PROTOCOL

Cardiovascular Risk Prevention With a Mediterranean Dietary Pattern Reduced in Saturated Fat (CADIMED):

Randomized Controlled Intervention Study with Metabolomic Analysis and Gut Microbiota

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1. SUMMARY

Cardiovascular disease (CVD) is the leading cause of death worldwide. The most important cardiovascular risk factors are modifiable, and it has been shown that an adequate diet is essential for the prevention of CVD due to its effect on blood pressure, lipids, obesity, inflammation and endothelial function. Among the main dietary macronutrients, saturated fatty acids (SFA) are of interest for their effects on the elevation of low-density lipoprotein cholesterol (LDL-C) and total cholesterol, which are associated with increased cardiovascular risk due to endothelial dysfunction and atherosclerosis. However, recent research suggests that not all SFA exerts the same effects and that certain foods such as red and processed meats contain a more unfavourable SFA profile, which can have important repercussions in relation to cardiovascular risk. The CADIMED study is an eight-week randomized, controlled intervention study comparing the effect of an intervention that promotes the reduction of red and processed meat intake in a sample of 156 adult subjects (>18 years) and dyslipidaemia. **Objectives**: 1) To develop a dietary intervention that promotes the reduction of consumption of SFA from red and processed meats in patients with elevated LDL-C; 2) To determine the efficacy of this dietary intervention aimed at eliminating red and processed meat, in the context of a Mediterranean dietary pattern, on LDL-C and palmitic acid after eight weeks of intervention (main objective); 3) To determine the metabolic alterations parallel to the modifications in the intake of SFA from red and processed meat, using a complete lipid and metabolomic profile and other markers of cardiovascular risk (biomarkers of inflammation, endothelial damage, insulin resistance and diabetes; changes in blood pressure; anthropometry and C-reactive protein); 4) To assess the effect of the intervention on dietary intake of various nutrients and other lifestyle factors; 5) To evaluate the effect of the intervention on metabolites derived from the gut microbiota; 6) Establish a working panel with patient and public participation (PPP) as well as health professionals, to improve research management and dissemination of project results.

Keywords: cardiovascular disease, Mediterranean diet, saturated fats, LDL cholesterol, red meat, palmitic acid.

2. BACKGROUND

Worldwide, cardiovascular disease (CVD) is the leading cause of mortality; the World Health Organization estimates that, in 2019, 32% of deaths worldwide were caused by CVD, mainly acute myocardial infarction.[1] The most important cardiovascular risk factors are modifiable and are associated with poor diet, lack of physical activity, tobacco and alcohol use. Among them, it has been shown that an adequate diet is essential for the prevention of CVD given its effects on blood pressure, lipids, obesity, inflammation and endothelial function.[2] Of the dietary macronutrients, saturated fatty acids (SFA) are of great interest due to their effects on the elevation of low-density lipoprotein cholesterol (LDL-C) and total cholesterol, which are associated with increased cardiovascular risk due to endothelial dysfunction and atherosclerosis.[3] Because of this, recommendations from different organizations and institutions, such as the *American Heart Association* and the *European Society of Cardiology*, indicate that AGS should represent less than 10% of total energy intake,4 preferably between 5% and 6%, to prevent CVD.[5] A meta-analysis of clinical trials by the Cochrane group has shown that reducing SFA consumption significantly decreases LDL-C and up to 17% cardiovascular events.[6]

However, current clinical practice guidelines on the prevention and treatment of CVD propose a healthy diet generally low in SFA but without offering detailed or specific advice for effective intervention.[4,7] This is important as different food sources of SFA could pose different health risks.[8] Foods contain complex mixtures of SFA and evidence suggests that some of them, such as lauric, myristic, and palmitic acid, may pose different risks.[9,10] Red meat contains a large proportion of palmitic acid [long-chain SFA (16:0)] and, although the current evidence is inconsistent and based mostly on observational studies, an increased risk of CVD associated with a higher intake of red and processed meat, compared to plant protein sources, has been documented, probably mediated by changes in the lipid profile. inflammatory or insulin resistance.[11,12] However, other SFA-rich foods, such as milk and cheese, contain large proportions of shorter-chain [lauric (12:0) and myristic (14:0)] fatty acids and have not been shown to be associated with CVD.[13] It has been suggested that dairy products have other elements such as calcium, which could counteract the unfavourable physiological effects of SFA.[14]

The principal investigator of this project has recently led a preliminary intervention study for the reduction of dietary saturated fat in a population with cardiovascular risk.[19] The final results found a non-significant decrease in SFA intake and beneficial changes in blood lipid profile were observed among those participants who reduced their consumption of red and processed meats the most, compared to those who reduced other sources of SFA such as dairy.[19] Ultimately, the scant current evidence suggests that different sources of SFA and individual proportions of SFA in red and processed meat may influence cardiovascular profile differently, although robust intervention studies are still needed to confirm this. Likewise, changes in the fatty acid and blood lipid profile resulting from a dietary approach based on a Mediterranean diet pattern along with the elimination of red and processed meat consumption are largely unknown. Therefore, it is important to investigate how these dietary modifications affect cardiovascular risk factors in the context of a Mediterranean diet in which

the consumption of unsaturated fats from olive oil or nuts is positively associated with less CVD.[15]

On the other hand, it is worth mentioning that despite recent efforts in public health, the reduction of SFA consumption through education or information programs in the population has not been sufficient. Previous research establishes that it is possible to reduce the intake of products rich in SFA by providing foods that replace them, and/or through intensive and adapted dietary advice, with the participation of specialized personnel and behavioural support.[15-18] However, there is little evidence on the effectiveness of other less intensive and low-cost interventions that can reach a larger number of people and have a significant effect on the population. Simple, food-specific dietary guidance (e.g., red meat) related to cardiovascular risk may have greater potential to be easily disseminated and adopted by a large number of primary care patients who need help to improve the quality of their diets and thus decrease their cardiovascular risk.

Based on what has been described, the CADIMED study aims to develop and test the efficacy of a dietary intervention to reduce the consumption of SFA from red and processed meat and to evaluate the effects on various biomarkers of cardiovascular risk in adults with dyslipidaemia. The determination of the lipid profile, metabolomic profile and the composition of the intestinal microbiota will allow a better understanding of the questions raised and will help to establish simple dietary guidelines based on a better approach for the treatment of dyslipidaemia and the prevention of CVD, relevant to the third Sustainable Development Goal (SDG) of the United Nations. The low cost and scalability of the intervention can help the Spanish health system, specifically primary care, particularly by helping the management of patients at risk of CVD, reducing the affected population and healthcare costs. In addition, the decrease in the consumption of red and processed meat also implies effects on global warming and environmental sustainability, contributing to achieving the 12th SDG.

3. HYPOTHESIS

A dietary intervention based on the exclusion of red and processed meat within a Mediterranean dietary pattern will have a better effect on lipid profile and cardiovascular risk markers compared to the usual recommendation to follow a healthier lifestyle.

4. OBJECTIVES

4.1. General objective:

To develop and determine the efficacy of a dietary intervention that promotes the elimination of SFA consumption from red and processed meats and to evaluate its impact on lipid and cardiovascular risk markers in patients with dyslipidaemia.

4.2. Specific objectives:

a. To develop the necessary elements for the application of a dietary intervention that promotes the reduction of the consumption of SFA from red and processed meats in patients with dyslipidaemia
b. To test the efficacy of a dietary approach aimed at avoiding red and processed meat, in the context of a Mediterranean dietary pattern, on LDL-C and palmitic acid (main results) after eight weeks of intervention, compared to the usual approach of healthier lifestyle recommendations.
c. To determine the metabolic alterations parallel to the changes in SFA intake, from red and processed meat, by means of a complete lipid and metabolomic profile, as well as the determination of other intermediate markers of CVD.
d. To assess the effect of the intervention on dietary intake and other lifestyle factors.
e. To evaluate the effect of the intervention on the composition of the gut microbiota and its derived plasma metabolites that could be influenced given the expected metabolic benefits;
f. Establish a working panel with patient and public participation (PPP) as well as health professionals, to improve research management and dissemination of project results.

5. METHODOLOGY

5.1. Experimental design

The CADIMED study is a randomized, controlled (two parallel arms) dietary intervention study, where the effect of an eight-week behavioural intervention consisting of a Mediterranean dietary pattern with elimination of red and processed meat will be evaluated, compared to the general advice for decreasing SFA intake in patients with elevated LDL-C.

To this end, 156 patients ≥18 years of age with dyslipidaemia and who express their desire to improve their diet and reduce the risk of CVD) will be recruited. The study will be registered in ClinicalTrials.gov and a Good Clinical Practice and Trial Quality Assurance strategy will be established (see section 11 for other ethical and legal considerations).

5.2. Study population

Inclusion criteria:

1) Signed informed consent;

2) Age ≥18 years to ≤75 years;

3) Indicated to start lifestyle intervention based on recent values (e.g., last 3-6 months) of LDL-C≥116 mg/dL and <190 mg/dL; as long as pharmacological intervention has not been advised and started (*ESC/EAS Guideline, Rev Esp Cardiol. 2020*);

4) Device access (e.g. *smartphone, tablet)* with internet and digital skills for the use of the mobile application;

5) Motivation and willingness to be assigned to any of the groups to improve their diet, as well as commitment to carry out the assigned protocol.

Exclusion criteria:

1) Patients with familial hypercholesterolemia and/or on treatment with lipid-lowering drugs (including statins, ezetimibe, PCSK9 inhibitors, fibrates, bile acid chelators, Omega 3 fatty acids, nicotinic acid/Vit B3), dietary supplements or functional foods for the treatment of dyslipidaemia (plant sterols, monacolin, red rice yeast, fibre supplements 3-10g (plantago), policosanol, berberine and soy protein/lecithin) in the last 3 months;

2) Cardiovascular risk that makes the use of drugs necessary, for example, in patients with recent and/or established CVD; type 1 and 2 diabetes;

3) Uncompensated thyroid function disorders; relevant comorbidities (including liver failure and cholestasis, chronic kidney disease, Cushing's syndrome, nephrotic syndrome, obesity grade III – BMI>40kg/m2); cancer; psychiatric disorders and/or that in the opinion of the researcher makes it difficult to comply with or follow up on the study;

4) Excessive alcohol consumption: >40 g/d (4 UBEs/day) in men and >20-25 g/d (2-2.5 UBEs/day) in women (*Ministry of Health, July 2020*)

4) Pregnancy, lactation, perimenopause (amenorrhea <1 year), and women who change, start, or end hormonal contraceptive treatment during the study;

5) Participation in other research or dietary pattern (e.g. vegetarian and vegan) that could interfere with the demands of the study;

6) Failure to provide informed consent or failure to complete the initial assessment.

5.3. Procedure and data collection

All data collected from patients will be stored in a password-protected database and encrypted/pseudonymized with a unique code for each patient. The code will consist of two letters (initials of the health centre where the patients come from) and three numbers that will be assigned in ascending order. Only the research team and the health authorities, who have a duty of confidentiality, will have access to all the data collected for the study. Only information that cannot be identified may be transmitted to third parties.

1) Patient recruitment and pre-selection: Initial recruitment will be done through health professionals in primary or secondary care practices, who will help distribute information and participation in the study. Interested persons will contact the research team via telephone or email to confirm that they meet the inclusion requirements through a brief initial pre-selection questionnaire (Annex 1 – Pre-selection Questionnaire). Volunteers who meet the inclusion requirements will be personally scheduled for an initial visit with the research team and the clinical team at the Virgen de las Nieves hospital (Granada).

2) Informed Consent: During the first initial visit with the research team, study information will be given to patients (Patient Information Sheet and Informed Consent) and the informed consent will be read and signed. The opportunity to resolve issues related to the study and participation will be offered and those who complete the informed consent will become part of the study.

3) Questionnaires and measures: The research team will administer during the initial visit a general questionnaire to obtain the patient's demographic characteristics, including age, sex, education, socioeconomic class, alcohol intake, physical activity, diet and smoking (Annex 2 – Data collection notebook). The clinical team (nurse from the Virgen de las Nieves Hospital with links to the project) will be responsible for taking clinical data related to the medical history and/or use of relevant medications. Height, weight and blood pressure will then be measured at the beginning and at the follow-up in a standardized manner.

In addition, help will be offered to access and use a mobile application (designed especially for the study) to start collecting data on diet. The app will include each patient's unique code and a built-in diet questionnaire that will need to be completed at least twice a week (ideally every day), during the first and last week of the intervention to obtain valid measures of usual intake. In addition, a questionnaire on the frequency of food consumption during the previous 4 weeks will be completed to assess the eating pattern.

4) Blood samples: the nurse in charge of the project will take blood samples during the 1st initial visit and at the end of the follow-up of each patient, with an overnight fast of 8 hours. The samples will be processed in the laboratory of the Virgen de las Nieves Hospital for subsequent analysis in the same hospital; in addition to the transfer/storage of the remaining samples in our laboratory of the Institute of Nutrition and Food Technology (INYTA) (details on page 11-12).

5) Stool samples: stool samples will be taken at the beginning and end of follow-up for which patients will be provided with the necessary material and instructions for collection (e.g. taking antibiotics) and storage of the samples. The samples will be delivered to the research team during the 2nd initial visit for processing and storage in our laboratory at the Institute of Nutrition and Food Technology (INYTA).

6) Randomization and assignment of the intervention group. During the 2nd initial visit, and once it has been confirmed that patients meet all the inclusion requirements, the intervention group will be randomly assigned and the corresponding explanation to the patient will be carried out.

Table 1. Data collection at CADIMED study visits

	Evaluation Preview	1st visit initial	2nd visit initial	Visit End
a. Pre-selection questionnaire (via telephone)	x			
b. Signed consent		x		
c. General questionnaire (demographics, lifestyle)		x		x
d. Anthropometric measurements, blood pressure, clinical data		x		x
e. Physical activity questionnaires, 24-hour food and diet patterns		x	x	x
f. Blood sample collection		x		x
g. Stool sample collection			x	x
h. Randomization and Intervention Allocation			x	

5.4. Intervention

Participants will be randomly assigned, in a 1:1 ratio, to one of two groups:

Intervention group: Mediterranean diet without red and/or processed meat

They will receive motivation and behavioural support through the use of the mobile application, which will provide them with reminders to self-monitor their changes in eating; while the rest of the clinical care will be usual. After randomization of the patients, the research team will give them more information about the use of the mobile app, with specific

indications to avoid red and processed meat in the context of a healthy Mediterranean dietary pattern, taking into account the key elements proposed in the dietary guidelines for CVD prevention. The app will provide details of Mediterranean diet recommendations, as well as recipes, tips and alternatives to replace meat when cooking at home or eating out. Participants will record their daily diet through the mobile application, during the first and last week of the intervention. The rest of the clinical care will be usual.

Control group: Mediterranean Diet

Similarly, the control group will be helped to download and use the mobile application, although they will only have access to a restricted version of it, by which they will receive brief and general information to maintain a healthy lifestyle following the general guidelines on the Mediterranean diet (without specific recommendations to reduce red and processed meat). Participants will also record their daily diet through the app, during the first and last week of the intervention. The rest of the clinical care will be usual.

5.5. Randomization

Randomization will be computer-generated, with sex, age, obesity, and diet (fiber intake) as stratification variables using random blocks of size 2 and 4. The computer program will reveal the group assigned at the same time to the researcher, without the option of viewing the groups that will be assigned to future patients.

It will not be possible to blind patients to the intervention group due to the nature of the intervention; however, the primary outcomes of efficacy measures will be objectively measured (blood lipids) and are not subject to interpretation by investigators. To the extent possible, the study team performing all measurements will remain blind to group assignment during the study.

5.6. Definition of variables: main and secondary results

With the determinations and measurements derived from the CADIMED study, it is proposed to investigate the following results (comparing the intervention group and the control group):

1) *Main results*: changes in LDL-C and fatty acid profile in erythrocytes after 8 weeks of intervention
2) *Secondary outcomes*:
    a. Changes in other metabolites derived from lipid and metabolomic profile analysis, including circulating fatty acids, HDL-C, total cholesterol, triglycerides, and endogenous and exogenous metabolites related to cardiovascular risk and meat consumption (e.g., TMAO, etc.);
    b. Changes in other related markers of cardiovascular risk, including biomarkers of inflammation and endothelial damage, insulin resistance, and diabetes (glucose, insulin, HbA1c, C-reactive protein, IL6, TNF-alpha), blood pressure, weight, and body and waist mass index;

c. Changes in the gut microbiota and derived metabolites
d. Changes in dietary intake (measured by 24-h recall): SFA intake, total energy intake, total fat, sugar, fiber, salt, general dietary patterns, red and processed meat consumption.
e. Changes in other lifestyle-related factors: collected through physical activity, smoking, and alcohol questionnaires

3) *Evaluation of the process*: analyses of both quantitative and qualitative measures will be included to understand whether the intervention has worked and through which processes. These measures include: the rate of recruitment of volunteers and the rate of patients completing the study; acceptance of the intervention through questionnaires at the end of follow-up; the use of the mobile application to carry out changes in the diet as well as the self-monitoring of intake; measures that evidence the contamination of the control group; changes in patients' ability and knowledge about diet, SFA, and cardiovascular disease.

5.7. Data analysis

Sample size

A recent randomized, controlled, peer-to-population trial testing similar dietary approaches to reduce SFA intake showed a long-term change in LDL-C of approximately 15 mg/dL (SD26).[19] If 78 participants are recruited in each group (n = 156), it is expected to detect a minimum difference of 15 mg/dl between the intervention group and the control, with a power or detection capacity of 90%, an error $\alpha = 0.05$ and a dropout rate of 20% using intention-to-treat analysis, so the final sample size includes an additional 20% of participants to mitigate dropouts.

Statistical analysis of primary and secondary outcomes

Baseline characteristics will be summarized in descriptive statistics in each study group, statistical analysis will be performed with STATA IC 16.0 software (College Station, TX: StataCorp LLC 2019). Descriptive statistics will be presented with standard deviation (*SD*) and 95% confidence intervals. Continuous variables will be presented as mean and standard deviation unless the use of the median and interquartile range is necessary; Categorical variables will be expressed as frequencies and percentages.

Efficacy results will be analysed by intent to treat, i.e., all participants will be assessed according to the group to which they are randomly assigned, regardless of their adherence. The analysis of effectiveness measures will use data from all participants for whom a baseline and monitoring measure exists. A linear regression model will be used to calculate the difference in measures with 95% confidence intervals, with adjustment of the initial values of the dependent variable and the place of recruitment. Before the analysis, the assumptions of linear regression, specifically homoscedasticity and the normality of the residual distribution, will be explored. Where these assumptions are not met, appropriate non-parametric analyses shall be used. The sensitivity of the results to confounders due to

differences in baseline characteristics will also be examined. A second sensitivity analysis will use the techniques of "baseline observation carried forwards" (using the initial measure of the variable) and "multiple imputation" (using information about the assigned group and the characteristics of the participants) to impute missing values in the variables of the main outcome. An exploratory analysis of subgroups of results will be carried out according to sociodemographic characteristics.

5.8. Determination of metabolomic profiles and gut microbiota

This project will employ a metabolic and metagenomic profiling approach (gut microbiota) using state-of-the-art analytical technologies to provide a deeper understanding of metabolic responses to dietary intervention (reduction of red/processed meat intake) in the context of a Mediterranean dietary pattern. Therefore, lipid profiling and a complete metabolomic and metagenomic analysis will be performed to investigate specific changes caused by the intervention compared to the control. The determinations will be made at the hospital (general biochemistry) as well as at the Institute of Nutrition and Food Technology (INYTA). Once the main determinations explained below have been carried out and with prior consent, the surplus samples with their corresponding code will be stored at -80ºC in the Biobank of the Public Health System of Andalusia (BBSSPA), for future analyses related to the project, including aliquots with leukocytes for DNA extraction and erythrocytes for studies of antioxidant defense enzymes.

5.8.1. Lipid metabolism, endothelial function and inflammatory markers

Fasting venipuncture blood samples will be obtained at the beginning and end of follow-up: two 10 mL tubes of K2E EDTA, and two 10 mL tubes of serum separator gel. First, part of the sample will be processed in the hospital according to its own established protocols to obtain blood biochemistries for the blood lipid fractions: total cholesterol, LDL-C (primary outcome), HDL-C, non-HDLc, triglycerides, glucose, insulin, HbA1c and CRP.

The rest of the samples will be processed at INYTA to obtain plasma and stored (-80 °C) until they are processed for analysis. The new LC-MS will be used to determine a specific plasma fatty acid profile, including palmitic acid (primary outcome), among others. Other plasma biomarkers of endothelial function, such as ICAM-1, VCAM-1, ELAM-1, and MPO, will be determined using Luminex®'s xMAP technology and MULTIPLEX kits; as well as endothelin-1 and angiotensin II using ELISA kits. Inflammatory biomarkers in plasma will also be determined with Luminex® xMAP and HSTCMAG-28SK kits, including IL-6, IL-8/CXCL8, IL-10, IL-23, TNF-α, IFN-γ. ELISA *kits* will be used to quantify lipopolysaccharides (LPS) and LPS-binding lipoprotein (LBP).

5.8.2. Metabolomic determinations

Plasma metabolomic profiles will be determined in a non-targeted manner using an LC-MS platform, although targeted analyses will also be proposed to determine endogenous and

exogenous metabolites specifically related to cardiovascular risk and meat consumption (e.g., TMAO, etc.). Non-targeted approaches are used to simultaneously measure hundreds/thousands of metabolic traits that then allow a metabolic signature to be profiled. For metabolite extraction, plasma samples will be mixed with an organic solvent preparation for protein precipitation, cooled, centrifuged, and filtered before being analysed. During this step, quality control (QC) samples will be prepared by taking 10 µL aliquots from all samples. Additional CC samples will be collected from subgroups of each group for further fragmentation analysis. The analysis will be carried out using two different LC methods coupled to an Orbitrap Q-Exactive instrument operating in positive and negative ionization modes, respectively, in order to relatively separate and quantify plasma metabolites. Pre-processing of the raw data generated will include removal of non-significant information, alignment, filtering, QC titration, drift correction, and normalization of the intensity of each ion, and will be carried out using MD-DIAL, XCMS, or a combination of proprietary R-based codes. A final table will be obtained containing: 1) mass/load ratio (m/z, mass error $\leq$ 3 ppm); 2) retention time (RT, min) and 3) chromatographic peak area for each of the metabolites. Additional fragmentation data will be acquired to improve information on metabolites. From current public metabolomic databases and from our own collection, metabolites will be mapped based on their m/z signal and fragmentation data. Identification will be carried out using appropriate chemical standards where possible. The information will be reported following the method suggested by Summer et al.

5.8.3. Determination of the composition of the gut microbiota

It will be carried out by 16S rRNA analysis of the samples obtained and previously processed and stored at -80º C. DNA will be obtained from the stool sample using the QIAamp DNA stoolmimi kit (Qiagen, Barcelona, Spain) and the PureLink™ Microbiome DNA purification Kit (Invitrogen). The quality and quantity of the same will be checked spectrophotometrically (NanoDrop ND-100). The variable V3-V4 region of the 16S rRNA gene will be amplified using specific oligonucleotides, by PCR. A second amplification will then be performed using barcode oligonucleotides. The purified PCR product will be sequenced on illumina's MiSeq platform.

5.8.4. Statistical analysis of metabolomic and metagenomic data

A "Knowledge Discovery from Databases" (DCBD) process will be followed, which will consist of:

- Selection of variables of interest and groups of subjects for analysis.

- Identify distinct clinical feature sets that are grouped into particular individual groups (phenotypic sets) without regard to their microbiota/metabolomic background.

- Examine the response associated with the intervention in each individual identified phenotypic set.

- Identify metabolomic networks composed of DMRs and metabolites that interact with each other.

- Integration of all previously identified patterns into a multi-layered space using genetic algorithms. The idea of this final step is, once the interesting variables derived from each of the previous approaches (phenotype, microbiota and metabolomics) have been identified, to incorporate them all together for the construction of a predictive model that allows the classification of good and bad responders to treatment.

To this end, different statistical methodologies will be used to identify anomalies, groups or patterns of metabolites, including principal component analysis (PCA) for the initial clustering. To differentiate metabolite patterns between the intervention and control groups, before and after the intervention, a novel multivariate approach, orthogonal partial least squares multiblock analysis of variance (AMOPLS), will be used. The AMOPLS consists of the application of a core-based multiblock OPLS approach on the pure matrices and those augmented by the residues arising from the ANOVA decomposition of the design response matrix and provides, as a product, a global view of the important effects on the model, which allows its interpretation. Analysis of variance and/or linear regression will test the main effects of: 1) time (follow-up *vs.* baseline); 2) intervention group *vs*. control; 3) interaction time x intervention group. Univariate analyses will be used to compare baseline means between the intervention and control groups using Student's t-tests. A false discovery rate (q) will be calculated to reduce false positives with a cut-off point < 0.1.

For the extraction of bacterial DNA from faeces, the commercial QIAamp Stool Mini Kit (Qiagen, Venlo, The Netherlands) will be used. The quality and quantity of the extracted DNA will be spectrophotometrically checked (NanoDrop ND-100). The variable region V4 515F-806R of the 16S rRNA gene will be amplified using specific s-oligonucleotides, by PCR. A second amplification will then be performed using barcode oligonucleotides. The purified PCR product will be sequenced on illumina's MiSeq platform, and the protocols to be followed will be those recommended by the manufacturer. Data analysis. The analysis will be carried out with the QUIIME2 v.2019.4 program. The data will be filtered with the q2-dada2plugin implemented in the R software (DADA2 method), obtaining the corresponding ASV (Amplicon sequence variance) correspondents. The taxonomic assignment will be determined with respect to the GreenGenes database (v.13.8). Both alpha diversity (Shannon index) and beta diversity (Unifrac index) will be determined. For the detection of differences in taxonomic composition, the ANCOM method implemented in QUIIME2 will be used. A prediction of the metabolic functions of the metagenomes will also be made using the PICRUSt2 tool.

For all these analyses , statistical software such as SIMCA® (Umetrics, Umea, Sweden), Stata and R will be used. Artificial intelligence methods, such as ARM (association rule mining) and GA (generic algorithms) will be used to combine and analyze the phenotypic and metabolomic data in order to understand the most predictive patterns that are associated with better outcomes in this population.

6. USEFULNESS AND APPLICABILITY

Patients at risk of cardiovascular disease will benefit the most from the results of this project. Clinical guidelines for reducing cardiovascular risk emphasize the importance of dietary change and recommend that all patients with dyslipidaemia be supported to change their diet. However, dietary interventions are uncommon and there is limited evidence of their effectiveness in the setting of routine primary or secondary care. At present, there are no comprehensive resources to enable physicians and health care professionals to offer brief dietary advice on the management of dyslipidaemia. This project will develop and test a dietary intervention that is easy to implement by clinical staff without much prior knowledge, but also intuitive so that patients with elevated LDL cholesterol can follow and maintain a healthier diet. If we get the expected results, this project will provide an effective approach to offer dietary advice within the Spanish healthcare system. The low cost and increased scalability of this intervention could substantially help the Spanish healthcare system to manage the large number of patients at risk of CVD who could benefit from this intervention. This in turn could translate into lower costs derived from medical care in the Spanish population.

In addition, the results of this project will inform food-based dietary guidelines on the most promising approach to the treatment of dyslipidaemia at the population level. Therefore, the findings of this study will be relevant to other countries where red and processed meat intake is above dietary recommendations, e.g. the UK or the US. The intervention materials and mobile app that will be developed in this project (potential intellectual property) will provide a low-cost, widely disseminated approach to providing dietary advice to all people who need it.

Finally, if a reduction in red and processed meat leads to health benefits, this will also have implications for global warming and environmental sustainability, as consuming less red meat could help protect the environment as well as improve human health. Therefore, this project will also contribute to achieving the Sustainable Development Goals (Sustainable Development Goals) by promoting healthy eating habits that are compatible with human and planetary health.

7. WORK PLAN

The CADIMED project is divided into four phases of work with a total duration of three years and two blocks aimed at the communication and dissemination of the results, as well as project management, which will happen simultaneously throughout the three years (see Table 2). The first year will be dedicated to the development of the intervention, recruitment of the working panel with health professionals and patients/interested persons, development of study materials and protocols along with the ethical approval of the intervention study. Approximately 15 months will be spent on participant recruitment and intervention. The last year will be devoted to metabolomic determinations. The communication and dissemination of results has been planned towards the end of the second year and during the third year. The management activities of the project will be regular (monthly, quarterly or annual) to ensure its development and the achievement of results and indicators.

The PI will be responsible for the coordination and administration of the project and will be supported by the rest of the research team and other collaborators. Steering committee meetings will be held every 6 months to provide progress on the project.

In addition, a research assistant will be hired to assist with patient recruitment and sample and data collection, as well as to assist with the overall management of the project. Specifically, the research assistant will support the PI in the development of the dietary intervention, including the materials that will be distributed to participants as part of the intervention; mobile application development; recruitment of PPIs and health professionals in the study; contact and management of participants; baseline and follow-up measurements; determinations in biological samples; Collaboration in data management and data analysis. The PI and the research group will have the capacity to train this person in the required skills and competencies.

Table 2. CADIMED Study Timeline

ACTIVITY	YEAR 1				YEAR 2				YEAR 3				MONTH
Phase I – Development of the intervention													
Preparation of materials for the intervention	■	■											1-6
Phase II – Working Panel													
Recruitment of health professionals	■	■	■	■									1-12
PPP Recruitment	■	■	■	■									1-12
Phase III – CADIMED Randomized, Controlled Intervention Study													
Study protocols, consent forms, ethical approval	■	■	■										1-9
Recruitment of participants, implementation of the intervention, data collection				■	■	■	■	■					9Mon-24
Trial data management and statistical analysis								■	■	■			21-30
Phase IV – Lipid, Metabolomic, and Metagenomic Profiling													
Lipid profile and other CVR markers								■	■	■	■		21-33
Metabolomic and metagenomic profiles								■	■	■	■		
Data management and statistical analysis								■	■	■	■		21-33
Communication, dissemination and exploitation of data													
Communication, dissemination and exploitation plan								■					21
Participation in outreach and engagement activities							■					■	18, 36
Manuscript preparation and submission									■			■	24, 36
Preparation of results and attendance at congresses									■			■	24, 36
Preparation of materials for use in the health system										■			27
Project Management													
Meetings with team members	■	■	■	■	■	■	■	■	■	■	■	■	Monthly
Data Management Plan Development				■									9
Administrative meetings and project reports		■				■				■			6, 18, 30
Trial steering committee meetings, coordination and evaluation seminars		■				■					■		6, 18, 33

8. DISSEMINATION PLAN

1) Academic results

- This project will produce high-quality, open-access publications, including potential journals such as Circulation, Annals of Internal Medicine, PloS Medicine or BMC Medicine, which are the main journals with a recent interest in these topics ranging from basic to clinical metabolic research. At least 2 other important publications are expected to emerge from this project: one describing the results of all metabolomics evaluations; and another with dietary and other CVD-related outcomes.

- The PI and research team have a proven track record in this field internationally (UK, USA), with frequent invitations to national and international conferences.

2) Public Health

- A guide with recommendations will be developed using the results of this project and will use the established links to promote knowledge transfer within the public health and regional health system (e.g. Health Ministry) as well as in Hospital Endocrinology Units and in Primary Care.

3) Media

- The results are expected to be disseminated in news published by the National Societies of Endocrinology and Nutrition (e.g. SEEDO, SEEN, SEÑ).

- National and regional health-related television programs.

- Dissemination of research activities in the communication unit of the University of Granada, including science fairs, or related activities in museums.

- Dissemination on social networks, including Researchgate, Facebook and Twitter.

- International coverage: the research team has extensive international collaboration networks, with multiple projects and publications in collaboration with international researchers.

4) Participants and members of the public (PPI)

- Diverse PPI members will be actively involved by writing summaries of the results and assisting with the communication strategy. PPI members will also participate in the preparation of infographics that will be publicly available in the Endocrinology Units of the Hospitals and in Primary Care.

9. BUDGET

For the realization of this project, funds are contemplated from two calls:

1. Call Ministry of Science and Innovation, Knowledge Generation Projects 2021, PID2021-127312OA-I00 (see table 3 with budget granted). This budget will be used to cover most of the expenses derived from the execution of the project, with the exception of the analysis of the microbiota.

2. Ramon y Cajal 2020 Call, RYC2020-028818-I, Ministry of Science and Innovation. It has a budget of €42,000 for the execution of research activities, which will be used to pay for microbiota analyses.

Table 3. Budget granted from the Ministry of Science and Innovation Call

Knowledge Generation Projects 2021 **PID2021-127312OA-I00** **Budget granted**	
GOODS AND SERVICES	BUDGET (€)
Material Fungible:	
Blood determinations: plasma lipid profiles, biochemistry, biomarkers of CVD and inflammation. *Sample of 156 patients x 2 points= 352 x €52/each=€18300*	18300
Metabolomic Determinations *Sample of 156 patients x 2 points= 352 x 100€/each=35200€*	35200
Other materials: printing, letters, creation and maintenance of databases	2000
Software Licenses:	
Mobile App Software	5000
EXPENSES OF HIRING PERSONNEL SPECIFICALLY FOR THE PROJECT including *salaries, employer Social Security contributions, legally established indemnities and other expenses derived from the hiring duly justified*	
Research support staff:	
Research Assistant (e.g. Undergraduate Student in Nutrition)	64500
TRAVEL, ACCOMMODATION AND MEALS:	
Travel and subsistence allowances for International Congresses	
FENS (Federation of European Nutrition Societies). 3 dias (Europa)	1300
OTHER EXPENSES	
Open Access main results	2500
Auditing	1200
DIRECT COSTS	130,000
INDIRECT COSTS *(21%)*	27,300
TOTAL GRANTED	157,300

10. INFRASTRUCTURE AND EQUIPMENT

Both the University of Granada and the Institute of Nutrition and Food Technology (INYTA), in the Department of Biochemistry and Molecular Biology II, as well as the collaboration with the Virgen de las Nieves hospital (Dr. Carmen Tenorio), make the project have all the necessary structural and technological resources to guarantee the adequate development of this research project and meet the needs of those who work in this institution. The link with the Virgen de las Nieves clinical team will facilitate access to the participants of the CADIMED study, as well as the necessary resources to obtain medical records, samples and blood biomarkers. In addition, the department has invested in the technical resources necessary to carry out the metabolomic analyses, including a new LC-MS, with high analysis speed and sensitivity, obtained with funds from the Ministry of Science, Innovation and Universities with ERDF funding (exp. No. EQC2019-006110-P). Currently, the department has a very advanced information system, with high-speed internet, a data centre equipped with state-of-the-art servers, a data storage area based on fibre optics, automatic backup systems and uninterrupted power supply. These advanced technological structures are complemented by a wide range of office, statistical, technological and document management software, as well as open access to any publication through the open access library.

Research Team

Dr. Carmen Piernas, PI of this project, has eight years of experience in postdoctoral research and holds a PhD in Nutrition and Epidemiology from the University of North Carolina at Chapel Hill (USA). The PI of this project has worked in the *Health Behaviours* team in the Department of Primary Care and Health Sciences of the University of Oxford (UK), so she brings important experience and skills in nutrition, epidemiology and behavioural sciences, as well as has demonstrated a strong leadership capacity to carry out projects successfully, from the conception of the research idea, to the application for funding and the conduct of the research. It was the PI of the *Primary Care Shopping Intervention for Cardiovascular Disease Prevention* (PCSHOP) study that was conducted at the University of Oxford; this study aimed to test new behavioural approaches to achieve reductions in SFA intake among patients with elevated LDL-C and has provided fundamental preliminary results for its advancement in this area of research and finding effective dietary approaches in CVD prevention. The project has resulted in several relevant publications, the main one in *PLOS Medicine*. During the management of the PCSHOP study, Dr. Piernas gained important skills and knowledge to successfully plan and conduct randomized clinical trials; he also led more recent behavioural dietary interventions (SwapSHOP and SaltSwap studies) in which he participated in the development of two mobile apps that help give dietary advice and facilitate self-management of dietary changes.

Dr. Piernas joins the CTS-461 group "Biochemistry of Nutrition, Therapeutic Implications" (www.bionit.ugr.es), co-directed by ***Dr. Concepción Aguilera*** within the Department of Biochemistry and Molecular Biology II and the Institute of Nutrition and Food Technology (INYTA), located at the Biomedical Research Centre of the University of Granada (UGR)

and the Biosanitary Institute of Granada (IBS. Granada) accredited by the ISCIII. This group is an excellent environment for the successful implementation of the CADIMED project. Prof. Aguilera and her group have demonstrated extensive experience in the fields of omics, in particular genetics, epigenetics and metabolomics. Prof. Concepción Aguilera and her team members are leading highly competitive research on the molecular basis of obesity and metabolic syndrome, and are one of the most fruitful institutions in medical sciences at the University of Granada, for which they have obtained funding for several projects, including nutritional interventions, within the biomedical sciences:

1. Association between genetic variants, biomarkers of oxidative stress, inflammation and cardiovascular risk in children with obesity. GENOBOX study. 2012/2015. Carlos III Health Institute – Ministry of Science and Innovation (EU funding): €143,330
2. Puberty and metabolic risk in obese children: epigenetic alterations and pathophysiological and diagnostic implications (PUBMEP). 2017/2020. Carlos III Health Institute – Ministry of Science and Innovation (EU funding): €131,890
3. Omics and artificial intelligence as tools to understand the molecular mechanisms of insulin resistance in children with obesity during puberty (IBEROMIC). 2021/2023. Carlos III Health Institute – Ministry of Science and Innovation (EU funding): €173,030
4. Clinical evaluation of an olive oil enriched with bioactive compounds with antihypertensive and cardioprotective properties. Research and development of new products for a healthier shopping basket that improves child nutrition (MEDKIDS). CIEN (CDTI – Ministry of Industry and Energy). 2019/2023. Total funding: €247,026
5. Evaluation of an olive oil spread, enriched with phytosterols and bioactive compounds with cholesterol-lowering and cardioprotective properties. Research and development of new products for a healthier shopping basket that improves child nutrition (MEDKIDS). CIEN (CDTI – Ministry of Industry and Energy). 2019/2023. Total funding: 140,000 €

This project has also formed a new multidisciplinary research group that has a wide range of experts, from nutritional sciences to behavioural sciences and biochemistry/molecular biology, as well as clinical experts in endocrinology and nutrition. This team will cover the knowledge, skills and competencies necessary to ensure that the project is viable, credible and obtains the proposed results:

Dr. Carmen Tenorio Jiménez, Medical Specialist in Endocrinology and Nutrition (Virgen de las Nieves University Hospital). His group has relevant experience in dietary interventions in patients with risk factors for CVD and obesity. Lead and coordinate the clinical team to recruit more health care professionals to help perform the intervention.

Dr. Martín López de la Torre, Medical Practitioner, Head of the Endocrinology and Nutrition Service (Virgen de las Nieves University Hospital). His group has relevant experience in dietary interventions in patients with related risk factors. She will support Dr. Tenorio in coordinating the clinical team.

Dr. Carolina Gómez Llorente, Associate Professor of the Department of Biochemistry and Molecular Biology II, Faculty of Pharmacy, Institute of Nutrition and Food Technology, Biomedical Research Center, University of Granada. He has relevant experience in the study of the gut microbiota as well as the integration of related clinical data.

Dr. Oscar Rangel Huerta, *Norwegian Veterinary Institute* (*Veterinærinstituttet*), Oslo, Norway. His expertise is related to metabolomic determinations as well as statistical analyses of metabolomic data.

Dr. Nerys Astbury, *Nuffield Department of Primary Care Health Sciences*, University of Oxford, UK. She is a senior nutrition researcher with expertise in behavioral science and dietary interventions in primary care and other clinical settings, involving patients with cardiovascular disease, diabetes, and obesity.

Dr. Aurora Perez Cornago, *Nuffield Department of Population Health*, University of Oxford, United Kingdom. She is a senior researcher (university research professor) with a background in nutrition and epidemiology. He has researched the relationship between meat consumption and cardiovascular diseases and has worked on large clinical trials for the prevention of cardiovascular diseases, such as PREDIMED.

Dr. Marta Guasch Ferre, researcher, *Department of Nutrition, Harvard T.H. Chan School of Public Health;* Instructor of Medicine*, Channing Division of Network Medicine, Harvard Medical School, Boston* (Mass, USA). She has a background in nutrition, with experience in clinical trials and epidemiological studies around diet and chronic non-communicable diseases, including cardiovascular diseases.

Dr. Lourdes Chávez Alfaro, Medical Practitioner (General Medicine), PhD student in Nutrition and Food Sciences, University of Granada. He has relevant training and experience in clinical studies, as well as in the development and application of interventions to improve habits in at-risk patients. They will be in charge of carrying out the recruitment of patients, development and application of the intervention, laboratory analysis and preparation of samples for analysis, preparation and updating of the database, statistical analysis and will participate in the dissemination and preparation of the results publications.

11. ETHICAL AND LEGAL CONSIDERATIONS

The development of the project will be carried out in compliance with the Declaration of Helsinki of the World Medical Association 1964 and ratifications of the following assemblies (Tokyo 75, Venice 83, Hong Kong 89, Somerset West 96, Scotland 00, Seoul 08 and Fortress 13) on ethical principles for medical research on human beings, RD 1090/2015, of 24 December. of clinical trials, specifically the provisions of Article 38 on Good Clinical Practice, and the Convention on Human Rights and Biomedicine), signed in Oviedo on 4 April 1997 and successive updates.

The investigators participating in this study undertake that all clinical data collected from the study subjects will be separated from the personally identifiable data in order to ensure the anonymity of the patients; respecting Organic Law 3/2018, of 5 December, on the Protection of Personal Data and guarantee of digital rights, Law 41/2002, of 14 November (basic regulation of patient autonomy and rights and obligations in terms of clinical information and documentation), as well as Law 3/2001, of 28 May, (regulating informed consent and patients' medical records), Law 3/2005, of 7 March, amending Law 3/2001 and Decree 29/2009 of 5 February, which regulates access to electronic medical records.

All data related to the project will be coded/pseudonymized, protecting the patient's identity, specifically for this project. Only the research team and the health authorities, who have a duty of confidentiality, will have access to all the data collected for the study. Only information that cannot be identified may be transmitted to third parties, maintaining the encryption/pseudonymization. Once the study is completed, the data will be pseudonymised for future use (always related to this line of research), with express permission for this in the informed consent of the participants.

The handling of samples will follow the provisions of Law 14/2007 of 3 July on Biomedical Research, in RD 1301/2006, of 10 November, which establishes the quality and safety standards for the donation, procurement, evaluation, processing, preservation, storage and distribution of human cells and tissues and approves the coordination and operation rules for their use in humans and in the RD 1716/2011, of 18 November, which establishes the basic requirements for the authorisation and operation of biobanks for the purposes of biomedical research and the treatment of biological samples of human origin, and regulates the operation and organisation of the National Register of Biobanks for biomedical research. The samples will be processed in a coded way, protecting the patient's identity. Once the study is completed, the samples will be stored coded in the Biobank of the Public Health System of Andalusia (BBSSPA), with prior signed consent, for future analyses related to the project.

The processing, communication and transfer of data will be carried out in accordance with the provisions of the General Data Protection Regulation (Regulation (EU) 2016/679 of the European Parliament and of the Council, of 27 April 2016) and Organic Law 3/2018, of 5 December, on the Protection of Personal Data and Guarantee of Digital Rights.

The data collected will only be used for the purposes of the research study described in the protocol and kept for the time necessary to achieve the objectives of the study and in accordance with applicable legislation.

Personal data will be processed exclusively for the research work referred to in this document. The processing of the data of this study is legitimized by the signed consent to participate. No data will be transferred to third parties unless legally obliged. The patient may exercise their rights of access, rectification, deletion and portability of their data, limitation and opposition to their processing, in accordance with the provisions of the General Data Protection Regulation (GDPR 2016/679) before the principal investigator of the project, and may obtain information in this regard by contacting the research and clinical team.

The processing of personal data will be carried out using techniques to maintain anonymity through the use of codes, so that personal identity is completely hidden during the investigation process.

Based on the results of the research work, scientific communications may be prepared to be presented at congresses or scientific journals, but they will always be done with grouped data and nothing that can identify the patient will ever be disclosed.

12. BIBLIOGRAPHY

1. World Health Organization. Cardiovascular diseases (CVDs). WHO: Copenhague; 2021. Disponible en: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

2. Verschuren WMM, Boer JMA, Temme EHM. Optimal diet for cardiovascular and planetary health. Heart 2022;108:1234-9.

3. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation 2004;109:27-32.

4. Visseren FLJ, Mach F, Smulders YM, et al. ESC 2021 Guidelines on the Prevention of Cardiovascular Disease in Clinical Practice. Rev Esp Cardiol 2022;75:364-910.

5. American Heart Association. Saturated Fat. AHA: Texas: 2021. Disponible en: https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/fats/saturated-fats

6. Hooper L, Martin N, Jimoh OF, et al. Reduction in saturated fat intake for cardiovascular disease. Cochrane Database Syst Rev 2020; 8:CD011737.

7. Pavia AA, Alcocer MA, Ruiz ED, et al. Mexican clinical practice guidelines for the diagnosis and treatment of dyslipidemias and atherosclerotic cardiovascular disease. Arch Cardiol Mex 2022;92:1-62.

8. Astrup A, Magkos F, Bier DM, et al. Satured fats and health: a reassessment and proposal for food-based recommendations: JACC State-of-the-Art review. J Am Coll Cardiol 2020:76:844-57.

9. Chowdhury R, Warnakula S, Kunutsor S, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. 2014 160:398-406.

10. Zong G, Li Y, Wanders AJ, et al. Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. *BMJ* 2016; 355:i5796.

11. Guasch M, Satija A, Blondin SA, et al. Meta-analysis of randomized controlled trials of red meat consumption in comparison with various comparison diets on cardiovascular risk factors. Circulation 2019;139:1828-45.

12. Key TJ, Appleby PN, Bradbury KE, et al. Consumption of meat, fish, dairy products, and eggs and risk of ischemic heart disease. Circulation 2019;139:2835-45.

13. Visioli F, Poli A. Fatty acids and cardiovascular risk. Evidence, lack of evidence, and diligence. Nutrients 2020;12:3782.

14. de Oliveira Otto MC, Mozaffarian D, Kromhout D, et al, Dietary intake of saturated fat food source and incident cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. Am J Clin Nutr 2012;96:397-404.

15. Estruch R, Ros E, Salas J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med 2018; 378:e34.

16. Reidlinger DP, Darzi J, Hall WL, et al. How effective are current dietary guidelines for cardiovascular disease prevention in healthy middle-aged and older men and women? A randomized controlled trial. Am J Clin Nutr 2015;101:922-30.

17. Vafeiadou K, Weech M, Altowaijri H, et al. Replacement of saturated with unsaturated fats had no impact on vascular function but beneficial effects on lipid biomarkers, E-selectin, and blood pressure: results from the randomized, controlled Dietary Intervention and VAScular function (DIVAS) study. Am J Clin Nutr 2015;102:40-8.

18. Lin JS, O'Connor E, Whitlock EP, et al. Behavioral counseling to promote physical activity and a healthful diet to prevent cardiovascular disease in adults: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med 2010;153:736-50.

19. Piernas C, Aveyard P, Lee C, et al. Evaluation of an intervention to provide brief support and personalized feedback on food shopping to reduce saturated fat intake (PC-SHOP): a randomized controlled trial. PLoS Med 2020; 17:e1003385.

ANNEX 1. PRE-SELECTION QUESTIONNAIRE

The participant:	**Yes**	**No**
You have read and signed your informed consent		
You are 18 years of age or older but less than 76 years of age		
Has recent LDL-C values of 116 to 189 mg/dL with no indication/initiation of pharmacological intervention		
Is motivated to participate in the study and make changes to their diet		
You have access to a smartphone mobile phone with internet and digital skills for the use of the mobile application		
Are participating in another study or are following an incompatible diet (vegetarians, vegans)		
Are pregnant or have pregnancy plans during the time allotted for the study		
Is breastfeeding		
Is in perimenopause (amenorrhea < 1 year)		
Started, stopped, or made changes to hormonal contraceptive/replacement therapy dosing (last 3 months) or plan changes in the next two months		
You are taking or need lipid-lowering medications/supplements (see Table 1)		
Have a history of cardiovascular, thyroid, renal, hepatic, psychiatric disease, familial hypercholesterolemia, type 1 or type 2 diabetes (see Table 2)		
It has a BMI ≥ 40 kg/m2		
Are male and consume > 40 g/day (4 UBEs/day) of alcohol or are female and consume > 25 g/day (2.5 UBEs/day)		

Table 1. LIPID-LOWERING MEDICATIONS/SUPPLEMENTS (used within the last 3 months)		
	Yes	**No**
Statins (atorvastatin, rosuvastatin, simvastatin...)		
Ezetimiba		
Inhibidores de la PCSK9 (evolocumab, alirocumab, bococizumab…)		
Fibratos (fenofibrate, gemfibrozilo, clofibrate...)		
Bile acid chelators (cholestyramine, colestipol hydrochloride...)		
Omega-3 fatty acids		
Nicotinic acid/niacin/vitamin B3		
Plant sterols/phytosterols		
Monacoline		
Red Yeast Rice		
Fiber supplements, 3 to 10 g (P*lantagus psyllium*)		
Policosanol		
Berberine		
Soy protein/lecithin		
Hormonal contraceptives		

Table 2. MEDICAL HISTORY		
	Yes	**No**
Kidney disease (chronic kidney disease, nephrotic syndrome...)		
Liver disease (liver failure, cholestasis...)		
Thyroid disease		

Cardiovascular disease (myocardial infarction, cerebral vascular event, heart failure, arrhythmia, angina...)		
Systemic arterial hypertension		
Type 1 diabetes		
Type 2 diabetes		
Cushing's Syndrome		
Familial hypercholesterolemia		
Mental health disturbance		
Autoimmune disease		
Cancer		
Perimenopause (amenorrhea < 1 year)		
Current breastfeeding		
Current pregnancy		

ANNEX 2. DATA COLLECTION NOTEBOOK

A. GENERAL DATA QUESTIONNAIRE

Age: ___ years

Gender: ☐ Men ☐ Women

Employment status: What is your current employment status, or last occupation?

Active worker (incl. leave of absence)	☐
Temporary incapacity for work	☐
Unemployed (available and looking for work)	☐
Student	☐
Permanent incapacity for work	☐
Dedicated to household chores	☐
Performing social work or charitable activities without pay	☐
I've never had a paid job	☐
Other	☐

Educational Level: What is your maximum level of completed studies?

Cannot read or write	☐
Incomplete primary schools: they can read and write without having finished primary education	☐
Completed Primary: Complete LOGSE Primary or five approved EGB courses	☐
First stage of Secondary Education: School Graduate, Elementary Baccalaureate, EGB or ESO completed	☐
Baccalaureate studies: upper baccalaureate, BUP, baccalaureate new plan, PREU or COU	☐
Intermediate vocational training: industrial official, FPI, intermediate level training cycles	☐
Higher level vocational training: industrial master's degree, FPII, higher level training cycles	☐
First-cycle university studies: university diploma, architecture and technical engineering	☐
Second-cycle university studies: bachelor's, bachelor's, architecture and engineering	☐
University studies of doctorate, postgraduate, master's, MIR or equivalent	☐
Another possibility.	☐

Economic situation: Can you tell which of the following ranges best represents your entire household's net monthly income, after deductions for taxes, Social Security, etc.?

Less than 570 euros	☐
From 570 to less than 800 euros	☐
From 800 to less than 1050 euros	☐
From 1050 to less than 1,300 euros	☐
From 1,300 to less than 1,550 euros	☐
From 1,550 to less than 1,800 euros	☐
From 1,800 to less than 2,200 euros	☐
From 2,200 to less than 2,700 euros	☐
From 2,700 to less than 3,600 euros	☐
From 3,600 to less than 4,500 euros	☐

From 4,500 to less than 6,000 euros	☐
From 6,000 euros onwards	☐
He doesn't know	☐
No answer	☐

Ethnicity or cultural group: To which ethnicity or cultural group do you belong?

White – Caucasian	☐
Asian	☐
Maghreb	☐
Amerindian	☐
Gypsy	☐
Black	☐
Other	☐
He doesn't know	☐
No answer	☐

Tobacco use:

Smoker	☐
Number of cigarettes/tobacco per day:	
Ex-smoker	☐
Non-smoker	☐
He doesn't know	☐
No answer	☐

Alcohol Consumption: How often do you drink alcohol?

4 to 7 times a week (Number of unidades_____)	☐
2 to 3 times a week (Number of unidades_____)	☐
2 to 4 times a month (Number of unidades_____)	☐
1 or fewer times per month (Number of unidades_____)	☐
Never	☐
He doesn't know	☐
No answer	☐

Health-related quality of life: In general, you would say that your health is:

Excellent or very good	☐
Good	☐
Regular	☐
Bad	☐
He doesn't know	☐
No answer	☐

Family history: select those diseases that your parents, grandparents, children and/or siblings have:

High blood pressure (high blood pressure)	☐
Obesity	☐
Diabetes (blood sugar)	☐
Myocardial infarction	☐
Cerebral vascular disease	☐
Hypercholesterolemia (high cholesterol)	☐
Elevated triglycerides	☐

B. PHYSICAL EXAMINATION AND CLINICAL DATA		
	Initial assessment	**Final Evaluation**
Date		
Weight (kg)		
Size (cm)		
BMI (kg/m2)		
Waist circumference (cm)		
Blood pressure (mmHg)	PAS1: ____ PAD1: ____ PAS2: ____ PAD2: ____ PAS3: ____ PAD3: ____	PAS1: ____ PAD1: ____ PAS2: ____ PAD2: ____ PAS3: ____ PAD3: ____
Heart rate (bpm)		
Relevant medical history		
Use of relevant medications		

C. LABORATORY DATA		
	Initial assessment	**Final Evaluation**
Date		
Total cholesterol		
c-LDL		
c-HDL		
Triglycerides		
Glucose		
Insulin		
HbA1c		
PCR		

D. BIOMARKERS OF ENDOTHELIAL FUNCTION, INFLAMMATORIES AND COMPOSITION OF THE GUT MICROBIOTA		
	Initial assessment	**Final Evaluation**
Date		
ICAM-1		

VCAM-1		
ELAM-1		
MPO		
Endothelin-1		
Angiotensin II		
IL-6		
IL-8/CXCL8		
IL-10		
IL-23		
TNF-α		
IFN-γ		
LPS		
LBP		

E. QUESTIONNAIRE ON PHYSICAL ACTIVITY AND SEDENTARY LIFESTYLE	
The following questions are related to how long you do different types of physical activity. Try to answer, even if you don't consider yourself an active person.	
At work	
Think first about the time you spend at work, whether it is paid or unpaid employment, studying, maintaining your home, or the main activity you do daily. In these questions, "intense physical activities" refer to those that involve significant physical exertion and cause a great acceleration in breathing or heart rate. On the other hand, "moderate-intensity physical activities" are those that involve moderate physical exertion and cause a slight acceleration in breathing or heart rate.	
Q1. Does your job require intense physical activity that involves significant acceleration of breathing or heart rate (such as lifting, digging, or construction work) for at least 10 minutes at a time?	
☐ Do not → go to P4.	
☐ Yes	**Q2.** How many days a week? ____ days a week **Q3**. How long each day? ____ minutes each day
Q4. Does your job require moderate-intensity activity that involves a slight acceleration of breathing or heart rate (such as brisk walking or carrying light weights) for at least 10 minutes at a time?	
☐ Do not → go to P7.	
☐ Yes	**Q5.** How many days a week? ____ days a week **Q6.** How long each day? ____ minutes each day
To get around	
We will leave aside physical activities at work to know how you move from one place to another. For example, how they go to school, shopping, the market, work, etc.	
Q7. Do you walk or bike for at least 10 consecutive minutes on your commute?	
☐ Do not → go to P10.	

☐ Yes	**Q8.** How many days a week? ____ days a week **Q9.** How long each day? ____ minutes each day
In your free time	
The following questions exclude physical activity at work and for commuting. Now we will ask about other physical activities that you practice in your free time.	
Q10. In your free time, do you play strenuous sports that involve significant acceleration of breathing or heart rate (such as running, playing soccer) for at least 10 minutes at a time?	
☐ Do not → go to P13.	
☐ Yes	**Q11.** How many days a week? ____ days a week **Q12.** How long each day? ____ minutes each day
P13. In your free time, do you engage in any moderate-intensity activity that involves a slight acceleration of breathing or heart rate (such as brisk walking, cycling, swimming, playing volleyball) for at least 10 minutes at a time?	
☐ Do not → go to P16.	
☐ Yes	**P14.** How many days a week? ____ days a week **P15.** How long each day? ____ minutes each day
Sedentary behavior	
The next question refers to the time you usually spend sitting or lying down at work, at home, on the commute or with your friends. It includes time spent at a work table, sitting with friends, traveling by bus or train, playing cards, watching TV, using a mobile phone or video games, but it does not include time spent sleeping.	
P16. How much time do you typically spend sitting or lying down on a typical day? ___ hours : ___ minutes	

F. FEEDING QUESTIONNAIRE	
1. Do you use olive oil as your main cooking fat?	☐ Yes ☐ No
2. How much olive oil do you consume in total per day? (including the one used for frying, meals out, salads, etc.)	____ tablespoons
3. How many servings of vegetables do you eat per day? (garnishes or accompaniments = 1/2 portion) 1 portion = 200 g	____ ration
4. How many pieces of fruit do you eat per day?	____ Parts
5. How many servings of red meat, hamburgers, sausages or sausages do you consume per day? (1 serving = 100-150 g)	____ Servings
6. How many servings of butter, margarine or cream do you consume per day? (single serving: 12 g)	____ Servings
7. How many carbonated and/or sugary drinks (soft drinks, colas, tonics, bitters) do you consume per day?	____ Drinks
8. Do you drink wine? How much do you consume per week?	____ glasses per week
9. How many servings of legumes do you eat per week? (1 plate or portion = 150 g)	____ Servings
10. How many servings of seafood do you eat per week? (1 dish, piece or portion = 100-150 g of fish, or 4-5 pieces, or 200 g of seafood)	____ Servings
11. How many times do you consume commercial (non-homemade) pastries such as cookies, custards, sweets or cakes a week?	____ times per week
12. How many servings of nuts do you eat a week? (1 serving = 30 g)	____ Servings
13. Do you prefer chicken, turkey or rabbit meat instead of beef, pork, hamburgers or sausages? (chicken meat = 1 piece or serving of 100-150 g)	☐ Yes ☐ No
14. How many times a week do you eat cooked vegetables, pasta, rice, or other dishes seasoned with tomato, garlic, onion, or leek sauce simmered with olive oil (sofrito)?	____ times per week

G. FOOD FREQUENCY QUESTIONNAIRE

Please read the instructions carefully:

For each food, check the box that indicates the frequency of consumption, on average, during the past month. You will find foods that are consumed by season, if you did not consume them during the past month, try to remember their frequency of consumption in that season.

		AVERAGE CONSUMPTION IN A MONTH								
I. DAIRY		**Never or less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2-4	5-6	1	2-3	4-6	6+
1.	Whole milk (1 cup, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
2.	Semi-skimmed milk (1 cup, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
3.	Skim milk (1 cup, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
4.	Condensed milk (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
5.	Cream or heavy cream (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
6.	Milkshakes (1 glass, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
7.	Full-fat yogurt (1, 125 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
8.	Yogur discremated (1, 125 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
9.	Petit Suisse (1.55 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.	Cottage cheese or curd (1/2 cup)	☐	☐	☐	☐	☐	☐	☐	☐	☐
11.	Portioned or creamy cheese (1.25 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
12.	Other cheeses: cured, semi-cured (Manchego, Bola, Emmental...) (50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
13.	White or fresh cheese (Burgos, goat...) (50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
14.	Natillas, flan, puding (1, 130 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
15.	Ice cream (1 cone)	☐	☐	☐	☐	☐	☐	☐	☐	☐
II. EGGS, MEAT, FISH (100-150g dish or serving, except where indicated)		**Never the less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2-4	5-6	1	2-3	4-6	6+
16.	Chicken eggs (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
17.	Chicken or turkey WITH skin (1 serving or piece)	☐	☐	☐	☐	☐	☐	☐	☐	☐
18.	Skinless chicken or turkey (1 serving or piece)	☐	☐	☐	☐	☐	☐	☐	☐	☐
19.	Beef (1 portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
20.	Pork (1 serving)	☐	☐	☐	☐	☐	☐	☐	☐	☐
21.	Lamb meat (1 portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
22.	Rabbit or hare (1 portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
23.	Liver (beef, pork, chicken) (1 serving)	☐	☐	☐	☐	☐	☐	☐	☐	☐
24.	Other organ meats (brains, heart, sweetbreads) (1 portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
25.	Serrano ham or shoulder ham (1 slice, 30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
26.	York ham, cooked ham (1 slice, 30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
27.	Processed meats (salami, chorizo, black pudding, mortadella, sausages, sausage, sobrasada) (50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
28.	Patés, foie gras (25 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
29.	Burger (1, 50 g), meatballs (3 units)	☐	☐	☐	☐	☐	☐	☐	☐	☐
30.	Bacon, bacon, bacon (50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
31.	White fish: grouper, sole, sea bream, hake, whiting... (1 dish, piece or portion)	☐	☐	☐	☐	☐	☐	☐	☐	☐
32.	Oily fish: sardines, tuna, bonitl, mackerel, salmon (1 dish, piece or portion 130 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
33.	Salted fish: cod, salted fish (1 portion, 60 g dry)	☐	☐	☐	☐	☐	☐	☐	☐	☐
34.	Oysters, clams, mussels and the like (6 units)	☐	☐	☐	☐	☐	☐	☐	☐	☐
35.	Squid, octopus, baby squid, cuttlefish (1 portion, 200 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐

36.	Crustaceans: prawns, prawns, langoustines, etc. (4-5 pieces, 200 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
37.	Canned fish and seafood (sardines, anchovies, bonito, tuna) (1 small can or half a normal can, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
38.	Fish and seafood in oil (sardines, anchovies, bonito, tuna) (1 small can or half a normal can, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐

III. VEGETABLES (200g dish or portion, except where indicated)		**Never the less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2-4	5-6	1	2-3	4-6	6+
39.	Swiss chard, spinach	☐	☐	☐	☐	☐	☐	☐	☐	☐
40.	Cabbage, cauliflower, broccoli	☐	☐	☐	☐	☐	☐	☐	☐	☐
41.	Lettuce, endives, escarole (100 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
42.	Crudo tomato (1.50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
43.	Carrot, pumpkin (100 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
44.	Green beans	☐	☐	☐	☐	☐	☐	☐	☐	☐
45.	Aubergines, zucchini, cucumbers	☐	☐	☐	☐	☐	☐	☐	☐	☐
46.	Peppers (150 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
47.	Asparagus	☐	☐	☐	☐	☐	☐	☐	☐	☐
48.	Andalusian gazpacho (1 glass, 200 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
49.	Other vegetables (artichoke, leek, thistle, celery)	☐	☐	☐	☐	☐	☐	☐	☐	☐
50.	Onion (1/2 piece, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
51.	Garlic (1 clove)	☐	☐	☐	☐	☐	☐	☐	☐	☐
52.	Parsley, thyme, bay leaf, oregano, etc. (1 pinch)	☐	☐	☐	☐	☐	☐	☐	☐	☐
53.	Commercial French fries (1 bag, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
54.	Homemade fries (1 portion, 150 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
55.	Roasted or cooked potatoes	☐	☐	☐	☐	☐	☐	☐	☐	☐
56.	Mushrooms, chanterelles, mushrooms	☐	☐	☐	☐	☐	☐	☐	☐	☐

IV. FRUITS (one piece or portion)		**Never the less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2-4	5-6	1	2-3	4-6	6+
57.	Orange (1), grapefruit (1), or tangerines (2)	☐	☐	☐	☐	☐	☐	☐	☐	☐
58.	Banana (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
59.	Apple or pear (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
60.	Strawberries (6 units, 1 dessert plate)	☐	☐	☐	☐	☐	☐	☐	☐	☐
61.	Cherries, picotas, plums (1 dessert dish)	☐	☐	☐	☐	☐	☐	☐	☐	☐
62.	Peach, apricot, nectarine (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
63.	Sandía (1 tajada, 200-250 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
64.	Melon (1 slice, 200-250 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
65.	Kiwi (1 piece, 100 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
66.	Grapes (one bunch, 1 dessert plate)	☐	☐	☐	☐	☐	☐	☐	☐	☐
67.	Olives (10 units)	☐	☐	☐	☐	☐	☐	☐	☐	☐
68.	Fruits in syrup or juice (2 units)	☐	☐	☐	☐	☐	☐	☐	☐	☐
69.	Dates, dried figs, raisins, prunes (150 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
70.	Almonds, peanuts, hazelnuts, pistachios, pine nuts (30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
71.	Walnuts (30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
72.	How many days a week do you eat fruit for dessert?	☐ 0 ☐ 1 ☐ 2 ☐ 3			☐ 4 ☐ 5 ☐ 6 ☐ 7					

V. LEGUMES AND CEREALS (150 g plate or serving)	**Never the less than once a month**	**Per month**	**Per week**	**Up to date**

			1-3	1	2-4	5-6	1	2-3	4-6	6+
73.	Lentils (1 dish, 150 g cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
74.	Beans (pinted, white or black) (1 plate, 150 g cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
75.	Chickpeas (1 plate, 150 g cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
76.	Peas, broad beans (1 plate, 150 g cooked)	☐	☐	☐	☐	☐	☐	☐	☐	☐
77.	White bread, sliced bread (3 slices, 75 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
78.	Black or wholemeal bread (3 slices, 75 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
79.	Breakfast cereals (30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
80.	Whole grains: muesli, rolled oats, all-bran (30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
81.	White rice (60 g raw)	☐	☐	☐	☐	☐	☐	☐	☐	☐
82.	Pasta: noodles, macaroni, spaghetti, oysters (60 g raw)	☐	☐	☐	☐	☐	☐	☐	☐	☐
83.	Pizza (1 serving, 200 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
VI. OILS AND FATS (one tablespoon or individual serving. For frying, spreading, dipping on bread, dressing, or salads, use in total:)		**Never the less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2-4	5-6	1	2-3	4-6	6+
84.	Olive oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
85.	Extra virgin olive oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
86.	Pomace olive oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
87.	Corn oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
88.	Sunflower oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
89.	Soybean oil (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
90.	Mixture of the above (1 tablespoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
91.	Margarine (single serving, 12 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
92.	Butter (single serving, 12 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
93.	Lard (10 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
94.	Brand of olive oil you regularly use:									
VII. PASTRIES AND PASTRIES		**Never the less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2-4	5-6	1	2-3	4-6	6+
95.	Maria biscuits (4-6 units, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
96.	Wholemeal or fibre biscuits (4-6 units, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
97.	Chocolate biscuits (4 units, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
98.	Homemade pastries and biscuits (50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
99.	Croissant, ensaimada, tea cakes or other commercial industrial pastries (1.50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
100.	Donuts (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
101.	Muffins (1-2 units)	☐	☐	☐	☐	☐	☐	☐	☐	☐
102.	Cakes (1, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
103.	Churros, porras and similar (1 portion, 100 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
104.	Chocolates y bombones (30 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
105.	Cocoa powder or soluble (1 teaspoon for dessert)	☐	☐	☐	☐	☐	☐	☐	☐	☐
106.	Nougat (1/8 loaf, 40 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
107.	Mantecados, marzipan (90 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
VIII. MISCELLANEOUS		**Never the less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2-4	5-6	1	2-3	4-6	6+
108.	Croquettes, fritters, dumplings, precooked (1)	☐	☐	☐	☐	☐	☐	☐	☐	☐
109.	Soups and creams (1 plate)	☐	☐	☐	☐	☐	☐	☐	☐	☐
110.	Mustard (one teaspoon for dessert)	☐	☐	☐	☐	☐	☐	☐	☐	☐
111.	Commercial mayonnaise (1 tablespoon, 20 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
112.	Fried tomato sauce, ketchup (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
113.	Spicy: tabasco, pepper, paprika (1 pinch)	☐	☐	☐	☐	☐	☐	☐	☐	☐
114.	Salt (1 pinch)	☐	☐	☐	☐	☐	☐	☐	☐	☐

115.	Jams (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
116.	Sugar (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
117.	Honey (1 teaspoon)	☐	☐	☐	☐	☐	☐	☐	☐	☐
118.	Snacks other than chips, worms, popcorn, corn, etc. (1 bag, 50 g)	☐	☐	☐	☐	☐	☐	☐	☐	☐
119.	Other foods frequently consumed:									
-		☐	☐	☐	☐	☐	☐	☐	☐	☐
-		☐	☐	☐	☐	☐	☐	☐	☐	☐
-		☐	☐	☐	☐	☐	☐	☐	☐	☐

IX. BEVERAGES		**Never the less than once a month**	**Per month**	**Per week**			**Up to date**			
			1-3	1	2 - 4	5-6	1	2-3	4-6	6+
120.	Carbonated with sugar: drinks with cola, lemonades, tonics, etc. (1 bottle, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
121.	Low-calorie carbonated drinks, light drinks (1 bottle, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
122.	Fresh orange juice (1 glass, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
123.	Natural juices of other fruits (1 glass, 200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
124.	Bottled or canned fruit juices (200 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
125.	Decaffeinated coffee (1 cup, 50 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
126.	Coffee (1 cup, 50 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
127.	Tea (1 cup, 50 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
128.	Wort (100 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
129.	Rosé wine glass (100 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
130.	Muscat wine glass (50 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
131.	Young red wine glass, of the year (100 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
132.	Glass of aged red wine (100 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
133.	White wine glass (100 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
134.	Glass of cava (100 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
135.	Beer (1 jug, 330 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
136.	Liqueurs, anise or anisetae... (1 glass, 50 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐
137.	Spirits: whiskey, vodka, gin, cognac (1 glass, 50 cc)	☐	☐	☐	☐	☐	☐	☐	☐	☐

H. 24-HOUR REMINDER

Instructions:

Before you begin, please read the following observations that will help you optimize data collection.

The objective of this survey is to know their daily consumption of food and beverages. Write down, as accurately as possible, all foods and drinks consumed in the last 24 hours or the previous day. You can start with yesterday's breakfast and continue until you complete the memory of the entire day's diet. Write down the foods consumed between meals.

Write down the **type**/**variety** of the food (whole or skimmed milk, white or wholemeal bread, type of meat, type of fish, type of oil, etc.) and estimate the **amount** consumed in household measurements or servings (large, medium, small); the information shown on the packaging of many foods may be useful for this purpose. Write down the oil used in culinary preparations, bread, sugar or drinks consumed (water, soft drink, milk, coffee, juice, alcoholic beverages, etc.). Also, record the culinary **preparation** method (cooked, fried, roasted, etc.).

To make it easier to remember, initially write down the menu consumed at each meal and then describe the ingredients in detail. To help memory, it is practical to remember where you ate, with whom, at what time, who prepared the meal, etc; These details will help you "get into the situation."

Has the food you're about to record been different for any reason?
☐ Yes ☐ No

If yes, please indicate why:

_____

Indicate if you take supplements (type and amount):

_____

Date corresponding to the day of remembrance:	
Breakfast	
Menu (food and preparation)	**Ingredients** (type and quantity)
Drinks:	
Sugar:	
Mid-morning	
Menu (food and preparation)	**Ingredients** (type and quantity)
Food	
Menu (food and preparation) • First • Second • Dessert	**Ingredients** (type and quantity)
Drinks:	
Bread:	
Oil (Type):	
Snack	
Menu (food and preparation)	**Ingredients** (type and quantity)
Dinner	
Menu (food and preparation) • First • Second • Dessert	**Ingredients** (type and quantity)
Drinks:	
Bread:	
Oil (Type):	
Between meals	
Menu (food and preparation)	**Ingredients** (type and quantity)

LOG OF PROTOCOL CHANGES

Version	Date	Changes
1.0	11 November 2022	
1.1	3 February 2023	1. Inclusion of the SAS nursing team 2. Size of the randomization blocks: change from 8 and 16 to 2 and 4 due to the improvement in the balance and distribution of characteristics in the intervention groups 3. Inclusion of sensitivity analyses: baseline observation carried forwards (using the initial measure of the variable) and multiple imputation (using information about the assigned group and the characteristics of the participants) to impute missing values in the variables of the main outcome

Title: Prevention of Cardiovascular Risk with a Mediterranean Dietary Pattern reduced in Saturated Fat (CADIMED Study).

Principal Investigator: Dr. Carmen Piernas (carmenpiernas@ugr.es); Tel 615210894

Name and Surname PARTICIPANT: .....

Name and Surname INFORMANT: .....

MARK WITH A CROSS	YES	NO
I have read the information document that accompanies this consent (Patient Information), understand the risks and benefits involved, as well as the requirements for participation in the study		
I have received enough information about the study, I have talked to the study staff and I have been able to ask questions about it		
I understand that my participation is voluntary and I am free to participate or not participate in the study. If you choose not to participate, this decision will not affect the medical care you receive		
I understand that I can withdraw from the study at any time, without explanation. In this case, no new data will be added to the database.		
In accordance with LO15/1999, I understand that I can exercise the rights of access, modification, opposition and cancellation of my data. To do this, you must contact the Principal Investigator of the project. You can also request the destruction of the samples.		
I have been informed that the donation/information obtained will only be used for the specific purposes of the study and studies related to cardiovascular disease		
I understand that the study information will be confidential and that no unauthorized person will have access to the data or samples.		
I give my permission to be informed of my data and other personal data that may be obtained in the course of the investigation, including any unexpected discoveries that may occur, provided that this information is necessary to avoid serious damage to my health		
I freely consent to participate in this project		
I give my consent for the surplus samples to be kept anonymously in the Biobank of the Andalusian Public Health System for future use in related research		

Patient's signature

Name and surname .....
Date: .....

Signature of the reporter

Name and surname: .....
Date: .....