Title: Impact of a Mediterranean-type diet (gReenheArTediEt - RATE) on cardiovascular disease risk factors: a randomized clinical trial.

Project nº: 22/2023/CEFCM

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PROTOCOL

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Introduction

Cardiovascular disease (CVD) encompasses various disorders of the heart and blood vessels[1], with a underlying atherosclerotic process (coronary artery disease, cerebrovascular disease, aortic and arterial diseases including hypertension and peripheral vascular disease), or in its absence, congenital heart disease, rheumatic heart disease, cardiomyopathy, and cardiac arrhythmias would be highlighted[2]. CVD continues to be identified as the leading cause of death worldwide. It is estimated that in the year 2019, approximately 17.9 million individuals died from this non-communicable disease, accounting for 32% of all global deaths[1]. In that same year, CVD mortality reached 4.1 million individuals in Europe (1.9 million deaths in males and 2.2 million deaths in females), representing an increase of two hundred thousand deaths annually compared to 2017[3,4]. Among CVDs, coronary artery disease and stroke are listed as the first and second leading causes of death in Europe[3]. In Portugal, a similar trend is observed regarding the high incidence of CVD mortality (29.9%); however, unlike other European countries where the leading cause of death is coronary artery disease, stroke is indicated as the incident with the highest prevalence at the national level[5].

It is considered that CVD is the result of the interaction between genetic predisposition (non-modifiable risk factors) and environmental influences (modifiable risk factors). Environmental factors predominate over others and often lead to negative outcomes through the adoption of poor lifestyles, such as unbalanced diet, physical inactivity, smoking, and excessive alcohol consumption. These are considered risk factors with direct effects on CVD pathogenesis and indirectly contribute to an increased risk for chronic diseases, such as hypertension, dyslipidemia, overweight, obesity, and diabetes mellitus[2,6]. In Portugal, overweight and obesity are the risk factors with the highest prevalence in the population (62.1%). Regarding metabolic risk factors, hypertension and high low-density lipoprotein cholesterol (LDL-c) have a prevalence of 43.1% and 31.5%, respectively[7].

In 2004, Yusuf et al. demonstrated, in a randomized trial, the effects of environmental risk factors (smoking, diet, and physical exercise) and metabolic risk factors (diabetes mellitus, obesity, and hypertension) on the contribution to myocardial infarction (MI), one of the outcomes of coronary artery disease. Participants who had a high daily intake of fruits and vegetables and regularly engaged in physical exercise had a significantly lower risk of MI[8]. According to the Global Burden of Disease study in 2017, the dietary habits of the Portuguese population were the third leading risk factor contributing to the loss of healthy years of life, particularly due to metabolic diseases, circulatory system disorders, and neoplasms. Other dietary risk factors, such as alcohol consumption, high plasma glucose, high body mass index (BMI), hypertension, and high LDL-c also contribute significantly to the loss of healthy years of life. Within dietary habits, low consumption of whole grains, low consumption of fruits, and low consumption of nuts and seeds were highlighted as the three main risk factors for this loss, suggesting the importance of modifying comprehensive lifestyle risk factors to prevent CVD[9].

Currently, the scientific literature has demonstrated the importance of diet in preventing CVD and how it can minimize its risk factors. Dietary Approaches to Stop Hypertension (DASH), the Mediterranean dietary pattern, vegetarian diet, EAT-Lancet diet, among other dietary strategies, have been published in various scientific journals[10-16]. The Seven Countries Study, conducted in the 1960s, stands out, as the revisitation of the Mediterranean dietary pattern identified important beneficial effects in preventing various non-communicable chronic diseases[17-20]. This dietary pattern is characterized by a high intake of fruits, vegetables, legumes, whole grains, nuts, and olive oil as the main source of fat. In moderation, there is consumption of dairy products, white meats, eggs, and fish, and finally, low consumption of red meats, processed meats, sugars, and sugary products[21]. A recent systematic review and meta-analysis of observational studies found that individuals diagnosed with metabolic syndrome who had a higher adherence to the Mediterranean diet (MD) exhibited lower triglyceride plasma concentration and smaller waist circumference, as well as higher levels of high-density lipoprotein (HDL) cholesterol[22]. In addition to the significant reduction in triglyceride levels, weight loss and decreased insulin resistance are associated with high adherence patterns[23]. Other studies and systematic reviews also corroborate the relevance of MD for cardiovascular health[14,24,25].

Other dietary patterns that have garnered interest within the scientific community due to the accumulation of scientific evidence on their environmental benefits and potential in preventing non-communicable chronic diseases are plant-based diets, primarily due to: a) early satiety resulting from increased dietary fiber intake, b) improved insulin sensitivity due to weight loss, c) low intake of cholesterol and saturated fatty acids, among others[13]. However, the methodology for defining "plant-based diet" or "vegetarian diet" is not consistent, which hinders the comparison of studies and, consequently, the corroboration of results. Regarding these dietary patterns, favorable risk factors for CVD have been associated, such as lower blood pressure levels, lower total cholesterol (TC) and LDL cholesterol levels, decreased BMI and body fat, as well as a decreased risk of diabetes mellitus[26-31]. On the other hand, some scientific studies present contradictory results, emphasizing the importance of conducting more studies for robust conclusions[32].

A randomized clinical trial evaluated the improvement of cardiovascular risk profile through the implementation of an ovo-lacto-vegetarian diet and the Mediterranean diet (MD). Both diets were effective in weight loss and reduction of body fat, with no significant differences observed. However, the plant-based diet showed better results in decreasing LDL cholesterol, while the MD led to lower triglyceride levels[33]. Another study with a similar purpose found that a low-fat vegan diet improved body weight, lipid concentrations, and insulin sensitivity compared to the MD. In this study, blood pressure decreased in both diets, but to a greater extent in the MD[34].

Currently, alongside health concerns, another topic that has received significant attention from the cientific community is the environmental degradation derived from current food production. With the Projected global population growth reaching 10 billion by 2050, the effects of food production on greenhouse gas emissions, nitrogen and phosphorus pollution, biodiversity loss, and water and land use are expected to reach such levels that will destabilize the Earth's system.

Considering that diet has both environmental value and the potential to promote cardiovascular health, the RATE aims to explore more sustainable dietary options that promote both health and environmental

sustainability. The intervention will be based on the principles of the Mediterranean diet, with a higher consumption of plant-based sources compared to other mentioned dietary patterns. Additionally, it will incorporate the benefits of chronobiology, which are important for weight and adiposity control.

Objectives

The aim is to compare the intervention and control groups, after the nutritional and physical exercise intervention, in the following aspects:

- Quantify the effect of nutritional and physical activity intervention on improving important cardiovascular disease risk factors: body weight, body composition (% body fat), and waist circumference.

As a secondary objective, we aim to compare the intervention and control groups regarding the following variables:

- Characterize the lipid profile, inflammatory markers, and glycated hemoglobin (HbA1c);
- Evaluate cardiovascular risk and health;
- Assess adherence to the Mediterranean-type diet;
- Evaluate body composition;
- Evaluate quantitative and qualitative muscle component and muscle performance (frailty, hand grip strength, and sarcopenia).

To achieve this objective, a randomized clinical study will be conducted with the following tasks:

Task 1 - Recruitment of Participants

Task 2 – Intervention

Task 3 - Evaluation of the impact of intervention

Task 3.1 - Anthropometric Measurements

Task 3.2 - Analysis of Blood Biomarkers

Task 3.3 - Quantitative muscle component and the qualitative and muscular performance

Task 3.4 - Adherence to the dietary regimen

Task 3.5 - Evaluation of quality of life

Task 3.6 - Evaluation of hand grip strength

Task 3.7 - Evaluation of cardiovascular risk level

Methodology

This project will be carried out at the Algarve Biomedical Center – Research Institute (ABC-RI) and at the University of Algarve, Gambelas campus.

Task 1 - Recruitment of Participants

This project consists of a randomized clinical trial, whose target population is adult volunteers at risk of cardiovascular disease. Participants will be recruited among men and women aged 50 years or older, who are autonomous in daily life activities, with a cardiovascular disease risk classification according to SCORE 2 and SCORE2 O.P, and who do not meet any of the exclusion criteria (Acute Myocardial Infarction occurrence, being under nutritional counseling

by a nutritionist; adopting a vegetarian or strict vegetarian dietary pattern and/or undergoing nutritional supplementation).
Individuals will be invited to participate voluntarily in this study, receiving detailed information about the research to allow for an informed and free decision. Individuals who agree to participate will sign an informed consent.

Task 2 – Intervention

The nutritional intervention under study was designed based on the principles of the Mediterranean dietary pattern. However, the gReenhAerTediEt - RATE was developed with the aim of optimizing health, specifically cardiovascular health parameters, while also considering food sustainability (principles of the Mediterranean pattern and the Eat-Lancet Diet).

The intervention group will be asked to have three (breakfast, lunch, and dinner) to four (breakfast, lunch, afternoon snack, and dinner) meals per day, adjusting the number of meals according to their daily routines. Participants will be encouraged not to count calories, promoting an emphasis on moderation. Regarding the main meals, legumes will be encouraged as the protein source for dinner. For lunch, the protein component may include white meats, fish, or eggs. Red and/or processed meats will be excluded in this intervention. Additionally, a minimum of 12 hours of overnight fasting will be encouraged for all participants in this group.

In the initial phase, a two-hour group session titled "Discovering New Flavors" will be conducted. The aim of this session is to promote the consumption of healthy foods that should be included in participants' daily diet. Oat flakes, high-fiber bread varieties, whole-grain crackers, corn tortillas, hummus, and guacamole will be presented as suggestions for spreads or to be consumed with vegetable sticks. In order to create a different dynamic and increase interaction, some recipe suggestions will be prepared together as a group (such as lentil curry, beetroot hummus, and guacamole). The intention of this activity is also to simplify and demonstrate how easy it is to prepare these types of recipes.

Subsequently, the participants will start individual nutritional counseling. For the first consultation, participants will be asked to keep a three-day non-consecutive food diary (i.e., two weekdays and one weekend day) on paper in advance. Sociodemographic data, biochemical data, personal and family history, pharmacotherapy, and physical activity practices will be provided by the A3-COR project, in which the present study is included. In this first consultation, dietary intake will be assessed through the analysis of the food diary, while subsequent consultations will involve a 24-hour dietary recall. Anthropometric evaluation will include parameters such as triceps and biceps skinfold thickness, arm circumference, and arm

muscle area. Other anthropometric measurements will also be provided by the A3-COR project. After assessing the nutritional status, nutritional counseling will follow the aforementioned guidelines. Objectives to be achieved by the participants are defined for each phase of the intervention (Table 1). Lastly, in the first consultation, a recipe book, a leaflet with the discussed dietary recommendations, and other guidance documents on food sustainability will be provided.

Task 3 - Evaluation of the impact of intervention

To investigate the impact of the nutritional intervention, participants will be evaluated at two established time points: at the beginning (day 1) and at the end of the intervention period (day 90). This evaluation will be conducted by healthcare professionals and will consist of collecting a 3-day non-consecutive food diary, administering two validated questionnaires to assess sarcopenia and frailty, and finally, evaluating the biochemical profile. At an intermediate point (between days 40 and 50), a dietary adherence questionnaire (MEDAS) will be administered via telephone.

Task 3.1 - Anthropometric Measurements

Anthropometric measurements, obtained according to standardized protocols, include the measurement of height, body weight, waist circumference (WC), bicipital and tricipital skinfolds, and collection of body composition data. Height and weight will be measured with a stadiometer and a scale, respectively, with participants barefoot. Waist circumference will be measured at the midpoint between the last rib and the iliac crest using a flexible measuring tape. Skinfolds will be assessed according to the methodology established by the International Society for the Advancement of Kinanthropometry (ISAK)[35]. Body composition data, including fat mass and lean body mass, will be collected through bioelectrical impedance analysis (BIA). Body mass index (BMI) will also be calculated by dividing weight by height squared. Lastly, for the evaluation of the arm muscle area (AMA), we will use the formula suggested by Frisancho[36].

Task 3.2 - Analysis of Blood Biomarkers

For the evaluation of lipid profile (triglycerides (TG), total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL)), glycated hemoglobin (HbA1c), and C-reactive protein (PCR), the COBAS b 101 system, a portable device that allows for the assessment of various analytical parameters, will be used. For this study, three capillary blood samples will be collected via a finger prick using a single-use lancet. The assessment of these parameters will be relevant to establish possible associations with changes in participants' dietary habits.

Task 3.3 - Quantitative muscle component and the qualitative and muscular performance

For the evaluation of the qualitative component and muscle performance, the FRAIL Scale and SARC-F tools will be used to assess frailty and sarcopenia, respectively. These tools have been validated for the Portuguese population[37].

Task 3.4 - Adherence to the dietary regimen

To evaluate adherence to the nutritional intervention in the study, participants will be asked to keep a three-day non-consecutive food diary (two weekdays and one weekend day) before and after the intervention. Throughout the interim consultations, the 24-hour recall questionnaire will also be used for continuous assessment of adherence to nutritional therapy.

Task 3.5 - Evaluation of quality of life

To determine health-related quality of life, the Medical Outcomes Study 36-item Short-Form Health Survey (MOS-SF-36) questionnaire will be used[38]. The SF-36 consists of 36 items that cover eight dimensions of health study and detect both positive and negative aspects. This instrument is considered a generic measure of health as it aims to measure health concepts that represent basic human values relevant to the functionality and well-being of each individual. This instrument has been translated, adapted, and validated for the Portuguese population (SF-36 v2).

Task 3.6 - Evaluation of hand grip strength

The maximum hand grip strength will be measured using the Lafayette Digital and Dynamometer 5030D1 manual dynamometer in the dominant limb. The participant will be seated with the elbow flexed at 90º, the shoulder flexed at 0º, and the wrist in a neutral position. Three grip measurements will be taken with 30 seconds of rest in between, and the best of the three measurements will be recorded.

Task 3.7 - Evaluation of cardiovascular risk level

For the evaluation of cardiovascular risk level, the SCORE2 or SCORE2 O.P38,39 algorithm will be used[39,40]. The SCORE2 algorithm is applied to individuals between the ages of 40 and 69 and is based on risk factors associated with CVD such as European region risk level, gender, age, systolic blood pressure, non-HDL cholesterol, and smoking habits. A risk percentage is assigned between <2.5% and >25%, with stratification into low, moderate, and high risk categories, with the specific percentages varying by risk region39. For inclusion in the present study, individuals with a risk ≥ 5% (moderate and high risk) will be considered. The SCORE2

O.P. algorithm is similar to the previous one but has been adapted and validated for individuals between the ages of 70 and 89. In this algorithm, the risk percentages range from <10% to >40%, with the distribution among low, moderate, and high risk categories varying by risk region38. For inclusion in the present study, individuals with a risk ≥ 7% (moderate and high risk) will be considered.

References

WHO. Fact sheets: Cardiovascular diseases (CVDs). World Health Organization. Published June 11, 2021. Accessed February 23, 2023. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

2.A. Alwan, T. Armstrong, D. Bettcher, et al. *Global Atlas on Cardiovascular Disease Prevention and Control*. (Shanthi Mendis, Pekka Puska and Bo Norrving, eds.). World Health Organization; 2011.

3.Timmis A, Vardas P, Townsend N, et al. European Society of Cardiology: cardiovascular disease statistics 2021. *Eur Heart J*. 2022;43(8):716-799. doi:10.1093/eurheartj/ehab892

4.Wilkins E, Wilson L WK, Bhatnagar P, et al. *European Cardiovascular Disease Statistics 2017*.; 2017. www.ehnheart.org

5.INE. *Estatísticas Da Saúde 2019*.; 2019. www.ine.pt

6.Weidinger F, Lüscher T, Achenbach S, et al. *Cardiovascular Realities 2022: An Illustrated Atlas of Key European Statistics*.; 2022.

7.Instituto Nacional de Saúde Doutor Ricardo Jorge I. *Prevalência de Fatores de Risco Cardiovascular Na População Portuguesa*.; 2019.

8.Yusuf PS, Hawken S, Ôunpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. *Lancet*. 2004;364(9438):937-952. doi:10.1016/S0140-6736(04)17018-9

9.Gregório M, Guedes L, Mendes de Sousa S. *PROGRAMA NACIONAL PARA A PROMOÇÃO DA ALIMENTAÇÃO SAUDÁVEL*. www.dgs.pt

10.Ge L, Sadeghirad B, Ball GDC, et al. Comparison of dietary macronutrient patterns of 14 popular named dietary programmes for weight and cardiovascular risk factor reduction in adults: Systematic review and network meta-analysis of randomised trials. *The BMJ*. 2020;369. doi:10.1136/bmj.m696

11.Filippou CD, Tsioufis CP, Thomopoulos CG, et al. Dietary Approaches to Stop Hypertension (DASH) Diet and Blood Pressure Reduction in Adults with and without Hypertension: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Advances in Nutrition*. 2020;11(5):1150-1160. doi:10.1093/advances/nmaa041

12.Naude CE, Brand A, Schoonees A, Nguyen KA, Chaplin M, Volmink J. Low-carbohydrate versus balanced-carbohydrate diets for reducing weight and cardiovascular

risk. *Cochrane Database of Systematic Reviews*. 2022;2022(1). doi:10.1002/14651858.CD013334.pub2

13.Mishra S, Xu J, Agarwal U, Gonzales J, Levin S, Barnard ND. A multicenter randomized controlled trial of a plant-based nutrition program to reduce body weight and cardiovascular risk in the corporate setting: The GEICO study. *Eur J Clin Nutr*. 2013;67(7):718-724. doi:10.1038/ejcn.2013.92

14.Vitale M, Masulli M, Calabrese I, et al. Impact of a mediterranean dietary pattern and its components on cardiovascular risk factors, glucose control, and body weight in people with type 2 diabetes: A real-life study. *Nutrients*. 2018;10(8). doi:10.3390/nu10081067

15.Knuppel A, Papier K, Key TJ, Travis RC. EAT-Lancet score and major health outcomes: the EPIC-Oxford study. *The Lancet*. 2019;394(10194):213-214. doi:10.1016/S0140-6736(19)31236-X

16.Ibsen DB, Christiansen AH, Olsen A, et al. Adherence to the EAT-Lancet Diet and Risk of Stroke and Stroke Subtypes: A Cohort Study. *Stroke*. 2022;53(1):154-163. doi:10.1161/STROKEAHA.121.036738

17.Keys A, Menotti A, Karvonen MJ, et al. *THE DIET AND 15-YEAR DEATH RATE IN THE SEVEN COUNTRIES STUDY*. Vol 124.; 1986.

18.Zeraattalab-Motlagh S, Jayedi A, Shab-Bidar S. Mediterranean dietary pattern and the risk of type 2 diabetes: a systematic review and dose–response meta-analysis of prospective cohort studies. *Eur J Nutr*. 2022;61(4):1735-1748. doi:10.1007/s00394-021-02761-3

19.Rosato V, Temple NJ, la Vecchia C, Castellan G, Tavani A, Guercio V. Mediterranean diet and cardiovascular disease: a systematic review and meta-analysis of observational studies. *Eur J Nutr*. 2019;58(1):173-191. doi:10.1007/s00394-017-1582-0

20.Torres-Peña JD, Garcia-Rios A, Delgado-Casado N, et al. Mediterranean diet improves endothelial function in patients with diabetes and prediabetes: A report from the CORDIOPREV study. *Atherosclerosis*. 2018;269:50-56. doi:10.1016/j.atherosclerosis.2017.12.012

21.Direção Geral da Saúde. *PADRÃO ALIMENTAR MEDITERRÂNICO: PROMOTOR DE SAÚDE*.; 2016.

22.Bakaloudi DR, Chrysoula L, Kotzakioulafi E, Theodoridis X, Chourdakis M. Impact of the level of adherence to mediterranean diet on the parameters of metabolic syndrome: A systematic review and meta-analysis of observational studies. *Nutrients*. 2021;13(5). doi:10.3390/nu13051514

23.Asbaghi O, Choghakhori R, Ashtary-Larky D, Abbasnezhad A. Effects of the Mediterranean diet on cardiovascular risk factors in non-alcoholic fatty liver disease patients: A systematic review and meta-analysis. *Clin Nutr ESPEN*. 2020;37:148-156. doi:10.1016/j.clnesp.2020.03.003

24.Franquesa M, Pujol-Busquets G, García-Fernández E, et al. Mediterranean diet and cardiodiabesity: A systematic review through evidence-based answers to key clinical questions. *Nutrients*. 2019;11(3). doi:10.3390/nu11030655

25.Filippou CD, Thomopoulos CG, Kouremeti MM, et al. Mediterranean diet and blood pressure reduction in adults with and without hypertension: A systematic review and meta-analysis of randomized controlled trials. *Clinical Nutrition*. 2021;40(5):3191-3200. doi:10.1016/j.clnu.2021.01.030

26.Yokoyama Y, Levin SM, Barnard ND. Association between plant-based diets and plasma lipids: A systematic review and meta-analysis. *Nutr Rev*. 2017;75(9):683-698. doi:10.1093/nutrit/nux030

27.Qian F, Liu G, Hu FB, Bhupathiraju SN, Sun Q. Association between Plant-Based Dietary Patterns and Risk of Type 2 Diabetes: A Systematic Review and Meta-analysis. *JAMA Intern Med*. 2019;179(10):1335-1344. doi:10.1001/jamainternmed.2019.2195

28.Kahleova H, Tura A, Hill M, Holubkov R, Barnard ND. A plant-based dietary intervention improves beta-cell function and insulin resistance in overweight adults: A 16-week randomized clinical trial. *Nutrients*. 2018;10(2). doi:10.3390/nu10020189

29.Kahleova H, Rembert E, Alwarith J, et al. Effects of a low-fat vegan diet on gut microbiota in overweight individuals and relationships with body weight, body composition, and insulin sensitivity. A randomized clinical trial. *Nutrients*. 2020;12(10):1-16. doi:10.3390/nu12102917

30.Djekic D, Shi L, Brolin H, et al. Effects of a Vegetarian Diet on Cardiometabolic Risk Factors, Gut Microbiota, and Plasma Metabolome in Subjects With Ischemic Heart Disease: A Randomized, Crossover Study. *J Am Heart Assoc*. 2020;9(18):e016518. doi:10.1161/JAHA.120.016518

31.Kahleova H, Petersen KF, Shulman GI, et al. Effect of a Low-Fat Vegan Diet on Body Weight, Insulin Sensitivity, Postprandial Metabolism, and Intramyocellular and Hepatocellular Lipid Levels in Overweight Adults: A Randomized Clinical Trial. *JAMA Netw Open*. Published online 2020. doi:10.1001/jamanetworkopen.2020.25454

32.Kaiser J, van Daalen KR, Thayyil A, Cocco MT de ARR, Caputo D, Oliver-Williams C. A Systematic Review of the Association between Vegan Diets and Risk of Cardiovascular Disease. *Journal of Nutrition*. 2021;151(6):1539-1552. doi:10.1093/jn/nxab037

33.Sofi F, Dinu M, Pagliai G, et al. Low-calorie vegetarian versus mediterranean diets for reducing body weight and improving cardiovascular risk profile. *Circulation*. 2018;137(11):1103-1113. doi:10.1161/CIRCULATIONAHA.117.030088

34.Barnard ND, Alwarith J, Rembert E, et al. A Mediterranean Diet and Low-Fat Vegan Diet to Improve Body Weight and Cardiometabolic Risk Factors: A Randomized, Cross-over Trial. *Journal of the American Nutrition Association*. 2022;41(2):127-139. doi:10.1080/07315724.2020.1869625

35.Esparza-Ros F, Vaquero-Cristóbal R,, Marfell-Jones M. *International Standards for Anthropometric Assessment*. (UCAM Universidad Católica de Murcia, ed.). International Society for the Advancement of Kinanthropometry. ; 2019.

36.Frisancho AR. Triceps skin fold and upper arm muscle size norms for assessment of nutrition status. *Am J Clin Nutr*. 1974;27(10).

37.Faria A, Sousa-Santos A, Mendes J, Sousa A, Amaral TF. Desenvolvimento das versões portuguesas dos questionários FRAIL Scale e SARC-F: ferramentas de rastreio para a fragilidade física e sarcopenia. *Acta Portuguesa de Nutrição*. 2021;26. doi:10.21011/apn.2021.2614

38.Lopes Ferreira P. Development of the Portuguese version of MOS SF-36. Part II -- Validation tests. *Acta Med Port*. 2000;13(3):119-127.

39.SCORE2-OP working group and ESC Cardiovascular risk collaboration. SCORE2-OP risk prediction algorithms: estimating incident cardiovascular event risk in older persons in four geographical risk regions. *Eur Heart J*. 2021;42:2455-2467.

40.SCORE2 working group and ESC Cardiovascular risk collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. *Eur Heart J*. 2021;41:2439-2454.

Statistical Analysis Plan

The analysis will be carried out using the Statistical Package for the Social Sciences (SPSS), version 27.0. All normally distributed numerical variables will be presented in the format of mean ± standard deviation. The normality of the variables will be tested using the Kolmogorov-Smirnov test. Group differences will be determined using paired t-tests. ANOVA with repeated measures will be used to detect changes between and within groups. Results will be considered statistically significant at $p<0.05$.

Informed Consent Form (ICF)

Consent informed, clarified and free for clinical investigation

in accordance with the Helsinki Declaration and the Oviedo Convention

Study Title: Impact of a Mediterranean Diet (GREENHEARTEDIET - RATE) on cardiovascular disease risk factors: a randomized clinical trial.

Background: This study will be conducted under the Algarve Active Ageing - Cardiac and Osteoarthritis Rehabilitation (A3-COR): personalized physical exercise protocol in the rehabilitation after acute myocardial infarction or at risk of cardiovascular disease and knee osteoarthritis, a multicenter clinical intervention study.

Study Explanation: This is a non-pharmacological intervention clinical study in which the following will be evaluated: 1) Sociodemographic data and medical history with a close-ended questionnaire to characterize the participant (sex, race, nationality, marital status, cohabitation, socio-economic and educational level, current employment status, dietary habits, medication, and medical history; 2) Body composition measurements with a bioimpedance analyzer, body circumferences, and skinfold thickness; 3) Biochemical data including C-reactive protein level, glycated hemoglobin, and lipid profile; 4) Health-related quality of life using the Short-Form 36 questionnaire - version 2; 5) Dietary pattern assessments using the PREDIMED questionnaire, 24-hour dietary recall, and food diary; 6) Frailty assessment using the FRAIL Scale questionnaire; 7) Sarcopenia assessment using the SARC-F questionnaire; 8) Grip strength measurement using a dynamometer.
Participants will be allocated to one of the following groups: nutritional intervention group (DIET+EXERCISE) and control groups (NO DIET+EXERCISE; NO DIET+NO EXERCISE). There will be two assessment points for all participants: at the beginning of the study and at the end (12 weeks after the intervention). Both control groups will receive a single educational meal session, while the intervention group will receive monthly nutritional follow-up with the prescription of the nutritional intervention (RATE) being studied.

The nutritional component of the study, guided by a nutritionist, aims to evaluate the effect of a nutritional intervention on improving important cardiovascular disease risk factors such as body weight, percentage of body fat, and waist circumference. This intervention will consist of nutritional recommendations for a healthy diet, including the predominance of plant-based foods over the consumption of animal-based and processed foods. No food groups included in

the food pyramid will be excluded.

This assessment does not pose any risks to the health and physical and mental integrity of the participants. The collection of blood samples through finger-prick may be considered a possible, and only, discomfort for the participants.

Funding: This study will be funded by the A3COR Project - Algarve Active Ageing-Cardiac and Osteoarthritis Rehabilitation (Operation Code: ALG-01-0145-FEDER-072590), financed by the Algarve Regional Operational Program - CRESC ALGARVE 2020, and by the School of Biotechnology of the Catholic University of Portugal. This study does not include the payment of travel expenses or compensation to the participants.

Voluntary participation: You have the freedom to refuse to participate in this study or withdraw your consent, suspending participation at any time. Participation is voluntary and your refusal to participate will not involve any penalty or loss of benefits.

Confidentiality and anonymity: The collected data will be confidential and for the exclusive use of this study. The data will be anonymized, and its processing will comply with the Data Protection Regulation, with no publication of data that allows identification. All collected information will be deleted immediately after the completion of the study and publication of the respective scientific article. The collection, encoding, processing, storage, and destruction of participant data are the responsibility of the research team. All contacts are made in a private environment.

The researcher responsible for the study is Ana Rita Mendes Barbosa, a registered nutritionist with the Portuguese Order of Nutritionists, with professional license number 4946N. In case of doubts, you can contact through ana.rita.mendes.barbosa@gmail.com.

Name of investigator requesting consent: _____

Signature:_____ Date:__/__/_____

I declare that I have read and understood this document, as well as the verbal information provided to me by the person who signs above. I have been assured the possibility to refuse participation in this study at any time without any consequences. Therefore, I agree to participate in this study and allow the use of the data voluntarily provided by me, trusting that they will only be used for this research and any potential secondary studies, and in the confidentiality and anonymity guarantees given to me by the researcher.

Name:_____

Signature:_____ Date: __/__/_____