PROTOCOL OF THE CLINICAL TRIAL

CONTROLLED STUDY ON THE EFFECT OF MEDITERRANEAN DIET, RICH IN OLIVE OIL, ON THE REDUCTION OF THE CARDIOVASCULAR RISK OF PATIENTS WITH ISCHEMIC HEART DISEASE: THE CORDIOPREV STUDY

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I.- SUMMARY

Background: There are two dietary patterns, with potential beneficial effects on the prevention and treatment of arteriosclerosis: *the low-fat diet* and the *Mediterranean diet,* rich in olive oil. The low-fat diet has been recommended as the most appropriate to prevent ischemic heart disease, as several studies have shown that such a diet reduces coronary risk. In Mediterranean countries ischemic heart disease is less prevalent than in other industrialized countries, perhaps due to the consumption of the so-called Mediterranean diet. This has higher fat content, but comes mostly from olive oil, so it is mainly monounsaturated fat. There are no randomized studies showing that a high-fat diet is beneficial for the clinical course of coronary heart disease.

Hypothesis: To investigate whether the *Mediterranean diet,* rich in olive oil or *the low-fat diet,* are better at preventing complications and mortality in patients with high-risk coronary heart disease. The hypothesis will be null if no differences are observed between the two intervention groups.

Experimental design: A seven-year, randomized, simple-blind, controlled study in patients diagnosed with coronary heart disease. It is intended to evaluate whether when coronary patients consume a Mediterranean diet, rich in olive oil (34% of calories in the form of fat),the clinical evolution of their disease is modified, with respect to a low-fat diet. We intend to investigate, in a randomized unicentric clinical trial, in 1002 patients with coronary heart disease, whether a dietary intervention with Mediterranean Diet for seven years causes a difference in the rate of cardiovascular recurrence compared to a low-fat diet. The dietary intervention will be carried out by a team of registered dietitians. The primary target is a composite target for cardiovascular events that includes myocardial infarction, revascularization, ischemic stroke, peripheral arterial disease, and cardiovascular death.

Statistical analysis: The analysis will be performed under the principle of "intention to treat". The primary statistical comparison will be made using the log-rank test and Cox proportional risk models. Sensitivity analyses will be carried out under various assumptions. Statistical programs as SPSS and R shall be used.

II.- BACKGROUND AND CURRENT STATUS

1.- DIET AND CORONARY ARTERIOSCLEROSIS

Arteriosclerosis is the leading cause of death in Western countries and, according to estimates made for the year 2020, its global prevalence will increase in the next 25 years (1). The development of this disease is linked to the presence of several risk factors, among which are high blood pressure, high levels of low-density cholesterol (LDL) and decreased high-density cholesterol (HDL), diabetes mellitus, tobacco consumption and a sedentary lifestyle. Of these, the first three are closely related to the type of diet, and their appearance is associated with the presence of overweight or obesity (2,3). In the 50s the effect of the different nutrients of the diet on plasma cholesterol was studied. It was observed that the ones that most influenced it were fats, although each type of fatty acid had a different effect. After the studies of Keys et al (4) it was established that saturated fatty acids (SAT) raise total plasma cholesterol, so they were considered atherogenic, while polyunsaturated fatty acids (PUFA) lower it. By this action it was argued that foods rich in PUFA, especially vegetable oils and fish, would be the most beneficial products for the prevention of arteriosclerosis. In contrast, monounsaturated fatty acids (MUFA)

would have a neutral effect, as they do not influence plasma cholesterol levels. These facts led to the development of several intervention studies, to confirm whether the biochemical effect on cholesterol corresponded to a beneficial effect in the prevention of coronary heart disease. The results of these studies concluded that the increase in the PUFA/SAT ratio in the diet induces a decrease in mortality, especially manifest in patients with previous ischemic heart disease. This effect was related to the decrease in plasma cholesterol.
Below, the most important dietary intervention work carried out with diets rich in polyunsaturated fats is reviewed.

1.- Los Angeles Veterans Study (5).
It is a secondary prevention study, of 8 years duration, with a diet poor in cholesterol and very rich in PUFA (the total fat consumption was 40% of the global caloric intake and the P/S ratio was 6 times higher in the intervention group). When comparing the two groups of individuals (422 in the control group and 424 in the intervention group) a reduction in coronary episodes was demonstrated, in cases of sudden death and in cerebral infarctions. There was no change in non-cardiovascular mortality and the beneficial effects were more marked in younger individuals and in hypercholesterolemic patients. There were 44 non-fatal coronary episodes and 70 deaths from coronary causes in the control, compared to 36 non-fatal episodes and 48 deaths in the intervention group.

2.- Study of the Mental Hospitals of Finland (6).
It is a primary prevention study started in 1958 and carried out in two Finnish hospitals, in which a common diet, rich in animal fat, was compared with another rich in polyunsaturated fat (the diets were similar to the study of the veterans of the Ángeles). A total of 922 hospitalized patients participated and the duration of the study was 6 years. There were no significant differences for mortality from coronary heart disease (6 in the intervention and 12 in the control), but there were differences in the number of coronary episodes (39 in the intervention and 85 in the control), with a reduction of 43%. The beneficial effects corresponded to a decrease in plasma cholesterol.

3.- The Oslo diet-heart study (7).
It consists of a secondary prevention study, in which 412 men who had had a myocardial infarction the previous year were randomized. Two diets rich in fat (39%), one habitual and one rich in polyunsaturated fat (8.5% SAT; 10.1% MUFA and 20.7% PUFA) were compared. At 5 years, fatal and non-fatal infarctions were reduced and at 11 years there had been 32 deaths due to infarction in the experimental group and 52 in the control group ($p=0.04$), without changing the total mortality. The beneficial effects corresponded to a decrease in plasma cholesterol.

4.- Medical Research Council Study (MRC) (8).
It is a secondary prevention study in which the addition of 85 grams of soy oil (46% of total fat intake) to the diet of 199 men who had suffered a heart attack was compared. The control group consisted of another 194 males, who received fat of animal origin (44% of total fat intake). It lasted 8 years and coronary heart disease was reduced by 14%, although it did not reach a level of significance. Along with the beneficial clinical effect, a decrease in plasma cholesterol was observed.

The beneficial effects derived from these studies reinforced the lipid hypothesis, according to which the consumption of diets rich in PUFA reduces serum cholesterol and, therefore, reduces coronary risk. However,

throughout these years several uncertainties arose that have led to view with caution the increase in the content of PUFA in the diet. With the introduction of methods to assess HDL cholesterol, it was observed that the consumption of these fatty acids reduces this lipid fraction, which can be considered potentially harmful (9). In addition, several experimental studies were conducted on animals that demonstrated that a diet rich in PUFA can favor the development of solid tumors (10). On the other hand, with the knowledge that oxidation is a fundamental preliminary step in the development of arteriosclerosis, the concern arose that diets rich in PUFA could favor the oxidation of structures enriched in PUFAs, such as lipoproteins and cell membranes. All this, together with the absence of human populations that have consumed diets rich in PUFA for a long time, has led to recommending that the consumption of PUFA in the diet should be less than 10% of the global caloric intake and, even, it is recommended not to exceed 7% of said contribution (11), including both PUFA of plant origin, as well as those of animal, terrestrial or maritime origin. These facts mean that diets rich in PUFA are not considered the ideal alternative to replace the excess of SAT present in the diet of industrialized populations. For all these reasons, many of the expert groups, among which stands out the National Cholesterol Education Program (NCEP-USA), have developed general dietary recommendations to prevent arteriosclerosis, based on the consumption of a diet with a reduction in fat content. The objective is to reduce the caloric intake provided by these nutrients, especially with the reduction of SAT fat, enhancing the intake of complex carbohydrates. In this way, it is sought to lower the plasma level of LDL cholesterol. These recommendations have been reinforced by several intervention studies, which have shown that low-fat diets are associated with lower coronary risk. These studies are as follows:

1.- The Oslo Study (12).

It is a primary prevention study, with 1232 participants, undergoing an intervention for 5 years. Fat consumption was reduced from 44% of caloric intake to 28% and saturated calories from 18% to 8%. This work is one of the best exponents of reducing the contribution of fat (with a simultaneous elevation of the P/S ratio from 0.39 to 1.01) reducing plasma cholesterol and the risk of suffering a coronary episode. This risk was significantly reduced (22 non-fatal coronary episodes in the control and 13 in the intervention), although mortality did not change significantly.

2.- The South Wales Diet and Reinfaction Trial (DART study) (13)**.**

It consists of a prospective secondary prevention study, conducted in 2033 men who had had a myocardial infarction, under the age of 70. Three diets were compared, one with a reduction in the total fat intake (32% of the caloric intake, with an increase in the P/S from 0.4 to 0.78), another in which the consumption of fish was enhanced (it went from 0.7 grams/day of eicosapentaenoic acid to 2.3 gr./day) and a third in which cereals were added (from 9 fiber/day to 18). This study is considered an example of good randomization and demonstrated a reduction in total mortality and coronary causes in the group with a low-fat diet, which consumed fish.

3.- Life Style Heart Study (14).

Multifactorial intervention study, secondary prevention, which investigates the effect of a diet low in fat, together with physical exercise and relaxation techniques, on the evolution of lesions assessed by angiography. They studied 48 patients with coronary heart disease documented by angiography. The intervention included a low-fat diet, relaxation, physical exercise and quitting smoking. In the intervention group, cholesterol was reduced by 24%

and LDL by 37%. The effects were demonstrated by quantitative angiography and were reflected in the improvement of the percentage of stenosis. These effects were more marked in the group with more adherence to the diet.

4.- Study with cardioprotective diet in India (15)

It consists of a secondary, randomized, double-blind prevention study with 204 cases (with a diet with 24% fat) and 202 controls (with a diet with 28% fat). The study began 24-48 hours after suffering an acute myocardial infarction. In addition to the change in fat content, fruits, vegetables, legumes, nuts and fish were recommended in the intervention diet, until achieving 400 grams of vegetables and fruits were completed, compared to 185 grams in the controls. The intervention period lasted 1 year, after which it was shown that the diet used significantly reduced plasma cholesterol and total mortality (21 vs 38). Overall, the control group suffered 24% of non-fatal heart attacks vs 15% in the intervention group, with 17% deaths in the control group vs 10% in the intervention arm.

5.- Study of the Indo-Mediterranean diet (16)

Secondary prevention study, conducted in patients with ischemic heart disease, with a total of 1000 participants (501 controls and 499 in the intervention group. The former received a diet of type NCEP I, while the latter were advised to consume fruits, cereals, legumes, fruits and vegetables. In these, the intake of soybean oil and mustard was enhanced, with a contribution of 1.8 gr of linolenic acid, vs 0.8 gr. A decrease of a compound endpoint was observed in the intervention group (39 events vs 76) concluding that eating a diet rich in alpha-linolenic acid reduces cardiovascular risk.

6.- St Thomas` Atherosclerosis Regression Study (17,18).

This study investigated the relationship between dietary nutrients and the progression of arteriosclerosis, assessed with quantitative angiography. Coronary patients were included, followed for 39 months. The diet used was low in fat (27% of total energy) and was compared with a usual diet, of the same caloric content, but with a higher proportion of saturated and monounsaturated fat. It was shown that the main determinant of disease progression was the presence of saturated fat in the diet. The work was developed in 24 controls and 26 in the diet group. In addition, a third group of 24 people who were treated with cholestyramine was added. In the control group, lesions increased by 6%, in the diet group the changes were –1% and in the pharmacological treatment group –20%.

7.- Study of Lyon, with Mediterranean diet rich in linolenic (19).

Secondary prevention study, with a low-fat diet, with the peculiarity of being the only study in which a high percentage of MUFA was used. It was designed in a randomized way and the two diets used were low in fat, similar to those recommended by NCEP. The differences between both were that the intervention diet included margarine made of canola, and therefore with high content of oleic acid and linolenic. The work was interrupted when a significant difference in mortality was found between the two groups. In total, the follow-up was 27 months, after which 17 coronary deaths were collected in the control group and 3 in the intervention group, with an overall mortality of 20 and 8, respectively. A few months later, a difference in survival curves began to be observed, which the authors attributed to the antiarrhythmic effect of linolenic acid.

2.- JUSTIFICATION OF THE DIETS STUDIED

One of the main arguments, for the recommendation of diets rich in carbohydrates, is that such diets would combine in double benefit of reducing coronary risk and favoring weight loss. On the contrary, the use of diets with foods of high caloric density, such as olive oil, could favor obesity. However, controlled studies have not supported this idea (9), since it has been observed that when the fatty intake of the diet is restricted, there is, in the long run, a phenomenon of replacing it with carbohydrates, with which after a few months it stops losing weight or is limited to about 2.5 kg (20). This could explain why the prevalence of obesity has increased in the United States in the last 20 years, despite a reduction in caloric consumption from fats (21). The problem of obesity is highly complex and diet covers a possibly limited role (9). From a theoretical point of view, the possible advantage of a diet low in fats, with respect to a Mediterranean-type diet, will depend on the overall caloric intake and its balance with the components that determine caloric expenditure, which is related to lifestyle. Perhaps this justifies that in the Mediterranean countries the prevalence of obesity has been lower than in the United States, for many years, even though the diet in such countries, such as Spain, has had a higher percentage of fatty caloric intake than in the American (22-24).

Several decades ago, Keys A and collaborators published data from the 7-countries study, which highlighted that the diet rich in saturated fat is associated with higher cardiovascular mortality, while the consumption of monounsaturated fat was correlated with a lower frequency of death from this cause (25). Following such findings appeared the concept of Mediterranean diet, which highlighted the high consumption of monounsaturated fat provided by olive oil. But the type of Mediterranean Diet that Keys observed has changed substantially in recent decades. However, in the current epidemiological landscape, the large differences in the prevalence of coronary heart disease among European populations continue to be striking, since on the shores of the Mediterranean Sea death from ischemic heart disease has a much lower rate. Among the reasons for this difference has been argued the different lifestyle between Northern and Southern Europe, including different dietary customs. Given these facts, it has been proposed that the alternative to the high consumption of saturated fat, of the Anglo-Saxon countries, does not have to be exclusively the consumption of complex carbohydrates, but that foods rich in monounsaturated fat could beneficially replace this excessive consumption of saturated fat. However, while several studies are demonstrating the benefit of the low-fat diet, as we have discussed above, there is only one intervention study with MUFA-rich diets that demonstrates that coronary risk is reduced, the Lyon Study (19). In addition, a low-fat diet was used and canola margarine was used as a source of MUFA, instead of olive oil. Canola oil is richer in linolenic acid, which has led to suggest that the benefits obtained in this study are not properly derived from the high consumption of MUFA, but from the overall low-fat content and the greater presence of linolenic acid.

An argument in favor of the use of the Mediterranean diet, rich in olive oil, are the results of the Study of the 7 Countries, mentioned above. However, this study does not have the solidity of an intervention study and, in addition, it was done in populations in which the type of diet and lifestyle cannot be extrapolated to what we consider among ourselves as a Mediterranean Diet (25). On the contrary, some epidemiological studies, such as that of Framingham (26), have shown that MUFA consumption is correlated with higher coronary mortality, which

should not be surprising when you consider that in the United States most of the MUFA in the diet comes from meat products, which invariably implies that the more MUFA consumption, the greater the consumption of SAT. Other authors have related the lower mortality of Mediterranean countries with the greater dietary presence of foods rich in antioxidants, of which olive oil is one of them, although it is not the most important (27). All these facts suggest that if you want to recommend a diet whose main source of fat is olive oil, you have to develop an intervention study. Oliver (28) recently spoke in this regard when he wrote that, despite biological and epidemiological evidence, a randomized study has never been done on the potential effects of dietary supplementation with olive oil.

In support of carrying out this study, there are, at present, sufficient reasons to think that the diet rich in MUFA, from olive oil, is a good alternative to prevent arteriosclerosis. This evidence is of two types: Indirect evidence and population evidence, as we will analyze below.

2.- I: INDIRECT EVIDENCE SUPPORTING THE BENEFIT OF A DIET RICH IN MONOUNSATURATED FAT

a.- Lipid effects of the diet rich in MUFA

Elevated plasma levels of LDL cholesterol are positively correlated with the risk of developing coronary heart disease and there are multiple studies (29-35), which show that a diet rich in MUFA reduces its plasma levels. This effect, which is also shared by PUFA-rich diets, is especially relevant when they replace saturated fat in the diet. The biological mechanism of such action is not well known but is related to recovery in the expression of LDL receptors. Some studies have compared the effect of diets rich in MUFA with diets rich in fat, showing that, when you go from a diet low in fat to one rich in MUFA, LDL cholesterol does not change or decreases (33,35), so it can be said that a diet rich in MUFA, such as the Mediterranean Diet, is equal to or more beneficial than the diet rich in carbohydrates, to replace the diet rich in SAT, typical of Western countries.

But the most unique peculiarity of MUFA, and what distinguishes them from PUFA, is their effect on HDL cholesterol (24-26). There are enough intervention studies to accept that isocaloric enrichment of the diet with olive oil, will raise plasma levels of HDL cholesterol. Given the important inverse relationship between this lipid fraction and the risk of ischemic heart disease, this argument is of great force to recommend the Mediterranean-type diet in the prevention and treatment of coronary heart disease. On the contrary, the consumption of a diet rich in carbohydrates would imply a decrease in HDL cholesterol, both consumed in the form of sugars and complex carbohydrates. In addition, this effect will be prolonged as long as the low-fat diet is maintained (9, 31,35). This action of diets rich in carbohydrates is a cause for concern since several genetic syndromes, which occur with low HDL, are associated with premature coronary heart disease and several epidemiological studies have shown that the decrease in HDL cholesterol is associated with an increased coronary risk (36). On the other hand, the circumstances that decrease HDL, such as sedentary lifestyle, tobacco consumption, obesity, alcohol abstinence and the male gender, are also associated with increased cardiovascular risk. On the contrary, its elevation, as in pharmacological intervention studies, predicts, regardless of changes in LDL, the risk of ischemic heart disease (3). Animal studies, infusing HDL, protect against the development of lesions that can lead to arteriosclerosis (37).

All these facts generate great concern about the decrease in HDL cholesterol, with diets low in fat. It can be argued that certain populations that eat low fats, as in China or Japan, have low frequency of coronary mortality, but these populations have peculiarities that distinguish them, such as the low rate of obesity, increased physical exercise, low consumption of saturated fat and zero intake of trans fatty acids (9,38).

In recent years there has also been great interest in the possibility that the lipid benefit of olive oil depends not only on fat but also on its micronutrient content. An example of this is the recent work in which an oil rich in phenolic compounds induced a specific effect, increasing plasma levels of coronary HDL cholesterol (39). Therefore, seen as a whole, diets rich in MUFA, and therefore in olive oil, would be the ones that would provide the best lipid profile, since together with a decrease in LDL cholesterol would raise HDL cholesterol, while the opposite would happen with diets poor in fat, and this without considering the effect on HDL that could be generated by the richness of olive oil in phenolic compounds.

b.- Antioxidant effect of diets rich in MUFA

In the last decade, experimental evidence has accumulated indicating that LDL oxidation may be the initial phenomenon leading to the development of atheroma plaque (40). LDL circulates in the blood and passes through the endothelium, being in the sub-intimal space where the phenomenon of oxidation would occur. The oxidation of these particles depends on their composition and their richness in antioxidants. The oxidative process probably starts in the polyunsaturated fatty acids of its phospholipids, then spreading to the rest of the molecule and finally affecting the apoproteins. This induces a change in the biological characteristics of the particles and, among other facts, favors their uptake by macrophages, which will be activated and transformed into foamy cells. Diets rich in MUFA induce an enrichment of lipoproteins in oleic acid, which determines an increase in their resistance to oxidation, concerning diets rich in PUFA. Such evidence, demonstrated in animal experiments, has also been tested in humans (41,42). When the *in vitro* oxidation of LDL, obtained after consuming diets with different fatty acids, was studied, the lipoproteins obtained after the diet rich in olive oil were more resistant to oxidation than those obtained after the consumption of two diets rich in PUFA, whether these came from sunflower oil or fish (43). This phenomenon was independent of the vitamin E content of the particles and correlated with the enrichment in MUFA after the olive oil diet. In addition. it was demonstrated that the greatest resistance to oxidation of LDL was also produced by comparing the particles obtained after the diet rich in olive oil with those obtained after diets rich in saturated fatty acids of vegetable origin (palm oil) (43). These facts allow us to affirm that the Mediterranean type diet, rich in MUFA from olive oil, is a possibly superior alternative to the diet low in fats, in the prevention of oxidation and, possibly, the onset of arteriosclerosis.

But in addition, in recent years it has been shown that the consumption of olive oil is accompanied by antioxidant effects that depend on the non-fatty components of virgin olive oil. In a human intervention study (43) it has been observed that a breakfast made with olive oil, with 400 ppm of phenolic compounds, induces a postprandial decrease in lipoperoxides and F-2 isoprostanes, improving the endothelium-dependent microvascular response and increasing the bioavailability of nitric oxide. Moreover, serum from people who had followed a Mediterranean Diet reduces the proliferative layer of smooth muscle cells from human coronary arteries (44). This leads to

thinking that the olive oil of the diet can modify the behavior of the cellular elements involved in the formation of atheroma plaque.

c.- Diet rich in MUFA and thrombosis

The formation of a thrombus, in which platelets participate and the activation of coagulation and fibrinolysis, is a key fact in the development of clinical episodes of coronary heart disease. Both coagulation and fibrinolysis are supported by enzymatic cascades, in which multiple components interact, which conclude with the formation of thrombin, in the case of coagulation, or plasmin, in the case of fibrinolysis. Fatty acids in the diet can influence these mechanisms, as well as the platelet aggregation process. The consumption of a diet rich in fish reduces this aggregation, which is attributed to an increase in the synthesis of series-3 eicosanoids, with modification of platelet function (45). Similar effects have been described with diets rich in olive oil (46,47) through a change in the production of prostanoids and thromboxane. Several components of coagulation (von Willebrand Factor, Factor VII and fibrinogen) and fibrinolysis (t-PA and PAI-1) are known to predict the risk of coronary heart disease or new episodes with pre-existing vascular disease (48). The possible influence of fatty acids in the diet on these components is less known, although there are already indicative data that Factor VII levels rise with diets rich in saturated fatty acids and that in the postprandial phase their plasma levels increase when taking a diet rich in any fat (49,50). However, the consumption of a diet rich in MUFA, both in healthy people and in diabetic patients, showed that plasma levels of von Willebrand Factor decrease, reducing the risk of thrombosis (50-51). This opens up an interesting perspective on the antithrombotic effect of a diet rich in olive oil.

Another mechanism mentioned above, and which has great importance in the development of vascular thrombosis, is fibrinolysis. This process depends on the activation of plasminogen and is regulated by the balance between the tissue activator (tPA) and its most potent natural inhibitor, the tissue activator inhibitor type 1 (PAI-1). Studies with diets rich in fish oils, of the n-3 series, have shown that plasma levels of PAI-1 do not vary or rise, suggesting that such diets do not improve the fibrinolytic capacity of plasma. However, studies of our group have shown that the presence of olive oil, within a typical Mediterranean diet, reduces PAI-1 levels while reducing plasma insulin levels, suggesting that such a diet may improve fibrinolytic capacity and prevent vascular thrombosis (52, 53). These findings are of extraordinary interest, since the levels of PAI-1 and t-PA correlate with the risk of suffering a coronary episode and with the severity of angiographically assessed arteriosclerosis (48). In the same study, we found, additionally, that the presence of cholesterol in the diet did not reduce the beneficial effect determined by olive oil. It should be noted that the effect on fibrinolysis was observed when comparing the Mediterranean diet with a diet rich in complex carbohydrates, which allows us to consider that, concerning fibrinolysis, the diet rich in olive oil is the most appropriate for potential protection from vascular thrombosis.

d.- Diets rich in MUFA and blood pressure

Arterial hypertension is an important and independent cardiovascular risk factor, since it favors the appearance of cerebral vascular accidents and coronary heart disease. The development of high blood pressure is related to diet in a complex way since multiple nutrients can influence its appearance. Specially well known is its positive relationship with salt consumption and its negative association with foods rich in calcium, potassium and magnesium. Also, dietary fats can modify blood pressure values. The diet rich in SAT fat is accompanied by a

tensional increase, largely related to the higher caloric density of these diets and the relationship between SAT consumption and obesity. The unsaturated fatty acids, with the best-known action on blood pressure, are the PUFA of the n-3 series. The diet rich in fish, or supplemented with this type of PUFA, induces a tensional decrease, attributable to an increase in the synthesis of eicosanoids, with vasodilator action (54). These effects have not been as consistent when the diet has been enriched with PUFA of the n-6 series.

There are few randomized, controlled studies in which blood pressure has been measured in people on MUFA high-fat diets, although epidemiological data indicate that MUFA consumption is negatively correlated with blood pressure figures (55). Intervention work (56) has been able to demonstrate that a Mediterranean-type diet, rich in MUFA fat, reduces both systolic and diastolic pressure in young and healthy people. In other studies, in hypertensive women, these findings have been confirmed, providing evidence that the Mediterranean diet can modify the metabolism of prostanoids (57). Moreover, such findings have been observed in large-scale epidemiological studies, in which olive oil was the best predictor of the benefitS of Mediterranean Diet over blood pressure (58). Another interesting fact, highlighted, is that the isocaloric substitution of seed oils with olive oil reduces the need for antihypertensive drugs (59). However, there are still important uncertainties in this field, since a study by a Spanish group has shown that the type of olive oil consumption can be key, given that the generation of polar compounds in frying oil could favor the tensional increase (60).

e.- Diets rich in MUFA and carbohydrate metabolism:

Diabetes mellitus is a process that greatly increases the risk of developing ischemic heart disease and arteriosclerosis of different vascular territories. The onset of clinical diabetes, as well as the development of insulin resistance, are situations related to increased caloric intake and the onset of obesity. To control both processes, it has been recommended to use diets rich in complex carbohydrates, always with a reduction in the overall caloric intake if obesity exists. However, studies in diabetics have shown that diets rich in monounsaturated fatty acids increase peripheral insulin sensitivity and improve metabolic control (61). Based on these facts, some expert organizations have considered that greater consumption of MUFA would be a good alternative to classic diets, rich in carbohydrates (62), for better control of diabetic patients. This would place the Mediterranean diet in an excellent situation as an alternative to the use of the Low-Fat diet. But in addition to this potential effect for diabetic patients, data from our laboratory indicate that a Mediterranean-type diet, rich in olive oil, reduces plasma levels of insulin and glucose, both basal and after an oral glucose overload (63-64). This suggests that the MUFA provided by olive oil may increase peripheral sensitivity to the hormone.

f.- Diet rich in olive oil and endothelial protection

In the last decade, a special interest has been paid to the initial phenomena of atherogenesis, which begin with the oxidation of LDL, the development of a local inflammatory response, with endothelial dysfunction. The latter would include the loss of vasodilator capacity dependent on the endothelium and the activation of thrombotic phenomena. Since the diet rich in olive oil protects from oxidation, it stands to reason that it could also dampen inflammation and endothelial dysfunction. Studies from our group have shown that a Mediterranean Diet pattern improves endothelium-dependent vasodilation, reduces plasma levels of adhesion molecules of endothelial origin, in relation to their antioxidant potential (65). The possibility that such effects are attributable to MUFA fat or the

minority components of olive oil, remains to be elucidated. In *vitro studies* have shown that the addition of oleic acid to cellular preparations reduces the expression of adhesion molecules, while we have observed, on the other hand, that the acute intake of virgin olive oil decreases the expression of NFkB, a transcription factor, which mediate the inflammatory response, in circulating mononuclear cells of healthy people (66). However, the antioxidant effect could depend on the richness in phenolic compounds of the oil (67). These facts are of special interest because they are accompanied by a set of benefits of great preventive potential in patients with multiple risk factors, cataloged.

g.- Benefits of olive oil related to its non-fatty components

Olive oil is the only fat of vegetable origin that can be consumed without the need for industrial processing since, in its virgin and extra-virgin varieties, it is a genuine juice, a product resulting from the milling of the olive. This determines that virgin olive oil has a series of minority components, of a non-fatty nature, which in the rest of the oils are lost, mostly, during the process known as rectification or refining of the oil. Among such components are important substances for their potent neutralizing action of simple oxygen radicals, such as superoxide anion and lipid peroxides. Flavonoids deserve special mention, among which are anthocyanins, catechins, flavones and flavanones. On the other hand, olive oil presents an appreciable amount of tocopherols, such as alpha- and gamma-tocopherol, and phenolic compounds, which can potentially contribute to the protection of the body against oxidation, especially tyrosol and hydroxytyrosol. Studies from our group have shown that the intake of oils with different content of phenolic compounds induces specific effects, such as antioxidants and endothelial protection (43). But these components can also influence lipid levels (39) and inflammatory markers of endothelial origin (67).

2.- II: EPIDEMIOLOGIC EVIDENCE OF THE BENEFIT OF OLIVE OIL AND CHOICE OF DIET MODEL

In recent years, multiple observational studies have been published, both of cases and controls, as well as of the transverse and cohort type, showing that the Mediterranean Diet reduces cardiovascular risk, as reflected in a recent review (68). A prospective study (PREDIMED) is currently being developed in our country comparing the comparative effect of the consumption of nuts and olive oil, compared to low-fat diets. The first data are very encouraging, as both foods reduce the expression of traditional risk factors (69).

When defining the Mediterranean diet, which we intend to study, it is necessary to remember that this term is usually applied to the diet defined by Willet et al. (70), when it was elaborated the concept of the *pyramid of the Mediterranean diet.* To do this, it took into account the data on food consumption from the eastern Mediterranean, corresponding to the decade of the 60s, which is very different from the subsequent reality (22-24). The main characteristic of the aforementioned Mediterranean diet is the possibility that it had a high percentage of fat (up to 35%), provided that the SAT was at a low limit (7-8%) and that the main source of fat was olive oil. As a special fact, in some Mediterranean regions, such as the island of Crete, fat consumption reached up to 40%.

The diet that is currently followed in countries like ours is far from corresponding to this diet, since, with the introduction of the typical habits of Anglo-Saxon countries, the consumption of total fat has gone in a few years from 30% to 40% of the total caloric, as Moreiras and Varela have already pointed out (22,23). But in addition, the increase in fat has occurred at the expense of a higher intake of SAT fat of animal origin, dairy products and polyunsaturated fat, from seed oils (23). On the contrary, in the last thirty years the consumption of olive oil, the most frequent source of MUFA in the Mediterranean diet, has been reduced. An idea about this is given by the fact that olive oil consumption could be around 60 grams oil/inhabitant/day, in the 60s, while the 1990s was at 37 grams/person/day (24).

Given these facts, and with the initial results of the PREDIMED study, the diet we propose to study is a diet that adapts to the Mediterranean diet model described by Willet (70), with a relatively high-fat content, but with a low percentage of saturated fat and, above all, with a fat intake superior to the NCEP recommended diet of and to the one used in the Lyon Study (19). This diet is currently easy to perform in our country since it adapts to our traditional habits and is very attractive to the population, given its high-fat content **(Table 3).**

III.- HYPOTHESES AND OBJECTIVES OF THE WORK

There are two dietary patterns or pyramids, with potential beneficial effects on the prevention and treatment of arteriosclerosis. One of them is the diet low in fat, rich in complex carbohydrates. Several randomized studies have shown that this diet reduces the risk of suffering new coronary episodes and dying from ischemic heart disease, so several international organizations recommend it for these patients. The other dietary alternative is the Mediterranean diet, rich in monounsaturated fat from olive oil. There is no randomized study that shows that this diet is beneficial for the secondary prevention of patients with ischemic heart disease.

1. HYPOTHESES

We intend to evaluate whether when high-risk coronary patients consume a Mediterranean diet, rich in olive oil, the clinical evolution of their disease is modified, compared to a low-fat diet. The selected patients will be high-risk patients with acute myocardial infarction, unstable angina or high-risk chronic ischemic heart disease. The null hypothesis will be considered if the group of patients fed the Mediterranean diet has the same clinical events as the patients belonging to the low-fat diet group.

2. OBJECTIVES

1.- Main objective:

The primary outcome of the CORDIOPREV study is a composite main event, including myocardial infarction, revascularization, ischemic stroke, documented peripheral arterial disease and cardiovascular death, for 7 years, comparing the Mediterranean Diet with the Low Fat Diet.

2.- Secondary objectives:

The pre-specified secondary outcomes are:

1. Compounded event of cardiac events (cardiac death, myocardial infarction, unstable angina, revascularization, heart failure, heart transplant and heart failure)
2. Expanded compound event of cardiovascular disease progression (cardiac death, myocardial infarction, unstable angina, revascularization, heart failure, heart transplantation, heart failure, stroke and peripheral artery disease)
3. Unqualified clinical events within the primary endpoint, nor in secondary objectives 1 and 2,especially those associated with cardiovascular disease
4. LDL cholesterol concentration, atherogenic ratios related to total cholesterol/HDL and LDL/HDL lipids and other lipid markers
5. Metabolic control of carbohydrates (assessed by glycemic and insulin responses to glucose tolerance tests)
6. Metabolic control of lipids and postprandial lipemia
7. Blood pressure
8. Cancer
9. Incidence, control and regression of type 2 diabetes mellitus
10. Incidence, control and regression of metabolic syndrome
11. Arrhythmias
12. Incidence of all components of the primary endpoint
13. Evolution of arteriosclerosis: Evaluation of arteriosclerosis to different vascular beds. Silent arteriosclerosis.
14. Evolution of cognitive decline
15. Study of the microbiota.
16. Global metabolomics in plasma, as well as techniques aimed at specific sets of metabolites such as lipid species through lipidomics, proteins through proteomics, etc. will be applied.
17. Specific metabolomics in plasma fractions, specific bioparticles such as lipoproteins or specific cells, lipidomics, proteomics, targeted metabolomics, etc.
18. Transcriptomics studies using transcriptomics techniques such as gene expression microarrays, quantitative PCR, GeneChip, etc.
19. Different physiological processes or metabolic pathways related to inflammation and oxidative stress will be studied
20. Genetics, genomics, epigenetics
21. Endothelial function and vascular homeostasis.
22. Metabolism of advanced glycation end products.
23. Mineral metabolism.
24. Studies of cardiac function by echocardiography.
25. Microparticles
26. Anthropometric changes. Metabolic disease

27. Differential impact in certain subgroups: Sex, age, anthropometry, genetics, genomics, metabolism of the immediate principles, cardiovascular risk factors, cancer, vascular function.

Additional secondary objectives will be carried out in the light of current and/or future knowledge on ischemic heart disease risk factors, prognostic factors and pathophysiological pathways, including, but not known, endothelial function, inflammation, cell biology, molecular biology, proteomics, metabolomics, genomics, transcriptomics, genetics and epigenetics. All objectives included in research projects funded by competitive public grants within the Cordioprev Project are automatically defined as secondary objectives of the Cordioprev study

IV.- EXPERIMENTAL DESIGN

1.- STUDY POPULATION

A.- JUSTIFICATION OF THE SAMPLE SIZE

The sample size and the power calculation for the final main objective and the final duration of the study (7 years) were calculated based on the following assumptions: an incidence rate in the low fat diet group of 4 events/100 person-years that would be equivalent to a 24.9% cumulative absolute incidence after 7 years, a risk ratio of 0.7 and a statistical power of 80%, with α of 2 tails =0.05. With these assumptions, the necessary sample size was established from 491 patients in each of the two groups.

B.- CRITERIA FOR THE SELECTION OF PATIENTS

I.- INCLUSION CRITERIA:

1.- Informed consent: All participants will accept their inclusion in the study, signing the protocol approved by the Ethical Committee for Clinical Research of the Reina Sofía University Hospital. In this consent, it will be stated that their inclusion in the groups will be random.

2.- Diagnostic criteria

Patients with acute coronary heart disease (unstable angina, acute myocardial infarction) and chronic coronary disease of some risk will be selected according to the following criteria:

a) Acute myocardial infarction: Existence of at least two of the following three manifestations: chest pain with anginal characteristics (or anginal equivalents), typical changes of the electrocardiogram (appearance of new Q waves and/or ST-segment and/or T wave changes), and elevation of myocardial enzymes (CPK and/or CPK/MB > 2 times the normal upper limit of the laboratory. The criterion of the value of the MB shall prevail, in case of discrepancies, over that of the total CPK.

b) Unstable angina

Hospital admission for chest pain of anginal characteristics of at least 15 minutes duration, both at rest and after exertion, which has increased in frequency and duration in recent days or weeks. The last episode must have occurred within 48 hours before admission and must be accompanied by at least one of the following electrocardiographic or analytical alterations:

- St descent of at least 2 contiguous leads.0,5 mm
- Ascent of the ST of at least 2 adjoining leads.1 mm
- T wave inversion of at least 2 contiguous leads2 mm
- Troponin or CPK-MB positive.

c) High-risk chronic ischemic heart disease.

It will include those patients who have been admitted for a coronary event and/or stable angina, at least once in the previous 2 years and who have undergone a diagnostic coronary angiography with evidence of severe coronary disease, defined as the existence of an epicardial vessel larger than 2.5 mm in diameter, with stenosis greater than 50%.

II- EXCLUSION CRITERIA

1.- Age less than 20 years or greater than 75 years, with a life expectancy that is not less than 5 years.

2.- Severe heart failure, functional class III or IV of the NYHA classification, except for self-limiting episodes of acute heart failure at the time of the acute ischemic event.

3.- Known severe systolic left ventricular dysfunction (With ejection fraction equal to or less than 35%).

4.- Patients with limitation to follow the protocol: People without personal or family capacity to subscribe to the indicated diet will be excluded, for any reason.

5.- Serious or difficult to control risk factors: Patients with hypertension and diabetes in which there is organic involvement that limits their survival (chronic renal failure with creatinine persistently >2.5 mg/dl) and disabling clinical manifestations of cerebral arteriosclerosis will be excluded.

6.- Chronic diseases not related to coronary risk: Severe psychiatric diseases, chronic processes in need of treatment that may modify lipid metabolism (chronic renal failure, chronic liver disease, neoplasms under treatment, chronic obstructive pulmonary disease with pulmonary respiratory failure with home oxygen therapy, endocrinopathies susceptible to decompensation and diseases of the digestive tract that course with episodes of diarrhea).

7.- Participants in other studies: Patients participating in other studies at the time of selection or within 30 days before the start of the study will be excluded.

C.- SELECTION AND RANDOMIZATION OF PATIENTS

The doctor who treats the patient will suggest his participation in the study, once it has been verified that he meets the inclusion criteria and that he does not have the exclusion criteria. If the patient decides to participate, the corresponding data collection notebook (CRD) will be opened. If the information is favorable, the patient will be asked to sign the informed consent. Then baseline studies (discussed below) will be undertaken and it will be randomized and assigned to one of the two diets of the study. So that the ascription to both diets is well balanced, the randomization will be done by the following variables:

1.- Man or woman

2.- Be under 60 years of age or older.

3.- Have or not suffered a previous myocardial infarction.

This distribution will result in 8 different groups.

Table 2. Randomization groups, resulting from applying the selection criteria.

Randomization groups			
Men <60 years, no previous IAM	Men <60 years, with previous IAM	Women <60 years, no previous IAM	Women <60 years, with previous IAM
Men >60 years, no previous IAM	Men >60 years, with previous IAM	Women >60 years, no previous IAM	Women >60 years, with previous IAM

Randomization procedure.

Fixed randomization will be carried out, stratified in blocks, keeping the entire randomization process blindly by the researchers who have to participate in the collection of the data and the treatment of the patients. This randomization process will be supervised by an international organization of recognized prestige in the field of Epidemiology and Clinical Research (Andalusian School of Public Health) that will know in detail the entire process of randomization of patients.

A working group will be set up to carry out the whole process of randomizing patients, being independent of the working group responsible for treating and monitoring patients.

Randomization Groups: (1; 2)

- Men <60 years without previous AMI
- Men <60 years with previous AMI

- Men >60 years without previous AMI
- Men >60 years with previous AMI
- Women <60 years without previous AMI
- Women <60 years with previous AMI
- Women >60 years without previous AMI
- Women >60 years with previous AMI

Practical procedure

1. Patient meets inclusion criteria.
2. Informed consent. Collecting all information at time 0
3. Randomization (Andalusian School of Public Health)
4. Instauración of treatment by the group of dietetics.
5. Continue with the follow-up.

Procedures for keeping blinded the clinical team

1. Food issues should only be discussed with the dietetics team who will otherwise be unaware of the evolution of the study at all times.
2. The medical team will know at all times the type of diet consumed by the patient and will avoid any questions to the patient about it. All questions about diet compliance, adherence to it, degree of satisfaction, etc. will be asked by the dietetics team.
3. The statistical analysis team will remain blind about randomization, about the randomization codes, so that it does not know what type of diet each group of patients consumes. (These codes will only be known to the randomization team.)

D .- Baseline studies. Study variables

The treatment of the samples collected and the different tests to be carried out in all the subjects included in the study is carried out in lipids and arteriosclerosis of the Reina Sofía University Hospital in Córdoba.la Unidad

All individuals will be completed with a CRD that will include the medical history and the appropriate complementary determinations, in order to define the presence of risk factors and the existence of inclusion and exclusion criteria. This study will be performed by the doctor responsible for your general care (internal medicine specialist). The following parameters shall be included in this study:

a.- Clinicians

1.- Family history of premature coronary heart disease (before the age of 55 in men and 65 in women) in first and second-degree relatives

2.- Calculation of the BMI, by the formula indicated above.

3.- Blood pressure: It will be collected in the right arm, after 5 minutes of rest in a supine position, with the arms supported at the height of the heart. It will be done with a mercury sphygmomanometer and the systolic pressure will be defined by the appearance of the first sound (Phase 1 of Korotkoff) and the diastolic by its disappearance (Phase 5 of Korotkoff). Two measurements will be made at intervals of 2 minutes and if they differ by more than 5mm a third will be made and the average will be obtained. Patients who follow previous antihypertensive treatment will be considered hypertensive, regardless of their tension figures at the beginning of the study. The average of the last two determinations shall be considered final. Individuals with strains equal to or greater than 140/90 (75) will be considered hypertensive.

4.- Tobacco consumption. The average type and daily amount of consumption will be assessed.

5.- Diabetes mellitus. This diagnosis will be made in view of the existence of the classic symptoms of diabetes, together with an unequivocal increase in blood glucose, not necessarily fasting (>200 mg/dl). Likewise, patients with fasting blood glucose equal to or greater than 126 mg/dl, on two different occasions and if the glycemia, after overloading of oral glucose, reaches 200 mg at 2 hours (62) will be considered as such. To do this, all patients will be given an oral glucose overload of 75 grams, (with blood drawn at baseline, 30, 60, 90, 120 minutes). Patients who are treated for the disease will be diagnosed with diabetes without having to meet these requirements.

5.- Alcohol consumption. The quantity and type of beverages will be collected, quantifying it in grams, according to the average alcohol content in the different alcoholic beverages (beer 4% alcohol content; young wine 9%, fine wine 16%; red wine 12%; spirits 50%). The drinking of >30 g/day of alcohol shall be considered excess consumption.

6.- Taking medication. All drugs, in particular antihypertensives (ACE inhibitors, calcium antagonists, beta-blockers), nitrites, antiplatelet agents and lipid-lowering agents, shall be indicated.

7.- Obesity: It will be considered obese when the subjects present BMI equal to or higher than 30 kg/m2.

8.- Physical inactivity: The presence of physical inactivity will be considered if you walk <1 km /day or perform daily work without any physical exercise.

b.- Complementary explorations:

1. Biochemical: The following determinations will be made in a venous blood draw, after 12 hours of fasting, in supine decubitus and without compression. After extraction, the samples will be processed conventionally, concentrating them and transporting them in ice for immediate study. Aliquots of the samples obtained shall be kept at –80 °C. Glucose, Urea, Creatinine, Bilirubin, Urea, ions, amylase, AST, ALT, GGT, Alkaline Phosphatase, LDH, CK, ApoA, ApoB, Total Cholesterol, LDL, HDL, triglycerides, LpA, Proteins, Albumin, Reactive ProtC, Calcium, Phosphate, Hba1c, TSH, T4 (if applicable), Folate, VitB12, Iron, Ferritin, Hematimetrito complete and coagulation study will be determined.

A urine study will also be carried out

2. Reception, processing and storage of samples: blood, urine, feces

3. Echocardiography: It will be practiced in the initial study of the patient and in the evolution

4. Isolation of mononuclear cells
5. Cytokine quantification
6. Determination of inflammation and coagulation parameters
7. Test insulin resistance.
8. Posprandrial lipaemia.
9. Study of microparticles
10. Study of carotid arteriosclerosis: Thickness of the medial carotid intima, quantification of plaques.
11. Impedanciometría
12. Endothelial Function: Brachial FMD and Microvascular Laser-Doppler
13. Microbiota in feces
14. Study of brachial artery vasodilation by FMD by ultrasound.
15. Extraction of nucleic acids (DNA and RNA) from mononuclear cells
16. Ankle-arm index performance
17. Oxidative stress studies
18. Evaluation of the metabolism of advanced glycation end products
19. Electrocardiogram.

2.- DEFINITION OF THE MAIN EVENT AND ITS COMPONENTS

The main event of the study is a composite event that includes the following cardiovascular events: myocardial infarction, revascularization, ischemic stroke, documented peripheral arterial disease, and cardiovascular death.

The main event components are defined as:

Acute myocardial infarction:

Detection of an increase and/or fall in values [cardiac biomarker] (preferably cTn, or Ck-Mb when not possible) with at least 1 value >99th percentile and b) At least 1 of the following:

1. Symptoms of myocardial ischemia
2. New or assumed significant new ST-segment–T (ST-T) wave changes or new branch blocks on the ECG
3. Development of pathological Q waves on the ECG
4. Image evidence of new viable myocardial loss or new abnormality of regional wall movement
5. Identification of an intracoronary thrombus by angiography or autopsy.

Revascularization:

Any physical procedure to restore circulation, either by percutaneous coronary intervention, or bypass graft surgery.

Ischemic stroke:

Acute neurological deficit lasting more than 24 hours caused by thrombosis or arterial embolism of the cerebral arteries, in which a brain imaging technique (CT scan or MRI) demonstrates a stroke.

Documented peripheral arterial disease:

Clinically symptomatic patients, who also showed documented disease in the different territories:

1. Carotid: DUS, CT or MRI showing significant stenosis (>70%), or revascularization.

2. Lower limb: symptomatic patients with at least 1 of the following criteria: Clinical diagnosis of arterial occlusive disease based on imaging tests (duplex ultrasonography, magnetic resonance angiography, computed tomographic angiography or catheter-based radiocontrast angiography), or clinical indication for endovascular or open surgical procedure (revascularization or amputation).

3. Mesenteric: clinical and radiology compatible

Cardiovascular death:

Coronary deaths from heart disease (i.e., acute myocardial infarction, unstable angina pectoris, and other forms of chronic ischemic heart disease), ischemic stroke, arrhythmias, dysrhythmias, congestive heart failure, pulmonary edema and pulmonary embolisms, and sudden death shall be considered as such. For a death to be considered sudden, the patient must have been seen by someone less than 24 hours before the supposed time of death, with no clinical worsening of his baseline condition detected. Sudden nonviolent deaths will be considered cardiovascular deaths unless other causes are evident. The reason for this assumption is the overwhelming percentage (90%) of these deaths that in necropsy studies are of cardiovascular origin in Spain, and supported by the AHA/ACC consensus(1-3)

3.- DESCRIPTION OF FOOD MODELS

The objective of this study is to compare two types of diets, the Mediterranean diet with 35% calories in the form of fat (22% monounsaturated fat, 6% polyunsaturated fat, <10% saturated fat), 15% protein and 50% carbohydrates, versus a low-fat diet with <30% total fat <10% SFA, 12-14% MUFA, 6-8% PUFA),15% protein and 55% carbohydrates. In both diets the cholesterol content will be adjusted to less than 300 mg/day. The goal is not to compare two types of diet defined by a given percentage of nutrients, but two different eating patterns or two different food pyramids: the food pattern of the Mediterranean diet pyramid versus the low-fat diet (80).

Both therapeutic diets must provide a diversity of foods of all types: vegetables, fruits, cereals, potatoes, legumes, dairy products, meats and fish.

1. Dietary model following the Low-Fat diet group.

The following rules should be followed with regard to the choice of specific foods or food groups.

1.1 **Cereals and derivatives** (bread, cereals, pasta, potatoes, rice, legumes). Between 6 and 11 daily servings of cereals and derivatives, preferably whole grains, should be consumed. Each serving corresponds to the following quantities: 1 slice of bread, 30 g of dry cereals, 1/2 cup of potatoes, rice, noodles, legumes, etc.

These foods are rich in carbohydrates, provide protein and in general have a low content of saturated fat and cholesterol. Pasta, legumes, potatoes, rice and vegetables can be combined with small amounts of lean meat, fish or poultry to prepare a tasty second course.

1.2 **Vegetables** (lettuce, corn, pea, green beans, broccoli, carrots, cabbage, celery, tomato, spinach, pumpkin, eggplant, mushrooms, etc.). It should be consumed between 3 and 5 servings a day. One serving of vegetables equals about 150-200g (one plate of salad, one plate of cooked vegetables, one tomato, etc). Fresh, frozen and/or packaged vegetables without added fats or sauces can be used.

1.3 **Fruits** (Orange, apple, pear, bananas, grape, plum, peach, strawberries, melon, kiwi, mango, papaya, etc.) It should be consumed between 2 and 4 servings a day. Each serving corresponds to 1 medium piece of fruit, 1/2 cup of cut fruit or 3/4 cup of fruit juice.

1.4 Skimmed dairy (skimmed milk, skimmed yogurt, low-fat cheeses). Between 2 and 3 servings a day should be taken. A serving corresponds to one cup of skimmed milk or 30 g of low-fat cheese or fresh cheese.

1.5 **Lean meats, poultry meat or fish.** It should be consumed about 150 gr a day. The lean cuts of the veal are the sirloin, the round steak, the butt and in the case of the pig, the central part of the ham, the loin or the sirloin. All visible fat must be removed before consumption and before cooking. In the case of chicken and turkey the skin should be removed before cooking. Choose white fish (pollock, cod, sole, hake, etc.)and avoid oily fish (tuna, bonito, mackerel, anchovy, etc.)and/or canned in oil.

The amount of 150g equals 2-3 fillets of loin, 1-2 fillets of chicken breast, 2-3 slices of fish or 1 fillet of fish.,

Seafood contains less fat than meat, but some types such as prawns contain a lot of cholesterol so its inclusion should be limited as far as cholesterol consumption is recommended (<300 mg/day).

1.6 **Fat and oils**. Use a maximum daily of 2-3 tablespoons (1 tablespoon≈10 ml), including both the oil used for cooking and that used for raw preparations. Use vegetable oils such as sunflower, corn or soy. You can replace a portion of the daily fat/oil (1 tablespoon) with normal olive oil or margarine.

1.7 **Nuts.** The consumption of nuts and peanut butter must be limited due to their richness in fat.

1.8 **Eggs.** Limit your intake to no more than 2-4 eggs a week.

1.9 **Miscellaneous.** Snacks of the type of crunchy cookies without any fat, toasted rice, bread doughnuts, etc. are suitable. Popcorn should be made with a small amount of vegetable oil.

1.10 **Desserts and sweets.** Fruits, low-fat fruit yogurt, fruit ice cream, sorbets, jellies and fat-free ice milk are suitable options. Limit sweets prepared with milk, chocolate and tropical oils.

1.10 **Culinary techniques.** Use cooking methods that do not include fats (boiling, baking, grilling, steaming, shaving, etc.), remove visible fat (or skin) from meats before cooking and defat soups and broths. Salt must be limited in the preparation of soups, broths and other dishes replacing it with spices, aromatic herbs or lemon juice.

Since the main sources of saturated fats in the American diet are high-fat meats and dairy products, patients should limit the consumption of butter, whole milk, and whole milk products such as ice cream, cream, fatty cheese, fatty meats such as hamburger, processed meats (sausages, bacon, etc.), high-fat sausages (salami, chorizo, sausage, ham derivative) and poultry skin.

2. Mediterranean Food Model.

The following rules should be followed to achieve the recommendations of a Mediterranean diet:

2.1 **Cereals and derivatives** (bread, cereals, pasta, potatoes, rice, legumes). Consume about 6 servings a day, preferably whole grains. Each serving corresponds to the following quantities: 1 slice of bread, 30 g. of dry cereals, 1/2 cup of potatoes, rice, noodles, legumes, etc.

Use bread with meals. Legumes and cereals should be the basis of the first dishes in the main meals (chickpeas, lentils, beans, rice, etc.). At least 3 dishes of legumes will be consumed per week.

2.2 **Vegetables** (lettuce, corn, pea, green beans, broccoli, carrots, cabbage, celery, tomato, spinach, pumpkin, eggplant, peas, mushrooms, etc.). It should be consumed between 3 and 5 servings a day, at least one serving will be raw vegetables or in the form of a salad seasoned with extra virgin olive oil A serving of vegetables is equivalent to about 150-200g (a salad dish, a cooked vegetable dish, a tomato, etc.). Preference will be given to fresh and seasonal vegetables.

2.3 **Fruits** (orange, apple, pear, banana, grape, plum, peach, strawberries, watermelon melon and any fruit of the time). It should be consumed between 2 and 4 servings a day. Each serving corresponds to 1 medium piece of fruit, 1/2 cup of cut fruit or 3/4 cup of fruit juice. As a general rule, the equivalent of at least one piece of fruit should be consumed at each of the two main meals, as a dessert, and no other desserts should be used.

2.4 Dairy (preferably fermented dairy products such as yogurt and cheese). Consume about 2 and 3 servings a day. A serving corresponds to a cup of milk, plain yogurt or fresh cheese.

2.5 **Fat and oils.** Use extra virgin olive oil for culinary preparations, especially together with bread, toasted or natural, breakfast and snack, in the preparation and dressing of vegetables and salads, in the cooking process of all dishes (legumes, vegetables, fried fish, etc.), for the preparation of certain typical dishes of Mediterranean Andalusian cuisine, such as *gazpacho* and *salmorejo*. The recommended portion of extra virgin olive oil should be taken daily, calculated to be about 50 milliliters/person/day (≈4-5 tablespoons), for men with normal weight, with a caloric intake of about 2,000 Kcal/day. In the case of women with normal weight, and overweight men and women, the consumption of extra virgin olive oil will keep the appropriate proportion for the overall caloric intake. The following table gives several examples of different diets and their corresponding proportions of consumption of extra virgin olive oil. No other fat should be consumed and especially other types of oils, butter, margarine or fatty derivatives should be eliminated.

Table 3. Examples of various diets, with different caloric content and the corresponding consumption of extra virgin olive oil.

	Men with normal weight	Overweight men	Women with normal-health	Overweight women
Daily kcals	2,000	1,500	1,700	1,200
Daily consumption of extra virgin oil	50 milliliters	45 milliliters	50 milliliters	36 milliliters

2.6 **Poultry or fish.** Fish should be consumed several times a week (4-5 times) and 2 times/week (150 gr in each serving) skinless poultry meat. Preferably, oily fish (tuna, bonito, mackerel, anchovy, etc.) will be consumed. In the case of cooking the fish by frying, this will always be done in virgin olive oil.

2.7 **Eggs.** Limit your intake to no more than 2-4 eggs a week.

2.8 **Nuts.** Natural nuts (not roasted or fried) will be consumed at least 3 times a week, considering that one serving is equivalent to a stab or 30g of walnuts, almonds, hazelnuts, chestnuts, etc.

2.9 **Red and processed meats.** Lean red beef is sirloin, round steak, butt and in the case of pork, the central part of ham, loin or sirloin. All visible fat should be removed before consumption and before cooking. Consume only occasionally or sporadically, i.e. several times a month, no more than once a week and on average twice a month (taking into account that 1 serving of red meat equals about 100-150g and 1 serving of processed meat equals 50g).

2.10 **Miscellaneous. Desserts and sweets.** Fruits are the best dessert to take on all occasions. The remaining desserts made with skimmed dairy, low-fat fruit yogurt, fruit ice cream, sorbets, jellies and fat-free ice milk are suitable options to take only sporadically in those circumstances where it is not possible to take a piece of fresh fruit. Eliminate sweets prepared with milk, chocolate and tropical oils.

Consume only homemade sweets made with virgin olive oil, flour and spices (for example, *pestiños*, homemade muffins, etc.)

2.11 **Culinary customs and condiments.** As a general rule, fresh foods will be consumed avoiding canned foods. In the Mediterranean diet the axis of culinary techniques is found in the use of olive oil. Virgin olive oil will be used for fried foods (fish, eggplants, potatoes, eggs, etc.), to sauté fresh vegetables once cooked, to add it to fresh vegetables and salads. Virgin olive oil will also be used in the preparation of typical dishes of Mediterranean cuisine (*salmorejo, gazpacho*, etc.), sweets (fritters, syringes, muffins, *pestiños*, etc.) and in the preparation of all dishes and stews (stew, beans, lentils, paella, etc.). There are also a number of condiments very typical of the Mediterranean cuisine and diet so that in the seasoning of most dishes garlic, onion and tomato are used, especially in the form of sofrito (saucemade with tomato and onion, which often includes garlic and some aromatic herbs, and simmered with olive oil). Use the sofrito at least 2 times a week.

2.12 **Wine:** In those people who already consume wine regularly in their diet, a maximum of 2 glasses/day for men and 1 glass/day for women would be allowed.

General scheme of consumption of the different foods in both diets

Foods	Low-Fat	Mediterranean
Olive oil	No	≅ 50 ml/day
Vegetable oils (sunflower, soybeans) (1)	<30 ml/day	No
Cereals and derivatives (2)	6-11 servings/day	6 servings/day Bread in all meals Legumes (≥3 times/sem)
Vegetables (3)	3-5 servings/day	3-5 servings/day fresh
Fruits (4)	2-4 servings/day	3-4 servings/day fresh
Skimmed dairy (5)	2-3 servings/day	2-3 servings/day
Red meat (6)	<150 g/day	150 g/2 times a month
Chicken meat (6)	<150 g/day	150 g/2 times/week
Fish (6)	<150 g/day	150 g/4-5 times/week
Nuts	Sporadically, of any	30g/≥3 times/week Natural nuts (not roasted or
Eggs	2-4 units/week	2-4 units/week
Desserts	Fruit, jellies, sorbets, ice cream with skimmed milk	Fresh seasonal fruits
Other Desserts	4-5 times/week. Yogurt, sorbets, jellies, skimmed Cream	Very occasional. In celebrations
Alcohol	No	Allow 2 glasses wine/day in men 1 glass wine/day in women
Culinary techniques	Steaming, grilling, baking, boiling.	Use of sofrito ≥2 times/week

	Remove fat and skin from meat. Degreasing broths and soups.	

(1) You can replace a portion of the daily fat/oil (1 tablespoon) with normal olive oil or margarine.
(2) One serving is equivalent to 15-20 g/of bread, 30 g of dry cereals or half a cup of legumes, rice, noodles or potatoes.
(3) One serving is equivalent to about 150-200g (one plate of salad, one plate of cooked vegetables).
(4) One serving is equivalent to one medium piece of fruit, half a cup of cut fruit or three quarters of fruit juice.
(5) One serving corresponds to one cup of milk , yogurt or fresh cheese.
(6) To be chosen by the patients one of them to consume in the day.

4.- MONITORING OF THE POPULATION.

During the follow-up of the patient, periodic revisions or controls of two types, dietary and clinical check-ups, will be carried out.

A.- Scheduled dietary and nutritional controls:

The dietary controls aim to ensure that the nutritional contribution is adequate and that the adherence of the patient to the assigned diet is ensured. For this purpose, a dietitian will be available. The scheduled controls are as follows:

1.- Bimonthly telephone follow-up: It will consist of a telephone call, made by the dietitian, to reinforce the monitoring of the diet and solve specific problems that may arise. The duration of the telephone conversation will be about 10 minutes.

2.- Semiannual face-to-face follow-up

This consultation will be carried out by the dietitian, as a complement to the clinical consultation (B.- Clinical controls), every 6 months. In it, the dietitian will measure the level of dietary adherence achieved by the patient through the 14-point Mediterranean diet adherence questionnaire (to be administered in both intervention groups) and the 9-point low-fat diet adherence questionnaire (to be administered only in the low-fat diet group). The dietitian will give personalized recommendations to improve those dietary items that the patient does not meet and reinforce those that the patient does. Thus, the appropriate corrections will be made, immediately, to ensure good adherence to the protocol. The duration of this review, which will include tips/educational material to reinforce nutritional monitoring, will be 20 minutes. In this consultation, the anthropometric parameters included in the study will be evaluated.

Semiannual visits that coincide with the annual check-up (1,2,3,4,5,6 and 7 years) will also include the administration of the food consumption frequency questionnaires, the Minnesota physical activity questionnaire and the SF36 quality of life questionnaire. These visits will last approximately 1 hour.

In all follow-up visits, food will be delivered to the patient to encourage adherence to the assigned diet: extra virgin olive oil in the Mediterranean diet group (approximately 1 L per week) and packs of low-fat products in the low-fat diet group.

3.- Four-year group visits

Group visits will be organized separately for each intervention group (Mediterranean diet and low-fat diet) and will include a maximum of 20 participants. These sessions will be conducted by the dietitian who will explain the chosen topic, resolve doubts and reinforce the dietary recommendations according to the assigned diet. The structure of each session will be flexible and adapted to the learning needs of the participants. Typically, these sessions will last approximately 1 hour and will include: a) PowerPoint presentation, b) group discussion, c) practical activities, and d) reinforcement of dietary recommendations. The patient will receive printed educational material on the content of the group session (cooking recipes, shopping list, etc.).

B.- Clinical controls

1.- The Control by the responsible doctors will be done by an internal medicine specialist, who will pay attention to any medical aspect related to the follow-up of the patient. It will be semiannual and will monitor all eventualities and evolutionary changes related or not to the fundamental disease, especially with regard to the monitoring and control of risk factors, acute intercurrent processes and the appearance of any other process that may influence the nutritional protocol. The patient will keep a diary in which he will collect any new phenomenon that modifies his normal life, both of clinical significance and the need to take any unscheduled drug or the consumption of drugs on demand, such as nitrates. In this consultation, the data collection notebooks will be completed, to facilitate the cardiological review. It will be completed with a blood draw, fasting, to complete the biochemical evaluation. It is estimated that the time consumed in the follow-up query will be forty minutes.

5.- WORK PLAN

With the material of each patient, a CRD will be developed, which will include the clinical, nutritional and dietary protocols of each one.

A.- Chronological development of the project

A minimum recruitment rate of 9 patients per week has been calculated so that the time required will be a maximum of 24 months. This recruitment will be done with patients diagnosed at the Reina Sofía University Hospital. If 50% of the patients have not been included at 12 months, other regional centres will be used. An intermediate analysis is expected to be carried out within 40 months of starting the (it is estimated that by then it will be at an average follow-up of 2.5 years) to investigate whether the recurrence rate is within the forecast. If this prediction is not met, the sample size shall be recalculated in accordance with the criteria set out above).

B.- Task of researchers and organization of work.

1.- Coordinator:

He will be responsible for the CRDs. It will coordinate the progress of the study, ensuring that each one meets the defined objective. Special interest will be given to the control of the most critical points of the study, such as the application of the inclusion and exclusion criteria, completion of data collection notebooks, collection and handling of biological samples, archiving of the material and personal monitoring of the patients. In this sense, it will arbitrate a system that allows it to know, in the shortest possible time, any eventuality that arises in the evolution of the patients, especially if they have been treated in the conventional care system. It will ensure that

the handling of the samples is adequate and that the study as a whole is integrated. In case of any assistance need, it must be available to solve it. The expected occupancy is calculated in 3 hours daily.

2.- Internal Medicine Consultation. This consultation will be organized with internal medicine specialists, and they will be responsible for the clinical follow-up of the patients. For this they must have experience in the management of this type of patients, and therefore have the capacity and dedication to interpreting any clinical process, related or not to the study. The scheduled consultations will be quarterly and will channel and attend to those that may arise on demand. It is intended that each specialist who participates is the doctor responsible for a group of patients. Different forms will be made on health and changes in clinical status. All documentation relating to any occurrence of a clinical event shall be completed. The expected of this consultation is 40 minutes.

3.- Nursing consultation: It will have the same periodicity as the medical consultation and the blood extractions, the reception of biological samples and the functional tests that are required according to the planned schedule will be carried out.

4.- Dietitian. Conducting telephone control approximately every 2 months and a personal control, in consultation, also every 6 months, in addition to the different group sessions.

5.- Team of auxiliary personnel: It includes a laboratory technician, secretariat staff and management control. Each one will carry out the tasks of his profession, from the introduction of the information in the database, help in the consultation of the doctor, extraction, management and archiving of biological samples.

6.- STATISTICAL PLAN

6. 1 Trial Identifications

TRIAL FULL TITLE	**ESTUDIO CONTROLADO SOBRE EL EFECTO DE LA DIETA MEDITERRANEA, RICA EN ACEITE DE OLIVA, EN LA REDUCCION DEL RIESGO CORONARIO DE PACIENTES CON CARDIOPATÍA ISQUÉMICA (CORDIOPREV STUDY)**
CLINICALTRIALS NUMBER[1]	NCT00924937
SAP VERSION	V7
SAP VERSION DATE	01/06/2018
STATISTICIAN TEAM	Javier Delgado Lista Juan Francisco Alcalá Diaz Jose Lopez Miranda
TRIAL PRINCIPAL INVESTIGATORS	Jose Lopez Miranda Francisco Perez Jimenez

[1]https:// Clinicaltrials.gov /

6.2 Ambit of Use

This document is provided as a guideline to perform statistical analysis in the Cordioprev Study.

6.3 Introduction

6.3.1 Preface

There are two dietary patterns with potential beneficial effects in the prevention and treatment of arteriosclerosis. One of them is the low-fat diet, rich in complex carbohydrates. Several randomized studies have shown that this diet reduces the risk of suffering new coronary episodes and of dying from ischemic heart disease, which is why several international organizations have recommended it for these patients. The other dietary alternative is the Mediterranean diet, rich in monounsaturated fat from olive oil. There is no randomized study that demonstrates that this diet is beneficial for secondary prevention in patients with ischemic heart disease in a large, long-term study.

6.3.2 Purpose of the study

The aim of this study is to evaluate whether a Mediterranean diet rich in olive oil (34% of calories in the form of fat, with 22% MUFA, 6% PUFA and <10% SAT) changes the clinical course of the disease in high-risk coronary patients compared to a diet low in fat, (28% of calories in the form of fat, 12% as MUFA, 8% PUFA and 8% SAT). The patients selected will be high-risk patients with acute myocardial infarction, unstable angina and high-risk chronic ischemic heart disease not amenable to revascularization. The null hypothesis will be considered if there are no differences in the clinical events between the two groups.

6.4 Study Objectives and Endpoints

6.4.1 Study Objectives

This study aims to evaluate the difference in the evolution of persons with coronary heart disease (CHD) when following the Mediterranean or the Low-Fat diets. The main objective will be evaluated by the presence of major cardiovascular events, while several secondary objectives will evaluate other outcomes in health.

6.4.2 Endpoints

6.4.2.1.- Main objective:

The primary result of the CORDIOPREV study is a major compound event, which includes myocardial infarction, revascularization, ischemic stroke, documented peripheral artery disease and cardiovascular death, for 7 years, comparing the Mediterranean Diet with the Low Fat Diet.

6.4.2.2.- Secondary objectives:

The prespecified secondary objectives are:

1. Extended compound event of cardiac events (cardiac death, myocardial infarction, unstable angina, revascularization, heart failure, heart transplant and cardiac arrest)
2. Extended compound event of cardiovascular disease progression (cardiac death, myocardial infarction, unstable angina, revascularization, heart failure, cardiac transplantation, cardiac arrest, stroke and peripheral artery disease)
3. Clinical events not qualifying as primary endpoint nor in the secondary objectives 1 and 2, especially those associated with cardiovascular disease
4. LDL cholesterol concentration, atherogenic ratios related to total cholesterol lipids/ HDL and LDL/HDL and other lipid markers
5. Metabolic control of carbohydrates (assessed by glycemic and insulin responses to glucose tolerance tests)
6. Metabolic control of lipids and postprandial lipemia
7. Blood pressure
8. Cancer
9. Incidence, control and regression of type 2 diabetes mellitus
10. Incidence, control and regression of metabolic syndrome
11. Arrhythmias
12. Individual evaluation of all components of the primary outcome.
13. Evolution of arteriosclerosis: Evaluation of arteriosclerosis at different vascular beds. Silent arteriosclerosis
14. Evolution of cognitive decline.
15. Microbiota study.
16. Global metabolomics in plasma, as well as techniques targeting specific sets of metabolites such as lipid-based lipid species, protein by proteomics, etc.
17. Specific metabolomics in plasma fractions, specific bioparticles such as lipoproteins or specific cells, lipidomics, proteomics, targeted metabolomics, etc.
18. Changes in Gene Expression using transcriptomic techniques such as gene expression microarrays, quantitative PCR, GeneChip, etc.
19. Different physiological processes or metabolic pathways related to inflammation and oxidative stress will be studied
20. Genetics, genomics, epigenetics
21. Endothelial function and vascular homeostasis.
22. Metabolism of advanced glycation end products.
23. Mineral metabolism.
24. Cardiac function studies by Echocardiography.
25. Microparticles
26. Anthropometric changes. Metabolic disease
27. Differential impact on certain subgroups: Sex, age, anthropometry, genetics, genomics, metabolism of immediate principles, cardiovascular risk factors, cancer, vascular function.

6.4.2.3 Other secondary objectives

Additional secondary objectives will be carried out in light of current and/or future knowledge of ischemic heart disease risk factors, prognostic factors and pathophysiological pathways, and will include, but not be limited to, endothelial function, inflammation, cell biology, molecular biology, proteomics, genetics and epigenetics.

All objectives included in research projects funded by competitive public grants within the Cordioprev Project are automatically defined as secondary objectives of the Cordioprev study

6.5 Methodology of the Study

6.5.1 General Design
Experimental design: Randomized trial with two active arms. No control group.
Blinding: Only patient, Personnel performing randomization and dietists will be aware of the arm
Randomization: Stratified by sex and medical history
Type of main analysis: Intention to treat analysis.
6.5.2 Non-Inferiority Clause
If the study does not meet the superiority significance criterion, the non-inferiority of the Mediterranean Diet will be tested and established if the upper 95% CI for the estimated hazard ratio falls below 1.10.
6.5.3 Inclusion-Exclusion Criteria and General Study Population
The inclusion and exclusion criteria are detailed in Table 1. To sum up, patients are eligible if they are over 20 years old, but under 76, had established CHD without clinical events in the last six months, are believed to be following a long-term dietary intervention and have no severe illnesses or an expected life expectancy of under five years. All the patients gave their written informed consent to participate in the study.

Inclusion Criteria in the CORDIOPREV study:
1.- Informed Consent: All participants will agree to be included in the study by signing the protocol approved by the Reina Sofia University Hospital Clinical Research Ethics Committee. In this written statement of consent, it will state that patients will be chosen for inclusion in the groups on a random basis.
2.- Diagnostic Criteria:
The patients are selected with acute coronary syndrome (unstable angina, acute myocardial infarction) and high-risk chronic coronary heart disease, according to the following criteria:
a) Acute myocardial infarction: The existence of at least two of the following three signs: angina-type chest pain (or anginal equivalents), typical ECG changes (appearance of new Q waves and/or changes in ST segments and/or T waves), and a rise in myocardial enzymes (CPK and/or CPK / MB> of twice the normal laboratory limits). The CPK-MB value criterion will prevail in case of discrepancies over the total CPK.
b) Unstable angina: Admission to hospital for angina-type chest pains lasting at least 15 minutes, both at rest and after exercise, which have increased in frequency and duration in recent days or weeks. The latest episode must have occurred at least 48 hours before admission and must be accompanied by at least one of the following electrocardiographic or analytical changes:
- ST depression of at least 0.5 mm in 2 contiguous leads.
- ST elevation of at least 1 mm in 2 contiguous leads.
- T-wave inversion of at least 2 mm in 2 contiguous leads.
- CPK-MB or troponin result.
c) Chronic high-risk ischemic heart disease: patients will be included who have been hospitalized for a coronary event and/or stable angina, at least once in the past 2 years and who have undergone diagnostic coronary angiography with evidence of severe coronary disease, which is defined as the existence of an epicardial vessel greater than 2.5 mm in diameter with stenosis of over 50%.

Exclusion Criteria
1.- Patients under 20 years of age or over 75 years old with a life expectancy of over 5 years.
2.- Severe heart failure, NYHA functional class III or IV, except for self-limited episodes of acute heart failure at the time of the acute ischemic event.
3.- Severe left ventricular systolic dysfunction (with ejection fraction equal to or under 35%).
4.- Patients with restricted capacity to follow the protocol: those unable to follow the prescribed diet for whatever reason, due to personal or family circumstances.
5.- Risk factors that are severe or difficult to control: patients with hypertension and diabetes, where there is organ involvement that limits their survival, will be excluded (chronic renal failure with creatinine which is persistent>2.5 mg/dl) and disabling clinical manifestations of cerebral atherosclerosis.
6.- Chronic diseases unrelated to coronary risk: severe psychiatric illnesses, chronic conditions requiring treatment that could modify the lipid metabolism (chronic renal failure, chronic liver disease, neoplasia under treatment, chronic obstructive pulmonary disease involving respiratory pulmonary failure with home oxygen therapy, endocrine diseases susceptible to decompensation and diseases of the digestive tract that involves episodes of diarrhea).
7.- Participants in other studies: patients taking part in other studies will be excluded, at the time of selection or up to 30 days before the study begins.

Table 1. Inclusion and exclusion criteria for CORDIOPREV study.

6.5.4 Randomisation and Blinding

Performed by a fixed randomization stratified in blocks keeping blind the whole process of randomization to the researchers who participate in the collection of data and in the treatment of patients. This process of randomization will be performed by an International organization of recognized prestige in the field of Epidemiology and Clinical Research (Andalusian School of Public Health), located in Granada (Spain). A working group has been established which will oversee carrying out the whole process of randomization of patients and which will be independent and act blindly from the working group in charge of treating and monitoring patients.
Randomization has been carried out so that the assignment to both diets will be balanced and based on these variables: sex (male, female), age (under and over 60 years old), and previous myocardial infarction (yes, no). Eight groups have been derived from the combinations of these variables and independent allocation blocks have been created. The procedure for assigning a diet have been as follows: when there was a candidate for randomization, the study dietitians phoned the person in charge of the study in the Andalusian School of Public Health, which communicated the assigned diet to the dietitian. The dietitians have been the only members of the intervention team to be aware of the dietary group of each participant.
The assignment to the diet group was made by telephone by a representative of the Andalusian School of Public Health. To this end, a mobile phone was set up.

RANDOMIZATION BLOCKS.
The following randomization groups will be used:

1. Men <60 years without previous AMI
2. Men <60 years with previous AMI
3. Men >60 years without previous AMI
4. Men >60 years with previous AMI
5. Women <60 years without previous AMI
6. Women <60 years with previous AMI
7. Women >60 years without previous AMI
8. Women >60 years with previous AMI

Internal control.
There were periodic teleconferences between the two parties reviewing the randomizations made in the different periods, where, if a mistake was found, a decision on the final randomization group were taken. As a rule, the randomization group that would apply by the stratification blocks would be applied. Exceptional changes could be done, always with the creation of a document explaining the motivations.

5.5 Study Variables
Baseline and annual visits included: 1) collection of biological samples: 2) a short questionnaire about lifestyle variables, medical conditions, and use of medication, 3) the MEDAS screener, which was performed in the two intervention groups; 4) a 9-point Low-Fat diet adherence score, which was only administered in the Low-Fat diet group(4) 5) a 137-item validated food frequency questionnaire(5, 6), and 6) a validated Spanish version of the Minnesota Leisure-Time Physical Activity questionnaire(7, 8) and a validated SF36 quality of life questionnaire(9). Weight, height and waist circumference were measured using standardized procedures. Regular medical visits to ascertain clinical, prescription drugs or other health changes were carried out, or alternatively on-demand, when the patients attended dietary visits and collected information on any changes in their health or treatment.

Table S3: Interventions and follow-up

		Number of yearly measurements							
Item/Measurements	**Brief description**	**Baseline**	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**

Eligibility questionnaire	Inclusion/Exclusion Criteria	1	0	0	0	0	0	0	0
General questionnaire [a]	Personal and family history, medical conditions, medications, anthropometry, BP, smoking, alcohol intake	1	0	0	0	0	0	0	0
Informed Consent		1	0	0	0	0	0	0	0
Randomization		1	0	0	0	0	0	0	0
Follow-up questionnaire [a]	Symptoms and conditions, marital status, job, medications, anthropometry, BP	0	2	2	2	2	2	2	2
Review prior/concomitant medications and Event Questionnaire [b]									
Tolerance questionnaire	Adverse experiences	0	1	1	1	1	1	1	1
Socio-economic questionnaire	Socio-demographic and economic characteristics, marital status, job, level of education	1	0	0	0	0	0	0	0
Circadian chronotype questionnaires	Horne-Östberg Morningness-Eveningness Questionnaire (Spanish version), sleep diaries	1	0	0	0	1*	0	0	0
Mini-mental state examination (MMSE)	Validated tool for screening cognitive function	1	0	0	0	0	1	0	0
Geriatric Depression Scale (GDS) or Yesavage	Validated tool for identifying depression in the elderly (Spanish version)	1	0	0	0	0	0	0	0
Health related quality of life	Validated 36-Item Short Form Health Survey (SF-36)	1	0	0	1	0	1	1	1
Physical activity questionnaire	Validated Minnesota questionnaire	1	1	1	1	1	1	1	1
Food frequency questionnaire (FFQ)	Validated 137-item FFQ	1	1	1	1	1	1	1	1
14-item MedDiet questionnaire	MedDiet adherence tool	1	6	6	6	6	6	6	6

9-item Low Fat Diet questionnaire	Low-fat Diet adherence tool	1	6	6	6	6	6	6	6
Dietary reinforcement	Face-to-face and telephone interviews	1	6	6	6	6	6	6	6
Group education sessions	Separate sessions for each group (20 participants/session)	0	3	3	3	3	3	3	3
Dispensing study foods		1	2	2	2	2	2	2	2
Blood sample	Lipids, glucose, renal function, transaminases, blood count and others	1	1	1	1	1	1	1	1
Urine sample		1	1	1	1	1	1	1	1
Stool sample		1	1	1	1	1	1	1	1
Insulin resistance test/Oral Glucose Tolerance Test (OGTT)		1	1	1	1	1	1	1	1
Postprandial lipaemia		1	0	0	1	0	1	0	0
Intima-media thickness (IMT) study	Carotid-Wall Intima–Media Thickness measurement	1	0	0	0	0	1	0	1
Brachial Artery Reactivity study	Ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery	1	0	0	0	0	1	0	0
Ankle-brachial index (ABI)		1	0	1	0	0	1	0	1
Microvascular endothelial function	Laser doppler flowmetry	1	1	1	1	1	1	1	1
Echocardiography		1	0	0	1	0	0	0	0

Table of interventions and follow-up. BP, blood pressure. (a) includes direct measurements of weight, height and waist circumference, and (b) only if necessary. *Circadian chronotype questionnaires were made at the beginning and one time during the study. They began to be collected in year 3 onwards, and the vast majority were obtained between the 3rd and 4th year (627), the rest were collected in the 5th year (135) and in the 6th year onwards (95)

6 Sample Size

The sample size and power calculation have been calculated on the following assumptions: an incidence rate in the control group (Low Fat) of 4 events/100 person-years that will amount to 24.9% of absolute

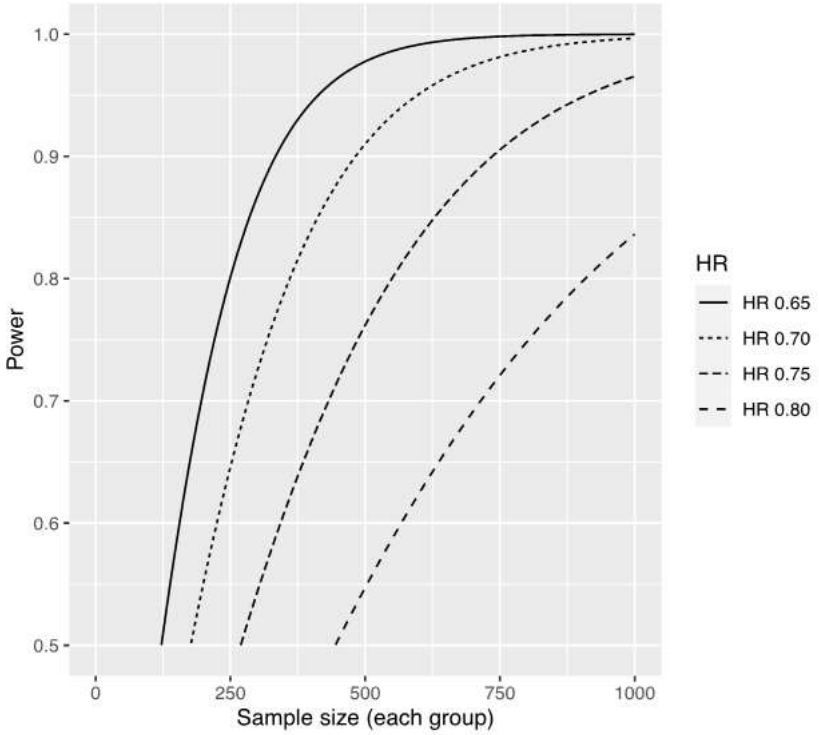
cumulative incidence after 7 years, a hazard ratio of 0.7 and a statistical power of 80%, with two-tailed alpha=0.05. Under these assumptions, the required sample size was 491 patients in each of the two groups. The size of the sample for the CORDIOPREV study was initially calculated for a cardiovascular disease recurrence of 35% in the Low Fat diet group and of 25% in the Mediterranean Diet group in 5 years, with an annual loss of 2%, an alpha error = 0.05 and a power of 0.90 (minimum sample size: 874 patients) of a composite endpoint that included cardiac death, myocardial infarction, unstable angina, revascularization, heart failure, heart transplantation, cardiac arrest, stroke, peripheral artery disease or any other manifestation of cardiovascular disease. In the meeting of the external advisory board in the third year of the study, and according to recent cardiovascular studies, the advisory board recommended limiting the definition of cardiovascular events to those finally included in the study, and which are defined above. This fact was in accordance with a clause in the original protocol, where an interim analysis evaluating the need for a change of sample size was included. Subsequently, based on the lower number of expected events, the final calculations for sample size and power were set as above.

7 General Considerations

7.1 Timing of Analyses

Power curves for the original assumptions of the number of events can be found below.

Yearly interim analyses will begin after a median of 2 years of follow-up. The interim analyses are planned for years 3, 4,5 and 6(O'Brien Fleming, pre-specified ps for the anticipated end of the study study 0·001, 0·004, 0·019 and 0·043).



Power curves under several assumptions for anticipated effect estimates (hazard ratios: 0.65, 0.70, 0.75 and 0.80) for the comparison of a Mediterranean Diet Intervention group versus the low-fat diet group.

7.2 Analysis Populations

7.2.1 Full Analysis Population

Analysis for Primary outcome will be performed in all subjects who were randomized (n=1002).

Analysis for secondary analysis will be performed in all subjects with all needed data for performing the statistical analysis. Subjects with missing or implausible data on the database on a per-subject basis could be excluded from the specific analysis after a reasoned informed approved by the Trial Steering Committee.

7.2.2 Per Protocol Population

Per Protocol main analysis included all subjects who will adhere to their randomized dietary model with a mean adherence higher or equal to 80% in all performed dietary assessments during the study, excluding

baseline data, based in the dietary screeners (MEDAS or Low-Fat score). Additional Per Protocol analysis will be evaluated by tentative cut-off points.

7.2.3 Safety Population
All subjects who started dietary intervention, until its censoring time.

7.3 Subgroup Analysis
Pre specific subgroup analysis will be performed based on:

- Sex
- Age
- Anthropometry
- Genetics
- Genomics
- Metabolism Of Immediate Principles
- Cardiovascular Risk Factors
- Cancer
- Vascular Function.

7.4 Missing Data
Missing data will be handled through multiple imputation protocol. Multiple imputation is a relatively flexible, general-purpose approach to dealing with missing data that reduces the risk of bias associated with excluding or including incomplete data sets in analysis. We will use multiple imputation by chained equations (MICE) which uses a separate conditional distribution and model for each imputed variable and allows imputation of data at a specific visit by including (imputed) data obtained at other visits. We will use R-software. Additional adjustments due to newer imputations systems could be used.

7.5 Interim Analyses and Data Monitoring
7.5.1 Purpose of Interim Analyses
Interim analysis will be performed to assess refining sample size at the third year of intervention, and to inform Data Monitoring Committees and assess the need for study stop.
7.5.2 Planned Schedule of Interim Analyses
An intermediate analysis will be done within 40 months of recruiting the first patient, to decide whether to change the sample size. In this analysis it will be assessed the random distribution of the patients selected, according to the risk factors. The control will be carried out by a person unaware of the randomization process. Yearly interim analyses will begin after a median of 2 years of follow-up. These interim analyses are planned for years 3, 4,5 and 6(O'Brien Fleming, pre-specified ps for the anticipated end of the study 0·001, 0·004, 0·019 and 0·043).

8 Efficacy Analyses

8.1 Primary Efficacy Analysis
Kaplan-Meier curves will be used for the graphical assessment of the risk provided by the different intervention groups as well as for calculating the probability of presenting new cardiovascular events after treatment and to study the differences between the two groups of patients the Log-Rank test will be used. The time until a new episode of the disease occurs will be assessed using Cox's proportional risk model. The risk to the development of cardiovascular events in each group will be calculated using Cox's proportional risk models by adjusting by multiple variables, performing forced models based on risk factors and risk markers. For the final analysis and the different interim analyses, cases will be censored on reaching the assessed follow-up time, on reaching life expectancy (as defined in the inclusion/exclusion criteria), on the date of abandon of the dietary intervention of participants who do not authorize the follow-up by electronic health records or telephone, or on the date of the first endpoint (major cardiovascular event), whichever occurred first.

For multivariate analysis of this data, it is predicted to use the SPSS statistical package and R software.

8.2 Secondary Efficacy Analyses
Continuous variables will be compared using preferentially Student "t" test and analysis of variance (ANOVA). If these variables do not follow a normal distribution, the relevant transformation of the data will be used for analysis. The association between categorical variables will be analyzed using the chi-square test.

In multivariate analysis, continuous variables could be categorized by levels of pathological significance (e.g. blood pressure, BMI, cholesterol, triglycerides) and introduced into the model as indicator variables. Continuous variables without defined cut-off points could be categorized by quartiles or quintiles, also entering them as indicators. To analyze the linear trend of the effect of an ordinal variable, it will be entered as continuous.
In those studies where a time-dependent study will be investigated, the log-rank test and the Cox's proportional risk models will be preferentially used.

8.3 Exploratory Efficacy Analyses
Due to the expected large amount of data to be generated, and the tight characterization of the population, with more than 1000 patients and several years of intervention, many hypothesis generator analyses will be made, in the light of present or future evidence.

8.4 Adjudication of events
In any medical visit, participants will be asked about any clinical event from the last visit. If there is any, a Final Event Report form (IFE) will be made, which will be eventually evaluated by a Clinical End Point Committee member.

Clinical End Point Committee: Cardiology: Eduardo de Teresa Galvan; Joaquin Ruiz de Castroviejo. Neurology: Juan José Ochoa, Roberto Valverde; Oncology: Enrique Aranda; Internal Medicine: Juan Antonio Ortiz Minuesa; Maria Angeles Blanco Molina. Cardiovascular Surgery: Ignacio Muñoz-Carvajal.

8.5 Definition of the components of the Primary Outcome:
The primary outcome of the CORDIOPREV study was to compare the appearance of several cardiovascular events after a median follow-up of 7 years in secondary prevention with 2 dietary models: a Mediterranean diet (rich in olive oil) or a low-fat diet. The composite outcome includes the following cardiovascular events: myocardial infarction, revascularization, ischemic stroke, documented peripheral artery disease, and cardiovascular death. These final components of the primary endpoint were adopted following the recommendation of the External Advisory Board during the third year of the study, based on contemporary relevant cardiovascular studies, in which only "hard events" were considered, reducing the number of components initially planned (namely, cardiac death, myocardial infarction, unstable angina, revascularization, heart failure, heart transplantation, cardiac arrest, stroke and peripheral artery disease). Following the adoption of the revised primary composite endpoint, the originally planned outcome was considered a secondary endpoint. As a result of the reduction of the components which defined the primary endpoint, and to maintain the power of the study, we extended the study follow-up from the 5 years originally planned to 7 years to have the power to detect a significant difference in the primary outcome between the 2 arms or until reaching futility on a 6-month basis analysis.
The components of the primary endpoint are defined as:
Acute myocardial infarction: Detection of a rise and/or fall of [cardiac biomarker] values (preferably cTn) with at least 1 value >99th percentile of the URL and b) At least 1 of the following:
1) Symptoms of myocardial ischemia
2) New or presumed new significant ST-segment–T wave (ST-T) changes or new LBBB on the ECG
3) Development of pathological Q waves on the ECG
4) Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
5) Identification of an intracoronary thrombus by angiography or autopsy.

Revascularization: Any physical procedure to restore circulation, either by percutaneous coronary intervention, or bypass graft surgery.
Ischemic stroke: Acute neurological deficit lasting more than 24 hours caused by thrombosis or arterial embolism of brain arteries, in which a brain imaging technique (computed tomography or magnetic resonance imaging) demonstrates a cerebral infarction.
Documented peripheral artery disease: Clinically symptomatic patients, which also showed documented disease in the different territories:
Carotid: DUS, CTA or MRA showing significant stenosis (>70%), or revascularization.
Lower limb: symptomatic patients with at least 1 of the following criteria: Clinical diagnosis of arterial occlusive disease based on imaging tests (duplex ultrasonography, magnetic resonance angiography, computed tomographic angiography, or catheter-based radiocontrast angiography), or clinical indication for endovascular or open surgical procedure (revascularization or amputation).
Cardiovascular Death: coronary heart disease deaths (i.e., acute myocardial infarction, unstable angina pectoris, and other forms of chronic ischemic heart disease), ischemic stroke, arrhythmias, dysrhythmias,

congestive heart failure, pulmonary edema and pulmonary embolisms. For a death to be considered sudden, the patient must have been seen by someone less than 24 hours before the presumed time of death, with no clinical worsening detected with respect to his or her baseline condition. Non-violent sudden deaths were considered cardiovascular deaths unless other causes were evident. The rationale for this assumption is the overwhelming (90%) percent of such deaths that in necropsy studies are found to be cardiovascular in origin in Spain and supported by the AHA/ACC consensus.

9 Safety Analyses

In any medical/dietist visit, participants will be asked about any secondary adverse event of diets. If there is any, a Final Event Report form (IFE) will be made, which will be eventually evaluated by a Clinical End Point Committee member.

Bibliography

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Intermediate Analysis.

Annual statistical intermediate analyses of the patients included in the study up to that time will be performed from year 3. If any difference in the occurrence of complications or survival between the groups will be detected, the study would be discontinued. Cut-off points based on O'Brien Fleming's rules will be used. In addition, an intermediate analysis shall be carried out 40 months after the first patient is recruited to decide whether to modify the sample size. In this analysis, the random distribution of the selected patients will be checked, according to the risk factors.

7. ETHICAL ASPECTS

The study has been prepared to respect the fundamental principles established in Helsinki Declaration (1964), in the Council of Europe Convention on Human Rights and Biomedicine (1997), in the human genome and human rights

UNESCO regulation (1997), as well as in compliance with the requirements established in Spanish legislation in the field of biomedical research, the protection of personal data and bioethics. The research project has been evaluated by the Ethics and Clinical Research Committee of the Reina Sofía University Hospital. All patients will be informed about the project and will be asked for written informed consent before entering it.

8. SCIENTIFIC-TECHNICAL DISSEMINATION PLAN

The research team undertakes to disseminate the results obtained in this study in the appropriate scientific forums and media.

The Results Dissemination Plan includes two important and complementary facets at the same time.

- First, the results of this research project will be presented at the National and International Congresses of the Scientific Societies related to the following fields: Arteriosclerosis, Nutrition and Internal Medicine. They will also be presented at the Annual Congress of the AHA, the highest international organization in the field of cardiovascular research in which our group has been making scientific contributions annually since 1992, at the International Congresses of Arteriosclerosis, at the Congress of Atherosclerosis and at the National Congress of Arteriosclerosis.
- Secondly, the results will be published in international journals related to the following fields: Arteriosclerosis, Nutrition and Metabolism. (Am J Clin Nutr; Arterioscler Thromb and Vasc Biol; Atherosclerosis; J Nutr; J Clin Endocrinol Metab, etc.). These forecasts are supported by the publications made by the research group in the last 5 years (54 original articles in the journals of maximum impact in the area (Am J Clin Nutr; J Mol Endocrinol; J Lipid Res; J Clin Endocrinol Metab; Atherosclerosis; Ann Int Med; Diabetes; Diabetology, Diabetes Care, Current Opinion in Cardiology, Current Opinion in Lipidology).
- Finally, the results will be disseminated in multiple conferences in other national and international forums (e.g. EXPOLIVA, MEDOLIVA, etc.) in which members of our research group participate every year.

2. Social and health dissemination.

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VI.- ABBREVIATIONS AND ACRONYMS

AHA: American Heart Association

CPK: Creatin fosfoquinasa

CRD: Data collection notebook

FA: Alkaline phosphatase

GOT: Oxaloacetic glutamic transaminase or Aspartate amino transferase

GPT: Transaminasa glutámico pirúvica o Alanino amino transferasa

HDL: High-density lipoproteins

AMI: Acute myocardial infarction

ACEinhibitors : Angiotensin-converting enzyme inhibitors

BMI: Body mass index (Kilograms: size:meters squared)

Kcal: Kilocalrías

Kg: kilograms

LDL: Low-density lipoproteins

MUFA: fat monoinsaturate

PA-I: Tissue plasminogen activator inhibitor type I

PUFA: grasa poliinsaturada

t-PA: Tissue plasminogen activator

SAT: saturated fat