), and high fat meal (50% fat, 40% carbohydrates, 10% protein). Postprandial effects have been assessed within 360 minutes after each meal, including the energy expenditure component (resting energy expenditure, thermic effects of feeding, respiratory quotient, and substrate oxidation) and arterial stiffness indices (augmentation index and pulse wave velocity). In addition, blood sampling has been performed to measure glucose, insulin, free fatty acids, and lipid profile.

Results: The study has started since September 2020 and will continue until January 2021. The assessment of the intervention outcomes will be carried out six hours after the end of the intervention.

Conclusion: The differences in the postprandial responses c affect the metabolic and vascular parameters due to different meal compositions, thereby providing beneficial data for the establishment of new strategies in terms of nutritional education and metabolic/vascular improvement.

Introduction

Obesity is a multifactorial, complex, and preventable disorder, which is predicted to affect 20% of the world’s adult population by 2030, while 38% of this population is predicted to become overweight. Epidemiological studies have demonstrated that obesity and overweight are important risk factors for cancer, diabetes, premature death, and cardiovascular diseases (1). Obesity could be caused by genetic factors, high energy intake, low energy expenditure, reduced physical activity, low sympathetic activity, decreased fat oxidation, and differences in hormonal responses (2). Genetic factors play a pivotal role in the prediction of obesity, and the rising trend of obesity has also highlighted the importance of environmental factors in this regard (2). The leading causes of obesity include increased energy intake (especially from energy-dense and high-fat foods) and reduced physical activity (3).

Dietary macronutrient composition remarkably influences body weight adjustment. In response to acute changes in the dietary macronutrient composition (e.g., increased fat intake), human subjects have reported the increased oxidation of carbohydrates and total energy expenditure (TEE). Moreover, the short-term changes in the energy intake of human subjects have been shown to stimulate hormonal and

metabolic alterations (4). Postprandial TEE and thermic effect of feeding (TEF) are the main objectives in the management of energy balance, with TEF contributing to postprandial TEE under the possible effect of dietary composition (5-7).

According to human studies, the consumption of animal protein in breakfast leads to less significant changes in the plasma levels of glucose and insulin, while it increased TEF (8-10), and reduced food intake during the day (8, 11). On the other hand, the vascular function is considered to be a major determinant of coronary artery health, and its disruption could increase the risk of cardiovascular diseases (CVDs) (12). Previous findings have indicated that the vascular function differs between obese and normal adults in the fasting state (13), while the acute effects of dietary macronutrient composition on the vascular function have not been well documented.

Postprandial hyperglycemia (14, 15), and hypertriglyceridemia (16, 17), have emerged as independent cardiovascular risk factors, promoting interest in the cardiovascular effects of acute dietary exposure. In addition, some findings have demonstrated that postprandial triglyceride (TG) levels may be influenced by the type of lipids, while other studies have shown that saturated fatty acids may induce a slight increase in the levels of postprandial TGs compared to unsaturated fatty acids (18, 19). A research in this regard indicated that high-fat meals could reduce postprandial cardiovascular reactivity (20). Another study also demonstrated that diets with high trans fatty acids may increase serum free fatty acids (FFAs), and high-FFA diets may lead to hyperinsulinemia (21).

Arterial stiffness is an independent risk factor for CVDs, and its intensity may increase in the presence of other CVD risk factors (22, 23). However, data are scarce regarding postprandial arterial stiffness (24-26), particularly in obese individuals. Aortic pulse wave velocity (PWV) is the 'gold standard' for the evaluation of aortic stiffness and subclinical organ damage (27). Despite the significant association between postprandial lipemia and vascular response, as well as vascular function and arterial stiffness, it remains unknown whether postprandial lipemia directly leads to acute detectable responses in arterial stiffness (28, 29).

The present study has been initiated to evaluate the effects of dietary macronutrient distribution on the metabolic responses (energy expenditure components, postprandial lipemia, and glycemia) and arterial stiffness indices (PWV and pulse wave analysis) of normal and obese adults.

Materials And Methods

Research Hypothesis

We have hypothesized that dietary macronutrient composition has different effects on the metabolic responses and arterial stiffness indices of normal and obese adults.

Experimental Design

The study protocol is based on the SPIRIT guidelines (30). Figure 1 depicts the diagram of enrollment, intervention, and assessments. Table 1 shows the schedule of enrollment, intervention, and data collection for each participant. This is a parallel clinical trial, and the subjects have been consuming three different test meals (high-carbohydrate, high-fat, and high-protein) on different days with a one-week interval between each intervention.

Participants and Data Collection

Data collection has been performed by two nutritionists and one nurse, who have been trained on the proper use of various devices and are able to complete the questionnaires accurately. Since this is a pilot study, we cannot assess the attrition rate, and it is also not necessary to define and calculate the sample size.

Study Setting and Sample Population

This pilot, parallel clinical trial has been underway at Imam Reza Hospital of Mashhad, located in the northeast of Iran. The participants were recruited by local advertising with posters distributed at Mashhad University of Medical Sciences.

Inclusion Criteria

The inclusion criteria of the study are as follows: 1) age of 18-40 years; 2) adults with normal weight (body mass index [BMI]: 18.5-23.5 kg/m^2, body fat percentage: 12-22%, waist circumference<90 cm) and obese adults (BMI>27.5 kg/m^2, body fat percentage>27%, waist circumference>95cm); 3) apparently healthy men; 4) written informed consent to participate and 5) willingness to undertake the required fasting periods.

Exclusion Criteria

The exclusion criteria of the study are as follows: 1) professional athletes; 2) greater changes in the body weight than 10% within the past six months; 3) current smoking habits; 4) use of medications or supplements affecting the metabolism (e.g., thyroid drugs, caffeine); 5) history of CVDs, hypertension, diabetes mellitus, hyperlipidemia, and neurological and/or neuropsychological disorders; 6) consumption of toxic substances; 7) use of supplements for weight loss or weight gain and 8) inability to partake in the intervention due to intolerance/dietary preferences.

Test Meals

Three interventions have been implemented in the current research, involving the consumption of high-fat, high-carbohydrate, and high-protein meals as the test meals in a randomized order. On the day of the intervention, the TEE of the subjects was determined via indirect calorimetry. The test meals contained

25% TEE for men, and the high-protein meal consisted of bread, cheese, boiled eggs, and skimmed milk (30% protein, 50% carbohydrates, and 20% fat). The high-carbohydrate meal consisted of skimmed milk, white bread, butter, jam, and honey (70% carbohydrates, 20% fat, and 10% protein). The high-fat meal contained bread, butter, cream cheese, high-fat milk, and jam (50% fat, 10% protein, and 40% carbohydrates). The subjects would be asked to ingest the test meals within a maximum of 15 minutes on each test day.

Statistical Analysis

Descriptive statistics will be used to compare and determine the primary characteristics of the study groups, and the normal distribution of the variables will be evaluated using the Kolmogorov-Smirnov test. In total, 20 subjects have been assigned to the obese and normal groups. The baseline comparisons between the groups will be performed using the independent samples t-test or Mann-Whitney U as appropriate.In addition, repeated measures analysis of variance (ANOVA) will be used to assess the timing effect of the research parameters in various phases of the study, as well as the possible overall significant differences of the parameters between the lean and obese men. In all the statistical analyses, the P-value of less than 0.05 will be considered significant.

Measurement Tools

Anthropometric Parameters and Body Composition

The anthropometric measurements will be performed at the outset of the fasting state by a trained nutritionist. In addition, body weight will be measured to the nearest 0.1 kilogram, with the participants in light clothing. The height of the subjects will be measured using a stadiometer in the standing position to the nearest 0.1 centimeter. BMI was defined as weight (kg) divided by the square of height (m^2), and waist circumference will be measured at the midline between the iliac crest and lowest ribs to the nearest 0.5 centimeter. Finally, the body composition of the participants will be determined using bioelectrical impedance analysis (AVIS 333).

Dietary Measurements

Dietary intakes will be assessed using the valid and reliable food frequency questionnaire (31), and the collected data will be expressed as gram per day using household measures. In addition, the modified food consumption database of the US Department of Agriculture will be used to calculate the daily nutrient intake of each subject (32).

Physical Activity

The level of physical activity in the participant will be evaluated using a validated questionnaire (33) and calculated based on the metabolic equivalent tasks. Based on the questionnaire, the participants will be divided into three groups of low, medium, and high physical activity.

Screening Questionnaires

A screening questionnaire will be designed based on a researcher-made questionnaire and the available standardized questionnaires in order to acquire the general data of the participants. Accordingly, the subjects will be excluded for medical reasons such as hypertension, CVDs, hypothyroidism/hyperthyroidism, intolerance, allergies, diabetes mellitus, and other acute/chronic diseases.

Indirect Calorimetry

At this stage, the participants will be asked to stay awake and motionless in the supine position, and air samples will be collected using a mask (MetaLyzer 3B-R3 device). Moreover, respiratory gas exchange measurements will be recorded for 20 minutes in the fasting state, followed by hourly measurements for six hours after the consumption of the test meals. Furthermore, fasting REE will be measured in a quiet area at room temperature, with the participants in the supine position. The respiratory quotient will also be calculated based on the oxygen consumption and carbon dioxide production, and TEF will be measured as the difference of the postprandial subtracted by the fasting REE. The resting energy expenditure and substrate utilization will be also be estimated via indirect calorimetry after resting for 30 minutes.

Pulse Wave Analysis

All the patients will receive an ultrasound examination of the carotid artery in the supine position, with the head turned to 45° from the side to be scanned and the operator placed on their right side. In addition, brachial blood pressure evaluation and pulse wave analysis will be conducted in the supine position using the Sphygmocor XCEL device. Blood pressure will be measured after the minimum rest of 15 minutes on the right upper arm in a quiet area. The augmentation index (AIx), central blood pressure (cBP), and heart rate (HR) will also be analyzed in accordance with the guidelines of the pulse wave analysis device manufacturer. After the measurement of the HR, cBP and AIx will be estimated using built-in algorithms.

Carotid-radial PWV

Carotid-radial pulse wave velocity (PWV_b) is a measure of arterial stiffness, which will be determined based on the sequentially measured electrocardiogram-gated left carotid and radial waveforms (applanation tonometry) using the foot-to-foot method to determine the pulse travel time in our study. Moreover, the travel distance of the pulse wave will be calculated as the difference in the distance

between the suprasternal notch and each recording site using a tape measure over the body surface. The measurements will be performed at least twice, and the mean PWV_b will be applied to the analysis. PWV_b will be assessed at baseline and 30, 90, 150, 210, 270, and 330 minutes after the test meals.

Blood Samples

Each test day will be implemented at 7 AM-2 PM; this period was selected since it was assumed to represent a common interval between breakfast and lunch. Serum samples will be collected before meal consumption and 60, 180, and 360 minutes after the meal initiation to evaluate metabolic activity markers. A maximum of three milliliters of blood will be collected from each patient at each of the given times (12 ml/day) for the analysis of insulin, glucose, FFAs, TG, low-density lipoprotein, high-density lipoprotein, and total cholesterol. The blood samples will be collected at room temperature and immediately centrifuged, and the serum samples will be frozen at the temperature of -20°C.

Experimental Protocol

The day before each of the three test days, the subjects prepared their own meals and a standardized daily menu plan, which consisted of 15% protein, 55% carbohydrates, and 30% fat based on their daily energy requirements. The diets ensured equally filled glycogen stores and similar macronutrient balance in each subject on every test day. The subjects will not be allowed to engage in severe physical activity within two days prior to the test days. On the test day, the subjects arrive at the research center at Imam Reza Hospital at 7 AM with minimum activity (by car/bus) and after fasting from food, drinks (except water), caffeine, and alcohol for the past 12 hours.

Before the tests, all the subjects rested in the supine position for 30 minutes, lying on a bed in the semirecumbent position until the end of the tests. Initially, all the measurements will be performed in the fasting state and postprandially within six hours. At the next stage, the participants will randomly receive the test meals and given 15 minutes to take the meals under the supervision of the researcher until the test meal is completely consumed. Afterwards, the participants will complete a series of tests, including indirect calorimetry, PWV, and pulse wave analysis. In addition, blood samples will be collected within six hours. All the tests will be carried out in the exact same conditions (e.g., temperature-regulated room and quiet area). Following the test meals, the participants would fast again, refraining from food and drinks (except water) for another six hours while the testing stage will continue. The testing conditions will be repeated on the next two test days. Figure 2 depicts the schedule of the study days.

Results

The study protocol has been approved by the Research Ethics Committee of Mashhad University of Medical Sciences, and the pilot study will be completed. The study has initiated since September 2020 and will continue until January 2021. In total, 20 participants (10 obese men and 10 lean men) would complete the interventions and assessments. Data analysis and reporting of the results were performed in the summer of 2020.

Discussion

The current research aimed to investigate the effects of the single ingestion of various test meals on the metabolic responses and vascular reactivity of healthy and obese adults. To the best of our knowledge, no previous research has concurrently assessed the postprandial effects of three meals with various compositions (high-protein, high-fat, and high-carbohydrate) on metabolic and vascular parameters. Based on the study design, the subjects will receive three types of meal on different test days to determine the differences in the metabolic parameters and arterial stiffness indices between the normal and obese subjects.

Considering the design of the present study, the balance in the confounding variables will be ensured in the study groups, and the bias induced by the characteristics of the subjects will be removed. However, the main challenge in this experiment will be the attrition rate and subject compliance as they must attend the study setting on three days, and the study process would continue for almost seven hours for each subject on each test day. Consequently, some of the subjects might fail to complete the three test days due to lengthiness, tardiness, and other reasons. Notably, the researchers will thoroughly describe the principles and requirements to the subjects prior to the experiment in order to ensure their compliance.

Conflicting results have been proposed regarding the effects of meal composition on metabolic and vascular responses. Based on the hypothesis of the current research, our findings could describe the effects of meal composition on metabolic and vascular responses. Given the increasing prevalence of CVDs and their close correlation with dietary habits, lipemia, and glycaemia after meals, investigating the effects of meal composition on vascular functions and metabolic responses would be of great interest. Furthermore, the subject matter could be incorporated into the design of novel lifestyle and dietary interventions.

Conclusion

If a significant result is achieved regarding the measured parameters in the pilot study, we will design a study with a larger sample size consisting of both genders. The results of these studies may provide the proper tools to adapt various meal types to different body compositions in order to improve metabolic responses and vascular reactivity.

Abbreviations

RCT: Randomized clinical trial; TEE: Total energy expenditure; TEF: Thermogenic effects of food; CVD: Cardiovascular disease; PWV: Pulse wave velocity; REE: Resting energy expenditure; BMI: Body mass index; AI: augmentation index; BP: Blood pressure; HR: Heart rate.

Declarations

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Authors' Contributions

S. F., R. R., N. P., M. M., and M. S. designed the research; S. F., R. R., N. P., M. M., L. J., J. G. N., G. R., Z. T., and M. S. drafted the manuscript; J. G. N. designed the figures. All the authors read and approved the final manuscript.

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Availability of Data and Materials: N/A.

Ethics Approval and Consent to Participate

This project was approved by the Ethics Committee of Mashhad University of Medical Sciences and registered with the code IR.MUMS.MEDICAL.REC.1398.185. Informed consent has been obtained from the patients to participate in the study.

Consent for publication: Not applicable.

Conflicts of interest: None declared.

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Tables

Table 1.

SPIRIT 2013 figure showing schedule of enrolment, intervention and data collection for each participant. * randomly assigned meal.

	STUDY PERIOD					
	Enrollment	**Allocation**	**Post-allocation**			
Timepoint	**preintervention**	**Time 0**	**baseline**	**Intervention Visit 1**	**Intervention Visit 2**	**Intervention Visit 3**
Enrolment	√					
Eligibility screen	√					
Informed consent		√				
Allocation						
Interventions				√*	√*	√*
High protein				√*	√*	√*
High charbohydrate				√*	√*	√*
High fat						
Data collection						
Food frequency Questionnaire		√				
International physical activity questionnaires		√				
Anthropometric variable		√				
FAT and Lean mass		√				
Energy expenditure			√	√	√	√
Resting metabolic rate			√	√	√	√
Charbohydrate oxidation			√	√	√	√

Fat oxidation	√	√	√	√
Protein oxidation	√	√	√	√
Blood pressure	√	√	√	√
Heart rate	√	√	√	√
Augmentation index	√	√	√	√
Pulse wave velocity	√	√	√	√
Serum glucose and insulin levels	√	√	√	√
Lipid profile	√	√	√	√

Figures

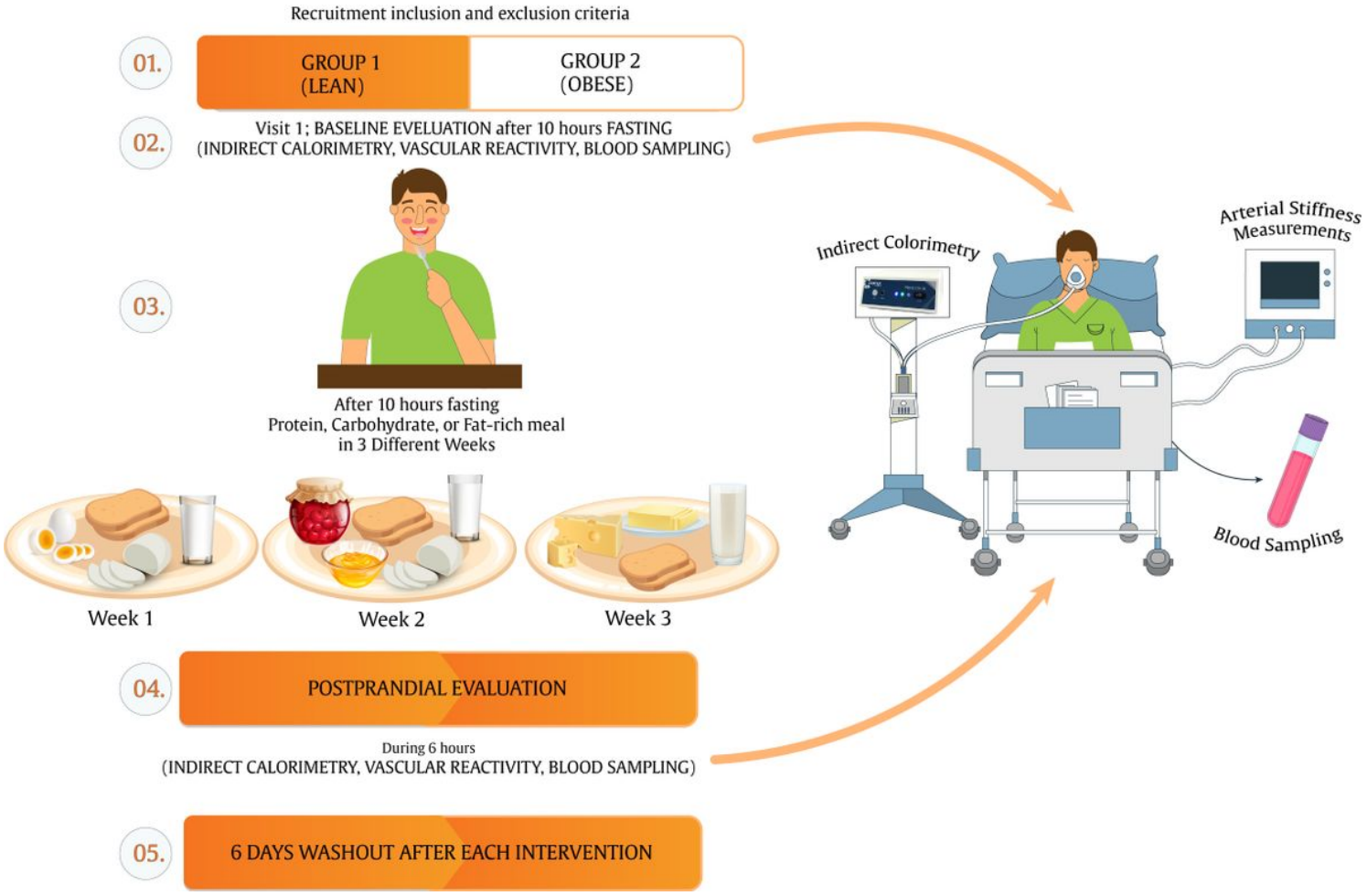

Figure 1

diagram of enrollment, intervention, and assessments

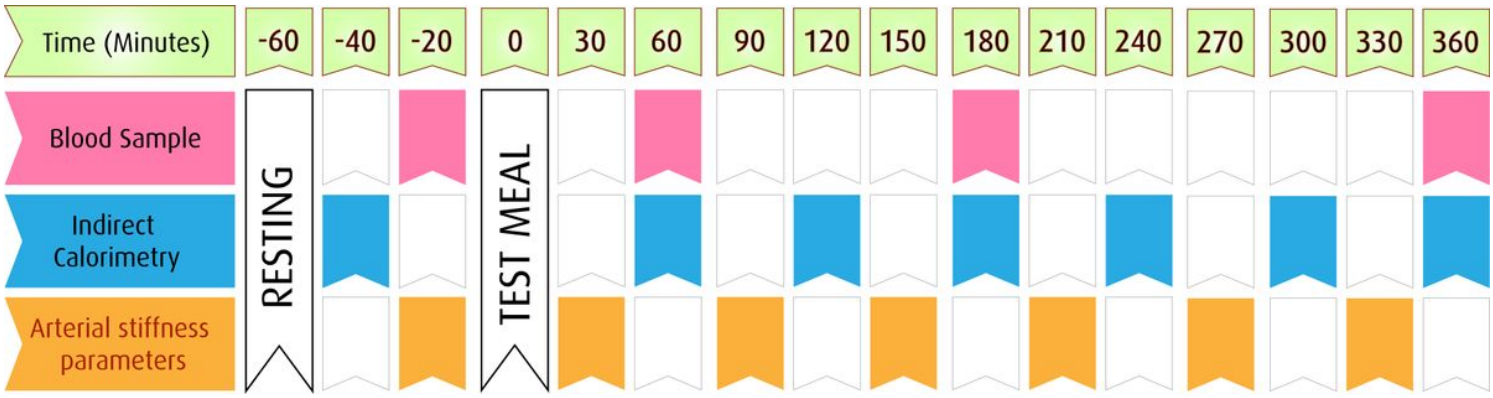

Figure 2

schedule of the study days