Association between alcohol consumption and subclinical femoral atherosclerosis in smoking and non-smoking men: the AWHS study

Addiction. 2020 115(9):1754-1761

Moreno-Franco B, et al.

Moreno-Franco Belén (Orcid ID: 0000-0003-0604-5042)

Association between alcohol consumption and subclinical femoral atherosclerosis in smoking and non-smoking men: the AWHS Study.

Moreno-Franco B[a,b], Perez-Esteban A[a], Civeira F[a,b], Guallar-Castillón P[c,d], Casasnovas JA[a,b], Mateo-Gállego R[a,b], Jarauta E[a,b], Malo S[a], Laclaustra M[b,e].

[a] Universidad de Zaragoza, Zaragoza, Spain

[b] Instituto de Investigación Sanitaria Aragón, Hospital Universitario Miguel Servet, CIBERCV, Zaragoza, Spain

[c] Department of Preventive Medicine and Public Health. School of Medicine. Universidad Autónoma de Madrid-IdiPaz, CIBERESP, Madrid, Spain

[d] IMDEA-Food Institute. CEI UAM+CSIC, Madrid, Spain

[e] Agencia Aragonesa para la Investigación y el Desarrollo, ARAID, Zaragoza, Spain

Running head: Alcohol, tobacco and atherosclerosis

Word count, text: 3220

Declaration of interests: None

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/add.15012

*** Corresponding author:**
Dr. Martín Laclaustra
Instituto de Investigación Sanitaria Aragón
Hospital Universitario Miguel Servet
Paseo Isabel La Católica 1-3
50009, Zaragoza, Spain
martin.laclaustra@unizar.es

ABSTRACT

Background and Aims: Many addictive substances, such as tobacco and alcohol, influence atherosclerosis development. Whether tobacco's pro-atherosclerotic effect is influenced by alcohol consumption is unknown. We aimed to estimate the impact of alcohol intake on the presence of subclinical atherosclerosis in femoral arteries in smoking and non-smoking middle-aged men. **Design, Setting, Participants:** Cross-sectional analysis of a subset of the Aragon Workers Health Study (AWHS), comprising 2099 men with mean age 50.9 years without previous cardiovascular disease. **Measurements:** The presence of plaques in femoral arteries was assessed by high-resolution sonography. Self-reported alcohol consumption over the previous year was measured with a food frequency questionnaire. The sample was divided into four groups according to their daily grams of alcohol consumption ≤1 (abstainers), ≥2 to <30, ≥30 to <60, and ≥60 g/day. Participants were divided on ever-smoking (current and former), versus never-smoking strata in the main analysis. **Findings:** We did not find a significant association between the different levels of alcohol intake and the likelihood of developing femoral artery atherosclerosis in never-smokers. Ever-smoking was positively associated with femoral atherosclerosis overall (OR=3.00; 95%CI:2.40,3.74; p<0.001) and within each level of alcohol consumption. Atherosclerosis was lower in ever-smokers who consumed 2g/day or more but less than 30g/day with respect to those ever-smokers who were abstainers (OR=0.70; 95%CI:0.49,0.99; p<0.05). However, among them, atherosclerosis prevalence was still higher than among never-smokers who consumed alcohol in the same amount (2g/day or more but less than 30g/day) (OR=2.73; 95%CI:2.07,3.61; p<0.001). **Conclusions**: Among middle-aged men, moderate alcohol consumption appears to

be associated with lower prevalence of femoral artery subclinical atherosclerosis compared with alcohol abstinence only in ever-smokers.

Keywords: Alcohol; Smoking; Atherosclerosis; Cardiovascular disease.

INTRODUCTION

Atherosclerosis is the pathological process that causes most cardiovascular disease (CVD). Consumption of some addictive substances, such as smoking tobacco and drinking beverages with alcohol, influences its development, even below addiction thresholds.

Among CVD risk factors, smoking increases atherosclerosis progression and the risk of atherosclerosis-related events by inducing oxidative stress, endothelial dysfunction, vascular inflammation, platelet aggregation, and worsening of serum lipid profile [1, 2]. Smoking more strongly affects lower limb arteries and it is the most important cause of peripheral artery disease [3]. Smoking cessation slowly decreases the acquired risk for CVD but the risk does not decrease close to that of never-smokers until ten years cessation [4].

Alcoholic beverages consumption also influences cardiovascular health, but the mechanisms, magnitude, and dose-dependency of the effects are still controversial [5, 6]. The Global Burden of Disease Study concluded that the level of alcohol consumption that minimizes health impact is zero [7]. However, the CALIBER study showed that moderate alcohol intake was associated with a lower incidence of cardiovascular events [8] . A recent review published by Wood et al. [9] concluded that the upper threshold of alcohol consumption for a lower risk of CVD defined as an aggregate of myocardial infarction, other coronary heart disease, and stroke was 100g/week. This potential atherosclerosis protection from alcoholic beverages consumption is attributed to antioxidant effects which may be due, at least in part, to their actual alcohol content but also to non-alcoholic compounds, mainly polyphenols [10, 11]. Thus, the protective effect of alcoholic beverages, if present, would be greater in tobacco users, who suffer from increased oxidative stress.

Subclinical atherosclerosis can be measured in several body locations including carotid, femoral, and coronary arteries. This allows to find evidence of vascular disease much earlier than when symptoms appear. Several studies have recently demonstrated that the presence of

femoral plaques is a strong marker of coronary lesions [12]. The Aragon Workers' Health Study (AWHS) [12] and the Progression of Early Subclinical Atherosclerosis (PESA) [13] study, have shown that atherosclerotic femoral plaques are the most prevalent subclinical atherosclerotic alteration available non-invasively in middle-aged population and they are the atherosclerotic subclinical alteration with the strongest association with traditional CVD risk factors, especially with tobacco consumption [12], higher than carotid plaques. Alcohol consumption has been studied as a protective factor for subclinical atherosclerosis, mostly in cerebral and coronary territories [8], but it has not been yet studied in subclinical femoral atherosclerosis. Thus, data about the effects of alcohol on early atherosclerosis are scarce.

Given the controversial results that were previously published, we aim for two things: first, to address early associations, which can support the causal nature of the influence of the exposures; second, to assess the possibility that alcohol partially counteracts strong pro-oxidant pro-atherosclerotic exposures such as tobacco. To that end we selected the femoral arteries, as the territory most heavily affected by smoking. Thus, we aim to describe, for the first time, the possible influence of alcohol – and its dose – on subclinical atherosclerosis in femoral arteries among ever-smoking and never-smoking individuals. We studied this association on a sample of male workers from a factory in Spain. To broaden and contrast our results, we also analyzed these associations in carotid arteries.

METHODS

Participants

This cross-sectional study is based on AWHS, which is a prospective cohort, aimed to investigate the determinants of the development and progression of subclinical atherosclerosis. Although the association of dietary factors, including alcohol, and smoking with atherosclerosis was considered as an aim of AWHS in the protocol since the beginning, the details of the particular analysis presented in this report were not publicly pre-registered and consequently the results may be regarded as exploratory. Healthy middle-aged workers of the Opel Spain automobile assembly factory [14], attending their annual health exam between February 2009 and December 2012 were invited to participate in the study. From 2011 to 2014 participants between 39 and 59 years of age were additionally invited to undergo subclinical atherosclerosis measurements, and to complete diet, behavior, and lifestyle

questionnaires. Of the 2617 participants who attended these extended examinations, we excluded women due to the small number (n=132), and those with missing data on subclinical atherosclerosis imaging exams (n=317), with missing data on CVD risk factors or relevant variables (BMI, blood pressure) (n=69). The final sample comprised 2099 men. All participants gave their written informed consent. The study was approved by the Clinical Research Ethics Committee of Aragon (CEICA).

Measures

Alcohol consumption

Usual diet over the preceding year was assessed using a 136-item semi-quantitative food frequency questionnaire (FFQ). This was previously validated in Spain [15], and included a validated alcohol consumption assessment. In this questionnaire, each participant provided information on the foods consumed over the year prior to the day of the interview. The variable "daily grams of alcohol" was calculated from the sum of the intake of red, rosé, and white wine, beer and distillates. As this is an FFQ-derived variable, it is unevenly distributed. Besides, the frequency of values 0 and 1 was high enough to compromise analysis of this variable as continuous. Thus, the sample was divided into four groups according to their consumption in: ≤1 (abstainers), ≥2 to <30, ≥30 to <60 and ≥60 g/day. In Spain, ≥30 g/day corresponds to 3 or more glasses of wine (100 ml), more than 3 beers (250 ml) or 2 spirit shots (50 ml) per day, and it was preferentially used here as a threshold versus 40 g/day (WHO risk of acute drinking problems classification) [16] because the number of participants above 40 g/day was scarce in our sample.

Atherosclerosis imaging

The presence of plaques in 2 vascular territories (carotid and femoral arteries) was assessed with a Philips IU22 ultrasound system (Philips Healthcare, Bothell, WA). 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 [17] and a specifically designed protocol for the femoral arteries [18]. Inspection sweeps were obtained in right and left sides for the carotid (common, internal, and bulb) and femoral territories. We recorded plaque presence, number and thickness. Plaque was defined as a focal structure

protruding ≥0.5 mm into the lumen or reaching a thickness ≥50% of the surrounding intima. All measurements were analyzed using electrocardiogram gated frames corresponding to end-diastole (R-wave) [19].

Demographic, clinical, and laboratory variables

Age, sex, clinical, and laboratory data were obtained during the annual medical examination, using standardized procedures, certified with ISO 9001-2008. The physical exam included height, weight, and waist circumference, and blood pressure. Total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides, and fasting serum glucose concentrations were determined by enzyme analysis using the ILAB 650 analyzer from Instrumentation Laboratory. Low density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula [20], when the triglyceride levels were <400 mg/dl. In all participants, non-HDL-c was calculated by subtracting the HDL-c value from total cholesterol. We defined arterial hypertension as having systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or self-reported use of antihypertensive medication [21]. Dyslipidemia was defined as having total cholesterol ≥240 mg/dl, LDL-c ≥160 mg/dl, HDL-c <40mg/dl, or self-reported use of lipid-lowering drugs [22]. Diabetes was defined as fasting plasma ≥126 mg/dl or self-reported treatment with hypoglycemic medication [21]. Smoking habits were categorized as current smoking if the participant reported having smoked in the last year, former smoking if the participant had smoked at least 50 cigarettes in his lifetime but not in the last year, and never smoking. Ever smoking (current and former), versus never smoking was studied in the main analysis. Smoking was quantified with self-reported number of cigarettes per day.

Analysis

Descriptive statistics were reported as mean, standard deviation, and percentage. Presence of atherosclerotic plaques in femoral and carotid arteries was fitted separately with logistic regression models which included all groups of variables combining alcohol and smoking exposures (created through terms for category interactions between both variables), and adjusted for age, hypertension, dyslipidemia, and diabetes. Linear combinations of coefficients were used to calculate odds ratios (OR) for plaque presence of each exposure with respect to the ever-smoker/alcohol abstainer group and to test for relevant differences. In

particular, ORs for the presence of plaque for each group of alcohol consumption with respect to the abstainers group were tested within each smoking stratum, and ORs for the presence of plaque for being ever-smoker with respect to never-smokers were tested within each alcohol consumption stratum. An overall interaction between both variables was also tested with the linear combination of all interaction terms in the regression. P values below 0.05 were considered statistically significant. To provide a detailed description of the influence of both variables on subclinical atherosclerosis, an analysis with smoking dose categories was performed. R statistical software (ver. 3.4.4) was used for the analysis.

RESULTS

The sample included 2099 men with a mean age of 50.9 (SD 3.9) years. The number of participants reporting alcohol intake ≤1 (abstainers), ≥2 to <30, ≥30 to <60, and ≥60 g/day were 246 (11.7%), 1239 (59.0%), 501 (23.9%), and 113 (5.4%) respectively. Higher alcohol consumers were older. Systolic and diastolic blood pressure, total cholesterol, non HDL-c, triglycerides, and HDL-c rose with higher alcohol consumption. Also, higher alcohol consumption was associated with belonging to the ever-smoking category (see Table 1), with unadjusted OR for each increasing category 1.38 (95% CI: 1.19, 1.60; p for trend<0.001).

At least one femoral plaque was present in 166 of the 474 never-smokers (35.0%), and in 1024 of the 1625 ever-smokers (63.0%) (fully adjusted OR=3.00; 95% CI: 2.40, 3.74; p<0.001)). We found no association between different levels of alcohol intake and the likelihood of developing subclinical atherosclerosis in femoral arteries in never-smoking participants (see Table 2 and Figure 1). The odds for having a femoral plaque was 3.70 (95% CI: 2.00, 6.98) times higher among the ever-smoker/alcohol abstainer group than among the never-smoker/alcohol abstainer group. The OR was 2.59 (95% CI: 1.49, 4.48) in those ever-smokers who consumed 2 or more g/day but less than 30g of alcohol/day compared to the ever-smoker/alcohol abstainer group. Testing the difference between these ORs, the latter represents a significantly lower atherosclerosis prevalence (fully adjusted OR=0.70; 95% CI: 0.49, 0.99; p=0.044) than the ever-smoker/alcohol abstainer group although still higher than never-smokers who consumed alcohol in the same amount (fully adjusted OR=2.73; 95% CI: 2.07, 3.61; p<0.001). At any alcohol consumption level, odds for femoral plaques were always higher among ever-smokers than among never-smokers (fully adjusted ORs ranging between 2.73 and 6.32; p≤0.001) (see Table 2 and Figure 1). Although this study was not

sized to provide adequate statistical power for it, a test for an overall interaction term between all drinking and smoking categories was performed and significance was not reached (p=0.165).

A detailed observation of the combined influence of alcohol and tobacco on femoral atherosclerosis considering smoking dose suggests that the U-shaped association tends to appear at higher smoking consumption levels (see Figure 2 and Supplemental Table 1).

At least one carotid plaque was present in 123 of the 474 never-smokers (25.9%), and in 647 of the 1625 ever-smokers (39.8%) (OR=1.89). We found no association between different levels of alcohol intake and the likelihood of developing subclinical atherosclerosis in carotid arteries in any of the two groups (see Supplemental Table 2). We did not seek a detailed smoking dose analysis given the weaker association of carotid atherosclerosis with smoking and the absence of association with alcohol.

DISCUSSION

In this study we could not demonstrate a protective influence of alcohol on femoral subclinical atherosclerosis among never-smokers. However, among ever-smokers, which are participants with increased prevalence of femoral subclinical atherosclerosis it appears that a pattern of moderate alcohol consumption, between 2-30g/day, is associated with a lower prevalence of subclinical atherosclerosis in femoral arteries, independently of other risk factors. The prevalence was still greater than that of never-smokers with the same level of alcohol consumption. No previous studies have analyzed this association on early atherosclerosis that can explain, at least in part, the overall beneficial effect on latter CVD observed in some observational studies.

Information on the association of alcohol consumption and femoral atherosclerosis is scarce. Most studies analyzing the role of alcohol consumption have mainly focused on the presence of atherosclerosis in coronary and cerebral territories, or on coronary and cerebrovascular clinical outcomes, and very few have focused on carotid territory [23, 24]. However, in the CALIBER study [8], among 1.93 million adults, alcohol consumption of 140g/week was associated with lower risk of myocardial infarction, ischemic stroke, and peripheral arterial disease. The ARIC study found a protective effect of alcohol on incident peripheral arterial

and coronary heart disease [25, 26] but none on brain disease [27]. Research of alcohol effect on subclinical femoral atherosclerosis was lacking. With respect to dose-effects, a recent analysis [9] of 599,912 current drinkers with an average age of 57 years belonging to 83 prospective studies determined a positive and progressively increasing association with alcohol consumption for all-cause mortality, with the lowest risk for those consuming below 100g/week. However, for the aggregate of CVD outcomes (myocardial infarction, other coronary heart disease, and stroke), there was a J-shaped association. In another systematic review and meta-analysis of longitudinal cohort studies comparing non-drinkers with alcohol drinkers, light to moderate alcohol consumption was associated with a reduced risk of coronary heart disease [28]. These studies payed very limited attention to exposure to smoking (CALIBER analyses were just adjusted for smoking), and none considered the proportion of exposure to it, nor to the possibility of an effect restricted to smokers. This is especially important because alcohol consumption, is frequently coincident with the use of tobacco [29, 30], and if the effect occurs only among smokers, observing the overall effect could depend on tobacco being consumed by a large proportion of the studied population.

Current smoking and cumulative exposure are significant risk factors for CVD [2]and in particular, very strong factors for peripheral arterial disease [31]. We have found a protective role of moderate alcohol intake in the femoral territory, which is the one most affected by smoking, and interestingly, it was only observable in current or former smokers. Smoking is an important modifiable risk factor, and it is addressed as a primary intervention aim in most guides [32, 33]. Probably, the potential benefits of moderate alcohol consumption may only be apparent among groups with a substantial smoking exposure. Eradicating tobacco use is a long-term goal of public health strategies. Thus, in future societies in which this achievement has been attained, the benefits of moderate drinking may fade off as the population becomes mainly non-smoker.

There are several possible explanations for our findings. It is possible that moderate alcohol consumption specifically counteracts the harmful effects of tobacco. Tobacco contains many oxidative substances [34] and there are many basic science studies that support the antioxidant properties of alcoholic beverages [35]. There has been a limited success in isolating a single antioxidant component in them [36, 37] as both alcohol [38] as well as other compounds [10, 11] seem to be responsible of those properties. The fact that the association is observed only in smokers and only in femoral arteries, which are affected much more than

carotid ones by tobacco, suggests that this could be one of the main mechanisms that explain our results. Furthermore, moderate alcohol consumption was associated with a lower risk of incident peripheral arterial disease in the CALIBER [8] and the ARIC studies [25], in contrast with a null effect in cerebral vascular disease in the later [27]. Similar results have been replicated in other populations [39, 40], demonstrating protective effects in peripheral territories. It is also possible that moderate alcohol consumption reflects a healthier lifestyle, like a Mediterranean diet, even in the presence of smoking, and that it is an epiphenomenon without causal relationship with atherosclerosis. This seems unlikely because the beneficial influence of moderate alcohol consumption does not appear in never-smokers, and appears not only in moderate smokers, but also in heavy smokers. It is also possible that moderate alcohol consumption acts in the prevention of all types of atherosclerosis if there is considerable risk, regardless of the causal risk factors. It is well known that alcohol consumption is associated with an increase in HDL-c, which in turn is a protective factor for CVD. However, in our study, the observed benefit is independent of the presence of dyslipidemia, and it is not observed in carotid territory, which does not support a general anti-atherosclerotic influence.

To the best of our knowledge, this is the first study to evaluate the impact of moderate alcohol consumption on subclinical femoral atherosclerosis, and to take smoking influence into consideration. Furthermore, it has the strength of having been carried out with standardized protocols and using high quality data collection methods to obtain information on subclinical atherosclerosis in both carotid and femoral territories. However, it has also several limitations. First, the cross sectional design does not allow us to assume causality and temporality of the associations found. Second, we only included men, so our result cannot be translated to women. Third, although alcohol intake assessment includes personal interviews by trained interviewers, we have to assume that the use of FFQ can lead to some misclassification [41] and is less precise than other tools, even though scientific literature supports that this questionnaire classifies usual intakes correctly. Unfortunately, we have not been able to break down the current group of abstainers into lifelong non-drinkers and ex-drinkers, so the combination of these groups can lead to an overestimation of the protective influence of moderate alcohol consumption. Also, never-smokers group size was smaller than ever-smokers one which means that finding associations among those participants suffers from smaller statistical power. Nonetheless, the dose-response for alcohol association with atherosclerosis was nearly flat and there were no visible trends where non-significance could

be attributed to lack of power. As an example, assuming that in the never-smoker stratum "abstainers" and "≥2 <30 g/d" categories are of the same size as we have (63 and 316 respectively), and that the prevalence of femoral plaque among "abstainers" is 0.35, this sample would have 80% power to detect a difference in plaque prevalence of approximately 0.15 less among the "≥2 <30 g/d" category. Only a 0.03 difference was observed between these groups.

Alcohol use has been ranked as the seventh leading risk factor for premature death and disability [7]. The recommendation on alcohol consumption varies, and it is subject to continuous review. From the point of view of public health, the prevention of alcohol related problems, derived fundamentally from certain behaviors such as compulsive alcohol consumption, is a social policy issue, especially in teenagers and young adults. Our study suggests that it may have a neutral effect on atherosclerosis in future tobacco-free societies, making it easier to supply more straightforward recommendations for alcohol.

Future studies of alcohol potential protective effect should address its potential interaction with other risk factors, in samples at high risk for atherosclerosis in which expected progression could be slowed down in any territory. Also particular attention devoted to interaction with factors that impact oxidative stress will clarify whether the nature of the risk factor is relevant. In any case, public health implications that we outlined above (potential protection fade off in tobacco free societies) remain relevant, no matter the mechanistic explanation.

CONCLUSIONS

In conclusion, we observed that moderate alcohol consumption has no association with subclinical femoral or carotid atherosclerosis among never smokers but a protective influence on femoral atherosclerosis among smokers.

ACKNOWLEDGEMENTS

This study was supported in part by CIBERCV and grants PI17/10704, PI18/01929, from the Carlos III Research Institute (co-supported by the European Regional Development Fund "Investing in your future"). Martin Laclaustra research activity is funded by Agencia

Aragonesa para la Investigación y el Desarrollo (ARAID). The authors thank the study participants, doctors, and health care professionals at the Opel factory. The authors also thank the AWHS technical staff.

References

1. Siasos G., Tsigkou V., Kokkou E., Oikonomou E., Vavuranakis M., Vlachopoulos C., *et al.* Smoking and atherosclerosis: mechanisms of disease and new therapeutic approaches. *Curr Med Chem* 2014; **21**: 3936-48.
2. McEvoy J.W., Blaha M.J., DeFilippis A.P., Lima J.A., Bluemke D.A., Hundley W.G., *et al.* Cigarette smoking and cardiovascular events: role of inflammation and subclinical atherosclerosis from the MultiEthnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol* 2015; **35**: 700-9.
3. Song P., Rudan D., Zhu Y., Fowkes F.J.I., Rahimi K., Fowkes F.G.R., *et al.* Global, regional, and national prevalence and risk factors for peripheral artery disease in 2015: an updated systematic review and analysis. *Lancet Glob Health* 2019; **7**: e1020-e30.
4. Duncan M.S., Freiberg M.S., Greevy R.A., Jr., Kundu S., Vasan R.S., Tindle H.A. Association of Smoking Cessation With Subsequent Risk of Cardiovascular Disease. *JAMA* 2019; **322**: 642-50.
5. Fernandez-Sola J. Cardiovascular risks and benefits of moderate and heavy alcohol consumption. *Nat Rev Cardiol* 2015; **12**: 576-87.
6. Leifer E.S. The Risks and Benefits of Moderate Alcohol Consumption: There Remains Much to Learn. *JACC Heart Fail* 2017; **5**: 845-47.
7. Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 2018; **392**: 1015-35.
8. Bell S., Daskalopoulou M., Rapsomaniki E., George J., Britton A., Bobak M., *et al.* Association between clinically recorded alcohol consumption and initial presentation of 12 cardiovascular diseases: population based cohort study using linked health records. *BMJ* 2017; **356**: j909.
9. Wood A.M., Kaptoge S., Butterworth A.S., Willeit P., Warnakula S., Bolton T., *et al.* Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. *Lancet* 2018; **391**: 1513-23.
10. de Gaetano G., Costanzo S., Di Castelnuovo A., Badimon L., Bejko D., Alkerwi A., *et al.* Effects of moderate beer consumption on health and disease: A consensus document. *Nutr Metab Cardiovasc Dis* 2016; **26**: 443-67.
11. Artero A., Tarin J.J., Cano A. The impact of moderate wine consumption on health. *Maturitas* 2015; **80**: 3-13.

12. Laclaustra M., Casasnovas J.A., Fernandez-Ortiz A., Fuster V., Leon-Latre M., Jimenez-Borreguero L.J., *et al.* Femoral and Carotid Subclinical Atherosclerosis Association With Risk Factors and Coronary Calcium: The AWHS Study. *J Am Coll Cardiol* 2016; **67**: 1263-74.

13. Fernandez-Friera L., Penalvo J.L., Fernandez-Ortiz A., Ibanez B., Lopez-Melgar B., Laclaustra M., *et al.* Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical Atherosclerosis in a Middle-Aged Cohort: The PESA (Progression of Early Subclinical Atherosclerosis) Study. *Circulation* 2015; **131**: 2104-13.

14. Casasnovas J.A., Alcaide V., Civeira F., Guallar E., Ibanez B., Borreguero J.J., *et al.* Aragon workers' health study--design and cohort description. *BMC Cardiovasc Disord* 2012; **12**: 45.

15. Martin-Moreno J.M., Boyle P., Gorgojo L., Maisonneuve P., Fernandez-Rodriguez J.C., Salvini S., *et al.* Development and validation of a food frequency questionnaire in Spain. *Int J Epidemiol* 1993; **22**: 512-9.

16. Organization W.H. International Guide for Monitoring Alcohol Consumption and Related Harm World Health Organization. Geneva, Switzerland.2000.

17. Muntendam P., McCall C., Sanz J., Falk E., Fuster V. The BioImage Study: novel approaches to risk assessment in the primary prevention of atherosclerotic cardiovascular disease--study design and objectives. *Am Heart J* 2010; **160**: 49-57 e1.

18. Junyent M., Gilabert R., Zambon D., Pocovi M., Mallen M., Cofan M., *et al.* Femoral atherosclerosis in heterozygous familial hypercholesterolemia: influence of the genetic defect. *Arterioscler Thromb Vasc Biol* 2008; **28**: 580-6.

19. Inaba Y., Chen J.A., Bergmann S.R. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. *Atherosclerosis* 2012; **220**: 128-33.

20. Friedewald W.T., Levy R.I., Fredrickson D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499-502.

21. Pearson T.A., Palaniappan L.P., Artinian N.T., Carnethon M.R., Criqui M.H., Daniels S.R., *et al.* American Heart Association Guide for Improving Cardiovascular Health at the Community Level, 2013 update: a scientific statement for public health practitioners, healthcare providers, and health policy makers. *Circulation* 2013; **127**: 1730-53.

22. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143-421.

23. Schminke U., Luedemann J., Berger K., Alte D., Mitusch R., Wood W.G., *et al.* Association between alcohol consumption and subclinical carotid atherosclerosis: the Study of Health in Pomerania. *Stroke* 2005; **36**: 1746-52.

24. Bo P., Marchioni E., Bosone D., Soragna D., Albergati A., Micieli G., *et al.* Effects of moderate and high doses of alcohol on carotid atherogenesis. *Eur Neurol* 2001; **45**: 97-103.

25. Ogilvie R.P., Lutsey P.L., Heiss G., Folsom A.R., Steffen L.M. Dietary intake and peripheral arterial disease incidence in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Clin Nutr* 2017; **105**: 651-59.

26. Fuchs F.D., Chambless L.E., Folsom A.R., Eigenbrodt M.L., Duncan B.B., Gilbert A., *et al.* Association between alcoholic beverage consumption and incidence of coronary heart disease in whites and blacks: the Atherosclerosis Risk in Communities Study. *Am J Epidemiol* 2004; **160**: 466-74.

27. Ding J., Eigenbrodt M.L., Mosley T.H., Jr., Hutchinson R.G., Folsom A.R., Harris T.B., *et al.* Alcohol intake and cerebral abnormalities on magnetic resonance imaging in a community-based population of middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) study. *Stroke* 2004; **35**: 16-21.

28. Ronksley P.E., Brien S.E., Turner B.J., Mukamal K.J., Ghali W.A. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. *BMJ* 2011; **342**: d671.

29. Mateo-Gallego R., Uzhova I., Moreno-Franco B., Leon-Latre M., Casasnovas J.A., Laclaustra M., *et al.* Adherence to a Mediterranean diet is associated with the presence and extension of atherosclerotic plaques in middle-aged asymptomatic adults: The Aragon Workers' Health Study. *J Clin Lipidol* 2017; **11**: 1372-82 e4.

30. Schroder H., Marrugat J., Elosua R., Covas M.I. Tobacco and alcohol consumption: impact on other cardiovascular and cancer risk factors in a southern European Mediterranean population. *Br J Nutr* 2002; **88**: 273-81.

31. Willigendael E.M., Teijink J.A., Bartelink M.L., Kuiken B.W., Boiten J., Moll F.L., *et al.* Influence of smoking on incidence and prevalence of peripheral arterial disease. *J Vasc Surg* 2004; **40**: 1158-65.

32. Piepoli M.F., Hoes A.W., Agewall S., Albus C., Brotons C., Catapano A.L., *et al.* 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth

Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J* 2016; **37**: 2315-81.

33. Goff D.C., Jr., Lloyd-Jones D.M., Bennett G., Coady S., D'Agostino R.B., Gibbons R., *et al.* 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014; **129**: S49-73.

34. Pryor W.A., Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxynitrate, and peroxynitrite. *Ann N Y Acad Sci* 1993; **686**: 12-27; discussion 27-8.

35. Snopek L., Mlcek J., Sochorova L., Baron M., Hlavacova I., Jurikova T., *et al.* Contribution of Red Wine Consumption to Human Health Protection. *Molecules* 2018; **23**.

36. Di Castelnuovo A., Costanzo S., di Giuseppe R., de Gaetano G., Iacoviello L. Alcohol consumption and cardiovascular risk: mechanisms of action and epidemiologic perspectives. *Future Cardiol* 2009; **5**: 467-77.

37. Rotches-Ribalta M., Andres-Lacueva C., Estruch R., Escribano E., Urpi-Sarda M. Pharmacokinetics of resveratrol metabolic profile in healthy humans after moderate consumption of red wine and grape extract tablets. *Pharmacol Res* 2012; **66**: 375-82.

38. Costanzo S., Di Castelnuovo A., Donati M.B., Iacoviello L., de Gaetano G. Wine, beer or spirit drinking in relation to fatal and non-fatal cardiovascular events: a meta-analysis. *Eur J Epidemiol* 2011; **26**: 833-50.

39. Garcia-Diaz A.M., Marchena P.J., Toril J., Arnedo G., Munoz-Torrero J.F., Yeste M., *et al.* Alcohol consumption and outcome in stable outpatients with peripheral artery disease. *J Vasc Surg* 2011; **54**: 1081-7.

40. Xie X., Ma Y.T., Yang Y.N., Li X.M., Liu F., Huang D., *et al.* Alcohol consumption and ankle-to-brachial index: results from the Cardiovascular Risk Survey. *PLoS One* 2010; **5**: e15181.

41. Michels K.B. A renaissance for measurement error. *Int J Epidemiol* 2001; **30**: 421-2.

Table 1. Baseline characteristics of study participants according to levels of alcohol intake.

	Overall	Abstainers	≥2 <30	≥30<60	≥60	p-value
N	2099	246	1239	501	113	
Age, years	50.9 (3.9)	50.7 (4.1)	50.6 (4.0)	51.5 (3.6)	51.8 (3.1)	<0.001
BMI, kg/m^2	27.6 (3.3)	27.4 (3.5)	27.6 (3.2)	27.7 (3.3)	28.1 (3.6)	0.341
Waist circumference, cm	97.3 (8.8)	96.6 (9.0)	97.3 (8.8)	97.1 (8.8)	98.6 (9.1)	0.254
Systolic blood pressure, mm Hg	125.3 (13.9)	124.3 (13.3)	124.8 (13.6)	126.4 (15.1)	129.2 (12.7)	0.002
Diastolic blood pressure, mm Hg	82.4 (9.4)	80.9 (8.9)	82.1 (9.4)	83.1 (9.8)	85.0 (8.7)	<0.001
Total cholesterol, mg/dl	220.0 (36.4)	212.1 (39.1)	218.9 (35.8)	224.9 (35.7)	227.7 (35.4)	<0.001
HDL-c, mg/dl	53.1 (11.4)	49.1 (10.8)	53.0 (10.9)	54.5 (12.1)	56.2 (11.4)	<0.001
Non-HDL-c, mg/dl	167.0 (35.2)	162.9 (37.3)	166.0 (34.9)	170.4 (34.9)	171.5 (34.4)	0.013
LDL-c, mg/dl	137.8 (31.4)	135.1 (33.3)	137.8 (31.2)	139.0 (31.1)	138.6 (31.3)	0.445
Triglycerides, mg/dl	150.0 (96.9)	147.9 (126.2)	143.1 (85.3)	161.8 (103.0)	176.9 (107.1)	<0.001
Fasting glucose, mg/dl	97.7 (17.5)	96.6 (19.3)	97.4 (17.2)	98.5 (18.2)	99.7 (13.5)	0.297
Ever-smokers, %	77.4 [1625]	74.4 [183]	74.5 [923]	84.6 [424]	84.1 [95]	<0.001
Hypertension, %	37.4 [784]	34.1 [84]	34.9 [433]	40.3 [202]	57.5 [65]	<0.001
Dyslipidemia, %	49.2 [1032]	52.0 [128]	47.2 [585]	52.5 [263]	49.6 [56]	0.177
Diabetes, %	5.7 [120]	8.1 [20]	4.8 [59]	7.2 [36]	4.4 [5]	0.078

BMI: body mass index; HDL-c: High-density lipoprotein cholesterol.
Values are mean (SD) or % [number].

Table 2. Odds ratio (95%CI) for the presence of femoral plaque according to levels of alcohol intake and smoking habit.

	Never smokers			**Ever-smokers**			
	n/N	**OR (95%CI)**	**p*1**	**n/N**	**OR (95%CI)**	**p*1**	**p*2**
Abstainers	23/63	1.00 (ref)	ref	124/183	3.70 (2.00,6.98)	ref	**<0.001**
≥2 <30 g/d	104/316	0.95 (0.53,1.72)	0.854	550/923	2.59 (1.49,4.48)	**0.044**	**<0.001**
≥30<60 g/d	33/77	1.14 (0.56,2.32)	0.718	280/424	3.17 (1.79,5.61)	0.426	**<0.001**
≥60 g/d	6/18	0.69 (0.21,2.13)	0.533	70/95	4.39 (2.16,8.93)	0.556	**0.001**

Adjusted odds ratios were calculated with logistic regression models adjusted for age, hypertension, dyslipidemia and diabetes.

ORs in the table use as reference the never-smoker/alcohol abstainer group. Note: p-values shown are not for testing these ORs but for specific intra-strata comparisons.

N: Number of participants in the smoking and alcohol consumption group.

n: Number of participants with at least one plaque.

p*1: Level of significance of the OR for the presence of plaque for each group of alcohol consumption with respect to the abstainers group, within each smoking stratum.

p*2: Level of significance of the OR for the presence of plaque for being ever smoker with respect to never-smokers, within each alcohol consumption stratum.

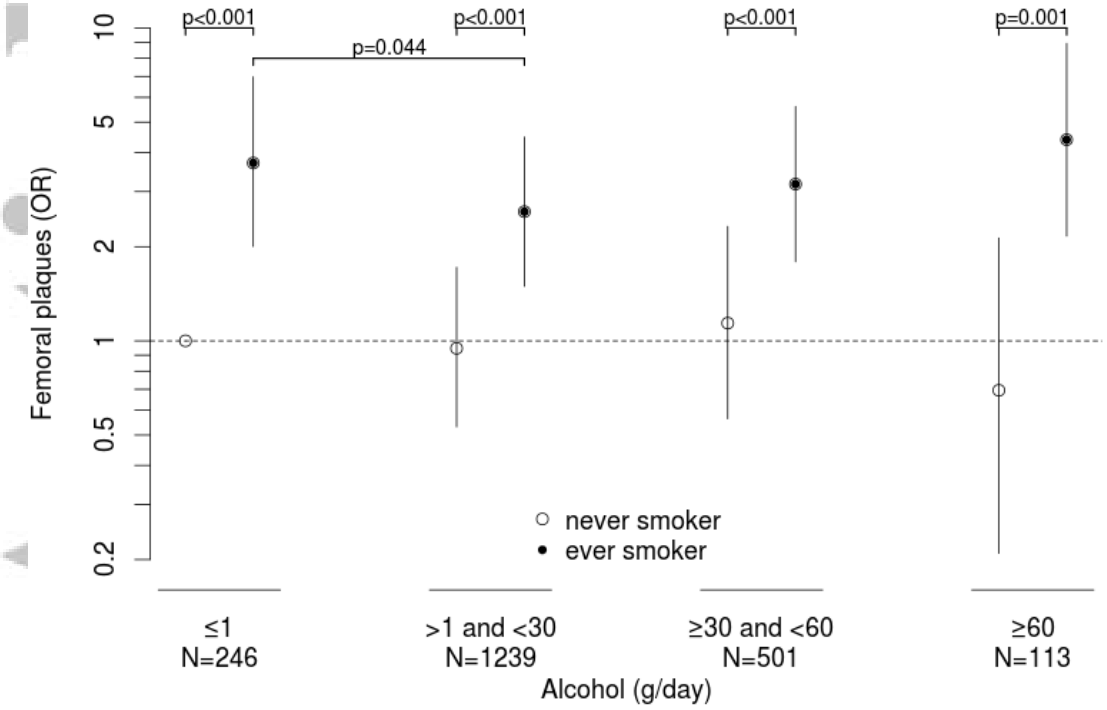

Figure 1. Odds ratio (95%CI) for the presence of femoral plaque according to levels of alcohol intake and smoking habit.

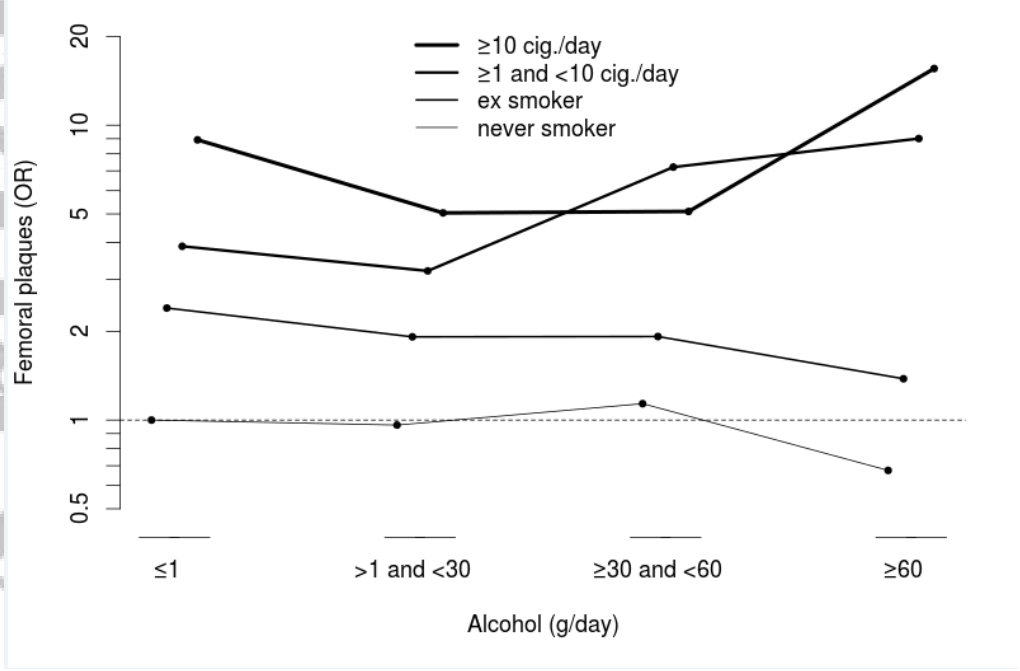

Figure 2. Odds ratio for the presence of femoral plaque according to levels of alcohol intake and smoking exposures.