

MEDITERRANEAN DIET AND INVASIVE BREAST CANCER RISK AMONG WOMEN AT HIGH CARDIOVASCULAR RISK IN THE PREDIMED TRIAL. A RANDOMIZED CLINICAL TRIAL.

Toledo E^(1,2,3), MD; Salas-Salvadó J^(2,4), MD; Donat-Vargas C^(1,3), PharmD; Buil-Cosiales P^(2,3,5), MD; Estruch R^(2,6), MD; Ros E^(2,7), MD; Corella D^(2,8), PhD; Fito M^(2,9), PhD; Hu FB^(10,11), MD; Arós F^(2,12), MD; Gómez-Gracia E^(2,13), MD; Romaguera D^(2,14,15), PhD; Ortega-Calvo M^(2,16), MD; Serra-Majem L^(2,17), MD; Pintó X^(2,18), MD; Schröder H^(8,19), PhD; Basora J^(2,4), Sorli JV^(2,8), Bulló M, PhD^(2,4), Serra-Mir M^(2,7), RD; Martinez-Gonzalez MA^(1,2,3), MD.

1: Department of Preventive Medicine and Public Health, University of Navarra-School of Medicine, Pamplona, Navarra, Spain

2: Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Madrid, Spain

3: IdiSNA, Navarra Institute for Health Research, Pamplona, Navarra, Spain

4: Human Nutrition Unit, Faculty of Medicine and Health Sciences, Biochemistry Biotechnology Department, Universitat Rovira i Virgili, and Hospital Universitari de Sant Joan de Reus, IISPV, Reus, Spain

5: Servicio Navarro de Salud-Osasunbidea, Pamplona, Navarra, Spain

6: Department of Internal Medicine, Institut d'Investigacions Biomèdiques August Pi I Sunyer, Hospital Clinic, University of Barcelona, Barcelona, Spain

7: Lipid Clinic, Department of Endocrinology and Nutrition, Institut d'Investigacions Biomèdiques August Pi I Sunyer, Hospital Clinic, University of Barcelona, Barcelona, Spain

8: Department of Preventive Medicine, University of Valencia, Valencia, Spain

9: University of Barcelona, Barcelona, Spain; Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, and Primary Care Division of Barcelona. Institut Català de la Salut and IDiap-Jordi Gol, Barcelona, Cataluña, Spain

10: Department of Nutrition, Harvard School of Public Health, Boston, MA, USA

11: Harvard Medical School and Channing Lab, Brigham Women's Hospital, Boston, MA, USA

12: University Hospital of Alava, Vitoria, Basque Country, Spain

13: Department of Preventive Medicine, University of Malaga, Malaga, Spain

14: Instituto de Investigación Sanitaria de Palma (IdISPa), Hospital Universitario Son Espases, Palma de Mallorca, Spain

15: School of Public Health, Imperial College London, UK

16: Department of Family Medicine, Primary Care Division of Sevilla, Sevilla, Spain

17: Research Institute of Biomedical and Health Sciences, University of Las Palmas de Gran Canaria, Las Palmas, Spain

18: Lipids and Vascular Risk Unit, Internal Medicine, Hospital Universitario de Bellvitge-IDIBELL-UB, Hospitalet de Llobregat, Barcelona, Spain

19: Centro de Investigación Biomédica en Red Epidemiología y Salud Pública, Instituto de Salud Carlos III, Madrid, Spain

* The PREDIMED (Prevención con Dieta Mediterránea) study investigators are listed in the Supplementary Appendix.

CORRESPONDING AUTHOR

Prof. Miguel A. Martínez-González

Department of Preventive Medicine and Public Health

University of Navarra-School of Medicine

Edificio de Investigación, 2a planta

C/ Irunlarrea, 1

31008 Pamplona, Navarra, Spain

Phone: +34 948425600 ext 806463

Fax: +34 948425740

Email: mamartinez@unav.es

WORD COUNT: 3,496

ABSTRACT

Importance

Breast cancer is the leading cause of female cancer burden and its incidence has increased by more than 20% worldwide since 2008. Some observational studies have suggested that the Mediterranean diet may reduce the risk of breast cancer.

Objective

To evaluate the effect of two interventions with Mediterranean diet versus the advice to follow a low-fat diet (control) on breast cancer incidence.

Design

The PREDIMED study is a 1:1:1 randomized, single-blind, controlled field trial conducted in Spain.

Setting

Primary health care centers.

Participants

From 2003 to 2009, 4,282 women aged 60-80 years and at high cardiovascular disease were recruited after invitation by their primary health care providers.

Intervention

Participants were randomly allocated to: a Mediterranean diet supplemented with extra-virgin olive oil, a Mediterranean diet supplemented with mixed nuts, or a control diet (advice to reduce dietary fat).

Main Outcome and Measure

Breast cancer incidence was a pre-specified secondary outcome of the trial for women without a prior history of breast cancer (n=4,152).

Results

After a median follow-up of 4.8 years, we identified 35 confirmed incident cases of breast cancer. Observed rates (per 1000 person-years) were 1.1 for the Mediterranean diet with extra-virgin olive oil group, 1.8 for the Mediterranean diet with nuts group, and 2.9 for the control group. The multivariable-adjusted hazard ratios versus the control group were 0.32 (95% CI:

0.13 to 0.79) for the Mediterranean diet with extra-virgin olive oil group, and 0.59 (95% CI: 0.26 to 1.35) for the Mediterranean diet with nuts group. In analyses with yearly cumulative updated dietary exposures, the HR for each additional 5% calories from extra-virgin olive oil was 0.72 (95% CI, 0.57 to 0.90).

Conclusions and Relevance

This is the first randomized trial finding an effect of a long-term dietary intervention on breast cancer incidence. Our results suggest a beneficial effect of a Mediterranean diet supplemented with extra-virgin olive oil in the primary prevention of breast cancer. These results come from a secondary analysis of a previous trial, are based on few incident cases and, therefore, need to be confirmed in longer-term and larger studies.

Trial registration

Controlled-Trials.com number, ISRCTN35739639.

INTRODUCTION

Breast cancer, the most frequently diagnosed malignant tumor and the leading cause of cancer death among women, has increasing incidence rates. In 2012, 1.7 million women were diagnosed with breast cancer. Since the 2008 estimates, breast cancer incidence has increased by more than 20% worldwide, while mortality has increased by 14%.¹ In European countries, breast cancer is the most common incident cancer and the first or second (after lung cancer) malignancy implicated in mortality among women.²

Diet has been extensively studied as a modifiable component of lifestyle that could influence breast cancer development. Epidemiological evidence of the effect of specific dietary factors is still inconsistent and the only convincing evidence relates to an increased risk in women with high alcohol consumption.³

The inconsistent association between foods or nutrients and breast cancer risk may be partly due to the fact that individuals do not consume foods or nutrients in isolation but mixtures of foods with different nutrient constituents that may interact synergistically to influence biological pathways leading to or protecting from cancer. Thus, assessing diet as a whole, based on overall dietary patterns, provides more useful information on the role of diet in breast cancer risk. The Mediterranean dietary pattern has attracted considerable attention because historically breast cancer rates have been lower in Mediterranean countries than in Northern or Central European countries or the US.^{4,5} The Mediterranean diet (MeDiet) is characterized by the abundance of plant foods, fish, and especially olive oil.⁵ In the Lyon Diet Heart Study, participants allocated to a cardioprotective Mediterranean-type diet showed a 61% lower risk of cancer (all subtypes) than those participants allocated to a control diet close to the step 1 American Heart Association prudent diet.⁶ Recent prospective cohort studies have evaluated the association between adherence to a MeDiet pattern and specifically breast cancer risk.^{7,8} However, the epidemiological evidence is still limited and conflicting.^{9,10} Moreover, no randomized trial has ever assessed the effect of the MeDiet on the primary prevention of breast cancer.

To further examine the effects of the MeDiet on breast cancer risk, we have analyzed the effect of the MeDiet supplemented with extra-virgin olive oil (EVOO) or nuts in the randomized intervention of the PREDIMED trial on the incidence of breast cancer.

METHODS

Trial design

This study was conducted within the frame of the PREDIMED (*PREvención con Dieta MEDiterránea*) trial [www.predimed.es; registered at Controlled-Trials.com (ISRCTN35739639)].^{11,12} Briefly, PREDIMED is a large, multicenter, randomized trial designed to test the effects of the traditional MeDiet on the primary prevention of cardiovascular disease (CVD). The trial was stopped in December 1, 2010 after a median follow-up of 4.8 years because of evidence of early cardiovascular benefit of both MeDiet groups compared to the control group.

Participants

Eligible participants for the PREDIMED trial were men aged 55 to 80 years and women aged 60 to 80 years free of CVD at enrolment, who had either type 2 diabetes or at least three of the following major cardiovascular risk factors: smoking, hypertension, elevated low-density lipoprotein cholesterol, low high-density lipoprotein cholesterol, overweight or obesity, or family history of premature coronary heart disease.¹¹ Study candidates were selected from databases of primary care facilities. Of those candidates meeting enrolment criteria, 89% agreed to participate and provided written informed consent.

Randomization, masking, interventions and measurements

During the period October 2003 to June 2009, 7,447 men and women were enrolled in the PREDIMED trial, of which 4,282 were women. Participants were randomly allocated in a 1:1:1 ratio to one of the three intervention groups: MeDiet supplemented with EVOO, MeDiet supplemented with mixed nuts, or control diet (advice to reduce dietary fat). The coordinating

center constructed a computer-generated randomization table. Allocation was concealed by opaque, sequentially numbered, and sealed envelopes and stratified by sex and age. For the present study, one woman was excluded due to a prior diagnosis of breast cancer and other 7 women were excluded because of probable (non-confirmed as malignant) breast tumors. Investigators assessing the occurrence of new breast cancer cases were blinded to the intervention.

Participants in the two intervention groups were given supplementary foods for free: EVOO (1 litre/wk for the participant and their families) or mixed nuts (30 g/day: 15 g walnuts, 7.5 g hazelnuts, and 7.5 g almonds) according to their randomization group. The purpose of supplementation was to both ensure a high consumption of these key components of the traditional MeDiet and promote a better overall adherence to the intervention.

At baseline and quarterly thereafter, dietitians ran individual and group sessions, with up to 20 participants, separately for each group. In the appropriate individual sessions, a 14-item dietary screening questionnaire was used to assess adherence to either of the MeDiets, and a 9-item dietary screening questionnaire was used to assess adherence to the control diet. The answers to the questionnaires were used as a tool to personalize the intervention for each participant and to negotiate changes to upgrade adherence to either the MeDiet or the control diet.

Participants in the control group also received dietary training at the baseline visit and completed the 14-item dietary screener used to assess baseline adherence to the MeDiet. Thereafter, during the first 3 years of the trial, they received a leaflet explaining the low-fat diet on a yearly basis. However, the realization that the more infrequent visit schedule and less intense support for the control group might be limitations of the trial prompted us to amend the protocol in October 2006. Thereafter, participants assigned to the control diet received personalized advice and were invited to group sessions with the same frequency and intensity as those in the Mediterranean-diet groups, with the use of a separate 9-item dietary screener. During the study, participants in the control group received gifts of non-food items as incentives.

Attained changes in diet are shown in eTable 1.

Energy restriction was not specifically advised nor was physical activity promoted in any group. The intervention did not target drug prescriptions; thus, it was implemented within the regular medical care of the participants.

Outcome

Cases were defined as the first invasive breast cancer (International Classification of Diseases for Oncology codes C50.1 to C50.9). Availability of results from a cytological or histological examination was considered as confirmation. Even though information on biological parameters was not requested for a case to be accepted, medical records have been reviewed to extract this information. Incident cases through December 1, 2010 were identified from two sources: review of all the medical records of each participant by a panel of physicians (masked to the intervention), both at the primary health care level and at the hospital level, and death certificates (ICD-9 code 174 or ICD-10 code C50). A clinical events committee blinded to the intervention and the dietary information of participants adjudicated all end points using pre-specified criteria.

Cancer incidence was defined as a secondary outcome in the original study protocol. Five specific cancer locations were always included as relevant outcomes in all interim analyses and in all reports prepared every year for the Data and Safety Monitoring Board of the PREDIMED trial: breast cancer, lung cancer, prostate cancer, colorectal cancer and gastric cancer. These results on breast cancer are the first results for any cancer that have been analyzed and submitted for publication in the PREDIMED trial.

Follow-up ended at the time of diagnosis of an invasive breast cancer, death, last follow-up contact or December 1st 2010, whichever occurred first.

Covariates

At baseline and once yearly during follow-up, a validated 14-item MeDiet screener¹³, a general medical questionnaire, a 137-item validated food-frequency questionnaire¹⁴, and the Minnesota Leisure-Time Physical Activity Questionnaire^{15,16} were administered. Information from the food-frequency questionnaire was used to calculate intake of energy and nutrients. Other lifestyle-related variables such as smoking, health conditions and socio-demographic variables were assessed by a 47-item general questionnaire¹². In addition, trained study personnel directly measured weight, height and waist circumference.

Sample size

Sample size was estimated for the primary endpoint, namely CVD. It was reassessed in 2008 and set at 7400 participants with the assumption of a 6-year follow-up and underlying CVD event rates of 8.8% and 6.6% in the control and intervention groups, respectively.¹²

Statistical analysis

Our main analyses were performed on an intention-to-treat basis. We used Cox regression models with robust estimates for the variance to assess the effect of the intervention on malignant breast cancer incidence. First, we fitted a crude model and then we adjusted for age (3 groups: ≤ 60 years, $>60-70$ years, >70 years), recruitment center, baseline body mass index (defined as the weight in kg divided by the square of height in meters, categorized into quartiles), waist-to-height ratio (dichotomous), use of hormone-replacement therapy, leisure-time physical activity (categorized into quartiles), total energy intake (categorized into quartiles), alcohol consumption (categorized into quartiles), age at menopause, smoking habit, diabetes, use of statins, family history of cancer, and baseline adherence to the MeDiet (high vs. low). In an ancillary analysis, we merged both MeDiet groups and assessed their effect compared to the control group. For the primary analysis, we excluded seven women with a non-pathologically confirmed incident breast cancer. In sensitivity analyses, we included these women as cases or as non-cases. We repeated our analyses after excluding women who were diagnosed of malignant breast cancer during the first year of follow-up and considering only

malignancies positive for estrogen receptors (ER+). We did subgroup analyses stratifying by age, smoking status, alcohol intake, prevalent type-2 diabetes, overweight, use of hormone-replacement therapy, and family history of cancer. However, the small number of cases in some of the strata precluded fitting the models for some of these subgroups. Analyses were repeated with Poisson regression models with robust estimates for the variance. Finally, we also completed a per protocol analysis where we used time-dependent Cox models to assess the association between attained consumption of EVOO during follow-up (cumulative average across all the available food-frequency questionnaires) and subsequent incidence of breast cancer.

RESULTS

From October 2003 through June 2009, 4,282 women were randomly assigned to one of the three intervention groups (eFigure 1). Their baseline characteristics are shown in Table 1. The mean age of participants was 67 years and mean body mass index was 30 kg/m². Most women had menopause before 55 years of age and less than three percent used hormone-replacement therapy. Baseline characteristics were well balanced in the three groups.

During a median follow-up time of 4.8 years, we identified 35 confirmed incident cases of malignant breast cancer. Among them, 33 had available information on estrogen receptors and 31 were positive. Out of 27 cases with information on progesterone receptors, 21 were positive, and out of 21 with information on erbB-2 receptors, 12 were positive. One hundred and twenty-two participants had no information for breast cancer incidence during follow-up.

Women allocated to the MeDiet supplemented with EVOO showed a 62% relatively lower risk of malignant breast cancer than those allocated to the control diet (95% CI: 0.16 to 0.87) (Figure 1). Participants in the MeDiet supplemented with nuts showed a non-significant risk reduction compared with women in the control group (RR 0.62, 95% CI: 0.29 to 1.36). When both MeDiet groups were merged together, we observed a 51% relative risk reduction (95% CI: 0.25 to 0.94) (Table 2). When we excluded women diagnosed with malignant breast cancer during

the first year after enrolment, the results hardly changed. Similarly, the results did not substantially change after including women with breast cancer with no cytological or histological confirmation either as cases or as non-cases or when we considered only ER+ malignancies. In the stratified analyses, all but just two point estimates showed an inverse association between the MeDiet+EVOO intervention and the incidence of breast cancer (Table 3).

When we assessed the three arms of the trial together, participants who attained a higher EVOO consumption during follow-up exhibited the lowest risk [HR 5th vs. 1st quintile: 0.18 (95% CI: 0.06-0.57)] (Figure 2). In these analyses with yearly cumulative updated dietary exposures, the HR was 0.72 (95% CI, 0.57 to 0.90) for each additional 5% calories from EVOO.

DISCUSSION

In this secondary analysis of the PREDIMED trial we found a significant inverse association between a MeDiet supplemented with EVOO and breast cancer. A high consumption of EVOO ($\geq 15\%$ of total energy intake) seems to be instrumental for obtaining this significant protection. A non-significant risk reduction was observed with the MeDiet supplemented with nuts.

The strengths of this study are its randomized design, the achieved changes in the participants' dietary habits according to the intervention¹⁷, little residual confounding with almost no changes in estimates after adjusting for many potential confounders, and the thorough and blind revision of medical information to assess outcomes. The Adjudication Committee, whose members were blinded to the intervention group, assessed the events with specific criteria dispelling potential misclassification biases. We also acknowledge some limitations. First, breast cancer was not the primary endpoint of the PREDIMED trial. Thus, the present work is only a pre-specified secondary analysis of a large nutritional intervention trial and we cannot warrant that all women had mammographies free from suggestive findings at baseline. However, the randomization was able to yield well-balanced and comparable groups and, given the large sample size, a balance in other characteristics can be safely assumed. Second, the number of observed breast cancer cases was small. The potential for missing some incident breast cancer cases is basically null

regarding clinically relevant events. In any case, this remote possibility will affect only women lost to follow-up, and most of them belonged to the control group. Therefore, undetected cases of breast cancer would more likely have increased even further the rate in the control group. Accordingly, our results would tend to underestimate the beneficial effect of the intervention. The low rate of breast cancer among women in the PREDIMED trial should not be surprising. If the MeDiet is actually protective against breast cancer, a very low incidence is to be expected in a study with these characteristics, especially when overall adherence to the MeDiet was good already at baseline. Third, we do not have information on an individual basis on whether and when women in our trial underwent a mammogram. Potentially, cancers could be missed without mammograms. However, because of the randomized design and the large sample size, we feel that we can safely assume an even distribution of subclinical cases in the three groups under the null hypothesis. Also, we prioritized specificity in our protocol for case ascertainment and we feel that our protocol for confirmation of cases ensures a high degree of specificity.¹⁸ Fourth, our participants were white postmenopausal women at high cardiovascular risk. Thus, our results may not be generalized to other age groups or ethnicities. Fifth, information on reproductive factors known to be associated with breast cancer risk was not available for further adjustment. Nevertheless, due to the randomized allocation of participants, it is not likely that these factors may have introduced substantial confounding. Fifth, our study cannot disentangle if the observed beneficial effect was attributable mainly to EVOO or to its consumption within the context of the traditional MeDiet. Sixth, according to the study event definitions, we collected information on malignant tumors. Thus, we did not register non-invasive tumors such as in situ tumors. Therefore, we cannot include non-invasive cases in our analyses. Seventh, up to October 2006 when the study protocol was amended¹², the intervention in the control group was less intense than in the intervention group. Consequently, some differences in social support, positive expectations and empowerment could have existed between the intervention and the control groups. Nevertheless, only five cases of breast cancer had been identified up to that date. In addition, it seems unlikely that the magnitude of the risk reduction in breast cancer can be explained only in terms of increased social support.

No prior nutrition intervention trial has addressed the effect of the MeDiet specifically on breast cancer. In the Lyon Diet Heart Study, a randomized trial, a protective effect of a cardioprotective Mediterranean-type diet against overall cancer incidence was observed supporting the hypothesis of an anticancer effect of the MeDiet.⁶ The potential beneficial effect of the MeDiet may be explained by several mechanisms¹⁹, e.g., a reduction of DNA oxidative damage²⁰. Specifically for breast cancer, results from observational studies have been inconsistent. A recent meta-analysis²¹ reported no association between adherence to the MeDiet and breast cancer in cohort studies, revealing a pooled estimate $RR = 1.01$ (95% CI: 0.88 to 1.16), whereas results from case-control studies suggested an 18% risk reduction (95% CI: 0.69 to 0.97). However, most cohort studies^{8,9,10,22} included in this meta-analysis were conducted outside the Mediterranean geographical area and it cannot be assumed that a proper MeDiet was followed outside this region. The EPIC study is the only large cohort study that has included countries from the Mediterranean area.⁷ In that study, the HR for postmenopausal women was 0.93 (95% CI: 0.87 to 0.99) when comparing high (10-16 points) versus low (0-5 points) adherence to the MeDiet. If this information (as a prior) were integrated with our present results using a simple Bayesian approach recommended in epidemiology¹⁸, the posterior relative risk would be 0.92 (95% CI: 0.87 to 0.98). The differential effects of the two MeDiet interventions on breast cancer may be attributed to a higher consumption of EVOO among participants allocated to the MeDiet supplemented with EVOO as our ancillary analyses showed (Figure 2). EVOO consumption accounted for 22% of total caloric intake in the MeDiet supplement with EVOO, while nuts represented 10% of the total calories in the MeDiet supplemented with nuts. The stronger inverse association with EVOO may be also ascribed to its high polyphenol content.

Epidemiological studies on the association between EVOO consumption and breast cancer incidence are scarce. A meta-analysis of case-control studies concluded that olive oil consumption, including not only EVOO but also other types of common olive oil (with a lower content of bioactive polyphenols), was inversely associated with breast cancer.²³ Remarkably,

these case-control studies had been conducted in Mediterranean countries and they consistently found an inverse association between olive oil consumption and breast cancer risk. This finding, however, was not replicated in the EPIC cohort.²⁴ Nonetheless, it is noteworthy that none of these studies differentiated between types of olive oil. Several biological mechanisms could explain the putative anti-carcinogenic properties of EVOO. All types of olive oil provide a high supply of monounsaturated fatty acids, mainly oleic acid, as well as squalene, whereas EVOO also contains various biologically active compounds, such as the polyphenols oleocanthal, oleuropein, hydroxytyrosol, and lignans. In vitro studies have suggested an anti-proliferative effect of oleic acid by affecting the expression of human oncogenes.²⁵ The hydrocarbon squalene has been reported to exert a beneficial effect on intracellular oxidative stress and DNA oxidative damage in mammary epithelial cells.²⁶ Olive oil polyphenols may have a potential role in breast cancer prevention.²⁷ Oleocanthal has been associated with inhibition of tumor growth and proliferation, migration, and invasiveness of breast cancer cells in vitro or in vivo breast cancer models.²⁸ Oleuropein has been associated with increased apoptosis of cultured breast cancer cells through different pathways.^{29,30} Also, hydroxytyrosol has been reported to reduce intracellular reactive oxygen species in human breast epithelial cells and to prevent oxidative DNA damage in both human breast epithelial cells and human breast cancer cells.³¹ Lignans are phytoestrogens that have been associated with a lower risk of breast cancer in postmenopausal women.³²

In the PREDIMED trial, participants in the control group did not reduce their total fat intake substantially –albeit their saturated fat intake stayed below 10% during follow-up–, even though they were advised to follow a low-fat diet. This fact can be ascribed to the rooted tradition of adherence to the MeDiet, particularly among older people. On the other hand, several prospective studies have suggested that higher fat intakes, especially animal fat, may be associated to a higher risk of breast cancer.³³⁻³⁶ Moreover, in the Women's Health Initiative study, total fat consumption was associated to a higher risk of breast cancer.³⁷ Also, in the Women's Health Initiative study, women who reported the highest levels of fat intake at baseline and therefore may have achieved the greatest reduction in fat intake, showed a

significantly lower risk of breast cancer.³⁸ Among women with early-stage breast cancer in the Women's Intervention Nutrition Study, lower fat intakes were associated with lower estrogen-negative breast cancer recurrence.³⁹ Taking all this evidence into account, greater reductions in the incidence of breast cancer could have been observed in the control group had these women followed a truly low-fat diet.

In conclusion, the results of the PREDIMED trial suggest a beneficial effect of a MedDiet supplemented with EVOO in the primary prevention of breast cancer. Preventive strategies represent the most sensible approach against cancer. The intervention paradigm implemented in the PREDIMED trial provides an ideal scenario for breast cancer prevention since it is conducted in primary health care centers and also offers beneficial effects on a wide variety of health outcomes.⁴⁰ Nevertheless, these results need confirmation by long-term studies with a higher number of incident cases.

ACKNOWLEDGEMENTS

We thank the participants in the trial for their enthusiastic and maintained collaboration, and the Independent Data and Safety Monitoring Board (F. Xavier Pi-Sunyer, M.D., Columbia University; Carlos Alberto González, M.D., Catalan Institute of Oncology; Joan Sabaté, MD, DrPH, Loma Linda University), and the collaborators at the different study sites. None of them received any compensation.

Author's contributions

ET was the principal responsible for the statistical analyses; ET, CD-V and MAM-G had full access to the data; ET and MAM-G critically interpreted the results; DR contributed to the interpretation of results; ET prepared the first draft of the manuscript together with CD-V; MAM-G thoroughly reviewed the first draft; FBH made fundamental contributions to the intervention and study implementation; JSS, PBC, RE, DC, MF, FA, EGG, MOC, LSM, XP, HS, JB, JVS, and MAM-G were responsible for the participants' recruitment and follow-up; JSS, RE, ER, DC, and MAM-G designed the trial; JSS, RE, ER, DC, MF, FA, EGG, LSM, XP,

MAM-G raised funding for the trial; MF and HS were in charge for the central dataset; FA was the chair of the End-point Adjudication Committee; JSS, ER, MB, and MS-M managed obtaining the supplemental foods from food industries; MAM-G had final responsibility for the decision to submit for publication. All authors have made substantial contributions to the intellectual content and they are responsible for the content and writing of the paper, agree to the submission of this manuscript, made an active contribution to the conception and design and interpretation of the data, critically reviewed the content of the manuscript important intellectual content, proofread and approved its final version, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Disclosure of potential conflicts of interest

FA, outside the submitted work, also reports personal fees from Menarini and from Astra-Zeneca.

Dr. Hu, outside the submitted work, reports grants from California Walnut Commission and from Metagenics.

Dr. Salas-Salvadó reports grants from ISCIII, and Nut and Dried Fruit Foundation during the conduct of the study, and received consultancies from Danone Research and EROSKI, outside the submitted work; JS-S is also a non-paid member of the Scientific Committee of the Nut and Dried fruit Foundation.

Dr. Ros, during the conduct of the study, reports grants from ISCIII and non-financial support from California Walnut Commission, from Patrimonio Comunal Olivarero, from La Morella Nuts, and from Borges S.A. ER, also, outside the submitted work, reports grants from California Walnut Commission, non-financial support from Nuts for Life, from La Asturiana S.A, from International Nut Council and from California Walnut Commission and, personal fees and others from Nuts for Life and La Asturiana S.A.

Dr. Estruch, outside the submitted work, also reports grants from California Walnut Commission, Sacramento CA (USA), non-financial support from Patrimonio Comunal Olivarero (Spain), non-financial support from La Morella Nuts (Spain), non-financial support from Borges S.A. (Spain), grants from Novartis Farmaceutica, SA, from Cerveceros De España, from SANOFI-AVENTIS, from FIVIN SPAIN from Instituto Cervantes Albuquerque (USA, April 2012), from Harvard School of Public Health (Boston, USA, September 2010), from The Culinary Institute Of America (CIA) (Hyde Park, New York, USA, October 2009), from International Family Doctors Association, (October 2010), from Instituto Cervantes (Milan, Italy, October 2012).

ET, CD-V, PB-C, DC, MF, FBH, EG-G, DR, MO-C, LS-M, XP, HS, JB, JVS, MB, MS-M, MAM-G have nothing to disclose.

Funding/Support

The supplemental foods used in the study were generously donated by Patrimonio Comunal Olivarero and Hojiblanca from Spain (EVOO); the California Walnut Commission from Sacramento, CA (walnuts); and Borges S.A. (almonds) and La Morella Nuts (hazelnuts), both from Reus, Spain. The PREDIMED trial was supported by the official funding agency for biomedical research of the Spanish government (Instituto de Salud Carlos III) through grants provided to research networks specifically developed for the trial: RTIC G03/140 (Coordinator: Dr Estruch) and RTIC RD 06/0045 (Coordinator: Dr Martínez-González). All investigators of the PREDIMED trial belong to Centro de Investigación Biomédica en Red (CIBER), an initiative of Instituto de Salud Carlos III. We also acknowledge grants from the National Institutes of Health (1R01HL118264-01 and 1R01DK102896); Fondo de Investigación Sanitaria– Fondo Europeo de Desarrollo Regional (PI04/0233, PI05/0976, PI07/0240, PI10/01407, PI10/02658, PI11/00049, PI11/02505 and AGL2010-22319-C03-03); Consejería de Salud de la Junta de Andalucía (PI0105/2007), and by the Generalitat Valenciana, Spain (ACOMP/2013/165 and ACOMP/2013/159). None of the funding sources played any role in the design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication.

REFERENCES

1. International Agency for Research on Cancer. Latest world cancer statistics. Global cancer burden rises to 14.1 million new cases in 2012: Marked increase in breast cancers must be addressed. In: Organization IAFRoCWH, editor. Lyon, France/Geneva, Switzerland: International Agency for Research on Cancer/World Health Organization; 2013.
2. Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012. *Eur J Cancer* 2013;49(6):1374-403.
3. WHO International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. Volume 96: Alcohol consumption. <http://monographs.iarc.fr/ENG/Monographs/vol96/mono96.pdf> (accessed February 26 2015).
4. Trichopoulou A, Lagiou P, Kuper H, Trichopoulos D. Cancer and Mediterranean dietary traditions. *Cancer Epidemiol Biomarkers Prev* 2000;9(9):869-73.
5. Willett WC, Sacks F, Trichopoulou A, et al. Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr* 1995;61(6 Suppl):1402S-6S.
6. de Lorgeril M, Salen P, Martin JL, Monjaud I, Boucher P, Mamelle N. Mediterranean Dietary Pattern in a Randomized Trial: Prolonged Survival and Possible Reduced Cancer Rate. *Arch Intern Med* 1998;158(11):1181-1187.
7. Buckland G, Travier N, Cottet V, et al. Adherence to the mediterranean diet and risk of breast cancer in the European prospective investigation into cancer and nutrition cohort study. *Int J Cancer* 2013;132(12):2918-27.

8. Fung TT, Hu FB, McCullough ML, Newby PK, Willett WC, Holmes MD. Diet quality is associated with the risk of estrogen receptor-negative breast cancer in postmenopausal women. *J Nutr* 2006;136(2):466-72.
9. Cade JE, Taylor EF, Burley VJ, Greenwood DC. Does the Mediterranean dietary pattern or the Healthy Diet Index influence the risk of breast cancer in a large British cohort of women? *Eur J Clin Nutr* 2011;65(8):920-8.
10. Couto E, Sandin S, Lof M, Ursin G, Adami HO, Weiderpass E. Mediterranean dietary pattern and risk of breast cancer. *PLoS One* 2013;8(2):e55374.
11. Martinez-Gonzalez MA, Corella D, Salas-Salvado J, et al. Cohort profile: design and methods of the PREDIMED study. *Int J Epidemiol* 2012;41(2):377-85.
12. Estruch R, Ros E, Salas-Salvado J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013;368(14):1279-90.
13. Schröder H, Fitó M, Estruch R, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. *J Nutr*. 2011;141(6):1140-5.
14. Fernández-Ballart JD, Piñol JL, Zazpe I, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. *Br J Nutr*. 2010;103(12):1808-16.
15. Elosua R, Marrugat J, Molina L, Pons S, Pujol E. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish men. The MARATHOM Investigators. *Am J Epidemiol*. 1994;139(12):1197-209.
16. Elosua R, Garcia M, Aguilar A, Molina L, Covas MI, Marrugat J. Validation of the Minnesota Leisure Time Physical Activity Questionnaire In Spanish Women. Investigators of the MARATDON Group. *Med Sci Sports Exerc*. 2000;32(8):1431-7.

17. Zazpe I, Sanchez-Tainta A, Estruch R, et al. A large randomized individual and group intervention conducted by registered dietitians increased adherence to Mediterranean-type diets: the PREDIMED study. *J Am Diet Assoc* 2008;108(7):1134-44.
18. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3rd ed. Philadelphia, USA: Lippincott Williams & Wilkins; 2008.
19. Grosso G, Buscemi S, Galvano F, et al. Mediterranean diet and cancer: epidemiological evidence and mechanism of selected aspects. *BMC Surg*. 2013;13 Suppl 2:S14.
20. Mitjavila MT, Fandos M, Salas-Salvadó J, et al. The Mediterranean diet improves the systemic lipid and DNA oxidative damage in metabolic syndrome individuals. A randomized, controlled, trial. *Clin Nutr*. 2013;32(2):172-8.
21. Schwingshackl L, Hoffmann G. Adherence to Mediterranean diet and risk of cancer: A systematic review and meta-analysis of observational studies. *Int J Cancer* 2014;135(8):1884-97.
22. Tognon G, Nilsson LM, Lissner L, et al. The Mediterranean diet score and mortality are inversely associated in adults living in the subarctic region. *J Nutr* 2012;142(8):1547-53.
23. Psaltopoulou T, Kosti RI, Haidopoulos D, Dimopoulos M, Panagiotakos DB. Olive oil intake is inversely related to cancer prevalence: a systematic review and a meta-analysis of 13,800 patients and 23,340 controls in 19 observational studies. *Lipids Health Dis* 2011;10:127.
24. Buckland G, Travier N, Agudo A, et al. Olive oil intake and breast cancer risk in the Mediterranean countries of the European Prospective Investigation into Cancer and Nutrition study. *Int J Cancer* 2012;131(10):2465-9.

25. Menendez JA, Papadimitropoulou A, Vellon L, Lupu R. A genomic explanation connecting "Mediterranean diet", olive oil and cancer: oleic acid, the main monounsaturated fatty acid of olive oil, induces formation of inhibitory "PEA3 transcription factor-PEA3 DNA binding site" complexes at the Her-2/neu (erbB-2) oncogene promoter in breast, ovarian and stomach cancer cells. *Eur J Cancer* 2006;42(15):2425-32.
26. Warleta F, Campos M, Allouche Y, et al. Squalene protects against oxidative DNA damage in MCF10A human mammary epithelial cells but not in MCF7 and MDA-MB-231 human breast cancer cells. *Food Chem Toxicol* 2010;48(4):1092-100.
27. Casaburi I, Puoci F, Chimento A, et al. Potential of olive oil phenols as chemopreventive and therapeutic agents against cancer: a review of in vitro studies. *Mol Nutr Food Res* 2013;57(1):71-83.
28. Akl MR, Ayoub NM, Mohyeldin MM, et al. Olive phenolics as c-Met inhibitors: (-)-Oleocanthal attenuates cell proliferation, invasiveness, and tumor growth in breast cancer models. *PLoS One* 2014;9(5):e97622.
29. Hassan ZK, Elamin MH, Omer SA, et al. Oleuropein induces apoptosis via the p53 pathway in breast cancer cells. *Asian Pac J Cancer Prev* 2013;14(11):6739-42.
30. Elamin MH, Daghestani MH, Omer SA, et al. Olive oil oleuropein has anti-breast cancer properties with higher efficiency on ER-negative cells. *Food Chem Toxicol* 2013;53:310-6.
31. Warleta F, Quesada CS, Campos M, Allouche Y, Beltran G, Gaforio JJ. Hydroxytyrosol protects against oxidative DNA damage in human breast cells. *Nutrients* 2011;3(10):839-57.

32. Buck K, Zaineddin AK, Vrieling A, Linseisen J, Chang-Claude J. Meta-analyses of lignans and enterolignans in relation to breast cancer risk. *Am J Clin Nutr* 2010;92(1):141-53.
33. Sieri S, Chiodini P, Agnoli C, et al. Dietary fat intake and development of specific breast cancer subtypes. *J Natl Cancer Inst.* 2014;106(5). pii: dju068.
34. Smith-Warner SA, Stampfer MJ. Fat intake and breast cancer revisited. *J Natl Cancer Inst.* 2007;99(6):418-9.
35. Thiébaud AC, Kipnis V, Chang SC, S et al. Dietary fat and postmenopausal invasive breast cancer in the National Institutes of Health-AARP Diet and Health Study cohort. *J Natl Cancer Inst.* 2007;99(6):451-62.
36. Wirfält E, Mattisson I, Gullberg B, Johansson U, Olsson H, Berglund G. Postmenopausal breast cancer is associated with high intakes of omega6 fatty acids (Sweden). *Cancer Causes Control.* 2002;13(10):883-93.
37. Freedman LS¹, Potischman N, Kipnis V, et al. A comparison of two dietary instruments for evaluating the fat-breast cancer relationship. *Int J Epidemiol.* 2006;35(4):1011-21.
38. Prentice RL, Caan B, Chlebowski RT, et al. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA.* 2006;295(6):629-42.
39. Chlebowski RT, Blackburn GL, Thomson CA, et al. Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. *J Natl Cancer Inst.* 2006;98(24):1767-76.
40. Ros E, Martinez-Gonzalez MA, Estruch R, et al. Mediterranean diet and cardiovascular health: Teachings of the PREDIMED study. *Adv Nutr* 2014;5(3):330S-6S.

FIGURE 1. Incidence of invasive breast cancer, according to the intervention group.

Rate ratios were obtained from Cox regression models.

* RR denotes rate ratio and CI confidence interval.

† EVOO denotes extra virgin olive oil.

‡ MeDiet denotes Mediterranean Diet adherence score (minimum adherence = 0 points; maximum adherence = 14 points).

FIGURE 2. Incidence of breast cancer, according to attained consumption of extra virgin olive oil during follow-up.

Results obtained from Cox regression models.

* EVOO denotes extra virgin olive oil.

eFigure 1. Flow-chart of participants in the PREDIMED trial, 2003-2010.

* 7 participants were evaluated from the main analysis because their incident breast cancer could not be confirmed (2 MeDiet + EVOO; 2 MeDiet + nuts; 3 control).

† EVOO denotes extra virgin olive oil.

‡ MeDiet denotes Mediterranean Diet adherence score (minimum adherence = 0 points; maximum adherence = 14 points).

§ BC denotes breast cancer.

TABLE 1. Baseline Characteristics of Female PREDIMED Trial Participants by Intervention Group.

Characteristic	Mediterranean diet with EVOO N= 1476	Mediterranean diet with nuts N= 1285	Control diet N= 1391
Age – y (mean ± SD)	67.6±5.8	67.4±5.6	68.1±6.0
Smoking – no. (%)			
Never smoker	1276 (86.5)	1123 (87.4)	1216 (87.4)
Former smoker	88 (6.0)	68 (5.3)	78 (5.6)
Current smoker	112 (7.6)	94 (7.3)	97 (7.0)
Body mass index† (mean ± SD)	30.4±3.9	30.2±4.1	30.7±4.2
Waist to height ratio (mean ± SD)	0.64±0.07	0.63±0.07	0.64±0.07
Hypertension‡ – no. (%)	1269 (86.0)	1114 (86.7)	1197 (86.1)
Type-2 diabetes§ – no. (%)	701 (47.5)	533 (41.5)	618 (44.4)
Dyslipidemia¶ – no. (%)	1112 (75.3)	1003 (78.1)	1065 (76.6)
Family history of premature CHD – no. (%)	392 (26.6)	328 (25.5)	372 (26.7)
Family history of cancer – no. (%)	807 (54.7)	680 (52.9)	709 (51.0)
Use of hormone-replacement therapy – no. (%)	42 (2.9)	34 (2.7)	37 (2.7)
Age at menopause >55 y– no. (%)	102 (6.9)	62 (4.8)	78 (5.6)
Physical activity - METS-min/week (mean ± SD)	179±168	177±165	161±166
MeDiet Adherence score (mean ± SD)	8.7±1.9	8.7±1.9	8.4±1.9
Total energy intake (kcal/d) (mean ± SD)	2163±568	2184±565	2100±539
Alcohol consumption – no. (%)			
Abstainers	765 (51.8)	594 (46.2)	760 (54.6)
>0-<15 g/day	645 (43.7)	642 (50.0)	575 (41.3)
≥15 g/d	66 (4.5)	49 (3.8)	56 (4.0)

Plus-minus values are means ± SDs.

† The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

‡ Hypertension was defined as systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or use of antihypertensive therapy.

§ Diabetes was defined as fasting blood glucose ≥ 126 mg/dl (7.0 mmol/l) on two occasions, or 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) after a 75-g oral glucose load, or use of antidiabetic medication.

¶ Dyslipidemia was defined as low-density lipoprotein cholesterol >160 mg/dl, high-density lipoprotein cholesterol ≤ 40 mg/dl in men or ≤ 50 mg/dl in women, or use of lipid-lowering therapy.

| A family history of premature coronary heart disease (CHD) was defined as diagnosis of the disease in a male first-degree relative before the age of 55 years or in a female first-degree relative before the age of 65 years.

|| Mediterranean diet (MeDiet) adherence score (minimum adherence = 0 points; maximum adherence = 14 points)

EVOO denotes extra virgin olive oil.

TABLE 2. Hazard ratios (95% CI) for the risk of invasive breast cancer, according to the intervention group

	Control diet (n=1391)	Mediterranean diet with EVOO (n=1476)	Mediterranean diet with nuts (n=1285)	Both Mediterranean diets vs. control diet (n=2761)
Cases/Person-years	17/5829	8/7031	10/5492	18/12523
Rate (/1000 person-years)	2.9	1.1	1.8	1.4
Crude rate ratio	1 (ref)	0.38 (0.16 to 0.87)	0.62 (0.29 to 1.36)	0.49 (0.25 to 0.94)
Multivariable adjusted rate ratio*	1 (ref)	0.32 (0.13 to 0.79)	0.59 (0.26 to 1.35)	0.43 (0.21 to 0.88)
After excluding women with follow-up < 1 year†				
Crude rate ratio	1 (ref)	0.37 (0.15 to 0.90)	0.64 (0.28 to 1.45)	0.48 (0.24 to 0.98)
Multivariable adjusted rate ratio*	1 (ref)	0.33 (0.13 to 0.85)	0.65 (0.27 to 1.53)	0.46 (0.22 to 0.96)
After including non-confirmed cases as cases				
Crude rate ratio	1 (ref)	0.38 (0.16 to 0.87)	0.62 (0.29 to 1.36)	0.49 (0.25 to 0.94)
Multivariable adjusted rate ratio*	1 (ref)	0.32 (0.13 to 0.79)	0.59 (0.26 to 1.35)	0.43 (0.21 to 0.88)
After including non-confirmed cases as non-cases				
Crude rate ratio	1 (ref)	0.38 (0.16 to 0.87)	0.62 (0.29 to 1.36)	0.49 (0.25 to 0.94)
Multivariable adjusted rate ratio*	1 (ref)	0.32 (0.13 to 0.79)	0.59 (0.26 to 1.35)	0.43 (0.21 to 0.88)
Including only ER+ malignancies				
Crude rate ratio	1 (ref)	0.31 (0.11 to 0.85)	0.65 (0.27 to 1.57)	0.46 (0.22 to 0.98)
Multivariable adjusted rate ratio*	1 (ref)	0.24 (0.08 to 0.71)	0.58 (0.23 to 1.47)	0.38 (0.17 to 0.86)

Results obtained from Cox regression models.

*: adjusted for age (3 groups), centre (continuous), body mass index (quartiles), waist to height ratio (dichotomous), use of hormone-replacement therapy, leisure-time physical activity (quartiles), total energy intake (quartiles), alcohol consumption (quartiles), age at menopause (<55y/>55y) and baseline adherence to the Mediterranean diet (high vs. low).

†: 4 cases were excluded: 1 in the Mediterranean diet with EVOO group, 1 in the Mediterranean diet with nuts group and 2 in the control group.

TABLE 3. Hazard ratios (95% CI) for the risk of invasive breast cancer by intervention group in subgroup analyses

	Cases/ person-years	Control diet	Mediterranean diet with EVOO	Mediterranean diet with nuts	Both Mediterranean diets vs. control diet
Age					
<=67 y (n=2095)	15/9099	1 (ref)	0.16 (0.04 to 0.68)	0.16 (0.04 to 0.71)	0.16 (0.05 to 0.50)
>67 y (n=2057)	20/9254	1 (ref)	0.56 (0.15 to 2.03)	1.52 (0.53 to 4.39)	0.92 (0.34 to 2.47)
Smoking					
Never (n=3615)	31/16082	1 (ref)	0.33 (0.13 to 0.86)	0.63 (0.27 to 1.49)	0.46 (0.22 to 0.96)
Ever (n=537)	4/2271	-	-	-	-
Alcohol intake					
<=25 g/d (n=2119)	22/9460	1 (ref)	0.35 (0.11 to 1.12)	0.84 (0.32 to 2.20)	0.54 (0.22 to 1.29)
>25 g/d (n=2033)	13/8893	1 (ref)	0.30 (0.07 to 1.30)	0.34 (0.08 to 1.45)	0.32 (0.10 to 1.06)
Diabetes					
No (n=2300)	16/9967	1 (ref)	0.37 (0.10 to 1.37)	0.49 (0.13 to 1.81)	0.42 (0.15 to 1.20)
Yes (n=1852)	19/8385	1 (ref)	0.22 (0.05 to 0.88)	0.61 (0.18 to 2.00)	0.37 (0.13 to 1.07)
Body mass index					
<30 kg/m ² (n=1995)	17/8809	1 (ref)	0.32 (0.09 to 1.09)	0.27 (0.07 to 1.16)	0.29 (0.11 to 0.83)
>=30 kg/m ² (n=2157)	18/9543	1 (ref)	0.28 (0.07 to 1.12)	0.99 (0.35 to 2.81)	0.57 (0.22 to 1.49)
Use of hormone-replacement therapy					
No (n=4039)	33/17905	1 (ref)	0.31 (0.12 to 0.80)	0.64 (0.28 to 1.46)	0.44 (0.21 to 0.92)
Yes (n=113)	2/448	-	-	-	-
Family history of cancer[†]					

No (n=1702)	10/7558	1 (ref)	0.19 (0.05 to 0.70)	0.10 (0.004 to 2.25)	0.15 (0.03 to 0.70)
Yes (n=2196)	22/9658	1 (ref)	0.37 (0.11 to 1.21)	0.78 (0.29 to 2.12)	0.53 (0.22 to 1.30)
Baseline adherence to the Mediterranean diet					
Low (<9 points) (n=1936)	21/8398	1 (ref)	0.31 (0.10 to 0.96)	0.39 (0.12 to 1.26)	0.34 (0.14 to 0.86)
High (≥9 points) (n=2216)	14/9955	1 (ref)	0.33 (0.07 to 1.63)	1.00 (0.25 to 4.03)	0.62 (0.16 to 2.33)

Results obtained from Cox regression models.

*: adjusted for age (3 groups), centre, body mass index (quartiles), waist to height ratio (dichotomous), use of hormone-replacement therapy, leisure-time physical activity (quartiles), total energy intake (quartiles), alcohol consumption (quartiles), age at menopause (<55y/>55y) and baseline adherence to the Mediterranean diet (high vs. low).

†: information was not available for 254 women