1. TITLE PAGE

CAFFEINATED COFFEE CONSUMPTION AND RISK OF ATRIAL FIBRILLATION IN TWO SPANISH COHORTS

Bazal P.[a, b], Gea A.[a, c], Navarro AM [a, b], Salas-Salvadó J [c,e], Corella D [c,f], Alonso-Gómez A [c,g], Fitó M [c,h], Muñoz-Bravo C [i] , Estruch R [c,j], Fiol M [c,k], Lapetra J [c,l], Serra-Majem L [c,m], Ros E [c,n], Rekondo J [c,g]; Muñoz MA[o,p], Basora J [e], Sorli JV [c,f], Toledo E [a, c], Martínez-González M.A [a, c, d], Ruiz-Canela M. [a, c]

[a] University of Navarra, Department of Preventive Medicine and Public Health, School of Medicine, IdiSNA, Pamplona, Spain; [b] Servicio Navarro de Salud-Osasunbidea, Pamplona, Spain; [c] CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid, Spain, [d] Department of Nutrition, Harvard TH Chan School of Public Health, Boston, USA; [e] Human Nutrition Unit, University Hospital of Sant Joan de Reus, IISPV, Rovira i Virgili University, Reus (Tarragona), Spain; [f] Department of Preventive Medicine, University of Valencia, Valencia, Spain, [g] Department of Cardiology, University Hospital Araba, Vitoria, Spain; [h]Cardiovascular Risk and Nutrition (Regicor Study Group), Hospital del Mar Medical Research Institute (IMIM), Barcelona, Spain; [i]Department of Public Health, University of Malaga, Malaga, Spain; [j] Institut d'Investigacions Biomèdiques August Pi i Sunyer, Hospital Clinic; [k] Illes Balears Health Research Institute (IdISBa), Palma, Spain; [l]Department of Family Medicine. Unit Research. Distrito Sanitario Atención Primaria Sevilla, Spain. [m] Research Institute of Biomedical and Health Sciences (IUIBS), University of Las Palmas de Gran Canaria & CHUIMI Canarian Health Service, Las Palmas, Spain; [n] Endocrinology & Nutrition Service, Institut d'Investigacions Biomèdiques August Pi Sunyer, Hospital Clínic, Barcelona, Spain; [o]Gerència territorial de Barcelona. Institut Català de la Salut. Barcelona. Spain;

[p]Fundació Institut Universitari per a la recerca a l'Atenció Primària de Salut Jordi Gol i Gurina (IDIAPJGol), Barcelona, Spain

Author contributions

PB, AG and R-CM contributed to the conception or design of the work. ET and MAM-G contributed to the acquisition, analysis, and interpretation of data for the work. AMN contributed to the analysis of the work. JS-S, DC, MF, CM-B, RE, MF, JL, LS-M, ER, JR, MAM, JB and JVS contributed to the acquisition.

PB, AG and R-CM drafted the manuscript. All critically revised the manuscript and gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

Correspondence to Alfredo Gea,

Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra, Irunlarrea 1, 31008 Pamplona, Navarra, Spain.

E-mail: ageas@unav.es

Telephone: +0034 948425600 (ext. 806637)

PREDIMED: http://www.isrctn.com/ISRCTN35739639

SUN: https://clinicaltrials.gov/ct2/show/NCT02669602

2. ABSTRACT

a) Aims: The association between caffeinated coffee consumption and atrial fibrillation (AF) remains unclear. Recent studies suggest an inverse association only between a moderate caffeinated coffee consumption and AF, but others have found no association. The aim of our study was to prospectively assess the association between caffeinated coffee consumption and AF in two Spanish cohorts, one of adults from a general population and another of elderly participants at high cardiovascular risk.

b) Methods and Results: We included 18,982 and 6,587 participants from the “Seguimiento Universidad de Navarra” (SUN) and "Prevención con Dieta Mediterránea" (PREDIMED) cohorts, respectively. Participants were classified according to their caffeinated coffee consumption in three groups: ≤3 cups/mo, 1-7 cups/wk, and >1cup/d. We identified 97 AF cases after a median follow-up of 10.3 years [IQR 6.5-13-5], in the SUN cohort and 250 cases after 4.7 years median follow-up [IQR 2.8-5.8] in the PREDIMED study. No significant associations were observed in the SUN cohort although a J-shaped association was suggested. A significant inverse association between the intermediate category of caffeinated coffee consumption (1-7 cups/week) and AF was observed in PREDIMED participants with a HR=0.53 (95% CI 0.36-0.79) when compared with participants who did not consume caffeinated coffee or did it only occasionally. No association was found for higher levels of caffeinated coffee consumption (>1 cup per day), HR=0.79 (95% CI 0.49-1.28). In the meta-analysis of both PREDIMED and SUN studies, HR for intermediate consumption of caffeinated coffee was 0.62 (95%CI 0.46-0.85)

without evidence of heterogeneity. Similar findings were found for the association between caffeine intake and AF risk.

c) Conclusion: Intermediate levels of caffeinated coffee consumption (1-7 cups/wk) were associated with a reduction in AF risk in two prospective Mediterranean cohorts.

Abstract word count: 283

Manuscript word count 4055

Keywords: Atrial Fibrillation, coffee, caffeine, Mediterranean diet.

3. INTRODUCTION

Coffee is a plant-derived beverage highly consumed in western countries. Coffee consumption has been associated with healthy benefits including a decrease in the risk of both cardiovascular and overall death [1–4]. However, the effect of coffee intake on cardiac arrhythmia is still unclear[5]. Traditionally, beverages with caffeine have been related to an increase of the burden of supraventricular arrhythmias, including atrial fibrillation (AF)[6] . This pro-arrhythmic effect has been found in some *in vitro* and animal models experiments [7,8]. Additionally, caffeine is one of the most frequent triggers for AF as reported by patients [9]. On the other hand, several population-based studies have found no increased risk of AF associated with coffee or caffeine intake [10,11]. Moreover, some studies have found a U-shaped or linear association between coffee and AF risk, suggesting a protective effect of low-to-moderate consumption of coffee [12–16]. However, some of these studies did not make a distinction between regular and decaffeinated coffee [10,15–17]. Caffeine may have both a proarrhythmic property by sympathomimetic effects and antiarrhythmic properties as an antioxidant [18]. Among adults, coffee is the main source of caffeine intake and therefore its consumption is related to a high intake of plant phytochemicals also with antioxidant properties [19]. The PREvención with DIeta MEDiterránea (PREDIMED) trial showed a protective effect of the Mediterranean diet supplemented with extra-virgin olive oil on the risk of AF [20]. It is estimated that in Mediterranean countries the intake of caffeine from coffee is about 198 mg/day per person [19]. In this context, it is important to know whether the consumption of coffee can also be recommended as a strategy to reduce the incidence of AF.

Our aim was to assess the association between the consumption of caffeinated coffee and AF in two Mediterranean Spanish cohorts, one of young adults, from the SUN cohort, and another of elderly subjects, from the PREDIMED study. Our hypothesis was that caffeinated coffee consumption could be inversely associated with the incidence of AF in a long-term prospective assessment.

4.METHODS

Population

We included participants from two Spanish studies, the SUN project and the PREDIMED trial, assessed as prospective observational cohorts.

The SUN Project is an open multipurpose young cohort made of university graduates. It was designed to assess the relationship between diet and lifestyle factors and different health disorders. All the information is collected using biennial mailed or web-based questionnaires [21,22]. Since the beginning of the study in December 1999 until 2017, 22,564 participants were recruited (39% men), with a mean age of 37.5 years (SD 11,9 years), and 23 and 54 years, the 10th and 90th percentiles, respectively.

The PREDIMED study was a multicentre intervention trial conducted in 11 recruiting centres[23]. A total of 7,447 men and women were recruited. Participant's age ranged between 55-80 years. All participants were initially free of cardiovascular disease but at high cardiovascular risk. As inclusion criteria they had to be diagnosed with type 2 diabetes or present ≥3 of any of the following cardiovascular risk factors: current smoking, hypertension, hypercholesterolemia, low high-density lipoprotein cholesterol,

overweight/obesity, or family history of premature coronary heart disease [23]. We analysed the PREDIMED trial as a prospective observational cohort, including the intervention group as a covariate in the models.

For the present assessment, we excluded 2,202 participants in the SUN cohort, 143 with prevalent AF cases at baseline and 2059 with no follow up. 916 participants in the PREDIMED study were also excluded, 75 prevalent AF cases at baseline and 841 with no follow up, including all participants from a center, center E (n=667), where the presence of AF was not assessed. We also excluded all participants with no information about coffee consumption (834 participants in SUN cohort and 50 in PREDIMED study) and those who did not meet predefined energy intake limits: <400 kcal or > 6,000 kcal in men and <400 kcal and >5,000 kcal in women (261 participants in the SUN cohort and 2 participants in the PREDIMED study).

Ethics

Approval from the Research Ethics Committees of the University of Navarra for the SUN cohort and recruitment centers of the PREDIMED trial was obtained. All participants provided written informed consent in PREDIMED study. In the SUN cohort, a response to the initial questionnaire was considered as an informed consent to participate in the study. Regarding the obtainment of informed consent of potential participants, we duly informed these potential candidates of their right to refuse to participate in the SUN study or to withdraw their consent to participate at any time without reprisal, according to the principles of the Declaration of Helsinki. After ensuring that

the candidate had understood the information, we sought their potential freely-given informed consent, and their voluntary completion of the baseline questionnaire. These methods were accepted by the Research Ethics Committee as to imply an appropriately-obtained informed consent.

Coffee consumption

A previously validated food-frequency questionnaire was used to measure baseline caffeinated and decaffeinated coffee consumption, both in the SUN (136 items) and in PREDIMED (137 items) [24,25]. Coffee consumption was measured using 9 possible categories: never/almost never, 1-3 cups/month, 1/week, 2-4/week, 5-6/week, 1/day, 2-3/day, 4-6/day and >6/day). For analyses in the present study, participants were classified according to three categories of coffee consumption: ≤3 cups/month (occasional or non-drinkers), 1-7 cups/week (light drinkers or intermediate level of consumption), >1cup/day (moderate-to-high drinkers).

Outcome

We included all incident AF events from baseline until December 2010 (maximum follow-up: 7 years) in the PREDIMED study and until the longest available follow-up in the SUN project (maximum follow-up: 16 years).
In the SUN cohort, incident AF diagnosis was assessed in the biennial follow-up questionnaires. As we have previously described[26], the following AF confirmation algorithm was applied in the SUN project: participants with a self-reported AF were asked to send the diagnostic ECG or a medical report with the specific diagnosis and the date of the first diagnosis of AF. All the ECGs

and medical records were reviewed by a cardiologist (PB). If no answer was obtained or the received information was incomplete, further information was required by the cardiologist using a brief questionnaire during a phone call. In these scenarios, AF cases were confirmed if the participant could answer at least two out of the three following questions: 1) name of the arrhythmia; 2) medication frequently used for the treatment of AF (anticoagulation, antiarrhythmics, etc); and 3) suggestive symptoms of AF (irregular palpitations associated or not to dyspnoea or asthenia). The information about the date of diagnosis was obtained from the information collected in the medical records. If this information was missing, it was obtained from self-reported questionnaire or from the phone call. If the information about the date of diagnosis was discordant we selected the earliest date as the most probable accurate date.

In the PREDIMED study, information about incident AF cases was obtained from medical records and yearly ECGs, and each case was assessed and confirmed by an Adjudication committee blinded to the intervention group. Additional information about the diagnosis of AF has been discussed elsewhere [27].

Covariates

Participants in the SUN cohort answered at baseline a questionnaire including information on socio-demographic factors, physical activity [28], and other health-related habits and a 136-item semi-quantitative food-frequency questionnaire.

Information related to other caffeine beverages (tea, sodas and energy drinks) was collected from the food-frequency questionnaire.

In the PREDIMED study, all participants completed at baseline a general questionnaire including lifestyle and clinical information, a validated Spanish version of the Minnesota Leisure-Time Physical Activity [28] and a 137-item semi-quantitative food frequency questionnaire.

Weight and height were biennially self-reported in the SUN project while in PREDIMED study those variables were measured by trained personnel, at baseline and yearly, using calibrated scales. Body mass index (BMI) was then calculated with the information obtained as the result of the weight (kilograms) by the square of height (meters).

At the SUN project hypertensive status was established by previous validated self-reported medical diagnosis of hypertension [29]. Blood pressure was measured in triplicate after 5 min of rest by using a validated semiautomatic sphygmomanometer in PREDIMED study, and diagnose of hypertension in this case was confirmed by a trained physician.

Other variables such as previous diagnoses and family medical history were also obtained from the baseline questionnaire in both the SUN and PREDIMED studies.

Statistical analyses

Baseline characteristics were described using means and standard deviations for continuous variables and percentages for categorical variables, using ANOVA and Chi-squared tests, respectively, to compare different groups of caffeinated coffee consumption.

Cox proportional hazards regression models were used to assess the association between caffeinated coffee consumption and the risk of incident AF during follow-up. We estimated Hazard Ratios (HRs) and 95% Confidence Intervals (CI) using seldom or never drinkers (≤ 3 cups/month) as the reference category. Person-years of follow-up were calculated from the date of baseline visit to the date of AF diagnosis, death, or the end of follow-up, whichever occurred first. First, we performed an age and sex adjusted model. In the SUN cohort age was used as the underlying time variable. In the PREDIMED cohort we used time in the trial and adjusted for age. Second, we additionally adjusted for other caffeinated beverages and decaffeinated coffee, alcohol consumption (nondrinkers, moderate drinkers defined as >0 and ≤ 14 units of drink/week in men and >0 and ≤ 7 units of drink/week in women and heavy drinkers), smoking habit (non-smokers, former and current smokers), height (m), body mass index (kg/m^2), sleep apnea (yes/no), physical activity (METS-min/day), hypertension or use of antihypertensive agents (yes/no), and previous diagnosis of diabetes and heart failure and other cardiovascular events (myocardial infarct, coronary disease or stroke) in SUN project and for non-atherosclerotic coronary disease in PREDIMED study.

Non-linear dose-response analysis was modelled using restricted cubic splines with 3 knots. In this analysis, we used the residuals of coffee consumption after adjusting for total energy following the residual method[30]. We adjusted for the same variables used in the multivariable model previously described.

We used simple imputation for 480 missing values in smoking habit, 50 in physical activity and 45 in height at baseline in the SUN cohort. In the PREDIMED study we imputed 2 participants with missing values for physical activity, and 4 participants with missing values for baseline hypertension were classified as non-hypertensives.

Different sensitivity analyses were performed repeating the Cox multivariable regression analyses under different assumptions: 1) after the imputation of the exposure variable, caffeinated coffee consumption. We imputed to zero and additionally performed a simple imputation; 2) changing the energy intake limits criteria; 3) excluding early incident cases of AF (<1 year of follow-up, to allow for an induction period longer than one year); 4) and excluding late incidence cases (diagnosed >5 years of follow-up).

In order to combine the results of SUN and PREDIMED, we performed a meta-analysis using random effect methods, to estimate the combination of the HRs and 95% CI from both studies.

As ancillary analyses, we analysed the association between decaffeinated coffee or caffeine with AF in multivariable Cox regression models adjusting for the same covariates as described previously for coffee with caffeine.

5. RESULTS

We included in our study a total of 18,983 participants from the SUN cohort, and 6,479 participants from the PREDIMED study. All of them were free from AF at baseline (Figure 1). In the SUN cohort a majority of participants (39%, n=7,529) were light to moderate coffee drinkers (1-7 cups/week) whereas in

the PREDIMED study, most participants (61%, n=3,978) were light or never/seldom drinkers of coffee (≤3 cups/month) (Tables 1 and 2). Compared with never-seldom drinkers (≤3 cups/month), participants with a higher caffeinated coffee consumption were more likely to be men, former or current smokers, physically active, alcohol drinkers, and with higher dyslipidemia with similar between-group differences in the SUN and PREDIMED cohorts. An exception was physical activity which was higher in never/seldom (≤ 3 cups/month) coffee drinkers in the SUN cohort. Participants with the lowest consumption of caffeinated coffee showed higher prevalence of hypertension at baseline in both cohorts (Tables 1 and 2).

After a mean follow up of 10 years in the SUN cohort, 97 incident cases of AF (191233 person years) were identified, whereas during 4.4 years of follow-up in the PREDIMED study, 250 cases of AF (28567 person years) were identified. The category of never/seldom coffee drinkers showed the highest proportion of incident cases in both cohorts: 4.3% in PREDIMED (n=173) and 0.6% in SUN (n=30).

Table 3 shows the association between different levels of coffee consumption and risk of incident AF in both the PREDIMED and the SUN studies. An intermediate level of caffeinated coffee consumption (1-7 cups/week) was inversely associated with the risk of AF in PREDIMED study, compared with participants drinking <3 cups/month (47% relative risk reduction (95% CI 21%-64%). A non-significant inverse association was observed for participants drinking more than 1 cup per day in the PREDIMED study HR=0.79 (95% CI 0.49-1.28). In the SUN cohort, we found similar results in the point estimates of the relative risk, but no association was statistically

311 significant; HR= 0.74 (95% CI 0.44-1.25) for intermediate levels of coffee
312 consumption and HR=1.01 (0.59-1.73) for >1 cup/day (Table 3). We repeated
313 the analyses in the SUN cohort with additional categories of caffeinated coffee
314 consumption with similar results (Supplementary Table 1). No relevant
315 changes were observed in sensitivity analyses (Supplementary Table 2).
316 Figure 2 shows the non-linear association between the consumption of
317 caffeinated coffee and the risk of AF. In both the PREDIMED trial and the
318 SUN cohort we observed a J-shaped association, although it was only
319 statistically significant for the intermediate level of consumption in the
320 PREDIMED trial.
321 A random-effect meta-analysis combining the results of both studies showed
322 a 40% relative risk reduction (95% CI 18%-56º%) of intermediate levels (1-7
323 cups/week) of caffeinated coffee consumption and AF risk compared with
324 participants drinking <3 cups/month (Figure 3) with no evidence of
325 heterogeneity (I^2 = 0.2%, p =0.317).
326 We also analyzed the association between decaffeinated coffee and AF, both
327 in the SUN and PREDIMED studies (Supplementary Table 3). No significant
328 association was observed for 1-7 cups/week and >1cup/day of decaffeinated
329 coffee consumption with respect to AF risk when it was compared to ≤ 3
330 cups/month.
331 Finally, when we explored the association between caffeine intake and AF
332 risk, a significant inverse association with AF in participants from the
333 PREDIMED study consuming 10-30 mg/day or >30 mg/day in comparison
334 with lower intake of caffeine (0-0.55 mg/day) was found (supplementary Table
335 4)

6. DISCUSSION

In two large Spanish cohorts a light caffeinated coffee consumption (1-7 cups/week) was associated with a decreased incidence of AF as compared to not drinking coffee or doing it only occasionally. This association was only statistically significant in the PREDIMED study but we also found a statistically significant inverse association in the combined analysis of the SUN and PREDIMED studies. No significant associations were observed for moderate-to-high coffee consumers (>1 cup/day). Consistency in all sensitivity analyses supports the robustness of the results.

Previous studies were not consistent in the association between coffee consumption and risk of AF. Several studies suggested a lack of association between coffee consumption or caffeine intake and AF [10,31,32]. In fact, the last consensus document for AF prevention of the European society of Cardiology stated that coffee consumption does not seem to increase the risk of AF [33]. However, one study suggested a possible linear dose-response relationship between coffee [15], and more recently, one study showed a protective effect only for a moderate consumption of total coffee (1-3 cups/day)[16]. Some studies have also found an inverse association for caffeine consumption [17,34,35] and AF. This association was first suggested in a large cohort of Danish women with an average consumption of caffeine of 135 mg/d [14]. Later, this inverse association between low-dose caffeine and the risk of AF was confirmed in a meta-analysis including seven observational studies [12]. Our study supports these results, suggesting a J-shaped relation between coffee consumption and AF, with the highest benefit for a light coffee consumption

(up to 1 cup per day).

A possible reason to explain the differences between studies could be the heterogeneity in the population assessed as well as the different ways to obtain and measure the main exposure (coffee) and the diagnosis of AF. In fact, most of the previous studies where a protective effect was found assessed the effect of caffeine itself as the main exposure excluding other potential protective compounds that coffee may contain [12,14,17,34]. On the other hand, AF diagnosis in many of the studied cohorts was based on medical records and no active follow up was made precluding the diagnosis of asymptomatic AF cases. This heterogeneity could make difficult, if not preclude, the comparison between studies. However, the growing evidence seems to be consistent with the absence of a harmful effect and a probable benefit of coffee intake.

The potential benefit of caffeinated coffee in AF susceptibility has been related to the antagonism with adenosine receptor and the anti-oxidative effects of other coffee compounds, mainly polyphenols [18]. We have also observed an inverse association between caffeine consumption and AF risk, suggesting that the inverse association could be at least partially attributed to the effect of caffeine. In fact, we found no association between decaffeinated coffee and AF. However, this lack of association could be explained by the loss of some cardioprotective compounds during the caffeine extraction process. Caffeine consumption could also be a surrogate of caffeinated coffee, especially in older participants from the PREDIMED study where the consumption of other beverages with caffeine was very low. Thus, we should be cautious before concluding whether the effect can be attributed to the

properties of caffeine or to other compounds available in the coffee. Further studies analyzing the association between other sources of caffeine and AF risk could be helpful to better understand this potential protective effect of caffeine.

Our results suggest a protective effect of coffee in old adults with higher prevalence of cardiovascular risk factors, both highly represented in the PREDIMED trial. This inverse association could be related to the inverse association between coffee intake and other cardiovascular risk factors, specially hypertension and diabetes [1,5,36]. However, our results were also statistically significant after adjusting for cardiovascular risk factors and there may be other mechanisms related specifically to the risk reduction in AF that we have observed. In the SUN cohort we observed a weaker association between coffee and AF that could be explained because participants were younger, AF was less intensively screened and the number of diagnosed cases was smaller. More research is required to explore more effectively whether the effect of regular coffee on AF is related to age or whether the effect is mediated by some specific cardiovascular risk factors.

Some remarkable strengths in our study are the high quality data collected in the PREDIMED trial from trained dieticians and the confirmation of AF cases by an end-point adjudication committee. In the SUN cohort, participation of university graduates (>50% of them health professionals) reduces the risk of information bias. Another strength of this study is the replication of data in two independent cohorts with significant differences between both regarding age, educational level, and cardiovascular risk factors. Although non-significant results were found in the younger cohort, a J-shaped association was

411 replicated in both studies and this reinforces the generalization of our results
412 to Mediterranean populations.
413 Our study has several limitations. First, in the SUN cohort we applied a non-
414 validated protocol to confirm the outcomes self-reported by participants
415 whereas in the PREDIMED all cases were confirmed according to an End-
416 point Adjudication Committee. The protocol used in the SUN cohort allowed
417 us to confirm some AF cases from participants who did not provide the ECG
418 or medical report of diagnosis of AF. A similar protocol was used by Bodar in
419 the Physicians' Health Study[16]. Moreover, a disease characteristic and
420 disease-specific treatment are among the most important factors identified in
421 a recent questionnaire designed to measure patient knowledge of AF [37]. In
422 the SUN cohort, the participation of university graduates (55% from health
423 professions), and the confirmation of cases by a cardiologist minimizes the
424 possibility of false positives. Second, In the SUN cohort, we were not able to
425 conduct an active search of AF cases with ECG or holter precluding the
426 diagnosis of silent AF. This limitation is common in other cohort studies[10,16].
427 Moreover, pulse palpation in trained participants could be effective for AF
428 screening[38] although this intervention could not be conducted in an
429 observational study such as the SUN cohort. Due to these reasons, we have
430 probably underestimated the real AF incidence, especially in the SUN cohort.
431 In any case, specificity can be assumed to be sufficiently high and suboptimal
432 sensitivity in outcome ascertainment due to non-differential measurement
433 error in prospective cohort studies is known not to introduce any substantial
434 bias in the estimation of relative risks[39]. Future studies with systematic and
435 comprehensive AF screening strategies can be helpful to increase specificity

and increase statistical power to confirm the association between coffee consumption and AF incidence that we have identified in our cohort.

Third, the low proportion of high coffee drinkers in both cohorts and this may explain the lack of association found for high coffee consumption. However, a higher risk for this group shown in the point estimates of the HRs in our studies, is similar to other large cohort studies suggesting a U-shaped relation between coffee intake and AF.

Four, we cannot exclude the possibility of reverse causality in the association between coffee consumption and AF. Under this assumption those participants with higher rates of risk factors or illnesses will try to avoid coffee consumption due to the previously commented belief of a deleterious effect of coffee on cardiovascular health. In fact, we observed a higher percentage of participants with hypertension among never/seldom coffee drinkers in the SUN study and higher proportion of anti-hypertensive use among never/seldom coffee drinkers in the PREDIMED study. However, the proportion of smokers was higher among heavy coffee consumers. The highest proportion of participants without hypertension or anti-hypertensive medication was found in participants with the highest consumption of coffee, and our results showed non-significant results for this group. Notwithstanding, most risk factors for AF were homogeneously distributed across categories of coffee consumption. In addition, when we removed the cases occurring during the first year in our sensitivity analyses, the results remained similar. These

findings make unlikely the existence of reverse causality. However, we cannot exclude a possible residual confounding despite the multivariable adjustment. Five, we have combined the results of two very different populations according to the lack of statistical heterogeneity identified in our cohorts. Although the meta-analysis that we used to combine both cohorts improved the statistical power within our study, the combined risk estimate cannot be interpreted as sufficiently generalizable as to represent the association between coffee and AF risk in any Mediterranean population independent of age and other cardiovascular risk factors. Further studies would be needed to suggest this generalizability.

In conclusion, in two large Mediterranean cohorts, light consumption of caffeinated coffee (1-7 cups/week) was associated with a reduction in AF incidence. Further studies are necessary to replicate this result and to better clarify the effect of decaffeinated coffee and other sources of caffeine.

7. FUNDING

The author(s) received no financial support for the research, authorship, and/or publication of this article and had full access to all of the data in this study.

The SUN Project was supported by the Spanish Government-Instituto de Salud Carlos III, and the European Regional Development Fund (RD 06/0045, PI14/ 01798, PI14/01764, PI17/01795), the Navarra Regional Government, and the University of Navarra. An Advanced Research Grant from the European Research Council to Miguel A Martínez-González (agreement #340918-PREDIMED-Plus) is duly acknowledged.

PREDIMED trial was supported by the official funding agency for biomedical research of the Spanish government (Instituto de Salud Carlos III) RTIC G03/140 (Coordinator: Dr Estruch) and RTIC RD 06/0045 (Coordinator: Dr Martínez-González). We also acknowledge grants from the National Institutes of Health, United States (1R01HL118264-01); 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).

8.REFERENCES:

1. O'Keefe JH, Bhatti SK, Patil HR, et al. Effects of habitual coffee consumption on cardiometabolic disease, cardiovascular health, and all-cause mortality. *J Am Coll Cardiol* 2013; 62: 1043–1051.
2. Torres-Collado L, Garcia-de-la-Hera M, Navarrete-Muñoz EM, et al. Coffee consumption and mortality from all causes of death, cardiovascular disease and cancer in an elderly Spanish population. *Eur J Nutr*. 2019; 58 (6): 2439-2448. DOI: 10.1007/s00394-018-1796-9.
3. Gunter MJ, Murphy N, Cross AJ, et al. Coffee Drinking and Mortality in 10 European Countries: A Multinational Cohort Study. *Ann Intern Med* 2017; 167: 236–247.
4. Mukamal KJ, Hallqvist J, Hammar N, et al. Coffee consumption and mortality after acute myocardial infarction: the Stockholm Heart Epidemiology Program. *Am Heart J* 2009; 157: 495–501.

5. Voskoboinik A, Koh Y, Kistler PM. Cardiovascular effects of caffeinated beverages. *Trends Cardiovasc Med*. 2019; 29(6): 345-350. DOI: 10.1016/j.tcm.2018.09.019.
6. Hughes JR, Amori G, Hatsukami DK. A survey of physician advice about caffeine. *J Subst Abuse* 1988; 1: 67–70.
7. Mehta A, Jain AC, Mehta MC, et al. Caffeine and cardiac arrhythmias. An experimental study in dogs with review of literature. *Acta Cardiol* 1997; 52: 273–83.
8. Zhang Y, Tuft RA, Lifshitz LM, et al. Caffeine-activated large-conductance plasma membrane cation channels in cardiac myocytes: characteristics and significance. *Am J Physiol Circ Physiol* 2007; 293: H2448–H2461.
9. Groh CA, Faulkner M, Getabecha S, et al. Patient-reported triggers of paroxysmal atrial fibrillation. *Hear Rhythm* 2019; 16: 996–1002.
10. Larsson SC, Drca N, Jensen-Urstad M, et al. Coffee consumption is not associated with increased risk of atrial fibrillation: results from two prospective cohorts and a meta-analysis. *BMC Med* 2015; 13: 207.
11. Dixit S, Stein PK, Dewland TA, et al. Consumption of Caffeinated Products and Cardiac Ectopy. *J Am Heart Assoc*; 2016; 5. DOI: 10.1161/JAHA.115.002503.
12. Caldeira D, Martins C, Alves LB, et al. Caffeine does not increase the risk of atrial fibrillation: A systematic review and meta-analysis of observational studies. *Heart* 2013; 99: 1383–1389.
13. Gronroos NN, Alonso A. Diet and risk of atrial fibrillation – epidemiologic and clinical evidence –. *Circ J* 2010; 74: 2029–38.

14. Conen D, Chiuve SE, Everett BM, et al. Caffeine consumption and incident atrial fibrillation in women. *Am J Clin Nutr* 2010; 92: 509–514.
15. Mostofsky E, Johansen MB, Lundbye-Christensen S, et al. Risk of atrial fibrillation associated with coffee intake: Findings from the Danish Diet, Cancer, and Health study. *Eur J Prev Cardiol* 2016; 23: 922–30.
16. Bodar V, Chen J, Gaziano M; JM, et al. Coffee Consumption and Risk of Atrial Fibrillation in the Physicians' Health Study. 2019; 8 (15): e011246. DOI: 10.1161/JAHA.118.011346.
17. Abdelfattah R, Kamran H, Lazar J, et al. Does Caffeine Consumption Increase the Risk of New-Onset Atrial Fibrillation? *Cardiology* 2018; 140: 106–114.
18. Voskoboinik A, Kalman JM, Kistler PM. Caffeine and Arrhythmias: Time to Grind the Data. *JACC Clin Electrophysiol* 2018; 4: 425–432.
19. Mattioli AV. Link between coffee and atrial fibrillation debunked? *Expert Rev Cardiovasc Ther* 2019; 17: 75–77.
20. Martínez-González MÁ, Toledo E, Arós F, et al. Extravirgin olive oil consumption reduces risk of atrial fibrillation: the PREDIMED (Prevención con Dieta Mediterránea) trial. *Circulation* 2014; 130: 18–26.
21. Seguí-Gómez M, Fuente C de la, Vázquez Z, et al. Cohort profile: The 'Seguimiento Universidad de Navarra' (SUN) study. *Int J Epidemiol* 2006; 35: 1417–1422.
22. Carlos S, De La Fuente-Arrillaga C, Bes-Rastrollo M, et al. Mediterranean Diet and Health Outcomes in the SUN Cohort. *Nutrients*; 2018; 10(4). DOI: 10.3390/nu10040439.
23. Martínez-González MÁ, Corella D, Salas-Salvadó J, et al. Cohort profile:

561 design and methods of the PREDIMED study. *Int J Epidemiol* 2012; 41:
562 377–85.
563 24. Fernández-Ballart JD, Piñol JL, Zazpe I, et al. Relative validity of a semi-
564 quantitative food-frequency questionnaire in an elderly Mediterranean
565 population of Spain. *Br J Nutr* 2010; 103: 1808–16.
566 25. de la Fuente-Arrillaga C, Vázquez Ruiz Z, Bes-Rastrollo M, et al.
567 Reproducibility of an FFQ validated in Spain. *Public Health Nutr* 2010;
568 13: 1364–1372.
569 26. Bazal P, Gea A, de la Fuente-Arrillaga C, et al. Olive oil intake and risk
570 of atrial fibrillation in the SUN cohort. *Nutr Metab Cardiovasc Dis* 2019;
571 29: 450–457.
572 27. Martínez-González MÁ, Toledo E, Arós F, et al. Extravirgin Olive Oil
573 Consumption Reduces Risk of Atrial FibrillationCLINICAL
574 PERSPECTIVE. *Circulation*; 2014; 130: 18-26.
575 28. Elosua R, Marrugat J, Molina L, et al. Validation of the Minnesota
576 Leisure Time Physical Activity Questionnaire in Spanish men. The
577 MARATHOM Investigators. *Am J Epidemiol* 1994; 139: 1197–209.
578 29. Alonso A, Beunza JJ, Delgado-Rodríguez M, et al. Validation of self
579 reported diagnosis of hypertension in a cohort of university graduates in
580 Spain. *BMC Public Health* 2005; 5: 94.
581 30. Willett W. *Nutritional epidemiology*. Oxford University Press,
582 31. Frost L, Vestergaard P. Caffeine and risk of atrial fibrillation or flutter: the
583 Danish Diet, Cancer, and Health Study. *Am J Clin Nutr* 2005; 81: 578–
584 582.
585 32. Dixit S, Stein PK, Dewland TA, et al. Consumption of Caffeinated

586 Products and Cardiac Ectopy. *J Am Heart Assoc* 2016; 5: e002503.
587 33. Gorenek Chair B, Pelliccia Co-Chair A, Benjamin EJ, et al. European
588 Heart Rhythm Association (EHRA)/European Association of
589 Cardiovascular Prevention and Rehabilitation (EACPR) position paper
590 on how to prevent atrial fibrillation endorsed by the Heart Rhythm
591 Society (HRS) and Asia Pacific Heart Rhythm Society (AP. *Eur J Prev*
592 *Cardiol* 2017; 24: 4–40.
593 34. Casiglia E, Tikhonoff V, Albertini F, et al. Caffeine intake reduces
594 incident atrial fibrillation at a population level. *Eur J Prev Cardiol* 2018;
595 25: 1055–1062.
596 35. Cheng M, Hu Z, Lu X, et al. Caffeine intake and atrial fibrillation
597 incidence: dose response meta-analysis of prospective cohort studies.
598 *Can J Cardiol* 2014; 30: 448–54.
599 36. Navarro AM, Martinez-Gonzalez MA, Gea A, et al. Coffee consumption
600 and risk of hypertension in the SUN Project. *Clin Nutr*. 2019; 38 (1):389-
601 397. DOI: 10.1016/j.clnu.2017.12.009.
602 37. Hoe R, Lin W, Ann M, et al. Validation of a questionnaire measuring
603 patient knowledge of atrial fibrillation in an Asian cohort. *Heart Asia*
604 2019; 11: 11143.
605 38. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the
606 management of atrial fibrillation developed in collaboration with EACTS.
607 *Eur Heart J* 2016; 37: 2893–2962.
608 39. Greenland S, Lash TL. Bias analysis. In: Rothman KJ, Greenland S, Lash
609 TL, eds. Modern Epidemiology. 3rd ed. Philadelphia, PA: Lippincott
610 Williams and Wilkins; 2008:359

9.TABLES

Table 1. Baseline characteristics according to caffeinated coffee intake in SUN study

	Overall	<3 cups/month	1-7 cups/week	>1 cup/day	p-value
n	18983	5091	7529	6363	
Age	37.3 (11.9)	36 (13)	37 (12)	38 (11)	<0.01
Sex					0.2
Men (%)	38.8	37.9	39.5	38.7	
Women (%)	61.2	62	60.5	61.3	
Caffeine (mg/day)	44.9 (40.9)	9.6 (19.1)	30.8 (16.1)	89.8 (33.7)	<0.01
Decaffeinated coffee (%)					<0.01
<3 cups/month	72.4	63.8	69	83.4	
1-7 cups/week	23.9	29.4	28.1	14.5	
>1 cup/day	3.7	6.7	2.9	2.1	
Soda drinks (%)					<0.01
<3 cups/month	57.9	58.5	56.9	58.4	
1-7 cups/week	40.7	39.5	42.1	40.1	
>1 cup/day	1.4	2	1	1.5	
Smoking (%)					<0.01
Current smoker	27.4	19.1	25.7	36.3	
Former smoker	24.5	20.4	25	27.1	
Diabetes (%)	1.8	1.9	1.8	1.6	0.39
Dyslipemia (%)	19.9	18.2	20.2	20.9	<0.01
Hypertension (%)	10.3	12.6	10.6	8.1	<0.01
CV disease (%)	1.3	1.6	1.3	1.1	0.04
Heart failure (%)	0.35	0.51	0.35	0.22	0.03
Height (cm)	168 (8.6)	168 (9)	169 (9)	168 (9)	0.36
BMI	23.5 (3.5)	23.3 (3.5)	23.4 (3.5)	23.7 (3.6)	< 0.01
BMI >30 (%)	4.6	4.20	4.62	4.81	<0.01
Physical activity (METS-min/day)	190.5 (198.5)	204(219)	189 (196)	179 (182)	<0.01
Sleep apnea (%)	1.7	1.7	1.8	1.7	0.82
Total alcohol intake (g/d)	6.8 (10.5)	5 (9.1)	6.9 (10.1)	8 (11.6)	<0.01
Alcohol patterns(%)					<0.01
Non drinkers	20.5	30.9	17.8	15.3	
Moderate drinkers	66.6	60.6	69.5	68	
Heavy drinkers	12.9	8.6	12.6	16.7	

Data are mean (SD) unless otherwise stated. SBP: systolic blood pressure. DBP: diastolic blood pressure. *P value from comparisons was calculated with Pearson's chi-square test and ANOVA, as appropriate. CV disease: prevalent cardiovascular disease, compound of stroke and artery heart disease

Table 2. Baseline characteristics according to caffeinated coffee intake in the PREDIMED study

	Overall	≤ 3 cups/month	1-7 cups/week	>1 cup/day	p-value
n	6479	3,978	1,643	858	
Age	67.0 (6.1)	67.6 (6.2)	66.5 (5.9)	65.1 (6.1)	<0.01
Sex					<0.01
Men (%)	42.3	37.3	47.5	55.6	
Women (%)	57.7	62.7	52.5	44.4	
Caffeine (mg/day)	21.8 (29.3)	5.7 (12.8)	30.1 (14.4)	81.1 (22.3)	<0.01
Soda drinks (%)					0.64
<3 cups/month	84.4	84.8	83.9	83.7	
1-7 cups/week	14.7	14.2	15.3	15.5	
>1 cup/day	0.9	1	0.7	0.8	
Decaffeinated coffee (%)					<0.01
<3 cups/month	47.3	25.7	74.9	94.6	
1-7 cups/week	36.2	48.7	22.5	4.1	
>1 cup/day	16.5	25.6	2.6	1.3	
Intervention Group (%)					0.21
MedDiet+NUTS	33	32.2	35	33	
MedDiet+EVOO	34.6	34.5	34.1	35.8	
Smoking (%)					<0.01
Current smoker	13.8	10.1	16.4	25.9	
Former smoker	24.4	22.6	26.8	28.6	
Diabetes (%)	48.1	48.8	46.2	48.7	0.19
Dyslipemia (%)	72.0	71.1	74.6	71.6	0.03
Hypertension SBP mmHg	83.3	85.9	82.6	72.14	<0.01
DBP mmHg	148 (18.7)	148.7 (19.1)	148 (18.1)	145.3 (17.5)	<0.01
Anti-hypertensive medication (%)	92.7 (13.8)	92.8 (14.0)	93.3 (13.6)	91.2 (14.0)	<0.01
Height (cm)	72.4	75.8	70.3	61.0	<0.01
BMI	159.9(8.8)	159.3 (8.8)	160.6 (8.9)	162.1 (8.7)	<0.01
BMI >30 (%)	30.0 (3.8)	30.1 (3.9)	29.9 (3.7)	30 (3.9)	0.15
Physical activity METS-min/day	47.6	47.8	46.6	48.5	0.84
Cardiopathy (%)	226.0 (8.8)	217 (224)	236 (240)	251 (283)	<0.01
Sleep apnea (%)	3.2	2.9	3.2	4.2	0.14
Total alcohol intake (g/d)	1.9	1.7	1.5	3.3	<0.01
	8.23 (13.9)	6.6 (12.1)	10.7 (16.3)	11.1 (15.8)	<0.01

Alcohol patterns(%)					<0.01
Non drinkers	37.4	42.7	29.9	27.5	
Moderate drinkers	42.1	40.4	44.3	45.9	
Heavy drinkers	20.5	17.0	25.8	26.6	

623 Data are mean (SD) unless otherwise stated. SBP: systolic blood pressure. DBP: diastolic blood pressure. *P
624 value from comparisons was calculated with Pearson's chi-square test and ANOVA, as appropriate.
625 Cardiopathy: heart failure and non-atherosclerotic coronary disease.

Table 3. Risk of atrial fibrillation (HR, 95% CI) according to caffeinated coffee intake

	SUN			PREDIMED		
	≤ 3 cups/month (5,091)	**1-7 cups /week (7,529)**	**>1 cup/day (6,363)**	**≤ 3 cups/month (3,978)**	**1-7 cups /week (1,643)**	**>1 cup/day (858)**
Number cases	30	33	34	173	45	32
Person-years	49,566	75,419	66,247	17,602	7,237	3,727
Crude	1 (ref.)	0.71 (0.43- 1.17)	0.89 (0.54-1.47)	1 (ref.)	0.59 (0.42-0.83)	0.83 (0.55-1.22)
Age, sex adjusted	1 (ref.)	0.70 (0.45-1.15)	0.86 (0.52-1.41)	1 (ref.)	0.59 (0.42-0.83)	0.87 (0.58-1.31)
Multivariable adjusted (*)	1 (ref.)	0.74 (0.44-1.25)	1.01 (0.59-1.73)	1 (ref.)	0.53 (0.36-0.79)	0.79 (0.49-1.28)

(*) Multivariable model:
- PREDIMED study: adjusted for age, sex, smoking habit, body mass index, height, physical activity, sleep apnea, diabetes, diastolic and systolic blood pressure, hypertension, alcohol intake, heart failure, other caffeinated beverage, decaffeinated coffee, intervention group and depression.
- SUN study: adjusted for age, sex, smoking habit, body mass index, height, physical activity, sleep apnea, diabetes, hypertension, alcohol intake, ischemic cardiopathy, prevalent heart failure, other caffeinated beverages and decaffeinated coffee.

Figure 1 Flow chart of participants in the SUN project and in the PREDIMED trial

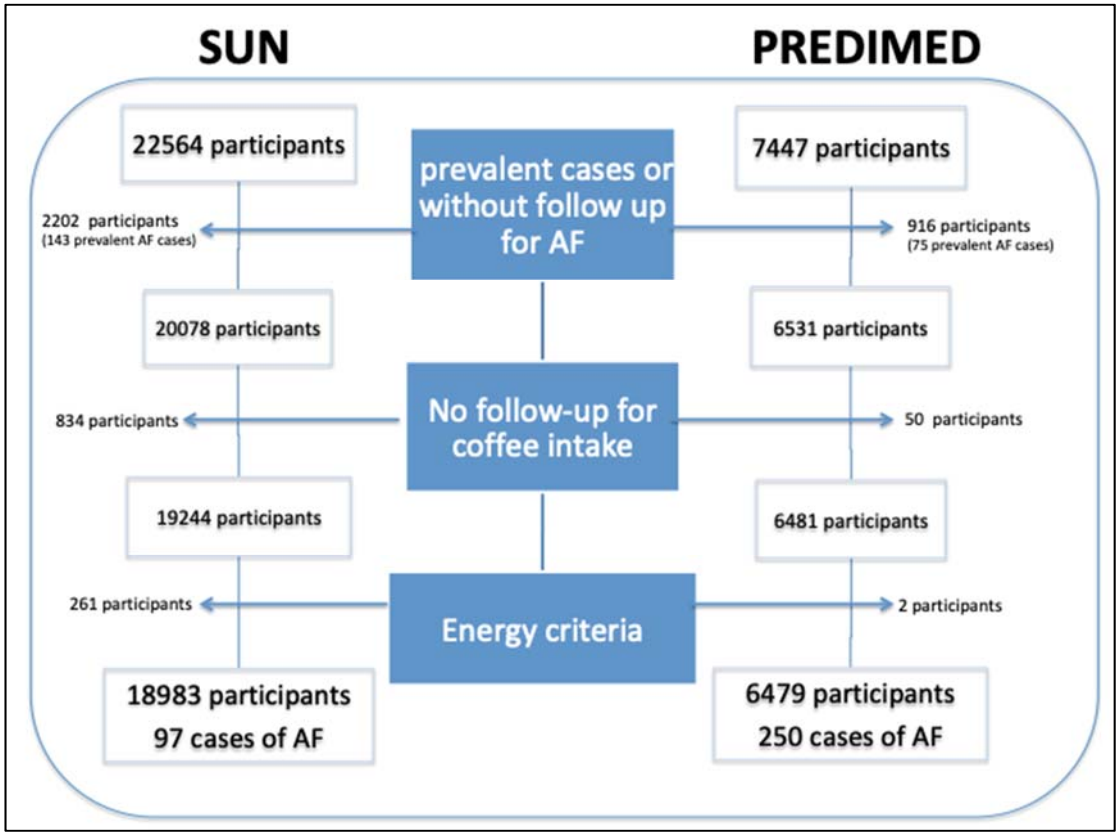
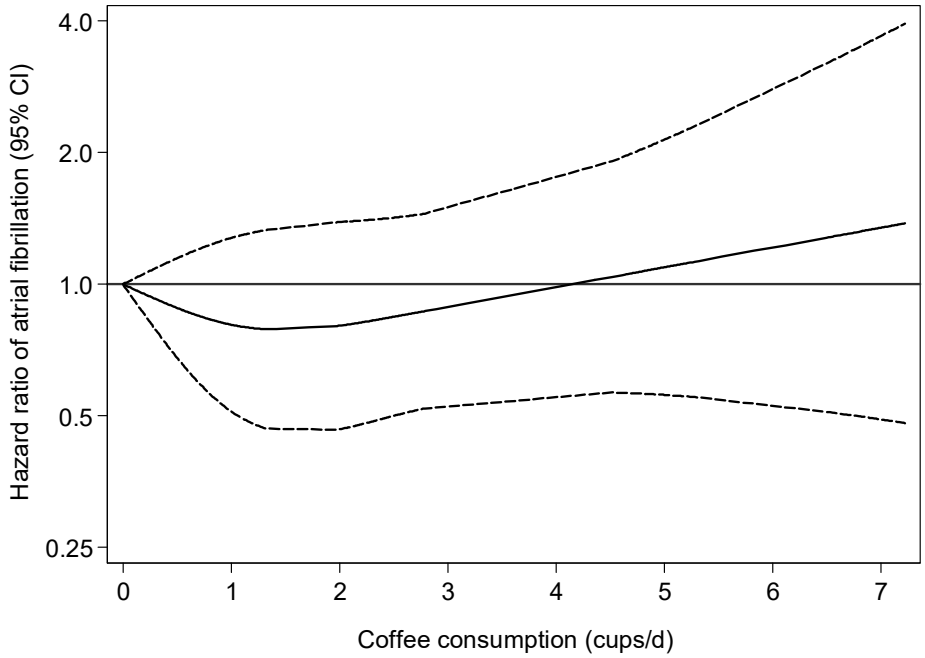
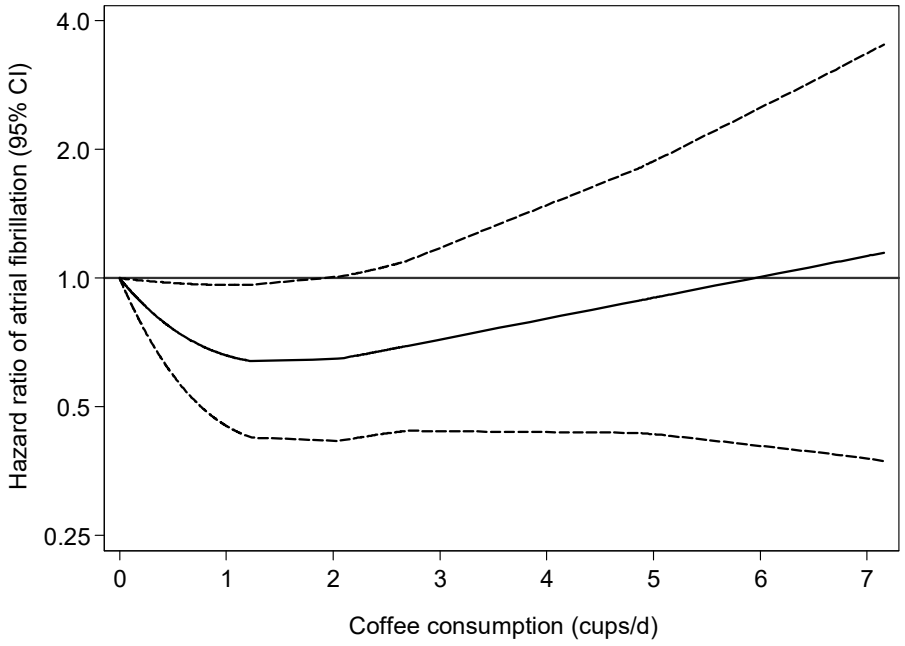


Figure 2. Risk of atrial fibrillation according to caffeinated coffee consumption in a) the SUN Project and b) the PREDIMED trial

a) SUN Project

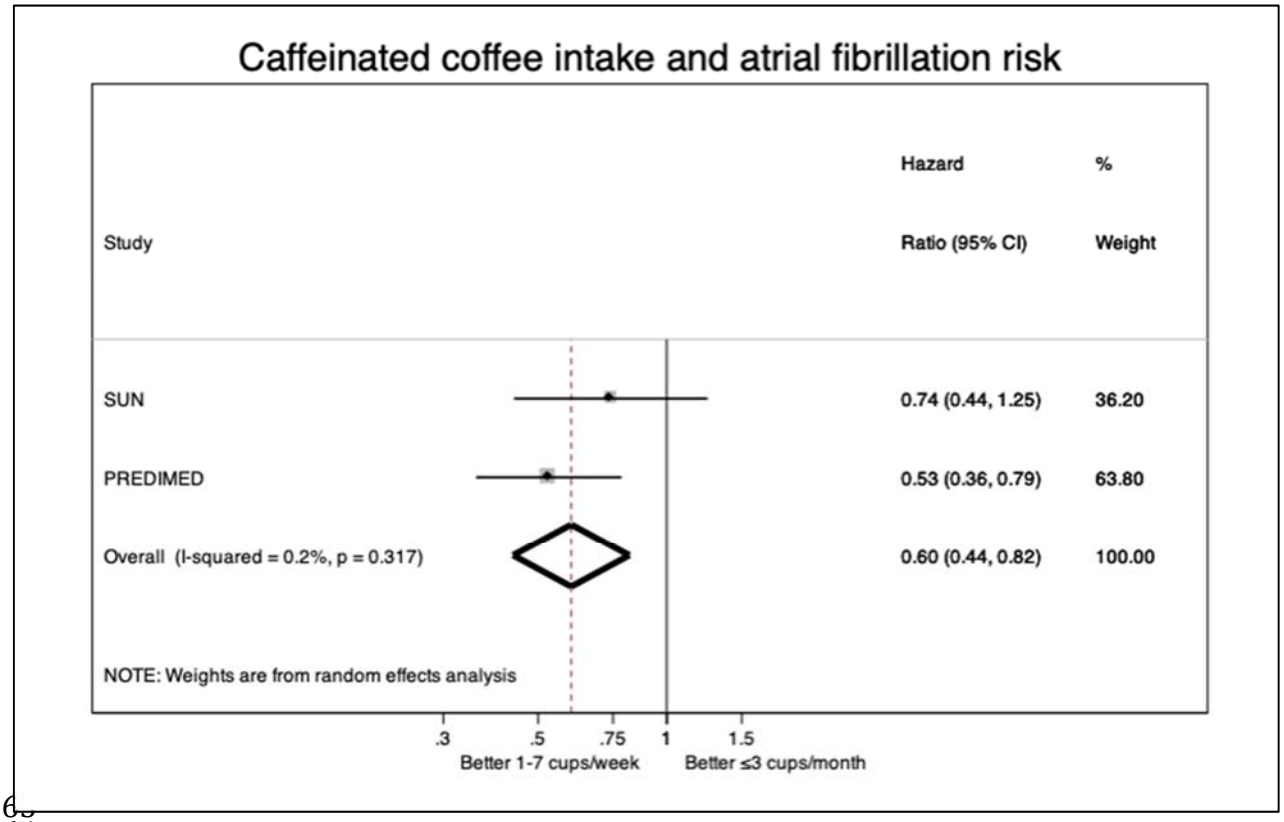


b) PREDIMED trial



(*) Multivariable model adjusted:
- PREDIMED study: adjusted for age, sex, smoking habit, body mass index, height, physical activity, sleep apnea, diabetes, diastolic and systolic blood pressure, hypertension, alcohol intake, heart failure, other caffeinated beverage, decaffeinated coffee, intervention group and depression.
- SUN study: adjusted for age, sex, smoking habit, body mass index, height, physical activity, sleep apnea, diabetes, hypertension, alcohol intake, ischemic cardiopathy, prevalent heart failure, other caffeinated beverages and decaffeinated coffee.

Figure 3. Intermediate levels of caffeinated coffee consumption (1-7 cups/week) and risk of atrial fibrillation



(*) Multivariable model adjusted:
- PREDIMED study: adjusted for age, sex, smoking habit, body mass index, height, physical activity, sleep apnea, diabetes, diastolic and systolic blood pressure, hypertension, alcohol intake, heart failure, other caffeinated beverage, decaffeinated coffee, intervention group and depression.
- SUN study: adjusted for age, sex, smoking habit, body mass index, height, physical activity, sleep apnea, diabetes, hypertension, alcohol intake, ischemic cardiopathy, prevalent heart failure, other caffeinated beverages and decaffeinated coffee.

672