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The SUN cohort study (Seguimiento University of Navarra)

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Abstract

Objective: Cohort study (the SUN project) to identify dietary determinants of hypertension, diabetes, obesity and coronary heart disease (CHD), among other conditions.
Design: The SUN project is a Mediterranean dynamic prospective follow-up (cohort) study assessing participants every 2 years. The recruitment started in 2000 and it is permanently open (this is a dynamic cohort). A preliminary case–control study of myocardial infarction was also conducted, selecting incident cases of myocardial infarction and matched hospital controls.
Setting: The SUN study is conducted in Spain with university graduates, most of them being former students of the University of Navarra. The case–control study was conducted in three tertiary hospitals in the city of Pamplona (Spain).
Subjects: The case–control study included 171 cases and 171 controls. We have currently enrolled more than 17 500 participants in the SUN cohort. Approximately 7500 of them were enrolled less than 2 years ago, and have not received yet the invitation to complete the 2-year follow-up assessment. The follow-up rate for the first 10 000 participants approaches 90%.
Results: The case–control study found inverse associations between olive oil (or adherence to a Mediterranean food pattern) and myocardial infarction. In the SUN cohort, after 28.5 months of follow-up a higher adherence to a Mediterranean food pattern rich in olive oil was associated with a reduced risk of hypertension, though the results were statistically significant only among men.
Conclusions: The SUN study and the accompanying case–control study support the benefits of a Mediterranean diet and olive oil against CHD and hypertension.

Keywords
Monounsaturated fatty acids
Hypertension
Coronary heart disease
Longitudinal studies
Mediterranean diet
Olive oil
Follow-up

Food items contain hundreds of chemical components that tend to be interrelated, thus making the study of their relationship with health outcomes particularly difficult. Therefore, the task of assessing the effect of food patterns on health is much more difficult than that of other exposures usually assessed in epidemiology. Dietary habits may be described according to the intake of nutrients, in terms of specific foods or food groups or in terms of overall food patterns. In general, the maximum information will be obtained when the analyses are based on all approaches[1,2]. Nutritional epidemiology began studying the effect of specific nutrients mainly defined from a biochemical point of view. Thus, the classical analytical approach was to assess the exposure to single nutrients or isolated food items, whereas a growing interest exists nowadays in studying overall food patterns because food items and nutrients could have synergistic or antagonistic effects when they are consumed in combination[3–5]. Overall patterns do represent the current practices found in the assessed population and, therefore, provide useful epidemiological information. Therefore, the assessment of the exposure to a particular existing food pattern in the real world may provide a direct and real base for elaborating food guidelines. In addition, this approach overcomes the problems between nutrient correlations. For example, it is very usual to find a substantial inverse correlation between fibre intake and saturated fatty acids (SFA), and the single-nutrient approach would not allow to ascertain whether there is a protection against coronary heart disease (CHD) afforded by fibre or a harm due to SFA intake. In this context, the Mediterranean food pattern has attracted considerable interest because of the belief that it may provide a substantial reduction in CHD risk[6].

Most information suggesting substantial benefits of the Mediterranean food pattern on cardiovascular risk comes from case–control studies, which are specially vulnerable to recall bias and selection biases, as may result from differential participation of cases and controls, selective survival of participating cases, and selection of a diet-related disease for the control group. Large cohort studies with repeated dietary assessment can give the most definitive answers. Unless similar cohorts are started now in Southern Europe, the needed evidences to produce

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soundly based guidelines will probably still remain controversial in the next 10–20 years. A large cohort study on diet and cancer, the European Prospective Investigation into Cancer and Nutrition (EPIC) was started in several European countries during the 1990s. However, it was not specifically designed to study the Mediterranean diet, nor has it selected a population which can be easily tracked for morbidity endpoints different from cancer because the follow-up in the EPIC study is based on cancer registries. A follow-up is planned only for fatal cardiovascular diseases (EPIC-Heart).

The most useful information available in public health about cardiovascular disease comes from cohorts which selected populations living in stable towns (Framingham), former students of a particular university (Harvard alumni), or highly educated professionals (Nurses' Health Study, Health Professionals' Follow-up Study). They have achieved an almost complete follow-up for fatal and non-fatal cases. The experience shows that these studies are invaluable. Nevertheless, a Mediterranean dietary pattern is uncommon in these US populations. For example, although increasing, olive consumption is still low, and the main source of monounsaturated fatty acids (MUFA) is red meat; the main source of ethanol intake is beer and liquors, instead of wine; and the main sources of some micronutrients are vitamin supplements instead of natural fresh fruits and vegetables. The only large available Mediterranean nutritional follow-up study is the Greek cohort of the EPIC study[7]. It has assessed only fatal cases of CHD (54 cases observed in 22 043 participants followed-up during 44 months). Using mortality as an endpoint can be confounded by factors related to case-fatality rate. Therefore, no available evidence about the association of a Mediterranean food pattern and the incidence of CHD (including both fatal and non-fatal) cases exists. Large cohorts assessing the incidence of CHD and other common diseases are needed. The Mediterranean food pattern has been many times postulated as a healthful alternative to reduce cardiovascular risk. Nevertheless, it is difficult to understand why a large cohort study with these characteristics has never been conducted in any Mediterranean country.

This SUN (Seguimiento Universidad de Navarra) project is developing a large long-term prospective study of dietary determinants of CHD, diabetes, and hypertension among Spanish subjects.

The SUN study: background, design and methods

The sharp contrast in dietary habits between the USA and Spain provides an exceptional opportunity to assess what aspects of the Mediterranean food pattern may be protective. That is the reason why we decided to start a prospective follow-up study (the SUN cohort) based on mailed questionnaires sent to alumni of the University of Navarra every 2 years.

We follow the design of a dynamic cohort, in which the recruitment of new participants is permanently open. We are inviting to participate all Spanish alumni of the University of Navarra and several other professional collectives with a university degree (Table 1 shows the cumulative number of participants). We select only those university graduates who are willing to commit themselves for returning questionnaires every 2 years. A semi-quantitative food-frequency questionnaire previously validated in Spain[8] is used together with other questionnaires designed to collect lifestyle characteristics. University graduates have the advantage of possessing a high cultural level that helps them to better understand and answer mailed questionnaires.

The SUN study was approved by the Institutional Review Board of the University of Navarra. Among its objectives are the study of the association between dietary and other lifestyle variables and the incidence of cardiovascular disease, hypertension, obesity, and diabetes[9–12]. Beginning on December 1999, all university graduates from the University of Navarra, and university graduates from professional associations, received a letter of invitation to participate in the study, a questionnaire to respond and a postage-prepaid envelope to return the questionnaire. This baseline questionnaire gathered information about sociodemographic variables, lifestyle factors, clinical variables and included a detailed food-frequency questionnaire[8]. A pilot study was conducted in 1999[10]. Currently, up to November 2005, more than 17 500 participants have been recruited and have completed the baseline assessment. Some of them (approximately 7500) were enrolled less than 2 years ago, and, therefore, they have not received yet the invitation to answer the 2-year follow-up questionnaire. The follow-up rate for the first 10 000 participants who have been already included in the cohort for a longer period than 2 years approaches 90%.

In cross-sectional analyses of the baseline data of our first participants, we identified those factors associated to a higher adherence to a Mediterranean food pattern[11,12]. We also conducted an analysis to identify the respective role of MUFA and fruit/vegetable consumption in the prevalence of high blood pressure[13]. In addition, a previous case–control study of first non-fatal myocardial

Table 1 Cumulative number of participants in the SUN cohort study

Year	2000	2001	2002	2003	2004	2005
Total (cumulative) number of enrolled participants	4717	8012	11 664	12 493	16 183	17 813

The recruitment of the cohort is permanently open (this is a dynamic cohort).
The cumulative number of participants for each year corresponds to 31 December of each year, with the exception of 2005 that corresponds to 15 October.

infarction (MI) (see below) was conducted using the same dietary assessment tool that we are currently using in the cohort study[14–22].

Preliminary case–control study of Mediterranean diet and MI

We conducted a case–control study in three tertiary hospitals of Pamplona, Spain, between 1999 and 2001. Study physicians enrolled 171 patients younger than 80 years of age with a first non-fatal MI and 171 control patients matched by age, sex, hospital and calendar month. We excluded patients with any prior major cardiovascular disease. Participants were interviewed about medical factors and lifestyle and completed the same food-frequency questionnaire previously validated in Spain that we are using in the SUN cohort[8]. Cases were defined as women and men less than 80 years of age with a first MI (International Classification of Diseases code 410) admitted to one of the three tertiary hospitals of Pamplona, Spain, within the period October 1999 through February 2001. For inclusion in the study, patients had to fulfil the criteria for definite MI of the MONICA project (two or more electrocardiograms showing definitive changes, electrocardiograms showing probable changes plus abnormal cardiac enzymes, or typical symptoms plus abnormal enzymes). We excluded patients with previous history of angina pectoris, a previous diagnosis of CHD, or other prior diagnosis of major cardiovascular disease. Participation rate was 95%. Institutional Review Board approval was obtained from the Navarre Medical School, and patients provided informed consent before participation.

Eligible controls were patients admitted to surgery, trauma, or urology wards of the same hospitals for treatment of conditions believed to be unrelated to diet. We applied the same exclusion criteria for controls as for cases. We matched one control to each case by age (within 5 years), sex, calendar time (hospitalised during the same month), and hospital. Eight controls refused to participate; each was replaced by another patient of similar characteristics for matching variables.

The same physician who interviewed a case patient also interviewed the respective matched control. The physician clarified any questions the patient might have had in completing the questionnaire and subsequently conducted a face-to-face interview about coronary risk factors (i.e. smoking, diabetes, high blood pressure, high blood cholesterol, and recent weight changes) and family history of cardiovascular disease. The physician took systolic and fifth-phase diastolic blood pressure readings and measured weight and height. In the food-frequency questionnaire, nine options for frequency of consumption were possible. The type of fat used in frying was specifically assessed. A dietician updated the nutrient databank using the latest available information included in the food composition tables for Spain. Participants were asked to report their usual time spent practising the following activities: walking, jogging, running, athletics, cycling, swimming, racquet sports, soccer, team sports other than soccer, dancing, aerobics, hiking, climbing, gardening, skiing, skating, fishing, martial arts, and water sports. To quantify the volume and intensity of leisure-time physical activity, we computed an activity metabolic equivalent (MET)[23–25].

We report below the most salient findings of this case–control study. Odds ratios (OR) and 95% confidence intervals (CI) were estimated for MI using conditional logistic regression with 171 case–control matched pairs. We assumed that OR from this case–control study provide a valid estimate of the relative risk. Energy-adjusted intakes were computed using the residuals method[1]. We adjusted relative risks for well-established risk factors for MI, including dietary exposures.

Olive oil and MI

The exposure to the upper quintile of energy-adjusted olive oil (median intake: 54 g/day) was associated with a statistically significant 82% relative reduction in the risk of a first MI (OR = 0.18, 95% CI 0.06–0.63) after adjustment for dietary and non-dietary confounders. These data suggest that olive oil may reduce the risk of CHD[14]. Our findings were consistent with the subsequent report of the GISSI cohort, where the outcome was restricted only to fatal cases[22]. Further large cohort studies with non-fatal events as outcome and randomised trials are still needed to confirm our findings[2,6].

Mediterranean dietary pattern and MI

We defined a Mediterranean food pattern including six food items that we considered protective: (1) olive oil, (2) fibre, (3) fruits, (4) vegetables, (5) fish, and (6) alcohol. For each of these six dietary factors, we calculated the distribution according to quintiles within the study and assigned each participant a score of 1–5 corresponding to the quintile of intake, with 1 representing the lowest and 5 representing the highest quintile. We also estimated the quintiles of two other elements assumed to be associated with a higher risk: (7) meat/meat products, and (8) some items with high glycaemic load (white bread, pasta and rice). For these two elements we inversely ranked the score, with 1 representing the highest and 5 representing the lowest quintile. Finally, we summed up the eight quintile values for each participant. We found that the higher the score, the lower the odds of MI. A significant linear trend was apparent after adjustment for the main cardiovascular risk factors. For each additional point in the Mediterranean pattern (observed range 9–38) the OR (95% CI) was 0.92 (0.86–0.98). Our data support the hypothesis that a Mediterranean food pattern (that emphasises olive oil, fibre, fruits, vegetables, fish and alcohol, and reduces meat/meat products) can be an effective measure for reducing the risk of MI. However,

our results support the exclusion of refined cereals with a high glycaemic load as healthy elements of this pattern[17].

Subsequent reports from case–control studies and the Greek EPIC cohort are consistent with these results[7,26].

Olive oil and reduced risk of hypertension among men

A major goal of dietary recommendations is to reduce blood pressure. In early epidemiological studies, mainly conducted in the United States, MUFA showed a deleterious association with blood pressure, or no relationship at all. However, more recent studies, conducted in Mediterranean countries, have shed new light on this issue[27]. It is conceivable that the lower blood pressure levels and the relatively lower prevalence of hypertension found in Spain, in spite of a high average alcohol consumption, may be explained by a high consumption of olive oil. Based in an increasing amount of evidence, the classical Mediterranean food pattern has been proposed as a healthy choice for the prevention of cardiovascular disease[28]. Part of its beneficial impact can be mediated through a favorable effect on blood pressure. A major characteristic of Mediterranean diet is a high supply of energy coming from MUFA, mainly from olive oil. When olive oil is the major dietary source of monounsaturated fat, as it happens in Mediterranean countries, it could exert a favorable effect on blood pressure. We tested this hypothesis using a prospective design in the SUN cohort.

The retention proportion for the first 7650 participants in the SUN cohort was 90%. The median follow-up time was 28.5 months. Out the first 6863 participants in our cohort with data at baseline and at the first follow-up questionnaire (after >2-year follow-up), 658 were excluded due to prevalent hypertension at baseline and 632 because of extremely low or high caloric intakes (<400 kcal/day for women, <600 kcal/day for men, >3500 kcal/day for women, >4200 kcal/day for men). Finally, 5573 participants were available for analysis, 3384 women and 2189 men. We assessed the validity of a self-reported diagnosis of hypertension in a sample of 79 individuals reporting a medical diagnosis of hypertension and 41 not reporting such diagnosis. The positive and negative predictive values for the self-reported diagnosis of hypertension were 82 and 85%, respectively[29].

During follow-up, 161 incident cases of hypertension were identified among them (cumulative incidence 2.9%). The incidence was much lower in women than in men (1.7 vs. 4.7%). The cumulative incidence of hypertension was computed for each quintile of olive oil consumption. To avoid confounding for other variables simultaneously associated with the outcome (hypertension) and the main exposure, we used non-conditional logistic regression modelling after adjusting for age, gender, body mass index, physical activity during leisure time, total energy intake, alcohol consumption, sodium intake, and calcium intake. In addition, models for each gender were run separately. Tests for a linear trend in the relationship between olive oil consumption and risk of hypertension were obtained by assigning the median value for each quintile of olive oil consumption and modeling this variable as continuous in the logistic model.

The risk of developing hypertension during follow-up was lower among participants with a higher baseline consumption of olive oil, but the results were not statistically significant ($P = 0.13$ for the trend test). Considering men and women together, the adjusted OR (95% CI) of hypertension for the two upper quintiles of olive oil consumption (compared with the first quintile) were 0.55 (0.31–0.98) for the fourth quintile and 0.63 (0.36–1.07) for the fifth quintile. Among men, the adjusted OR (95% CI) of hypertension for the second to fifth quintiles of olive oil consumption were 0.55 (0.28–1.10), 0.75 (0.39–1.43), 0.32 (0.15–0.70), and 0.46 (0.23–0.94), respectively. The linear trend test was statistically significant only in men. No association was found between olive oil consumption and the risk of hypertension in women[30].

Our results are consistent with a cross-sectional analysis conducted in Greece[31], with the results of a small trial conducted in Italy[32] and with the recent results from the OmniHeart Randomized Trial[33], and they support a protection afforded by olive oil against the risk of developing hypertension.

References

1 Willett W. *Nutritional Epidemiology*. New York: Oxford University Press, 1998.
2 Martínez-González MA, Estruch R. Mediterranean diet, antioxidants, and cancer: the need for randomised trials. *European Journal of Cancer Prevention* 2004; **13**: 327–35.
3 Jacques PF, Tucker KL. Are dietary patterns useful for understanding the role of diet in chronic disease? *American Journal of Clinical Nutrition* 2001; **73**: 1–2.
4 Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Current Opinion in Lipidology* 2002; **13**: 3–9.
5 Jacobs DR Jr, Steffen LM. Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. *American Journal of Clinical Nutrition* 2003; **78**(3 Suppl): 508S–13S.
6 Martínez-González MA, Sánchez-Villegas A. The emerging role of Mediterranean diets in cardiovascular epidemiology: monounsaturated fats, olive oil, red wine or the whole pattern? *European Journal of Epidemiology* 2004; **19**: 9–13.
7 Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *New England Journal of Medicine* 2003; **348**: 2599–608.
8 Martín-Moreno JM, Boyle P, Gorgojo L, Maisonneuve P, Fernández-Rodríguez JC, Salvini S, *et al*. Development and validation of a food frequency questionnaire in Spain. *International Journal of Epidemiology* 1993; **22**: 512–9.
9 Martínez-González MA, Sánchez-Villegas A, De Irala J, Martí A, Martínez JA. Mediterranean diet and stroke:

objectives and design of the SUN project. *Nutritional Neuroscience* 2002; **5**: 65–73.
10 Sánchez-Villegas A, de Irala-Estévez J, Martínez-González MA, *et al*. Dieta Mediterránea y enfermedad cardiovascular: resultados del estudio piloto del proyecto SUN. *Revista de Medicina de la Universidad de Navarra* 2002; **46**: 9–16.
11 Sanchez-Villegas A, Martínez JA, De Irala J, Martínez-González MA. Determinants of the adherence to an 'a priori' defined Mediterranean dietary pattern. *European Journal of Nutrition* 2002; **41**: 249–57.
12 Sánchez-Villegas A, Delgado-Rodriguez M, Martínez-González MA, De Irala-Estevez J. Gender, age, socio-demographic and lifestyle factors associated with major dietary patterns in the Spanish Project SUN (Seguimiento Universidad de Navarra). *European Journal of Clinical Nutrition* 2003; **57**: 285–92.
13 Alonso A, de la Fuente C, Martin-Arnau AM, de Irala J, Martinez JA, Martinez-Gonzalez MA. Fruit and vegetable consumption is inversely associated with blood pressure in a Mediterranean population with a high vegetable-fat intake: the Seguimiento Universidad de Navarra (SUN) study. *British Journal of Nutrition* 2004; **92**: 311–9.
14 Fernández-Jarne E, Martínez-Losa E, Prado-Santamaria M, Brugarolas-Brufau C, Serrano-Martínez M, Martínez-González MA. Risk of first non-fatal myocardial infarction negatively associated with olive oil consumption: a case–control study in Spain. *International Journal of Epidemiology* 2002; **31**: 474–80.
15 Fernández-Jarne E, Alegre Garrido F, Alonso Gutierrez A, de La Fuente Arrillaga C, Martínez-González MA. Ingesta de ácidos grasos omega 3 y riesgo de infarto de miocardio: un estudio de casos y controles. *Medicina Clinica* 2002; **118**: 121–5.
16 Martínez-González MA, Fernández-Jarne E, Martínez-Losa E, Prado-Santamaria M, Brugarolas-Brufau C, Serrano-Martínez M. Role of fibre and fruit in the Mediterranean diet to protect against myocardial infarction: a case–control study in Spain. *European Journal of Clinical Nutrition* 2002; **56**: 715–22.
17 Martínez-González MA, Fernández-Jarne E, Serrano-Martínez M, Marti A, Martínez JA, Martín-Moreno JM. Mediterranean diet and reduction in the risk of a first acute myocardial infarction: an operational healthy dietary score. *European Journal of Nutrition* 2002; **41**: 153–60.
18 Hernandez-Diaz S, Martínez-Losa E, Fernández-Jarne E, Serrano-Martínez M, Martínez-González MA. Dietary folate and the risk of nonfatal myocardial infarction. *Epidemiology* 2002; **13**: 700–6.
19 Martínez-González MA, Alonso A, Fernández-Jarne E, de Irala J. What is protective in the Mediterranean diet? *Atherosclerosis* 2003; **166**: 405–7.
20 Alonso A, Fernández-Jarne E, Serrano-Martínez M, Martínez-González MA. Fish and shellfish consumption in relation to death from myocardial infarction among men in Shanghai China. *American Journal of Epidemiology* 2003; **157**: 85.
21 Fernández-Jarne E, Martínez-Losa E, Serrano-Martínez M, Prado-Santamaria M, Brugarolas-Brufau C, Martínez-González MA. Type of alcoholic beverage and first acute myocardial infarction: a case–control study in a Mediterranean country. *Clinical Cardiology* 2003; **26**: 313–8.
22 Barzi F, Woodward M, Marfisi RM, Tavazzi L, Valagussa F, Marchioli R, GISSI-Prevenzione Investigators. Mediterranean diet and all-causes mortality after myocardial infarction: results from the GISSI-Prevenzione trial. *European Journal of Clinical Nutrition* 2003; **57**: 604–11. Erratum in: *European Journal of Clinical Nutrition* 2003; **57**: 1034.
23 Chasan-Taber S, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E, *et al*. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology* 1996; **7**: 81–6.
24 Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, *et al*. Compendium of physical activities: an update of activity codes and MET intensities. *Medicine and Science in Sports and Exercise* 2000; **32**: S498–S504.
25 Martínez-González MA, Varo JJ, Santos JL, De Irala J, Gibney M, Kearney J, *et al*. Prevalence of physical activity during leisure time in the European Union. *Medicine and Science in Sports and Exercise* 2001; **33**: 1142–6.
26 Panagiotakos DB, Pitsavos C, Chrysohoou C, Stefanadis C, Toutouzas P. Risk stratification of coronary heart disease in Greece: final results from the CARDIO2000 epidemiological study. *Preventive Medicine* 2002; **35**: 548–56.
27 Alonso A, Ruiz-Gutierrez V, Martínez-González MA. Monounsaturated fatty acids, olive oil and blood pressure: epidemiological, clinical and experimental evidences. *Public Health Nutrition* 2006; in press.
28 Alonso A, Beunza JJ, Delgado-Rodriguez M, Martinez-Gonzalez MA. Validation of self reported diagnosis of hypertension in a cohort of university graduates in Spain. *BMC Public Health* 2005; **5**: 94.
29 Martinez-Gonzalez MA, Fernandez-Jarne E, Serrano-Martinez M, Wright M, Gomez-Gracia E. Development of a short dietary intake questionnaire for the quantitative estimation of adherence to a cardioprotective Mediterranean diet. *European Journal of Clinical Nutrition* 2004; **58**: 1550–2.
30 Alonso A, Martinez-Gonzalez MA. Olive oil consumption and reduced incidence of hypertension: the SUN study. *Lipids* 2004; **39**: 1233–8.
31 Psaltopoulou T, Naska A, Orfanos P, Trichopoulos D, Mountokalakis T, Trichopoulou A. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. *American Journal of Clinical Nutrition* 2004; **80**: 1012–8.
32 Ferrara LA, Raimondi S, D'Episcopo l, Guilda l, Dello Russo A, Marotta T. Olive oil and reduced need for antiyhypertensive medications. *Archives of Internal Medicine* 2000; **160**: 837–42.
33 Appel LJ, Sacks FM, Carey VJ, Obarzanek E, Swain JF, Miller ER 3rd, *et al*. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart Randomized Trial. *Journal of the American Medical Association* 2005; **294**: 2455–64.