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Original article

Olive oil consumption is associated with a lower risk of cardiovascular disease and stroke

Carolina Donat-Vargas [a, b, c, d, *], Helena Sandoval-Insausti [b, c, e], José L. Peñalvo [f], Maria Concepción Moreno Iribas [i, l], Pilar Amiano [c, j, k], Maira Bes-Rastrollo [g, h, l], Esther Molina-Montes [c, m, n, o], Belén Moreno-Franco [p, q], Antonio Agudo [r], Cristina Lasheras Mayo [s], Martín Laclaustra [p, q], Carmen De La Fuente Arrillaga [g, h, l], Maria Dolores Chirlaque Lopez [c, t], Maria-José Sánchez [c, o, u, v], Miguel Angel Martínez-Gonzalez [e, g, h, l], Pilar Guallar-Castillón [a, b, c]

[a] IMDEA-Food Institute, CEI UAM+CSIC, Madrid, Spain
[b] Department of Preventive Medicine and Public Health, School of Medicine, Universidad Autónoma de Madrid-IdiPaz, Madrid, Spain
[c] CIBERESP (CIBER of Epidemiology and Public Health), Madrid, Spain
[d] Unit of Nutritional and Cardiovascular Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
[e] Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA
[f] Unit of Non-Communicable Diseases, Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium
[g] University of Navarra, Department of Preventive Medicine and Public Health, School of Medicine, Pamplona, Spain
[h] CIBEROBN (CIBER of Obesity and Nutrition), Madrid, Spain
[i] Research Network on Health Services in Chronic Diseases (REDISSEC), Pamplona, Spain
[j] Ministry of Health of the Basque Government, Sub-Directorate for Public Health and Addictions of Gipuzkoa, San Sebastián, Spain
[k] Biodonostia Health Research Institute, Epidemiology and Public Health Area, San Sebastián, Spain
[l] Navarra Institute for Health Research (IdiSNA), Pamplona, Spain
[m] Department of Nutrition and Food Science, University of Granada, Granada, Spain
[n] Biomedical Research Center, Institute of Nutrition and Food Technology (INYTA) "José Mataix", University of Granada, Granada, Spain
[o] Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), Granada, Spain
[p] University of Zaragoza, Zaragoza, Spain
[q] CIBERCV and Instituto de Investigación Sanitaria Aragón, Hospital Universitario Miguel Servet, Zaragoza, Spain
[r] Unit of Nutrition and Cancer, Catalan Institute of Oncology (ICO), And Nutrition and Cancer Group, Epidemiology, Public Health, Cancer Prevention and Palliative Care Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Spain
[s] Department of Functional Biology, Faculty of Medicine, University of Oviedo, Spain
[t] Murcia Regional Health Council, IMIB-Arrixaca, Murcia University, Murcia, Spain
[u] Andalusian School of Public Health (EASP), Granada, Spain
[v] Department of Preventive Medicine and Public Health, University of Granada, Granada, Spain

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SUMMARY

Background & aims: The specific association of olive oil consumption with coronary heart disease (CHD) or stroke has not been totally established.
Objective: to examine whether olive oil consumption is associated with subclinical atherosclerosis, the risk of total cardiovascular disease (CVD), CHD, and stroke.
Methods: Three cohorts were included: AWHS (2318 men), SUN Project (18,266 men and women), and EPIC-Spain (39,393 men and women). Olive oil consumption was measured at baseline using validated questionnaires.

Abbreviations: AWHS, Aragon Workers' Health Study; CACS, Coronary Artery Calcium Score; CHD, Coronary Heart Disease; CI, Confidence Intervals; CVD, Cardiovascular Disease; HDL-C, High Density Lipoprotein-Cholesterol; FFQ, Food Frequency Questionnaire; EPIC, European Prospective Investigation into Cancer and Nutrition; MUFA, Monounsaturated Fatty Acid; LDL-C, Low Density Lipoprotein-Cholesterol; OR, Odds Ratio; SUN, Seguimiento Universidad de Navarra; TC, Total Cholesterol; HR, Hazard ratio.

* Corresponding author. Department of Preventive Medicine and Public Health, School of Medicine, Universidad Autónoma de Madrid-IdiPaz, Madrid, Spain.
E-mail addresses: carolina.donat.vargas@ki.se (C. Donat-Vargas), helenagabar@gmail.com (H. Sandoval-Insausti), jpenalvo@itg.be (J.L. Peñalvo), mmorenoi@navarra.es (M.C. Moreno Iribas), epicss-san@euskadi.eus (P. Amiano), mbes@unav.es (M. Bes-Rastrollo), memolina@ugr.es (E. Molina-Montes), mbmoreno@unizar.es (B. Moreno-Franco), a.agudo@iconcologia.net (A. Agudo), lasheras@uniovi.es (C.L. Mayo), martin.laclaustra@unizar.es (M. Laclaustra), cfuente@unav.es (C. De La Fuente Arrillaga), mdolores.chirlaque@carm.es (M.D. Chirlaque Lopez), mariajose.sanchez.easp@juntadeandalucia.es (M.-J. Sánchez), mamartinez@unav.es (M.A. Martínez-Gonzalez), mpilar.guallar@uam.es (P. Guallar-Castillón).

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Stroke
Subclinical atherosclerosis
Primary prevention

Results: In the AWHS, 747 participants had a positive coronary artery calcium score (CACS>0), and the OR (95% CI) was 0.89 (0.72, 1.10) in those with virgin olive oil consumption ≥30 g/day (*v.* <10 g/day). In the SUN Project (follow-up 10.8 years) 261 total CVD cases occurred, and the HR was 0.57 (0.34, 0.96) for consumptions ≥30 g/day (*v.* <10 g/day). In the EPIC-Spain (follow-up 22.8 years) 1300 CHD cases and 938 stroke cases occurred; the HRs for stroke according, 0 to <10 (ref), 10 to <20, 20 to <30, and ≥30 g/day of olive oil consumption, were 0.84 (0.70, 1.02), 0.80 (0.66, 0.96), 0.89 (0.74, 1.07). A weaker association was observed for CHD. The association was stronger among those consuming virgin olive oil, instead of common (refined).

Conclusions: Olive oil is associated with lower risk of CVD and stroke. The maximum benefit could be obtained with a consumption between 20 and 30 g/day. The association could be stronger for virgin olive oil and might operate from the early stages of the disease.

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1. Introduction

The Mediterranean diet ranks first as the healthiest dietary pattern in the world [1]. Olive oil consumption is the hallmark of this traditional dietary pattern. In Mediterranean countries, olive oil contributes between one-third and two-thirds of total vegetable fat consumed [2–4]. It is affordable, widely used as a dressing (even in some fast-food establishments) and makes food tastier.

Its high monounsaturated fatty acids (MUFA) content appears to have anti-hypertensive, anti-inflammatory, and anti-thrombotic effects [5–7]. Likewise, the polyphenols enriching virgin olive oil have also shown important cardioprotective effects through several mechanisms [8].

In the two published meta-analysis of epidemiological studies [9,10] (one of them including the PREDIMED trial [11]), olive oil consumption showed an inverse association with all-cause mortality, cardiovascular mortality, cardiovascular events, and stroke. Neither, however, found a clear association between olive oil consumption and coronary heart disease (CHD). However, these meta-analyzes have been based on a limited number of studies on cardiovascular events.

Recently, the beneficial association of olive oil consumption on cardiovascular disease (CVD) risk has also been assessed in non-Mediterranean populations [12]. Results from two large U.S. cohorts showed a stronger inverse association of olive oil (relatively low intake, the mean consumption in the highest category was only 11 g/day) with CHD risk than with stroke [12].

The aim of this article is to assess the association between olive oil consumption and CVD and its natural history using data from three different Spanish cohorts. Therefore, we assessed the association between olive oil consumption and 1) subclinical atherosclerosis using the Aragon Workers' Health Study (AWHS); 2) cardiovascular events after a mean follow-up of 10.8 years using the 'Seguimiento Universidad de Navarra' (SUN) Project; and 3) cardiovascular events after a mean follow-up of 22.8 years using the European Prospective Investigation into Cancer and Nutrition (EPIC)-Spain cohort.

The driver of this research is to seek more knowledge on whether there is association of olive oil consumption with particular expressions of CVD (CHD and stroke), whether particular olive oil varieties are more beneficial and how it may associate with early stages of the disease.

Using the specific data available in each cohort, the impact of olive oil in the development of both coronary calcium and plaques in arteries will be evaluated. The distinction between CHD and stroke and between common (refined) and virgin olive oil (non-refined and better quality) will be also assessed.

2. Participants and methods

2.1. Study design and population

2.1.1. AWHS

The AWHS design has been described in detail elsewhere [13,14]. Study participants are workers of the Opel Spain automobile assembly plant located in Figueruelas (Zaragoza, Spain). They were recruited during an annual physical examination in 2009–2010 (participation rate 95.6%). Between January 2011 and December 2014, all participants aged 39–59 (34% of the initial sample) and free of CVD at baseline were invited to undergo noninvasive subclinical atherosclerosis imaging as well as questionnaires on cardiovascular and lifestyle factors.

Among the 2617 workers who attended this extended examination, we excluded 132 women, 31 participants who reported a previous history of CVD or when this information was not available, and 136 with unreliable information on total energy intake. We performed a cross-sectional analysis with a sample of 2318 male participants. Among them, information was available for: 1876 on coronary calcium; 2183 on carotid plaques, and 2187 on femoral plaques (Supplemental Fig. 1).

The study was approved by the Ethics Committee of the central Institutional Review Board of Aragón (CEICA). All participants provided written informed consent.

2.1.2. The SUN project

The SUN Project is a cohort of young university graduates from Spain with ages between 18 and 91 years. Information was self-reported using biyearly mailed questionnaires, with an overall follow-up rate >90% [15].

The recruitment began in December 1999 and is currently ongoing. As of July 2018, a total of 22,468 participants had been recruited and followed-up for at least 2 and a half years, but information on follow-up was missing for 1746 participants. We additionally excluded 346 participants with prevalent CVD, and 2110 with total energy intake outside of pre-defined limits (<500 or >3500 kcal/d for women, and <800 or >4000 kcal/d for men). The remaining 18,266 participants were included in our analyses. The follow-up time was defined as the interval between the date of recruitment to the date of death or cardiovascular event, or to the date of returning the last follow-up questionnaire. Mean follow-up was 10.8 ± 4 years (Supplemental Fig. 2).

The study was approved by the Ethics Committee of the University of Navarra. Voluntary completion of the first self-administered questionnaire was considered to imply informed consent.

2.1.3. The EPIC–Spain cohort

The methodological design of the EPIC-Spain cohort has previously been reported [16,17]. EPIC-Spain consisted of 41,446 healthy volunteers, aged 29–69, and recruited between 1992 and 1996 in five Spanish regions (Asturias, Granada, Gipuzkoa, Murcia, and Navarra). Most of participants were blood donors, civil servants, as well as the general population. The study included participants from diverse social and educational levels.

Of the initial 41,446 participants, we excluded 234 participants with prevalent CHD, 20 with missing data on CHD, 147 with prevalent stroke, 274 with missing data on stroke, as well as 188 individuals who lacked data on the date of CVD diagnosis. We also excluded 785 participants with total energy intake beyond p1 or p99, and those with missing values on confounding variables: 109 on body mass index (BMI), 20 on smoking status, 170 on hypercholesterolemia, 36 on hypertension, and 70 on diabetes. The final analyses were performed with 39,393 participants (Supplemental Fig. 3).

The study was approved by the Ethics Committee of the International Agency for Cancer Research (Lyon, France). Before enrollment, all participants gave informed consent.

2.2. Olive oil and dietary information assessment

In both studies, the AWHS and the SUN Project, a similar self-administered questionnaire was used at baseline to obtain dietary information. It was a 136-item semi-quantitative food frequency questionnaire (FFQ) previously validated in Spain [18,19]. Each item in the FFQ included a typical portion size, and consumption frequencies were measured in 9 categories that ranged from “never or almost never” to “more than 6 times/day”. Participants were asked about the intake of olive oil (total in the SUN Project but differentiating by type in the AWHS) during the previous year considering the amount of olive oil used for cooking or frying as well as the use of olive oil as a salad dressing or a spread on bread or other food.

In the EPIC study, a validated computerized dietary history was used to collect information on habitual food intake [16]. The dietary history, administered by trained staff, registered household measurements and standard unit, as well as a list of 662 different foods and recipes from each region and 35 sets of photographs to help in identifying portion sizes (g/day) of each food consumed.

2.3. Baseline information on covariates

In the AWHS an interviewer obtained information on educational level, smoking, and physical activity that was assessed using the Health Professionals' Follow-up questionnaire [20]. Height and weight were measured using standard procedures. Blood samples were obtained after 6-fasting hours. Dyslipidemia was considered when having total cholesterol (TC) $\geq$200, high-density lipoprotein-cholesterol (HDL-C) <40 mg/dl, triglycerides $\geq$150 mg/dl or self-reported use of lipid-lowering drugs. Blood pressure was measured using standard procedures and hypertension was defined for systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg or self-reported use of antihypertensive medication. Diabetes was defined as a serum glucose $\geq$130 mg/dl or self-reported treatment with hypoglycemic medication.

In the SUN cohort information on covariates included educational level, smoking, height, weight, practice of physical activity according to a validated physical activity questionnaire [20], as well as the prevalence of cardiovascular risk factors. Blood pressure was self-reported as categories. For systolic blood pressure participants could select one of the following 8 ranges: <100, 101–110, 111–120, 121–130, 131–140, 141–150, 151–160, 161–175, >175 mmHg. For diastolic blood pressure participants could select one of the following 8 ranges: <60, 61–70, 71–80, 81–90, 91–100, 101–110, 111–120, 121–130, >130 mmHg. Hypertension was defined if the diagnosis or use of antihypertensive medication was self-reported, or the reported systolic blood pressure was $\geq$131 mmHg or the diastolic $\geq$81 mmHg. Hypercholesterolemia was considered when having total cholesterol (TC) $\geq$200.

In the EPIC-Spain cohort information on educational level, smoking, and physical activity was collected through interview-administered questionnaires [17]. Weight and height were measured using standardized procedures. Participants were also asked if they suffered from hypercholesterolemia, hypertension, diabetes mellitus, and if they had experienced myocardial infarction or stroke.

2.4. Outcome ascertainment

2.4.1. AWHS

Subclinical atherosclerosis imaging was conducted among AWHS participants. Coronary calcium was obtained with a multidetector-row CT scanner (Mx 8000 IDT 16, Philips Medical Systems, Best, the Netherlands) using a low-dose, prospectively ECG-triggered, and a high-pitch spiral acquisition protocol. Coronary calcium was quantified with calcium scoring software (Workspace CT viewer, Philips Medical Systems) that follows the Agatston method [21]. Agatston's method is a summed score obtained from all coronary calcified lesions, accounting for both, the total area as well as the maximum density of coronary calcium. A high coronary artery calcium score (CACS) is a strong indicator of extensive disease with a significant amount of calcium deposits. CACS is the reference standard and the most commonly used coronary artery calcium score in clinical practice [21]. The outcome A CACS>0 was considered as positive outcome.

The presence of plaques in carotid and femoral arteries was determined using an ultrasound system IU22 Philips (Philips Healthcare, Bothell, Washington). Ultrasound images were acquired with linear high-frequency 2-dimensional probes (Philips Transducer L9-3, Philips Healthcare), using the Bioimage Study protocol for the carotid arteries [22] and a protocol that was specifically designed for the femoral arteries [23]. Inspection sweeps were obtained on the right and left side of the carotid (common, internal, external, and bulb) and femoral territories. The presence of a plaque was defined as a focal structure protruding $\geq$0.5 mm into the lumen artery or reaching a thickness $\geq$50% of the surrounding intima. All measurements were analyzed using electrocardiogram (ECG)-gated frames and obtained at the end of the diastole (R-wave) [24].

2.4.2. The SUN project

The primary outcome was CVD, defined as a hard clinical CVD event, i.e., death from cardiovascular cause, incident non-fatal acute coronary syndrome (infarction with or without ST-segment elevation), or incident non-fatal stroke in participants without CVD at baseline. The participants' diagnoses reported in their follow-up questionnaires (Q2-Q16) were confirmed and adjudicated by a team of physicians of the SUN project, blinded to the exposure, after revision of the medical records of participants. Probable cardiovascular disease was considered when it was not possible to access the medical records. Deaths were reported by next-of-kin, work colleagues, or postal authorities. CVD deaths were confirmed reviewing the medical records with the permission of participants' next-of-kin.

2.4.3. The EPIC–Spain cohort

During follow-up (from baseline 1992–1996 to 31st December 2017), we assessed incident coronary heart disease and incident

stroke by record linkage with uniform hospital discharge databases [codes 410–414 for CHD and codes 430–438 for stroke of the ninth revision of the International Classification of Diseases (ICD-9); codes I20–I25 for CHD and codes I60–I69 for stroke of the ICD-10] and with primary care datasets (codes K74, K75, and K76 for CHD and codes K89, K90, and K92 for stroke using the International Classification of Primary Care). A team of trained health professionals validated CHD and stroke events against hospital records, primary care records, and autopsy reports. CVD was defined as confirmed CHD and/or confirmed stroke according to the aforementioned procedure.

2.5. Statistical analysis

To characterize exposure similarly across studies, participants were categorized into 10-g categories of olive oil consumption (g/day): 0 to <10, 10 to <20, 20 to <30, and ≥30. The association between olive oil consumption and subclinical atherosclerosis was assessed by logistic regression obtaining odds ratios (OR) and their 95% confidence intervals (CI). Cox proportional hazards regression models were used to estimate hazard ratios (HR) and their 95% CI in the SUN Project and the EPIC cohort. Linear trend tests were calculated using the median of each category of olive oil consumption as a continuous variable.

Two different models were created and used across studies. The first was adjusted for age, sex (if appropriate), and total energy intake, whereas the second model was additionally adjusted for baseline covariates that can operate as confounders such as: educational level, smoking, number of cigarettes per day, physical activity, BMI, alcohol consumption, dietary fiber, and the prevalence of dyslipidemia/hypercholesterolemia, hypertension, and diabetes. Additionally, analyses were stratified by the calendar year of recruitment. In the EPIC-Spain cohort analyses were stratified by the region or recruitment.

In the SUN Project and in the AWHS, to maximize the use of available information, missing values on categorical variables were included in a separate category, while missing values on continuous variables were imputed by predicted values from a multivariable regression model containing the corresponding explanatory variables.

In the AWHS, we also examined the risk of subclinical atherosclerosis according to types of olive oil (common or virgin) categorizing the participants' consumption into 0 to <10, 10 to <30 and ≥ 30 g/day. In the EPIC-Spain cohort we were also able to classify into participants who mostly consumed common olive oil and who consumed mostly virgin olive oil.

Statistical analyses were performed using STATA (Intercooled STATA software version 16; StataCorp LLC, TX, USA). P-values were two-sided and considered statistically significant when <0.05.

3. Results

In the AWHS cohort, all participants were males with a mean age of 51 ± 4 years. The mean of total olive oil consumption was 32.8 ± 14.3 g/day. The corresponding means for common and virgin olive oil consumption were 12.7 ± 16.0 and 20.1 ± 20.5 g/day. Baseline characteristic of participants were similar across 10-g categories of total olive oil consumption with few exceptions. Participants with higher total olive oil intake consumed less alcohol, more vegetables and more salt (Supplemental Table 1).

Among the total number of participants, 39.8% had a CACS>0; 38.2% at least one plaque in the carotid arteries, and 56.5% at least one plaque in the femoral arteries. The inverse association between total olive oil consumption and positive coronary calcium was stronger for consumptions between 20 and <30 g/day (OR; 95% CI 0.44; 0.17, 1.13) than for higher consumptions (OR 0.69; 0.35, 1.35), when compared to consumptions <10 g/day. This association was clearer when not adjusting for cardiovascular risk factors that could mediate the association (i.e., BMI, dyslipidemia, hypertension, and diabetes) (OR 0.41; 0.16, 1.04, comparing total olive oil consumption between 20 and <30 g/day *v.* <10 g/day; data not presented in tables). Similarly, the lowest ORs for total olive oil consumption with the presence of carotid or femoral plaques were reached for consumptions between 20 and <30 g/day (Table 1). When differentiating by type of olive oil, associations only remained protective (OR < 1) for virgin olive oil with maximum protection with consumptions between 10 and <30 g/day (Table 2).

In the SUN cohort, 39.5% of participants were men and their mean age was 38 ± 12. The mean of total olive oil consumption was 18.5 ± 14.9 g/day. Participants with a higher total oil consumption also had a higher total energy intake, were more frequently smokers, had higher alcohol consumption and higher intakes of fiber, fruit and vegetables and salt, as well as their prevalence of hypertension at baseline was slightly lower (Supplemental Table 2).

Participants were followed up for a mean of 10.8 ± 4 years (198,315 total person-year at risk). During the follow-up, 261 total CVD cases were reported, among which 150 were confirmed by a physician. The multivariable adjusted HRs of confirmed CVD according to 10-g categories of total olive oil consumption (0 to <10, 10 to <20, 20 to <30 and ≥ 30 g/day) were 0.97 (0.65, 1.47), 0.83 (0.51, 1.37) and 0.73 (0.38, 1.40), when compared with those consuming <10 g/day. When probable CVD cases (n = 111) were also considered (self-reported events were not confirmed by an expert), the associations were stronger reaching statistical significance in those with higher consumption, HR 0.57; 0.34, 0.96 (Table 3). Although virgin olive oil intake could not be specifically evaluated, only 2% of participants said they never consume virgin olive oil. While 70% of those participants consuming >20 g/day stated to mainly consume virgin olive oil (at least 75% of their total olive oil consumption).

In the EPIC cohort, 37.0% of participants were men, and their mean age was 49 ± 8. The mean of total olive oil consumption was 20.0 ± 14.9 g/day. When differentiating by types, the mean of common (refined) olive oil consumption was 16.4 ± 15.5 g/day, while the mean of virgin olive oil was 3.8 ± 10.0 g/day. Participants with a higher consumption of total olive oil were more frequently men, consumed more energy, were more educated, had a higher consumption of alcohol, fiber, fruit, and vegetables, and lower consumption if salt, as well as, had more frequently hypercholesterolemia, but less frequently hypertension and diabetes (Supplemental Table 3).

Participants were followed up for a mean of 22.8 ± 3.4 years (908,246 total person-year at risk). At the end of follow-up 2159 confirmed CVD cases occurred. Among them 1300 were CHD events, and 938 were strokes.

The multivariable adjusted HRs of total CVD according to 10-g categories of total olive oil consumption were 0.90 (0.79, 1.02), 0.87 (0.76, 0.98), and 0.95 (0.84, 1.08), respectively. This inverse association was stronger when stroke was evaluated, and the greatest benefit was also observed for consumptions between 20 and <30 g/day with risk reduction of 20% (HR 0.80; 0.66, 0.96) when compared to those in the lowest category consumption, <10 g/day (Table 4). The association with CHD was weaker and the statistical significance was lost. When the association for those who mostly consumed virgin olive oil was evaluated, the inverse associations for the three outcomes were always stronger, although without reaching statistical significance (Table 5).

Table 1
Odds Ratios (95% Confidence Intervals) of subclinical atherosclerosis risk according to olive oil consumption among the AWHS participants.

Olive oil consumption (g/day)	n	Cases	MV-adjusted OR (95% CI)[a]	MV-adjusted OR (95% CI)[b]
CACS >0 (n = 1876)				
0 to <10	41	20	Ref.	Ref.
10 to <20	212	84	0.63 (0.32, 1.26)	0.65 (0.32, 1.35)
20 to <30	43	14	0.41 (0.16, 1.01)	0.44 (0.17, 1.13)
≥ 30	1580	629	0.63 (0.33, 1.20)	0.69 (0.35, 1.35)
P for trend			0.574	0.872
Presence of at least one plaque in the carotid arteries (n=2183)				
0 to <10	39	18	Ref.	Ref.
10 to <20	263	113	0.89 (0.45, 1.78)	0.94 (0.46, 1.94)
20 to <30	59	17	0.43 (0.18, 1.01)	0.49 (0.20, 1.20)
≥ 30	1822	686	0.69 (0.36, 1.32)	0.74 (0.37, 1.46)
P for trend			0.046	0.082
Presence of at least one plaque in the femoral arteries (n=2187)				
0 to <10	41	27	Ref.	Ref.
10 to <20	259	140	0.62 (0.31, 1.24)	0.62 (0.29, 1.33)
20 to <30	56	28	0.49 (0.21, 1.14)	0.57 (0.22, 1.43)
≥ 30	1831	1041	0.68 (0.35, 1.31)	0.75 (0.36, 1.55)
P for trend			0.893	0.411

CACS: coronary artery calcium score.
[a] Adjusted for age (years), and total energy intake (Kcal/day).
[b] Additionally adjusted for education level (middle school, high school, secondary, university), smoking (never, former, current), number of cigarettes per day, physical activity (total METs-h/week), BMI (kg/m^2), alcohol intake (0, 1–19, ≥20 g/day), fiber intake (g/day), fruit and vegetables (g/day), sodium intake (g/day), systolic and diastolic blood pressure (mmHg), dyslipidemia (yes/no), hypertension (yes/no), and diabetes (yes/no).

4. Discussion

These analyses carried out in three Spanish studies add evidence to the suggested inverse association of olive oil consumption with CVD. Overall inverse associations are consistently observed when examining total olive oil consumption in all cardiovascular endpoints in the three cohorts. Results from the EPIC study suggest a maximum benefit between 20 and 30 g/day, while consumptions above 30 g/day may not provide further advantage. The protective effect could be greater for virgin olive oil while also operating from early stages of the disease.

We believe that the associations we are reporting can be considered as properly causal [25] given i) the appropriate temporal sequence, ii) the availability of large randomized trials with clinical CVD events using olive oil in the intervention, iii) the abundance of mechanistic findings, iv) the consequent high biological plausibility [6,8,26–29], as well as v) the consistency of our results with previous meta-analyses [9,10]. Very specially, recent findings from large cohorts with excellent methodology and repeated measurements of intake [12] support this causality.

In the AWHS and EPIC cohorts the range of consumption between 10 and 20 g/d showed the strongest inverse association, but not in the SUN cohort, where an inverse dose–response was observed. Remarkably, when we differentiated between the common variety and the virgin olive oil variety (rich in polyphenols) in the AWHS and EPIC cohorts, the inverse association was substantially clearer for the virgin olive oil variety. Thus, in the AWHS, higher intakes of common olive oil (≥30 g/day) were no longer protective against subclinical atherosclerosis or even reversing the trend; but this did not happen with virgin olive oil, which remained inversely associated throughout the whole range of consumption. In the EPIC cohort, the consumption of common olive oil was considerably more frequent than the consumption of the virgin variety, but it was not possible to quantify the variety of consumption as accurately as in the AWHS. Notwithstanding, the EPIC results also evidenced stronger and clearer inverse association among those participants who mainly consumed the virgin variety.

Similarly, in the SUN cohort it was not possible to quantify the consumption of olive oil variety, however, only 2% of participants reported they never consumed virgin, while 70% of those in the highest olive oil consumption category reported to mainly consume virgin (at least 75% of their total consumption). The fact that the virgin variety accounted for the most part of the olive oil consumption may explain the observed dose-dependent association between olive oil and CVD. This association should be expected to be linear for the observed range of consumption when the olive oil consumed is of higher quality and rich in polyphenols. But it should be expected to be non-linear, reaching a plateau at high levels (or even reversing the trend) when the olive oil consumed is devoid of polyphenols (i.e., the common variety). Thus, in the EPIC cohort, the total consumption of olive oil may not be associated in such a linear way with CVD because the total olive oil consumed was mostly of the common variety. More than 200 minor compounds have been described to enrich virgin olive oil, some of them with demonstrated cardioprotective effects [8]. However, during the refining process used to obtain the common variety, most of these minor compounds might be lost and chemical solvents are added. Therefore, the beneficial minor compounds are basically found in sufficient amounts only in the virgin variety of olive oil, which is obtained only by mechanical means through crushing and pressing olives. Common olive oil, which is a mixture of virgin and refined oil (80% refined oil) has fewer bioactive compounds, justifying the clearer beneficial results observed for virgin olive oil.

However, the observation of no further advantage with the highest levels of total olive oil consumption in the EPIC and AWHS cohorts could also be due to residual confounding; that is, to differential characteristics of the subjects with the greatest oil consumptions that somehow might blur the association. On the other hand, it seems biologically plausible that a saturation effect might be present, and no additional benefit may happen once the provision of crucial nutrients is achieved. So far, we really do not know. Further studies carefully quantifying and differentiating between the different varieties of olive oil are required to clarify the dose–response associations between each variety of olive oil and CVD/atherosclerosis and to disentangle if there is a threshold beyond which no more benefit is likely to be provided.

The contribution of our work is the following. First, olive oil has a protective association with stroke which accounts for a high

Table 2
Odds Ratios (95% Confidence Intervals) of subclinical atherosclerosis risk according to types of olive oil consumption among the AWHS participants.

CACS >0 (n = 1876)				
Common olive oil consumption (g/day)	n	Cases	MV-adjusted OR (95% CI)[b]	MV-adjusted OR (95% CI)[c]
0 to <10	1076	418	Ref.	Ref.
10 to <30	199	71	0.84 (0.61, 1.16)	0.78 (0.55, 1.09)
≥ 30	601	258	1.19 (0.96, 1.47)	1.11 (0.89, 1.38)
P for trend			0.117	0.375
Virgin olive oil consumption (g/day)[a]	n	Cases	MV-adjusted OR (95% CI)[b]	MV-adjusted OR (95% CI)[c]
0 to <10	729	309	Ref.	Ref.
10 to <30	157	60	0.80 (0.56, 1.15)	0.84 (0.57, 1.22)
≥ 30	988	377	0.82 (0.67, 1.01)	0.89 (0.72, 1.10)
P for trend			0.070	0.309
Presence of at least one plaque in the carotid arteries (n=2183)				
Common olive oil consumption (g/day)	n	Cases	MV-adjusted OR (95% CI)[b]	MV-adjusted OR (95% CI)[c]
0 to <10	1180	444	Ref.	Ref.
10 to <30	253	96	1.01 (0.76, 1.34)	1.01 (0.75, 1.36)
≥ 30	750	294	1.06 (0.88, 1.29)	1.03 (0.85, 1.26)
P for trend			0.533	0.755
Virgin olive oil consumption (g/day)	n	Cases	MV-adjusted OR (95% CI)[b]	MV-adjusted OR (95% CI)[c]
0 to <10	905	363	Ref.	Ref.
10 to <30	194	71	0.84 (0.61, 1.17)	0.85 (0.61, 1.20)
≥ 30	1082	399	0.86 (0.72, 1.04)	0.88 (0.73, 1.08)
P for trend			0.128	0.243
Presence of at least one plaque in the femoral arteries (n=2187)				
Common olive oil consumption (g/day)	n	Cases	MV-adjusted OR (95% CI)[b]	MV-adjusted OR (95% CI)[c]
0 to <10	1187	655	Ref.	Ref.
10 to <30	245	125	0.84 (0.63, 1.11)	0.79 (0.58, 1.08)
≥ 30	755	456	1.24 (1.02, 1.49)	1.17 (0.95, 1.44)
P for trend			0.031	0.146
Virgin olive oil consumption (g/day)	n	Cases	MV-adjusted OR (95% CI)[b]	MV-adjusted OR (95% CI)[c]
0 to <10	910	542	Ref.	Ref.
10 to <30	189	101	0.76 (0.55, 1.05)	0.74 (0.52, 1.06)
≥ 30	1086	591	0.80 (0.67, 0.96)	0.86 (0.70, 1.06)
P for trend			0.022	0.188

NOTE: Virgin olive oil also includes extra virgin; and common olive oil refers to refined olive oil.
CACS: coronary artery calcium score.
[a] For the virgin olive oil analysis, 2 participants are lost for having missing values in this variable.
[b] Adjusted for age (years), and total energy intake (Kcal/day).
[c] Additionally adjusted for education level (middle school, high school, secondary, university), smoking (never, former, current), number of cigarettes per day, physical activity (total METs-h/week), BMI (kg/m^2), alcohol intake (0, 1–19, ≥20 g/day), dietary fiber (g/day), fruit and vegetables (g/day), sodium intake (g/day), systolic and diastolic blood pressure (8 categories), hypercholesterolemia (yes/no), hypertension (yes/no), and diabetes (yes/no).

proportion of the burden of disease in Spain (especially among older women), with an incidence similar to that in the US [30]. Second, the protective association with stroke is similar to the previous two studies in other Mediterranean countries [31,32], although in contrast to what has been reported in the US (with a much lower consumption of olive oil) [12]. Third, the inverse associations were consistently more pronounced in virgin olive oil consumers, as already described in the previous EPIC-study evaluating olive oil and CHD (10.5 years of follow-up) [33]. Fourth, the protective association could be acting from the early stages of the disease. Finally, olive oil consumption is not a marker for socioeconomic status due to its widespread availability compared to other non-Mediterranean countries, and because the SUN cohort used restriction (admitting only highly educated subjects), which is an outstanding approach to avoid or at least reduce confounding by socioeconomic status and other potential factors. Restriction in epidemiology, as described by Rothman et al. [34], represents an excellent technique to prevent or at least reduce confounding by known factors, and obtain high-quality data from participants. The rationale is that a variable cannot produce confounding if it is prohibited from varying. Restricting the admissibility criteria for subjects to be included in a study is therefore an extremely effective method of preventing confounding.

The strengths include the use of three Spanish cohorts with few losses to follow-up and large sample size. In addition, validated instruments were used to collect dietary information, and analyses were controlled for the same confounding factors to make the results comparable. Also, several CVD outcomes were used when available and results on stroke were based on many numbers of cases. There are only three previous epidemiological studies that have examined olive oil consumption and stroke [12,31,32].

Table 3
Hazard Ratios (95% Confidence Intervals) of cardiovascular disease risk according to olive oil consumption among the SUN Project participants (n = 18,266).

Olive oil consumption (g/day)	n	Cases	Persons- year	Rate/1000 Pers-y	MV-adjusted HR (95% CI)[a]	MV-adjusted HR (95% CI)[b]
Confirmed Cardiovascular Disease						
0 to <10	5381	57	5,8163	0.98	1 (Ref.)	1 (Ref.)
10 to <20	5971	49	6,5255	0.75	0.92 (0.62, 1.37)	0.97 (0.65, 1.47)
20 to <30	4596	30	49,059	0.61	0.85 (0.52, 1.37)	0.83 (0.51, 1.37)
≥ 30	2318	14	25,838	0.54	0.79 (0.42, 1.47)	0.73 (0.38, 1.40)
P for trend					0.394	0.276
Probable Cardiovascular Disease						
0 to <10	5381	93	58,023	1.60	1 (Ref.)	1 (Ref.)
10 to <20	5971	98	65,087	1.51	1.02 (0.76, 1.37)	1.09 (0.81, 1.48)
20 to <30	4596	50	48,975	1.02	0.76 (0.53, 1.11)	0.79 (0.54, 1.15)
≥ 30	2318	20	25,814	0.77	0.59 (0.35, 0.98)	0.57 (0.34, 0.96)
P for trend					0.014	0.009

[a] Adjusted for age (years), sex, total energy intake (Kcal/day), and stratified by calendar year of recruitment (≤2001, 2002-03, 2004-05, 2006-07, 2008-10, 2011-15).
[b] Additionally adjusted for years of university (years), smoking (never, current, former), number of cigarettes per day, physical activity (METs-h/wk), BMI (kg/m^2), alcohol consumption (0, 1–19, ≥20 g/day), dietary fiber (g/day), fruits and vegetables (g/day), sodium intake (g/day), systolic and diastolic blood pressure (mmHg), hypercholesterolemia (yes/no), hypertension (yes/no), and diabetes (yes/no).

Table 4
Hazard Ratio (95% Confidence Intervals) of confirmed cardiovascular disease, coronary heart disease, and stroke risk according to olive oil consumption: the EPIC-Spain study (n = 39,393).

Olive oil consumption (g/day)	n	Cases	Persons- year	Rate/1000 Pers-y	MV-adjusted HR (95% CI)[a]	MV-adjusted HR (95% CI)[b]
Confirmed cardiovascular disease						
0 to <10	10,444	690	237,071	2.91	1 (ref.)	1 (ref.)
10 to <20	10,647	478	243,526	1.96	0.86 (0.76, 0.97)	0.90 (0.79, 1.02)
20 to <30	9418	436	215,438	2.02	0.80 (0.71, 0.90)	0.87 (0.76, 0.98)
≥ 30	8884	555	201,817	2.75	0.88 (0.78, 0.98)	0.95 (0.84, 1.08)
P for trend					0.007	0.235
Confirmed coronary heart disease						
0 to <10	10,444	407	240,536	1.69	1 (ref.)	1 (ref.)
10 to <20	10,647	302	245,676	1.23	0.91 (0.78, 1.06)	0.94 (0.80, 1.10)
20 to <30	9418	272	217,416	1.25	0.83 (0.71, 0.97)	0.90 (0.77, 1.05)
≥ 30	8884	319	204,618	1.56	0.89 (0.77, 1.04)	0.99 (0.84, 1.17)
P for trend					0.066	0.659
Confirmed stroke						
0 to <10	10,444	309	242,783	1.27	1 (ref.)	1 (ref.)
10 to <20	10,647	196	247,465	0.79	0.80 (0.67, 0.97)	0.84 (0.70, 1.02)
20 to <30	9418	177	219,181	0.81	0.73 (0.61, 0.88)	0.80 (0.66, 0.96)
≥ 30	8884	256	206,052	1.24	0.85 (0.72, 1.01)	0.89 (0.74, 1.07)
P for trend					0.025	0.127

[a] Adjusted for age (10 categories), sex, total energy intake (Kcal/day), and stratified by center (Asturias, Gipuzkoa, Granada, Murcia, and Navarra).
[b] Additionally adjusted for level of education (no studies, primary, secondary, university), smoking (never, current, former), number of packs per year, physical activity (METs-h/wk), BMI (kg/m^2), alcohol consumption (0, 1–19, ≥20 g/day), dietary fiber (g/day), fruits and vegetables (g/day), sodium intake (mg/day), prevalent hypercholesterolemia (yes/no), prevalent hypertension (yes/no), and prevalent diabetes (yes/no).

Table 5
Hazard Ratio (95% Confidence Intervals) of confirmed cardiovascular disease, coronary heart disease and stroke according to types of olive oil consumption: the EPIC-Spain study (n = 39,393).

Olive oil consumption	n	Cases	Persons- year	Rate/1000 Pers-y	MV-adjusted HR (95% CI)[a]	MV-adjusted HR (95% CI)[b]
Confirmed cardiovascular disease						
No consumption	5768	425	130,603	3.25	1 (ref.)	1 (ref.)
Mostly common	27,596	1485	630,278	2.36	0.85 (0.76, 0.95)	0.91 (0.82, 1.02)
Mostly virgin	6029	249	136,971	1.82	0.80 (0.67, 0.96)	0.86 (0.71, 1.03)
Confirmed coronary heart disease						
No consumption	5768	249	132,796	1.88	1 (ref.)	1 (ref.)
Mostly common	27,596	901	637,283	1.41	0.89 (0.77, 1.02)	0.96 (0.83, 1.11)
Mostly virgin	6029	150	138,168	1.09	0.80 (0.63, 1.01)	0.85 (0.66, 1.08)
Confirmed stroke						
No consumption	5768	193	134,084	1.44	1 (ref.)	1 (ref.)
Mostly common	27,596	638	642,396	0.99	0.80 (0.68, 0.94)	0.85 (0.72, 1.01)
Mostly virgin	6029	107	139,001	0.77	0.78 (0.60, 1.02)	0.84 (0.63, 1.10)

NOTE: Virgin olive oil also includes extra virgin; and common olive oil refers to refined olive oil.
[a] Adjusted for age (10 categories), sex, total energy intake (Kcal/day), and stratified by center (Asturias, Gipuzkoa, Granada, Murcia, and Navarra).
[b] Additionally adjusted for level of education (no studies, primary, secondary, university), smoking (never, former, current), number of packs per year, physical activity (METs-h/wk), BMI (kg/m2), alcohol consumption (0, 1–19, ≥20 g/day), dietary fiber (g/day), fruits and vegetables (g/day), sodium intake (mg/day), prevalent hypercholesterolemia (yes/no), prevalent hypertension (yes/no), and prevalent diabetes (yes/no).

Additionally, the variety of olive oil consumed has hardly been examined in the literature.

The limitations also need mentioning. Dietary information was self-reported and measurement errors and non-differential misclassification cannot be ruled out. And especially, to assess accurately oil consumption through a questionnaire, either self-reported or conducted by an interviewer is difficult. In addition, we could not differentiate weather the oil was consumed crude, heated, or even reheated; despite the stability of olive oil in the face of relatively high temperatures, the effect of crude oil may differ from heated/reheated oil. Also, even though the analyses were adjusted for potential confounders, a certain degree of residual confounding could remain. In addition, only one dietary measurement was collected at the beginning of the follow-up, which again could lead to non-differential misclassification. In the SUN cohort we could not distinguish between common and virgin olive oil, as well the limited number of CVD events in this relatively young sample did not allow us to analyze types of CVD with sufficient statistical power. In the EPIC cohort, the measurement of the exposure and the events were taken far apart, assuming that the exposure remained stable over time. Additionally, a causal association cannot be properly established because of the observational study designs. Finally, the analyses conducted in the AWHS only included male workers, while in the SUN cohort they were predominantly educated professionals. So that, generalizability of the results could be limited in both studies. Still, there is no reason to expect that the underlying biological mechanisms might be different in women or in less educated subjects.

5. Conclusion and perspectives

In these Spanish studies, higher consumption of total olive oil was associated with a lower risk of CVD and stroke. The maximum benefit could be obtained with a consumption between 20 and 30 g/day. Further advantage with higher levels of consumption was not consistently confirmed in all three cohorts. The association seems to be stronger for virgin olive oil and, plausibly, operating from early states of the disease, i.e., preventing the formation of coronary calcium and atheroma plaques in the arteries.

These results support the recommendation to increase and reinforce the consumption of olive oil, if possible, virgin, over the intake of other fats, the earlier in life the better, for primary prevention of cardiovascular disease. If no more benefits are provided above a threshold it needs to be further investigated. Therefore, additional research is needed to clarify what is the optimal dose to obtain the desired benefits and disclose the differences between common and virgin olive oil consumption in relation to cardiovascular risk and underlying mechanisms.

Transparency declaration

Carolina Donat-Vargas affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Data share statement

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

Authorship

All authors listed fully meet the criteria for authorship.

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Conflict of Interest

All authors have completed the Unified Competing Interest form and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years, no other relationships or activities that could appear to have influenced the submitted work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.11.002.

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