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

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Abstract (currently 273 words, we need to reduce to 250 words)

**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. Participants were classified as smokers and non-smokers at each period. Smoking intensity was assessed as number of cigarettes smoked in lifetime (“high” ≥100, “moderate” 20-99, and “low or never” <20 cigarettes in lifetime). Participants were classified as low and high frequency drinkers and light (LI < 2), medium (MI 3-9), and heavy (HI > 10 drinks) intensity drinkers respectively. Aortic pulse wave velocity (PWV) was assessed in all participants at 17 years. Estimates for PWV are provided as mean±SD and/or mean difference, 95% confidence intervals [CIs].

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 m/s, 95% CIs 0.087/0.334, p=0.001).

Participants who stopped smoking had similar PWV compared 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**

Higher use of cigarette smoking and consumption of alcohol are associated with increased cardiovascular 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 cardiovascular (CV) benefit for those consuming small or moderate amount of alcohol daily. This has however been questioned in recent studies and meta-analyses\(^3\) 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 to try these substances 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\(^7\). Most of these individuals, however, lack understanding of the health risks associated with cigarette smoking, both in the short and long term. 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 these unhealthy behaviors on contemporary arterial disease is not well documented.

The Avon Longitudinal Study of Parents and Children was set up in the 1991 to provide detailed environmental information on participants from childhood to adolescence and young adulthood and to study the impact on their health\(^8\). The cohort and study design are described in detail elsewhere (http://www.alspac.bris.ac.uk). The cohort has been followed with questionnaires through childhood and at regular annual clinics since the 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

Our study population consisted of adolescents who attended the vascular clinic assessment in ALSPAC 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 in adolescence 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 in lifetime). Cotinine levels were not measured. Adolescence 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 containing alcohol 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 (ie beer, wine and spirits) were evaluated at 17 years by questionnaires, which assessed the number of each of the aforementioned drinks consumed in the last 30 days. 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, respectively.

**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 (SD difference) 0.2 (0.1) m/s).

**Cardiovascular risk factors (Confounders and mediators)**

Blood pressure was measured in the right arm in the seated position using an Omron 705 IT oscillometric BP monitor following a 10 min resting period. Three measurements were taken.
The mean of the last two values was used for analysis. Heart rate was measured as the average of the last 2 readings recorded by the same device. Waist circumference, weight and height were measured using a standardized procedure and body mass index (BMI) was calculated as weight (kg)/height (metre)$^2$. At 17 years, fasting blood samples were taken; all samples were immediately centrifuged and stored at -80°C. Lipid profile (including total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides), inflammatory markers (C-reactive protein (CRP)) were measured as previously described$^9$. Liver function tests, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (GGT) were also measured using colorimetric assay (Roche c311 using the manufacturers calibration and quality control material). Socioeconomic status (SES) was assigned based on father’s occupation and was designated from 1 to 5 according to National Readership Survey (NRS) grading criteria, with class 5 denoting unskilled workers. (http://www.nrs.co.uk/nrs-print/lifestyle-and-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 per valid day of measurement.

**Statistical analysis**

Normally distributed descriptive data are expressed as mean± standard deviation (SD). Normal distribution of the data was assessed by the Kolmogorov-Smirnov test or graphically inspected with histograms and distributional plots. Data with a non-normal distribution were log-transformed to approximate normality prior to parametric testing and are expressed as median (interquartile range)$^{10}$ in descriptive analyses. 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. In particular, we adjusted the association of smoking and drinking behaviour with arterial stiffness for established CV risk factors (i.e. gender, age, family history of cardiovascular disease, LDL cholesterol, systolic blood pressure) and exposure variables which have previously been associated with increased CV risk and arterial stiffness (i.e. parental SES, high-sensitivity CRP, physical activity and parental smoking).

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). Longitudinal data for smoking and drinking behaviour were assumed to be missing at random. Participants with and without longitudinal data on smoking and drinking habits did not differ substantially in anthropometric, biochemical and liver function indices as well as in risk factor distribution (Supplementary Table 1, 2). CRP and LDL, passive smoking and drinking intensity at 17 years presented a substantial proportion of missing values (i.e. 32.4% for biochemical measurements, 52.2%
for passive smoking exposure and 14.1% for drinking pattern, respectively). We replaced missing data for all aforementioned variables by multiple imputation using the MCMC method. We imputed 30 datasets after an adequate burn-in period and we used all available variables in the multivariate model. 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**

At 17 years, 23.8% of participants were smokers. There was no difference in either smoking or alcohol use between males and females. In terms of drinking intensity, 154 (14.2%) participants drank 0-2 drinks/typical day, 822 (75.6%) participants drank 3-9/typical day and 111(10.2%) subjects exceeded 10 drinks/typical day. As far as drinking frequency was concerned, 724 (66.6%) participants were classified as low frequency drinkers (10 never drinkers, 203 monthly or less, 511 drank 2-4 times per month) and 363 (33.4%) as high frequency drinkers (316 drank 2-3 times per week and 47 participants drank ≥4 times per week.

The demographic characteristics, according to smoking and alcohol intensity at 17 years, are summarized in Table 1. 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 (i.e. increased ALT) (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 equal to 3.7% or 0.211 [0.087, 0.334] m/s, p=0.001 as 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_{interaction}=0.308) between gender and smoking exposure on vascular profile.

In 661 participants with complete measurements of smoking status at 13, 15 and 17 years, longitudinal analysis indicated that subjects who had never smoked (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_{linear trend}=0.004) (Figure 2) (Table 2). Notably, 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). When passive exposure to smoke was taken into account, increased duration of smoking was still associated with increased PWV at 17 years (mean increase in PWV 0.211[0.083, 0.340] m/s per category, p_{linear trend}=0.002) compared to never smokers. 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 high intensity drinking was associated with increased PWV compared to low intensity drinking (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] 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). An interaction effect between gender and alcohol consumption towards arterial stiffness was not established (p=0.230). Increased drinking intensity as a proxy to binge drinking (≥10 drinks on a 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 the effect of gender, SBP, SES, LDL, BMI, CRP (mean increase 0.231 [0.057, 0.405]m/s, p=0.01).

In terms of drinking preferences, adolescents in our study consumed more beer drinks compared to wine-based drinks (p<0.001) across a period of one month at 17 years. There was 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. Wine drinking categories were as follows: 558 subjects (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±0.684 m/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±0.690 m/s for heavy versus PWV: 5.62±0.597 m/s for no consumption, 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 in our study. In longitudinal analysis the drinking pattern was not associated with PWV (p>0.05 for all categories) (Table 2).

Higher alcohol intensity and smoking intensity had an additive effect on arterial stiffness (high alcohol and smoking versus never smokers and low alcohol; 5.89±0.857 versus 5.61±0.589, mean increase 0.277m/s [0.028, 0.525], 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], p=0.006)(Table 2).
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 the age of 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 aortic stiffness, but stopping this unhealthy behaviour during adolescence could restore arterial health. In addition to smoking, 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 aortic pulse wave velocity. These data demonstrate the importance of further public health measures, which focus on preventing the establishment of unhealthy behaviours in children and adolescents.

The links between smoking and excessive alcohol use with adverse CV outcomes in adults are well established\textsuperscript{12, 13}. 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 chose to measure carotid to femoral pulse wave velocity (PWV) to assess aortic stiffness 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\textsuperscript{14}. 

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Epidemiological studies have consistently demonstrated that acute and chronic cigarette use adversely affects vascular health in adulthood and promotes atherosclerosis progression\(^\text{15}\). 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 other reports that demonstrate 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\(^\text{16}\). 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\(^\text{17}\). Exposure to parental smoking at home was assessed as binary variable and this has precluded us from evaluating potential differences in the impact of the degree of passive exposure on aortic stiffness.

The pathophysiological mechanisms linking smoking to vascular disease are still not fully elucidated\(^\text{18, 19}\). Tobacco contains a number of toxic and vasoactive compounds, which can exert a direct adverse effect on vascular endothelium and can activate inflammatory and thrombotic pathways relevant to CV events\(^\text{18}\). Although cigarette smoking is discouraged in many countries with legislation and other initiatives, the results of our study confirm that smoking continues to be prevalent in adolescence in both sexes in the UK. Accounting for total number of cigarettes smoked, 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.
Interestingly, 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\textsuperscript{13}.

There are conflicting findings on the impact of alcohol consumption on the vasculature. Alcohol intake has been shown to prevent atherosclerosis in some animal studies\textsuperscript{20,21}, but not in all\textsuperscript{22,23}. Furthermore, results from clinical studies investigating the vascular effects of alcohol consumption are inconsistent\textsuperscript{24-26}. 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\textsuperscript{27}. Mild alcohol consumption, up to 2 alcoholic beverages per day, can independently improve endothelial function both in young healthy subjects and in those with type 2 diabetes\textsuperscript{28,29}. 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\textsuperscript{30}. In support of this, a recent Mendelian randomization meta-analysis implicated alcohol consumption as a causal factor for CV disease\textsuperscript{12}.

In our study, 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\textsuperscript{31}.  

\textsuperscript{13}Recent European CV prevention guidelines.

\textsuperscript{20}Recent European CV prevention guidelines.

\textsuperscript{21}Recent European CV prevention guidelines.

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\textsuperscript{31}Recent European CV prevention guidelines.
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. In contrast, excess alcohol consumption has been associated with elevation in blood pressure, autonomic dysregulation and derangements in coagulation and fibrinolytic pathways. In our study, we did not find any beneficial effect of alcohol consumption on arterial stiffness even at lower consumption levels. Once we analysed separately the different alcoholic beverages, we were able to demonstrate that light and moderate wine drinking groups were associated with lower PWV, consistent with a beneficial effect, whereas beer drinkers had higher PWV. However, these associations lost their significance in multivariable analysis which implies that drinking intensity rather than type of alcoholic beverage may be more influential on arterial stiffness.

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. Interestingly, lower heart rate was noticed in adolescents with high drinking intensity and this might represent autonomic dysregulation.

Our study has limitations, which should be considered when interpreting our results. The study 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; however, such a misclassification 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. Imputation of missing values 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.

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References

Uncategorized References


**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. Asterisk indicates statistically significant (p<0.05) difference in PWV as compared to the reference category (low lifetime smoking exposure and low drinking intensity). No correction for multiple comparisons has been performed.