Early vascular damage from smoking and alcohol in teenage years: The ALSPAC study

Marietta Charakida PhD[a], George Georgiopoulos PhD[a], Frida Dangardt PhD[a], Scott Chiesa PhD[a], Alun D Hughes PhD[a], Alicja Rapala BA[a], George Davey Smith DSc[b], Debbie Lawlor PhD[b], Nicholas Finer MB BS[a], John E Deanfield MB, BChir[a].

[a] Institute of Cardiovascular Science, University College London, London, UK
[b]MRC Centre for Causal Analysis in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Bristol UK

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Address for correspondence
Marietta Charakida MD, PhD
Senior Lecturer& Consultant in Paediatric Cardiology
Division of Imaging Sciences & Biomedical Engineering
Lambeth Wing, St Thomas' Hospital
London SE17EH, UK
Email: charakidadoc@gmail.com
Tel: +44(0)2071884970

Abstract

Aims: To determine the impact of smoking and alcohol exposure during adolescence on arterial stiffness at 17 years.

Methods and Results: Smoking and alcohol use were assessed by questionnaires at 13, 15 and 17 years in 1266 participants (425 males and 841 females) from the ALSPAC study. Smoking status (smokers and non-smoker) and intensity ("high" ≥100, "moderate" 20-99, and "low or never" <20 cigarettes in lifetime) were ascertained. Participants were classified by frequency (low or high) and intensity of drinking (light (LI < 2), medium (MI 3-9), and heavy (HI > 10 drinks)). Carotid to femoral pulse wave velocity (PWV) was assessed at 17 years (mean±standard deviation and/or mean difference [95% confidence intervals]). Current smokers had higher PWV compared to non-smokers (p=0.003). Higher smoking exposure was associated with higher PWV compared to non-smokers (5.81±0.725 versus 5.71±0.677 m/s, mean adjusted difference 0.211[0.087,0.334] m/s, p=0.001). Participants who stopped smoking had similar PWV to never smokers (p=0.160). High intensity drinkers had increased PWV (HI: 5.85±0.8 versus LI: 5.67±0.604 m/s, mean adjusted difference 0.266 [0.055, 0.476] m/s, p=0.013). There was an additive effect of smoking intensity and alcohol intensity, so that "high" smokers who were also HI drinkers had higher PWV compared to never-smokers and LI drinkers (mean adjusted increase 0.603 [0.229, 0.978] m/s,p=0.002).

Conclusion: Smoking exposure even at low levels and intensity of alcohol use were associated individually and together with increased arterial stiffness. Public health strategies need to prevent adoption of these habits in adolescence to preserve or restore arterial health.

Key words: smoking; alcohol; arterial stiffness; adolescence.

Introduction

Cigarette smoking and consumption of alcohol are associated with increased cardiovascular (CV) risk in adult life[1, 2]. Smokers are twice as likely to suffer a myocardial infarction compared to people who have never smoked. For alcohol, a J-shaped association has been reported, with CV benefit for those consuming small or moderate amount of alcohol daily. This has however been questioned in recent studies [3] which showed that higher intake or binge drinking are associated with increased cardiovascular morbidity and mortality[4]. Most adult users of alcohol or tobacco first start in their early teens [5, 6]. In the United States, each day approximately 6,000 adolescents, aged 12 to 18 years, smoke a cigarette for the first time, and 3,000 adolescents become daily smokers [7]. A large body of research suggests that adolescents who participate in one health-risk behavior are more likely to engage in additional risk behaviors[7]. According to the Center of Disease Classification (CDC), 75% of high school students report drinking alcoholic beverages at least once [7]. The early impact of unhealthy behaviors on contemporary arterial disease is not well documented.

The Avon Longitudinal Study of Parents and Children was established in 1991 to provide environmental information on participants from childhood to adolescence and young adulthood and to study the impact on their health (http://www.alspac.bris.ac.uk) [8]. The cohort has been followed with questionnaires through childhood and at regular annual clinics since age of 7 years. Detailed information about alcohol and smoking is available for participants through adolescence from questionnaires, and vascular phenotyping was performed at age 17 years. This provided a unique opportunity to assess the impact of exposure to these substances on arterial phenotype and CV risk factor profile in young adulthood.

Methods

Vascular study population

We studied adolescents who attended the ALSPAC clinics in Bristol at 17 years (Figure 1). The study was approved by the ALSPAC Ethics and Law Committee and written informed consent was obtained from all participants.

Tobacco use in adolescence

Information about tobacco use was obtained from questionnaires at ages 13, 15 and 17 years. Participants were classified as smokers or no-smokers at each period. Smoking exposure over the 5-year period was assessed. Participants who stopped smoking were also identified.

Numbers of cigarettes smoked over lifetime were assessed by questionnaire at 17 years and participants were classified in three groups to reflect smoking intensity: “high” (≥100 cigarettes), moderate (20-99 cigarettes) and low/never smokers (<20 cigarettes). Cotinine levels were not measured. Exposure to parental smoking (passive smoking) was also assessed by questionnaires.

Alcohol consumption in adolescence

Information about alcohol consumption was obtained from questionnaires at the ages of 13, 15 and 17 years and participants were classified as drinkers or non-drinkers at each period. At 17 years, more detailed information about patterns of alcohol use was obtained. The participants were asked to report the age when they started drinking alcohol, frequency of alcohol consumption/ month and intensity of alcohol consumption (number of drinks on a typical drinking day, with one drink equating to 8 g of alcohol). Heavy (HI), medium (MI) and light (LI) intensity drinkers were defined as subjects consuming > 10 drinks, 3-9 drinks and < 2 drinks respectively on a typical day that they were drinking alcohol. Preferences for different beverages (i.e. beer, wine and spirits) were evaluated at 17 years by questionnaires, which assessed the number of each of the aforementioned drinks consumed. Participants were classified as never, light, moderate and heavy beer, wine or spirit-drinking if they reported no consumption or consumption of 1-5, 6-20 and over 20 drinks in the last 30 days.

Aortic stiffness

Pressure-pulse waveforms were obtained using the Vicorder device by placing a 100 mm wide blood pressure cuff around the upper thigh to measure the femoral pulse pressure, and a 30 mm partial cuff around the neck at the level of the carotid artery. To measure carotid to femoral pulse wave velocity (PWV), high quality waveforms were recorded simultaneously for 3 seconds with the subject in the supine position, and the foot-to-foot transit time was determined using an in-built cross-correlation algorithm centred around the peak of the second derivative of pressure. All measurements were performed independently by one of two trained vascular technicians (inter-observer mean difference (standard deviation of difference) 0.2 (0.1) m/s).

Cardiovascular risk factors (Confounders and mediators)

Three seated right arm blood pressure measurements were made (Omron 705 IT oscillometric BP monitor) after a 10min rest and the mean of the last 2 were analyzed. Simultaneous heart rate was measured. Waist circumference, weight and height were measured and body mass index (BMI) was calculated. At 17 years, fasting blood samples were taken; all samples were immediately centrifuged and stored at -80^{o}C for lipid profile (total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides), inflammatory markers (C-reactive protein (CRP)) and liver function tests [9]. Socioeconomic status (SES) was designated from 1 to 5 according to National Readership Survey (NRS) grading criteria based on father's occupation, with class 5 denoting unskilled workers. (http://www.nrs.co.uk/nrs-print/lifestyleand-classification-data/social-grade). Physical activity (PA) was assessed by actigraph accelerometer at 15 years. Time spent in moderate to vigorous PA (MVPA) like cycling, swimming, running was calculated, the cut-point being four times resting metabolic rate (equivalent to brisk walking). Minutes of MVPA was calculated as the average minutes of such activity/valid day of measurement.

Statistical analysis

Standard analytical approaches to normally and non-normally distributed measures were used and values are expressed as mean ± standard deviation (SD) and median (interquartile range), respectively. Categorical variables are expressed as N, percentage. Unadjusted comparisons for continuous variables between groups of interest were performed using analysis of variance (ANOVA), with a test for linear trend carried out across pre-specified categories. Categorical variables were compared using the chi-squared test. Multivariable linear regression analysis was performed to assess the association between smoking, alcohol and cardiovascular risk factors and PWV. Numeric estimates for comparisons of interest are provided as mean difference in PWV along with 95% confidence intervals [CIs] around the mean difference. Multivariable models were built through an unbiased method of a priori variable selection, based on previous medical literature and conventional knowledge with adjustment for established CV risk factors and exposure to variables previously associated with increased PWV (Supplementary file). An interaction effect between gender and smoking/alcohol consumption behaviour was tested as well as a potential additive or multiplicative adverse effect of combination of bad behaviours on PWV at 17 years (Supplementary file).

To assess smoking and alcohol exposure across the range of 13-17 years, adolescents with complete questionnaires for smoking behaviour and alcohol consumption were included in longitudinal analyses (Supplementary file).

We replaced missing data for all variables of interest by multiple imputation using the MCMC method [10]. Variables with < 5% missing values were not imputed. Statistical analysis was performed by STATA package, version 11.1 (StataCorp, College Station, Texas USA). We deemed statistical significance at alpha=0.05.

Results

Participant characteristics (Table 1)

There was no difference in either smoking or alcohol use between males and females. At 17 years, 23.8% of participants were smokers. One hundred and fifty-four (14.2%) participants drank 0-2 drinks/typical day, 822 (75.6%) 3-9/typical day and 111(10.2%) > 10 drinks/typical day). Seven hundred and twenty-four (66.6%) participants were classified as low frequency drinkers (10 never drinkers, 203 monthly or less, 511 (2-4 times/ month) and 363 (33.4%)) were high frequency drinkers (316 drank 2-3 times/ week and 47 ≥4 times/week).

Smoking rates (p=0.012) but not alcohol consumption (p=0.130) in adolescence increased progressively from social class I (high status) to V (manual unskilled). Parental smoking was associated with smoking in adolescence (p=0.046). Physical activity did not differ across smoking and drinking intensity groups. Participants with higher alcohol intensity had increased weight but normal BMI, increased TC and mildly deranged liver function (Table 1). Following adjustment, drinking intensity was still related to increased ALT (p=0.014).

Patterns of smoking behaviour during adolescence and arterial stiffness

Current smokers had increased arterial stiffness compared to non-smokers (mean difference 0.176 [0.058, 0.293] m/s, p=0.003). Number of cigarettes smoked over lifetime (smoking intensity) was positively associated with PWV, with "high" intensity smokers having higher PWV compared to low/never-smokers (5.81±0.725 versus 5.71±0.677 m/s, mean difference 0.104 [0.01, 0.199] m/s, p=0.032). This association remained after adjustment for other CV risk factors (mean increase in PWV for high smokers 3.7% or 0.211 [0.087, 0.334] m/s, p=0.001 compared to low/never smokers) (Table 2) and passive smoking (p=0.004). Following imputation for missing values in covariates of interest (i.e. LDL, CRP, family history of CAD, physical activity and passive smoking), the association of smoking intensity and increased PWV remained significant (mean increase 0.122 [0.032, 0.212], p=0.008). There was no interaction ($p_{for\ interaction}$=0.308) between gender and smoking exposure on vascular profile.

Of 661 participants with measurements of smoking status at 13, 15 and 17 years, longitudinal analysis indicated that never smokers (n=269) had lower PWV at 17 years compared to those who had been smoking since 13 years (-0.313[-0.01, -0.618] m/s, p=0.044) and to current smokers (i.e. at 17 years) (-0.196 [-0.034, -0.357] m/s, p=0.018). The longitudinal effect of smoking across the period of 13 to 17 years on PWV was consistent across categories of increased duration (mean increase in PWV 0.143 [0.047, 0.239] m/s per category, $p_{for\ linear\ trend}$=0.004) (Figure 2) and when adjusted for passive smoking (mean increase in PWV 0.211[0.083, 0.340] m/s per category, $p_{for\ linear\ trend}$=0.002) compared to never smokers (Table 2). Subjects (n=91) who smoked between 13 and 17 years but who subsequently stopped, had comparable PWV to never smokers (mean difference -0.152 [-0.364, 0.06], p=0.160). Finally, when missing values for longitudinal smoking status as well as for confounders were imputed, the association of increased duration of smoking with PWV at 17 years remained significant (mean expected increase 0.047[0.01, 0.089] m/s per category, p=0.027) after adjustment. Adolescents who smoked since 13 or 15 years, presented an adjusted increase of 0.157 [0.01, 0.308] m/s in PWV compared to participants who had never smoked (p=0.042) (n=1225) (Table 2).

Patterns of alcohol consumption during adolescence and arterial stiffness

There was no association between age of starting drinking alcohol or frequency of drinking and PWV, whereas HI drinking was associated with increased PWV (HI: 5.85±0.8 versus LI: 5.67±0.604 m/s, mean difference 0.182 [0.019, 0.346] m/s, p=0.029). This association remained after adjustment for CV risk factors (relative mean increase 4.7% or 0.266[0.055, 0.476] m/s, p=0.013) (Table 2). Complete analysis for imputed observations of drinking intensity at 17 years and additional exposure variables, including physical activity, revealed a similar association with increased PWV (p=0.048) but additional adjustment for family history of CAD and parental smoking attenuated this effect (p=0.059). There was no interaction between gender and alcohol consumption (p=0.230). Increased drinking intensity as a proxy to binge drinking (≥10 drinks/ typical drinking day) correlated with increased arterial stiffness (5.85±0.8 versus 5.7±0.625 m/s, mean increase 0.147 [0.016, 0.279], p=0.028), even after controlling for gender, SBP, SES, LDL, BMI, CRP (mean increase 0.231[0.057, 0.405] m/s, p=0.01). In longitudinal analysis, drinking intensity was not associated with PWV (p>0.05 for all categories) (Table 2).

Over a month period, adolescents consumed more beer than wine (p<0.001) with no difference between consumption of beer- and spirit-based drinks (p=0.426). Three hundred twenty-seven (26.7%) adolescents did not drink beer while 414 (33.8%), 371 (30.3%) and 112 (9.15%) were light, moderate and heavy beer-drinkers. Almost half of participants, 558 (46.5%) did not consume wine, 458 (38.2%), 158 (13.16%) and 26 (2.17%) were light, moderate and heavy wine drinkers respectively. Finally, 264 (21.48%) participants abstained from spirits in the last month, while 518 (42.14%), 372 (30.27%) and 75 (6.1%) adolescents were light, moderate and heavy spirit-drinkers. Light and moderate wine drinking were associated, in univariate analysis, with decreased arterial stiffness (5.67±0.677 m/s for light and 5.61±0.649 m/s for moderate versus 5.77±0.684m/s for no consumption, p=0.02 and p=0.008 respectively). Moderate and heavy consumption of beer were related to increased PWV (5.66±0.672 m/s for moderate and 5.79±0.690m/s for heavy versus 5.62±0.597 m/s for non-consumers, p=0.001 and p<0.001, respectively) as compared to non-consumption. However, these associations lost their significance following adjustment for confounding exposure variables (p>0.05 for all categories). Spirit drinking pattern was not associated with arterial stiffness at 17 years.

Higher alcohol intensity and smoking intensity had additive effects on arterial stiffness (high alcohol and smoking versus never smokers and low alcohol; 5.89±0.857 versus 5.61±0.589, mean increase 0.277 [0.028, 0.525] m/s, p=0.029) compared to never smoking and low alcohol consumption. After adjustment, the combined index of alcohol and smoking intensity remained an independent predictor of PWV (mean increase 10.8% or 0.603 [0.229, 0.978] m/s in PWV, for adolescents with both high alcohol and smoking versus never smokers and low alcohol, p=0.002) (Table 2) (Figure 3). The association of the combined index of alcohol and smoking intensity with increased PWV remained significant after additional adjustment for parental smoking (p<0.001) or imputation for missing values in confounders at 17 years (mean increase 0.331[0.094, 0.568] m/s, p=0.006)(Table 2). There was a non-multiplicative combined effect of these unfavorable behaviors for the vascular profile of adolescents (p for interaction>0.1 in both unadjusted and adjusted models, likelihood ratio test p>0.1 for the addition of the interaction terms smoking*drinking intensity over the core model).

Discussion

This study demonstrates that smoking and alcohol use up to age 17 years have both independent and additive associations with arterial stiffness, a marker of vascular damage that predicts later CV disease and events. Smoking in youth, even at low levels, was associated with increased arterial stiffness, but stopping during adolescence could restore arterial health. In addition, patterns of alcohol use had a significant impact on arterial stiffness with higher intensity, rather than frequency of consumption, showing the greatest adverse effect on PWV. These data demonstrate the importance of public health measures, which focus on preventing the establishment of unhealthy behaviours in children and adolescents.

Impact of smoking and excessive alcohol use on adverse CV outcomes in adults is well established[11, 12]. While most adult users of alcohol or tobacco first tried these "drugs" during their early teens, the impact of smoking and alcohol consumption on the atherosclerotic process at this early stage of the life course is less clear. To evaluate the development of early atherosclerosis, we measured carotid to femoral PWV in a well-characterised cohort of 17 year olds. Previous studies have demonstrated that PWV is reproducible and, in adults, predicts CV outcomes independently from conventional risk factors[13].

Epidemiological studies have consistently demonstrated that acute and chronic cigarette use adversely affects vascular health in adulthood and promotes atherosclerosis progression[14]. We quantified exposure to cigarettes during adolescence and also assessed smoking frequency. Both current cigarette use and chronic smoking exposure had an adverse effect on arterial stiffness. This finding is consistent with reports in adults which show that the longer and earlier a person starts smoking, the higher the incidence of coronary artery disease and hypertension and the worse the impact on life expectancy [15]. Passive smoking has consistently been shown to adversely affect arterial health in the young, however no association with aortic stiffness in adolescence was demonstrated in our cohort[16]. Exposure to parental smoking at home was assessed as binary variable which precluded evaluation of potential differences in the degree in passive smoking.

The pathophysiological mechanisms linking smoking to vascular disease are still not fully elucidated[17, 18]. Tobacco contains many toxic and vasoactive compounds, which can exert damage on vascular endothelium and can activate inflammatory and thrombotic pathways relevant to CV events[17]. Although cigarette smoking is discouraged in many countries with legislation and other initiatives, our findings confirm that smoking continues to be prevalent in adolescence in both sexes in the UK. It was interesting that only a small percentage of our cohort was in the highest smoking category of >100 cigarettes in total. This is considerably less exposure than that of typical adult smokers, in whom life time exposure is measured in pack-years (≡20 cigarettes/day for 1 year). These data are consistent with a recent NHS survey, which demonstrated that the number of children trying cigarettes has fallen by three quarters since 2003. However, even at these low levels of smoking exposure, we could detect an adverse effect on arterial stiffness even when adjusted for other CV risk factors. Quitting smoking in adolescence could restore aortic stiffness, emphasizing the benefit and opportunity from implementing interventional and educational strategies in the young to preserve arterial health consistent with the recent European CV prevention guidelines[12].

There are conflicting findings on the impact of alcohol consumption on atherosclerosis. Alcohol intake has been shown to prevent atherosclerosis in some animal studies , but not in all,[19, 20] and results from clinical studies are also inconsistent[21, 22]. High alcohol intake (>34g/day) is associated with higher blood pressure, but observational evidence does not support an association of alcohol intake with blood pressure below this level[23]**.** Mild alcohol consumption (≤ 2 alcoholic beverages/ day), can independently improve endothelial function both in young healthy subjects and in those with type 2 diabetes[24]. In contrast, a Finnish study reported a direct linear adverse effect of alcohol consumption on carotid intima media thickness in the young, from as little as 2 alcoholic drinks per day [25]. In support of this, a recent Mendelian randomization meta-analysis implicated alcohol consumption as a causal factor for CV disease[11].

We used a combination of frequency and intensity questionnaires to describe better patterns of alcohol use. The age at which participants started consuming alcohol was not associated with vascular stiffness, suggesting that duration of exposure might not be that important at this young age and this was confirmed in our longitudinal analysis. In contrast, higher intensity rather than frequency of alcohol consumption had the greatest adverse relationship with arterial stiffness. This finding is of particular concern since excessive drinking, with the aim of getting drunk, is increasingly the norm for teenagers[26].

The mechanistic link between alcohol and arterial stiffness is not well explored. Light to moderate alcohol consumption is associated with an increase in HDL cholesterol, a decrease in inflammatory mediators and improvement in metabolic pathways [27, 28]. In contrast, excess alcohol consumption has been associated with elevation in blood pressure, autonomic dysregulation and derangements in coagulation and fibrinolytic pathways [27]. We did not find any beneficial effect of alcohol consumption on arterial stiffness even at lower consumption levels. When we separated different alcoholic beverages, light and moderate wine drinking groups and moderate to heavy beer drinkers were associated with lower and higher PWV, respectively as compared to non-consumers. However, these associations were not significant in multivariable analysis, implying that drinking intensity rather than type of alcoholic beverage may be more important. The association between alcohol use and CV risk factors was modest and only mild derangements in liver function tests were noted in participants with high alcohol intensity [29]. Interestingly, lower heart rate was noticed in adolescents with high drinking intensity and this might represent autonomic dysregulation[30].

Our study has limitations as it is observational, so that causal associations between smoking, alcohol exposure and arterial stiffness cannot be established. We relied on self-reported confidential questionnaires, collected every 2 years through adolescence, without any biomarker-based assessment of smoking or alcohol exposure. Although self-report of smoking and alcohol behaviour has been shown to be a valid measure compared to biochemical measures, we cannot exclude the possibility that some of our participants may have been misclassified, particularly those from the heavier exposure categories. This however, would be likely to result in underestimation of the effect sizes observed. The presence of unmeasured or residual confounders on our results cannot be excluded. In addition, a number of missing data were imputed in order not to reduce the power of our multivariable longitudinal analysis. This is a well- established statistical approach. Nevertheless, despite these limitations, we were able to demonstrate important associations between patterns of smoking and drinking use in adolescence with arterial stiffness.

In summary, in this large contemporary British cohort of adolescents, we have demonstrated that drinking intensity and smoking in adolescence, even at lower levels compared to those reported in adult studies, is associated with arterial changes relevant to atherosclerosis progression. The effect of these unhealthy behaviours was independent of one another and additive. Smoking cessation in adolescence was associated with normalization of aortic stiffness. These findings have significant public health implications and provide further support for public health measures to discourage young adults adopting smoking and drinking habits and the benefit of discontinuing these unhealthy behaviours.

Summary illustration

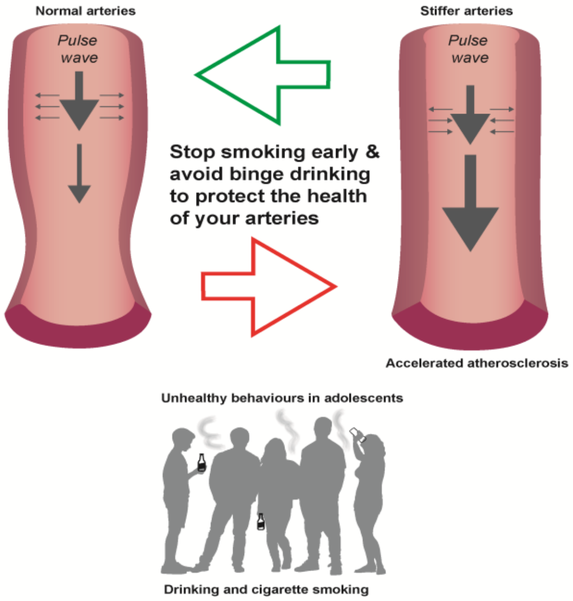

Illustration legend: Unhealthy behaviours in adolescents (drinking and cigarette smoking) are associated with increased carotid to femoral pulse wave velocity (stiffer arteries) and accelerated atherosclerosis. Stopping smoking in adolescence and reducing binge drinking has potential for reversibility of arterial stiffening.

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References

1. Willett WC, Green A, Stampfer MJ, Speizer FE, Colditz GA, Rosner B, Monson RR, Stason W, Hennekens CH. Relative and absolute excess risks of coronary heart disease among women who smoke cigarettes. New England Journal of Medicine 1987;**317**(21):1303-1309.

2. Roerecke M, Rehm J. Alcohol consumption, drinking patterns, and ischemic heart disease: a narrative review of meta-analyses and a systematic review and meta-analysis of the impact of heavy drinking occasions on risk for moderate drinkers. BMC medicine 2014;**12**(1):182.

3. Stockwell T, Zhao J, Panwar S, Roemer A, Naimi T, Chikritzhs T. Do "moderate" drinkers have reduced mortality risk? A systematic review and meta-analysis of alcohol consumption and all-cause mortality. Journal of studies on alcohol and drugs 2016;**77**(2):185-198.

4. Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K. Alcohol and coronary heart disease: a meta-analysis. Addiction 2000;**95**(10):1505-1523.

5. Azagba S, Baskerville NB, Minaker L. A comparison of adolescent smoking initiation measures on predicting future smoking behavior. Preventive Medicine Reports 2015;**2**:174-177.

6. Schulenberg JE, Maggs JL. A developmental perspective on alcohol use and heavy drinking during adolescence and the transition to young adulthood. Journal of Studies on Alcohol, Supplement 2002(14):54-70.

7. Eaton DK, Kann L, Kinchen S, Shanklin S, Ross J, Hawkins J, Harris WA, Lowry R, McManus T, Chyen D. Youth risk behavior surveillance—United States, 2007. MMWR Surveill Summ 2008;**57**(4):1-131.

8. Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Smith GD, Henderson J, Macleod J, Molloy L, Ness A. Cohort profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. International journal of epidemiology 2013;**42**(1):97-110.

9. Falaschetti E, Hingorani AD, Jones A, Charakida M, Finer N, Whincup P, Lawlor DA, Smith GD, Sattar N, Deanfield JE. Adiposity and cardiovascular risk factors in a large contemporary population of pre-pubertal children. European heart journal 2010;**31**(24):3063-3072.

10. Van Buuren S, Boshuizen HC, Knook DL. Multiple imputation of missing blood pressure covariates in survival analysis. Statistics in medicine 1999;**18**(6):681-694.

11. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. Bmj 2011;**342**:d671.

12. Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney M-T, Corrà U, Cosyns B, Deaton C. 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). Atherosclerosis 2016;**252**:207-274.

13. Ben-Shlomo Y, Spears M, Boustred C, May M, Anderson SG, Benjamin EJ, Boutouyrie P, Cameron J, Chen C-H, Cruickshank JK. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. Journal of the American College of Cardiology 2014;**63**(7):636-646.

14. Kubozono T, Miyata M, Ueyama K, Hamasaki S, Kusano K, Kubozono O, Tei C. Acute and chronic effects of smoking on arterial stiffness. Circulation Journal 2011;**75**(3):698-702.

15. Jonas MA, Oates J, Ockene J, Hennekens C. Statement on smoking and cardiovascular disease for health care professionals. American Heart Association. Circulation 1992;**86**(5):1664-1669.

16. Celermajer DS, Adams MR, Clarkson P, Robinson J, McCredie R, Donald A, Deanfield JE. Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. New England Journal of Medicine 1996;**334**(3):150-155.

17. Benowitz NL. Cigarette smoking and cardiovascular disease: pathophysiology and implications for treatment. Progress in cardiovascular diseases 2003;**46**(1):91-111.

18. Doonan RJ, Hausvater A, Scallan C, Mikhailidis DP, Pilote L, Daskalopoulou SS. The effect of smoking on arterial stiffness. Hypertension Research 2010;**33**(5):398-410.

19. Emeson EE, Manaves V, Emeson BS, Chen L, Jovanovic I. Alcohol inhibits the progression as well as the initiation of atherosclerotic lesions in C57Bl/6 hyperlipidemic mice. Alcoholism: Clinical and Experimental Research 2000;**24**(9):1456-1466.

20. Bentzon JF, Skovenborg E, Hansen C, Møller J, de Gaulejac NS-C, Proch J, Falk E. Red wine does not reduce mature atherosclerosis in apolipoprotein E–deficient mice. Circulation 2001;**103**(12):1681-1687.

21. Van Trijp MJ, Beulens JW, Bos WJ, Uiterwaal CS, Grobbee DE, Hendriks HF, Bots ML. Alcohol consumption and augmentation index in healthy young men: the ARYA study. American journal of hypertension 2005;**18**(6):792-796.

22. Mukamal KJ, Kronmal RA, Mittleman MA, O’Leary DH, Polak JF, Cushman M, Siscovick DS. Alcohol Consumption and Carotid Atherosclerosis in Older Adults The

Cardiovascular Health Study. Arteriosclerosis, thrombosis, and vascular biology 2003;**23**(12):2252-2259.

23. Marmot MG, Elliott P, Shipley MJ, Dyer AR, Ueshima H, Beevers DG, Stamler R, Kesteloot H, Rose G, Stamler J. Alcohol and blood pressure: the INTERSALT study. Bmj 1994;**308**(6939):1263-1267.

24. Puddey I, Zilkens R, Croft K, Beilin L. Alcohol and endothelial function: a brief review. Clinical and experimental pharmacology and physiology 2001;**28**(12):1020-1024.

25. Juonala M, Viikari JS, Kähönen M, Laitinen T, Taittonen L, Loo B-M, Jula A, Marniemi J, Räsänen L, Rönnemaa T. Alcohol consumption is directly associated with carotid intima–media thickness in Finnish young adults: the Cardiovascular Risk in Young Finns Study. Atherosclerosis 2009;**204**(2):e93-e98.

26. Seaman P, Ikegwuonu T. Drinking to belong: understanding young adults' alcohol use within social networks. York: Joseph Rowntree Foundation 2010.

27. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. Bmj 1999;**319**(7224):1523-1528.

28. Sierksma A, Patel H, Ouchi N, Kihara S, Funahashi T, Heine RJ, Grobbee DE, Kluft C, Hendriks HF. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-α, and insulin sensitivity. Diabetes Care 2004;**27**(1):184-189.

29. Mathews MJ, Liebenberg L, Mathews EH. The mechanism by which moderate alcohol consumption influences coronary heart disease. Nutrition journal 2015;**14**(1):1.

30. Evans BE, Greaves-Lord K, Euser AS, Tulen JH, Franken IH, Huizink AC. Alcohol and tobacco use and heart rate reactivity to a psychosocial stressor in an adolescent population. Drug and alcohol dependence 2012;**126**(3):296-303.

Figure 1: **Study population.**

Flow chart shows the number of participants who responded to questionnaires exploring smoking and alcohol use from 13-17 years.

Figure 2: The longitudinal association between smoking exposure and arterial stiffness

Increased smoking exposure was associated with higher aortic pulse wave velocity (PWV) compared to those who had never smoked. Participants who quit smoking had similar PWV compared to those who never smoked. *$p<0.05$

Figure 3: The combined effect of smoking over lifetime and intensity of drinking on arterial stiffness.

The combination of high intensity drinking with lifetime smoking exposure is shown. Pulse wave velocity (PWV) measurements are expressed as mean values and 95% confidence intervals around the mean on the X axis. The participants who had "high" drinking intensity and "high" smoking exposure had the highest pulse wave velocity compared to the "low lifetime smoking exposure" and "low drinking intensity". *: $p<0.05$

Table 1: Demographic characteristics according to lifetime smoking exposure and drinking intensity groups.

	"Low/Never" smoking exposure	"Moderate" smoking exposure	"High" smoking exposure	P-value	Low intensity drinkers	Medium intensity drinkers	High intensity drinkers	P value
N (% males)	710 (36.2)	257 (29.2)	281 (31.7)	0.089	154 (31.8)	822 (33.2)	111 (36.04)	0.768
Socioeconomic status (I/V)%	14.3/6.58	15.7/9.17	11.1/9.36	0.012	15.4/6.62	15.2/6.95	9.68/14	0.130
Anthropometric								
Weight (kg)	65.2(11.9)	66.3(12.4)	65.4(12)	0.450	66.2(12.2)	65.0(11.9)	68.3(13.2)	0.018
Height (cm)	171(8.83)	170(8.25)	170(8.92)	0.471	171.2(9.4)	171.3(9.1)	173.1(9.0)	0.010
BMI (kg/m^2)	22.3(3.44)	23(4.02)	22.5(3.71)	0.048	22.9(4.01)	22.4(3.53)	23(4.14)	0.107
*Heart rate (beats/min)	64(57.5-70.9)	65.4(59.6-71.6)	64(57.6-71.8)	0.335	66(58.3-71)	63.8(57.9-71)	65.5(57.8-71.3)	0.746
Risk factors								
Passive smoking, n (%)	187(26.3)	61(23.7)	81(28.8)	0.046	44(28.6)	206(25.1)	34(30.6)	0.588
Family History of CAD, n (%)	47(6.6)	15(5.8)	18(6.4)	0.88	10(6.5)	55(6.7)	3(2.7)	0.351
*Physical activity, mins	446(364-555)	419(365-535)	489(411-616)	0.059	422(352-553)	455(377-565)	418(359-497)	0.897
SBP (mmHg)	116(9.38)	115(8.99)	115(9.21)	0.772	115(9.79)	115(9.03)	116(9.84)	0.508

DBP (mmHg)	63.4(5.71)	64.3(5.74)	63.9(5.86)	0.081	64.2(6.41)	63.5(5.61)	64.3(6.33)	0.191
Lipids								
Tchol (mmol/L)	3.8(0.675)	3.83(0.668)	3.75(0.693)	0.541	3.88(0.803)	3.81(0.666)	3.62(0.617)	0.044
LDL (mmol/L)	2.13(0.615)	2.13(0.585)	2.08(0.654)	0.651	2.2(0.722)	2.13(0.609)	2(0.589)	0.119
HDL (mmol/L)	1.28(0.302)	1.32(0.336)	1.3(0.295)	0.246	1.29(0.32)	1.29(0.302)	1.26(0.325)	0.801
*Triglycerides (mmol/L)	0.79(0.620-1)	0.8(0.600-1)	0.77(0.625-1)	0.793	0.76(0.630-1.02)	0.82(0.620-1)	0.75(0.600-0.940)	0.464
Inflammatory markers								
*CRP (mg/L)	0.6(0.3-1.43)	0.660(0.360-1.28)	0.695(0.405-1.565)	0.371	0.635(0.390-1.55)	0.62(0.320-1.43)	0.69(0.330-1.4)	0.896
Liver function								
*AST (U/L)	19.3(16.6-22.6)	18.8(16.8-22.8)	19.3(16.5-22)	0.615	18.9(16.6-22.9)	19.2(16.5-22.4)	19.7(17-24.1)	0.422
*ALT (U/L)	15.1(11.7-19.2)	14.4(11.5-18.6)	15(11.8-19)	0.809	14.5(11.6-18.6)	14.9(11.6-18.7)	17.2(12.9-21.8)	0.024
*GGT (U/L)	17(13-21)	16(13-21)	16(13-21)	0.930	17(13-22)	17(13-21)	18(14-24)	0.178

Comparisons between groups were performed by one-way ANOVA for continuous variables and chi squared test for categorical variables. Non- parametric variables (*) were log transformed for analysis to approximate normality. Continuous variables are presented as mean(SD).Values for non-parametric data are presented as median(IQR).

Abbreviations: BMI: body mass index, CAD: coronary artery disease, SBP: systolic blood pressure, DBP: diastolic blood pressure, Tchol: total cholesterol, LDL: low density lipoprotein, HDL: high density lipoprotein, CRP: c-reactive protein, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase, SD: standard deviation, IQR: interquartile range

Table 2: Multi-adjusted associations of smoking and drinking behavior on arterial stiffness at 17 years

	N (%males)	Adjusted Coefficient (95% CIs)	*p-value	**p-value
	Cross-sectional analysis			
Smoking intensity	1248 (33.7%)	0.211m/s (0.087/0.334)	0.001	0.008
Drinking intensity	1087 (33.3%)	0.266 m/s (0.055/0.476)	0.013	0.059
Combined smoking & drinking	1072 (33.5%)	0.603 m/s (0.229/0.978)	0.002	0.006
	Longitudinal analysis			
†Smoking	661 (28.8%)	¥0.313 m/s (0.01/0.618)	0.044	0.042
‡Drinking	1023 (34.02%)	¥ 0.029 m/s (-0.210/0.269)	0.812	0.401

All the associations were adjusted for gender, SES, CRP, BMI, SBP and LDL in the cross sectional analysis, plus height in the longitudinal analysis (*p-value). Additional adjustment for parental smoking, family history of CAD and physical activity in the imputed sample is also provided (** p-value).

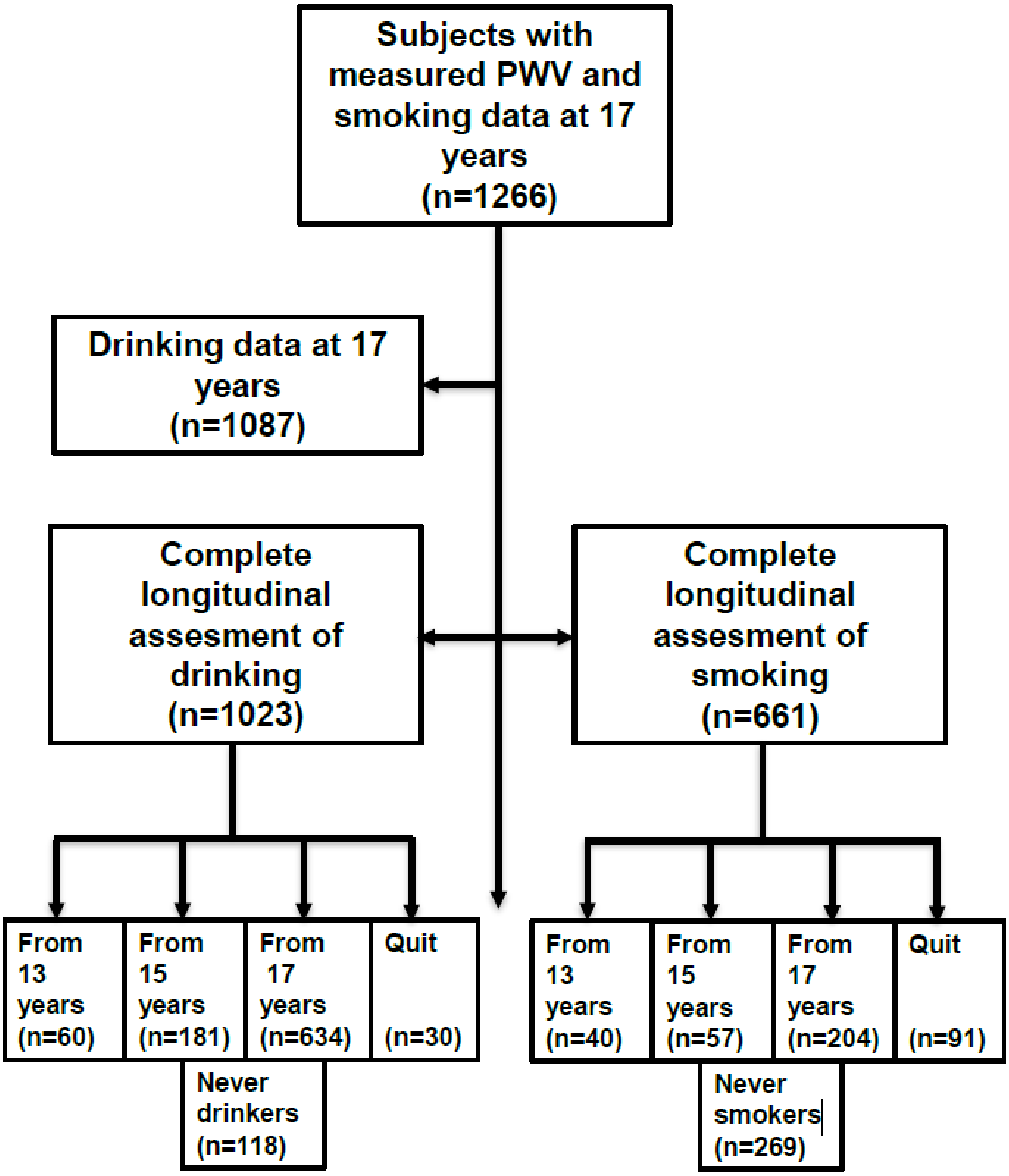
† A 5-group variable was used to encode smoking behaviour during the adolescence as follows: smokers since 13years, smokers since 15years, smokers at 17 years, never smokers and previous smokers that quit

‡ A 5-group variable was used to encode drinking behaviour during the adolescence as follows: drinkers since 13years, drinkers since 15years, drinkers at 17 years, never drinkers and previous drinkers that quit

¥ Coefficient corresponds to the comparison of never smokers/drinkers to smokers/drinkers since 13years

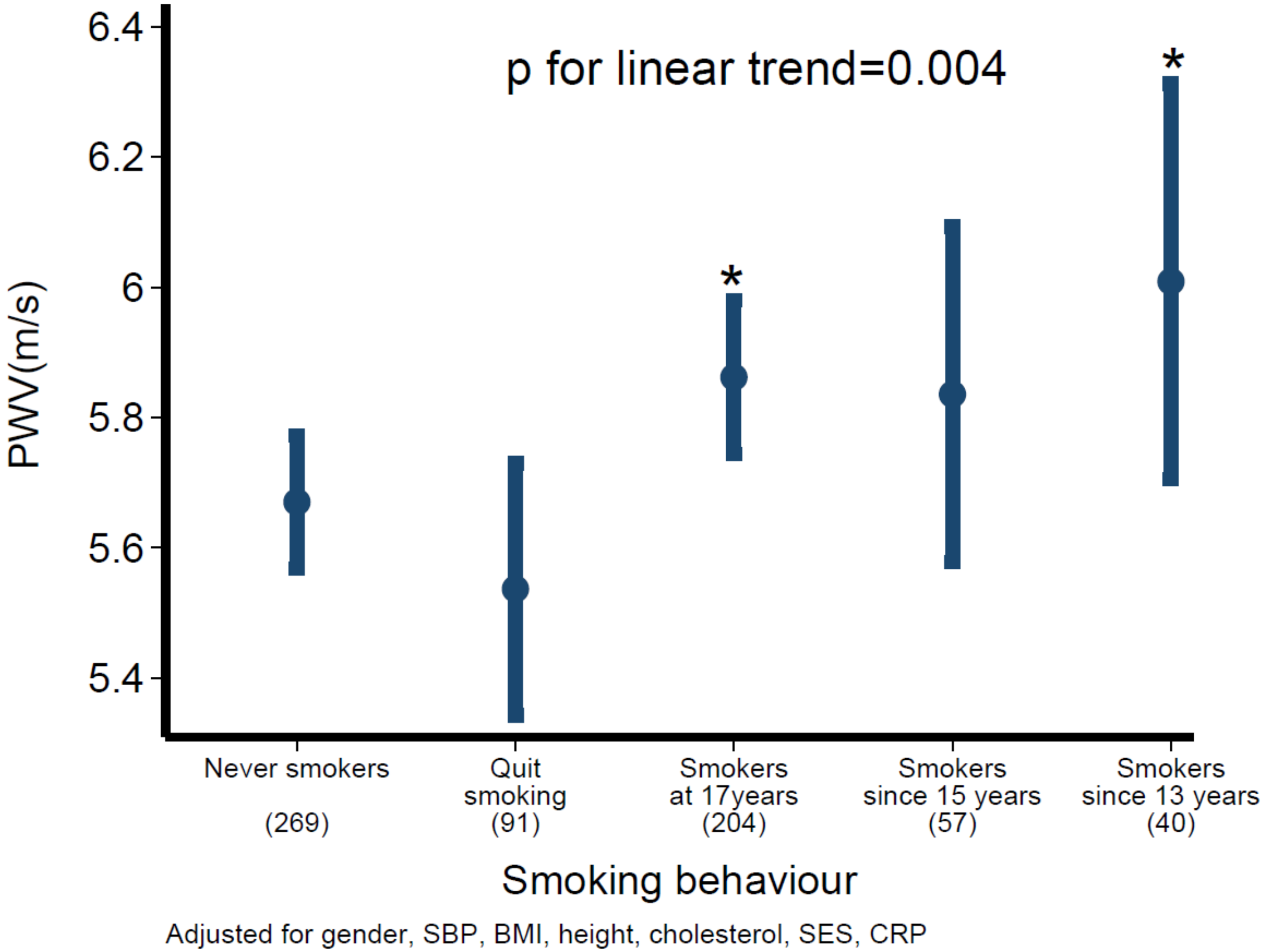
Abbreviations: SES; parental socio-economic status, CRP; C-reactive protein, BMI; body mass index, SBP; systolic blood pressure, LDL; low-density lipoprotein

Figure 1



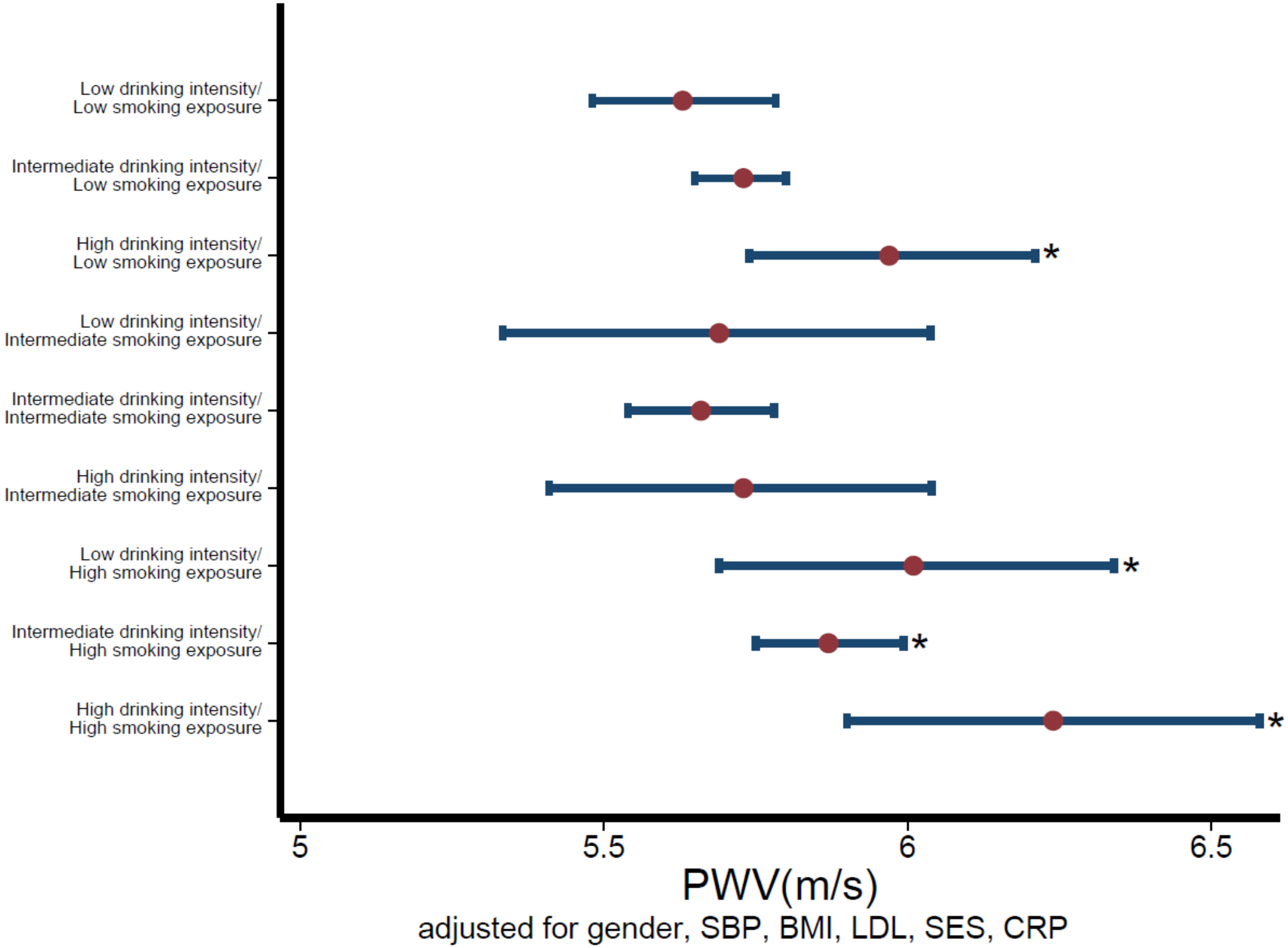
Subjects with measured PWV and smoking data at 17 years (n=1266)
Drinking data at 17 years (n=1087)
Complete longitudinal assesment of drinking (n=1023)
Complete longitudinal assesment of smoking (n=661)
From 13 years (n=60)
From 15 years (n=181)
From 17 years (n=634)
Quit (n=30)
Never drinkers (n=118)
From 13 years (n=40)
From 15 years (n=57)
From 17 years (n=204)
Quit (n=91)
Never smokers (n=269)

Figure 2



6.4
6.2
6
5.8
5.6
5.4
PWV(m/s)
p for linear trend=0.004
*
*
Never smokers
(269)
Quit
smoking
(91)
Smokers
at 17years
(204)
Smokers
since 15 years
(57)
Smokers
since 13 years
(40)
Smoking behaviour
Adjusted for gender, SBP, BMI, height, cholesterol, SES, CRP

Figure 3



Low drinking intensity/
Low smoking exposure
Intermediate drinking intensity/
Low smoking exposure
High drinking intensity/
Low smoking exposure
Low drinking intensity/
Intermediate smoking exposure
Intermediate drinking intensity/
Intermediate smoking exposure
High drinking intensity/
Intermediate smoking exposure
Low drinking intensity/
High smoking exposure
Intermediate drinking intensity/
High smoking exposure
High drinking intensity/
High smoking exposure
*
*
*
*
5
5.5
6
6.5
PWV(m/s)
adjusted for gender, SBP, BMI, LDL, SES, CRP