

Cotinine-assessed second-hand smoke exposure and risk of cardiovascular disease in older adults

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ABSTRACT

Objectives To examine whether second-hand smoke (SHS) exposure measured by serum cotinine is associated with increased coronary heart disease (CHD) and stroke risk among contemporary older British adults.

Design Prospective population-based study with self-reported medical history and health behaviours. Fasting blood samples were analysed for serum cotinine and cardiovascular disease (CVD) risk markers.

Setting Primary care centres in 25 British towns in 1998–2001.

Patients 8512 60–79-year-old men and women selected from primary care registers.

Main outcome measures Fatal and non-fatal myocardial infarction (MI; n=445) and stroke (n=386) during median 7.8-year follow-up.

Main exposure Observational study of serum cotinine assayed from fasting blood sample using liquid chromatography tandem mass spectrometry method, and self-reported smoking history.

Results Among 5374 non-smokers without pre-existing CVD, geometric mean cotinine was 0.15 ng/ml (IQR 0.05–0.30). Compared with non-smokers with cotinine \leq 0.05 ng/ml, higher cotinine levels (0.06–0.19, 0.2–0.7 and 0.71–15.0 ng/ml) showed little association with MI; adjusted HRs were 0.92 (95% CI 0.63 to 1.35), 1.07 (0.73 to 1.55) and 1.09 (0.69 to 1.72), p(trend)=0.69. Equivalent HRs for stroke were 0.82 (0.55 to 1.23), 0.74 (0.48 to 1.13) and 0.69 (0.41 to 1.17), p(trend)=0.065. The adjustment for sociodemographic, behavioural and CVD risk factors had little effect on the results. The HR of MI for smokers (1–9 cigarettes/day) compared with non-smokers with cotinine \leq 0.05 ng/ml was 2.14 (1.39 to 3.52) and 1.03 (0.52 to 2.04) for stroke.

Conclusions In contemporary older men and women, SHS exposure (predominantly at low levels) was not related to CHD or stroke risks, but we cannot rule out the possibility of modest effects at higher exposure levels.

INTRODUCTION

Second-hand tobacco smoke (SHS) exposure appears to increase the risks of coronary heart disease (CHD). Previous prospective studies of self-reported SHS exposure estimated the risk of CHD to be increased by 1.2- to 1.3-fold, independently of established CHD risk factors.^{1,2} These studies were subsequently supported by the results of a large international case–control study of CHD risk factors³ and by ecological studies of CHD mortality and morbidity rates in communities enacting bans on smoking in public places, which tended to find lower CHD rates after implementation of the

ban.^{4–7} Several cohort^{8–10} or case–control^{11–13} studies report increased risks of ischaemic stroke associated with self-reported SHS exposure, though others did not identify significantly increased risks for new-onset stroke¹⁴ in non-smokers, or cerebrovascular mortality¹⁵ in never-smokers.

Self-reported SHS exposure is an imprecise exposure measure. A recent prospective study used serum cotinine, a stable metabolite of nicotine, as a marker of SHS exposure and found that high levels of SHS exposure (serum cotinine levels $>$ 0.7 ng/ml) may increase CHD risk by 1.4- to 1.5-fold.¹⁶ Moreover, a recent cross-sectional study based on data from the Third National Health and Nutrition Examination Survey (NHANES III) showed that low-level SHS exposure (cotinine levels as low as 0.2 ng/ml) may influence inflammatory markers, though the association with CHD events was not studied.¹⁷ These studies suggest that the impact of SHS may have been underestimated in earlier studies and that large prospective studies using cotinine to examine the impact of SHS exposure on CHD and stroke events, particularly at lower cotinine levels and among populations with high CHD and stroke risk, are needed. We have therefore examined the associations between cotinine level, CHD and stroke in parallel studies of older British men and women who were initially studied in 1998–2001, when SHS exposures were markedly lower than those 20 years earlier.¹⁸

METHODS

Study design

In 1998–2000, a total of 4252 men from a single general practice (primary care centre) in each of 24 British towns who were already participating in a prospective study of cardiovascular disease (CVD) attended for follow-up measurements at age 60–79 years (77% response rate).¹⁹ In 1999–2001, a parallel study of 4286 women of the same age and in the same practices was established, with the addition of one study town (Bristol) and the omission of two others (Dewsbury and Maidstone) (60% response rate).²⁰ Almost identical protocols for data collection were used. Ethical approval was provided by all relevant local research ethics committees. All participants provided written informed consent to the investigations, which were carried out in accordance with the Declaration of Helsinki. Participants completed questionnaires including detailed questions about current and previous smoking history (cigarettes, pipes and cigars), other health behaviours, occupation and medication use. Participants reported whether their spouse



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or partner smoked and if they currently lived alone or with their partner. Those cohabiting and reporting that the partner smoked were coded as exposed to SHS at home, while those cohabiting and reporting that the partner did not currently smoke, and those living alone were coded as not exposed to SHS at home. Nurses made physical measurements and collected fasting venous blood samples (see online supplementary data).

Laboratory assays

Serum samples were assayed for cotinine in 2007–8 at ABS Laboratories Ltd. For non-smokers a liquid chromatography tandem mass spectrometry assay with a lower limit of detection of 0.02 ng/ml and with a limit of quantification of 0.1 ng/ml was used.²¹ Cotinine values at the limit of quantification (0.1 ng/ml) were assigned a value of 0.05 ng/ml, as in other studies,²² and statistical sensitivity analyses (using values of 0.025 and 0.075 ng/ml instead) assessed the validity of this assumption. Further details of the assay can be obtained from abslabs@biopark.org.uk. Assays for blood lipids and inflammatory markers are described in the online supplementary data.

Classification of smokers and non-smokers

Non-smokers reported no current cigarette, cigar or pipe smoking or any smoking in the past 5 years and had serum cotinine levels ≤ 15 ng/ml, consistent with other literature.²² Among 7161 self-reported non-smokers, 7085 (99%) had cotinine levels ≤ 15 ng/ml, the remaining 76 (1%) participants with cotinine > 15 ng/ml were recoded as smokers of 1–9 cigarettes/day, as their cotinine levels were similar to those of the other ($n=230$) light smokers. No participants reported taking nicotine replacement therapy (British National Formulary code 4.10).²³

CHD and stroke outcome

The outcome measures used were first fatal or non-fatal event of (a) myocardial infarction (MI) and (b) stroke occurring after the 1998–2000 survey (men) and 1999–2001 survey (women) and up to June 2008 (men) and September 2007 (women). Fatal cases were ascertained through the National Health Services Central Registers (death certificates with ICD-9 (International Classification of Diseases) codes 410–414 for MI and 430–438 for stroke and ICD 10 codes I21–I23, I252 for MI and I60, I61, I62, I629, I63–I66, I67, I672, I678, I679, I69, G450–453, G46 for stroke). Non-fatal events were recorded from 2-yearly reviews of patient primary care notes (including details of patient encounters with primary care and all correspondence and diagnoses from secondary care). MI was reported as heart attack or coronary thrombosis, diagnosed in accordance with WHO criteria; stroke was reported as cerebrovascular accident, cerebral thrombosis, haemorrhage or embolism.

Statistical methods

Serum cotinine was highly positively skewed and therefore analysed as a categorical variable. The threshold for the highest exposure group (> 0.7 ng/ml) was chosen because cotinine > 0.7 ng/ml has been reported to be associated with increased CHD risks¹⁶ and because of the generally low cotinine levels in non-smokers in our study, there were too few participants to further subdivide those above this level. The lowest group had undetectable exposure (≤ 0.05 ng/ml) and participants with intermediate exposure were split into two equal-sized groups. Analyses were also completed with cotinine as a continuous variable that was log transformed (to base 2) to approximate normality.

Means, medians or proportions of behavioural and demographic factors selected a priori were calculated for groups of passive smokers (defined by cotinine level) and active smokers (defined by cigarettes/day). Linear trends across cotinine categories were tested using linear regression analyses with \log_2 cotinine as a predictor, to represent the effect of a doubling of cotinine concentration, adjusted for age, gender and region of residence. Skewed variables were natural log-transformed and adjusted for time of measurement if they showed significant diurnal variation. Blood pressure (BP), body mass index (BMI) and forced expiratory volume in 1 s (FEV₁) were also adjusted for intraobserver variation. No diurnal or seasonal (October–March vs April–September) variation in cotinine levels was seen.

Cox proportional hazards regression models were used to estimate associations between serum cotinine and risk of CHD or stroke in non-smokers with complete data on covariates. Survival times were censored at date of MI or stroke, death from any cause, or end of follow-up period, whichever occurred first. Date of entry into the study was used as the time origin. The proportional hazards assumption was examined using time-varying covariates, calculating interactions of predictor variables and a function of survival time and including them in the models. Examination of time-varying covariates did not indicate violation of the proportionality assumption in the non-smoker sample. The HRs for categories of cotinine exposure compared with cotinine ≤ 0.05 were estimated and the overall association in non-smokers was tested with the continuous association between \log_2 cotinine and CHD or stroke risk, adjusted for gender, age (continuous variable) and region of residence. Models were adjusted for covariates associated with both CHD risk and SHS, first established biological risk factors and inflammatory markers as continuous variables: systolic and diastolic BP, total and high-density lipoprotein cholesterol, BMI, FEV₁, natural log triglycerides and white cell count, C-reactive protein (CRP) and interleukin 6 (IL-6). Models were then further adjusted for pre-existing diabetes (yes/no) and behavioural risk factors as categorical variables: physical activity (inactive (< 3 h moderate or vigorous activity/week) or active), alcohol intake (none/occasional, light, heavy), social class (I and II, III non-manual, III manual, IV and V manual and armed forces). Interactions with gender and age were tested using likelihood ratio (LR) tests. In a sensitivity analysis, regression models excluded past smokers from the non-smoker group. All hypothesis tests were two sided and significance levels are reported.

RESULTS

Among 8512 participants (4267 women, 4245 men), 7375 had questionnaire data on cigarette smoking and cotinine data. Participants with a history (self-report or medical record) of MI or stroke ($n=1168$), current smokers of ≥ 10 cigarettes/day ($n=276$) and recent ex-smokers (within 5 years) ($n=251$) were excluded from analyses. The analysis sample included 5374 non-smokers (of whom 2783, 52% were never-smokers) and, as a comparison group, 306 smokers of 1–9 cigarettes/day. In the analysis sample of 5680 participants, there were 217 new MI cases (192 in non-smokers) and 176 new stroke cases (165 in non-smokers) in mean follow-up times of 8.3 years (men) and 7.0 years (women). Geometric mean cotinine level was 104.57 ng/ml (IQR 66–227) in active smokers (1–9 cigarettes/day) and 0.15 ng/ml (IQR 0.05–0.30) in non-smokers (p no difference < 0.001). Of the 5374 non-smokers, 2256 (42%) had undetectable cotinine levels (≤ 0.05 ng/ml). Table 1 shows the characteristics of SHS-exposed non-smokers and of active

Table 1 Association between serum cotinine in non-smokers (≥ 5 years since quitting) or active smokers and cardiovascular disease risk factors in men and women (mean (SD) or %(n))*

Risk factors	N	Non-smokers (serum cotinine ng/ml)				Smokers 1–9 Cigarettes/day (n=306)	p(trend) [†] In non-smokers	p(difference) [‡] Cotinine ≤ 0.05 vs smokers
		≤ 0.05 (n=2256)	0.06–0.19 (n=1248)	0.20–0.70 (n=1184)	0.71–15 (n=686)			
Cotinine (ng/ml) [‡]	5680	0.05	0.14	0.34	1.69	104.57	–	–
Age (years)	5680	69.3 (5.5)	68.4 (5.5)	68.2 (5.5)	68.1 (5.4)	69.1 (5.3)	<0.001	0.526
Men % (n)	5680	38.3 (865)	45.1 (563)	51.6 (611)	55.5 (381)	39.4 (121)	<0.001	0.516
Southern region of residence, %	5680	43.4 (981)	32.2 (402)	27.4 (324)	20.7 (142)	30.4 (93)	<0.001	0.001
Manual occupational class, %	5345	41.0 (880)	45.5 (539)	56.5 (626)	65.7 (418)	55.3 (147)	<0.001	<0.001
Never/occasional alcohol use, %	5397	52.1 (1128)	48.0 (571)	41.7 (475)	40.1 (256)	54.5 (145)	<0.001	0.589
>43 Alcohol units/week, %	5397	0.4 (9)	0.3(4)	1.6 (18)	4.2 (27)	0.8 (2)	<0.001	0.518
Never-smokers, %	5680	56.7 (1278)	54.1 (675)	46.6 (552)	40.5 (278)	–	<0.001	–
Low level of physical activity, % (< 3 h moderate/vigorous /week)	5645	62.9 (1377)	59.6 (721)	61.2 (698)	62.4 (406)	74.6 (206)	0.002	<0.001
Pre-existing diabetes, %	5680	10.2 (230)	8.5 (106)	11.5 (136)	10.5 (72)	9.8 (30)	0.762	0.791
Body mass index (kg/m ²)	5647	26.9 (4.3)	27.4 (4.1)	27.8 (4.5)	27.8 (4.5)	25.8 (3.9)	<0.001	<0.001
Systolic Blood Pressure (mm Hg) [§]	5646	149.5 (24.3)	147.7 (24.5)	147.6 (24.1)	150.3 (24.1)	146.1 (25.4)	0.690	0.018
Diastolic Blood Pressure (mm Hg) [§]	5646	82.3 (11.5)	82.0 (11.4)	82.7 (12.1)	84.1 (11.9)	80.8 (11.6)	0.096	0.030
Total cholesterol (mmol/l)	5648	6.5 (1.2)	6.4 (1.2)	6.3 (1.1)	6.3 (1.2)	6.4 (1.2)	0.131	0.353
HDL cholesterol (mmol/l)	5629	1.6 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.4)	1.5 (0.5)	0.160	0.151
Triglyceride (mmol/l) ^{‡¶}	5495	1.6	1.6	1.6	1.6	1.7	0.100	0.016
C-reactive protein (mg/l) [‡]	5601	1.5	1.5	1.7	1.9	2.3	<0.001	<0.001
IL-6 (pg/ml) [‡]	5640	2.2	2.2	2.3	2.5	3.1	<0.001	<0.001
White cell count ($\times 10^9/l$) ^{‡¶}	5418	6.6	6.7	6.7	6.7	7.8	0.062	<0.001
FEV ₁ ^{§**}	5680	2.3 (0.5)	2.3 (0.5)	2.3 (0.5)	2.2 (0.6)	2.0 (0.6)	<0.001	<0.001

*Non-smoker sample excludes (i) current cigarette, pipe or cigar smokers, (ii) ex-smokers who quit < 5 years ago, (iii) pre-existing myocardial infarction or stroke. Means are adjusted for age, gender and region;

[†]p(trend) from linear regression models of ln(cotinine) in non-smokers, p(difference) from linear regression models contrasting cotinine < 0.05 with 1–9/day smokers. Models adjusted for age, gender and region;

[‡]geometric mean;

[§]adjusted for interobserver variation;

[¶]Adjusted for time of day;

**adjusted for height squared and age.

FEV₁, forced expiratory volume in 1 s; HDL, high-density lipoprotein; IL, interleukin.

smokers, both compared with the baseline group of non-smokers with undetectable SHS exposure (cotinine ≤ 0.05 ng/ml). Active smokers tended to be resident in the north of the UK, from manual social class and physically inactive compared with non-smokers with undetectable SHS exposure. Non-smokers with higher serum cotinine levels, indicating greater SHS exposure, were also resident in the north of the UK, from manual social class, physically inactive and younger, male, ex-smokers rather than never-smokers, and less likely to be non-drinkers or occasional alcohol drinkers compared with non-smokers with undetectable SHS exposure. Among non-smokers, cotinine level was positively and significantly associated with higher CRP, IL-6 and BMI and with lower FEV₁. Blood pressure, lipids and pre-existing diabetes showed no association with cotinine. Active smokers, had lower BMI, systolic BP, diastolic BP and FEV₁ but higher triglycerides, CRP, IL-6 and white cell count than non-smokers with undetectable SHS exposure.

In Cox proportional hazard models examining the association between cotinine level and CHD risk among non-smokers and adjusted for age, gender and region of residence (table 2, model 1), a doubling of cotinine level was not strongly associated with CHD risk: HR 1.02 (95% CI 0.95 to 1.11), $p=0.56$. Increasing cotinine categories showed no appreciable association with CHD risk when compared with participants with cotinine ≤ 0.05 ng/ml. Further adjustments for established cardiovascular risk factors and inflammatory markers (model 2) and pre-existing diabetes, social class and behavioural risk factors (model 3) did not materially alter the results. LR tests showed no evidence for interactions of cotinine on risk of MI by gender ($p=0.94$) or age ($p=0.65$). Exclusion of ex-smokers did not

materially affect the results. In contrast, active smokers, when compared with the same reference group (non-smokers with cotinine ≤ 0.05 ng/ml) had a HR for MI of 2.53 (95% CI 1.61 to 3.99), reducing slightly to 2.14 (95% CI 1.39 to 3.52) after adjustment for established risk factors (equivalent to model 3 in table 2).

In Cox proportional hazard models examining the association between cotinine level and stroke risk among non-smokers and adjusted for age, gender and region of residence (table 3, model 1), increasing cotinine levels showed a weak inverse association with stroke risk compared with participants with cotinine ≤ 0.05 ng/ml. A doubling of cotinine level was associated with a decrease in stroke risk of 0.91 (95% CI 0.83 to 1.01), $p=0.07$. Further adjustments for established cardiovascular risk factors, social class and inflammatory markers (models 2 and 3) did not materially alter the results. LR tests showed no evidence for interactions of cotinine on risk of stroke by gender ($p=0.92$) or age ($p=0.83$). Exclusion of ex-smokers did not materially affect the results. Active smokers, when compared with the same reference group (non-smokers with cotinine ≤ 0.05 ng/ml) had a HR for stroke of 1.13 (95% CI 0.60 to 2.13), reducing to 1.03 (95% CI 0.52 to 2.04) after adjustment (equivalent to model 3 in table 3).

Analyses presented were not influenced either by the cotinine threshold used to define active smoking (use of a more conservative cotinine threshold (9.5 ng/ml)²⁴ did not materially affect results), or by the duration of follow-up period (HRs did not differ between early and late follow-up periods).

Among participants with data on self-reported exposure to SHS in the home (n=4818), 485 (10%) lived with a spouse/

Table 2 Hazard ratios (HRs) for risk of myocardial infarction (MI) in men and women by serum cotinine or active smoking level

	Non-smokers (serum cotinine ng/ml) (n = 4608)*				Smokers (n = 242*) 1–9 Cigarettes/day	All non-smokers Cotinine ≤15 ng/ml†	p(trend)†
	≤0.05	0.06–0.19	0.20–0.70	0.71–1.5			
Mean cotinine (ng/ml)	0.05	0.14	0.34	1.71	100.02	0.15	
Number of participants	1943	1087	1018	560	242	4608	
Number of events	77	40	47	28	25	192	
Person-years	14641	8323	7902	4333	1701	35199	
CHD rates/1000	5.26	4.81	5.95	6.46	14.67	5.45	
HR (95% CI)							
Model 1	1.00	0.92 (0.63 to 1.35)	1.09 (0.76 to 1.58)	1.11 (0.72 to 1.73)	2.53 (1.61 to 3.99)	1.02 (0.95 to 1.11)	0.562
Model 2	1.00	0.92 (0.62 to 1.35)	1.09 (0.75 to 1.58)	1.09 (0.70 to 1.71)	2.07 (1.26 to 3.40)	1.02 (0.94 to 1.10)	0.644
Model 3	1.00	0.92 (0.63 to 1.35)	1.07 (0.73 to 1.55)	1.09 (0.69 to 1.72)	2.14 (1.39 to 3.52)	1.02 (0.94 to 1.11)	0.690
Excluding former smokers			(n = 2321)		(n = 239)		
Mean cotinine (ng/ml)	0.05	0.14	0.33	1.76	100.02	0.13	
Number of participants	1081	572	458	210	239	2321	
Number of events	34	17	16	7	25	74	
Person-years	8034	4349	3521	1582	1687	17486	
CHD rates/1000	4.23	4.35	4.54	4.42	14.82	4.23	
HR (95% CI)							
Model 1	1.00	0.90 (0.50 to 1.62)	1.03 (0.56 to 1.88)	1.01 (0.44 to 2.32)	2.52 (1.48 to 4.29)	1.01 (0.88 to 1.17)	0.858
Model 2	1.00	0.90 (0.49 to 1.60)	0.98 (0.53 to 1.81)	0.95 (0.41 to 2.20)	2.10 (1.17 to 3.76)	1.00 (0.87 to 1.16)	0.985
Model 3	1.00	0.91 (0.50 to 1.63)	0.99 (0.53 to 1.84)	0.94 (0.40 to 2.24)	2.17 (1.20 to 3.90)	1.00 (0.86 to 1.16)	0.975

*HRs for non-smokers from models with cotinine ≤0.05 as baseline. HRs for smokers from models comparing cotinine ≤0.05 with smokers of 1–9 cigarettes/day. Participants with pre-existing myocardial infarction are excluded.

†From Cox regression models of log₂(cotinine) level (representing a doubling in cotinine) and CHD in non-smokers, cotinine ≤15 ng/ml.

Model 1 = age+gender+region; Model 2 = model 1+BMI+SBP+DBP+total cholesterol+HDLc+triglycerides+FEV₁+CRP+IL-6+white cell count; Model 3 = model 2+pre-existing diabetes+physical activity (inactive or active)+alcohol intake (none/occasional, light, heavy)+smoking history (never/ time since quitting)+social class (non-manual, manual and armed forces). CHD, coronary heart disease; CRP, C-reactive protein; FEV₁, forced expiratory volume in 1 s; HDLc, high-density lipoprotein cholesterol; IL, interleukin.

partner who smoked and 4333 (90%) did not live with a partner who smoked; geometric mean cotinine in these groups were respectively 0.13 ng/ml (IQR 0.05–0.25) and 0.70 ng/ml (IQR 0.22–2.4). Non-smoking participants who lived with a smoker had a HR for CHD of 1.33 (95% CI 0.82 to 2.15) and a HR for stroke of 0.53 (95% CI 0.25 to 1.16) compared with those not living with a smoker, adjusted for age, gender and region. Further adjustments for established cardiovascular risk factors, social

class and inflammatory markers did not materially alter the results and there was no evidence of effect modification by gender or age. Restricting analyses to never-smokers, in the sample with full covariate data: 110 (7%) lived with a smoker and 1457 (93%) did not, geometric mean cotinine levels were respectively 0.12 ng/ml (IQR 0.05–0.21) and 0.74 ng/ml (IQR 0.25–2.46). Never-smoking participants who lived with a smoker compared with those who did not live with a smoker

Table 3 Hazard ratios (HRs) for risk of stroke in men and women by serum cotinine or active smoking level

	Non-smokers (serum cotinine ng/ml) (n = 4405)*				Smokers (n = 228) 1–9 Cigarettes/day	Non-smokers Cotinine ≤15 ng/ml†	p(trend)†
	≤0.05	0.06–0.19	0.20–0.70	0.71–1.5			
All participants							
Mean cotinine (ng/ml)	0.05	0.14	0.34	1.70	100.01	0.15	
Number of participants	1858	1031	975	541	228	4405	
Number of events	80	34	32	19	11	165	
Person-years	13990	7901	7593	4182	1602	33666	
Stroke rates/1000	5.72	4.30	4.21	4.54	6.87	4.90	
HR (95% CI)							
Model 1	1.00	0.78 (0.52 to 1.17)	0.73 (0.48 to 1.10)	0.74 (0.44 to 1.23)	1.13 (0.60 to 2.13)	0.91 (0.83 to 1.01)	0.051
Model 2	1.00	0.81 (0.54 to 1.22)	0.75 (0.50 to 1.15)	0.69 (0.41 to 1.16)	1.03 (0.52 to 2.04)	0.91 (0.83 to 1.00)	0.051
Model 3	1.00	0.82 (0.55 to 1.23)	0.74 (0.48 to 1.13)	0.69 (0.41 to 1.17)	1.03 (0.52 to 2.04)	0.91 (0.82 to 1.01)	0.065
Excluding former smokers			(n = 2232)		(n = 228)		
Mean cotinine (ng/ml)	0.05	0.14	0.33	1.74	100.01	0.13	
Number of participants	1036	550	441	205	230	2232	
Number of events	37	17	10	6	11	70	
Person-years	7717	4162	3401	1550	1615	16830	
Stroke rates/1000	4.79	4.08	2.94	3.87	6.81	4.16	
HR (95% CI)							
Model 1	1.00	0.87 (0.49 to 1.55)	0.62 (0.30 to 1.26)	0.86 (0.36 to 2.07)	1.07 (0.53 to 2.15)	0.95 (0.81 to 1.11)	0.526
Model 2	1.00	0.90 (0.50 to 1.62)	0.60 (0.29 to 1.22)	0.78 (0.32 to 1.89)	1.12 (0.50 to 2.47)	0.94 (0.80 to 1.10)	0.407
Model 3	1.00	0.91 (0.50 to 1.62)	0.61 (0.30 to 1.26)	0.81 (0.33 to 1.99)	1.21 (0.55 to 2.65)	0.94 (0.80 to 1.11)	0.476

*HRs for non-smokers from models with cotinine ≤0.05 as baseline. HRs for smokers from models comparing cotinine ≤0.05 with smokers of 1–9 cigarettes/day. Participants with pre-existing myocardial infarction or stroke are excluded.

†From Cox regression models of log₂(cotinine) level (representing a doubling in cotinine) and stroke in non-smokers, cotinine ≤15 ng/ml.

Model 1 = age+gender+region; Model 2 = model 1+BMI+SBP+DBP+total cholesterol+HDLc+triglycerides+FEV₁+CRP+IL-6+white cell count; Model 3 = model 2+pre-existing diabetes+physical activity (inactive or active)+alcohol intake (none/occasional, light, heavy)+smoking history (never/ time since quitting)+social class (non-manual, manual and armed forces). CHD, coronary heart disease; CRP, C-reactive protein; FEV₁, forced expiratory volume in 1 s; HDLc, high-density lipoprotein cholesterol; IL, interleukin.

had an age, gender and region adjusted HR for CHD of 2.17 (95% CI 0.97 to 4.84) strengthening to 2.41 (95% CI 1.04 to 5.59) on full adjustment, and a fully adjusted HR for stroke of 0.69 (95% CI 0.16 to 2.94). There was no evidence for effect modification by age, although data were too sparse to estimate interactions with gender.

DISCUSSION

Main findings

In this population of older men and women with low levels of SHS exposure (indicated by low mean levels of serum cotinine among non-smokers and also the small prevalence (just 10%) of self-report of living with a partner who smoked) there was little evidence for an association between SHS exposure and either MI or stroke, irrespective of whether these were never-smokers or ex-smokers. These findings did not vary by age or by gender. However, there was evidence of a raised risk of MI in never-smokers who lived with a smoker, though the confidence limits are wide and this was a post hoc analysis.

Relation to earlier studies

Previous studies and meta-analyses have shown that SHS exposure, particularly defined by partner smoking, is associated with a 25–30% increase in risk of CHD,^{1 2 25} an estimate recently supported by the INTERHEART Study.³ Evidence from cotinine-based studies suggests that the increase in CHD risk at high overall SHS exposure (particularly at exposure levels associated with cotinine concentrations >0.7 ng/ml) may exceed these earlier estimates, and be as high as 45–50%.¹⁶ Subsequently, cross-sectional studies relating cotinine levels to intermediate markers such as fibrinogen and white cell count have suggested that the effects of cotinine on cardiovascular risk might begin at SHS exposure levels associated with lower cotinine concentrations (<0.7 ng/ml).^{17 26}

Our investigation is the first to provide data on the association of both CHD and stroke risk with lower cotinine levels (<0.7 ng/ml) resulting from lower nicotine exposure due to public health measures. The low overall SHS exposure levels (24% of non-smokers have cotinine levels between 0.06 and 0.70 ng/ml) mean our cohorts are well placed to provide information about any continued cardiovascular risk in contemporary groups with low SHS exposure. In line with a case–control study based on self-reported lifetime SHS exposure in the context of falling SHS exposure levels,²⁷ our results provide little support for an increased risk of CHD or stroke associated with low levels of cotinine exposure. However, we are unable to exclude adjusted HRs for CHD as high as 1.53, when comparing cotinine levels between 0.06 and 0.70 ng/ml with cotinine ≤0.05 ng/ml. We had limited information on the effects of higher SHS exposure, associated with cotinine levels 0.71–15 ng/ml; only 13% of non-smokers had a cotinine level in this range. Higher SHS exposure appeared to be associated with a very modest increase in CHD risk and could only be imprecisely estimated. The results are, however, consistent with the increase in CHD risk of 45% associated with such cotinine levels in our earlier study, although we reported even greater elevations in risk over shorter follow-up periods.¹⁶

The pattern of overall association between cotinine level and CHD risk among non-smokers in this study (expressed as a 2% (–5% to 11%) increase in CHD risk for each doubling of cotinine concentration) is weaker than in the previous study, where the equivalent estimate was 16% (6% to 27%).¹⁶ This study population is appreciably older than in the previous study and includes both genders. Although within this study neither

gender nor age appreciably influenced cotinine–CHD associations, the relative strengths of associations between many major risk markers (especially active smoking) and CHD are recognised to attenuate with increasing age,^{28 29} so our estimates may be lower than estimates which would be observed in a middle-aged population. The point estimate of the effect of partner smoking on CHD risk in never-smokers is considerably stronger than in the published literature, while the association with stroke is weaker.¹ Both estimates were post hoc and based on small numbers and had wide confidence limits.

The literature for stroke is less conclusive than for CHD and fewer previous reports exist. Two case–control studies^{11 12} and three prospective cohort studies^{8–10} reported positive associations between self-reported SHS exposure and stroke risk, although not all studies do. One prospective cohort had limited power to detect an effect on cerebrovascular mortality as distinct from cardiovascular mortality¹⁵ and another had few cases of stroke among non-smoking women.¹⁴ Other studies did not find significantly elevated risks of subarachnoid haemorrhage³⁰ or silent cerebral infarction³¹ associated with SHS exposure from living with a spouse who smokes. Our previous prospective study showed no evidence of an association between cotinine level in non-smokers and stroke risk. In this study, no association was found between stroke and SHS exposure measured by cotinine, over a very wide range of exposure (cotinine from 0.05 to 15 ng/ml), or measured by living with a spouse who smoked. However, our analyses have limited precision and cannot exclude the possibility of a moderately strong association. The lack of association with stroke is consistent with our finding in this population of no association of cotinine with variation in BP, an association that was precisely estimated (table 1). By contrast, cotinine was associated with several CHD risk factors, including inflammatory markers, despite not showing a strong association with CHD events. We did not observe the expected associations between active smoking and stroke, perhaps because we only studied smokers of 1–9 cigarettes/day and not heavier smokers.

Strengths and weaknesses

A key strength of this study is the use of serum cotinine as an objective measure of SHS exposure. Cotinine is the major metabolite (70–80%) of nicotine, reflecting recent overall SHS exposure, which enables more accurate estimates of the associations between SHS exposure and CVD than the use of self-reported exposures, which may only partially capture exposures from different sources.^{32 33} Nicotine is metabolised into cotinine by cytochrome P450. Although genetic factors controlling nicotine metabolism may influence cotinine levels,³⁴ unless these are systematically associated with CHD or stroke risk (for which there is little current evidence), they should not bias the estimation of cotinine–CHD associations.

While it is possible that other environmental influences on cotinine levels exist, the evidence for them is weak at the present time and we have included adjustment for BMI and alcohol intake in our models, which did not have any important effect. Cotinine is well validated as a biomarker of nicotine exposure³³ and also prevents appreciable misclassification of active smokers who fail to self-report smoking. We reclassified 76 participants who reported being non-smokers but had cotinine levels >15 ng/ml (a standard cut-off point) as active smokers. Including the 76 participants in the analysis in the highest exposure group did not materially alter any of our results. This study was large, combining cohorts of older men and women from socially and geographically representative population studies. However, the

removal of a substantial number of subjects with pre-existing disease and low event rates limited the statistical power of the study to detect small but important elevations in CVD risk. The results are based entirely on older populations of retirement age, exposed to contemporary SHS levels and at relatively high CVD risk and may not be applicable to younger subjects or to populations with higher SHS exposure. In particular, this older population is less likely to be exposed to SHS in the workplace and may be less likely to be exposed in leisure public places than younger populations.

CONCLUSIONS

The results suggest that the lower levels of cotinine prevailing in the UK in 1998–2001, even before the introduction of legislation to reduce SHS exposure in public places, were not apparently associated with any marked increase in CHD or stroke risk in older people. Our findings relating to low levels of SHS exposure do not undermine previous epidemiological studies reporting positive associations between SHS exposure and CHD or stroke events, or the weight of laboratory studies suggesting causal associations between SHS and elevated CHD risk. However, because of the limited statistical power of our study and the older age range of the population, further evidence from pooled analyses of large population studies, including people of working age, are needed to establish the associations between low-level SHS exposure and CHD risk. In addition, studies specifically examining the continuing impact of domestic partner smoking, which is the main determinant of high SHS exposure in this population,¹⁸ would be valuable.

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