Lu, Liya (2017) *Association between active smoking, secondhand smoke and peripheral arterial disease.* PhD thesis.

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Association between active smoking, secondhand smoke and peripheral arterial disease

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Thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (PhD)

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March 2017

Abstract

Worldwide, cardiovascular disease (CVD) is the leading cause of death. It is widely accepted that both active smoking and exposure to secondhand smoke (SHS) are associated with CVD. About 20% of the global population smoke tobacco or tobacco-related products. The global prevalence of smoking is increasing, although it is decreasing in some high-income and upper middle-income countries. Globally, about a third of adults and 40% children are regularly exposed to SHS. According to the World Health Organisation (WHO), only 16% of the global population is protected by a comprehensive smoke-free legislation. Coronary heart disease (CHD), stroke and peripheral arterial disease (PAD) are all types of atherosclerosis and often co-exist in the same patients. Therefore, they share many common risk factors including cigarette smoking. However, previous epidemiological studies on CVD including those on cigarette smoking mainly focused on CHD and stroke and pay little attention to PAD. Evidence is increasing in support of the association between exposure to SHS and both CHD and stroke. In contrast, there is a paucity of studies on SHS and the risk of PAD. The overarching aim of this thesis was to collate the published evidence on the association between active cigarette smoking and PAD, and examine the association between exposure to SHS and PAD in the general population.

This thesis starts with a systematic review on the association between active cigarette smoking, SHS and PAD undertaken using four databases: Medline, Embase, PubMed and Web of Science to identify existing published evidence up to 30 April 2012 (Chapter 2). Prior to the published studies contained in this thesis, there had been no meta-analyses on the association between active cigarette smoking and PAD and only two studies published on the association between SHS and PAD. Therefore, this systematic review was followed by a meta-analysis on the association between active cigarette smoking and PAD. This meta-analysis identified 55 studies: 43 cross-sectional, 10 cohort and 2 case-control. Of the 68 results for current smokers, 59 (86.8%) were statistically significant and the pooled odds ratio (OR) was 2.72 (95% confidence interval [CI] 2.28-3.21). Of the 40 results for ex-smokers, 29 (72.5%) were statistically significant and the pooled OR was 1.67 (95% CI 1.54-1.81). Active cigarette smoking significantly increases the risk of PAD, compared with never smokers. The magnitude of association between active cigarette smoking and PAD was greater in current smokers than ex-smokers.

In contrast, prior to my studies in this thesis, only two studies on SHS were identified. Only one showed an overall association between self-report SHS and PAD in Chinese never smokers, with a clear dose-response relationship. The other study used serum cotinine as measure for SHS exposure and found neither an overall association nor a dose-response relationship but suggested a very high cotinine concentration as threshold.

Chapter 3 examines the association between SHS exposure and PAD in adult non-smokers in Scotland. This chapter includes two cross-sectional studies using the Generation Scotland: Scottish Family Health Study (GS: SFHS) and the Scottish Health Survey (SHeS), and one retrospective cohort study using the record linkage of the SHeS. In the cross-sectional study using SFHS, PAD was measured using ankle brachial pressure index (ABPI) but SHS exposure was self-report. Of the 5,686 never smokers, 134 (2.4%) had PAD (defined as an ABPI <0.9). Participants who reported overall high level of SHS exposure (exposed to ≥40 hours per week) were more likely to have PAD, compared with those who reported no exposure to SHS. After adjustment for potential confounders, the association between SHS and PAD persisted (adjusted OR 4.53, 95% CI 1.51-13.56, p=0.007), with suggestion of a dose-response relationship. In the other cross-sectional study using SHeS, SHS exposure was measured objectively using cotinine concentration but PAD was based on self-report symptoms of intermittent claudication (IC) using the Edinburgh Claudication Questionnaire. Of the 4,231 non-smokers (defined as self-reported non-smokers with a salivary cotinine concentration <15 ng/mL), 134 (3.2%) had IC. Participants with high exposure to SHS (cotinine ≥2.7 ng/mL) were at significantly higher risk of IC, after adjustment for potential confounders (adjusted OR 1.76, 95% CI 1.04-3.00, p=0.036). A dose-response relationship was suggested, whereby the risk of IC increased with increasing cotinine concentration. However, the association varied by age category. Participants aged <60 were more strongly associated with PAD. This may be explained by survival bias. For the third, retrospective cohort study in Chapter 3, I used record linkage of SHeS to Scottish Morbidity Record 01 (SMR01) records and death certificates to identify the first hospital admission/death following the SHeS in which PAD was recorded as the primary or secondary cause. Of the 4,045 confirmed non-smokers who were free of baseline IC were included. Over the follow-up period (mean follow-up 9 years), there were 568 deaths, none of which were coded as due to PAD, and 64 participants were hospitalised for PAD. High exposure to SHS was associated with increased risk of all-cause mortality (adjusted hazard ratio [HR] 1.42, 95% CI 1.09-1.86, p=0.011) among all non-smokers and increased risk of incident PAD (adjusted HR 2.82, 95% CI 1.14-6.96, p=0.024) among male non-smokers. Increased cotinine concentrations at baseline were associated with increased risk of all-cause mortality, with a dose-response relationship.

SHS contains both sidestream smoke, from burning cigarette tips, and exhaled mainstream smoke. Shortened telomere length is broadly viewed as a biomarker for biological ageing including atherosclerosis phenotypes such as PAD. Evidence is strong that active smoking increases telomere length attrition but whether such association occurs between SHS and telomere length is unknown. Therefore, Chapter 4 aimed to add to growing evidence that exposure to SHS is associated with disproportionately higher biomarkers of cardiovascular risk compared with active smoking and may accelerate normal biological ageing. This chapter includes two cross-sectional studies. The first study investigated the relationship between salivary cotinine and several preclinical cardiovascular biomarkers: C-reactive protein (CRP), high-density lipoprotein (HDL) cholesterol, TC/HDL cholesterol ratio and fibrinogen in 10,081 adults from the SHeS. CRP concentration and the TC/HDL cholesterol ratio increased, and HDL cholesterol concentration decreased with increasing cotinine concentration among both non-smokers and active smokers. There were step changes in the relationship between tobacco exposure and cardiovascular biomarkers at the interface of non-smokers exposed to SHS and active smokers. Non-smokers with high exposure to SHS had lower cotinine concentrations than light active smokers but comparable concentrations of CRP (p=0.709), HDL cholesterol (p=0.931) and the TC/HDL cholesterol ratio (p=0.405). Fibrinogen concentration was less clear-cut and only increased in moderate and heavy active smokers. The second study in this chapter explored the association between self-reported levels of SHS exposure and telomere shortening per annum using a subgroup of participants from the SFHS. Of the 1,303 non-smokers, telomere length decreased more rapidly with increasing age among participants with high level of SHS exposure, compared with both those with no exposure (adjusted coefficient -0.006, 95% CI -0.008- -0.004) (high vs no SHS: p=0.010) or low exposure (adjusted coefficient -0.005, 95% CI -0.007- -0.003) (high vs low SHS: p=0.005).

In summary, there is now substantial evidence of an association between active cigarette smoking and PAD. This thesis adds to the limited existing evidence on SHS as an independent risk factor for PAD. There was an overall association between exposure to SHS and PAD, with suggestion of a dose-response relationship. However, the association varied by age category. Individuals aged <60 were more strongly associated with the prevalence of IC. SHS was significantly associated with incident PAD only in men. This thesis further demonstrates that exposure to SHS carries a disproportionately higher cardiovascular risk than active smoking for a given level of smoke exposure. Telomere shortening per year of age may be an intermediate step between SHS and CVD including PAD. This also supports the association between SHS exposure and the atherosclerosis-related biomarkers, which play an important role in the pathophysiology of PAD. Further research is needed in the future to better understand the association between SHS and PAD, and the underlying mechanisms. The research in this thesis supports the need to protect the general public from exposure to SHS.

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Publications and conference presentations

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1. **Lu L,** Mackay DF, Pell JP. Meta-analysis of the association between cigarette smoking and peripheral arterial disease. *Heart* 2014;100:414-423.

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2. **Lu L,** Mackay DF, Pell JP. Association between level of exposure to secondhand smoke and peripheral arterial disease: Cross-sectional study of 5,686 never smokers. *Atherosclerosis* 2013;229:273-276.
3. **Lu L,** Mackay DF, Pell JP. Secondhand smoke exposure and intermittent claudication: a Scotland-wide study of 4,231 non-smokers. *Heart* 2013;99:1342-1345.

Chapter 4

4. **Lu L,** Mackay DF, Newby DE, Pell JP. Association between salivary cotinine and cardiovascular biomarkers among nonsmokers and current smokers: Cross-sectional study of 10,081 participants. Eur J Vasc Endovasc Surg 2014;48:703-710.

Conference presentations

Abstracts of the research work included in this thesis were accepted for presentation at the following conferences:

Chapter 3

1. Faulty of Public Health in Scotland Annual Conference. Dunblane, Scotland 2013.
2. World Congress of Cardiology. Melbourne, Australia 2014.

Chapter 4

3. World Congress of Epidemiology. Anchorage, Alaska, USA 2014.

Acknowledgment

I would like to express my gratitude to my PhD supervisors Professor Jill Pell and Dr. Daniel Mackay for their advice, guidance, support, encouragement, and feedback. My primary supervisor Professor Jill Pell has great experience and skills in the field of public health and epidemiology and has provided me great vision and direction throughout my PhD project. I am very thankful for my co-supervisor Dr. Daniel Mackay who has given me lots of guidance with patience and understanding on the statistical methods needed for my PhD project. I am also thankful to University of Glasgow and China Scholarship Council for providing me a joint scholarship.

I am grateful for the help and assistance from Lorraine Waddell, Margaret Ashton, Jacqueline Reilly, Professor Olivia Wu, Dr. Craig Melville (convenor), Dr. Cathy Johnman, Professor Daniel Smith (assessor), Dr. Jim Lewsey (assessor), Racheal Allan and many other staff in the Institute of Health and Wellbeing. I would like to thank my friends David Blenkinson, Dr. Wenhao Shen, Junhua Mao, Dr. Zia Ul Haq, Dr. Claire Niedzwieds, Dr. Adele Warrilow, Julie Amstrong, Yiqiao Xin, Dr. Jingbo Wei, Dr. Haixiang Xiao, Louise Trelogon, Camilla Baba (PhD candidate), Alex and Esther Glen for their support during my stay in Glasgow.

For Chapter 2, thanks are due to the authors of the studies included in the meta-analysis for providing me with relevant information where needed.
For chapter3 and 4, I would like to extend my thanks to the ISD team from the NHS Scotland for providing me with the SMR linked data and to Professor Paul Shiels and his research team from Institute of Cancer Sciences for providing me with the telomere length data in a subgroup of the SFHS. Thanks are due to all the staff who collected and sorted the data in the SFHS and SHeS.

This thesis would not have been possible without the support from my family and my friends. I am particularly grateful for my father Guangda Lu and my mother Qunrong Ning for always being so supportive and believing in me. I would like to particularly thank my husband Dr. Johannes Herrnsdorf for the financial and emotional support during the final stage of my PhD project. Special thanks for his help with the proofreading of this thesis.

Author’s Declaration

I declare that the contents of this thesis are my own work. The work of others has been indicated and appropriately referenced. Parts of the research work included in this thesis have been published or submitted with co-authors.

Liya Lu

Abbreviations

AAC	aortic arch calcification
ACS	acute coronary syndrome
ACE	acute coronary events
ABPI or ABI	ankle brachial pressure index
AMI	acute myocardial infraction
ANOVA	analysis of variance
BMI	body mass index
bp	base pair
CDC	Center for Disease Control and Prevention
CHD	coronary heart disease
CI	confidence interval
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
Ct	threshold cycle
CVD	cardiovascular disease
DALYS	disability-adjusted life-years
ETS	environmental tobacco smoke
FCTC	Framework Convention on Tobacco Control
GS:SFHS	Generation Scotland: Scottish Family Health Study
HDL-C	High-density lipoprotein cholesterol
HIC	high-income countries
HR	hazard ratio
IARC	International Agency for Research on Cancer
IC	intermittent claudication
ICD	International Classification of Disease
IHD	ischemic heart diseas
ISD	Information Services Division
LDL-C	low-density lipoprotein cholesterol
LMIC	low-income or middle-income countries
MDA	malondialdehyde
MI	myocardial infarction
NHANES	the National Health and Nutrition Examination Survey
NHS	National Health Service

Abbreviations

NICE	the National Institute for Health and Care Excellence
NNAL	4-[methylnitrosamino]-1-[3-pyridyl]-1-butanol
NNK	4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone
NRT	nicotine replacement therapy
OPCS	Office of Population Census and Surveys Classification of Surgical Operations and Procedures
OR	odds ratio
PAD	peripheral arterial disease
PAH	polycyclic aromatic hydrocarbons
PAOD	peripheral artery occlusive disease
PDGF	platelet-derived growth factor
PICO	the Population Intervention Comparison Outcome framework
PM	particulate matter
$PM_{2.5}$	fine particulate matter in diameter < 2.5 μm
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
PVD	Peripheral vascular disease
RR	relative risk
SCD	sudden cardiac death
SD	standard deviation
SES	socioeconomic status
SIMD	Scottish Index of Multiple Deprivation
SHS	secondhand smoke
SHeS	Scottish Health Survey
SMR	Scottish Morbidity Record
SRNT	Society for Research on Nicotine and Tobacco
TC	total cholesterol
TIA	transient ischemic attack
T/S ratio	telomere repeat copy number to single copy gene number ratio
VCAM-1	vascular cell molecule-1
VLDL	very-low-density lipoprotein
WHO	World Health Organisation
11-DH-TXB_2	11-Dehydrothromboxane B_2

1 Chapter 1: General introduction

1.1 Chapter outline

This chapter provides the context for the subsequent chapters by discussing exposure to secondhand smoke (SHS) as a risk factor for atherosclerotic diseases, measurement of SHS exposure and legislation to protect the general public from exposure to SHS. Furthermore, it discusses lower extremity peripheral arterial disease (PAD) in terms of its diagnosis, risk factors, management and public health burden. This chapter concludes with the aims and objectives that will be addressed in the subsequent chapters.

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Introduction

SHS refers to inhaling other people's cigarette smoke. It is a mixture of air-diluted 'sidestream' smoke from a burning cigarette tip, and the 'mainstream' smoke exhaled by the smoker. SHS contains over 4,000 chemicals of which more than 250 are known to be harmful to health (1). Worldwide, approximately a third of adults and 40% children are regularly exposed to SHS in 2004. Exposure to SHS is associated with around 603,000 deaths across 192 countries; equivalent to about 1% of global mortality. Of these, 379,000 deaths are from ischaemic heart disease (2). The World Health Organisation (WHO) has recognised no-safe level for SHS exposure. The first WHO public health treaty to be published focused on tobacco control and comprehensive smoke-free legislation was one of its recommendations. In spite of this, only around 18% of the global population is covered by comprehensive smoke-free legislation (3) .

SHS has been shown to be associated with increased risk of atherosclerotic disease (4-6). Atherosclerotic disease may manifest as coronary heart disease (CHD), stroke or PAD. The presence of one is associated with a higher risk of the others (7) but the relative frequency of the conditions varies between subgroups of the population; for example by age (8) and ethnicity (9-11). Also, whilst the three conditions share many common risk factors (12, 13), the relative importance of some risk factors varies between the conditions. In comparison with CHD and stroke, PAD has been relatively neglected as a focus of research. Both active smoking and SHS exposure are now well established as risk factors for CHD and stroke (4, 6, 14, 15). In contrast, whilst a number of studies have now addressed active smoking and PAD (16), there has been a paucity of studies on SHS exposure and PAD. In this thesis, I aim to help address this neglected area.

1.2 Secondhand smoke

SHS is the inhalation of tobacco smoke by people other than the active smoker. SHS can also be called 'environmental tobacco smoke (ETS)' or 'passive smoking' or 'involuntary smoking' (3). SHS is the leading source of indoor air pollution in developed countries (17).

Inhaling tobacco smoke is unavoidable for active smokers. However, non-smokers also inhale SHS when tobacco smoke permeates any environment. SHS fills enclosed spaces including workplaces, restaurants, bars, hospitals, public transport, educational institutes and other public places, home and vehicles, when people burn tobacco products such as cigarettes, bidis and water pipes. In my thesis, I focus on exposure of non-smoking adults to the SHS generated from cigarette smoking.

1.2.1 Prevalence of secondhand smoke exposure

Implementation and enforcement of smoke-free legislation is effective at reducing exposure to SHS (18). The World Health Organization Framework Convention on Tobacco Control (WHO FCTC) has been signed by 168 countries and is legally binding in 180 ratifying countries as of March 2015 (19). However, currently only 16% of the world's population is protected by comprehensive nationwide smoke-free legislation (3). Furthermore, whilst legislation usually covers all or most enclosed public places and workplaces, outdoor public places, homes and vehicles are generally not covered by legislation.

Worldwide, according to the fact sheet from WHO, almost half of the children regularly breathe in SHS. Over 40% of the children have one or two parents who are active smokers (20). For children, private vehicles and homes are the places they are most likely to be exposed to SHS. Globally, about a third of adults are regularly exposed to SHS (Table 1.1). Within Europe, exposure is highest in WHO-Region C (Belarus, Estonia, Hungary, Kazakhstan, Latvia, Lithuania, Republic of the Republic of Moldova, Russian Federation, Ukraine), where 66% of adults are exposed to SHS. In China, public awareness of the health risks associated with SHS exposure is low (21). A Chinese nationally representative household survey reported that, among non-smokers aged ≥15 years, 556 million (72.4%) were exposed to SHS, and 50% were exposed daily. Exposure in public places, households and indoor workplaces was 72.7%, 67.3% and 63.3% respectively (22).

Table 1.1 Proportion of non-smoking adults exposed regularly to second-hand tobacco smoke, by WHO region

WHO sub-region	Exposure in men (%)	Exposure in women (%)
Africa (D)	7	11
Africa (E)	4	9
The Americas (A)	16	16
The Americas (B)	13	21
The Americas (D)	15	18
Eastern Mediterranean (B)	24	22
Eastern Mediterranean (D)	21	34
Europe (A)	34	32
Europe (B)	52	53
Europe (C)	66	66
South-eastern Asia (B)	58	41
South-eastern Asia (D)	23	18
Western Pacific (A)	50	54
Western Pacific (B)	53	51
Global	33	31

Source: Adapted from Öberg *et al.* (23)

In Scotland, on 26 March 2006, a comprehensive smoke-free legislation was implemented to ban smoking in virtually all enclosed public places and workplaces (24). Repeated national cross-sectional surveys were conducted to compare exposure to SHS among Scottish adult non-smokers aged 18 to 74 years old before and after implementation of this legislation. Pre-legislation data were collected between 1 September and 20 November 2005 and between 9 January and 25 March 2006. Post-legislation data were collected between 1 September and 10 December 2006 and between 8 January and 2 April 2007. The surveys demonstrated significant reductions in exposure to SHS in places covered by legislation: workplaces (from 12.4% to 4.3%); in pubs and bars (from 33% to 1.7%), on public transport (from 3.3% to 0.9%); and in other enclosed public places (from 9.4% to 2.6%). In contrast there was no significant change in SHS exposure in private homes and cars which were not covered by the legislation. Non-smokers living in smoking households continued to have high levels of exposure to SHS in their own homes (17.4% and 17.1% pre- and post-legislation respectively) and the homes of others (21.3% and 20.8% pre- and post-legislation). Prior to the legislation, 8.1% of non-smokers reported exposure to SHS. Following the legislation, this fell but 6.8% still reported that they were exposed (25). Semple and his colleagues compared the levels of SHS exposure measured by fine particulate matter in diameter < 2.5 μm ($PM_{2.5}$) in 41 pubs in two Scottish cities (Aberdeen and Edinburgh) eight weeks before and then after the legislation. Levels of SHS were reduced after the legislation, with the average reduction in $PM_{2.5}$ by 86% (averaged 246 $\mu g/m^3$ and 20 $\mu g/m^3$ pre- and post-legislation) (26). Six years after the legislation, in the main report of Scottish Health Survey 2012, 17% non-smoking adults reported exposure to SHS in their own home or others' home and 11% reported exposure outside buildings in public places (27).

1.2.2 Chemical composition

SHS is a mixture of air-diluted 'sidestream' smoke from a burning cigarette tip, and 'mainstream' smoke exhaled by the smoker. Mainstream smoke is the smoke inhaled and exhaled by smokers directly from tobacco products. Sidestream is the smoke which goes into the ambient air from a burning cigarette, cigar, or other smoking device. Sidestream smoke is often the major source of SHS. SHS contains over 4000 chemicals including at least 250 harmful chemicals, such as tar and carbon monoxide (CO), and more than 50 carcinogens, such as polycyclic aromatic hydrocarbons (PAH) and arsenic (20). Sidestream smoke contains a range of chemicals similar to mainstream smoke. However, sidestream smoke contains higher concentrations of toxic gases and small (<2.5μm), respirable particles than mainstream smoke (28-31). The concentrations of the constituents in SHS can vary with time, environmental conditions and commercial cigarette brands (32). Table 1.2 summarises the concentrations of some representative constituents of SHS.

Table 1.2 Concentrations (in µg/m^3) of selected constituents of secondhand tobacco smoke in some experimental and real-life situations†

Constituent	18-m^3 chamber: mean for 50 best-selling US cigarettes (µg/m^3)	Living quarters (µg/m^3)	Tavern (µg/m^3)	Disco (µg/m^3)	Home (µg/m^3)
Respirable suspended particles	1440	240–480	420	801‡	-
Nicotine	90.8	8-87	71	120	51.8
CO (ppm)	5.09	-	4.8	22.1	-
Benzene	30	-	27	-	17.6
Formaldehyde	143	-	104	-	-
1,3-Butadiene	40	-	19	-	-
Acetaldehyde	268	-	204	-	-
Isoprene	657	50–200	150	-	83.3
Styrene	10	-	-	-	7.3
Catechol	1.24	-	-	-	-
3-Ethenyl pyridine	37.1	-	-	18.2	-
Ethylbenzene	8.5	-	-	-	8.0
Pyridine	23.8	-	-	17.6	6.5
Toluene	54.5	-	-	-	51.2
Limonene	29.1	-	-	-	22.0

- not reported
† Values represent the higher end of the exposure scale.
‡ Fine particles (< 2 µm size)

Source: Adapted from WHO International Agency for Research on Cancer (IARC). IARC monographs on the evaluation of carcinogenic risks to humans. Tobacco smoke and involuntary smoking. Volume 83. 2004. (32)

1.2.3 Measures of secondhand smoke exposure

The level of exposure to SHS can be measured directly or indirectly. Direct methods measure the concentrations of one or more SHS constituent. Biomarkers of tobacco smoke can be measured in biological specimens, of which saliva is the most commonly used. Biomarkers specifically used to detect tobacco exposure, including SHS exposure, are nicotine and its metabolites such as cotinine, and the metabolites of NNK (4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone), such as NNAL (4-[methylnitrosamino]-1-[3-pyridyl]-1-butanol). NNK is a tobacco-specific pulmonary carcinogen but the detection of its metabolites requires costly laboratory equipment (33).

Nicotine, cotinine and trans-3-hydroxycotinine can also be measured in blood samples. Of these, cotinine is the most commonly used due to its longer half-life. Cotinine is an alkaloid found in tobacco and also a metabolite of nicotine. The concentration of cotinine in biological samples increases after active smoking or exposure to SHS (34). In vivo, cotinine has a half-life of approximately 20 hours, and is typically detectable for up to one week after tobacco exposure. Cotinine can be detected in different biological samples such as saliva, serum and urine, and even hair (35). The concentration of cotinine in the blood, saliva, and urine is proportionate to the amount of exposure to tobacco smoke (36, 37). Therefore, cotinine is suitable for cumulative doses over short exposure periods. Venipuncture is invasive and urine collection requires privacy and may cause logistical problems if undertaken as part of large population studies or studies of children. Also, urine cotinine concentrations can be influenced by creatinine clearance. Salivary cotinine is non-invasive and samples can be disseminated and returned by post. Therefore, salivary cotinine is often the preferred option. A liquid chromatography tandem mass spectrometry assay is usually used to detect cotinine. Previous studies suggested a value of 0.05 ng/ml is the lower limit of detection for cotinine assay (38, 39)

Cotinine concentrations are used to measure exposure among both active smokers and non-smokers exposed to SHS. In addition, cut-offs are commonly applied to differentiate between the two groups, and identify smoking deceivers (active smokers who deliberately misclassify themselves as non-smokers). In reality, the cotinine concentrations of non-smokers with heavy exposure to SHS and light/occasional active smokers can overlap. Therefore, the cut-off levels need to be selected so as to minimize misclassification. The Society for Research on Nicotine and Tobacco (SRNT) Subcommittee on Biochemical Verification has recommended a cut-off point of 15 ng/mL in saliva or serum and 50 ng/ml in urine for the identification of 'current regular smokers' (SNRT Subcommittee on Biochemical Verification 2002) (40).

In addition to measuring biological samples, respirable suspended particles (aerodynamic diameter <10 μm) can be used to measure indoor SHS exposure. However, they are not specific to tobacco combustion and can be influenced by other ambient smoke sources, such as vehicle exhaust emissions and biofuel mass (41). Other components of tobacco smoke such as CO, nitrogen oxides, formaldehyde and thiocyanate can be measured (41, 42). But these biomarkers lack specificity. For example, CO can come from traffic emissions, gas heaters and cookers (43). Another approach that has been used is to measure SHS exposure in the home is measurement of nicotine concentrations in dust (44).

Indirect measures of SHS exposure are generally obtained from survey questionnaires. Questionnaires usually include self-report of the level and/or duration of exposure or self-report of specific situations associated with SHS exposure (*e.g.* living with a partner who smokes or working in a smoky environment) (45) (46, 47) as summarised in Table 1.3. Measures of tobacco smoke exposure based on self-reported questionnaires are used more often in large population-level epidemiological studies. However, many studies have suggested that self-report may underestimate the true current smoking prevalence due to some current active smokers deliberately misclassifying themselves as non-smokers. A systematic review identified 67 studies that assessed the relationship between self-reported smoking status and smoking status conferred from cotinine concentrations. The studies confirmed under-reporting of active smoking. The mean difference between smoking prevalence based on self-reported compared with cotinine measured -4.8%, -6.2% and -9.4% for saliva, serum and urine respectively (34).

The two approaches (self-report and cotinine measurement) can be combined to improve accuracy. Participants who classify themselves as non-smokers and have a salivary cotinine concentration <15.0 ng/ml can be safely assumed to be non-smokers and those who classify themselves as smokers are likely to be so. The decision as to whether to include, and if so how to classify, people who report themselves as non-smokers but have a salivary cotinine concentration ≥15.0 ng/ml will depend on the question being asked and the relative importance of sensitivity and specificity.

Table 1.3 contains a summary of methods of assessing SHS exposure.

Table 1.3 Types of methods for measuring exposure to secondhand smoke (33)

	Suggested indicators	Pros	Cons
Direct	Biomarker concentrations:		
	Cotinine	Specific to tobacco exposure Reflect recent tobacco exposure Highly sensitive	Short half-life in body fluids Only measures recent exposure
	Saliva	Easy, non-invasive to collect Good for multiple measurement	Potential issues with age, gender, race, oral pH, dehydration, or drug treatment
	Blood	No adjustment required for hydration	Invasive to collect Difficulty for infants and young children Pregnant women have increased clearance rate
	Urine	Non-invasive to collect	Need facilities with privacy during collection Need creatinine clearance adjustment Potential issues with renal disease and some prescription drugs
	Nicotine	Specific to tobacco exposure	
	Hair or nail	Easy, non-invasive to collect Reflect longer exposure	Age, gender, race and chemical hair treatments may affect hair nicotine concentrations Nail nicotine concentrations need further research
	Body fluids		Very short half-life
	CO and Carboxyhaemoglobin	Integrated exposures from all sources	Lack specificity Many indoor and vehicular sources are possible sources of CO

			Concentrations depend on CO level in inhaled air, duration of exposure and lung ventilation Carboxyhaemoglobin blood samples are invasive
	NNK metabolites	Specific to tobacco exposure Can be detected in urine, non-invasive Reflect longer exposure than cotinine	Costly Require analytical expertise
	Concentration of SHS components in the air:		
	Nicotine in the air	Specific to tobacco combustion Emitted in large quantities in sidestream SHS Can be used to measure and compare exposures from different sources Can be measured in indoor dust and household surfaces	High absorption rate to indoor surfaces Tendency to be re-emitted even in the absence of active smoking
	Respirable particles	PM measurements allow multiple assessment of real-time indoor air quality	Not specific to SHS
	Other markers	VOCs constitute a major proportion of the organic mass of SHS	VOCs are of low sensitivity and require laboratory techniques
Indirect	Report of SHS exposure (questionnaire) at:	Easy Low cost Feasible in large populations Can integrate into existing surveys Permits tracing long-term/lifelong exposure pattern	Misclassification errors may occur due to recall bias, intentional alteration, memory failure and the respondents' lack of knowledge Low sensitivity
	Home Presence of SHS Number of smokers Smoking of parents Amount (number of cigarettes smoked) cumulative time (hours exposure) Workplace		

Presence of SHS	
Number of smokers	
Amount (number of cigarettes smoked)	
Cumulative time (hours exposure)	
Other indoor public places	
Presence of SHS	
Number of smokers	
Amount (number of cigarettes smoked)	
Cumulative time (hours exposure)	

SHS secondhand smoke; pH potential of hydrogen; CO carbon monoxide; NNK 4-[methylnitrosamino]-1-[3-pyridyl]-1-butanone; PM particulate matter; VOCs volatile organic compounds

Source: Adapted from:
Avila-Tang E, Al-Delaimy WK, Ashley D et al. Assessing secondhand smoke using biological marker. Tob Control 2013; 22:164-171.

1.2.4 Health effects of secondhand smoke exposure

Since the early 1980s, there has been growing evidence on the adverse health effects of exposure to SHS. Exposure to SHS is associated with lung cancer, CHD, respiratory diseases and stroke in adult non-smokers (4, 6, 48, 49). Worldwide, 40% of children, 35% of female adult non-smokers and 33% of male adult non-smokers were estimated to have been exposed to SHS in 2004. This exposure resulted in 603,000 deaths, about 1% of worldwide mortality, and 10.9 million disability-adjusted life-years (DALYs) attributable to SHS. Of these deaths, 379,000 deaths were from ischaemic heart disease, 165,000 from lower respiratory infections in children under 5 years old, 36,900 from asthma and 21,400 from lung cancer. The largest number of deaths attributable to SHS occurred in women who had a partner who smoked or were exposed to SHS in enclosed places. More than 80% of the deaths in children under 5 years old attributed to SHS exposure occurred in Southeast Asia, Africa and Eastern Mediterranean regions (2). Prior to the introduction of smoke-free legislation, SHS was responsible for an estimated 11,317 deaths across the UK (50) and 865 in Scotland each year (51).

1.2.4.1 Short-term health effects

Exposure to SHS has immediate health effects including eye irritation, headache, cough, sore throat, dizziness and nausea. Even brief exposure to SHS brings about rapid cardiovascular changes including plate activation, endothelial dysfunction and arterial stiffening (52-60). As little as 30 minutes of exposure (comparable to exposure in a pub or bar) was associated with impaired endothelium-dependent vasodilation in coronary arteries in non-smokers comparable to habitual active smokers (Otsuka et al. 2001). One hour of SHS exposure increased the levels of 11-dehydro-thromoboxane B_2 (11-DH-TXB_2) and malondialdehyde (MDA) to the levels observed in active smokers (59). Lung function was reported significantly reduced after one hour of SHS exposure (61).

1.2.4.2 Long-term health effects

There are long-term health effects as a result of exposure to SHS as well. The 2006 the United States (US) Surgeon General's report concluded that there is no risk-free level of exposure to SHS (62). Evidence on the harmful effect of SHS exposure has been building over decades.

The association between smoking and cancer is strongest for lung cancer (63), from which 1.38 million people die every year (64). Smoking accounts for around 90% of lung cancer incidence (65). However, lung cancer can also occur among people who have never smoked. Studies have pointed to an association between SHS exposure and lung cancer. In 2000, a meta-analysis was conducted of 35 case-control studies and 5 cohort studies among lifetime non-smoking subjects. From a total of 5,140 lung cancer cases, lifetime non-smoking women and men experienced a 20% (pooled relative risk [RR] 1.20, 95% confidence interval [CI] 1.12-1.29) and a 48% (RR 1.48, 95% CI 1.13-1.92) excess risk of lung cancer respectively, as a result of exposure to SHS from their spouse's smoking habit. SHS exposure at work produced a 15% and 29% increased risk among lifetime non-smoking women and man respectively (RR 1.15, 95% CI 1.04-1.28 for women; RR 1.29, 95% CI 0.93-1.78 for men) (66). Another large scale study which combined European and US studies assessed 1,263 lung cancer cases among adult non-smoking patients. They found evidence for a dose-response relationship between duration of SHS exposure and long-term risk of lung cancer for three sources of exposure: spousal smoking (adjusted odd ratio [OR] 1.30, 95% CI 1.04-1.63, p for trend=0.04 for ≥ 31 years of exposure); workplace exposure (adjusted OR 1.25, 95% CI 1.03-1.51, p for trend=0.01 for ≥ 21 years of exposure); and social exposure (adjusted OR 1.26, 95% CI 1.01-1.58, p for trend=0.02 for ≥ 20 years of exposure) (48).

The effect of SHS on the risk of CHD has been demonstrated in a meta-analysis published in 1999. This meta-analysis included 10 prospective cohort studies and 8 case-control studies and reported a pooled RR of 1.25 (95% CI 1.17-1.32). Exposure at home showed a 17% excess risk and at work an 11% excess risk of CHD (RR 1.17, 95% CI 1.11-1.24 for exposure at home; RR 1.11, 95% CI 1.00-1.23 for exposure at work). There was suggestion of a dose relationship with increasing dose and duration of exposure to SHS from 1-19 cigarettes per day (RR 1.23, 95%CI 1.13-1.34) to ≥ 20 cigarettes per day (RR 1.31, 95%CI 1.21-1.42); and from 1-9 years of exposure (RR 1.18, 95%CI 0.98-1.42), and 10-19 years of exposure (RR 1.31, 95%CI 1.11-1.55) to ≥20 years of exposure (RR 1.29, 95%CI 1.16-1.43) (4). In 2004, Whincup et al. examined the risk of CHD events during 20 years of follow-up in the British Regional Heart Study which recruited participants from England, Wales and Scotland. Among 2,105 male non-smokers, 1,722 (81.8%) had a serum cotinine concentration >0.8 ng/mL. The risk of CHD among non-smokers exposed to high levels of SHS (adjusted hazard ratio [HR] 1.57, 95% CI 1.08-2.28), who had a mean cotinine concentration 4.9 ng/mL, was comparable to light active smokers (adjusted HR 1.66, 95% CI 1.04-2.68), who had a mean cotinine concentration 138.4 ng/mL (67). The comparable risk of CHD and the high prevalence of SHS exposure suggested the effect of SHS in earlier studies may have been underestimated. Published meta-analyses have concluded a significantly higher risk of lung cancer among active current smokers (pooled overall RR 8.96, 95%CI 6.73-12.11) (63) than non-smokers exposed to SHS (pooled RR all < 2.00 for either men or women exposed at home or at work) (66). Therefore, it has been concluded that for CHD, SHS exposure conveys a disproportionately high risk (in relation to level of nicotine exposure) that is not true for other smoking-related conditions, such as lung cancer.

Another common cardiovascular risk of being exposed to SHS is stroke. A meta-analysis of 20 studies, published in 2011, has indicated an increased risk of stroke among those exposed to SHS (pooled RR 1.25, 95%CI 1.12 to 1.38). The dose relationship was clear. The relative risk was 1.16 (95%CI 1.06-1.27) for exposure to 5 cigarettes per day, 1.31 (95%CI 1.12-1.54) for exposure to 10 cigarettes per day, 1.45 (95%CI 1.19-1.78) for exposure to 15 cigarettes per day and 1.56 (95%CI 1.25-1.96) for exposure to 40 cigarettes per day (6). These findings are consistent with the pattern observed for CHD. The 2010 US Surgeon General’s report concluded that current evidence was insufficient to infer a causal relationship between exposure to SHS and stroke (68).

Aortic arch calcification (AAC) is independently associated with CHD, stroke and PAD (69). A recent study in China has found the risk of AAC increased significantly with increasing duration of exposure to SHS at home in adulthood (adjusted OR 1.24, 95%CI 1.07-1.43 for 40 hours per week over >5 years, p for trend=0.005) and indoor workplaces exposure to SHS (adjusted OR 1.22, 95%CI 1.04-1.43 for 40 hour per week over >5years, p for trend=0.012) among never smoking women. Significant trends were also found for increasing severity of AAC with increasing duration of SHS exposure (70).

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide. Active cigarette smoking is a well-established risk factor for COPD (62). SHS has been linked to the development of COPD, but most studies included current smokers in their analysis. In the Guangzhou Biobank Cohort Study, researchers analysed data from 15,379 never smokers aged ≥ 50 years on self-reported SHS exposure and risk of COPD. They found that the risk of COPD increased with increasing cumulative lifetime exposure to SHS at home (adjusted OR 1.60, 95%CI 1.23-2.10 for 40 hours per week over >5 years), in indoor workplaces (adjusted OR 1.50, 95%CI 1.14-1.97 for 40 hours per week over >5 years); and total exposure (adjusted OR 1.48, 95 %CI 1.18-1.85 for 40 hours per week over >5 years, p for trend=0.001) (71).

Long-term exposure to SHS can induce asthma and exacerbate symptoms such as nasal symptoms, headaches, cough, wheezing, sore throat, hoarseness and eye irritations (32, 72). Nicotine, aldehydes and other toxic components in the tobacco smoke are associated with asthma and these symptoms. In a Finnish study, over a 2.5-year period, exposure to SHS increased the risk of asthma onset among adults aged ≥ 21 years, with an adjusted OR of 2.16 (95%CI 1.26-3.72) for exposure at work and an adjusted OR of 4.77 (95%CI 1.29-17.7) for exposure at home (73). A meta-analysis of 79 studies attributed a 21-85% increase in incident asthma and a 30-70% increase in incident wheezing to pre- or postnatal SHS exposure among children aged up to 18 years (74). In Scotland, before the introduction of smoke-free legislation, hospital admissions for children asthma were increasing by a mean of 5.2% per year among children aged <15 years. Following smoke-free legislation, admissions decreased at a mean rate of 18.2% per annum (75).

There are other long-term health effects of exposure to SHS such as cancers other than lung cancer (63) and mental health issues (76, 77). Evidence has emerged of a possible association between exposure to SHS and nasal sinus cancer (78). A cross-sectional study published in 2009 showed an association between high level of SHS exposure (cotinine 0.8-13.5 ng/mL) and increased risk of cognitive impairment (adjusted OR 1.44, 95% CI 1.07-1.94) among adult non-smokers aged >50 years (79). High SHS exposure (cotinine 0.7-15.0 ng/mL) among adult non-smokers was associated with increased risk of psychological distress (adjusted OR 1.49, 95% CI 1.13-1.97) and admission to psychiatric hospitals (adjusted HR=3.74, 95%CI 1.55-8.98) in prospective data (77). For children, home is the major source of exposure due to parents and other household members smoking cigarettes indoors. Prenatal exposure can have impact on both the mother and the foetus including female fertility, low birth weight, preterm birth, stillbirth and spontaneous abortion (80-83).

Therefore, SHS is a substantial threat to public health. There is no safe level of exposure (62). The most effective measure to prevent any harmful effects of SHS is to protect the general public from SHS exposure.

1.2.5 Smoke-free legislation

Because of the risk to health posed by exposure to SHS, tobacco control experts recommend the introduction of smoke-free legislation that prohibits smoking in indoor public places and protects workers and the general public. According to the WHO FCTC guidelines article 8, the countries which have ratified the WHO FCTC have a legal obligation to implement effective smoke-free legislations for protection from exposure to tobacco smoke in indoor workplaces, public transport, and indoor public places and as appropriate, other public places (84). Since the WHO FCTC entered into force in 2005, 180 parties have ratified their legal obligation (19). As of 2014, 125 of these 180 parties have actually implemented smoking bans in indoor public places, public transport, and as appropriate, other public places to protect their citizens from exposure to SHS. Of these, 111 have introduced national legislation to protect their citizens from exposure to SHS, and 65 have introduced administrative and executive orders or a combination of these orders and the national legislation. Twenty-nine parties use voluntary agreements (85). However, a factsheet from the WHO has indicated that only around 18% of the world's population is covered by comprehensive smoke-free legislation (3).

1.2.5.1 An overview of smoke-free legislation

On the 29 March 2004, Ireland was the first country in the world to institute an outright ban on smoking in general indoor workplaces, including offices, shops, factories, restaurants, bars, educational facilities, hospital facilities and public transports, with on the spot fines of up to €3,000. However, the legislation does not cover smoking in designated hotel rooms, private residential places and prisons (86). One year after the Irish smoke-free law, a 94% compliance rate among all eligible workplaces was recorded under the National Tobacco Control Inspection Programme and 96% of all indoor workers reported working in smoke-free environments (87). Air quality in pubs has improved significantly since the smoke-free law. A study of 24 pubs throughout Dublin measured the exposure levels of airborne particles (Particulate matter [PM]), which were mainly from tobacco smoke, before and after the smoke-free law over at least a three hour period at each premises with repeat measurements on the same day of the week and the same month, one year on. The average levels of small particles ($PM_{2.5}$) reduced by 87.6% while the average levels of large particles (PM_{10}) reduced by 53% (87, 88). Following introduction of the Irish smoke-free legislation, exposure to SHS dramatically declined in all venues: workplaces from 62% to 14%, restaurants from 85% to 3%, and bars/pubs from 98% to 5%. (18).

In the United Kingdom (UK), smoking prevalence peaked in 1948, at which time 82% of adult men smoked (89). Overall the smoking prevalence among both male and female adults has been declining since 1974 when the first national survey on smoking began. In 2011, smoking prevalence among adults in the UK had reduced to 20% (90).

Prior to the smoke-free legislation, it was estimated that exposure to SHS in public and private places caused 11,317 premature deaths among adult non-smokers in the UK each year (50). Since July 2007, smoking in virtually all enclosed public places and workplaces has been banned by comprehensive smoke-free legislation across all UK jurisdictions. Smoke-free legislation was first introduced in Scotland in 2006 followed by England, Wales and Northern Ireland in 2007. The Scottish smoke-free legislation prohibits smoking in wholly and substantially enclosed public places and workplaces, with few exemptions such as designated rooms, seating and playing areas of sports stadia and private dwellings (24). Compliance rates have been high. Since the implementation, exposure to SHS has declined in all public places covered by the legislation (91). A study of 41 Scottish pubs demonstrated an 86% reduction of $PM_{2.5}$ levels two months after implementation of the legislation (26). The legislation has resulted in reduced exposure among both bar workers and the general population. Among adult non-smokers in the general population, the geometric mean salivary cotinine concentration has fallen by 39% since the legislation (25). Similar results have been found in England.

During the first 9 months following introduction of the English legislation, 98.2% of 390,148 premises inspected were found to be smoke-free (92). Since October 1 2015, smoking is also no longer permitted in a private vehicle carrying children in England and Wales (93).

Comprehensive smoke-free legislation has been introduced in other jurisdictions such as Norway and Italy. In Brazil, since December 2011, the Federal Law 12546 bans smoking in enclosed spaces throughout the country. Tobacco advertising is restricted to posters in shops and banned on television and radio. In 2012, Brazil became the first country in the world to outlaw all flavours and additives in tobacco products because they lure people to start smoking (94-96). Brazil has reduced its smoking prevalence by 46% in the last 20 years. A study attributed 14% of the reduction to the implementation of smoke-free air laws, 14% to marketing restrictions, and 8% to health warnings (97).

Both direct studies of hospitality venues (25, 26, 98, 99) and surveys (87, 100) of the general population have shown that compliance with smoke-free legislation remains high in countries with good enforcement and sufficiently high fines. However, compliance in some countries has been poorer. In Greece, the level of indoor exposure to SHS remains high, with 72.2% of the population exposed in restaurants and 52.3% in workplaces, in spite of its comprehensive smoke-free legislation (101). In Russia, 21.9 million adults (34.9% of the adult population) are still exposed to SHS in their workplace (102).

Furthermore, there are some countries that still do not have comprehensive smoke-free law with national coverage, such as the US, China and Australia. Alternative methods such as regional laws have been proposed to eliminate the harmfulness of environmental tobacco exposure.

The US Congress has not yet enacted any nationwide federal smoking ban. Since California became the first state to introduce a statewide smoking ban, an increasing number of states have enacted statewide legislation. To date, 40 states and the District of Columbia have local laws in effect which require non-hospitality workplaces, restaurants and bars to be 100% smoke-free. According to the American Non-smokers’ Rights Foundation, 81.9% of the US population lives under the smoking bans, but only 49.3% under a ban that covers all workplaces, restaurants and bars (103). Although the US Surgeon General's report has concluded that the only way to fully protect non-smokers from exposure to SHS exposure is to prohibit smoking in all indoor areas, including private-sector worksites, restaurants, and bars (62), regional disparities remain in policy adoption (104).

In Australia, smoking bans have been determined on a state-by-state basis. Currently, all Australian states and territories have banned smoking in most enclosed public places and in vehicles carrying children under the age of 16 years. Tobacco products are not allowed to be sold to people under 18 years old (105). From July 2015, commercial outdoor dining areas in New South Wales, Australia are also smoke-free (106).

The FCTC came into force in China in 2006 (107). Action has been limited to several cities such as Beijing, Shanghai, and Guangzhou which have enacted local regulations to prohibit smoking in public places (108). National legislation is urgently needed to effectively reduce the increasing health and economic burden of smoking- and SHS-related diseases.

1.2.5.2 Public opinion

Opinion polls have shown considerable support for smoke-free air legislation. Worldwide, over 75% of young people support smoke-free laws (109, 110). In Ireland, after implementation, 93% of the population supported the smoke-free legislation, compared with 59% before (87). Public support for total bans also increased. Among smokers, 46% reported that the legislation made them more likely to quit. Among those who had quit smoking post-legislation, 80% indicated that the law had helped them quit smoking and 88% reported that the law made them less likely to smoke again (18). In Norway, the smoke-free legislation had the support of over 75% of the population by the end of the first year after implementation (111).

1.2.5.3 Health impact of smoke-free legislation

There is growing evidence that links the implementation of smoke-free legislation with a reduction in hospital admissions for outcomes related to exposure to SHS.

A meta-analysis published in 2013 examined the effectiveness of smoke-free legislation on the risk of acute myocardial infraction (AMI). This review of 18 studies (44 estimates of effect size since the first smoke-free law in 2004) demonstrated a significant reduction in the incidence of AMI following implementation of smoking bans in workplaces and public places, with a pooled estimate of RR of 0.87 (95% CI 0.84-0.91) (112). Another meta-analysis was conducted in 2012 to determine the association between smoke-free legislation and hospitalisations for cardiac, cerebrovascular and respiratory diseases. They included 45 studies of 33 smoke-free laws with a median follow-up of 2 years. The outcomes in this study included: AMI, acute coronary syndrome (ACS), acute coronary events (ACE), ischemic heart disease (IHD), angina, CHD, sudden cardiac death (SCD), stroke, transient ischemic attack (TIA), COPD, asthma, respiratory infections, and spontaneous pneumothorax. They found that comprehensive smoke-free legislation was significantly associated with lower hospital admission rates (or deaths) for 4 diagnostic groups: coronary events (RR=0.85, 95% CI 0.82–0.88), other heart disease (RR=0.61, 95% CI 0.44–0.85), cerebrovascular accidents (RR=0.840, 95% CI 0.75–0.94), and respiratory disease (RR=0.76, 95% CI 0.68– 0.85) (113). A study in Norway evaluated the effect of an indoor smoking ban on respiratory symptoms including morning cough, daytime cough, phlegm cough, dyspnea and wheezing among workers in the hospitality industry five months after enactment. There was a significant decrease in these symptoms, with the largest decrease observed among people who had quitted smoking and among those who reported a positive attitude towards the smoking ban (114). In a meta-analysis, researchers extracted data from five North American local smoking bans and six European national bans. Implementation of smoking bans was associated with a 10.4% reduction in preterm birth and a 10.1% reduction in hospital attendances for asthma (115).

In Scotland, 10 months after the implementation of the legislation, there was an overall 17% reduction of the number of hospital admission for acute coronary syndrome, with a 14% reduction among smokers, a 19% reduction among ex-smokers and a 21% reduction among never smokers, when compared with those numbers during the 10 months before implementation (116). As for admissions for childhood asthma in Scotland, there was a mean reduction of 18.2% per year relative to the rate on the legislation implementation day, in contrast to a mean rate of 5.2% admission per year before the legislation (75).

Therefore, there is now ample evidence that smoke-free legislation can reduce SHS exposure in both workers and the general population. The implementation of smoke-free legislation is associated with many benefits including reduced hospital admission for health conditions related to SHS exposure.

1.3 Peripheral arterial disease

Peripheral vascular disease (PVD) refers to diseases of blood vessels (arteries and veins) located outside the coronary, aortic arch vasculature or brain. PVD is commonly referred to as PAD, or peripheral artery occlusive disease (PAOD) resulting from atherosclerotic blockages in the arteries in the lower extremity. PAD is often defined, in studies, as an ankle brachial pressure index (ABPI or ABI) of less than 0.9. ABI is the ratio of the ankle to the arm blood pressure. The main cause of PAD is chronic atherosclerosis in the lower extremity (117). A variety of risk factors for PAD are almost identical to those of atherosclerotic disease elsewhere (12). Active cigarette smoking is the most important risk factor (13, 118).

1.3.1 Prevalence and classification of peripheral arterial disease

1.3.1.1 Prevalence

Worldwide, about 202 million people were diagnosed with PAD in 2010. Of these, 69.7% were living in low-income or middle-income countries (LMIC). More than one quarter of the people who had PAD were living in Southeast Asia and more than one fifth living in West Pacific Region. In the last decade, the prevalence of PAD has increased by 28.7% and 13.1% in LMIC and high-income countries (HIC) respectively (119).

Sex-specific prevalence of PAD increased with advancing age. In HIC, PAD affected 5.28% (95% CI 3.38-8.17%) in women and 5.41% (95% CI 3.41-8.49%) in men aged 45-49 years, and 18.38% (95% CI 11.16-28.76%) in women and 18.83% (95% CI 12.03-28.25%) in men aged 80-89 years. In LMIC, prevalence was higher in women than in men aged 45-49 years (6.31% [95% CI 4.86-8.15%] for women; 2.89% [95% CI 2.04-4.07%] for men). The prevalence in LMIC was 15.22% (95% CI 10.80-21.02%) in women and 14.94% (95% CI 9.58-22.56%) in men aged 80-89 years (119).

In the Edinburgh Artery Study, PAD affects 16.6% in people aged 55-74 years (120). Price and her colleagues have reported from the 5-year follow-up of the Edinburgh Artery Study, the incidence of symptomatic PAD is 5.1% in people aged 55-74 years (121).

1.3.1.2 Classification

PAD may be asymptomatic or symptomatic. In Europe and North America, an estimated 27 million people have PAD. Of these, 60% are asymptomatic (122). Symptomatic patients usually present initially with intermittent claudication (IC). This is defined as muscle discomfort (ache, cramp, numbness or sense of fatigue) felt by the patient, classically in the calf, which occurs during exercise, such as walking, and is relieved with rest. Clinically, PAD is commonly classified using Fontaine’s stages or Rutherford’s categories (Table 1.4) (123). Progression of the disease can result in rest pain: more severe pain that is not relieved by rest or can occur at rest. Finally, severe and prolonged ischaemia can cause ulceration or gangrene in the lower limb.

Table 1.4 Classifications of peripheral arterial disease by Fontaine's stages and Rutherford's categories

Fontaine's classification		Rutherford's classification		
Stage	clinical description	grade	category	clinical description
I	Asymptomatic	0	0	Asymptomatic
II a	Mild claudication	I	1	Mild claudication
II b	Moderate to severe claudication	I	2	Moderate claudication
III	Ischemic rest pain	I	3	Severe claudication
IV	Ulceration or gangrene	II	4	Ischemic rest pain
		III	5	Minor tissue loss
		III	6	Major tissue loss

Source: Adapted from:
Fontaine's classification: (124)
Rutherford's classification: (125, 126)

1.3.2 Development of PAD

PAD often co-exists with CHD and/or cerebrovascular disease (121). The presence of PAD increases an individual's risk of suffering angina, myocardial infarction (MI) or stroke (7). PAD is also more frequent among diabetic patients than among non-diabetic subjects (127) .

The development of PAD is a complex, multifactorial process of atherogenesis that involves three stages: initiation of the lesion of the arteries, progression of the lesion, and plaque complications. Many risk factors are common to those of atherosclerotic disease elsewhere in the body, including demographic and lifestyle factors as described below.

1.3.2.1 Demographic factors

The prevalence of PAD is age-dependent. In a German study, the prevalence of PAD increased from 3.0% in men aged 45-49 years to 18.2% in men aged 70-75 years. PAD affected 2.7% in women aged 45-49 years and 10.8% in women aged 70-75 years (128). Approximately 8 million people in the US are affected by PAD and 20% of adults older than 55 years require treatment for PAD (129).

In relation to cardiovascular diseases (CVDs), there are widely accepted sex differences in risk. In contrast, the influence of gender on the prevalence of PAD is controversial. Some studies have suggested an increased risk among men (130, 131). While some other studies have reported that, between the ages of 60 and 85 years, the prevalence of PAD is higher among women (8). The Rotterdam study also showed a higher prevalence of PAD among women aged ≥ 55 years. The frequency of PAD varies by ethnic subgroup (9-11). Criqui *et al*. documented that non-Hispanic blacks had significantly higher PAD prevalence compared to non-Hispanic whites (132).

1.3.2.2 Lifestyle factors

Lifestyle factors that are strongly associated with PAD include cigarette smoking, obesity, diabetic mellitus (diabetes), dyslipidaemia and hypertension.

Smoking

Smoking is one of the most important, modifiable risk factors for PAD (13). The association was first identified by Erb in 1911 who reported that the risk of IC was three times greater among smokers (133). Many studies have since been conducted corroborating his findings. According to WHO, almost 20% of the global population smoke tobacco (84) and 84% of these smokers live in developing and transitional economy countries (134).

Active smoking and SHS exposure can have detrimental effects on cardiomyocytes and peripheral vessels. Though the exact mechanism of how smoking induces atherosclerosis is not completely understood, experimental models suggest toxins in cigarettes, including oxidative free radicals, impair mitochondrial function and energy metabolism of the endothelial cells thus altering cell function and damaging vessel walls (135). This increases lipid permeability, platelet aggregation and adhesion, and formation of coagulation factors and decreases fibrinolysis, resulting in arterial stiffness and narrowing. The main component of cigarettes, nicotine, is a euphoriant affecting mood and behaviour and has been clearly implicated as the source of smoking addiction (136). Nicotine and its metabolite cotinine increase the level of platelet-dependent thrombin (137).

Thrombin is an important enzyme in the coagulation cascade and also a potent platelet agonist. Increased platelet activity is a key risk factor for atherosclerosis. Platelet-dependent thrombin may play a role in thrombus formation (138). Also nicotine stimulates sympathoadrenal activity, and therefore may lead to changes in blood flow (139). Another major component, CO, exacerbates vessel constriction by competitively attaching to haemoglobin (normally bound with oxygen) which reduces oxygen supply in the blood (140, 141). The lack of oxygenated blood and reduced blood flow through smaller vessels and capillaries together may lead to ischemia in the lower limbs.

In a meta-analysis published in 2013, on prevalent PAD and its risk factors, current smoking was the strongest lifestyle risk factor for PAD, with a pooled OR of 2.72 (95% CI 2.39-3.09) in HIC and 1.42 (95%CI 1.25-1.62) in LMIC, followed by diabetes, hypertension and hypercholesterolemia (119). Willigendael *et al.* suggested that in countries where approximately 30% of the population are smokers, 50% of PAD cases are attributable to smoking (16). The US National Health and Nutrition Examination Survey (NHANES) 1999-2004 which comprised 3,947 men and women aged over 60 years showed current smokers were 5.84 times more likely to develop PAD, and former smokers were 1.94 times more likely, compared to never smokers (142). Continuous and long-term exposure to active smoking is associated with a higher risk of developing PAD. People who have a long history of smoking constitute a high risk group for PAD. Merino and other researchers have reported that the incidence of new PAD at five years follow-up is around 20% overall but is significantly higher among those who had smoked more than 40 pack-year (143). There appears to be a dose relationship, whereby the risk of PAD increases with the number of cigarettes smoked.

The Edinburgh Artery Study is a cohort study in which the study population is comprised of 1,592 Edinburgh inhabitants aged 55 to 74 years. In a previous study, heavy smokers (defined as pack years ≥ 25) were demonstrated to have 3.94 fold risk of developing intermittent claudication compared with never smokers, and moderate smokers (defined as pack year <25) to have 1.87 fold higher risk (121). Since PAD is considered to be associated with previous cardiovascular events (13, 144) and smoking is one of the major factors responsible for CVDs (14, 15, 118) and diabetes mellitus (145, 146), being a smoker and at the same time having additional comorbid factors may even enhance the probability of developing PAD (13) and aggravate the condition of these diseases (12, 147). Among type 2 diabetic patients in a cohort followed up for 12-years, current smokers who smoked over 25 cigarettes per day were nearly 11 times more likely to develop PAD, and those who consumed less than 14 cigarettes a day were almost 5 times more likely, compared with never smokers (148). People who start smoking at an early age and those who continue to smoke long-term are at greatest risk of the disease. Some studies indicate that initiating smoking at an early age (less than 16 years) doubles the risk in comparison with those who start smoking later than 16 years of age (149).

As with current smokers, former smokers are also at increased risk of developing the disease in comparison with never smokers. Being a former smoker appears to be associated with a risk of PAD that lies between that of current and never smokers (13, 118). This suggests that cessation of smoking is associated with reduced risk (150, 151). However, ex-smokers comprise both recent quitters and those who have not smoked for many years. The effect of smoking cessation has been studied extensively for CVDs in terms of the rate of risk reduction, whether the reduction is linear or non-linear, whether it eventually reverts to the level of risk among never smokers and whether this is dependent on the duration of smoking (152-154). In contrast there is a relative paucity of information on the impact of smoking cessation on the risk of PAD. Among people who quit smoking ≥ 21 years previously the relative risk of PAD, in comparison with current smokers, was 0.41 among those who originally smoked for 11 years and 0.49 among those who had smoked 20 years (151). Quitting smoking is believed to reduce risk of PAD, even among diabetic smokers due to reduced chronic inflammation (150). Quitting or reducing smoking may also reduce the risk of disease progression among those who have already developed PAD (155). Hobbs et al. reviewed the published evidence relating to smoking cessation on the MEDLINE and the Cochrane Library including meta-analyses of randomised controlled trials. They suggested that permanent smoking cessation is probably the most clinically and cost effective intervention for PAD patients (156). It is also suggested by researchers that a comprehensive program including smoking cessation is important to prevent cardiovascular events among patients with PAD (157).

In conclusion, smoking impairs normal circulation by narrowing the blood vessels and decreasing the amount of oxygen in the blood, leading to ischemia in lower limbs, and thus PAD (158). Cigarette smoking is a stronger risk factor for PAD than for other CVDs (159) and also the most modifiable major factor. Hence, smoking cessation plays a vital role in reducing PAD risk, slowing down PAD progression and improving prognosis (158).

Other modifiable risk factors

Other modifiable risk factors for PAD include diabetes, hypertension, and dyslipidemia (160). A recent meta-analysis has demonstrated the association between these three risk factors and risk of PAD: diabetes (pooled OR 1.88, 95% CI 1.66-2.14 in HIC; pooled OR 1.47, 95% CI 1.29-1.68 in LMIC); hypertension (pooled OR 1.55, 95% CI 1.42-1.71 in HIC; pooled OR 1.36, 95%CI 1.24-1.50 in LMIC); and hypercholesterolemia (pooled OR 1.19, 95%CI 1.07-1.33 in HIC; pooled OR 1.14 95%CI 1.03-1.25 in LMIC) (119). Diabetes is associated with large and small vessel atherosclerotic occlusive diseases. In one study using the 1999–2004 NHANES, PAD prevalence among adults aged ≥ 40 was significantly higher among adults with undiagnosed (9.2%) and diagnosed diabetes (7.5%) than those with normal glucose concentrations (3.9%) (161). Researchers have suggested that the risk of developing PAD is proportional to the severity and duration of diabetes (162). Other studies have linked hypertension with an increased risk of PAD. The Framingham study suggested that the risk of IC was increased 2.5 fold among men with hypertension and 3.9 fold among women with hypertension (163). Dyslipidemia, such as hypertriglyceridemia, lipoproteinemia and hyperhomocysteinemia, has also been related to an increased risk of PAD (164-166). A study, among 14,916 healthy men aged >40 years, examined the predictive values of lipid and non-lipid biomarkers as risk factors for incident PAD. Among lipid biomarkers, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), High-density lipoprotein cholesterol (HDL-C), TC/HDL-C ratio, Apolipoprotein B-100 and fibrinogen were predictors of the development of PAD. The TC/HDL-C ratio was the strongest lipid predictor of risk (adjusted RR for the highest vs lowest quartile 3.9, 95% CI 1.7-8.6). C-reactive protein (CRP) was the strongest nonlipid predictor (RR for the highest vs lowest quartile 2.8, 95% CI 1.3-5.9) (167). In the Cardiovascular Health Study, higher body mass index (BMI) was found to be associated with PAD prevalence in older persons in good health who had never smoked (prevalence ratio 1.30, 95% CI 1.11-1.51) (168).The concurrence of multiple risk factors increases the risk for PAD. The RR for PAD increased from 2.3, 3.3 to 6.3 when smoking, diabetes and systolic hypertension concurred respectively (169).

1.3.2.3 Pathophysiology

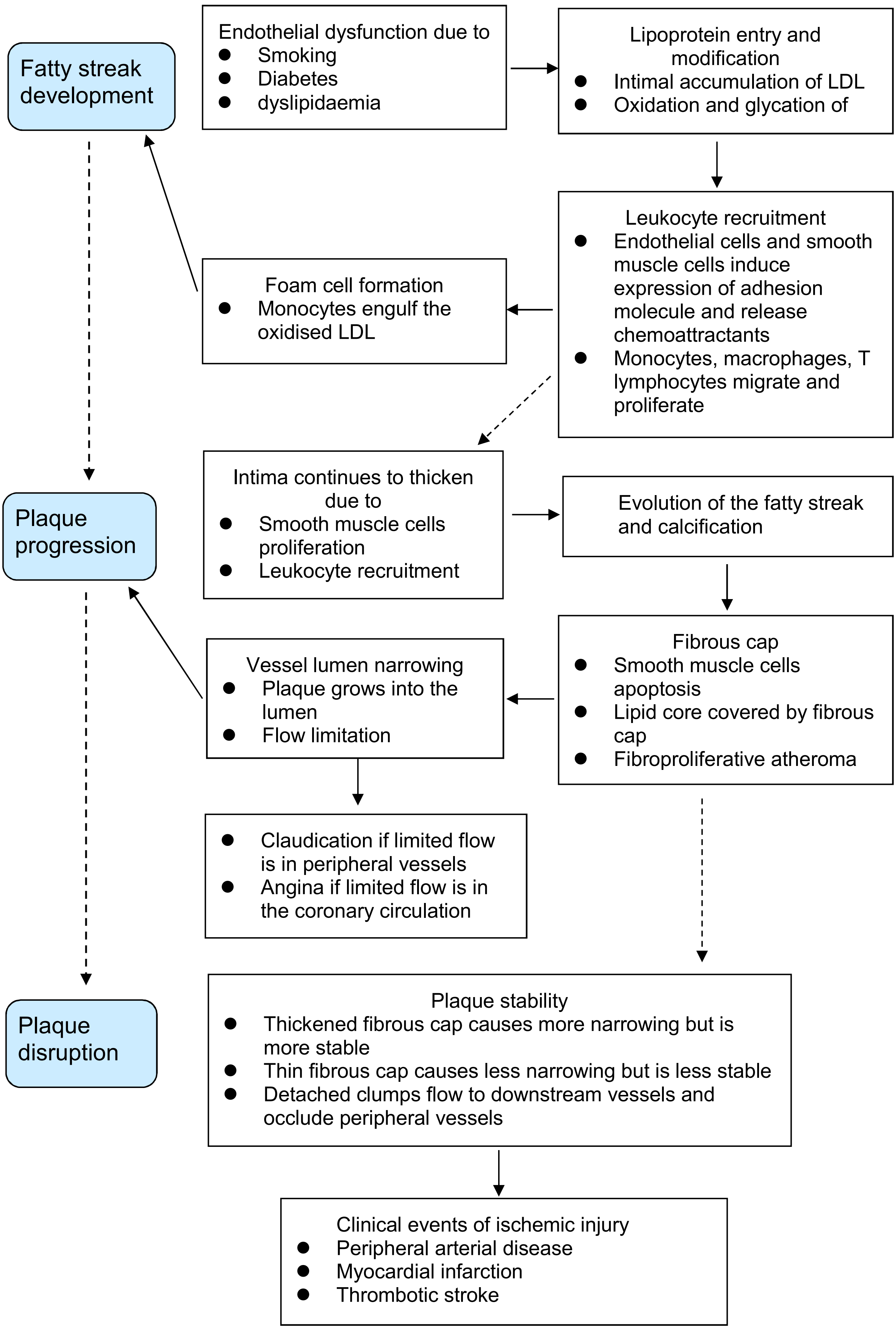
PAD is similar to atherosclerosis elsewhere in the body. Atherosclerosis can be briefly described as being divided into several stages: lesion initiation, progression of the lesion, and plague complications (170). Smoking, diabetes, dyslipidemia, high blood pressure and other risk factors can cause endothelial injury or endothelial dysfunction (171-174).

The lesion initiation stage is endothelial injury. This stage involves recruitment of monocytes to the intimal layer of the vessel wall, dependent on a number of adhesion molecules including selectins and vascular cell molecule-1 (VCAM-1) (170). Selectins are responsible for transient deposition of leukocytes on the epithelium. VCAM-1 is involved in binding monocytes and lymphocytes on the epithelial cells (175). After the leucocytes have immigrated into the intima, they collect lipids and become foam cells. The release of some growth factors, including platelet-derived growth factor (PDGF), leads to smooth muscle cell migration and proliferation. Foam cells of atheromatous plaques are originated from macrophages or smooth muscle cells via the very-low-density lipoprotein (VLDL) receptor and LDL modifications recognized by scavenger receptors. The foam cells form so-called “fatty streaks”, inflammatory lesions that affect the intima of the artery. The fatty streaks are largely constituted of smooth muscle cells, macrophages, monocytes, and T and B cells. The final stage of atherogenesis is that the fatty streaks then develop into fibroproliferative atheroma (plaque) that contains a number of smooth muscle cells filled with lipids. The advanced lesion contains intrinsic vascular wall cells (endothelial and smooth muscle cells) and inflammatory cells (monocytes, macrophages and T lymphocytes). This cellular component is then combined with a lipid core covered by a fibrous cap (170). Two factors play an important role in determining whether the plaque is stable or not: the thickness of the fibrous plaque and the amount of extracellular matrix including collagen synthesised by proliferated smooth muscle cells (176). T cells may inhibit collagen synthesis (177). Macrophages can digest the collagen of the fibrous cap and make the plaque unstable (178). The progressively built-up plague in the lining of artery leads to the narrowing of the vessels (174).

When the fibrous cap is disrupted, a coagulation cascade initiates. The platelets, fibrinogen and other coagulation factors form a platelet clump (170). If the platelet clump is attached to the vessel wall firmly, it can continue to build in size until it completely blocks the vessel lumen. However, if the platelet clump is not firmly attached to the vessel wall, it may separate into smaller clumps due to the blood flow (174). The detached clumps flow to downstream vessels and occlude peripheral vessels, and cause relevant clinical events of ischemic injury such as thrombotic stroke, MI and peripheral arterial disease (179). Figure 1.1 shows the stages of atherosclerosis briefly.

The compensatory mechanisms involve remodeling such as vasodilation, anaerobic metabolism and development of collateral vessels (174, 180). As PAD progresses, these compensatory mechanisms cannot offset the oxygen demands for the ischemic region. Tissues in the relevant region tend to experience necrosis (ulcer, gangrene, amputation) (13, 181, 182).

Figure 1.1 Atherosclerosis stages of development



1.3.3 Management

1.3.3.1 Public health burden

Since PAD often co-exists with CHD and other cerebrovascular disease and shares many risk factors, the prevalence of PAD is high and increasing. In the US, PAD affects 8 to 12 million individuals (12). It is estimated that the initial treatment episode of a patient requiring major lower limb amputation costs about $65,000 (£40,000), representing a huge cost to the health service (183). In the UK, there are approximately 3,500 lower limb amputations carried out every year as a result of PAD (184). Critical limb ischaemia has been estimated to cost the health service over £200 million per year (185).

Of the estimated 27 million people who have PAD in Europe and North America, 60% are asymptomatic (122). In the late stages of the disease, PAD progresses to ischemic ulcer and gangrene. Major amputation may be required eventually in at least a third of these patients (186). However, public awareness of PAD - diagnosis, risk factor knowledge, symptoms and amputation risk – is low (187). In the USA, only 25% of people with PAD are under treatment. In a 10-year follow-up, patients with PAD were found to have a higher risk of all-cause death (adjusted RR 3.1, 95%CI 1.9-4.9) death from CVDs (adjusted RR 5.9, 95%CI 3.0-11.4) and death from CHD (adjusted RR 6.6 95%CI 2.9-14.9), compared with patients without PAD (188).

1.3.3.2 Treatment

The treatment of PAD mainly aims to improve lifestyle risk factors, alleviate symptoms, halt or slow the progression of PAD, and reduce the risk of cardiovascular events (MI, stroke and death)(12). In some cases, surgery may be required. What type of treatment is suitable depends on the extent and severity of the disease condition (12, 189).

Smoking cessation has received a class I recommendation in the American College of Cardiology/American Heart Association (ACC/AHA) guidelines. Smoking cessation has been shown to not only reduce claudication symptoms but also reduced overall mortality and cardiovascular events (190). A Finnish study reported adjusted OR 0.86 (95% CI 0.75-0.99) of IC in ex-smokers, compared with current smokers (191). Cui et al. reported no significant difference within 19 years of smoking cessation but the risk of PAD was significantly reduced among those who had stopped smoking at least 20 years previously (OR 0.30, 95% CI 0.10-0.90) (155).

Modifying diet and/or taking up exercise may help slow down the progression of PAD when IC is the only symptom. Törnwall et al. reported an inverse association between incidence of IC and dietary intake of anti-oxidants such as vitamin C, α-Tocopherol and ß-carotene (191). A meta-analysis of three different randomised trials has found that surgical reconstruction combined with subsequent physical training increased the symptom-free walking distance in patients of IC (192). In the management of PAD, supervised exercise has been recommended by the National Institute for Health and Care Excellence (NICE) as one of the first steps.

Diabetes, hypertension and hyperlipidemia are the other three major risk factors for PAD (160). The American Diabetes Association suggested tight control of diabetes (hemoglobin A1c of <7%) among PAD patients to reduce complications(193). In the Diabetes Control and Complication Trial, type I diabetes patients experienced a 22% risk reduction of PAD events following intensive insulin therapy (194). Whether anti-hypertensive treatment reduces the progression of PAD is not fully known. But the Heart Outcomes Prevention Evaluation Study found that the anti-hypertensive therapies, angiotensin converting enzyme inhibitors and angiotensin receptor blockers, diminished the progression symptoms of PAD (195). Researchers have suggested a target LDL-C concentration of <100 mg/dL for PAD patients and a target LDL-C concentration of < 70 mg/dL if PAD coexists with other CVDs (196). Statins are commonly recommended for hyperlipidemia and antiplatelet medications may be recommended to reduce the risk of blood clot (197).

Endovascular therapy for PAD includes angioplasty and stenting. Owing to the advances of catheter and balloon design, the number of percutaneous procedures has increased (198). If endovascular therapy is not appropriate, arterial bypass surgery can be performed to divert blood which is not able to flow down a blocked artery through an artificial vessel to reach the tissues which need it.

1.4 Summary of the introduction

SHS is inhaled smoke from other people's cigarettes and contains over 250 harmful chemicals. Exposure to SHS has both immediate and long-term adverse effects on health, and the WHO has recognised no-safe level for SHS exposure. In spite of this, only 16% of the global population is currently protected from SHS exposure by comprehensive smoke-free legislation (3).

Among non-smoking adults, SHS has been linked with increased risk of many diseases, including coronary heart disease and stroke (4, 6). In a meta-analysis, exposure to SHS was associated with a 25% increased risk of CHD (4). In the British Regional Heart Study, the risk of CHD among non-smokers exposed to high levels of SHS was comparable to that among light active smokers, in spite of a 30-fold difference in cotinine concentration (67). This contrasts with the relationship between tobacco smoke exposure and lung cancer where there is a linear relationship between cotinine concentration and lung cancer risk across the continuum from SHS exposure to active smoking (63, 66). The disproportionately high risk of CHD associated with SHS exposure is thought to be due to constituents other than nicotine (199). SHS is a mixture of air-diluted 'sidestream' smoke from a burning cigarette tip, and the 'mainstream' smoke exhaled by the smoker but sidestream smoke contains more toxic gases and small (< 2.5 μm), respirable particles than mainstream smoke. These appear to be particularly injurious to the vascular system (199).

PAD refers to atherosclerosis in the limbs. It shares many risk factors with other atherosclerotic conditions and, therefore, often co-exists with CHD and cerebrovascular disease (200). Many studies have reported a significant association between active smoking and PAD (118, 200). In contrast, research studies are generally lacking on the association between SHS exposure and risk of PAD. PAD is a relatively common condition and its prevalence is increasing due to the ageing population (13, 200). It is associated with increased risk of major morbidity and mortality (200). Therefore, it is important to identify and address modifiable risk factors and, specifically, to determine whether exposure to SHS may play a role.

1.5 Aims and objectives of this thesis

The aim of this thesis is to establish whether tobacco exposure is associated with PAD.

Firstly, in order to inform my own studies, I undertook a systematic review of the published literature pertaining to the association between exposure to tobacco smoke (both active cigarette smoking and exposure to SHS) and PAD. I confirmed that there is already sufficient evidence of the association between active smoking and PAD and I performed a meta-analysis to summarise this evidence. I also confirmed the current lack of published evidence on the association between SHS exposure and PAD.

Therefore, I focused on the association between SHS exposure and PAD. I used existing sources of data collected from the Scottish general population to examine the association between exposure to SHS and PAD. I identified two potential data sources: the Generation Scotland: Scottish Family Health Study (GS:SFHS) and the Scottish Health Survey (SHeS). These studies used different approaches to both measuring SHS exposure and the definition of PAD. Therefore, I was able to determine whether the results were consistent using these complementary studies that, effectively, addressed some of the weaknesses of each other. I used both resources to undertake cross-sectional studies. I then addressed the limitations of using a cross-sectional design by using record linkage of the SHeS data to undertake a third, cohort study.

Having demonstrated an association between SHS exposure and PAD, I then aimed to explore whether the disproportionately high risk of CHD associated with SHS exposure also holds true for PAD. In the Physician's Heart Study, researchers have suggested that TC/HDL-C ratio and CRP are strong predictors of incident PVD, independent of heart disease (167). A review of 13 prospective studies on CRP as a predictor for PVD has shown a strong association between CRP and PVD (201). I then used the SHeS to examine whether exposure to SHS is associated with disproportionately higher biomarkers of cardiovascular risk compared with active smoking.

Finally, since PAD is an age-related condition (13, 200), I took advantage of the existence of leukocyte telomere length data in a subgroup of the SFHS, to explore whether SHS exposure was associated with evidence of biological ageing.

Therefore, this thesis comprises six complementary studies that address the following specific objectives:

1. To undertake a systematic review on the association between exposure to tobacco smoke (both active cigarette smoking and exposure to SHS) and PAD (Chapter 2, 1 study: systematic review).

2. To determine whether exposure to SHS (measured as self-reported in GS:SFHS and as salivary cotinine concentrations in SHeS) was independently associated with PAD (defined by ABPI in GS:SFHS and by the Edinburgh Claudication Questionnaire in SHeS), whether exposure to SHS was an independent predictor of incident hospitalisations of PAD and all-cause mortality, and whether the associations varied by sex (Chapter 3, 2 cross-sectional studies and 1 retrospective study).

3. To determine whether SHS carries a disproportionately higher cardiovascular risk than active smoking for a given level of smoke exposure (Chapter 4, 1 cross-sectional study)

4. To determine whether there is any association between SHS and leukocyte telomere shortening per year of age (Chapter 4, 1 cross-sectional study)

2 Chapter 2: A systematic review on active smoking, secondhand smoke and peripheral arterial disease

2.1 Chapter summary

Cigarette smoking is an important risk factor for CHD, stroke and PAD. Many studies have also demonstrated an association between exposure to SHS and CHD and stroke. In contrast, there have been fewer studies on the association between active cigarette and PAD, and very few studies on SHS and PAD. In this chapter, a systematic review on the association between exposure to tobacco smoke (both active cigarette smoking and exposure to SHS) and PAD was conducted.

Medline, Embase, PubMed and Web of Science databases were used to identify relevant articles published up to 30 April 2012. Prior to the published studies in this thesis, there had been no published meta-analyses on the association between active cigarette smoking and PAD. Only two published studies on the association between exposure to SHS and PAD were identified. Therefore, this systematic review was followed by a meta-analysis of the published studies on the association between active cigarette smoking and PAD. Overall and stratified random effects meta-analyses, cumulative meta-analyses and meta-regression analyses were conducted. Heterogeneity was tested using the I^2 test, and publication and small study bias were tested using funnel plots and Egger's test. Fifty-five eligible studies were identified: 43 cross-sectional, 10 cohort and 2 case-control. Of the 68 results for current smokers, 59 (86.8%) were statistically significant and the pooled OR was 2.71 (95% CI 2.28-3.21). There was a high level of heterogeneity (I^2 94.9%, p<0.001) and the Egger's test was significant (p=0.023). The association with active smoking was significant among both general (OR 3.08, 95% CI 2.56-3.69) and disease populations (OR 1.54, 95% CI 1.31-1.83). Of the 40 results for ex-smokers, 29 (72.5%) were statistically significant and the pooled OR was 1.67 (95% CI 1.54-1.81). There was moderate heterogeneity (I^2 54.7%, p<0.001) and the Egger's test was significant (p<0.001).

There is now substantial evidence of an association between active smoking and PAD. The magnitude of the association is greater than that reported for CHD. The risk is lower among ex-smokers but, nonetheless, significantly increased compared with never smokers. The results highlight the need for interventions both to encourage quitting among existing smokers and discourage commencement among never smokers. However, the paucity of evidence on the association between SHS and PAD suggests that research is needed to examine whether there is an association.

2.2 Introduction

Almost one-fifth of the global population smoke tobacco or tobacco-related products, and the prevalence is increasing (202). Tobacco-related diseases account for approximately one death every six seconds, and up to half of the world's one billion smokers will die of a tobacco-related disease (203).

As described in the introduction chapter, CHD, stroke and PAD are all types of atherosclerosis. They often co-exist in the same patients and share many common risk factors including cigarette smoking (121, 204). It is generally assumed that CHD, stroke and PAD are manifestations of the same atherosclerotic disease process (204). However, there are some important differences. It has been more than a decade since it was shown that the magnitude of the association between active smoking and PAD may be even greater than that observed for CHD (121, 205). In spite of this, previous epidemiological studies, including those on active smoking have focused on CHD and stroke and pay relatively little attention to PAD.

There have been numerous studies on the association between active smoking and CHD and stroke. In 2011, a meta-analysis of 75 cohort studies reported that active smoking increased the risk of CHD in both men (RR 1.72, 95% CI 1.57-1.88) and women (RR 1.92, 95% CI 1.66-2.23) (15). Overall, the risk among ex-smokers lies between that of current and never smokers (206), but falls with increasing time from cessation. It also varies according to the duration of smoking prior to cessation; such that the risk will never fall to that of never smokers if cessation follows a prolonged period of smoking (207, 208). In 2013, a meta-analysis of 81 prospective cohort studies estimated the effect of active smoking in women compared with men. The pooled RR of stroke associated with current smoking compared with non-smoking was 1.83 (95% CI 1.58-2.12) in women and 1.67 (95% CI 1.49-1.88) in men. The risk of stroke was lower in ex-smokers than in current smokers but still significantly increased compared with never smokers in both sexes (209).

Studies collated into published meta-analyses have also shown significant association between SHS and CHD and stroke. In 1999, a meta-analysis of 18 studies reported a RR of 1.25 (95% CI 1.17-1.32) for CHD. There was evidence of a clear dose response relationship, with the RR increasing from 1.23 (95% CI 1.13-1.34) among those exposed to 1-19 cigarettes per day to 1.31 (95% CI 1.21-1.42) among those exposed to ≥20 cigarettes per day (4). In 2011, a meta-analysis of 20 studies on SHS demonstrated an increased risk of stroke (RR 1.25, 95% CI 1.12-1.38). A dose response relationship was also shown with the RR increasing from 1.16 (95% CI 1.06-1.27) among those exposed to around 5 cigarettes per day to 1.56 (95% CI 1.25-1.96) among those exposed to around 40 cigarettes per day (6).

In contrast, there have been fewer individual studies on active smoking and PAD and very few individual studies on SHS exposure and PAD. An association between active smoking and PAD was first reported in 1911 (133). Smokers develop PAD ten years earlier than non-smokers (210), and their disease is more likely to progress to amputation (211). The only systematic review to examine the association between active smoking and PAD was published in 2004 and identified 4 relevant cohort studies and 13 cross-sectional studies. The authors estimated that in countries where 30% of the population are smokers, around 50% of the PAD cases can be attributable to smoking (16). However, there had been no meta-analysis. Therefore, in this chapter, I conducted an updated systematic review on the association between exposure to tobacco smoke (both active cigarette smoking and exposure to SHS) and PAD, and a meta-analysis of the association between active cigarette smoking and PAD.

2.3 Materials and methods

2.3.1 Systematic review

A systematic review of the published literature pertaining to the association between smoking and PAD was undertaken. The reporting of this systematic review and meta-analysis was in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines (212, 213). Studies were identified using the Medline, Embase, PubMed and ISI Web of Science databases. The electronic search strategy was developed on the basis of the Population Intervention Comparison Outcome (PICO) framework (214): i.) Population: participants who had either ABI measurements recorded or completed a claudication questionnaire such as the Edinburgh Claudication Questionnaire or had peripheral angiography performed; ii.) Intervention: exposure to active cigarette smoking or exposure to SHS; iii.) Comparison: exposure to active cigarette smoking or exposure to SHS versus (*vs.*) no exposure; iv.) Outcome: PAD. The following search terms were applied: (peripheral arter* OR peripheral athero* OR peripheral vascular OR claudication OR ABPI OR ABI OR ankle brachial) AND (smoking OR cigarette* OR tobacco OR nicotine OR smoke*). The final search was conducted on 30 April 2012. The electronic search was restricted to observational studies published as journal articles published in, or translated into, English and published between 1 January 1980 and 30 April 2012 inclusive and studies undertaken on humans (Appendix 1). The type of study design was restricted to observational study (cross-sectional study, case-control study, and cohort study).

The articles identified by the electronic search were reviewed manually by 2 researchers (my supervisor Professor Jill Pell and me). Inclusion was limited to original studies that: examined the risk of developing PAD rather than its outcomes; defined PAD based on ABI less than or equal to 0.90 (215), a claudication questionnaire or peripheral angiography; and quantified the association between active smoking, exposure to SHS and PAD and reported the result as an OR, RR or HR with CIs. Where the latter information was missing a single attempt was made to contact the corresponding author to obtain the relevant information. Interventional studies were excluded. The reference lists of the articles identified by the electronic search were checked for additional relevant studies. Observational studies that examined the association between active cigarette smoking and PAD and met the above inclusion and exclusion criteria were assessed to decide that whether or not they could be included in the meta-analysis. For the meta-analysis, studies were also excluded if some, or all, ex-smokers were included in the same category as current smokers or if some current smokers were excluded because they fell below a cut-off for the amount smoked. Studies were also excluded if the results were only expressed in terms of the dose relationship with the amount smoked and not with smoking per se. Where more than one article related to the same study, only the most recent relevant article was used. The reference lists of eligible articles were reviewed to identify additional studies that might be relevant.

The quality assessment for all studies included in the systematic review was conducted using the QualSyst tools for the quantitative studies. The QualSyst tool for assessment of the quality of quantitative studies is a generic validated checklist which is made up of 14 questions (Appendix 2) (216). The QualSyst tools have been used by many published systematic reviews and meta-analyses (217, 218). The checklist for reporting meta-analyses of observational studies in epidemiology has been proposed and supported by the recommendations of a consensus statement and the National Health Service (NHS) Centres for Reviews and Dissemination (219, 220). This checklist for assessing the quality of quantitative studies includes: objective sufficiently described research question, appropriate study design, sufficiently described subject characteristics, well defined outcome and exposure, appropriate sample size, appropriate analytic methods, estimate of variance, control for confounding, detailed reporting of results, conclusions supported by the results (Appendix 2).

The following information was extracted from each of the eligible studies: study size, design and continent, the sex of participants, decade of publication, definition of PAD, recruitment from the general population or a disease population, referent group and level of statistical adjustment. Where results were presented for relevant subgroups, these were used in preference to the overall results. Similarly, where results were presented both unadjusted and adjusted for potential confounders, the latter were used. Appendix 3 is the data extraction template (221).

2.3.2Meta-analysis

OR can be approximated to RR if an outcome is rare (222). In those studies that reported only RRs, these were treated as equivalent to ORs. The results were categorised according to whether they compared current smokers with never/non-smokers, ex-smokers with never smokers or ex-smokers with current smokers.

Random effects meta-analyses were undertaken to produce pooled estimates of effect size, both overall and stratified by study characteristics: study size, design and continent, the sex of participants, decade of publication, definition of PAD, recruitment from the general population or a disease population, referent group and level of statistical adjustment. Forest plots were used to display the results of the meta-analyses. I^2 tests were used to estimate the magnitude and statistical significance of between-study heterogeneity. A value of 50% or more indicated a substantial level of heterogeneity (223). Funnel plots of the log ORs against standard errors were employed to assess visually whether publication or small study bias was likely. This was tested more formally using the Egger's test of the intercept (224). A p value <0.05 was considered indicative of publication bias. Meta-influence plots were used to determine whether individual studies heavily influenced the pooled estimate and cumulative meta-analyses were used to determine the extent to which the pooled effect sizes had changed over time as evidence accumulated (225). In cumulative meta-analyses, the pooled estimate of effect size is updated each time the results of a new study are published. This allows detection of both temporal trends and publication bias (226).

Univariable and multivariable meta-regression analyses were used to determine whether recorded study characteristics had contributed to between-study heterogeneity (227): study size, design and continent, the sex of participants, decade of publication, definition of PAD, recruitment from the general population or a disease population, referent group and level of statistical adjustment. When there are many covariates in meta-regressions, chances of false-positive findings increase. Higgins and Thompson proposed a permutation test approach to assessing the true statistical significance of meta-regression findings. At least 1,000 permutations were suggested for sufficient precision (228). In this study, 20,000 random permutations were used to produce multiplicity adjustment p values for each meta-regression analysis. P values <0.05 were considered statistically significant. All analyses were undertaken using Stata 12.0 (Stata Corporation, College Station, Texas, USA).

2.4 Results

2.4.1 Systematic Review

The electronic search identified 8,132 published articles. Of these, 3,631 were removed as duplicates and the titles of the remaining 4,501 articles were screened (Figure 2.1). Abstracts were reviewed for 341, and 100 justified review of the full text. Among these, only two studies were on SHS (38, 45). One study reported results for both active smoking and SHS (38), one for SHS only (45). For the meta-analysis on the association between active smoking and PAD, the study which reported only on SHS was excluded. Therefore, among the 100 full texts reviewed, fifty-one satisfied the exclusion criteria, resulting in 49 studies eligible for inclusion. A further 8 eligible studies were identified following manual review of reference lists in the 49 selected studies. Only two studies reported results as HR which was insufficient to comprise a useful subgroup. Therefore, they were excluded. The remaining 55 studies were included in the meta-analysis. In this Section 2.4.1, narrative synthesis focused on the eligible studies which reported results for active smoking. The studies on SHS are summarised in Section 2.5.

The 55 studies were published between 1989 and 2011 (Table 2.1). They included a total of 69,521 current smokers and 54,821 ex-smokers who were compared with relevant referent groups (Table 2.1). Twenty (36.4%) studies were conducted in Europe(130, 159, 191, 229-245), 15 (27.3%) in North or South America (38, 246-259), 15 (27.3%) in Asia (151, 155, 260-272), 3 (5.5%) in Australia (273-275), and 1 (1.8%) in Africa (276). One (1.8%) was multi-national (277). Eight studies (14.5%) recruited only male subjects (151, 155, 191, 239, 255, 260, 273, 274), 2 (3.6%) only female (257, 258), and 45 (81.8%) both (38, 130, 159, 229-238, 240-254, 256, 259, 261-272, 275-277). Forty-three (78.2%) were cross-sectional studies (38, 130, 151, 155, 159, 230-233, 235-238, 240, 241, 243, 246-249, 251-254, 256-259, 261-273, 276, 277), 10 (18.2%) were cohort studies (191, 229, 234, 239, 245, 250, 255, 260, 274, 275) and 2 (3.6%) were case-control studies (242, 244). Forty-seven (84.5%) studies defined PAD using the ABI (38, 130, 151, 155, 159, 229-233, 236-241, 243-253, 256-259, 261-273, 275-277), and 7 (12.7%) based on symptoms of intermittent claudication, using either the Edinburgh, WHO/Rose or San Diego claudication questionnaires (191, 235, 242, 254, 255, 260, 274). One study (1.8%)

used both the ABI and a claudication questionnaire, and examined symptomatic and asymptomatic PAD separately (234).

Of the 55 eligible studies, 24 (43.6%) reported results for both current and ex-smokers (38, 130, 151, 155, 159, 229-231, 234-236, 238, 239, 244, 250, 254, 255, 259, 260, 270, 273-275, 277), 24 (43.6%) for current smokers only (232, 233, 237, 240-243, 245, 247-249, 251, 252, 256-258, 261, 265-267, 269, 271, 272, 276), and 7 (12.7%) for ex-smokers only (191, 246, 253, 262-264, 268). The 48 studies on current smokers provided 68 estimates of effect size. Of these, 59 (86.8%) suggested a statistically significant association between current smoking and PAD (Figure 2.2). Current smokers were compared with never smokers in 29 (52.7%) studies (38, 130, 151, 155, 159, 229-231, 234-236, 238, 239, 244, 247, 248, 250, 254-257, 260, 266, 270, 271, 273-275, 277), and with non (never plus ex) smokers in 19 (34.5%) studies (232, 233, 237, 240-243, 245, 249, 251, 252, 259, 261, 265, 267, 269, 271, 272, 276). Seven studies (three cross-sectional (155, 243, 273), three cohort(255, 260, 274), and one case-control (244)) reported evidence of a dose-relationship with the amount smoked or duration of smoking.

Of the 31 studies of ex-smokers, 29 (52.7%) studies compared ex-smokers to never smokers(38, 130, 159, 229-231, 234-236, 238, 239, 244, 246, 250, 253-255, 259, 260, 263, 264, 268, 270, 273-275, 277), and provided 40 estimates of effect size. Of these, 29 (72.5%) suggested a significantly increased risk of PAD among ex-smokers (Figure 2.3). Only two studies compared ex-smokers with current smokers (155, 191). Both reported a significantly reduced risk of PAD. Törnwall et al. reported the odds ratio for ex-smokers as 0.86 (95% CI 0.75-0.99) (191). Cui et al. reported no significant difference within ten years of cessation (OR 0.80, 95% CI 0.62-1.07), and 10-19 years post cessation (OR 1.00, 95% CI 0.40-2.20) but the risk of PAD was significantly reduced among those who had stopped smoking at least 20 years previously (OR 0.30, 95% CI 0.10-0.90) (155).

2.4.2 Meta-analyses

In comparison with non-smokers, the pooled ORs for PAD in current smokers were 3.08 (95% CI 2.56-3.69, p<0.001) in general population studies, 1.54 (95% CI 1.31-1.63, p<0.001) in disease population studies and 2.71 (95% CI 2.28-3.21, p<0.001) overall (Figure 2.2). Overall, there was significant heterogeneity between the studies (I^2 94.9%, p<0.001). Visual inspection of the funnel plot suggested some asymmetry (Figure 2.4) and the Egger's test was statistically significant (p=0.023). In both the univariable and multivariable meta-regression analyses, sample size, definition of PAD, recruitment from the general population or a disease population, the sex of participants, decade of publication, and the use of never versus non-smokers as the reference group were not significant predictors of estimated effect size (Table 2.2). Study design, level of statistical adjustment and the continent in which the study was conducted were all significantly associated with the magnitude of the effect size in multivariable analysis, but were no longer statistically significant after adjustment for multiple testing (Table 2.2). Furthermore, the association between current smoking and PAD was statistically significant in all but one of the thirty subgroup meta-analyses (Table 2.3). The cumulative meta-analysis suggested that the pooled estimate of effect size had remained relatively constant over time.

In comparison with never smokers, the pooled ORs for PAD in ex-smokers were 1.76 (95% CI 1.58-1.97, p<0.001) in general population studies, 1.52 (95% CI 1.36-1.69, p<0.001) in disease population studies and 1.67 (95% CI 1.54-1.81) overall (Figure 2.3). Between-study heterogeneity was moderate (I^2 54.7%, p<0.001). The funnel plot was slightly asymmetrical (Figure 2.5) and the Egger's test reached statistical significance (p=0.003). In the meta-regression analyses, study design was significantly associated with the magnitude of estimated effect size (Table 2.2). There was a significantly higher risk of PAD among ex-smokers than never smokers in 24 (92.3%) of the 26 subgroups (Table 2.3). The cumulative meta-analysis suggested that the pooled estimate had remained fairly constant over time. In the meta-influence graphs, no individual study had a disproportionately large effect on the pooled estimates of current smokers or ex-smokers.

Table 2.1 Characteristics of studies reporting the association between smoking and peripheral arterial disease

First author	Year	Country	Study design	Number of smokers				Sex	Age (years)	Study population	PAD definition	Referent group
				Current	Non	Ex	Never					
Skalkidis (244)	1989	Greece	Case-control	102	-	40	58	MF	≥49	General	ABI/Questionnaire	Never
Mangion (237)	1991	UK	Cross-sectional	95	200	-	-	MF	68-92	General	ABI/Questionnaire	Non
Vogt (257)	1993	USA	Cross-sectional	147	-	-	956	F	≥65	General	ABI	Never
Bowlin (260)	1994	Israel	Cohort	2,958	-	1,313	2,707	M	40-65	General	Questionnaire	Never
Leng (159)	1995	UK	Cross-sectional	404	-	582	593	MF	55-74	General	ABI/Questionnaire	Never
Ögren (239)	1996	Sweden	Cohort	129	-	104	155	M	55	General	ABI	Never
Hooi (234)	1998	Netherlands	Cohort	39	-	35	384	MF	40-78	General	ABI/Questionnaire	Never
Meijer (238)	2000	Netherlands	Cross-sectional	1,294	-	2,609	2,547	MF	≥55	General	ABI	Never
Törnwall (191)	2000	Finland	Cohort	22,334*	-	4,538*	-	M	50-69	General	Questionnaire	Current
Yeh (258)	2000	USA	Cross-sectional	63	-	-	414	F	≥50	General	ABI/Questionnaire	Never
McDermott (251)	2001	USA	Cross-sectional	44	246	-	-	MF	≥55	General	ABI/Questionnaire	Non
Passos (254)	2001	Brazil	Cross-sectional	337	-	268	880	MF	≥60	General	Questionnaire	Never
Adler (229)	2002	UK	Cohort	710	-	857	831	MF	25-65	Diabetic	AAI/Questionnaire	Never
Fowler (273)	2002	Australia	Cross-sectional	463	-	2,695	1,312	M	65-83	General	ABI/Questionnaire	Never
Murabito (252)	2002	USA	Cross-sectional	522	1032	-	-	MF	≥40	General	ABI/Questionnaire	Non
O'Hare (253)	2002	USA	Cross-sectional	-	-	6643	6,886	MF	60±16	Haemodialysis	ABI/Questionnaire	Never
Tseng (269)	2004	Taiwan	Cross-sectional	135	373	-	-	MF	64± 11	Diabetic	ABI	Non
Faglia (233)	2005	Italy	Cross-sectional	760	-	-	1,799	MF	59± 11	Diabetic	ABI	Never
Jensen (235)	2005	Norway	Cross-sectional	6,070	-	6,117	7,342	M&F	40-69	General	ABI/Questionnaire	Never
Kennedy (250)	2005	USA	Cohort	184	-	944	1,161	MF	≥65	General	ABI	Never
Zheng (259)	2005	USA	Cross-sectional	3,945	-	4,904	6,324	M&F	45-64	General	ABI	Never & Non
Allison (247)	2006	USA	Cross-sectional	870	-	-	3,344	MF	45-84	General	ABI	Never
Collins (248)	2006	USA	Cross-sectional	76	327	-	-	MF	≥50	General	ABI/Questionnaire	Never
Cui (155)	2006	Japan	Cross-sectional	492	-	519*	204	M	60-79	General	ABI	Never & Current
He (262)	2006	China	Cross-sectional	-	-	376	1,605	M&F	≥60	General	ABI/Questionnaire	Never
Norman (275)	2006	Australia	Cohort	68	-	191	214	MF	62±9	Diabetic	ABI	Never
Rajagopalan (277)	2006	Multinational	Cross-sectional	4,834	-	6,309	18,112	MF	≥18	Haemodialysis	ABI/Questionnaire	Never
Woo (270)	2006	China	Cross-sectional	273	-	1,190	2,529	MF	≥65	General	ABI	Never
Bendermacher(230)	2007	Netherlands	Cross-sectional	1,847	-	2,520	2,911	MF	≥55	General	ABI	Never
Gabriel (249)	2007	Brazil	Cross-sectional	54	59	-	-	MF	66± 13	CAD	ABI	Non
Li (263)	2007	China	Cross-sectional	-	-	592	1,055	MF	68± 11	Diabetic	ABI	Never
Luo (264)	2007	China	Cross-sectional	-	-	1,169	1,878	MF	68±11	Hypertensive	ABI	Never
Paul (276)	2007	South Africa	Cross-sectional	168	374	-	-	MF	>50	General	ABI	Non
Rhee (266)	2007	Asia	Cross-sectional	860	5,765	-	-	MF	≥50	Diabetic	ABI	Never
Sritara (267)	2007	Thailand	Cross-sectional	357	1,948	-	-	MF	52-73	General	ABI/Questionnaire	Non
Tapp (245)	2007	France	Cohort	723	3,082	-	-	MF	30-65	General	ABI/Questionnaire	Non

Yang (271)	2007	China	Cross-sectional	790	3,926	-	-	MF	40-75	Hypertensive	ABI	Non
Maeda (265)	2008	Japan	Cross-sectional	898	3,008	-	-	MF	61±12	Diabetic	ABI	Non
Schgoer (242)	2008	Austria	Case-control	244	622	-	-	MF	67± 11	General	ABI/Questionnaire	Non
Zheng (272)	2008	China	Cross-sectional	2,142	3,044	-	-	M&F	≥40	Hypertensive/CVD	ABI	Non
Agarwal (38)	2009	USA	Cross-sectional	1,570	-	2,530	3,451	MF	>40	General	ABI	Never
Cacoub (231)	2009	France	Cross-sectional	1,292	4,387	-	-	MF	≥55	General	ABI/Questionnaire	Never
Kröger (236)	2009	Germany	Cross-sectional	1,116	-	1,638	1,979	MF	45-75	General	ABI	Never
Sigvant (243)	2009	Sweden	Cross-sectional	2,585	2,341	-	-	MF	60-90	General	ABI/Questionnaire	Non
Tavintharan (268)	2009	Singapore	Cross-sectional	-	-	217	417	MF	40-80	Diabetic	ABI	Never
Ramos (240)	2009	Spain	Cross-sectional	1,379	4,793	-	-	M&F	35-79	General	ABI/Questionnaire	Non
Alzamora (130)	2010	Spain	Cross-sectional	624	-	992	1,975	MF	>49	General	ABI	Never
Chuengsamarn (261)	2010	Thailand	Cross-sectional	24	195	-	-	MF	≥15	Diabetic	ABI/Questionnaire	Non
Lakshmanan (274)	2010	Australia	Cohort	292	-	2,260	1,442	M	65-83	General	Questionnaire	Never
St-Pierre (255)	2010	Canada	Cohort	2,834	-	757	553	M	35-64	General	Questionnaire	Never
Aboyans (246)	2011	USA	Cross-sectional	-	-	614	1,169	MF	45-84	General	ABI	Never
Escobar (232)	2011	Spain	Cross-sectional	210	1,252	-	-	MF	>70	General	ABI/Questionnaire	Non
Lee (151)	2011	Korea	Cross-sectional	603	-	1,298	616	M	≥50	General	ABI	Never
Sanna (241)	2011	Italy	Cross-sectional	1,485	3,627	-	-	MF	M≥45; F≥55	General	ABI	Non
Tailor-Piliae (256)	2011	USA	Cross-sectional	76	941	-	-	MF	60-69	General	ABI	Never

N number; PAD peripheral arterial disease; M male; F female; MF male and female together; M&F male and female separately; ABI ankle brachial index; UK United Kingdom; USA United State of America; CAD coronary artery disease; CVD cardiovascular disease; * this number was not included in the meta-analyses

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Table 2.2 Multivariable meta-regression analyses of the study characteristics associated with estimated effect size

		Current smokers				Ex-smokers			
		Coefficient	95% CI	P value	Multiplicity adjusted p value	Coefficient	95% CI	P value	Multiplicity adjusted p value
Sample size	1-250*								
	250-500	1.37	0.62-3.03	0.437	1.000	0.93	0.34-2.56	0.884	1.000
	500-1,500	1.33	0.60-2.98	0.484	1.000	0.79	0.21-2.97	0.726	1.000
	>1,500	1.13	0.52-2.43	0.764	1.000	0.47	0.17-1.33	0.156	0.845
Study design	Cross-sectional*								
	Cohort	0.73	0.48-1.09	0.126	0.862	0.52	0.31-0.89	0.017	0.242
	Case-control	4.14	1.72-9.93	0.001	0.054	0.52	0.05-5.09	0.577	1.000
PAD definition	ABI*								
	Questionnaire	1.10	0.69-1.76	0.697	1.000	1.07	0.60-1.91	0.820	1.000
Study population	General*								
	Diabetic	1.23	0.73-2.06	0.431	0.999	0.96	0.56-1.65	0.890	1.000
	Others	0.92	0.55-1.54	0.752	1.000	0.78	0.48-1.29	0.337	0.987
Continent	America*								
	Asia	0.58	0.39-0.87	0.009	0.199	0.77	0.53-1.13	0.188	0.936
	Europe	0.84	0.59-1.20	0.337	0.997	0.84	0.56-1.28	0.426	0.999
	Africa	1.44	0.57-3.69	0.442	1.000	-	-	-	-
	Oceania	1.35	0.83-2.20	0.227	0.967	0.67	0.34-1.29	0.231	0.961
	Multi-continent	0.49	0.20-1.21	0.122	0.871	1.02	0.64-1.65	0.919	1.000
Sex	Male only*								
	Female only	0.85	0.56-1.28	0.433	1.000	0.70	0.43-1.15	0.164	0.898
	Male and female	0.73	0.51-1.04	0.085	0.737	0.71	0.47-1.09	0.121	0.773
Year	1989-1998*								
	1999-2008	0.88	0.54-1.43	0.606	1.000	0.80	0.47-1.36	0.420	0.997

	2009-2012	0.95	0.60-1.50	0.813	1.000	0.75	0.41-1.36	0.343	0.990
Statistical adjustment	Fully adjusted*								
	Age/ sex adjusted	0.66	0.29-1.52	0.333	0.995	0.84	0.35-2.01	0.693	1.000
	Unadjusted	0.36	0.16-0.79	0.011	0.205	-	-	-	-
	Unknown	0.73	0.51-1.04	0.085	0.737	1.17	0.79-1.75	0.432	0.998
Referent group	Never smokers*								
	Non-smokers	0.78	0.58-1.05	0.101	0.813	-	-	-	-

CI confidence interval; PAD peripheral arterial disease; ABI ankle brachial index; * referent category
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Table 2.3 Subgroup analyses of pooled odds ratios

		Numbers of participants*	Current vs never/non-smokers		Ex vs never smokers	
			OR (95% CI)	P value	OR (95% CI)	P value
Sample size	1-250	532	3.93(2.59-5.98)	<0.001	1.68(1.37-2.06)	<0.001
	250-500	2,784	2.30 (1.57-3.36)	<0.001	2.41 (1.63-3.56)	<0.001
	500-1,500	6,851	2.20 (1.80-2.68)	<0.001	1.66 (1.46-1.89)	<0.001
	>1,500	206,129	2.32 (1.79-3.00)	<0.001	1.64 (1.46-1.84)	<0.001
Study design	Cross-sectional	190,303	2.51 (2.06-3.06)	<0.001	1.70 (1.55-1.86)	<0.001
	Cohort	24,927	2.84 (2.20-3.67)	<0.001	1.55 (1.25-1.90)	<0.001
	Case-control	1,066	8.80 (5.99-12.91)	<0.001	2.30 (0.37-14.18)	0.369
PAD definition	ABI	199,695	2.56 (2.12-3.09)	<0.001	1.66 (1.52-1.81)	<0.001
	Questionnaire	16,601	3.59 (2.47-5.21)	<0.001	1.72 (1.41-2.10)	<0.001
Study population	General	141,481	3.08 (2.56-3.69)	<0.001	1.76 (1.58-1.97)	<0.001
	Diabetic	18,969	1.75 (1.13-2.69)	0.012	1.46 (0.96-2.21)	0.074
	Other	55,846	1.46 (1.25-1.71)	<0.001	1.51 (1.36-1.69)	<0.001
Continent	America	55,125	3.20 (1.97-5.19)	<0.001	1.71 (1.40-2.09)	<0.001
	Asia	44,957	1.79 (1.49-2.16)	<0.001	1.67 (1.45-1.91)	<0.001
	Europe	77,480	2.51 (2.02-3.10)	<0.001	1.71 (1.46-2.00)	<0.001
	Africa	542	4.29 (2.66-6.91)	<0.001	-	-
	Oceania	8,937	5.35 (3.69-7.74)	<0.001	1.89 (1.45-2.48)	<0.001
	Multi-continent	29,255	1.46 (1.31-1.63)	<0.001	1.55 (1.42-1.69)	<0.001
Sex	Male only	23,187	3.47 (2.60-4.63)	<0.001	2.01 (1.55-2.60)	<0.001
	Female only	1,580	2.59 (1.52-4.42)	<0.001	1.81 (1.46-2.25)	<0.001
	Male and female	191,529	2.33 (1.97-2.76)	<0.001	1.58 (1.45, 1.71)	<0.001

Year	1989-1998	11,001	3.50 (2.19-5.59)	<0.001	1.85 (1.29-2.65)	0.001
	1999-2008	151,761	2.46 (2.05-2.95)	<0.001	1.62 (1.47-1.79)	<0.001
	2009-2012	53,534	2.73 (2.11-3.52)	<0.001	1.77 (1.50-2.09)	<0.001
Statistical adjustment	Fully adjusted	166,413	2.98 (2.47-3.60)	<0.001	1.68 (1.54-1.84)	<0.001
	Age/sex adjusted	20,006	2.58 (1.78-3.73)	<0.001	1.70 (1.18-2.45)	0.005
	Unadjusted	3,067	0.86 (0.61-1.21)	0.373	-	-
	Unknown	26,810	2.10 (1.52-2.91)	<0.001	1.56 (1.17-2.09)	0.003
Referent group	Never smokers	151,698	3.22 (2.58-4.02)	<0.001	-	-
	Non-smokers	41,977	2.10 (1.71-2.58)	<0.001	-	-

vs versus; OR odds ratio; CI confidence interval; ABI ankle brachial index; ***** number of participants in the meta-analyses
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Figure 2.1 Study selection (PRISMA chart)

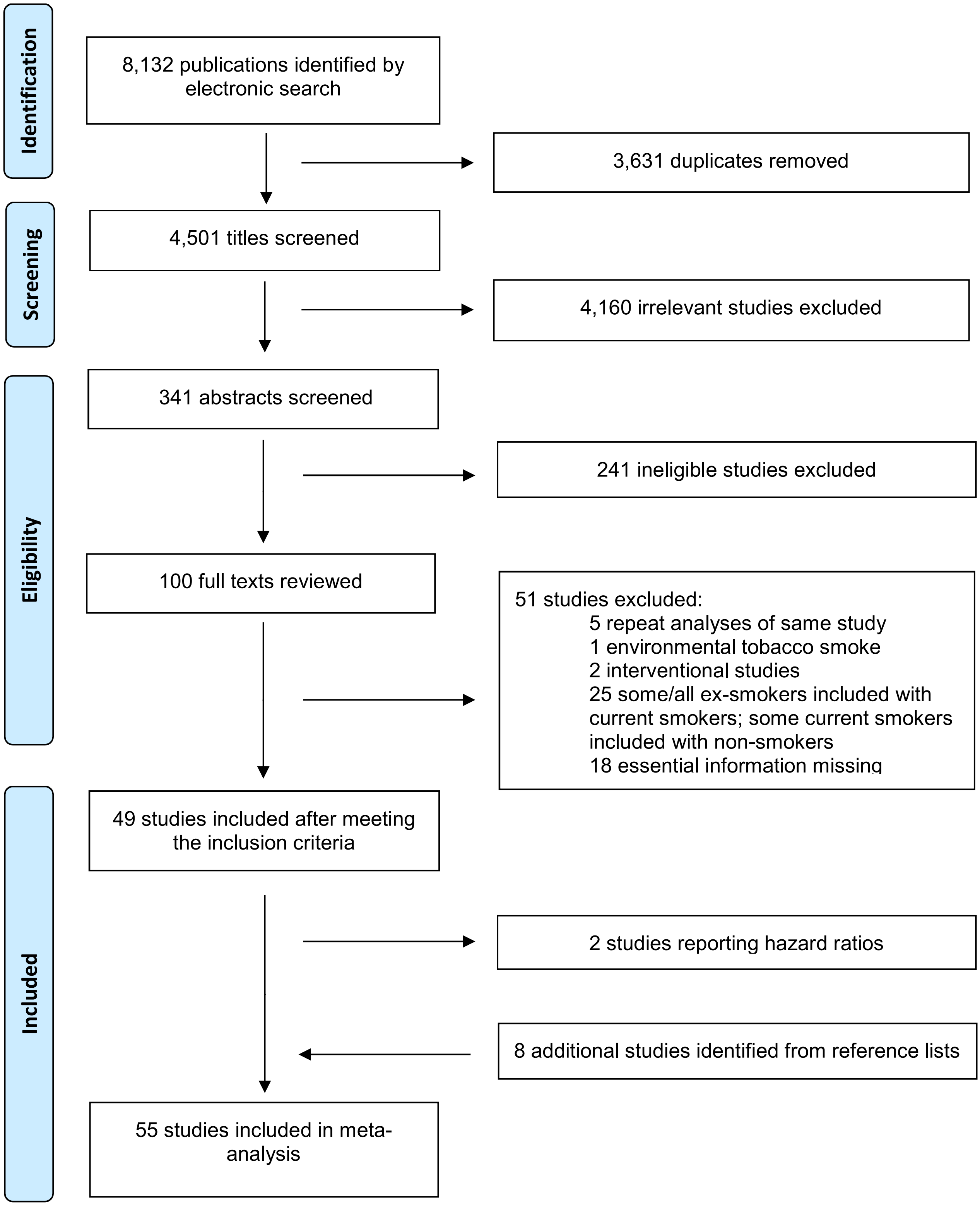

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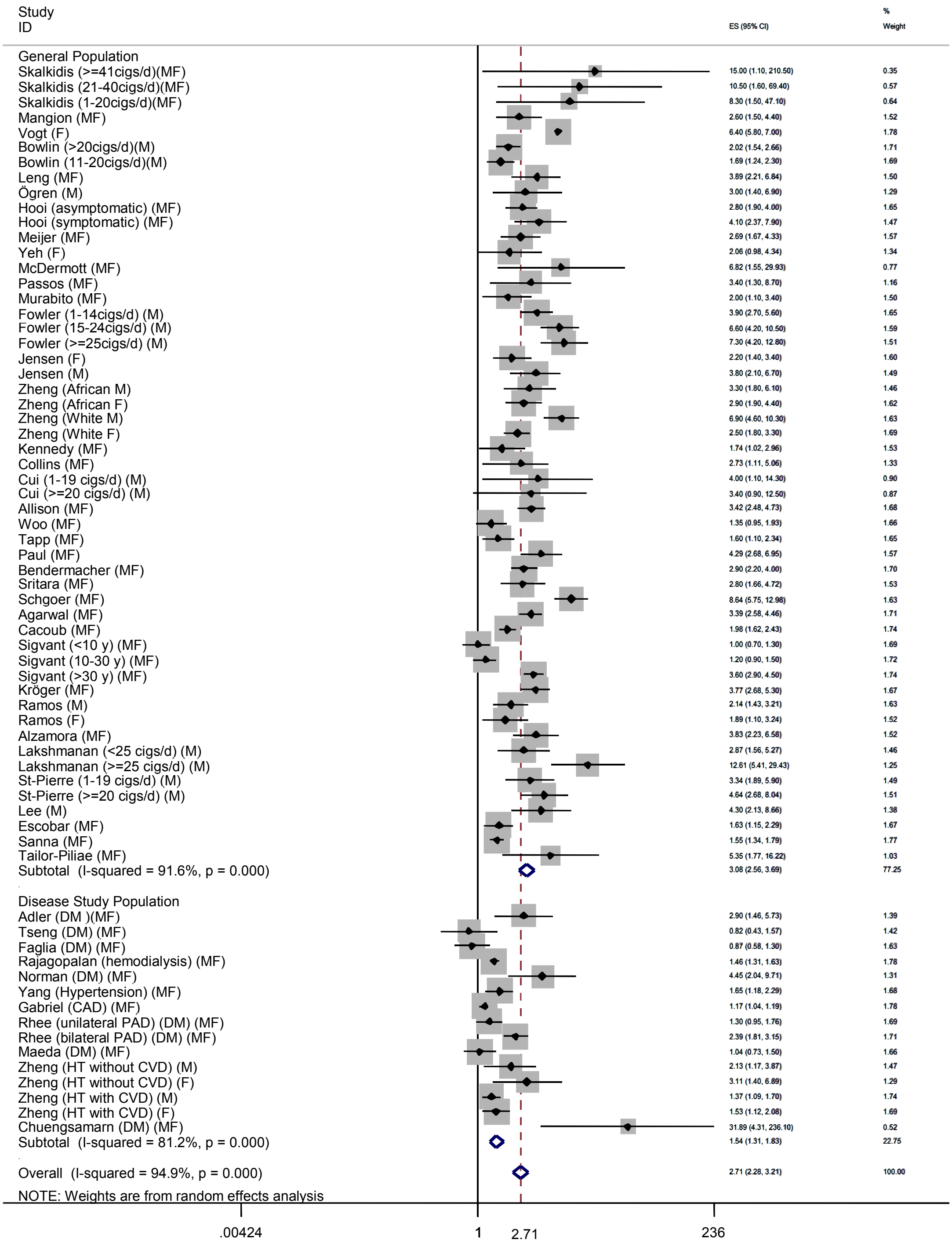
Figure 2.2 Forest plot of current smokers compared with never/non-smokers



ES effect size; CI confidence interval; MF Male and female; M Male; F Female; DM diabetic; HT Hypertensive; PAD peripheral arterial disease; CVD cardiovascular disease; CAD coronary artery disease; cigs/d cigarettes/day; y year

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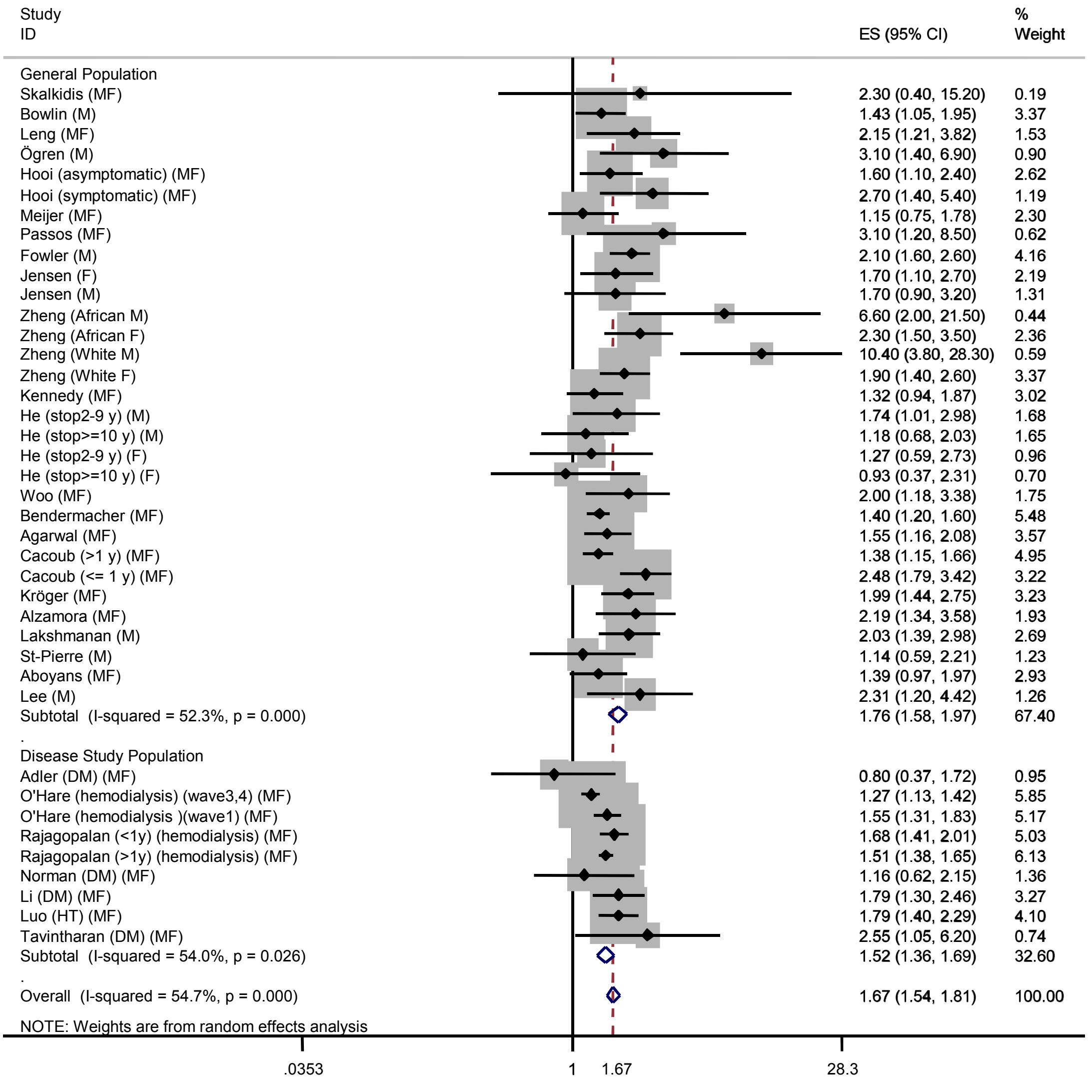
Figure 2.3 Forest plot of ex-smokers compared with never smokers



ES effect size; CI confidence interval; MF Male and female; M Male; F Female; DM diabetic; HT Hypertensive; PAD peripheral arterial disease; CVD cardiovascular disease; CAD coronary artery disease; cigs/d cigarettes/day; y year
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Figure 2.4 Funnel plot of studies examining the association between current smoking and risk of peripheral arterial disease

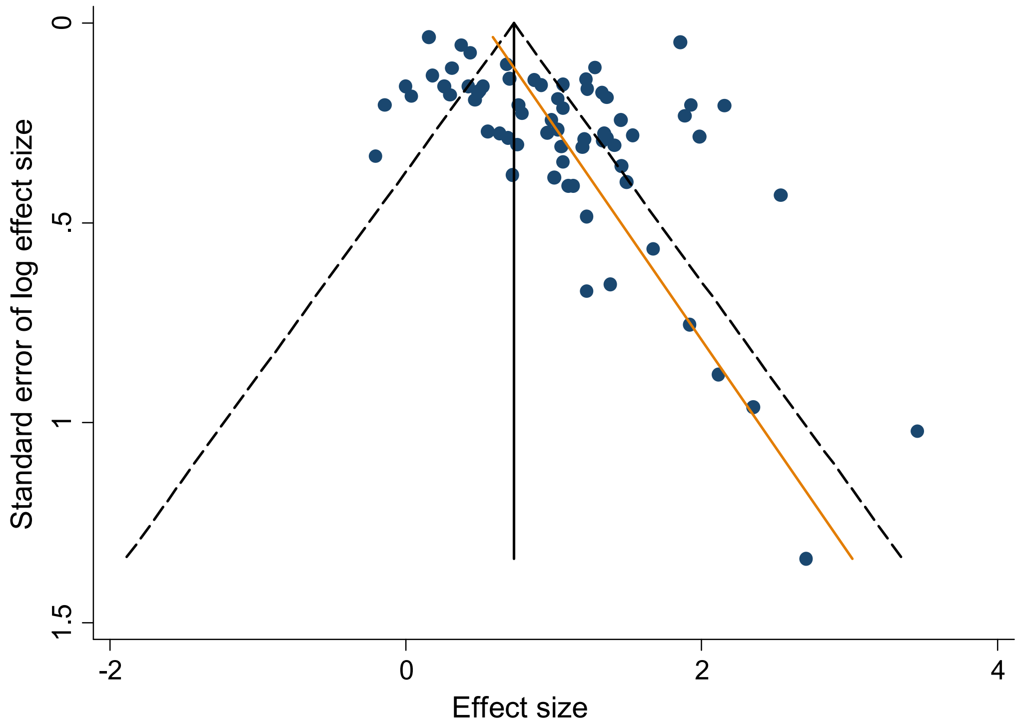
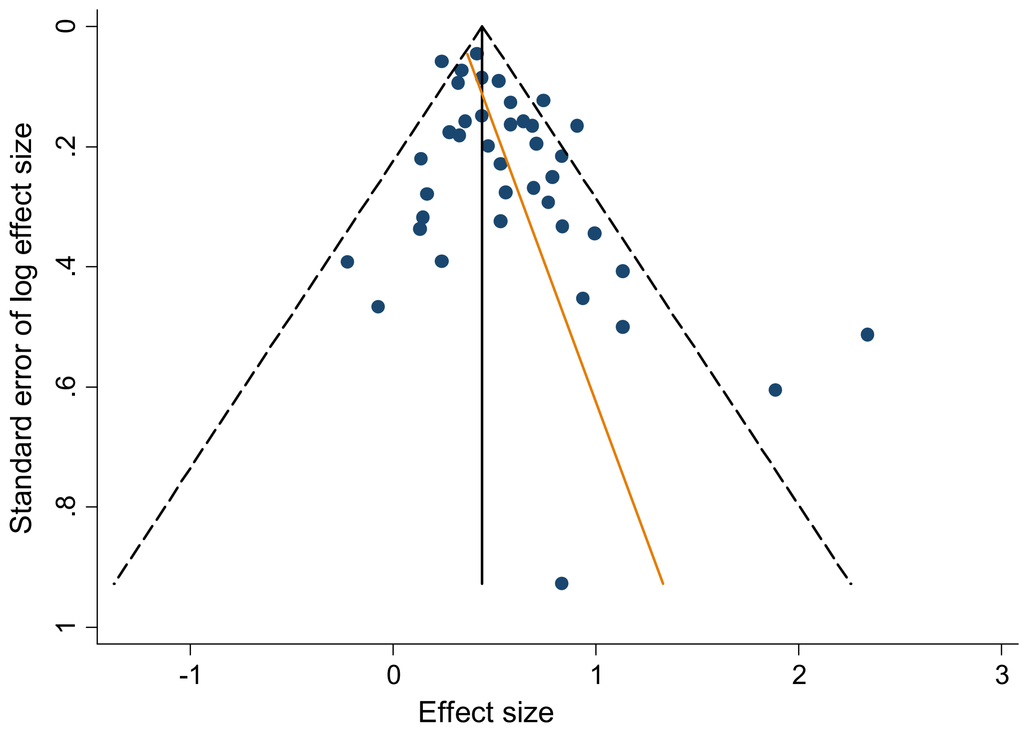


Figure 2.5 Funnel plot of studies examining the association between past smoking and risk of peripheral arterial disease



2.5 Secondhand smoke and peripheral arterial disease

Only two studies have been published on the association between exposure to SHS and PAD (38, 45). One study reported results for both active smoking and SHS (38) and therefore was included in the meta-analysis. The other study reported results for only SHS (45). Both studies were cross-sectional. One study was conducted among 1,209 Chinese women aged ≥60 years who had never smoked. SHS exposure was defined by self-report in the home and workplace. The study reported significant association, with adjusted ORs of PAD defined by WHO Rose Questionnaire, by ABI<0.9, and by either were 1.87 (95%CI 1.30-2.68), 1.47 (95%CI 1.07-2.03) and 1.67(95%CI 1.23-2.16) respectively. There was evidence of a dose-response relationship whereby the risk of overall prevalence of PAD increased with increasing amount of SHS exposure amount from 1-9 cigarettes per day (OR 1.40, 95% CI 0.87-2.28), 10-19 cigarettes per day (OR 1.76, 95% CI 1.20-2.61) to ≥20 cigarettes per day (OR 1.90, 95% CI 1.27-2.86) (p for trend=0.002). Dose-response relationship was also shown between risk of PAD and duration of SHS exposure from ≤20 minutes per day (OR 1.62, 95% CI 1.04-2.58), ≤40 minutes per day (OR 1.59, 95% CI 1.11-2.30) to >40 minutes per day(OR 2.68, 95% CI 1.49-4.88) (p for trend=0.001) (45). The other study examined 5,653 non-smokers aged >40 years in the USA using the pooled data from the NHANES. SHS exposure was measured using serum cotinine. They did not show an overall association between cotinine concentration and PAD defined by ABPI but suggested a possible threshold effect with significant association evident for cotinine concentrations >155 ng/mL (38).

2.6 Discussion

2.6.1 Main findings of this research

There are now a large number of published studies on the association between smoking status and PAD, and they provide consistent evidence of an increased risk among current smokers. The risk is lower among ex-smokers but, nonetheless, significantly increased compared with never smokers.

2.6.2 What is already known on this topic

The association between smoking and PAD was first recognised in 1911 (133). In 2004, Willigendael et al. published a systematic review on smoking and PAD and identified 4 relevant cohort studies and 13 cross-sectional studies. One of the cohort studies was conducted on the same study participants included in one of the cross-sectional studies (16). Using data from the cross-sectional studies, they derived weighted ORs of 2.3 for current smokers and 2.6 for ex-smokers. However, to my knowledge, the study in this chapter is the first meta-analysis of the association between active cigarette smoking and PAD. This meta-analysis included 38 studies published after Willigendael et al.'s review, and produced ORs of 2.7 and 1.7 respectively. Individual studies have suggested that the magnitude of the association with active smoking is even greater for PAD than CHD (121, 205). My results corroborate this. In this study, the pooled OR of 2.71 for current smokers compares with RRs of 1.72 and 1.92 for men and women respectively in a meta-analysis of smoking and CHD (15). In contrast to CHD, my meta-regression analyses did not provide any evidence that the magnitude of the association between smoking and PAD differs between men and women.

2.6.3 Strengths and limitations

The study in this chapter was reported in accordance with the PRISMA Statement, which consists of a four-phase flow diagram (Figure 2.1) and a 27-item checklist (Appendix 4). The PRISMA guideline is an evolution of the original QUOROM (Quality of Reporting of Meta-analyses) guideline. PRISMA focuses on ways to ensure the transparent and complete reporting of systematic reviews and meta-analyses (212). My systematic review was undertaken using four databases (Medline, Embase, PubMed and ISI Web of Science) to ensure that the largest possible number of eligible studies were identified. Despite this effort, 8 studies were added from the reference lists of the 49 identified studies. This suggests that the search strategy did not give an exhaustive list of relevant publications. However, the number of the identified studies is large enough to provide a meaningful result. The pooled estimates for current smokers were derived from a total of 47,187 current smokers who participated in 48 studies. The I^2 test was used to measure heterogeneity. Higgins et al. argue that since clinical diversity and methodological diversity always occur when different studies are brought together in a systematic review, statistical heterogeneity is inevitable (223). Random effects meta-analyses allow for heterogeneity by assuming that the underlying effects follow a normal distribution. Also, heterogeneity may be explored by conducting subgroup analyses and meta-regressions. In my study, random effects meta-analyses were used, in preference to fixed effect models, so that the weighting process took account of possible between-study heterogeneity due to differences in study population and methodology (223, 278). Because of the large number of studies on the association between active cigarette smoking and PAD now published, I was able to supplement the overall meta-analyses with stratified meta-analyses that generated pooled estimates for subgroups defined by study size, design, continent, sex, decade of publication, definition of PAD, use of a general population or disease study population, reference group and level of adjustment.

A systematic review involves defining review questions, developing inclusion and exclusion criteria, developing a comprehensive search strategy, assessing quality for all relevant studies to reduce bias, synthesising and presenting findings (279, 280). It synthesises the evidence based on the largest possible number of studies on a particular topic identified under a search strategy related to PICO (281). A systematic review often includes a meta-analysis (quantitative synthesis) using statistical techniques from data extracted from the eligible studies into a pooled estimated effect size to examine the strength of the association or the effectiveness of the intervention (282). Since heterogeneity inherently occurs among individual studies, in a meta-analysis, meta-regressions and subgroup analyses are often used to examine what factors may account for the heterogeneity (223, 278). Narrative synthesis is the descriptive aspect of the studies in a systematic review and primarily uses a textual approach to summarise the findings from the included studies. It is often used when a statistical meta-analysis or another specialist form of synthesis is not feasible. Therefore, meta-analysis is sometimes viewed as 'superior' technique to narrative synthesis for integrating data (283). However, to inform the development of policy and practice, systematic reviews can be used to answer a wide range of questions including the effectiveness of a particular intervention and why a particular intervention works or not (284). It is useful to include the synthesis of different types of evidence including qualitative evidence (285). Narrative synthesis can be applied to both quantitative and qualitative studies and can be used in different ways subject to the review question (280, 286). In my systematic review and meta-analysis, I mainly focused on examining the strength of the association between cigarette smoking and PAD and so included only quantitative studies. I used narrative approach to describe the data extracted from the eligible studies as study characteristics (Section 2.4.1 and Table 2.1).

A properly conducted systematic review is often viewed as the best research evidence for a focused clinical, social science-related or health science-related question (287). However, the summary provided in a systematic review or meta-analysis relies on the methods used in the individual studies to estimate the effect size (288). However, there are inevitable methodological shortcomings in the design and execution of the individual primary studies, as a result, risk of bias can be introduced by the evidence itself (288). In my systematic review, a comprehensive search strategy was used to identify the largest number of potentially relevant studies. The QualSyst tools (216) which have been adopted by many published systematic reviews and meta-analyses (217, 218) were used to assess the quality of the potentially relevant primary studies. However, primary studies with positive results in support of the authors' research hypothesis are more likely to be reported. Thus, these studies are more likely to be identified, summarised and pooled in a systematic review or meta-analysis than studies that reported smaller or non-significant effect sizes, which may lead to publication bias (278). In my meta-analysis, the visual inspections of the funnel plots and Egger's test suggested possible publication bias. However, the limitations of Egger's test are discussed in the paragraph below. During the full-text screening period for the eligible studies for inclusion, 18 studies were excluded due to missing essential information *e.g.* smoking status even after the attempts had been made to contact the corresponding authors. Excluding these studies may introduce potential bias such as selection bias. Furthermore, deciding which study to be included to some extent can be subjective. This may lead to potential selection bias. In this systematic review, interventional studies were excluded with the hope of reducing between-study heterogeneity due to different types of study design. Since primary studies can vary in their design, methodological quality, measures of the outcome and exposure, and study populations, combining these studies together may lead to potential bias. However, I applied subgroup meta-analyses to examine how applicable the association between active smoking and PAD were across different subgroups by study characteristics. Meta-regression analyses were used to explore the factors that may contribute to the between-study heterogeneity. There is also a growing concern about if and how risk of bias appraisals inform the synthesis process (279). Researchers have suggested that sensitivity analysis, narrative assessment and restricting the synthesis to studies at lower risk of bias are the most common methods to incorporate risk of bias assessments into the synthesis process (279). One limitation about this systematic review and meta-analysis was that it did not include a sensitivity analysis. Section 2.4.1 and Table 2.1 describe the study characteristics of the studies included in the meta-analysis. Quality assessments of individual primary studies for inclusion were performed.

My meta-analyses were based on the aggregated results of individual studies. I did not have access to individual participant data. I did not include studies published in languages other than English or studies on sources of tobacco other than cigarettes. The systematic review identified results expressed as both ORs (from case-control and cross-sectional studies) and RRs (from cohort studies). OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome that will occur in the absence of that exposure. RR is the ratio of incidence rates in the exposed and unexposed groups. RR represents the cumulative risk over a time span (289). RR asymptotically approaches the OR if an outcome is rare, *e.g.* if a disease is rare (222). Since the population prevalence of PAD is relatively low (38, 121), RRs approximate to ORs (289). Therefore, I treated them as equivalent in my meta-analysis. The Egger's test is widely used to test for the funnel plot asymmetry. However, Irwig et al. have pointed out the limitation of the Egger's test. They have demonstrated that the standard error of the log OR is correlated with the size of the OR because of sampling variability alone even in the absence of small-study effects. Funnel plots which were plotted using log ORs may appear asymmetric, leading to false-positive test results of the Egger's test (290). Different from RR, HR is commonly calculated from Cox proportional regression models in survival analyses when summarising time-to-event data and represents instantaneous risk over the study time period. However, researchers have suggested that using HR for causal inference is risky due to the change of HR over time and the built-in selection bias in HR (291). There are methods to make an approximate conversion between HR and OR (292). HR can be approximated to RR if the outcome is rare, the follow-up period of time is short and the ratio of event rates of the outcome in two groups is small (292). Of the two studies reporting HR that met the inclusion criteria, one followed up at 5 yearly intervals and up to 30 years (293). The other had a median (interquartile range) follow-up of 12.7 (12.4-13.8) years (294). Approximating HR to OR to be pooled in a meta-analysis is imprecise with associated uncertainty. Excluding these studies would mitigate this problem. However, it is important to consider the totality of available evidence. As a balanced approach, I planned to split those studies reporting HRs as a subgroup in the meta-analyses. However, only two studies expressed the results as HRs and were insufficient to comprise a subgroup. Both reported a significant association between active cigarette smoking and PAD, which yielded the same conclusion as the overall pooled estimates in the meta-analysis. Kollerits et al. examined 1,160 men aged 40-59 years and followed up at 5 yearly intervals and reported that current smoking was significantly associated with incident intermittent claudication (adjusted HR 2.20, 95% CI 1.24-3.92, p=0.01) (293). Conen et al. reported a significant dose-relationship, with HR increasing from 11.94 (95% CI 6.90-20.65) among current smokers smoking <15 cigarettes per day to 21.08 (95% CI 13.10-33.91) among current smokers smoking ≥15 cigarettes per day. A strong risk gradient for PAD was demonstrated across 10, 10 to 29, and ≥30 pack-years, with adjusted HRs 2.52 (95% CI 1.49-4.25), 6.75 (95 CI 4.33-10.52) and 11.09 (95% CI 6.94-17.72) respectively. Ex-smokers also revealed attenuated risk (adjusted HR 3.16, 95% CI 2.04-4.89) (294). Since these two studies reported consistent findings. Therefore, the limitation of not including them in the meta-analysis is unlikely to have introduced significant bias to the overall results. Most of the published studies were cross-sectional studies. Therefore, temporal relationships cannot be ascertained and caution should be heeded in inferring causation from association

(Appendix 5). However, the stratified analyses demonstrated significant associations even in the subgroup of cohort studies. One study only reported their results separately for unilateral and bilateral PAD (266). Since they used a common referent group in both analyses, their weighting was slightly inflated, but the overall impact on the pooled estimate from 55 studies will be small. In this meta-analysis, in general populations, the pooled OR was much higher for current smokers versus non-smokers than ex-smokers versus never smokers. However, in disease populations, the difference in the pooled ORs for these two was less pronounced. One possible explanation is that people with other smoking related diseases may have already quit because of these diseases. Therefore, the proportion of ex-smokers within the non-smoker group may be higher in disease populations than in the general population. Thus the association for current versus non-smokers will be reduced among disease populations compared to the general population.

In the meta-analysis, I combined the unadjusted and adjusted estimates from the individual studies to obtain the overall pooled estimates. There is a growing concern among researchers that the adjustment for confounders in the individual studies can be a considerable source of heterogeneity (295). In the individual studies, confounding can be reduced via study design or addressed statistically using multiple regressions, propensity score matching and stratified analyses (295). As mentioned above, I did not have access to individual participant data. Although there is currently no consensus about how to synthesise adjusted and unadjusted estimates, Quigley et al. suggested synthesising the adjusted and unadjusted findings separately as a common option to avoid this potential heterogeneity due to adjustment for confounders (296). In this meta-analysis, I synthesised the unadjusted estimates and adjusted estimates separately by subgroup analyses. The pooled estimates for PAD in current smokers based on the adjusted estimates and the overall pooled estimate for PAD in current smokers yielded the same conclusion. However, the pooled estimate for PAD in current smokers based on the unadjusted estimates was not statistically significant. The relatively low quality of some included studies due to the lack of control for confounding may be one possible explanation. However, all observational studies, irrespective of the level of adjustment, are to some extent vulnerable to built-in bias including selection bias, measurement bias and confounding such as residual confounding (297). On the other hand, it is often pointed out by researchers that attention should be paid to overadjustment bias. Overadjustment bias occurs as a consequence of the control (including statistical adjustment, stratification and restriction) for an intermediate variable or a descending proxy for an intermediate variable on the causal pathway between the exposure and the outcome (298) (Appendix 5). Overadjustment would either increase net bias or decrease precision, and usually bias results towards the null (298). In this systematic review, the individual studies used empirical methods including multivariable statistical adjustment and stratification to reduce confounding. The choice of confounders in the regression models was often based on prior knowledge and/or stepwise regression analyses or other commonly suggested statistical methods. If overadjustment occurs, the adjusted estimate would be much smaller than the unadjusted estimate. The estimate of risk of PAD among smokers is likely to be attenuated due to overadjustment. Synthesising adjusted estimates alone in a systematic review is likely to underestimate the true effect or association due to overadjustment bias. Further research on the mechanisms relating smoking to PAD is needed to clearly define the intermediate variables on the causal pathway.

Meta-regression analyses enabled me to explore possible sources of between-study heterogeneity. Unsurprisingly, the two case-control studies produced higher estimates of effect size, and contributed to the heterogeneity, but they did not impact greatly on the overall result. Similarly, estimates differed accordingly to the degree of statistical adjustment, but the association with smoking was statistically significant in the subgroup of studies that adjusted for all potential confounders available to them. Meta-regression may result in false-positive (type I error) findings with a small number of primary studies, with multiple covariates, or when there is a large magnitude of statistical heterogeneity (228, 299, 300). It is suggested by the Stata Journal that permutation test is useful to assess the true statistical significance of meta-regression. It is suggested that 5,000 or 20,000 permutations may be necessary for sufficient precision (278). Permutation tests suppress P values when they are used to explore heterogeneity and will result in more conservative probability estimates. In other words, it is possible that the P values may cross over to the level of non-significance (228, 301).

2.6.4 Implications of this research

Smoking is the most important modifiable risk factor for PAD and is, therefore, key to prevention. The lower risk among ex-smokers suggests that smoking cessation should be encouraged, but more research is required to determine whether, and when, the risk reverts to that of never smokers and whether, as with CHD (207, 208), this is dependent on the duration of smoking. There have been numerous studies on the association between SHS and CHD and stroke. In contrast, up to 2012 only two studies had been published on the association between SHS and PAD. In the light of the relative paucity of original studies in this area, the goal of my next chapter is to examine the association between SHS exposure and PAD.

It is more than 100 years since the first study was published reporting an association between active smoking and PAD (133). In spite of this, the global prevalence of smoking is increasing, especially in large, developing countries such as China (203). My results reinforce the need to pursue tobacco control.

3 Chapter 3: Secondhand smoke and peripheral arterial disease

3.1 Chapter summary

The global prevalence of smoking is increasing. It is widely accepted that both active smoking and exposure to SHS are associated with CHD and stroke. As described in my previous chapter, there is now also a substantial body of evidence that active smoking is a risk factor for PAD. In contrast, there is a paucity of studies on the association between SHS exposure and PAD. Prior to my publication of the studies contained in this chapter, there had been only two studies published on this subject. The aim of this chapter was, therefore, to add to the existing evidence on the association between SHS exposure and PAD among adult non-smokers.

On viewing existing cohorts and surveys, it was clear that, in contrast with CHD, most studies have not collected data on PAD. Similarly, in contrast with smoking status, most studies have not collected data on SHS exposure. Hence, very few studies conducted on the general population have collected information on both SHS exposure and PAD.

I identified two potential sources of data on the Scottish general population: the Generation Scotland: Scottish Family Health Study (GS: SFHS) and the Scottish Health Survey (SHeS). Ideally, I would have included studies with objective measurement of both PAD (for example ABPI <0.9) and SHS exposure (for example cotinine concentration). In reality, data from SFHS measured PAD objectively using ABPI but used self-reported exposure to SHS. In contrast, the SHeS measured SHS exposure objectively, using salivary cotinine concentration, but ascertained PAD based on self-report of symptoms of IC using the Edinburgh Claudication Questionnaire. Therefore, the studies had different limitations and, effectively, complemented each other. By analysing data extracted from both studies, I was able to determine whether the findings were consistent using their different approaches. A limitation of both the SFHS and SHeS was their cross-sectional design. Therefore, I also used record linkage of the SHeS data to identify incident cases of PAD in a third, retrospective, cohort study. The methodology and results for all three studies are contained in this chapter. Logistic regression analyses were used for the two cross-sectional studies and Cox proportional hazard analyses were used for the cohort study. Potential confounders (age, sex, deprivation quintile, BMI, physical active, alcohol consumption, and survey year) were adjusted for in different multivariate analyses.

In my study using the SFHS, of the 5,686 never smokers, 134 (2.4%) had PAD based on ABPI. Three percent of participants with PAD reported being exposed to SHS for ≥40 hours per week, compared with only 0.6% of those without PAD (x^2 test, p=0.010). Following adjustment for potential confounders, participants exposed to ≥40 hours per week of SHS were still more likely to have PAD (adjusted OR 4.53, 95% CI 1.51-13.56, p=0.007), with suggestion of a log-linear dose relationship among those exposed.

In my study using the SHeS, of the 4,231 confirmed non-smokers (defined as self-reported non-smokers with a salivary cotinine concentration <15 ng/mL), 134 (3.2%) had IC based on the Edinburgh Claudication Questionnaire. There was suggestion of a dose relationship, whereby the risk of IC increased with increasing cotinine concentration. After adjusting for potential confounders, participants with a cotinine concentration ≥2.7 ng/mL were still at significantly increased risk of IC (adjusted OR 1.76, 95% CI 1.04-3.00, p=0.036), compared with those with a cotinine concentration <0.7 ng/mL. Among all non-smokers, 5.6% (95% CI -0.8%-11.7%) of IC cases were attributable to cotinine concentrations ≥2.7 ng/mL and a further 3.6% (95% CI -6.6%-12.8%) to cotinine concentrations of 0.7-2.6 ng/mL.

Of the 4,045 confirmed non-smokers, in the SHeS, who had consented to passive follow-up by record linkage to routine hospital admission and death certificate records, 1,163 (28.8%) had either moderate or high exposure to SHS (cotinine concentrations ≥ 0.7 ng/mL) at baseline. High exposure to SHS was associated with increased risk of all-cause death (adjusted HR 1.42, 95% CI 1.09-1.86, p=0.011) among all non-smokers and increased risk of PAD events (adjusted HR 2.82, 95% CI 1.14-6.96, p=0.024) among male non-smokers. There was suggestion of a dose relationship as the risk of all-cause death increased with increasing cotinine concentration at baseline (adjusted p for trend=0.001).

As with coronary heart disease and stroke, SHS exposure is independently associated with both prevalent and incident cases of PAD among non-smokers. Our findings add to the published evidence in support of protecting the general public from SHS exposure.

3.2 Introduction

The first WHO public health treaty (The WHO Framework on Tobacco Convention) focused on tobacco control, and included recommendations to protect the public from SHS exposure. Only 16% of the global population are protected by comprehensive smoke-free legislation (3). Most smoke-free legislation only prohibits smoking in public and work places. Even in many signatory countries, exposure to SHS remains unacceptably high, due to either breaches of the legislation or exposure in places not covered by legislation, such as homes and vehicles. In Scotland, six years after implementation of smoke-free legislation (24), and in spite of observed increases in home voluntary restrictions (302), 25% of male non-smokers and 12% of female non-smokers reported exposure to SHS in one or more location (303). In large, developing countries, such as China, the prevalence of smoking is increasing rapidly (22, 304) and awareness of the harmful effects of SHS exposure is low (21, 305).

Active smoking is widely recognised as a risk factor for all atherosclerotic diseases, including PAD (14, 15, 118). Evidence is increasing that exposure to SHS may also increase the risk of atherosclerosis. The sidestream smoke present in SHS contains high levels of fine particles (<2.5μm diameters) and toxic gases (28-31). Exposure produces rapid changes in platelet activation and endothelium-dependent vasodilation (31). The level of 11-Dehydrothromboxane B_2 (11-DH-TXB_2) and the level of malondialdehyde (MDA) increase in both non-smokers and active smokers, after repeated daily exposure to SHS of 30 cigarettes for 60 minutes per day over 12 days, but the levels of these biomarkers increased more in non-smokers than in active smokers. After exposure, the levels remained significantly high in non-smokers (59). The effect of exposure to SHS was cumulative in non-smokers (306). Many studies have demonstrated an association between SHS exposure and both CHD and stroke. A meta-analysis, published in 1999 of 18 studies, reported a RR of 1.25 (95% CI 1.17-1.32) for CHD and a clear dose relationship whereby the risk increased with increasing exposure from 1-19 cigarettes per day (RR 1.23, 95% CI

1.13-1.34) to more than 20 (RR 1.31, 95% CI 1.21-1.42) (4). In 2011, a meta-analysis of 20 studies demonstrated an increased risk of stroke among those exposed to SHS (RR 1.25 95% CI 1.12-1.38). A dose relationship was shown across the spectrum of exposure from 5 (RR 1.16, 95% CI 1.06-1.27) to 40 (RR 1.56, 95% CI 1.25-1.96) cigarettes per day (6).

Prior to the work described in this chapter, only two published studies had examined the association between SHS exposure and PAD. One cross-sectional study was conducted among 1,209 Chinese participants aged ≥ 60 years who had never smoked. This study relied on self-reported exposure to SHS and reported an overall association and a dose relationship (45). The other study was also cross-sectional and undertaken among 5,653 non-smokers aged >40 years in the USA. This study had access to serum cotinine concentrations and reported an association with PAD at very high exposure levels (38).

To examine the association between level of exposure to SHS and risk of PAD, I identified two potential data sources: GS: SFHS and SHeS. The baseline data from GS:SFHS collected objective measurement of PAD using the ABPI and an ABPI <0.9 as the definition of PAD. Exposure to SHS was based on self-report. The baseline data from the SHeS contained salivary cotinine concentration measurement and identified prevalent PAD cases on the basis of IC identified using the Edinburgh Claudication Questionnaire. I also used record linkage of SHeS to identify incident PAD events (defined as hospitalisation for PAD or death due to PAD) and all-cause deaths.

3.3 Materials and methods

3.3.1 Data source

Generation Scotland: Scottish Family Health Study (GS: SFHS)

The GS: SFHS is a cross-sectional study of the general population. Proband is a term used to describe an individual who is the initial member of a family to come under study in the medical genetics or other medical fields (307). In GS: SFHS, probands aged between 35 and 55 years of age were recruited between 2006 and 2011 from two cities in Scotland (Glasgow and Dundee) where they were randomly selected from general practitioner records. The probands were invited to identify and recruit their adult (≥18 years of age) first degree relatives (308). All participants completed a questionnaire on demographic information (including age, sex, and postcode of residence) and lifestyle (including smoking status, exposure to SHS alcohol consumption and physical activity). Trained research staff measured height, weight, brachial blood pressure, as well as ankle systolic blood pressure in the dorsalis pedis and posterior tibial arteries in both legs using standard procedures, and obtained blood samples for assays (including lipid concentrations).

Scottish Health Survey (SHeS)

The SHeS uses multi-stage, stratified probability sampling of residents of private households across Scotland (309). The Survey was undertaken in 1995, 1998, 2003 and then annually from 2008. Different households were recruited in each Survey. Household response rates were 81% in 1995, 76% in 1998, 68% in 2003, and 61%-64% between 2008 and 2010. The Surveys used a two-stage interview process: a face to face interview undertaken by the trained staff in which they administered questionnaires on demographics (including age, sex, social status and postcode of residence) and lifestyle (including smoking status, alcohol consumption and physical activity) followed by a nurse visit in which they collected anthropometric measurements (including height, weight, and blood pressure) and biomedical measurements (including blood, urine and saliva samples). In each survey, all individuals aged ≥16 years were asked by the nurse to provide a saliva sample to measure cotinine concentrations. In my study, I collated data from the 1998, 2003, 2008 and 2010 Surveys as they provided consistent information on both IC and salivary cotinine.

Over 90% of the SHeS participants consented to passive follow-up via record linkage to routine administrative data. In Scotland, the Information Services Division (ISD) of the NHS collates and links Scotland-wide administrative data including data on hospitalisations and deaths. Data on SHeS participants were linked, at an individual-level, to several Scotland-wide datasets including: death certificates (collected by the General Registrar Office) and admissions to acute hospitals (Scottish Morbidity Record [SMR] 01). I used the disease and procedures codes to identify those hospital admissions and deaths due to PAD which I defined as any of the following codes recorded in any position:

- International Classification of Disease, Tenth Version (ICD-10) A48.0, I10.5, I73.9, I70.2, I70.9, I74.3, I74.5, I79.2, R02,
- International Classification of Disease, Ninth Version (ICD-9) 250.7, 440.20, 440.21, 440.22, 440.23, 440.24, 440.29, 443.9, 443.81, 707.10, 785.4, or
- Office of Population Censuses and Surveys Classification of Surgical Operations and Procedures (OPCS) X09.3, X09.4, X09.5, X09.8, X09, X10.1, X10.4, X10.8, X10.9, X11.1, X11.2, X11.8, X11.9, X12.1, L54.1, L63.1.

SMR data undergo regular quality assurance checks. These demonstrate that the data are over 90% accurate and around 99% complete (310). The linked data provided follow-up to the censor date of 31 December 2011.

The study designs, definitions of PAD and measurement of SHS are summarised in Table 3.1.

Table 3.1 Study Characteristics

	GS: SFHS	SHeS 1998, 2003, 2008, 2010	SHeS record linkage study
Data Source Summary			
Coverage	Glasgow, Dundee	Scotland	Scotland
Participants (n)	21,558	41,664	37,967
Age >45 years (n, (%))	12,135 (56.3)	17,179 (41.2)	17,128 (45.1)
Age range (years)	18-92	0-97	0-97
PAD definition	ABPI<0.9	Edinburgh Claudication Questionnaire	Hospitalisation or death
SHS exposure	Self-reported	Salivary cotinine	Salivary cotinine
Study Summary			
Study design	Cross-sectional	Cross-sectional	Cohort
Age (years)	≥18	>45	>45
Participants (n)	5,686 never smokers (3,056 aged >45)	4,231 non smokers (2,293 never smokers)	4,045 non smokers (2,216 never smokers)
PAD cases (n)	134* (47 among aged 18-45) (86 among aged >45)	134 (55 never smokers) (79 ex-smokers)	64 (37 never smokers) (27 ex-smokers)
SHS exposure (n)	1,769	1,366**	1,163**

GS: SFHS Generation Scotland: Scottish Family Health Study; SHeS   Scottish Health Study; Scottish Morbidity Records   SMR; PAD peripheral arterial disease; ABPI   ankle brachial pressure index
*1 missing age data

**either moderate or high exposure to secondhand smoke (cotinine 0.7-14.9 ng/mL)

3.3.2 Ethical approval

Both GS: SFHS and SHeS have an ethics approval which permits the provision of anonymised data extracts to other researchers for uses that are consistent with the original aims of the studies. Therefore, I did not require an additional NHS ethics approval to obtain anonymised data extracts for any of the studies.

The GS Access Committee approved provision of an extract of data from GS: SFHS. Access to SHeS was obtained via the UK Data Service. Students or members of staff at a UK institution of higher or further education can register using the user account issued by their institution. I registered as a student from University of Glasgow and was able to download an extract of SHeS data. The Privacy Advisory Committee of the ISD, NHS National Services Scotland approved provision of follow-up data via individual-level linkage to death certificates and hospital admission records (SMR01, SMR04 and SMR06).

3.3.3 Inclusion criteria and definitions

Generation Scotland: Scottish Family Health Study (GS: SFHS)

For the study using GS: SFHS, participants who classified themselves as never smokers were included. The ABPI was calculated for each leg as the ratio of the highest measurement of ankle systolic blood pressure (either dorsalis pedis or posterior tibial artery) to the brachial systolic blood pressure. The presence of PAD was defined as an ABPI <0.9 in one or both legs (311). The level of SHS exposure was self-reported. Participants classified their exposure in their workplace, home and other locations as: none, a little, some or a lot, and classified their overall duration of exposure (total hours per week) as: none, 1-19, 20-39 or ≥40 hours per week. Alcohol consumption was self-reported and classified as never, stopped >1 year previously, stopped ≤1 year previously or drink currently. Physical activity was defined as self-report of moderate or vigorous activity of at least ten minutes duration on at least four days each week. Body mass index (BMI) was categorized into normal weight (<25 kg/m^2), overweight (25-30 kg/m^2) and obese (≥30 kg/m^2) (312). In Scotland, there are 6,505 datazones, based on postcode of residence, with a mean population of 800. The Scottish Index of Multiple Deprivation (SIMD) for each datazone is derived from information on income, employment, health, education (including skills and training), housing, crime, and access to services (313). The SIMD has been used to derive quintiles of socioeconomic status for the Scottish population; ranging from 1 (most deprived) to 5 (least deprived). The postcode of residence was used to categorise study participants according to these general population quintiles.

Scottish Health Survey (SHeS)

I combined the 1998, 2003, 2008 and 2010 Surveys for use in both the cross-sectional study and the retrospective cohort study as they provided consistent information on salivary cotinine and diagnosis of PAD at baseline. The 1995 Survey used serum to measure cotinine and, therefore, the concentrations, at any given level of SHS exposure, would differ from measurement using saliva samples. For both the cross-sectional and retrospective cohort studies, inclusion was restricted to participants who, at the time of participation in the Survey, were aged >45 years old, classified themselves as non (never or ex) smokers and whose salivary cotinine concentration was <15.0 ng/ml, as higher concentrations usually indicate smoking deception (40). Participants who reported taking nicotine replacement products were excluded. SHS exposure was categorised into low (cotinine <0.7 ng/mL), moderate (cotinine 0.7-2.6 ng/mL) and high (cotinine 2.7-14.9 ng/mL).

For the cross-sectional study, the presence of prevalent IC at the time of the study was determined using the results of the Edinburgh Claudication Questionnaire (314) (Table 3.2). The information on deprivation quintile (SIMD) was incomplete in the 1998 survey among participants who did not consent to the passive follow-up. Therefore, I used social class, as an alternative to SIMD, to adjust for confounding due to socioeconomic status in the logistic regression analyses. Social class was categorised into: professional, managerial technical, skilled non-manual, skilled manual, semi-skilled manual and unskilled manual.

Table 3.2 The Edinburgh Claudication Questionnaire

The Edinburgh Claudication Questionnaire:
CAD/PVD

* A positive questionnaire diagnosis of claudication is made only if the ***"correct"*** answer is given to ***all questions***

Questions	Correct Answer
1. Do you get pain or discomfort in your legs(s) when you walk? ○ Yes ○ No ○ Unable to walk • If you answered "yes" to question 1, please answer the following questions	Yes
2. Does the pain ever begin when you are standing or sitting still?	No
3. Do you get it when you walk uphill or in a hurry?	Yes
4. Do you get it when you walk at an ordinary pace on the level?	Yes
5. What happens if you stand still? • Usually continues for more than 10 minutes? • Usually disappears in 10 minutes or less?	 No Yes
6. Where do you get this pain or discomfort? • Mark the places with an "X" on the diagram	

Source: Adapted from Leng GC, Fowkes FG. *J Clin Epidemiol*. 1992; 45:1101-1109.

Participants with IC at baseline were excluded from the retrospective cohort study. The SHeS records had already been linked to several Scotland-wide databases including death certificates and SMR01. As described in section 3.3.1 data source, incident cases of PAD were defined as a hospital admission or death with relevant codes recorded in any position. For participants who had consented to passive follow-up via record, ISD was able to provide SIMD data. These were used to derive quintiles of deprivation, ranging from 1 (most deprived) to 5 (least deprived) (313). Participants were categorised into these quintiles based on the postcode of residence. BMI was classified as underweight or normal weight (<25 kg/m^2), overweight (25-30 kg/m^2) and obese (≥30 kg/m^2) (312). Physical activity was defined as self-report of any kind of physical activity for at least 3 hours per week (315). Alcohol consumption status was self-reported as: never drinker, ex drinker, low-risk drinker (< 28 units/week, women < 21 units/week), increasing-risk drinker (men < 50 units/week, women < 35 units/week) and high-risk drinker (men ≥ 50 units/week, women ≥ 35 units/week) (316).

For the study using GS: SFHS datasets, an ABPI <0.9 was used to define the presence of PAD. This included both asymptomatic and symptomatic PAD. For the study using SHeS datasets, the Edinburgh Claudication Questionnaire was used to identify intermittent claudication, the typical form of symptomatic PAD. PAD can progress from asymptomatic to symptomatic. Therefore, the latter is suggestive of a more severe form of the disease, and generally occurs at an older age (13). Therefore, I included those participants aged younger than 45 years in my study using GS: SFHS datasets to identify more PAD cases including asymptomatic cases in order to increase statistical power. In contrast, I included only participants aged >45 in my study using SHeS to keep the age inclusion criterion consistent to the previous published study on SHS and PAD (38). Furthermore, many studies on smoking and cardiovascular diseases have used an age of >45 years as the inclusion criteria.

As mentioned in the above section, according to the WHO, BMI was categorised into normal weight, overweight and obese (312). The categorisations of SIMD quantiles and socioeconomic status variable were predefined in SHeS. In SFHS, the categorisation of the variable on alcohol consumption was already predefined in the datasets provided. In SHeS, categorisation of variable on alcohol consumption was also predefined. In order to maximise available power to ensure sufficient cases, I combined some groups based on units/week in accordance with NHS alcohol risk assessment health check into low-risk, increasing-risk and high-risk (316). In SFHS, physical activity was predefined as " how many days per week did you do physical activity?" Due to statistical power, I defined at least 4 days per week as physically active. In SHeS, physical activity was predefined as "average hours doing all physical activities per week: no time, less than 1, less than 3, less than 5, less than 7, 7 hours or more". According to the WHO, for adults aged 18-64, or 65+, physical activity comprises at least 150 minutes of moderate activity or at least 75 minutes vigorous activity (315). Based on the predefined categories in the SHeS, I defined at least 3 hours per week as physically active.

The interaction between SHS and other risk factors (including age, sex and socioeconomic status) related to CVD have been tested by previous studies (317-319). Researchers suggested that SHS exposure is inversely associated with socioeconomic status (320, 321) and socioeconomic status is known risk factors for CVD (322, 323). In my studies, interactions tests with age, sex, variables on socioeconomic status were performed.

3.3.4 Statistical analyses

<u>Generation Scotland : Scottish Family Health Study (GS: SFHS)</u>

Categorical data were summarized using frequencies and percentages. Chi-square tests were used for categorical variables and Chi-square tests for trend for ordinal variables. Univariate and multivariate logistic regression models were used to examine the association between SHS exposure and PAD using no exposure as the referent category. I developed several models with increasing level of statistical adjustment: unadjusted, partially adjusted (age, sex and deprivation quintile) and fully adjusted (partially adjusted model plus alcohol consumption, physical activity and BMI category). The confounders were chosen based on the available prior knowledge and in keeping with the published literature. The covariates were selected via a combination of a forward-stepwise selection approach (significance level <0.20 for inclusion) (326) on one hand and published evidence on the other hand. Missing data on categorical or ordinal variables were coded as dummy values and included in the adjusted models. I tested whether there were statistically significant interactions with age, sex and socioeconomic status using the likelihood ratio test (39, 324). Statistical significance was defined as a two-sided p-value <0.05 for both main effects and interactions. All statistical analyses were undertaken using Stata 12.0 (Stata Corporation, College Station, Texas, USA).

Scottish Health Survey (SHeS)

Categorical data were summarised using frequencies and percentages. Chi-square tests for trend were used for ordinal variables and chi-square tests for categorical variables. Univariate and multivariate logistic regression models were applied to examine the association between SHS and prevalent IC using cotinine <0.7 ng/mL as the referent category (67, 325). I adjusted for the potential confounding effects of age, sex and social class. The confounders were chosen based on the available prior knowledge and in keeping with the published literature. The covariates were selected via a combination of a forward-stepwise selection approach (324) on one hand and published evidence on the other hand. A margin plot was used to predict the probability of IC over salivary cotinine concentration. 'Marginsplot' is a command in Stata that graphs the results from 'margins' command. The 'Margins' command can calculate functions of fitted values after estimation commands including logistic regression. The 'Marginsplot' command in stata automatically adds CIs (326). For the study using logistic regression analyses based on SHeS data in this chapter, the Y-axis of the margin plot was the predicted probability of having IC. The X-axis was the value of cotinine concentration. The margin plot graphed the predicted probability of IC as a function of the cotinine concentration. Cotinine concentrations of 0, 5, 10 and 14.8 ng/mL were used as fitted values. In this study, cotinine The adjusted odds ratios and prevalences of raised cotinine concentrations were used to derive the attributable percentages (326). All statistical analyses were conducted using Stata 12.0 (Stata Corporation, College Station, Texas, USA).

For the retrospective cohort study using record linkage, differences in baseline characteristics across the SHS exposure groups of the study participants were summarised and assessed as above. Tests of Cox proportional-hazards assumptions were performed using Stata estat phtest (327). Separate Cox proportional hazard models were developed to examine the association between levels of SHS exposure and two separate outcomes: incident PAD (hospital admission or death) and all-cause mortality. I ran a series of models with increasing levels of statistical adjustments for potential confounders: unadjusted, partially adjusted (age and sex) and fully adjusted (partially adjusted plus deprivation quintile, BMI category, physical active, alcohol consumption and survey year) using cotinine <0.7ng/mL as the referent category (328). For both the cross-sectional study and the cohort study, missing data were coded as dummy values and included in the adjusted models. Statistical interactions with covariates (age, sex, and socioeconomic status) were tested using the likelihood ratio test (39, 324). Statistical significance was defined as a two-sided p-value <0.05 for both main effects and interactions. All statistical analyses were performed using Stata 12.0 (Stata Corporation, College Station, Texas, USA).

3.4 Results

Generation Scotland : Scottish Family Health Study (GS: SFHS)

Of the 21,558 participants in the Scottish Family Health Study, 6,168 were classified as never smokers. Among these, 5,686 (92.2%) had both brachial and ankle blood pressure measurements recorded and comprised the study population. One hundred and thirty-four (2.4%) had PAD (ABPI <0.9). Participants with PAD were significantly older and more likely to be female (Table 3.3). There were no significant differences in the prevalence of diabetes and dyslipidaemia between participants with and without PAD (Table 3.3). Three percent of the participants with PAD reported being exposed to at least 40 hours of SHS per week, compared with 0.6% of those without PAD (x^2 test, p=0.010) (Table 3.4).

Table 3.3 Characteristics of never smokers by presence or absence of peripheral arterial disease, Scottish Family Health Study

	PAD (ABPI <0.9) N=134 N (%)	No PAD (ABPI ≥0.9) N=5,552 N (%)	P value*
Age group (years)			0.002
18-45	47 (35.1)	2,556 (46.0)	
46-59	45 (33.6)	1,852 (33.4)	
≥60	41 (30.6)	1,118 (20.1)	
Missing	1	26	
Sex			<0.001
Male	30 (22.4)	2,161 (38.9)	
Female	103 (76.9)	3,365 (60.6)	
Missing	1	26	
Deprivation quintile			0.092
1 (most deprived)	16 (11.9)	495 (8.9)	
2	19 (14.1)	658 (11.9)	
3	21 (15.7)	810 (14.6)	
4	27 (20.2)	1,352 (24.4)	
5 (least deprived)	40 (29.9)	1,854 (33.4)	
Missing	11	383	
Alcohol consumption			0.466
Never	4 (3.0)	209 (3.8)	
Stopped >1 year	8 (6.0)	202 (3.6)	
Stop ≤1 year	4 (3.0)	67 (1.2)	
Current	114 (85.1)	4,959 (89.3)	
Missing	4	115	
Physically active			0.425
No	55 (41.0)	2,593 (46.7)	
Yes	70 (52.2)	2,639 (47.5)	
Missing	9	320	
Body mass index (kg/m^2)			0.873
<25.0	65 (48.5)	2,377 (42.8)	
25.0-29.9	34 (25.4)	2,045 (36.8)	
≥30.0	33 (24.6)	1,098 (19.8)	
Missing	2	32	
Hypertension	57 (42.5)	1,942 (35.0)	0.070
Diabetes	4 (3.0)	128 (2.3)	0.554
Total cholesterol (mmol/L)			0.740
≤6.2	102 (76.1)	4,361 (78.5)	
>6.2	20 (14.9)	781 (14.1)	
Missing	12	410	
HDL cholesterol (mmol/L)			0.766
≥1.0	112 (83.6)	4,760 (85.7)	
<1.0	10 (7.5)	376 (6.8)	
Missing	12	416	

PAD peripheral arterial disease; ABPI ankle brachial pressure index; N number; HDL high-density lipoprotein.
*x^2 test for trend
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Table 3.4 Self-reported exposure to secondhand smoke among never smokers by presence or absence of peripheral arterial disease, Scottish Family Health Study

		PAD (ABPI <0.9) N=134 N (%)	No PAD (ABPI ≥0.9) N=5,552 N (%)	P value*
Work	None	102 (76.1)	4,338 (78.1)	0.394
	A little	4 (3.0)	444 (8.0)	
	Some	5 (3.7)	126 (2.3)	
	A lot	3 (2.2)	36 (0.7)	
	missing	20	608	
Home	None	104 (77.6)	4,646 (83.7)	0.314
	A little	5 (3.7)	232 (4.2)	
	Some	5 (3.7)	126 (2.3)	
	A lot	3 (2.2)	100 (1.8)	
	missing	17	448	
Other locations	None	84 (6.3)	3,419 (61.6)	0.635
	A little	28 (2.1)	1,536 (27.7)	
	Some	7 (5.2)	238 (4.3)	
	A lot	4 (3.0)	49 (0.9)	
	missing	11	310	
Total hours per week	0	83 (61.9)	3,534 (63.7)	0.214
	1-19	33 (24.6)	1,634 (29.4)	
	20-39	3 (2.2)	61 (1.1)	
	≥40	4 (3.0)	34 (0.6)	
	missing	11	289	

PAD peripheral arterial disease; ABPI ankle brachial pressure index; N number
*x^2 test for trend
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On univariate logistic regression analysis, participants with PAD were found to be significantly more likely to report high levels of SHS exposure at work, and at other locations, and an overall duration of exposure of at least 40 hours (Table 3.5). When adjusted for age, sex and deprivation quintile as potential confounders, the significant associations with work, other locations and overall exposure persisted (Table 3.5). When further adjusted for age, sex, deprivation quintile, alcohol consumption, physical activity and BMI category, the association between PAD and SHS exposure at work, at other locations and overall exposure remained significant (adjusted OR=3.80, CI 1.12-12.89, p=0.032 for SHS exposure a lot at work; adjusted OR=3.56, CI 1.20-10.56, p=0.022 for SHS exposure a lot in other places; adjusted OR=4.53, CI 1.51-13.56, p=0.007 for overall SHS exposure at least 40 hours per week) (Appendix 6). When the adjusted odds ratios were plotted on a logarithmic scale, there was suggestion of a log-linear dose relationship among those exposed to SHS (Figure 3.1). There were no statistically significant interactions with any of the covariates.

Table 3.5 Logistic regression analyses of the association between secondhand smoke exposure and peripheral arterial disease, Scottish Family Health Study

		Unadjusted				Adjusted*			
		OR	95% CI	P value	P value for trend	OR	95% CI	P value	P value for trend
Work	None	1.00	-	-	0.395	1.00	-	-	0.278
	A little	0.38	0.14-1.05	0.061		0.43	0.16-1.19	0.105	
	Some	1.69	0.68-4.21	0.262		1.88	0.75-4.74	0.179	
	A lot	3.54	1.07-11.70	0.038		3.56	1.06-11.90	0.040	
Home	None	1.00	-	-	0.316	1.00	-	-	0.334
	A little	0.96	0.39-2.38	0.935		0.92	0.37-2.31	0.866	
	Some	1.77	0.71-4.42	0.220		1.78	0.70-4.48	0.224	
	A lot	1.34	0.42-4.30	0.622		1.18	0.36-3.83	0.786	
Other locations	None	1.00	-	-	0.635	1.00	-	-	0.609
	A little	0.74	0.48-1.14	0.176		0.79	0.51-1.24	0.313	
	some	1.20	0.55-2.62	0.652		1.34	0.60-2.99	0.474	
	A lot	3.32	1.17-9.42	0.024		3.30	1.13-9.67	0.029	
Total hours per week	0	1.00	-	-	0.214	1.00	-	-	0.078
	1-19	0.86	0.57-1.29	0.468		0.93	0.61-1.43	0.748	
	20-39	2.09	0.64-6.81	0.219		1.96	0.59-6.51	0.272	
	≥40	5.01	1.74-14.44	0.003		4.61	1.56-13.61	0.006	

OR odds ratio; CI confidence interval
*adjusted for age, sex and deprivation quintile
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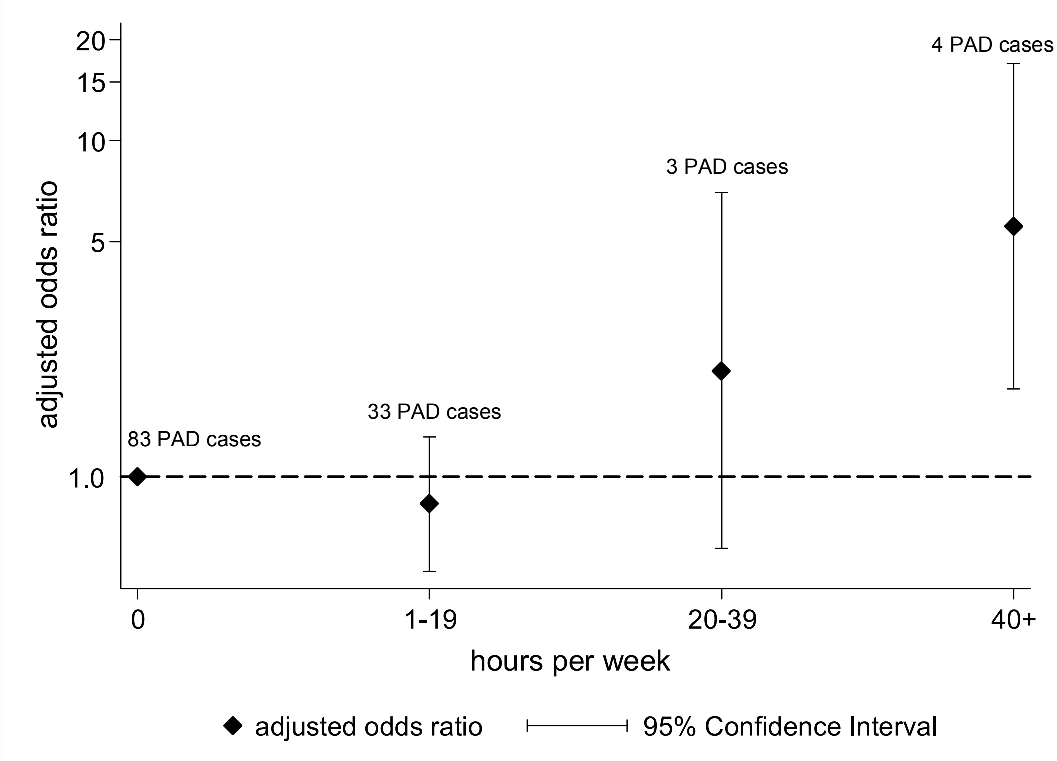
Figure 3.1 Adjusted odds ratios for the association between total number of hours exposed to second hand smoke per week and peripheral arterial disease, Scottish Family Health Study



PAD peripheral arterial disease

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When I re-ran the models using dummy values for missing data for the most incomplete variables (deprivation quintile, alcohol consumption and physical activity) the associations were still apparent (≥ 40 hours per week exposure: adjusted OR 4.29, 95% CI 1.43-12.83, p=0.009). After further adjustment for hypertension, diabetes and dyslipidaemia, they remained statistically significant (≥ 40 hours per week exposure: adjusted OR 5.36, 95% CI 1.74-16.54, p=0.003).

Scottish Health Survey (SHeS)

Of the 41,664 participants in the SHeSs, 8,519 were aged >45 years, classified themselves as non-smokers and completed the Edinburgh Claudication Questionnaire. Of these, 4,434 had provided a saliva sample. When these were compared with the 4,085 participants who did not, there was no significant difference in the prevalence of IC (x^2 test p=0.318). Of the 4,434 participants, 203 (4.5%) were excluded because they had a cotinine concentration ≥15.0 ng/mL which suggested that, contrary to their self-report smoking status, they were likely active smokers. Therefore, 4,231 participants comprised the study population.

Of the 4,231 participants, 2,293 (54.2%) classified themselves as never smokers and 1,938 (45.8%) as ex-smokers. Among the ex-smokers, 1,882 (97.1%) had quit smoking at least one year prior to each survey. Overall, 134 (3.2%) eligible participants had IC. Individuals with IC were older and had significantly higher salivary cotinine concentrations than those without IC (Table 3.6). In the univariate logistic regression model, there was a dose relationship such that IC increased with increasing cotinine concentration. Adjustment for age, sex and social class only attenuated the association slightly and it remained statistically significant (Table 3.7). Further adjustment for body mass index did not alter the relationship (cotinine 2.7-14.9 ng/mL: adjusted OR 1.74, 95% 1.02-2.96, p=0.042). When age was taken as a continuous variable in the fully adjusted logistic regression models, the association persisted (cotinine 2.7-14.9 ng/mL: adjusted OR 1.92, 95% 1.13-3.27, p=0.016, p for trend=0.016). The predicted margins of the cotinine level were based on four point estimates: 0, 5, 10 and 14.8 ng/mL, the latter being the maximum permissible value in the study population. The margin plot suggested a linear, positive dose relationship between cotinine concentration and IC (Figure 3.2).

Table 3.6 Characteristics of non-smokers by presence or absence of peripheral arterial disease, Scottish Health Survey

	PAD (N=134) N (%)	No PAD (N=4097) N (%)	P value*
Age group (years)			<0.001
45-60	33 (24.6)	1,886 (46.0)	
≥60	101 (75.4)	2,211 (54.0)	
Missing	0	0	
Sex			0.230
Male	68 (50.7)	1,864 (45.5)	
Female	66 (49.3)	2,233 (54.5)	
Missing	0	0	
Social class			0.078
Professional	6 (4.5)	233 (5.7)	
Managerial technical	34 (25.4)	1,118 (27.3)	
Skilled non-manual	17 (12.7)	524 (12.8)	
Skilled manual	37 (27.6)	1,284 (31.3)	
Semi-skilled manual	25 (18.7)	600 (14.6)	
Unskilled manual	14 (10.4)	253 (6.2)	
Missing	1	85	
Salivary cotinine (ng/mL)			0.017
<0.7	81 (60.5)	2,784 (68.0)	
0.7-2.6	35 (26.1)	997 (24.3)	
2.7-14.9	18 (13.4)	316 (7.7)	
Missing	0	0	

IC Intermittent claudication
*x^2 test for age and sex; x^2 test for trend for social class and cotinine concentration
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Table 3.7 Logistic regression analyses of the association between secondhand smoke exposure and peripheral arterial disease, Scottish Health Survey

		Unadjusted			Adjusted*		
		OR (95% CI)	P value	P value for trend	OR (95% CI)	P value	P value for trend
Salivary cotinine (ng/mL)							
All ages	<0.7	1.00	-	0.017	1.00	-	0.040
	0.7-2.6	1.21 (0.81-1.81)	0.361		1.21 (0.80-1.82)	0.368	
	2.7-14.8	1.96 (1.16-3.31)	0.012		1.76 (1.04-3.00)	0.036	
≥60 years of age	<0.7	1.00	-	0.502	1.00	-	0.659
	0.7-2.6	0.84 (0.50-1.39)	0.493		0.81 (0.48-1.35)	0.417	
	2.7-14.8	1.49 (0.81-2.74)	0.203		1.39 (0.75-2.57)	0.300	
<60 years of age	<0.7	1.00	-	0.001	1.00	-	<0.001
	0.7-2.6	3.15 (1.49-6.68)	0.003		3.41 (1.58-7.36)	0.002	
	2.7-14.8	4.00 (1.40-11.41)	0.009		4.46 (1.53-12.98)	0.006	

OR odds ratio; CI confidence interval
*adjusted for age, sex and social class for all ages; adjusted for sex and social class for ≥60 years of age or <60 years of age
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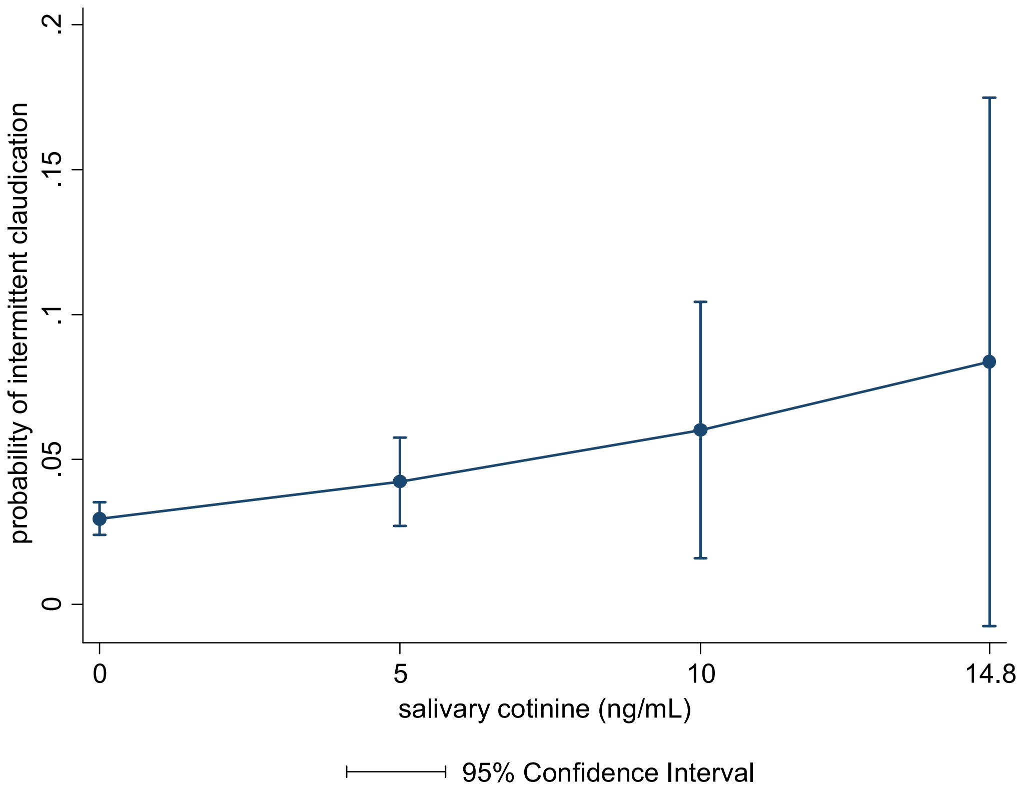
Figure 3.2 Associations between salivary cotinine concentration in non-smokers and probability of intermittent claudication (unadjusted), Scottish Health Survey.



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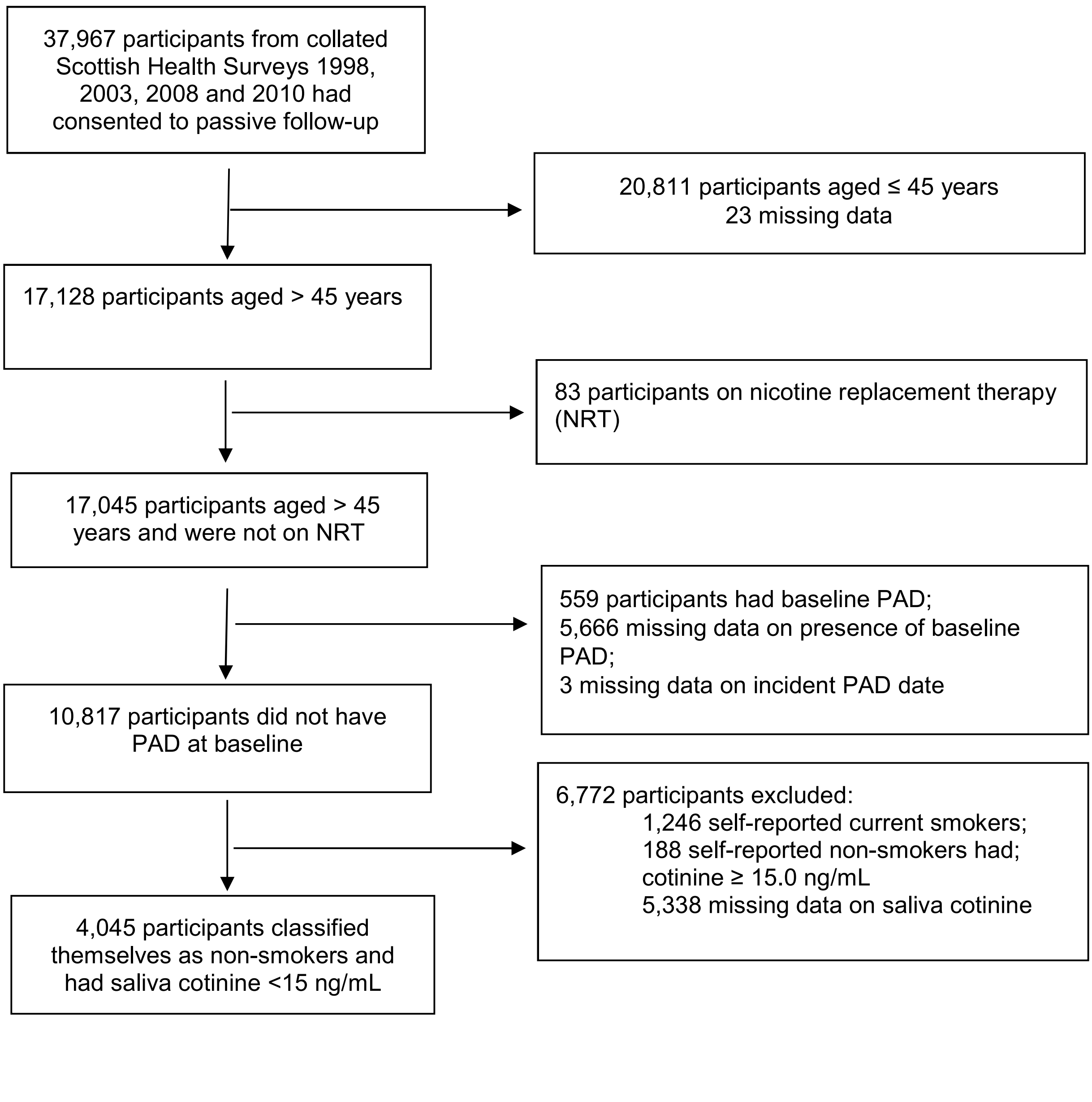
There was a statistically significant interaction with age (p=0.013). Among participants over 60 years of age, the association did not reach statistical significance. However, among those under 60 years of age, there was a statistically significant, dose relationship that persisted following adjustment for potential confounders (Table 3.7). Among all non-smokers over 45 years of age, the adjusted attributable percentages were 3.6% (95% CI -6.6%-12.8%) for cotinine concentrations of 0.7-2.6 ng/mL, and 5.6% (95% CI -0.8%-11.7%) for cotinine concentrations 2.7-14.9 ng/mL.

Of the 41,664 participants in the Scottish Health Surveys 1998, 2003, 2008 and 2010, 37,967 (91.9%) participants had consented to passive follow-up via record linkage to routine administrative data. Among these, 17,128 (45.1%) were aged > 45 years. Of these, 83 participants were excluded because of being on nicotine replacement therapy (NRT). Of the remainder, 10,817 participants completed the Edinburgh Claudication Questionnaire at baseline and were free of baseline IC. Of these participants, 6,772 were excluded because: 1,246 reported being current smokers, 188 reported being non-smokers but had a cotinine concentration ≥ 15.0 ng/mL, and 5,338 did not provide a saliva sample. Therefore, 4,045 participants classified themselves as non-smokers and had a cotinine concentration < 15 ng/mL and were, therefore, eligible for the record linkage, cohort study (Figure 3.3).

Figure 3.3 Flow diagram of participant inclusion and exclusion, Scottish Health Survey, routine administrative data



Of these, 2,216 (54.8%) classified themselves as never smokers and 1,829 (45.2%) as ex-smokers. Among the ex-smokers, 1,774 (97.0%) had quit smoking for at least 1 year prior to each survey and 1,620 (88.6%) had quit for at least 5 years prior to each survey. Overall, 1,163 (28.8%) had either moderate or high exposure to SHS at baseline. The mean age at recruitment was 61 (standard deviation (329) 10) years and there was a total of 29,040 person years of follow-up (mean follow-up 9 years). Over the follow up period there were 568 all-cause deaths, none of which were coded as due to PAD, and 64 people were hospitalised for PAD.

Compared with the no or low SHS exposure group, participants with high exposure were older, and more likely to be male, obese and social economically deprived; they drank more drank alcohol and were less physically active (Table 3.8). There was a statistical significant association between baseline exposure to SHS and all-cause mortality among all participants (Table 3.9, Figure 3.4) and among male subgroup of participants (Table 3.9). In univariate and multivariate Cox proportional hazard models, participants with high exposure to SHS (cotinine 2.7-14.9 ng/mL) were significantly more likely to die, with a clear dose-response relationship across the cotinine categories (Table 3.9). In relation to incident PAD, in terms of all participants, the association with baseline exposure to SHS did not reach statistical significance (Table 3.9, Figure 3.5). However, there was a significant interaction with sex ($p=0.025$). Male participants with high exposure to SHS were significantly more likely to experience PAD events when unadjusted or adjusted for age only, compared with the low exposure group (Table 3.9, Figure 3.6). After further adjustment for other potential confounders, the HR attenuated but was not statistically significant. Among female non-smokers, there were no significant associations between baseline exposure to SHS and either all-cause mortality or PAD hospitalisations. There were no significant interactions with other covariates. The proportional hazards assumptions were met in all of the models (Global test: all $p>0.050$) except for the adjusted models for all-cause mortality in the female only subgroup (Global test: $p=0.018$ for partially adjusted model and $p<0.001$ for fully adjusted model). The numbers of participants were too small to run subgroup piecewise analyses stratified by the other covariates.

Table 3.8 Baseline characteristics of non-smokers by cotinine concentrations, Scottish Health Survey, routine administrative data

	Cotinine (ng/mL)			
	0-0.6	0.7-2.6	2.7-14.9	
	N=2,882	N=850	N=313	P values*
	N (%)	**N (%)**	**N (%)**	
Age (years)				
45-59	1,338 (46.4)	406 (47.8)	124 (39.6)	0.042
≥ 60	1,544 (53.6)	444 (52.2)	189 (60.4)	
Missing	0	0	0	
Sex				
Male	1,250 (43.4)	423 (49.8)	167 (53.4)	<0.001
Female	1,632 (56.6)	427 (50.2)	146 (46.6)	
Missing	0	0	0	
Deprivation quintile				
1(most deprived)	315 (10.9)	137 (16.1)	75 (24.0)	<0.001
2	479 (16.7)	190 (22.4)	82 (26.2)	
3	622 (21.6)	202 (23.8)	55 (17.6)	
4	697 (24.2)	153 (18.0)	50 (16.0)	
5(least deprived)	644 (22.3)	141 (16.6)	40 (12.8)	
Missing	125	27	11	
Body mass index (kg/m^2)				
<25.0	670 (23.2)	151 (17.8)	46 (14.7)	<0.001
25.0-29.9	1,181 (41.0)	347 (40.8)	122 (39.1)	
≥ 30	757 (26.3)	272 (32.0)	122 (39.0)	
Missing	274	80	23	
Physically active				
No	1,442 (50.0)	450 (52.9)	177 (56.5)	<0.001

Yes	1,251 (43.4)	332 (39.1)	101 (32.3)	
Missing	190	68	35	
Alcohol consumption				
Never drinker	237 (8.2)	51 (6.0)	25 (8.0)	<0.001
Ex drinker	129 (4.5)	43 (5.1)	23 (7.3)	
Low-risk drinker	2,300 (79.8)	655 (77.1)	211 (67.4)	
Increasing-risk drinker	163 (5.7)	66 (7.8)	33 (10.5)	
High-risk drinker	51 (1.8)	34 (4.0)	19 (6.1)	
Missing	2	1	2	

* x^2 test

Table 3.9 Cox proportional hazard models of the association between secondhand smoke exposure, peripheral arterial disease and all-cause mortality, Scottish Health Survey, routine administrative data

	Cotinine (ng/mL)	Unadjusted			Partially adjusted†			Fully adjusted‡		
		HR (95%CI)	P value	P value for trend	HR (95%CI)	P value	P value for trend	HR (95%CI)	P value	P value for trend
Incident PAD										
All non-smokers[1]	0-0.6*	1.00	-	0.172	1.00	-	0.140	1.00	-	0.382
(64 events)	0.7-2.6	1.26 (0.71-2.25)	0.437		1.30 (0.73-2.33)	0.372		1.15 (0.64-2.06)	0.648	
	2.7-14.9	1.64 (0.77-3.49)	0.203		1.66 (0.77-3.51)	0.184		1.38 (0.65-2.95)	0.400	
Male non-smokers[1]	0-0.6*	1.00	-	0.100	1.00	-	0.084	1.00	-	0.280
(28 events)	0.7-2.6	0.82 (0.30-2.24)	0.702		0.91 (0.33-2.49)	0.848		0.76 (0.28-2.07)	0.595	
	2.7-14.9	2.89 (1.18-7.10)	0.021		2.82 (1.14-6.96)	0.024		2.10 (0.78-5.65)	0.141	
Female non-smokers[1]	0-0.6*	1.00	-	-	1.00	-	-	1.00	-	-
(36 events)	0.7-2.6	1.66 (0.81-3.38)	0.165		1.65 (0.81-3.37)	0.168		1.51 (0.73-3.15)	0.266	
	2.7-14.9	**	**		**	**		**	**	
All-cause mortality										
All non-smokers[1]	0-0.6*	1.00	-	0.004	1.00	-	0.001	1.00	-	0.043
(568 events)	0.7-2.6	1.25 (1.03-1.52)	0.022		1.34 (1.10-1.63)	0.003		1.24 (1.02-1.51)	0.034	
	2.7-14.9	1.30 (1.04-1.79)	0.024		1.42 (1.09-1.86)	0.011		1.21 (0.91-1.61)	0.194	
Male non-smokers[1]	0-0.6*	1.00	-	0.006	1.00	-	<0.001	1.00	-	0.004
(304 events)	0.7-2.6	1.26 (0.98-1.63)	0.077		1.47 (1.13-1.92)	0.004		1.40 (1.07-1.83)	0.014	
	2.7-14.9	1.52 (1.09-2.13)	0.014		1.54 (1.08-2.18)	0.016		1.54 (1.07-2.22)	0.020	

Female non-smokers[2]	0-0.6*	1.00	-	0.475	1.00	-	0.504	1.00	-	0.523
(264 events)	0.7-2.6	1.14 (0.85-1.53)	0.380		1.14 (0.85-1.53)	0.368		1.03 (0.76-1.40)	0.836	
	2.7-14.9	1.07 (0.68-1.70)	0.764		1.05 (0.67-1.65)	0.828		0.80 (0.51-1.27)	0.344	

HR hazard ratio; CI confidence interval; HDL high-density lipoprotein; PAD peripheral arterial disease
*reference; ** only one participant; † adjusted for age and sex for all non-smokers, adjusted for age for male or female non-smokers; ‡ partially adjusted plus deprivation quintile, body mass index, physical activity, alcohol consumption and survey yeara

[1]Test of proportional-hazards assumption all p≥0.050

[2]Test of proportional-hazards assumption all p<0.050

Figure 3.4 Survival proportion of all-cause mortality among all participants by cotinine concentrations using Kaplan-Meier method. Scottish Health Survey, routine administrative data

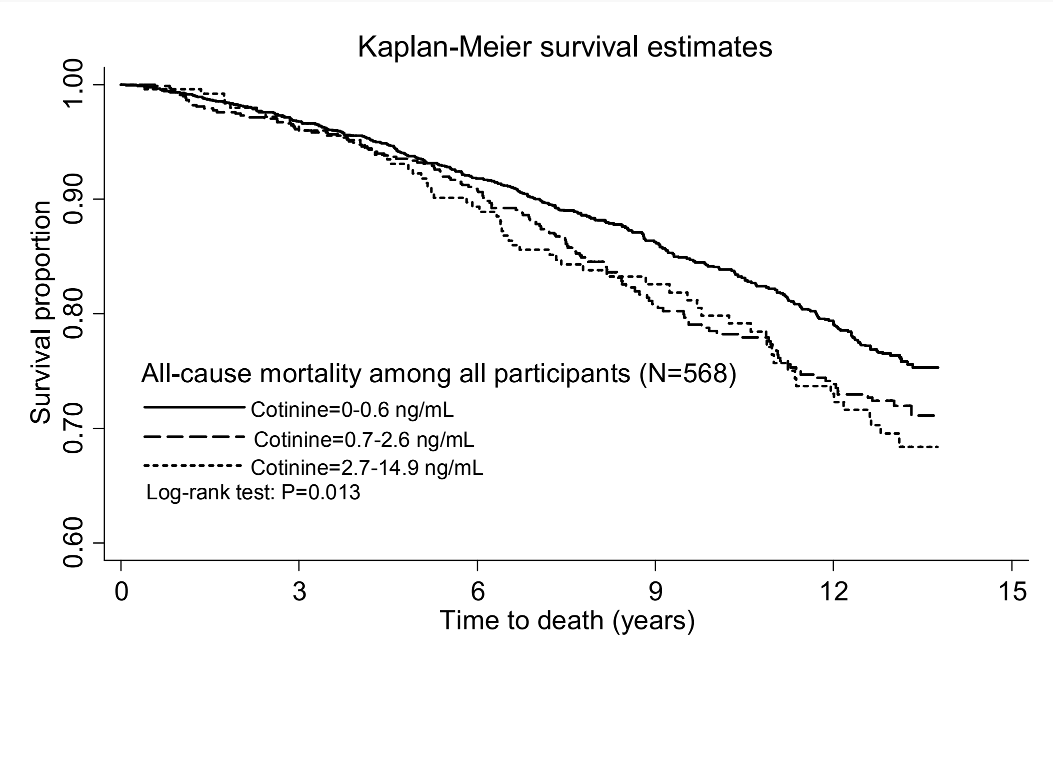
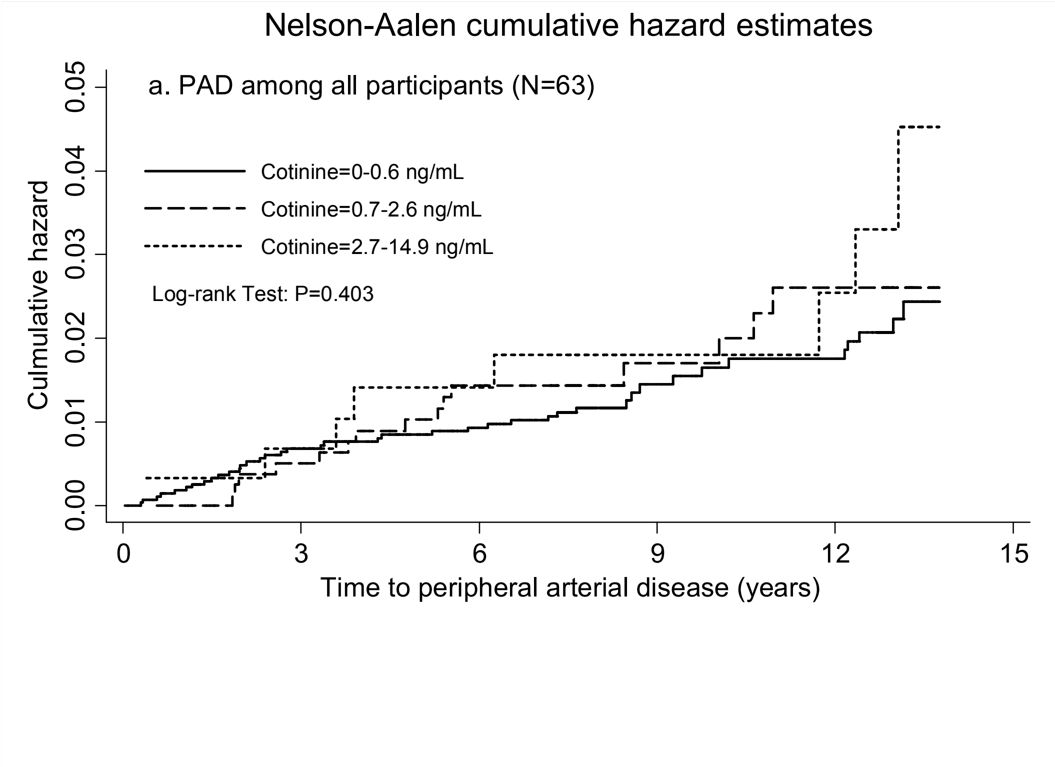
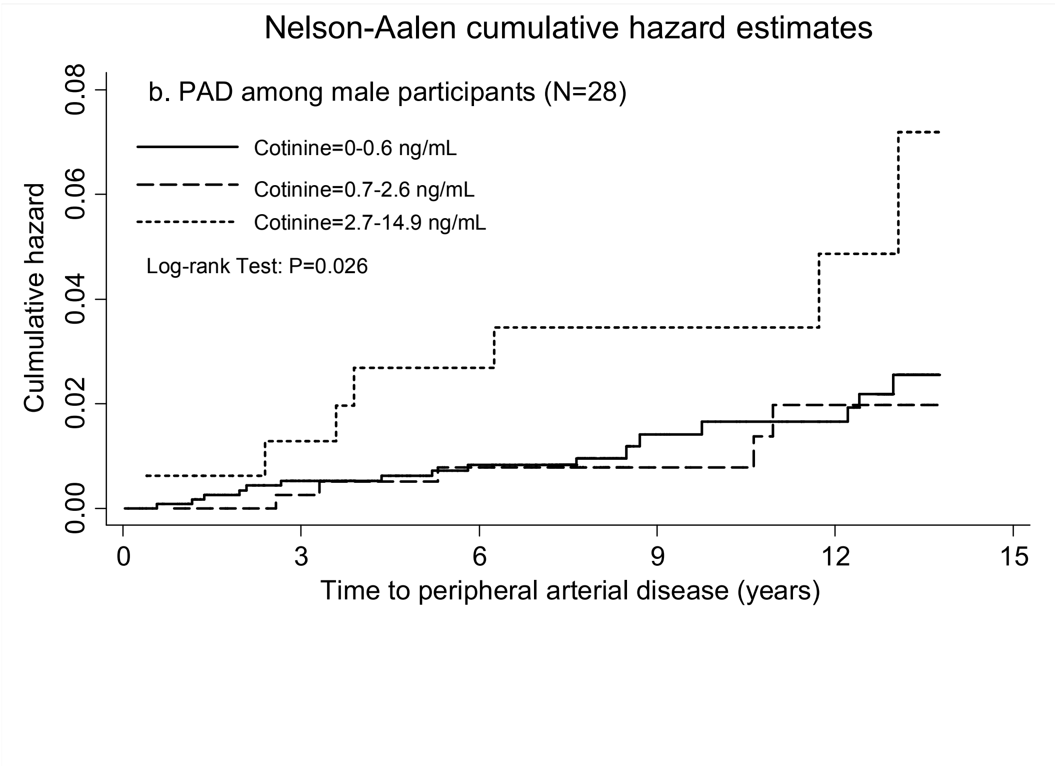


Figure 3.5 Cumulative hazard of peripheral arterial disease among all participants by cotinine concentrations using the Nelson-Aalen method. Scottish Health Survey, routine administrative data



PAD: peripheral arterial disease
N: Number of events

Figure 3.6 Cumulative hazard of peripheral arterial disease among male participants by cotinine concentrations using the Nelson-Aalen method. Scottish Health Survey, routine administrative data



PAD: peripheral arterial disease
N: Number of events

3.5 Discussion

Overall, my two cross-sectional studies demonstrated a statistically significant, independent association between the level of SHS exposure and PAD. Individuals who had objective evidence of PAD (ABPI <0.9) were significantly more likely to report high, overall levels of SHS exposure, even after adjusting for potential confounding factors (adjusted OR 4.53, 95% CI 1.15-13.56, p=0.007 for participants exposed to ≥ 40 hours per week of SHS) (Appendix 6). Increased cotinine concentration was significantly associated with IC based on the Edinburgh Claudication Questionnaire, with evidence of a dose-response relationship. After adjustment for potential confounders, participants with a cotinine ≥ 2.7 ng/mL were at a significantly increased risk of IC (adjusted OR 1.76, 95% CI 1.04-3.00, p=0.036), compared with those with a cotinine concentration < 0.7 ng/mL (Table 3.7). Overall, 9.2% of the cases of IC among non-smokers were attributable to raised cotinine concentrations. There was evidence of a statistical interaction whereby the association was stronger and statistically significant among individuals under 60 years of age and did not reach statistical significance in older individuals. In my cohort study, compared with no and low SHS exposure, increased cotinine concentration at baseline were associated with increased risk of all-cause mortality (adjusted HR 1.42, 95% CI 1.09-1.86, p=0.011), with a clear dose relationship. The association between high cotinine concentration at baseline and risk of incident PAD reached statistical significance in male participants only (adjusted HR 2.82, 95% CI 1.14-6.96, p=0.024). The cohort study might possibly be underpowered to assess the association between high cotinine concentration at baseline and risk of incident PAD among female non-smokers.

Attributable risk is generally used to assess the burden of disease at the level of populations and involves causal inference. Attributable risk % or attributable fraction is calculated from the attributable risk and provides information on the proportion of disease attributable to this particular exposure in the exposed group or the proportion of disease avoidable in the exposed group if this particular exposure is eliminated (222). In relation to the association between SHS and PAD, as mentioned in Chapter 2, very few studies have collected data on both SHS exposure and PAD. In SHeS, the presence of IC at the time of study was defined by the Edinburgh Claudication Questionnaire. In my cross-sectional study, the adjusted ORs and prevalences of raised cotinine concentrations were used to derive the attributable risk %. However, the association estimate does not reflect the causal estimate. This is a limitation of my study. In reality, usually there are many causes of a disease. It is unknown whether or not there is a temporal relationship between SHS and PAD. The results in my study are estimates of the burden of disease among Scottish adults who are exposed to SHS and may overestimate or underestimate the true value.

Due to the limited number of incident PAD cases among female non-smokers who were exposed to high level of SHS (Table 3.9), the cohort study in this chapter is possibly underpowered to assess in particular the overall association between SHS exposure and PAD among female non-smokers. As mentioned in Chapter 1, the influence of gender on the prevalence of PAD in controversial in previous studies (8, 130, 131). In the main report of SHeS 2012, around 25% male non-smokers and 12% female non-smokers reported the exposure to SHS (303). In my cohort study, of the 64 incident PAD cases, 28 were male non-smokers and 36 were female non-smokers. Among the 1,840 male non-smokers (Table 3.8), the incidence rate was 1.5%. Among 2,205 female non-smokers (Table 3.8), the incidence rate was 1.6%. However, as mentioned above (Table 3.8), participants with high SHS exposure were more likely to be male (7 male vs. 1 female). This may be a possible explanation for the observed association between high cotinine concentration and incident PAD among male non-smokers. Furthermore, the incident PAD cases were ascertained as severe cases that warranted hospitalisation or surgery or contributed to death. It is possible that a proportion of PAD cases were missed. It is still uncertain whether or not the magnitude of association between SHS and PAD is stronger in male non-smokers than female non-smokers. Future research is needed to explore the association and the possible explanations.

PAD shares many common risk factors with CHD and the two diseases commonly co-exist in the same individuals (13). As described in Chapter 2, there have been many studies demonstrating an association between active smoking and PAD (118). Exposure to SHS causes similar haemodynamic and inflammatory changes in vessels, (58, 330-335) and predisposes to the formation, progression and instability of atherosclerotic plaques (59, 335-340). There is now strong evidence for an association between SHS exposure and both CHD and stroke (4, 6). In contrast, before the publication of my studies, only two studies, both cross-sectional, had been published on the association between SHS exposure and PAD. A cross-sectional study examined 1,209 Chinese women aged ≥60 years who had never smoked. The investigators did not have access to cotinine concentrations but 40% of women reported exposure to SHS. Those women who had an ABPI <0.9 were significantly more likely to report SHS exposure (adjusted OR 1.47, 95% CI 1.07-2.03, p=0.018). They demonstrated a dose relationship with the number of cigarettes to which they were exposed each day for IC, ABPI <0.9 and either of the two (p values for linear trend = 0.009, 0.002 and 0.002 respectively). The findings were similar for the daily duration of exposure (p values for linear trend = 0.003, 0.048 and 0.001 respectively) (4). The second cross-sectional study, undertaken in the USA, examined 5,653 non-smokers. They dichotomised non-smokers into those exposed to SHS (serum cotinine 0.05-10 ng/mL) and those not (serum cotinine <0.05 ng/mL) and found no significant association with PAD defined as ABPI<0.9. However, on further analysis, they found a significantly higher risk of PAD in the top decile of exposure to SHS (equivalent to cotinine concentration >155 ng/mL), which they interpreted as evidence of a threshold effect (38).

My study using the SFHS is the largest published study to date on the association between SHS exposure and PAD. Because of its size I was able to restrict inclusion to never, rather than non (never or ex), smokers. The study included five times the number of never smokers included in the Chinese study and doubled the number in the USA study. As mentioned in my previous chapter, the risk of developing PAD is lower among ex-smokers when compared with current smokers, but still significantly increased when compared with never smokers. Studies have suggested that the prevalence of PAD among people who had stopped smoking declines overtime since smoking cessation (155, 191). Therefore, in the study using SFHS, I included only never smokers because there were sufficient participants who classified themselves as never smokers. A further strength was the ability to ascertain PAD objectively using ABPI measurements rather than self-reported symptoms. This approach also leads to more complete case ascertainment as it includes participants with early stage, asymptomatic disease. In contrast, a weakness of this study was the reliance on self-reported level of exposure to SHS.

The GS: SFHS did not obtain cotinine, or equivalent, measurements. The GS: SFHS study recruited probands and their adult first degree relatives from two big cities in Scotland (Glasgow and Dundee). These two cities have high levels of socioeconomic deprivation and therefore have higher incidence of atherosclerotic diseases and premature atherosclerotic disease (307, 341). When I tried to re-run the models by splitting into age groups, the numbers of participants with different SHS exposure levels were too small to show the effect size precisely. Forty eight cases of PAD would have been excluded if I had only included participants aged>45 years and only 3 self-reported never smokers had PAD. Symptomatic PAD increases with advancing age (238, 342) and most PAD cases are asymptomatic (13). Therefore, in this GS:SFHS study, I used a lower age cut-off (participants aged ≥ 18) to identify more asymptomatic PAD cases.

I was able to adjust for potential confounders such as age, sex socioeconomic status, physical activity, BMI and alcohol consumption (45, 325). It is acknowledged that active cigarette smoking reduces body weight loss by the nicotine receptor-mediated effects that lead to suppression of appetite (343). Reduced BMI among active smokers, which is related to cigarette smoking, may confound the association between cigarette smoking and CVD events (344, 345). Nicotine is the chemical compound that causes addiction to cigarette smoke. The harm of cigarette smoke on cardiovascular system is primarily from CO, tar and other carcinogens including PAH and arsenic (68). Studies have suggested that childhood exposure to SHS is positively associated with childhood BMI and obesity (346, 347). Exposure to SHS during pregnancy is also associated with low birth weight (348). However, it is not clear about the relationship between exposure to SHS and BMI among adults. There is published evidence supporting increased BMI as a risk factor for PAD (168). Researchers have suggested that BMI is not on the causal pathway of health related outcomes including mortality (349). In keeping with the previous published studies on the association between SHS and CHD, stroke and PAD (45, 67), I further adjusted BMI in the fully adjusted regression models in my studies. Previous studies have adjusted for diabetes and serum lipid concentrations but these are potential mediators rather than confounders. Exposure to SHS is associated with diabetes and lipid changes. Diabetic patients and dyslipidaemia patients can consequently have peripheral vasculopathy including PAD (diabetic foot ulceration) (13, 28, 145, 147, 334, 335, 350-354). As mentioned in the pathophysiology section in Chapter 1, it is possible that the causal pathway through which SHS acts could be through diabetes and lipids. Therefore, in the studies in this chapter, I did not include them as covariates. And in the next chapter, the associations between SHS exposure and some cardiovascular biomarkers are explored.

The SHeSs are pan-Scotland surveys and are intended to be representative of the general population living in households across Scotland. Each survey in the series has included an administered questionnaire on demographics (including age, sex and social status) and lifestyle (smoking status, alcohol consumption and physical activity) and measurements (height, weight, blood pressure, and if applicable, blood and saliva samples), with modules of questions on specific health conditions. A strength of the SHeS, in comparison with GS: SFHS, was the access to salivary cotinine measurements rather than reliance on self-reported exposure to SHS. However, smoking status was still self-reported and, because of the social undesirability of smoking, a proportion of current smokers are known to deliberately misclassify themselves as ex-smokers (termed "smoking deceivers"), especially if they already have a smoking-related condition (355). Thereby, in compliance with usual practice, I applied a maximum cotinine concentration of 15 ng/mL to people who classified themselves as non-smokers in order to exclude smoking deceivers (40). I also excluded participants taking nicotine replacement therapy. My previous cross-sectional study using GS:SFHS on SHS and PAD was restricted to participants aged ≥ 18 in order to identify asymptomatic PAD based on ABPI measurements. In a study using 38-year follow up data from the Framingham study aiming at developing an IC risk profile, the rate of IC increased with advancing age in both sexes, ranging from 0.9% and 0.4% for men and women aged 45-54 years, to 2.1% and 1.2% for men and women aged 55-64 years, to 2.5% and 1.5% for men and women aged 65-74 years, respectively (356). Thus, in my cross-sectional study on SHS and PAD, I applied an age cut-off of >45 years to identify more intermittent claudication cases based on the Edinburgh Claudication Questionnaire. A limitation of the studies using SHeS is that both ex-smokers and never smokers were included as non-smokers. Previous studies suggested that there can be a lag between past smoking behaviour among ex-smokers and the disease onset (357). Ex-smokers still carry the risk of developing cardiovascular events including PAD (118, 358). Ideally, I would have included only never smokers in the research. In reality, the analyses were possibly underpowered to show the association between SHS and PAD. Therefore, caution should be taken when interpreting the results.

In order to maximise statistical power, I included ex- as well as never smokers but the vast majority of ex-smokers (88.1%) had not smoked for at least five years. Furthermore, 79 (59.0%) PAD cases would have been excluded if I only included never smokers in my study using SHeS. When including both never and ex-smokers, the number of cases of PAD was 134, the same as in my study using GS:SFHS. In these analyses, I adjusted for potential confounders: demographic and socioeconomic risk factors and BMI. After further adjustment for other confounders such as physical activity, the association between cotinine and PAD attenuated and became statistically insignificant. A weakness of the SHeS was the lack of an objective measure of PAD, such as ABPI. Therefore, case ascertainment had to be based on self-report of disease symptoms. Nonetheless, cases were ascertained via a widely used and well validated questionnaire; the Edinburgh Claudication Questionnaire. In my cross-sectional study using the SHeS, I observed a dose relationship across the cotinine concentrations rather than a threshold effect as the suggested by the previous USA study, with a statistically significant association above a concentration as low as 2.7 ng/mL. The presented studies are exploratory and warrant further research. The association between SHS exposure and PAD would be more plausible if future longitudinal studies include only never smokers with repeat measures of SHS exposure and longer follow-up time.

Similar to the two existing studies on SHS exposure and PAD, a limitation of both of my first two studies was their cross-sectional design. Cross-sectional studies are relatively quick and easy to conduct. However, they suffer from three weaknesses. Firstly, the primary limitation is that risk factor/exposure and disease/outcome are ascertained simultaneously. That is, it is difficult to establish a temporal relationship and thereby confirm that the exposure predated the onset of the disease. Thereby, associations may occur as a result of reverse causation (359) (Appendix 5). If the exposure is an inherent risk factor such as gender or race and the outcome developed over time, the association between the exposure and the outcome is more plausible. Vice versa, if the exposure developed over time, causality is unknown (360).

Secondly, the main outcome measure obtained from a cross-sectional study is prevalence rather than incidence. Another weakness of cross-sectional studies is that their reliance on prevalent cases of disease makes them susceptible to survival bias (359). Survival bias is a type of selection bias and can occur in both cross-sectional studies and case-control studies. It occurs when individuals with favourable survivorship are included in the analysis because of exposure related to mortality from the disease being studies (361, 362).

Thirdly, alternative explanations (chance, bias and confounding) for the study results may need to be appropriately assessed (360). In the two cross-sectional studies in this chapter, SHS exposure and prevalent PAD were measured at one point in time. The observed associations between SHS exposure and prevalent PAD may result from those exposed to SHS being more likely to develop PAD, or less likely to die after developing PAD, or a combination of both. However, given that there is substantial evidence from the meta-analyses showing the association between SHS and cardiovascular risk including CHD (67) and stroke (45), in the specific case of SHS exposure and PAD, reverse causation is highly unlikely, and SHS exposure is unlikely to be protective against PAD case-fatality. Nonetheless, using record linkage of SHeS to undertake a cohort study enabled me to address these methodological limitations in the third study. If SHS exposure is associated with survival as well as incidence, survival bias may explain why the association appeared to be weaker in those over 60 years of age. If this is the case, then the magnitude of association among younger participants is likely to be a better measure of the true association.

The cohort study using record linkage of SHeS suggested a similar relationship between SHS exposure and PAD but the associations did not reach statistical significance among non-smoker participants overall. Only the subgroup of male non-smokers with high exposure levels of cotinine >2.7 ng/mL reached statistical significance. Due to the limited number of female non-smokers in the high SHS exposure group, this cohort study is possibly underpowered to assess the association between high cotinine concentration at baseline and risk of incident

PAD among female non-smokers. A major limitation of the cohort study was that case ascertainment was restricted to those participants with PAD that was sufficiently severe to warrant hospitalisation or surgery or contribute to death. Therefore, these incident PAD events were a highly selected subgroup of all participants with incident PAD. Any association between SHS and incident PAD, defined in this way, could be due to an association with all incident PAD, an association with disease progression or a combination of both. In order to maximise statistical power, I analysed never and ex-smokers together as non-smokers. The majority of ex-smokers (88.7%) had quit smoking for at least five years. Having access to cotinine concentrations was a strength in terms of it being an objective measure of baseline SHS exposure. However, a limitation of this study was the lack of repeat measures of SHS exposure. Therefore, it has to be assumed that baseline concentrations persist long-term, or at least that any changes over time are not systematically different between those exposed and not exposed at baseline.

However, my findings suggested a dose-response relationship whereby the risk of PAD increases with increasing cotinine concentration. The number of incident PAD events is small but this reflects the general lack of focus on SHS and PAD in existing studies and surveys. In my cohort study, the number of female participants who had incident PAD events was too small to be split into exposure groups.

All these three studies were observational studies which to some extent are vulnerable to built-in bias such as selection bias, information bias and confounding (363) (Appendix 5). Selection bias occurs when the method of selecting participants distorts the association between exposure and outcome in the target population (Appendix 5). That is, the study population does not represent the target population. Selection bias can be introduced at any stage of a study: design and implementation (361). In SFHS, participants were randomly selected from the general population from general practitioner records in Glasgow and Dundee. The SHeS used multi-stage, stratified probability sampling frame and represents the general population living in private households nationwide in Scotland. As mentioned in the results (Section 3.4), when the participants who had provided a saliva sample for cotinine assay were compared with those who did not, there was no significant difference in the prevalence of IC. In this respect, selection bias is unlikely to explain the observed associations. However, in the study using SFHS, in order to identify asymptomatic PAD defined by ABPI, I used a younger age cut-off. While, in the study using SHeS, to include symptomatic PAD based on the Edinburgh Claudication Questionnaire, I used an age cut-off of >45 years. Survival bias is a type of selection bias and is possible in cross-sectional studies. Survival bias could be a possible explanation about why the association between SHS exposure and PAD appeared to be weaker in those over 60 years of age. If this is the case, the magnitude of association among younger participants is likely to be a better measure of the true association.

In the cohort study, participants were restricted to confirmed non-smokers (self-reported non-smokers who had salivary cotinine concentration <15.0 ng/mL) who were free of IC at baseline and had consented to passive follow-up via record linkage to routine administrative data. Potential bias could be a question. Selection bias may distort the results about the association between SHS and PAD. It is not possible to extract the participant's records without their consent to passive follow-up, and therefore, selection bias is inevitable. In my analyses, HR was calculated from the Cox proportional hazards model. HR has a built-in selection bias (291). The subjects were follow-up over certain period of time until some events took place. It is possible that those participants who were observed to be event-free up to the defined time point were observed for a shorter time (e.g. participants from the 2008 SHeS and 2010 SHeS) than those who did have PAD. If so, the observed magnitude of association between SHS and incident PAD cases can be biased.

Information bias occurs during data collection. It is often known as observation or measurement or classification bias (363) (Appendix 5). It often occurs when the individual measurements or classifications of the disease or exposure are not accurate. As a result, exposed and/or diseased subjects can be misclassified as non-exposed and/or non-diseased and vice versa (361, 364). In the cohort study, the NHS Scotland's ISD links the administrative SHeS data to hospitalization record and death certificates. Over 90% of SHeS participants consented at each survey to the passive follow-up with data linkage (310). In this respect, information bias in the outcome measure was less of an issue. However, SHS exposure was only measured at baseline. Repeat measures of SHS exposure were not available in this cohort study. Information bias may be a potential question. This may distort the association between SHS exposure and PAD. In addition, when smoking status was self-reported, because of the social undesirability of smoking, a proportion of participants who might be current smokers misclassified themselves as non-smokers. This may introduce potential reporting bias. In the study using SFHS, SHS exposure was self-reported. If the participants know the harmful effect of SHS, especially if they already have SHS-related conditions, potential information bias may be a concern. It is possible that the estimate of the effect size is overestimated.

Confounding occurs when a variable is a known risk factor for the outcome and is associated with the exposure but is not a result of the exposure (363) (Appendix 5). Unlike a mediator, a confounder is not an intermediate step in the causal pathway between the exposure and the outcome. A confounder is unequally distributed among the groups being compared (359). Confounding can be reduced by restricting, matching and randomisation at the design stage, and stratifying, making multivariable statistical adjustment and doing standardised rate analysis at the analysis stage (363, 365). As mentioned in Section 1.3.2, Chapter 1, PAD shares many risk factors with CHD (121). I developed statistical models with increasing level of adjustment for the well-established confounders. However, other omitted confounders such as concentrations of homocysteine (366), CRP (367, 368), and cadmium (369) may affect the observed association between SHS and PAD. In the baseline data of SFHS, I did not have access to measurements of these risk factors. Baseline concentrations of CRP were measured and collected in the SHeS. In the studies using SHeS in this chapter, after further adjustment for other confounders, the association did not reach statistical significance. It is possible that confounding may play a role in the observed association. Furthermore, other unknown risk factors may introduce confounding bias. Residual confounding may also be an issue. Residual confounding refers to the distortion that remains after controlling for confounding in the design and/or analysis of a study. It occurs when: additional confounding factors were not considered or not measured; confounding was not controlled well enough; and there were errors in the measured confounders including misclassification of subjects with respect to confounding variables due to reporting or measurement errors (370, 371). Residual confounding is likely to affect the association between

SHS exposure and PAD. Since a confounder can be a risk factor or a preventive factor for the disease (297), residual confounding can either underestimate or overestimate the true association. The biggest concern about confounding is that a causal relationship could appear from confounding but in fact does not exist (222). My studies used secondary analyses of the SFHS and SHeS and data on a proportion of confounders were not collected. I restricted the eligible study populations to a certain age range in order to include the largest possible number of PAD cases. There might still be differences in age among the groups being compared.

Confounders can be identified by empirical methods or theoretical methods. An example of theoretical strategies is the directed acyclic graphs (DAGs) (372). Empirical strategies generally include forward, backward, and stepwise regression analyses, and a 10% change-in-estimate (CIE) criterion (373). Furthermore, there are other criteria to aid the selection of variables into the multivariable analysis including the likelihood ratio χ^2 test. The likelihood ratio χ^2 test is performed by comparing the log likelihoods of two models (a model with the additional variable and a model without the additional variable) and can be used to compare the fit of one model to the fit of the other (324, 374). Comparing the model χ^2 on addition of additional confounders can be another approach to assess the presence of confounding. In my studies, the confounders were chosen based on the available prior knowledge, in keeping with previous published evidence on the association between SHS and CHD (4). It is possible that potential confounders which were not detected with the stepwise selection approach could have been identified by comparing the model χ^2. This may result in biased estimates of the exposure-outcome association (370, 375). However, researchers suggested that theoretical confounders should always be adjusted for even if empirical and theoretical methods yield contradictory results (376, 377).

Chance is a random error that occurs unpredictably (222). Random error may produce the appearance of an association between an exposure and an outcome which in fact does not exist. It may also produce the absence of an association which in fact is real. Furthermore, it can lead to either underestimation or overestimation of a measurement value from the true population value. There are three sources of random error including sampling error, measurement error and individual biological variation (Appendix 5). Due to chance alone, different samples can produce different estimates. Caution must be taken whenever an inference is being made from a sample to a population (222).

If a study is unbiased, the CI generally presents the precision of an estimate of the association between the exposure and the outcome (Appendix 5). The number of subjects with the outcome, which is often influenced by the sample size, affects the width of the CI (297). In my studies, the findings may be affected by the small number of PAD cases. This may explain the wide range of CIs in the high exposure groups. However, these studies are so far the largest studies that have assessed the association between SHS and PAD. Further research is needed to examine the association between SHS and PAD to reduce the possibility of false positive association due to bias, confounding and chance.

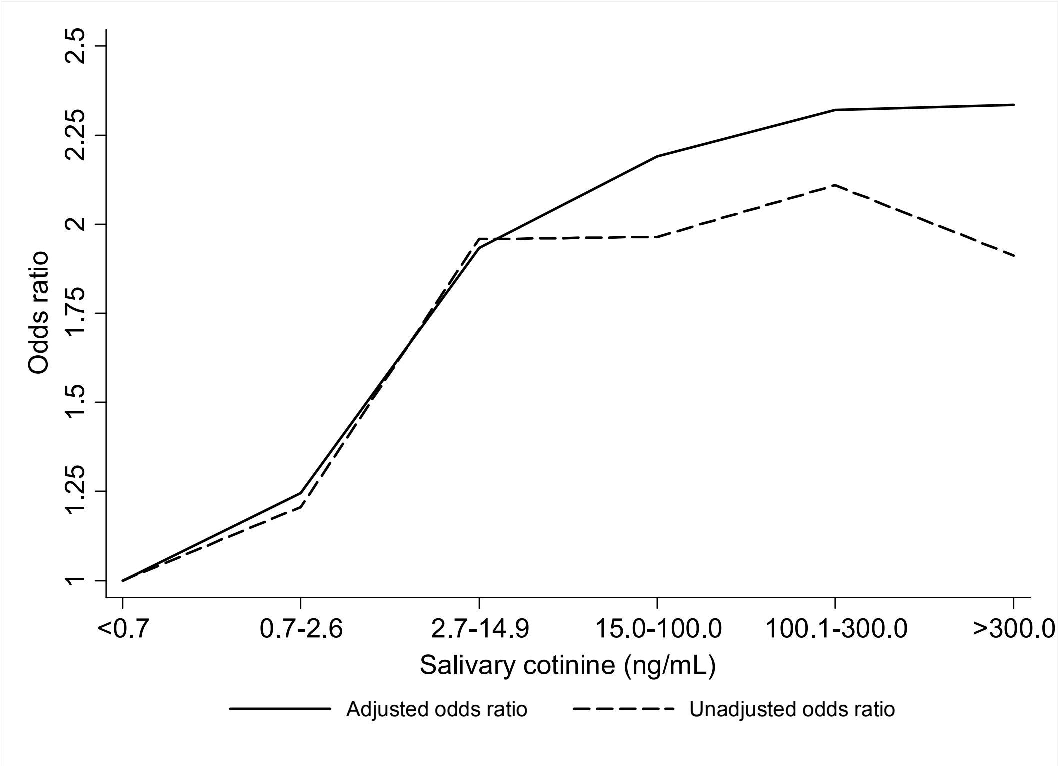
As discussed above, before the observed association between SHS exposure and PAD is assessed for the possibility that it is a causal relationship, other explanations for the observed association should be excluded, including chance, bias and confounding. One of the criteria to judge causation is reverse causality (Appendix 5). A review of the meta-analyses of randomised controlled trials suggested that permanent smoking cessation is probably the most clinical and cost effective intervention for PAD patients (156). As mentioned in Chapter 1, previous studies suggested that comprehensive smoke-free legislation was associated with lower hospital admission rates (or deaths) for coronary events, other heart disease and cerebrovascular accidents (112, 113). Pell et al. also found a 21% reduction of admissions for ACS among never smokers during the 10 months after the smoke-free legislation in nine hospitals in Scotland, compared with the 10 months before the legislation (116). In contrast, the studies on hospital admission of PAD are rare. It is important to assess the impact of smoke-free legislation on hospital admissions of PAD to avoid reverse causality.

The three studies described in this chapter used different measurements of SHS and different definitions of PAD. Whilst this prohibits direct comparison of the results between studies, it means that the limitations of one study are somewhat offset by the strengths of another. The consistency of the findings using these different approaches provides reassurance that the findings may reflect a true association and provide evidence that the harmful effects of SHS exposure extend beyond CHD and CVD to PAD and underpin the need to protect the general population from exposure.

Whilst possibly underpowered to assess the overall association between SHS exposure and PAD, the third study is the first to have attempted to do so using incident cases in a cohort design. In the future, new, larger cohort studies are required to study this hitherto neglected area; ideally using objective measurements of both SHS exposure and PAD.

Some previous studies on cardiovascular disease suggested that SHS exposure may carry a disproportionate risk compared with active smoking (29-31, 52, 67, 334, 378). In my cross-sectional study using the SHeSs, when I reran the models by including confirmed current smokers (self-reported current smokers with salivary cotinine ≥ 15.0 ng/mL) in the analysis. The margin plot suggested a positive dose relationship between cotinine and IC (Figure 3.6). The association between current smoking and IC was comparable to that of non-smokers with high SHS exposure dosage (≥ 2.7 ng/mL) (adjusted OR =1.81, CI 1.07-3.08, p=0.021 for non-smokers with high SHS exposure; adjusted OR =2.12, CI 1.52-2.96, p<0.001 for current smokers). Studies on the underlying mechanisms have been relatively few in number. In my next chapter, I will examine the relationship between the cotinine concentration and a number of cardiovascular biomarkers among non-smokers and current smokers for a given level of smoke exposure.

Figure3.7 Odds ratios for the association between salivary cotinine concentration among both non-smokers and current smokers and intermittent claudication, Scottish Health Survey



4 Chapter 4: Active smoking, secondhand smoke and cardiovascular biomarkers

4.1 Chapter summary

CVD is the global leading cause of death. Both active smoking and SHS are important risk factors for many age-related diseases including CVD. In my previous chapter, I have demonstrated the association between exposures to SHS and PAD. There is good evidence that both active smoking and SHS exposure are associated with well-established cardiovascular biomarkers such as CRP, fibrinogen, and low-density lipoprotein (LDL) cholesterol. Sidestream smoke contains higher levels of small particles and toxic gases than mainstream smoke. In spite of this, there is a lack of studies directly comparing non-smokers with high levels of secondhand exposure, and light and moderate active smokers. Therefore, in this chapter, I aim to examine the relationship between exposure to SHS and several cardiovascular biomarkers among non-smokers and specifically test the hypothesis that SHS carries a disproportionately higher cardiovascular risk than active smoking for a given level of tobacco exposure.

Shortened telomeres have been described as a biomarker for biological ageing including atherosclerosis phenotypes. Many studies have demonstrated an association between active smoking and telomere length attrition. In contrast, the association between SHS exposure and leukocyte telomere length attrition per year of age among adult non-smokers remains unknown. Therefore, in this chapter, I will also examine the relationship between exposure to secondhand smoke and telomere length.

I identified two potential sources of data among the Scottish general population: the SHeS and a subgroup of participants from the SFHS who were included in a previous study on biomarkers of aging. Ideally, I would have included studies with objective measurement of SHS exposure (for example cotinine concentration). The SHeS measured SHS exposure using salivary cotinine concentration, but the SFHS used self-reported exposure to SHS.

To examine the relationship between cotinine concentration and a number of cardiovascular biomarkers among non-smokers and active smokers, I undertook a cross-sectional study using the SHeSs conducted between 1998 and 2010. Inclusion was restricted to participants aged ≥ 16 years who had provided saliva and blood samples and were not taking a nicotine replacement therapy. Univariate and multivariate regression models were used to examine the relationships between cotinine concentration and CRP, high-density lipoprotein (HDL) cholesterol and fibrinogen concentrations, as well as TC/HDL cholesterol ratios.

Further in line with the hypothesis of the association between SHS exposure and leukocyte telomere length shortening per year of age, I undertook another cross-sectional study using a subgroup of 1,779 participants from the SFHS. These participants were chosen because they had participated in a previous sub-study on aging and therefore had already had their telomere length measured. The inclusion criteria, dictated from the previous study, were non-smokers aged ≥ 18 years who were not taking nicotine replacement therapy. Linear regression models were used to relate the telomere T/S ratio to age, where the T/S ratio is the telomere repeat copy number to the single copy gene ratio (379, 380).

In my study using the SHeSs, of the 10,018 eligible participants, 7,345 (73.3%) were confirmed non-smokers (cotinine <15.0 ng/mL) and 2,673 (26.7%) were confirmed current smokers (cotinine ≥15.0 ng/mL). CRP and TC/HDL cholesterol increased, and HDL cholesterol decreased, with increasing cotinine concentration across non-smokers and smokers (all p<0.001). However, there were step changes at the interface, whereby non-smokers with high exposure to SHS had lower concentrations of cotinine than light active smokers but comparable concentrations of CRP (p=0.709), HDL cholesterol (p=0.931) and TC/HDL cholesterol (p=0.405). Fibrinogen concentrations were significantly raised in moderate and heavy active smokers only (both p<0.001).

In my study using the subgroup from SFHS, 1,303 eligible participants were included because they were self-reported non-smokers, had provided self-reported SHS exposure status and had had telomere assays performed as part of a previous study of aging. Of these, 779 (54.4%) reported no SHS exposure, 495 (34.5%) low exposure (1-19 hours per week), 29 (2.0%) high exposure (≥20 hours per week). Compared with those with no SHS exposure, participants with high SHS exposure were older (p value for trend=0.025) and more likely to live in socioeconomically deprived areas (p value for trend <0.001). In the univariate linear regression analyses, the relative telomere T/S ratio declined with increasing age in years in all exposure groups. Telomere length decreased more rapidly with increasing age among those with high exposure to SHS when compared with both those with no exposure to SHS (adjusted p=0.010) and those with low exposure to SHS (p=0.005).

These findings suggest that:

1) Exposure to SHS is associated with disproportionately higher concentrations of biomarkers of cardiovascular risk compared with active smoking;

2) High SHS exposure may accelerate normal biological aging.

Premature telomere attrition may be an intermediate step between exposure to SHS and CVD including PAD. Further studies on the relevant mechanisms should be conducted and efforts on protecting the public from SHS exposure should be increased.

4.2 Introduction

Globally, CVD is the leading cause of death and is projected to cause 23 million deaths per annum by 2030 (381). The global prevalence of smoking is increasing, due to increasing prevalence in large, developing countries such as China. A 2013 WHO report indicated that only 16% of the world's population is covered by comprehensive smoke-free legislation (3). Active smoking is an established risk factor for CHD (15), stroke (14), and PAD (118). There is growing evidence that exposure to SHS is also a risk factor. Two meta-analyses reported relative risks of 1.25 (95% CI 1.17-1.32) and 1.25 (95% CI 1.12-1.38) for CHD (4) and stroke (6) respectively. To date, four cross-sectional studies have examined the association with PAD (38, 45, 46, 328), with three reporting significant associations (45, 46, 328). In my work described in the last chapter, I demonstrated that non-smokers with cotinine concentrations ≥2.7 ng/mL were significantly more likely to have intermittent claudication than those with cotinine concentrations <0.7 ng/mL (adjusted OR 1.76, 95% CI 1.04-3.00)(328).

Active smoking is associated with higher concentrations of cardiovascular biomarkers including: CRP (382), fibrinogen (383), and low-density lipoprotein (LDL) cholesterol (384). SHS contains mainly sidestream smoke, from burning cigarette tips, as well as exhaled mainstream smoke. Sidestream smoke contains higher concentrations of small respirable particles (<2.5 µm) and toxic gases than mainstream smoke inhaled by active smokers (28-31). Brief exposure to SHS produces rapid changes in inflammatory markers (52, 53), resulting in concentrations comparable to active smokers (334, 378, 385). Therefore, the sidestream smoke inhaled by non-smokers exposed to SHS may convey a disproportionately higher risk of cardiovascular disease. In the British Regional Heart Study, the risk of CHD events over 20 years of follow-up was comparable in non-smokers exposed to high levels of SHS (adjusted HR 1.57, 95% CI 1.08-2.28) and light active smokers (adjusted HR 1.66, 95% CI 1.04-2.68) in spite of cotinine concentrations being nearly 30-fold higher in the latter group (mean 4.9 versus 138 ng/mL) (67, 306).

The telomere is a region of repetitive DNA sequences (TTAGGG) at the end of a chromosome, which protects the end of the chromosome from deterioration and end-to-end fusion (386). The telomeres of somatic cells are eroded with each cycle of cell division. Telomere attrition normally limits cells to a fixed number of divisions and cumulative oxidative stress accelerates the attrition and, therefore, biological ageing (386, 387). Previous studies have demonstrated that common age-related diseases including CVD and a shorter life span are associated with shorter telomeres through mechanisms involving oxidative stress associated with cigarette smoking (388, 389). However, whether SHS accelerates telomere attrition with age is unknown.

I used the SHeS to explore the association between the level of secondhand and active smoke exposure, measured by salivary cotinine concentration, and a number of preclinical cardiovascular biomarkers: CRP, high-density lipoprotein (HDL) cholesterol, TC/HDL cholesterol and fibrinogen. To examine the association between levels of SHS exposure and telomere length shortening per annum, I conducted another cross-sectional study using a subgroup of individuals from the SFHS who had had telomere assays performed as part of a previous study of biological aging. This data source provides information on telomere length in blood leukocytes among the consented individuals from this subgroup. As mentioned in my previous chapter, data from the SFHS used self-reported exposure to SHS.

4.3 Materials and methods

4.3.1 Data source

Scottish Health Survey (SHeS)

I conducted another cross-sectional study using baseline data collected on SHeS. As described in the previous chapter, the Surveys are ongoing, repeated, cross-sectional studies used to monitor the health and health-related risk factors of the general population living in private households across Scotland (309). The surveys were undertaken in 1995, 1998 and 2003, and then annually from 2008 using a multi-stage, stratified sampling frame. Each survey recruited different households. Trained staff conducted face-to-face interviews and obtained measurements, including height and weight. All consenting individuals aged ≥16 years were visited by a nurse and invited to provide a salivary sample, for cotinine assay, and blood samples, for assays including lipids, CRP and fibrinogen. Cholesterol concentrations were measured using cholesterol oxidase assays on an

Olympus 640 analyser (Olympus, Canter Valley, Pennsylvania) prior to 2010 and, subsequently a Roche Modular P analyser (Roche, Basel, Switzerland). CRP concentrations were determined using the N Latex CRP mono-immunoassay on the Behring Nephelometer II analyser (Behring, Milan, Italy). Fibrinogen concentrations were measured using the Organon Teknika MDA 180 analyser (Organon, Oss, the Netherlands). Cotinine was assayed using a Hewlett Packard hp5890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA).

Generation Scotland : Scottish Family Health Study (GS: SFHS)

GS:GFHS is a family-based, cross-sectional study of the general population, with a specific focus on cardiovascular risk factors and disease (308). Probands aged between 35 and 55 years were randomly selected for invitation from the records of general practitioners based in Glasgow and Dundee. Between 2006 and 2011, 7,953 probands were recruited along with 16,007 consenting first degree relatives aged ≥18 years; producing a total of 23,960 participants (341). All participants completed a questionnaire that provided information on demographics (including age, sex and postcode of residence) and lifestyle (including smoking status, and number of hours of exposure per week to SHS). Trained staff measured height and weight. Trained staff collected blood samples from each consenting participants.

Ethical approval for the GS:SFHS was obtained from NHS Tayside Committee on Medical Research Ethics (REC Reference Number: 05/S1401/89). GS:SFHS has been granted Research Tissue Bank status by the Tayside Committee on Medical Research Ethics (REC Reference Number: 10/S1402/20) providing generic ethical approval for a wide range of uses within medical research. Permission to use the GS:SFHS data and access to the blood samples was provided following review by the GS:SFHS Access Committee.

In this chapter, I used existing data on a subgroup of 1,779 individuals from the SFHS, randomly selected to participate in a study on ageing. DNA was extracted from peripheral blood leukocytes using Maxwell automated purified system (Promega, WI, USA). Telomere lengths in the DNA samples were determined by quantitative-polymerase chain reaction (Q-PCR) blindly using a Roche Light Cycler LC480 (Roche Diagnostics, Indianapolis, Indiana, USA). (379)

Analyses were performed in triplicate for each sample using a single-copy gene amplicon primer set (acidic ribosomal phosphoprotein, 36B4) and a telomere-specific amplicon primer set (390). A cut-off 0.15 for the SD of the threshold cycle (Ct) for sample replicates was used as a quality control parameter for the amplification. The samples were reanalysed if an SD above 0.15 was encountered. The average SD across plates was 0.07. Relative telomere length was estimated from Ct scores using the comparative Ct method when telomere and control gene assays yielded similar amplification efficiencies. The ratio of telomere repeat copy number to single copy gene number (T/S) ratio in experimental samples relative to a control sample DNA was determined. This normalised T/S ratio was defined as the estimate of relative telomere length (Relative T/S). The inter-assay variation was tested by comparing the relative T/S estimates for positive controls on every assay plate. The average inter-assay coefficient of variance was 0.58% for telomere length and 0.23% for 36B4 (379, 380, 391, 392), which indicates a low variation.

4.3.2 Inclusion criteria and definitions

Scottish Health Survey (SHeS)

In this chapter, I collated data from the 1998, 2003, 2008, 2009 and 2010 surveys as they collected consistent information on cotinine, CRP, fibrinogen and lipid concentrations. Inclusion was restricted to participants aged ≥16 years who provided saliva and serum samples, and were not taking nicotine replacement products. Consistent with guidelines, non-smokers were defined as self-reported never or ex-smokers who had a salivary cotinine concentration <15.0 ng/mL (40). Current smokers were defined as self-reported current smokers who had a cotinine concentration ≥15.0 ng/mL. Among non-smokers, SHS exposure was classified into low (cotinine <0.7 ng/mL), moderate (cotinine 0.7-2.6 ng/mL) and high (cotinine ≥2.7 ng/mL). Current smokers were categorised into light (cotinine 15.0-100.0 ng/mL), moderate (cotinine 100.1-300.0 ng/mL) and heavy (cotinine >300.0 ng/mL). BMI was categorized into normal weight (<25 kg/m^2), overweight (BMI 25-30 kg/m^2) and obese (≥30 kg/m^2) (312). Alcohol consumption was based on self-report and classified as never drinker, ex drinker, low-risk drinker (men <28 units/week, women <21 units/week), increasing-risk drinker (men <50 units/week, women <35 units/week) and high-risk drinker (men ≥50 units/week, women ≥35 units/week) (316). Being physically active was defined as self-report of any kind of physical activity for at least three hours per week (315).

Generation Scotland : Scottish Family Health Study (GS: SFHS)

In this chapter, inclusion was restricted to non-smokers aged ≥ 18 years who had provided blood samples for telomere analysis. As mentioned in the previous chapter, in Scotland, an index of socioeconomic status based on postcode of residence at recruitment-the SIMD (313) was used. There are 6,505 datazones based on postcode of residence, with a mean population of 800. The SIMD for each datazone incorporates information on income, employment, health, education, housing, crime and access to services and is divided into quintiles for the Scottish population. Levels of SHS exposure were self-reported and categorised into no exposure, low exposure (1-19 hours per week) and high exposure (≥ 20 hours per week).

4.3.3 Statistical analyses

Scottish Health Survey (SHeS)

The characteristics of non-smokers and current smokers were summarised using frequencies and percentages for categorical data, medians and inter-quartile ranges for non-parametric continuous data (CRP) and mean and standard deviation for parametric continuous data (fibrinogen and lipids). The differences between the exposure groups were assessed using chi-square tests for categorical variables and analysis of variance (ANOVA) for continuous variables. Non-smokers and current smokers were included in the same model, in order to examine the effect of increasing cotinine concentration across the whole spectrum from non-smokers protected from SHS exposure to heavy active smokers. Univariate and multivariate median regression models were used to examine the association between cotinine concentration and serum CRP using non-smokers with low SHS exposure (cotinine <0.7 ng/mL) as the referent category. General linear regression models were used, in the same way, to examine the associations between cotinine concentration and fibrinogen and lipid concentrations. Three models were developed for each assay: unadjusted; partially adjusted (age and sex) and fully adjusted (age, sex, social class, body mass index, alcohol consumption and physical activity). Interactions with age, sex and socioeconomic status were tested by fitting interaction terms in the regression models. Statistical significance was defined as a two-sided p value <0.001 for main effects and <0.05 for interactions. All statistical analyses were undertaken using Stata 12.0 (Stata Corporation, College Station, Texas, USA).

Generation Scotland : Scottish Family Health Study (GS: SFHS)

Linear regression models were used to relate the telomere T/S ratio to age. The dose effect of SHS on telomere T/S ratio was presented as change per increasing year of age across each exposure group. The differences between the exposure groups were assessed by comparing the coefficients (slopes) of the linear regression lines by age. Statistical significance was defined as two-sided $p<0.05$. Interactions with covariates (age, sex and socioeconomic status) in the fully adjusted models were tested. All statistical analyses were performed using Stata 12.0 (Stata Corporation, College Station, Texas, USA).

4.4 Results

Scottish Health Survey (SHeS)

Of the 51,802 participants in the SHeS, 38,436 were aged ≥16 years. Of these, 180 were excluded because they were taking nicotine replacement therapy (nicotine chewing gum, patch or nasal spray). Of the remaining 38,256, 10,512 provided saliva and blood samples and had valid assay results. Three hundred and eighty four (3.7%) were excluded because they classified themselves as non-smokers but had a cotinine concentration ≥15.0 ng/mL, 94 (0.9%) because they classified themselves as current smokers but had a cotinine concentration <15.0 ng/mL, and 16 (0.1%) because of missing smoking status. The remaining 10,018 participants constituted the study population (Figure 4.1).

Of the 10,018, 7,345 (73.3%) were non-smokers and 2,673 (26.7%) were current smokers and, of the 2,725 ex-smokers, 2,604 (95.6%) had quit smoking at least one year prior to the survey and 2,251 (82.6%) had quit smoking for at least five years prior to the survey. Among the non-smokers, 2,208 (30.1%) had either moderate or high SHS exposure. Of the current smokers, 208 (7.8%), 980 (36.7%) and 1,485 (55.5%) were light, moderate and heavy smokers, respectively. Across the different cotinine groups, there were differences in age, sex, social class, BMI category, physical activity and alcohol consumption (Table 4.1).

CRP, fibrinogen, and cholesterol concentrations and TC/HDL cholesterol ratios differed for varying cotinine concentration (Table 4.2). CRP concentration and TC/HDL cholesterol ratio increased with increasing cotinine concentration but exhibited a step reduction between high exposure non-smokers and light active smokers (Table 4.2). Conversely, the HDL cholesterol concentration fell with increasing cotinine concentration but exhibited a step increase at the same point. There was no clear pattern relating to fibrinogen (Table 4.2).

Figure 4.1 Flow diagram of participant inclusion and exclusion. Scottish Health Survey

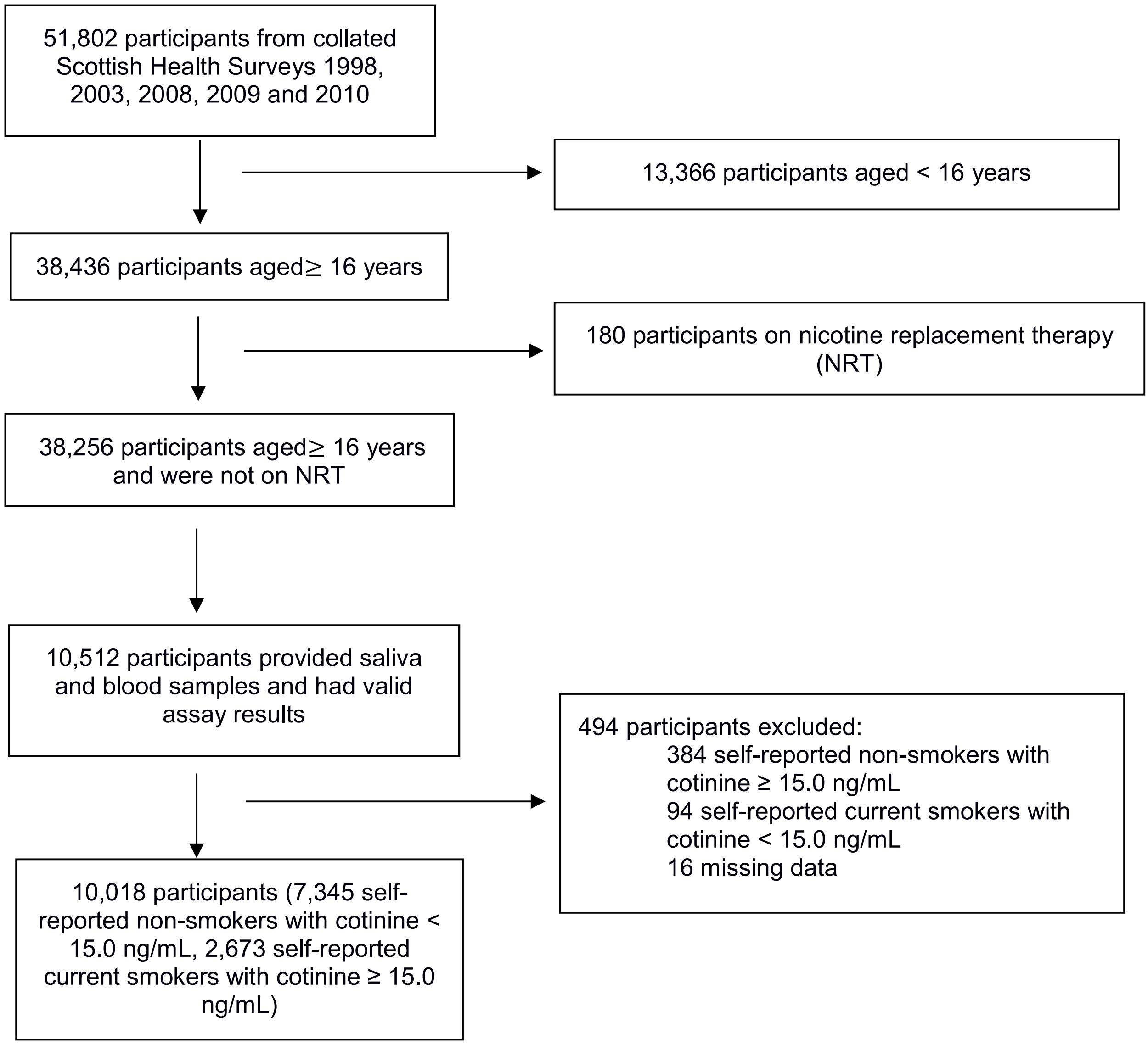


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Table 4.1 Characteristics of study population by cotinine concentrations. Scottish Health Survey

	Cotinine (ng/mL) Non-smokers			Current smokers			
	0-0.6 N=5,137	0.7-2.6 N=1,684	2.7-14.9 N=524	15.0-100.0 N=208	100.1-300.0 N=980	≥ 300.1 N=1,485	P values*
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	
Age (years)							
16-45	2,002 (39.0)	793 (47.1)	231 (44.1)	139 (66.8)	542 (55.3)	752 (50.6)	<0.001
46-59	1,422 (27.7)	444 (26.4)	114 (21.8)	43 (20.7)	233 (23.8)	466 (31.4)	
≥ 60	1,713 (33.3)	447 (26.5)	179 (34.1)	26 (12.5)	205 (20.9)	267 (18.0)	
Missing	0	0	0	0	0	0	
Sex							
Male	2,249 (43.8)	850 (50.5)	290 (55.3)	89 (42.8)	425 (43.4)	773 (52.1)	<0.001
Female	2,888 (56.2)	834 (49.5)	234 (44.7)	119 (57.2)	555 (56.6)	712 (47.9)	
Missing	0	0	0	0	0	0	
Social class							
Professional	440 (8.6)	94 (5.6)	19 (3.6)	13 (6.3)	28 (2.9)	32 (2.2)	<0.001
Managerial technical	1,758 (34.2)	361 (21.4)	119 (22.7)	57 (27.4)	231 (23.6)	269 (18.1)	
Skilled non-manual	710 (13.8)	223 (13.2)	67 (12.8)	26 (12.5)	132 (13.5)	164 (11.0)	
Skilled manual	1,308 (25.5)	592 (35.2)	175 (33.4)	61 (29.3)	290 (29.6)	501 (33.7)	
Semi-skilled manual	588 (11.4)	265 (15.7)	93 (17.7)	32 (15.4)	195 (19.9)	343 (23.1)	
Unskilled manual	203 (4.0)	104 (6.2)	31 (5.9)	13 (6.3)	79 (8.1)	137 (9.2)	
Missing	130	45	20	6	25	39	
BMI (kg/m^2)							
<25.0	1,564 (30.4)	469 (27.9)	143 (27.3)	81 (38.9)	386 (39.4)	676 (45.5)	<0.001
25.0-29.9	2,008 (39.1)	647 (38.4)	198 (37.8)	79 (38.0)	312 (31.8)	461 (31.0)	
≥ 30	1,174 (22.9)	430 (25.5)	145 (27.7)	36 (17.3)	198 (20.2)	231 (15.6)	
Missing	391	138	38	12	84	117	

Physically active							
Yes	2,624 (51.1)	847 (50.3)	235 (44.8)	123 (59.1)	468 (47.8)	729 (49.1)	0.018
No	2,301 (44.8)	757 (45.0)	256 (50.6)	76 (36.5)	469 (47.9)	692 (46.6)	
Missing	212	80	33	9	43	64	
Alcohol consumption							
Never drinker	322 (6.3)	75 (4.5)	33 (6.3)	4 (1.9)	31 (3.2)	47 (3.2)	<0.001
Ex drinker	204 (4.0)	64 (3.8)	24 (4.6)	4 (1.9)	54 (5.5)	96 (6.5)	
Low-risk drinker	4,139 (80.6)	1,321 (78.4)	371 (70.8)	145 (69.7)	695 (70.9)	1,086 (71.9)	
Increasing-risk drinker	351 (6.8)	148 (8.8)	63 (12.0)	26 (12.5)	112 (11.4)	155 (10.4)	
High-risk drinker	101 (2.0)	55 (3.3)	27 (5.2)	27 (13.0)	84 (8.6)	96 (6.5)	
Missing	20	21	6	2	4	5	

Values given as n (%) unless otherwise stated.
CI confidence interval; BMI body mass index
*x^2 test for age, sex, social class, body mass index, physical activity, alcohol consumption
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Table 4.2 Concentrations of C reactive protein, high-density lipoprotein cholesterol and fibrinogen, and total cholesterol/high-density lipoprotein cholesterol ratio by cotinine concentrations. Scottish Health Survey

		Cotinine(ng/mL)						
		Non-smokers			Current smokers			
		0-0.6 N=5,137	0.7-2.6 N=1,684	2.7-14.9 N=524	15.0-100.0 N=208	100.1-300.0 N=980	≥ 300.1 N=1,485	P values*
		Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	
CRP (mg/L)		1.30 (0.60-3.00)	1.50 (0.60-3.50)	1.80 (0.70-4.40)	1.30 (0.50-3.65)	1.85 (0.70-4.30)	2.10 (0.90-4.60)	<0.001
		Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
HDL cholesterol (mmol/L)	Unadjusted	1.52±0.42	1.47±0.39	1.43±0.39	1.50±0.43	1.42±0.40	1.37±0.38	<0.001
	Adjusted**	1.37±0.04	1.36±0.04	1.33±0.04	1.39±0.04	1.30±0.04	1.26±0.04	<0.001
TC/HDL cholesterol	Unadjusted	3.94±1.33	4.00±1.30	4.32±6.24	3.73±1.23	4.14±1.69	4.48±2.33	<0.001
	Adjusted**	3.05±0.11	3.10±0.12	3.42±0.25	3.06±0.11	3.35±0.12	3.53±0.14	<0.001
Fibrinogen (g/L)	Unadjusted	2.87±0.66	2.78±0.70	2.81±0.75	2.77±0.73	2.97±0.79	3.09±0.74	<0.001
	Adjusted**	2.07±0.14	2.02±0.14	2.00±0.16	2.18±0.17	2.20±0.15	2.37±0.12	<0.001

CRP C-reactive protein; TC total cholesterol; HDL high-density lipoprotein; IQR inter-quartile range; n number; SD standard deviation
*Kruskal-Wallis test for C-reactive protein; one-way ANOVA test for HDL cholesterol, TC/HDL cholesterol and fibrinogen
**Adjusted for age, sex and social class; Mean±standard error

The median regression analyses suggested a dose relationship whereby CRP concentration increased with increasing cotinine concentration among non-smokers and among smokers but there was a step between high exposure non-smokers and light active smokers. In the fully adjusted model, non-smokers with high levels of SHS exposure had CRP concentrations that were higher than non-smokers protected from SHS exposure and were comparable to those of light (p=0.709) and moderate (p=0.136) active smokers (Table 4.3, Figure 4.2). There were statistically significant interactions with age (p=0.001) and sex (p=0.002). There was evidence of a dose response relationship between cotinine and CRP concentration in all subgroups but the difference in CRP concentration between non-smokers protected from SHS exposure and those with high exposure levels only reached statistical significance in men (p=0.005) and those over 60 years of age (p<0.001). In all subgroups there was no significant difference in CRP concentration between high-exposure non-smokers and light active smokers.

In the fully adjusted linear regression analysis, HDL cholesterol concentration generally decreased with increasing cotinine concentration. Non-smokers with high levels of SHS exposure had HDL cholesterol concentrations that were lower than those of non-smokers protected from SHS exposure and were comparable to those of light active smokers (p=0.931) (Table 4.4, Figure 4.3). There was a significant interaction with sex (p<0.001). The difference in HDL cholesterol concentration between non-smokers protected from SHS and those with high exposure was statistical significant among women (p=0.006) but less significant among men (p=0.216). There were no significant differences in the HDL cholesterol concentrations between non-smokers with high SHS exposure and light active smokers among either men or women. TC/HDL cholesterol ratios generally increased with increasing cotinine concentration. In the fully adjusted model, only moderate and heavy active smokers had ratios that were higher than non-smokers protected from SHS exposure. The ratios of non-smokers with high exposure were similar to those of light (p=0.405) and moderate (p=0.827) active smokers (Table 4.4, Figure 4.4). There were significant interactions with both age (p<0.001) and sex (p=0.039) but the TC/HDL cholesterol ratios generally increased with increasing cotinine concentration in all subgroups and the ratio was not significantly different between non-smokers with high SHS exposure and light active smokers in any subgroup. The relationship between cotinine and fibrinogen was less clear-cut with only moderate and heavy active smokers having raised concentrations (Table 4.4, Figure 4.5).

Table 4.3 Median regression analyses of the association between cotinine concentration and C reactive protein concentration. Scottish Health Survey

	Cotinine (ng/mL)	C reactive protein								
		Unadjusted			Partially adjusted†			Fully adjusted‡		
		Coefficient (95%CI)	P value	P value for trend	Coefficient (95%CI)	P value	P value for trend	Coefficient (95%CI)	P value	P value for trend
Non-smokers	0-0.6*	-	-	<0.001	-	-	<0.001	-	-	<0.001
	0.7-2.6	0.200 (0.037-0.363)	0.016		0.200 (0.075-0.325)	0.002		0.125 (0.034-0.216)	0.007	
	2.7-14.9	0.500 (0.230-0.770)	<0.001		0.500 (0.255-0.745)	<0.001		0.300 (0.121-0.479)	0.001	
Current smokers	15.0-100.0	-0.000 (-0.299-0.299)	1.000		0.200 (-0.152-0.552)	0.265		0.250 (0.039-0.461)	0.020	
	100.1-300.0	0.600 (0.370-0.830)	<0.001		0.700 (0.511-0.889)	<0.001		0.475 (0.311-0.639)	<0.001	
	≥ 300.1	0.800 (0.628-0.972)	<0.001		1.000 (0.829-1.171)	<0.001		0.925 (0.788-1.062)	<0.001	

CI confidence interval
*reference category
† adjusted for age and sex; ‡ adjusted for age, sex, social class, body mass index, physical activity and alcohol consumption
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Table 4.4 Linear regression analyses of cotinine concentration associated with high-density lipoprotein cholesterol concentration, total cholesterol/ high-density lipoprotein cholesterol ratio and fibrinogen concentration. Scottish Health Survey

	Cotinine (ng/mL)	Unadjusted			Partially adjusted†			Fully adjusted‡		
		Coefficient (95%CI)	P value	P value for trend	Coefficient (95%CI)	P value	P value for trend	Coefficient (95%CI)	P value	P value for trend
HDL cholesterol										
Non-smokers	0-0.6*	-	-	<0.001	-	-	<0.001	-	-	<0.001
	0.7-2.6	-0.049 (0.072- -0.027)	<0.001		-0.027 (-0.048- -0.006)	0.013		-0.016 (-0.037-0.005)	0.134	
	2.7-14.9	-0.092 (-0.130- -0.055)	<0.001		-0.063 (-0.099- -0.028)	<0.001		-0.053 (-0.088- -0.018)	0.003	
Current smokers	15.0-100.0	-0.018 (-0.079-0.044)	0.568		-0.005 (-0.061-0.051)	0.863		-0.050 (-0.105-0.005)	0.076	
	100.1-300.0	-0.102 (-0.131- -0.073)	<0.001		-0.094 (-0.121- -0.066)	<0.001		-0.117 (-0.145- -0.090)	<0.001	
	≥ 300.1	-0.153 (-0.176- -0.129)	<0.001		-0.127 (-0.151- -0.104)	<0.001		-0.156 (-0.178- -0.134)	<0.001	
Total/HDL cholesterol										
Non-smokers	0-0.6*	-	-	<0.001	-	-	<0.001	-	-	<0.001
	0.7-2.6	0.057 (-0.016-0.130)	0.124		0.046 (-0.025-0.117)	0.207		0.008 (-0.061-0.077)	0.810	
	2.7-14.9	0.378 (-0.186-0.941)	0.189		0.334 (-0.236-0.905)	0.251		0.297 (-0.249-0.843)	0.287	
Current smokers	15.0-100.0	-0.207 (-0.384- -0.031)	0.022		-0.076 (-0.252-0.099)	0.395		0.055 (-0.111-0.221)	0.515	
	100.1-300.0	0.197 (0.081-0.314)	0.001		0.279 (0.163-0.396)	<0.001		0.360 (0.247-0.472)	<0.001	
	≥ 300.1	0.536 (0.407-0.664)	<0.001		0.532 (0.409-0.654)	<0.001		0.657 (0.525-0.789)	<0.001	
Fibrinogen										
Non-smokers	0-0.6*	-	-	<0.001	-	-	<0.001	-	-	<0.001

	0.7-2.6	-0.095 (-0.136- -0.054)	<0.001	-0.044 (-0.083- -0.005)	0.029	-0.060 (-0.097- -0.024)	0.001
	2.7-14.9	-0.065 (-0.139-0.009)	0.087	-0.032 (-0.102-0.038)	0.369	-0.056 (-0.125-0.014)	0.116
Current smokers	15.0-100.0	-0.096 (-0.205-0.012)	0.081	0.018 (-0.086-0.123)	0.734	0.027 (-0.072-0.124)	0.596
	100.1-300.0	0.102 (0.047-0.157)	<0.001	0.172 (0.118- -0.225)	<0.001	0.171 (0.118-0.224)	<0.001
	≥ 300.1	0.216 (0.171-0.261)	<0.001	0.291 (0.247-0.334)	<0.001	0.313 (0.270-0.356)	<0.001

CI confidence interval; HDL high-density lipoprotein
*reference; † adjusted for age and sex; ‡ partially adjusted plus social class, body mass index, physical activity and alcohol consumption
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Figure 4.2 Change in C reactive protein concentration per unit change in cotinine concentration (fully adjusted). Scottish Health Survey

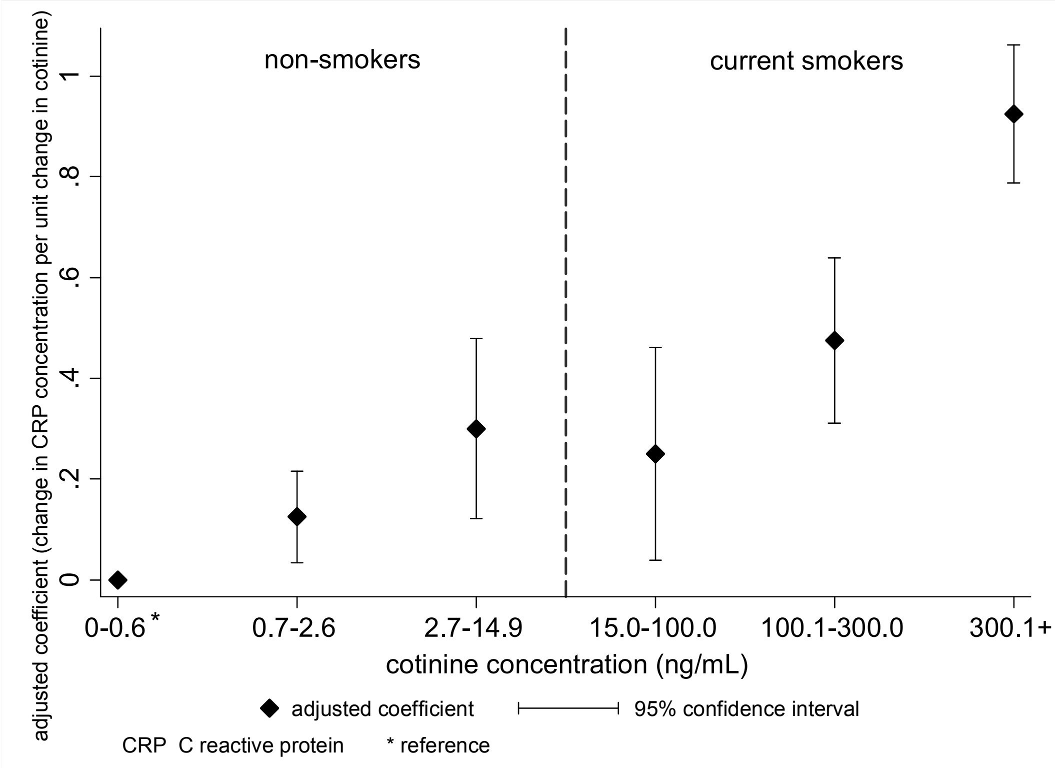


Figure 4.3 Change in high-density lipoprotein cholesterol concentration per unit change in cotinine concentration (fully adjusted). Scottish Health Survey

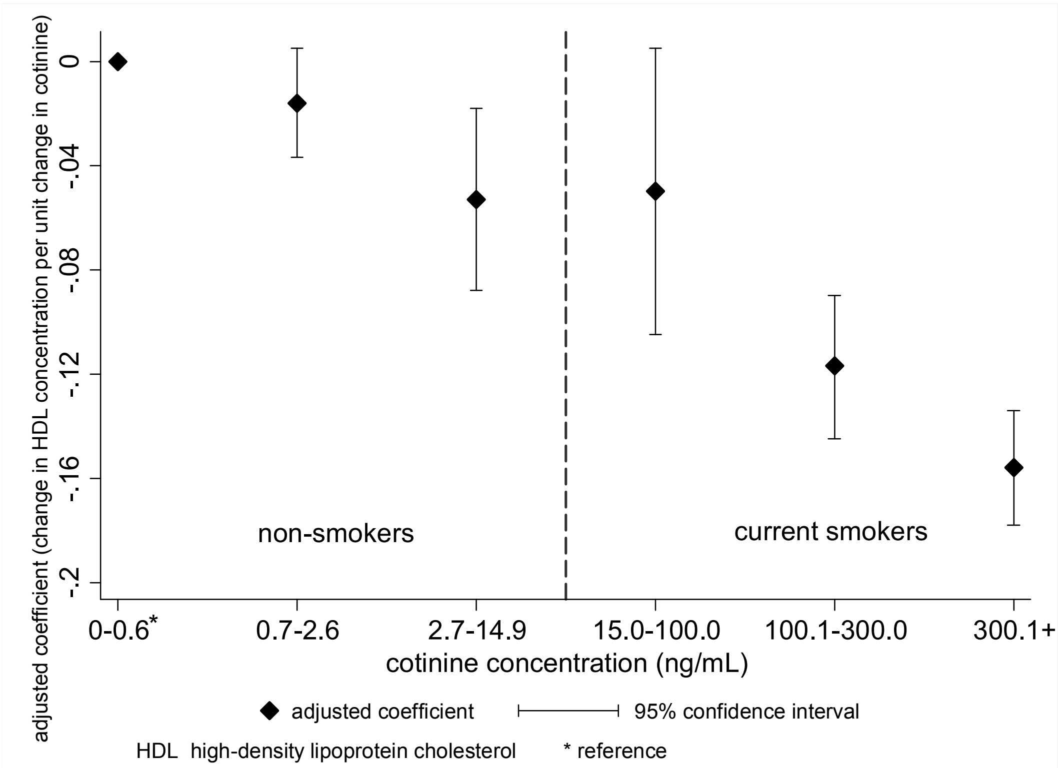


Figure 4.4 Change in total cholesterol/high-density lipoprotein cholesterol ratio per unit change in cotinine concentration (fully adjusted). Scottish Health Survey

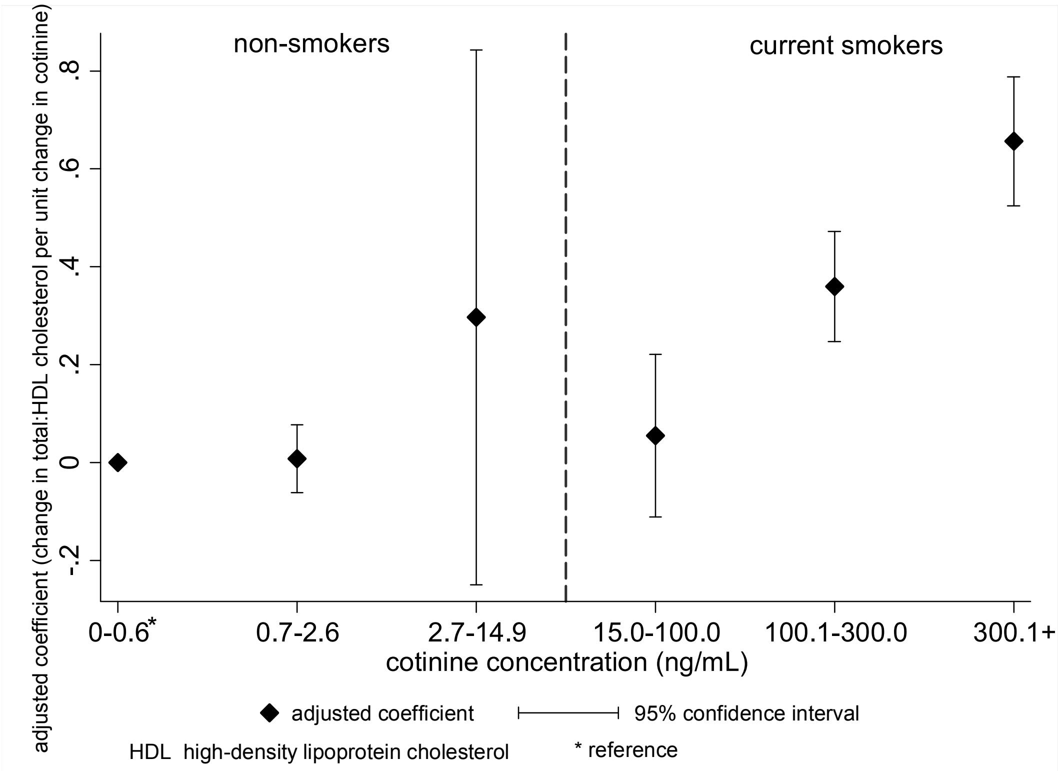
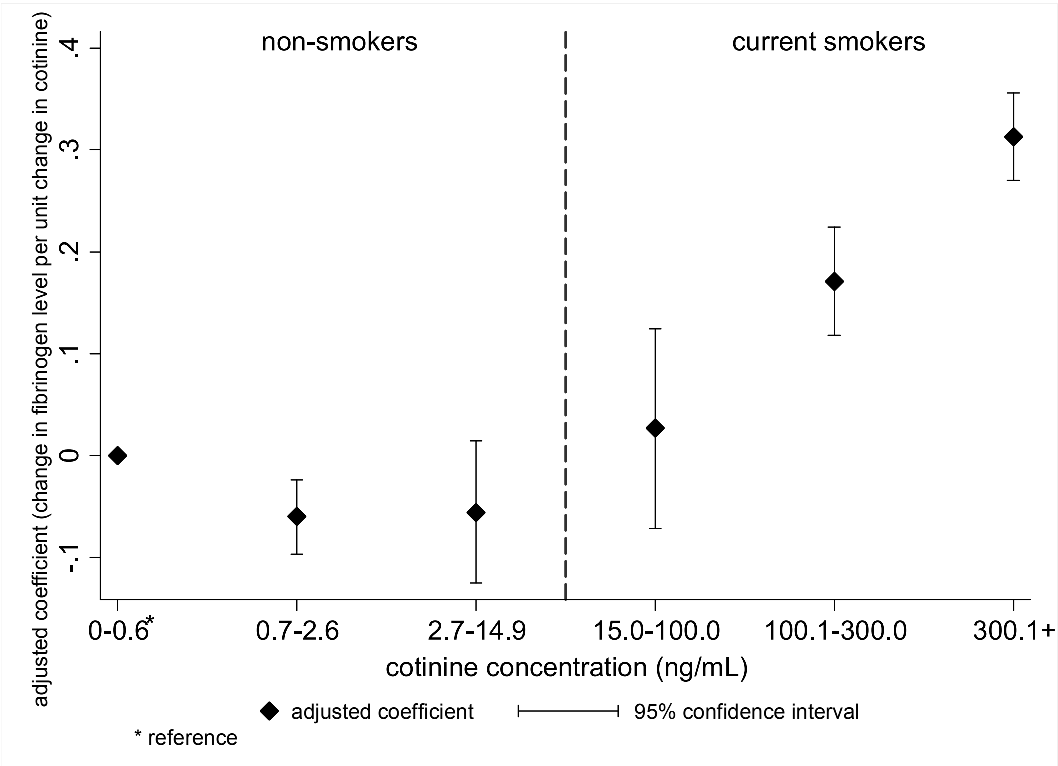


Figure 4.5 Change in fibrinogen concentration per unit change in cotinine concentration (fully adjusted). Scottish Health Survey



Generation Scotland : Scottish Family Health Study (GS: SFHS)

Of the 1,779 participants, 1,721 had provided adequate blood samples for telomere assays. Among these, 1,433 were self-reported non-smokers. Among these non-smokers, 1,303 provided self-reported SHS exposure status and were therefore included in my study. Among these, 861 (66.1%) were men and 442 (33.9%) women, 846 (64.9%) self-reported as never smokers and 457 (35.1%) self-reported as ex-smokers. Among the 1,303 eligible participants, 779 (59.8%) reported no SHS exposure, 495 (38.0%) low exposure (1-19 hours per week), 29 (2.2%) high exposure (≥20 hours per week) (Table 4.5). Participants with high SHS exposure were older at age (p value for trend=0.025), lived in more socioeconomically deprived areas (p value for trend<0.001), and were more likely to be obese (p value for trend=0.012) when compared with participants with no SHS exposure.

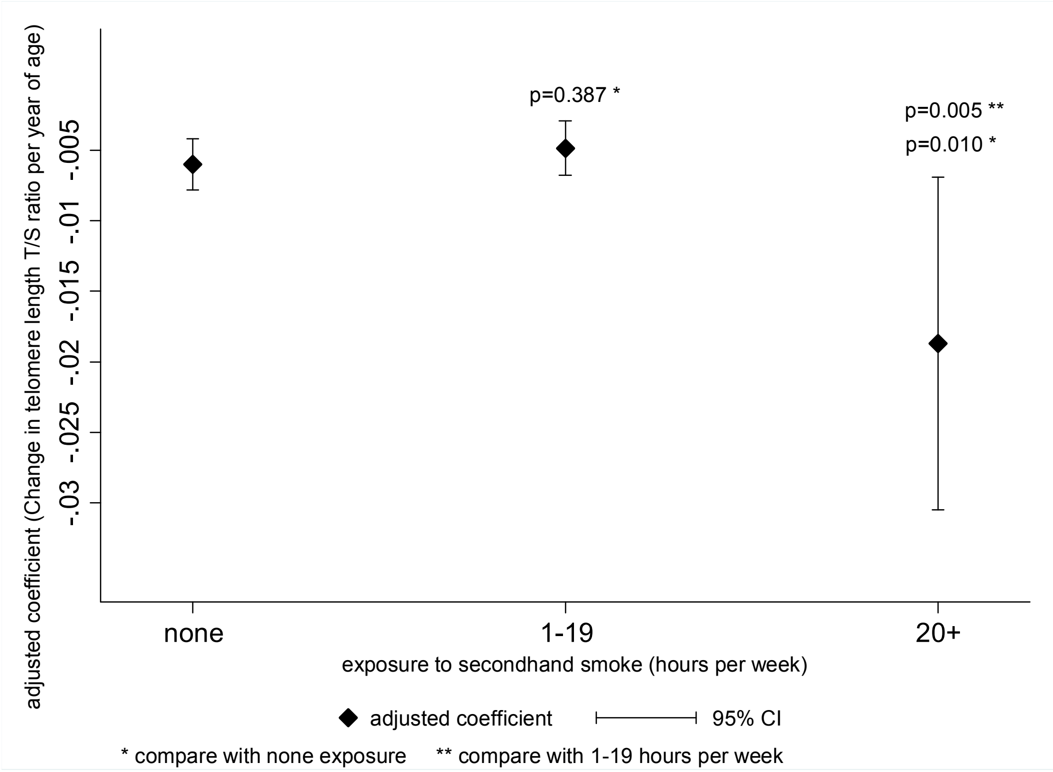
Table 4.5 General Characteristics of the 1,303 Non-smokers. A subgroup from Scottish Family Health Study chosen as part of a study on biomarkers of ageing

	Total hours per week			
	0 (n=779)	1-19 (n=495)	≥ 20 (n=29)	P value*
Age group, n (%)				<0.001
<45 years	368 (47.2)	327 (66.1)	20 (69.0)	
45-59 years	193 (24.8)	98 (19.8)	5 (17.2)	
≥60 years	218 (28.0)	70 (14.1)	4 (13.8)	
Missing	0	0	0	
Gender, n (%)				0.190
Male	529 (67.9)	312 (63.0)	20 (69.0)	
Female	250 (32.1)	183 (27.0)	9 (31.0)	
Missing	0	0	0	
Deprivation, n (%)				<0.001
1 (least deprived)	297 (38.1)	164 (33.1)	4 (13.8)	
2	211 (27.1)	123 (24.9)	6 (20.7)	
3	99 (12.7)	73 (14.8)	5 (17.2)	
4	52 (6.7)	64 (12.9)	8 (27.6)	
5 (most deprived)	50 (6.4)	44 (8.9)	4 (13.8)	
Missing	70	27	2	

* x^2 test

In the univariate linear regression analyses, relative telomere T/S ratio declined with increasing year of age in all exposure groups. Telomere length decreased more rapidly with increasing age among those with high exposure to SHS when compared with both those with no exposure to SHS (p=0.047) and those with low exposure to SHS (p=0.047). After adjustment for sex and deprivation, the significant association accentuated. With increasing age per annum, telomere length decreased more rapidly among those with high exposure to SHS when compared with both those with no exposure to SHS (p=0.010) and those with low exposure to SHS (p=0.005) (Figure 4.6).

Figure 4.6 Change in telomere length T/S ratio per year of age and levels of secondhand smoke exposure among non-smokers (adjusted for sex and deprivation). A subgroup from Scottish Family Health Study chosen as part of a study on biomarkers of ageing



4.5 Discussion

Pre-clinical biomarkers of cardiovascular disease

My study using the Scottish Health Survey data, corroborated previous studies by demonstrating that within both non-smokers and active smokers, there was a dose response relationship whereby increasing exposure to tobacco smoke was associated with higher risk concentrations of most cardiovascular risk biomarkers. It also added to existing evidence on SHS exposure by demonstrating a step change in the relationship between tobacco exposure and biomarkers at the interface between non-smokers and active smokers. Compared with light, and sometimes moderately, active smokers, non-smokers exposed to high levels of SHS had lower cotinine concentrations but comparable concentrations of most cardiovascular risk biomarkers. My findings suggest that SHS exposure carries a disproportionately higher cardiovascular risk than would be anticipated from extrapolation of the effects seen in active smokers.

There is strong evidence that both active smoking and exposure to SHS are associated with CVD (4, 6, 14, 15, 118). Inflammation plays a crucial role in the initiation and the progression of atherosclerosis in various vascular beds, from endothelial dysfunction to all stages of plague progression and to clinical events of ischemic injury including PAD (393-398). LDL and fibrinogen included in my study relate to the pathophysiology of atherosclerosis (397). CRP and fibrinogen are acute phase proteins and markers of inflammation and haemostasis (399-403). However, there is an ongoing debate on whether or not CRP may be a predictor of future adverse cardiovascular events or may be directly involved in the atherosclerotic process. One argument presents that the association between CRP and clinical outcomes is likely to be a consequence of confounding in the notion that CRP has been found to be associated with increasing age, sex, BMI and socioeconomic status (404-406). Recent published evidence does not support the causal role of CRP in the pathogenesis of atherosclerosis. Although there are several limitations on inferring causality using mendelian randomisation (407), a study of four Danish cohorts suggested that CRP polymorphisms are not associated with increased risk of ischemic vascular disease (408). A mendelian randomisation meta-analysis indicated CRP is unlikely to be a causal factor in CHD (409). Yet, in 2012, a meta-analysis of 52 prospective studies comprising 246,669 participants without known CVD investigated the value of adding information on CRP or fibrinogen levels to conventional risk factors for the prediction of cardiovascular events. This meta-analysis suggested that after initial screening with conventional risk factors alone, the additional assessment of the CRP or fibrinogen level in people at intermediate risk (a predicted risk of 10% to < 20% over a period of 10 years) for a cardiovascular event could help prevent one extra event over a period of 10 years for approximately every 400 to 500 people screened (410). Taken together, although the association between CRP and CVD are unlikely to be causal, CRP can be an integrative indicator of chronically elevated inflammation and relates to many cardiovascular risk factors (411). Researchers suggested that CRP and fibrinogen have a significant though limited incremental prognostic value in addition to conventional risk factors (412, 413). Cholesterol is a major risk factor for the development of atherosclerotic disease with adverse effects on endothelial function and vasomotion as well as directly promoting atherogenesis. In contrast to total, and especially LDL, cholesterol, the reverse cholesterol transport mediated by HDL particles protects against atherogenesis and endothelial dysfunction (414, 415). Similarly, there is mixed evidence on the association between LDL and cardiovascular events (416). However, several global risk assessment scores including the Framingham Risk Score (417) take total cholesterol and HDL cholesterol into the CVD risk calculation.

Studies have shown that active smoking is associated with increased CRP, fibrinogen and total and LDL cholesterol concentrations, and reduced TC/ HDL cholesterol ratios (418, 419). Fewer studies have been conducted on chronic exposure to SHS in humans and the results are controversial. In a cross-sectional study of 995 never smokers, CRP concentrations were 0.08 mg/dL (95% CI 0.02-0.10, p=0.03) higher in those exposed to SHS for more than 3 days per week compared with those who were not exposed (334). Using data from the Third National Health and Nutrition Examination Survey on 7,599 never smokers, Venn et al. reported that raised cotinine concentrations were associated with higher fibrinogen concentrations (adjusted mean difference 9.96 mg/dL, 95% CI 0.92-19.01, p=0.03 for cotinine >0.215 ng/mL) but not CRP concentrations (420). Two studies have examined the association between cotinine and CRP across both non-smokers and active smokers (325, 421). Both applied a cotinine concentration of 15.0 ng/mL to differentiate between active and non-smokers. Hamer *et al.* studied the 13,443 people aged ≥35 years who participated in the English and Scottish Health Surveys conducted between 1998 and 2004 (325). Non-smokers were categorised into three groups, according to cotinine concentration (<0.01, 0.06-0.7 and 0.71-14.99 ng/mL), but current smokers were included as a single group. Also in my study using the Scottish Health Survey, moderate and high SHS exposure groups comprised a single group in the Hamer study. Among non-smokers, higher concentrations of cotinine were associated with higher concentrations of CRP. Overall, active smokers had higher CRP and fibrinogen concentrations, and lower HDL cholesterol concentrations, than non-smokers, but the investigators were not able to compare non-smokers exposed to high levels of SHS with light active smokers. The adjusted HR for CHD mortality over eight years follow-up was 2.00 (95% CI 1.06-3.78) for never smokers with high SHS exposure and 1.74 (95% CI 1.24-2.46) for current smokers (325) .

The British Regional Heart Study recruited individuals aged ≥59 years from general practices in 24 towns. Jefferis *et al.* conducted a cross-sectional study using baseline data on 5,029 non and light (<10 cigarettes/day) active smokers. Among the non-smokers, higher cotinine concentrations were associated with higher CRP (p for trend <0.001) and fibrinogen (p for trend = 0.026) concentrations (421). Compared with non-smokers with cotinine >0.7 ng/mL, light active smokers had higher unadjusted mean CRP (2.29 vs 1.78 mg/L) and fibrinogen (3.49 vs 3.28 g/L), lower unadjusted mean HDL cholesterol (1.49 vs 1.53 mmol/L) and identical total cholesterol (both 6.37 mmol/L) but the investigators only calculated p values for a comparison between light active smokers and non-smokers with cotinine ≤0.05 mg/mL (421). The risk of CHD events over 20 years of follow-up was comparable in non-smokers exposed to high levels of SHS (adjusted HR 1.57, 95% CI 1.08-2.28) and light active smokers (adjusted HR 1.66, 95% CI 1.04-2.68) in spite of cotinine concentrations being nearly 30-fold higher in the latter group (mean 4.9 versus 138 ng/mL) (67).

In my study using the SHeS, the main novelty was the direct comparison between non-smokers with high levels of SHS exposure, and light and moderate active smokers using several biomarkers. I used data from a representative Scotland-wide survey and based SHS exposure on salivary cotinine concentrations rather than self-report. Salivary cotinine measurements have been shown to be more sensitive than serum or urine cotinine concentrations in classifying smoking status (34). Applying a cut-off of 15 ng/mL enabled us to differentiate between genuine non-smokers exposed to high levels of SHS exposure and smoking deceivers. Any cut-off could result in some misclassifications in both directions. However, the cut-off value I used complies with published guidelines and is consistent with previous studies on this topic (40, 325). I was able to exclude participants using nicotine replacement therapy from the study. I did not have data on use of other forms of tobacco, such as chewing tobacco, but this is used very uncommonly in Scotland. I combined ex- and never smokers to maximise statistical power but the vast majority of ex-smokers had not smoked for more than five years. I combined a number of survey years to increase statistical power. I did not have sufficient power to test for interactions for study year or perform subgroup analysis by year.

In the regression models, I was able to adjust for potential confounders including demographic, socioeconomic and lifestyle risk factors. In relation to the association between cotinine and CRP, HDL cholesterol concentrations, there were interactions with sex. After stratifying the analyses, the difference in CRP concentration or HDL cholesterol concentration between non-smokers protected from SHS exposure and those with high exposure levels was less pronounced in the subgroups. However, results of low statistical significance can still be clinically relevant. This requires cautious interpretation and further investigation to reduce the play of chance. There were no interactions with socioeconomic and lifestyle risk factors. To increase statistical precision, I used median regression models to estimate the change of median CRP value produced by one unit change in cotinine across different levels of SHS exposure and active smoking. In contrast to the two previous studies, I included all current smokers and classified them into three groups according to the amount smoked in order to examine the association between cotinine and CRP across the whole range of non-smokers and active smokers. I also split non-smokers with cotinine concentrations >0.7 ng/mL into two groups. I examined the overall trend in biomarkers by cotinine concentration and also specifically tested the difference between non-smokers with high SHS exposure and light active smokers. As with the previous investigations, my study was cross-sectional. Therefore, a temporal relationship cannot be demonstrated but reverse causality is unlikely (Appendix 5). It is acknowledged that there are numerous biomarkers relevant to the pathophysiology of atherosclerosis including markers of coagulation cascade (e.g. apolipoprotein B) (422) and markers of hemodynamic stress (e.g. B-type natriuretic peptides) (423). In my study, I had access to four biomarkers relevant to different pathways, but did not have access to other endothelial, inflammatory and haemostatic markers.

<u>Biomarker of biological aging</u>

In my study using a subgroup of participants from the SFHS, my findings suggested that high SHS exposure may accelerate normal biological ageing, as measured by leukocyte telomere length.

Telomeres consist of TTAGGG tandem repeats, which cap the chromosomes and protect them from DNA-damage repair pathways (424). In regard to the telomere as a biomarker of ageing in humans, Mather et al. suggested the evidence is inconclusive due to many of the previous studies being cross-sectional and possibly underpowered and more longitudinal studies are needed to unravel the association between telomere length and human ageing (425). However, it is widely accepted that telomere is a biomarker for cumulative oxidative stress, inflammation and consequently biological ageing (389). As atherosclerosis is an age-related disease, telomere attrition has been demonstrated to be associated with CVD phenotypes (426-428). In a genome-wide meta-analysis of almost 50,000 individuals, researchers calculated a genetic risk score with the lead variants of mean leukocyte telomere length in seven genetic loci. They found an association between this score and an increased risk for CVD (429). In a case-control study, relative telomere length with T/S ratios were measured among 241 male patients diagnosed with symptomatic PAD and 249 age- and diabetes- matched controls. They found that the mean relative telomere length was significantly shorter in patients with PAD than in the control groups. Per telomere length attrition by one standard deviation increased the odds for PAD by 44% (adjusted OR=1.44, 95%CI 1.19-1.75, p<0.001). Correlations between relative telomere length and HDL cholesterol, CRP and ABPI were observed (correlation coefficients: r=0.121, p<0.01 for HDL; r=-0.111, p<0.05 for CRP; r=0.178, p<0.01 for ABPI) (430).

Both active smoking and SHS exposure increase inflammation, thrombosis and oxidative stress (59, 306). Several studies have demonstrated an association between active smoking and telomere attrition. One study related blood leukocyte telomere attrition to active smoking and demonstrated this association among 1,122 healthy women aged 18-72 years. Telomeres were measured as the mean of the terminal telomere restriction fragment lengths. They showed that never smokers had longer age-adjusted telomeres than former smokers and both had longer telomeres than current smokers. Among current smokers, there was a dose relationship whereby each pack-year smoked equated to an additional 5 base pairs (bp), or 18% of the average annual loss in age-adjusted telomere length (389). In contrast, evidence on examining SHS is sparse. In a cross-sectional study conducted in the US among 77 traffic officers exposed to high levels of traffic pollutants and 57 office workers as controls, ever smoking was associated with shorter telomeres among controls (unadjusted OR=1.17, 95% CI 1.10-1.25, p=0.04). Telomeres were measured as relative T/S ratios. However, they did not find a significant association between telomere and pack-years smoked, or number of cigarettes per day. They also reported no significant association with exposure to SHS (431). However, this previous study reflected a lack of statistical power. Of the 26 never smokers among the 57 office workers included in their study, 12 reported exposure to SHS. Of the 40 never smokers among the 77 traffic officers, 16 reported exposure to SHS. In my study using the subgroup of SFHS, of the 1,303 non-smokers included, 524 reported SHS exposure. This enabled me to categorise non-smokers into groups with different level of SHS exposure.

My study is among the few published studies, and the largest to date, to examine the association between telomere and SHS. I was able to compare the attrition in telomere length T/S per year of age across different levels of SHS. In this subgroup from SFHS for ageing study, real-time (RT) PCR assays were used to determine telomere length. RT-PCR involves detecting the telomere-to-single copy gene (T/S) ratio, which is demonstrated to be proportional to the average telomere length in a cell (379, 380, 392). It is feasible to be used in large epidemiological studies. In order to maximise statistical power, non-smokers in this study included ex-smokers but the majority of the ex-smokers had not smoked for more than a year.

In the regression models, I was able to adjust sex and deprivation quintile. The association increased in magnitude. This may suggest that individuals exposed to both scocioeconomic deprivation and SHS exposure may be at particularly high risk of premature biological ageing. I did not have access to salivary cotinine data and therefore had to reply on self-report of SHS exposure. This study was cross-sectional and therefore the temporal relationship cannot be established. However, reverse causation is unlikely to be plausible. Future research is needed to explore the possible mechanisms by which the observed association between SHS exposure and accelerated shortening of telomeres can be explained. However, plausible mechanisms may include cumulative oxidative stress-mediated damage and the stimulation of inflammation after exposure to SHS that leads to telomere attrition and therefore to age-related diseases including CVD. One limitation of the study using SFHS is the small sample size. Ideally, sample size should not be too small or too large (432). Small sample size makes the interpretation of the results including the p values and CIs difficult. Generally large studies produce small p value and narrow CIs. A very small p value and very narrow CIs generally suggest that the result is precise and is less likely to be due to chance (433). In my study, the 95% CIs are wide. It is possible that the results are false-positive or the magnitude of association between SHS exposure and telomere length attrition is overestimated. However, this study using SFHS is a hypothesis-testing study. There is suggestion of an association. Because of the small size of this study using a subgroup from the SFHS, the results should be interpreted accordingly and should be corroborated in future by larger studies or meta-analyses. Future, larger studies should also compare the effects of SHS exposure and active smoking on telomere length in order to establish whether the disproportionately large effect on the biomarkers of cardiovascular risk studied in the first part of this chapter also applies to biomarkers of aging. There will be a possible plan to corroborate the findings from this cross-sectional study using record linkage to follow-up data of telomere and other CVD-related inflammatory markers measurements.

Since there can be a lag between past smoking behaviour among ex-smokers and the disease onset (357), ex-smokers still carry the risk of developing cardiovascular events including PAD (118, 358). Including only never smokers would have been ideal for the research. In reality, the analyses were possibly underpowered to show the association between SHS and these biomarkers. In my studies, in order to maximise available power, I included ex-smokers with never smokers, as non-smokers. However, most of the ex-smokers had stopped smoking at least for five years prior to the survey. Including ex-smokers in the studies is an important limitation of this thesis.

There may be other alternative analytical approaches. According to the SHeS report (www.gov.scot/resource/0040/00402630.pdf), the proportion of non-smokers who reported being exposed to SHS in public places has declined since 2008. As mentioned in Chapter 1, exposure to SHS has fallen markedly since the implementation of the smoke-free legislation in 2006 in Scotland. This may be a possible explanation that in my studies the majority of participants were either no or low exposure to SHS. In the study using SHeS, I would have conducted subgroup analyses split by pre-legislation period and post-legislation period provided that there are sufficient numbers of participants in each exposure group. Furthermore, I would have included more potential confounders. In particular, it is important to include only never smokers and compare the absolute differences of the strength of the association by cotinine concentrations between never smokers, ex-smokers and current smokers, provided that there are sufficient numbers of participants in each survey.

Both studies in this chapter are observational studies, which are susceptible to potential for built-in bias including confounding (Appendix 5). A confounder is an extraneous factor that is associated with the exposure and affects the outcome, but it is not an intermediate step in the causal pathway between the exposure and the outcome (363). Confounding can be minimised by restricting, matching and randomisation at the design stage, and by stratifying, making multivariable statistical adjustment and doing standardised rate analysis at the analysis stage (363, 365). In the study using SHeS, the effects of confounding were reduced both by stratifying the analysis as well as by developing multivariable statistical models with adjustments. After multivariable adjustment, the change of CRP or HDL concentration per unit change of cotinine concentration among non-smokers with high SHS exposure was still comparable to light active smokers. The change of TC/HDL cholesterol ratios per unit change of cotinine concentration among non-smokers with high SHS exposure was similar to those of light and moderate active smokers. Similarly, confounding was controlled by multivariable adjustments in the study using SFHS, telomere length still decreased more rapidly among non-smokers with high exposure to SHS when compared with no or low exposure groups. Admittedly, these findings may still have some confounding effect of the unknown or omitted confounders, which may affect the observed association between cotinine and these biomarkers, and the association between SHS exposure and telomere length attrition. Residual confounding occurs when additional confounding factors were not considered or not measured; confounding was not controlled well enough; and there were errors in the measured confounders including misclassification of subjects with respect to confounding variables due to reporting or measurement errors (370, 371). Therefore, residual confounding may also be an issue. Both studies in this chapter use existing secondary data and therefore data on a proportion of confounders were not collected.

Furthermore, stratifying the analyses by the confounding variables would be beneficial in reducing the confounding effects. However, the results of these subgroup analyses could, at least to some extent, be due to the play of chance (Appendix 5). Therefore, caution should be taken when interpreting the results. However, the presented studies are exploratory and warrant further research. The association between SHS and CVD-related biomarkers would be more plausible if in future longitudinal studies include only never smokers with repeat measures of SHS exposure and repeat follow-up measures of relevant biomarkers.

Both studies were cross-sectional and therefore the temporal relationship (Appendix 5) cannot be implied. One of the criteria to gauge causality is reverse causation (Appendix 5). However, in my studies, reverse causation is unlikely to be plausible. Reverse causation occurs when the probability of the outcome is causally related to the exposure under study (appendix 5). As mentioned in Chapter 1 (Section1.2.5), there is now substantial evidence that comprehensive smoke-free legislations is associated with reduced SHS exposure (25, 26, 91) and reduced hospital admissions of coronary events, other heart disease and cerebrovascular accidents (113). In contrast, there is a paucity of studies on the association smoke-free legislation and cardiovascular biomarkers and telomere. Both active smoking and SHS exposure increase inflammation, thrombosis and oxidative stress (59, 306). Previous studies demonstrated an association between active smoking and telomere attrition (389, 434, 435). However, there is very limited evidence on SHS and telomere. The limited research into the effect of passive exposure to tobacco focused on prenatal exposure among children and pregnant women (436, 437). As mentioned in Chapter 1, sidestream smoke contains higher concentrations of toxic gases and small (<2.5μm), respirable particles than mainstream smoke (28-31). LDL, fibrinogen and other inflammatory factors are involved in the atherosclerosis process (179, 397). Given these considerations, there is clearly a higher possibility that increased cardiovascular risk follows SHS rather than the other way around. As discussed above, including ex-smokers in the analyses is an important limitation. It should be pointed out that in future studies, it is important to divide participants into never-smokers with no exposure to SHS, never smokers with exposure to SHS, and active current smokers, and compare the changes of a number of cardiovascular biomarkers per unit change in cotinine concentration across these groups separately to avoid reverse causality.

From the findings in the two studies in this chapter, exposure to SHS carries a disproportionately higher cardiovascular risk than would be anticipated from active smoking. Telomere attrition relating to per year of age may be an intermediate step between exposure to SHS and CVD including PAD. These findings add to the limited published evidence supporting an association between SHS exposure and CVD-related biomarkers.

5 Chapter 5: Discussion

This chapter summarises the key findings, discusses the strengths and limitations of the methodology used in the studies in this thesis, and suggests recommendations on future research and public health and clinical implications, based on the findings of the studies in this thesis.

5.1 Review of key findings

5.1.1 A systematic review on the association between active smoking, exposure to SHS and PAD

In Chapter 2, I did a systematic review on the existing published evidence up to 30 April 2012 on the association between active smoking, SHS and PAD. The results corroborated the previous and only systematic review which was undertaken in 2004 (16). Based on a substantial number of eligible studies on active smoking, I was able to conduct a meta-analysis. There is now substantial evidence of an increased risk of PAD among current smokers. The risk is lower among ex-smokers but, nonetheless, significantly increased compared with never smokers.

In contrast, only two studies on the association between SHS and PAD were identified prior to my studies in this thesis. Both of these two studies were cross-sectional but only one showed an overall positive association. The first study was among 1,209 Chinese women aged ≥ 60 years who had never smoked. SHS exposure was defined as self-reported exposure either in the home or in the workplace. Participants who were exposed to SHS had an overall 1.47-fold increased risk of PAD defined by an ABPI <0.9. Dose-response relationships were also found in relation to both the number of cigarettes these participants were exposed to each day and the daily cumulative time of exposure (45). But this study did not provide detailed information on SHS exposure in public settings and the duration of SHS exposure such as overall exposure per week or overall years of exposure. The other study was conducted in the USA using data of 5653 non-smokers from the NHANES. They defined self-reported non-smoking status with a serum cotinine <10.0 ng/mL as SHS-exposed non-smokers. But neither an overall association between cotinine concentration and PAD nor a dose-relationship was found. By dividing the serum cotinine concentration in this study population into 20 equal quantiles, they suggested a threshold effect of cotinine > 155ng/mL, above which the risk for PAD was significantly increased (38).

Furthermore, this systematic review has suggested that very few studies have collected information on both SHS exposure and PAD. Therefore, in Chapter 3, I used data from the GS:SFHS and SHeS to examine the association between SHS and PAD among adult non-smokers.

5.1.2 SHS and the risk of PAD

In Chapter 3, I undertook two cross-sectional studies and one retrospective cohort study to examine the association between SHS exposure and PAD among adult non-smokers. For the cross-sectional studies, I used the baseline data from the SFHS and SHeS. The SFHS measured PAD objectively using ABPI <0.9 but used self-reported exposure to SHS. Information on venues of SHS exposure was provided: at home, at work and in other public places. Overall duration of SHS exposure was interpreted as hours per week. The SHeS collected information on symptomatic PAD based on the Edinburgh Claudication Questionnaire and measured SHS exposure objectively using salivary cotinine concentration. I also used record linkage of the SHeS in my third, retrospective cohort study to determine whether SHS exposure was an independent predictor for PAD incidence.

Overall, the two cross-sectional studies suggested a significant association between level of SHS exposure and PAD, after adjustment for potential confounding factors. In my study using the SFHS, self-report high level of SHS exposure at work, at other locations and overall exposure of ≥ 40 hours per week were significantly associated with PAD defined by ABPI < 0.9 among never smokers. In my studies using the SHeS, non-smokers with high concentration of salivary cotinine (≥ 2.7 ng/mL) were significantly more likely to have IC defined by Edinburgh Claudication Questionnaire. The association varied by age category, such that individuals aged <60 were more strongly and significantly associated with PAD. Survival bias may explain why the association turned out weaker among participants aged ≥ 60 years. Overall, among all participants included in this study, 9.2% of cases of IC were attributed to raised cotinine concentrations. Both studies suggested a dose response relationship whereby the risk of PAD/IC increased with increasing level of SHS exposure.

Cross-sectional studies are not adequate to establish a temporal relationship. To address this limitation, I conducted the third, retrospective cohort study. Compared with low SHS exposure, increased cotinine concentration at baseline was associated with increased risk of all-cause mortality, with a dose-response relationship. The risk of incident PAD increased statistically significantly only in male non-smokers with high cotinine concentration (≥ 2.7 ng/mL).

These findings added to the limited existing published evidence. My study using the SFHS included 5,686 never smokers and is so far the largest published study on this research topic. Using ABPI as the objective PAD evidence, I was able to include a close to complete number of ascertained PAD cases which otherwise is difficult because early stage PAD is asymptomatic. In contrast, symptomatic PAD, typically IC, increases with advancing age. Thus, I used a lower age cut-off in the study using the SFHS. In my studies using the SHeS, I included non-smokers (ex or never smokers) to maximise statistical power and identify more IC cases. However, in the record linkage data of the SHeS, the ascertainment of incident PAD was confined to cases serious enough to warrant hospitalisation or surgery or lead to death. Therefore, the association between SHS exposure and PAD could be due to an association with all incident PAD, an association with disease progression or a combination of both. However, the third study in Chapter 3 was the first attempt to examine the association between SHS exposure and PAD in a cohort design.

5.1.3 SHS and cardiovascular biomarkers

In Chapter 4, I conducted two cross-sectional studies. Firstly, using the SHeS, I examined the relationship between SHS exposure and active smoke exposure, measured by salivary cotinine, and several preclinical cardiovascular biomarkers: CRP, HDL cholesterol, TC/HDL cholesterol ratio and fibrinogen. Subsequently, I compared the changes of the concentrations of these cardiovascular biomarkers per unit change of cotinine concentration in non-smokers with high SHS exposure and in active smokers.

The findings corroborated previous studies. I demonstrated dose-response relationships between tobacco exposure and the concentrations of most cardiovascular risk biomarkers in both non-smokers and active smokers. Compared with non-smokers protected from SHS exposure (no or low SHS exposure), CRP concentration increased with increasing cotinine concentration among both non-smokers and active smokers. The TC/HDL cholesterol ratio showed a similar trend. HDL cholesterol concentration decreased with increasing cotinine concentration. But the association between cotinine and fibrinogen was less clear-cut. Fibrinogen concentration only increased in moderate and heavy active smokers (cotinine > 100.0 ng/mL). An important novelty of my study was the direct comparison between non-smokers with high level of SHS exposure (cotinine ≥ 2.7 ng/mL), and light/moderate active smokers. The changes of CRP concentration and the changes of TC/HDL cholesterol ratio with increasing cotinine concentration in non-smokers with high level of SHS exposure was comparable to those changes in light/moderate active smokers. The changes of HDL concentrations in non-smokers with high SHS exposure were similar to light active smokers. There was a step change in the relationship between tobacco exposure and cardiovascular biomarkers at the interface of non-smokers exposed to SHS and active smokers. This added to the limited existing evidence that SHS may carry a disproportionately higher cardiovascular risk than active smoking for a given level of SHS exposure.

Active smoking increases telomere attrition. However, there is a paucity of studies on SHS. Therefore, I conducted another cross-sectional study using a subgroup of participants from the SFHS to explore the association between SHS exposure and telomere attrition. I compared the attrition in telomere length T/S per year of age across different level of SHS exposure among adult non-smokers. Telomere length decreased more rapidly with increasing age among participants with high level of SHS exposure, compared with both those with no exposure and those with low exposure. In this study, participants with high level of SHS exposure were more likely to live in socioeconomically deprived areas. After further adjusting for other risk factors including socio-economic deprivation quintiles in the regression models, the association with telomere attrition inflated. This suggests that, if a high level of SHS exposure is combined with other factors including deprivation, then telomere attrition per year of age may accelerate.

In summary, previous published evidence supports that active smoking is strongly associated with PAD. In contrast, there was a paucity of studies on the association between SHS and PAD. My thesis added to the limited existing evidence on establishing the importance of SHS as a risk factor for PAD and then further demonstrated that exposure to SHS carries a disproportionately high cardiovascular risk compared to active smoking for a given level of smoke exposure. Telomere attrition per year of age may be an intermediate step between SHS and CVD including PAD. This also supports the association between SHS exposure and the atherosclerosis-related biomarkers, which play an important role in the pathophysiology of PAD.

5.2 Strengths and limitations of this thesis

This thesis comprises six complementary studies using different study designs: a systematic review and meta-analysis, cross-sectional studies and a retrospective cohort study. The strengths and weaknesses of each study have been discussed in each relevant chapter in this thesis. Therefore, this section mainly focuses on the overall strengths and limitations of the thesis.

5.2.1 Strengths

This thesis has made many contributions to the limited literature on the association between SHS exposure and PAD, particularly in Scotland. My systematic review on active smoking, SHS and PAD was reported in accordance with PRISMA guidelines. I used four databases, namely the Medline, Embase, Pubmed and ISI Web of Science databases, to ensure that all eligible studies were identified. The only published systematic review, prior to the studies in this thesis, was on the association between active smoking and symptomatic PAD, published in 2004 (16). I included many more studies published based on both objective PAD measured by ABPI and symptomatic PAD defined by a claudication questionnaire or peripheral angiography. Therefore, I was able to conduct a meta-analysis to quantify the association and to attempt to explain the between-study heterogeneity. However, my systematic review only identified two studies on the association between SHS and PAD. This showed that most studies have not collected data on both PAD and SHS exposure, while in Scotland, population-based data are available for analysing the association between SHS exposure and PAD.

In this thesis, all of the four cross-sectional studies and the cohort study were conducted based on existing secondary data in Scotland: the Scottish Family

Health Study and the Scottish Health Survey (SFHS and SHeS). These data sources provided very large numbers of participants from the general population living in Scotland. The samples covered a large geographic area across Scotland and therefore enable researchers to assess national trends. One advantage of analysing existing data is their rapid and low cost access. The quality of these data is good with a high percentage of completeness and accuracy (310, 329, 341). Both data sources include a wide range of information on socio-demographics, lifestyles, anthropometric measurements and samples of blood or saliva, despite the difference in data collection and certain measurements. The SFHS recruited probands aged between 35 and 55 years randomly selected from the general practitioner records in Glasgow and Dundee in Scotland, and their first degree adult relatives aged ≥ 18 years. Over 90% of the participants consented to link data with medical and related records (341) . The SHeSs are based on a stratified, clustered random probability sample of individuals living in private households across mainland Scotland and the large inhabited islands. Data were collected in two stages: a face-to-face interview followed by a nurse visit for anthropometric measurements and biomedical measurements. Over 90% of the participants consented to passive follow-up via record linkage to routine administrative data (310).

In Chapter 3, despite the measures of PAD and SHS exposure being not the same in SFHS as in SHeS, I was able to demonstrate the consistency of findings across the studies based on these data sources. For example, the SFHS defined PAD using ABPI while the SHeS used the Edinburgh Claudication Questionnaire. In the study using the SFHS, I used a lower age cut-off as (participants aged ≥ 18 years) in order to include cases of early-stage, asymptomatic PAD. In the study using SHeS, I applied a higher age cut-off (participants aged >45 years) because symptomatic PAD increases with advancing age. In my study using the SFHS, I included only never smokers due to the sufficient number of participants who classified themselves as never smokers via self-reported smoking status. This study included five times the number of never smokers as in the previous Chinese study, and showed consistent results. One of the other strengths of this thesis was the access to salivary cotinine concentration, an objective measurement of tobacco exposure, in SHeS. I was able to exclude the smoking deceivers based on the maximum cut-off concentrations suggested by the SRNT. Despite the different measurements of SHS exposure in the studies, ordinal data were summarised to show the increasing levels of exposure. Therefore, whether there was evidence of a dose relationship was determined by analysing these ordinal data. I was also able to adjust for potential confounding factors such as age, sex and socioeconomic status (SES). I tested the interactions with these confounding factors and conducted subgroup analyses where appropriate. In contrast to the previous Chinese study, I did not adjust for diabetes, blood pressure and lipid concentrations in my regression models because these are potential mediators rather than confounders.

Furthermore, with the linkage to data on hospitalisations and mortality from each SHeS, I was able to undertake a third, retrospective cohort study to examine whether SHS exposure increased the risk of incident PAD among a representative sample of the Scottish population. My study is also the first to have attempted to demonstrate the association between SHS and PAD in a cohort design. Worldwide, few large population-based linked data are available to study the association between SHS and PAD. Scotland is pioneering the use of linked health service data for population-based research. Data linkage allows for studies in retrospective or prospective cohort design to analysis past trends and forecast future scenarios. Compared to primary longitudinal survey in which participants are asked for the same information continuously, data linkage reduces the cost and the length of time of the survey, and respondent burden (438). The linkages of data maximise the value of existing data by reusing them to undertake new research and provide new statistics (310). The linkages also help to build up more reliable and more complete data by deleting duplicate records and correcting data artifacts (439). They offer the potential to monitor the quality of life in a community or region over time. Therefore, the outcomes of the research projects help to inform health policy decisions and service delivery (440).

In Chapter 4, to understand whether SHS exposure carries a disproportionately higher cardiovascular risk compared with active smoking, I used SHeS because the data collection was principally focused on CVD and the related risk factors including cotinine concentrations in Scotland. Pre-clinical biomarkers of cardiovascular disease including CRP, HDL, LDL, total cholesterols and fibrinogen concentrations were determined by standard assays in SHeS (309). I collated several surveys to increase statistical power. This enabled me to have sufficient number to classify participants into groups with different levels of tobacco exposure. My findings were consistent with previous studies by showing a dose response relationship between increasing level of tobacco exposure and higher risk of concentrations of most of these cardiovascular biomarkers. I was also able to directly compare between non-smokers with high levels of SHS exposure, and light and moderate active smokers in relation to the changes of these cardiovascular risk biomarkers. Very few published studies have done such a direct comparison. However, my study indicated that non-smokers exposed to a high level of SHS had comparable concentrations of most of these cardiovascular risk biomarkers, despite lower cotinine concentrations, compared with light, and sometimes moderate active smokers. In Chapter 4, I also used a subgroup of SFHS for ageing study, in which telomere T/S ratios in the DNA samples were detected, to examine the association between SHS and telomere attrition. This study was among the very few published studies, and so far the largest study, to determine the relationship between SHS and telomere. This data from SFHS enabled me to compare the attrition in telomere length per years of age across different levels of SHS. My findings demonstrated that exposure to high level of SHS may accelerate normal biological ageing assessed by telomere attrition. There has been growing evidence on telomere attrition associated with age-related diseases such as CVD phenotypes including PAD (426-428). Therefore, my two studies in Chapter 4 further demonstrated SHS as a risk factor for PAD by affecting pre-clinical biomarkers of cardiovascular disease and increasing telomere attrition per year of age.

5.2.2 Limitations

Study-specific limitations have been discussed in relevant chapters. This chapter will mainly describe the methodological limitations relating to the research in this thesis, and suggest how to improve the research on the association between SHS and PAD.

5.2.2.1 Systematic review and meta-analysis

In Chapter 2, the meta-analyses were based on the aggregated results of individual studies. I did not have access to the individual data of each study. The effect size from each study was of different adjustment levels. Therefore, I could not adjust for the same potential confounders across different studies. The choice of confounders in the regression models in the individual studies was generally based on prior knowledge and/or stepwise regression analyses or other commonly suggested statistical methods. If overadjustment occurs, the adjusted would be smaller than the unadjusted estimate. Then, the estimate of the risk of PAD is likely to be attenuated due to overadjustment. Since the population prevalence of PAD is relatively low (38, 121), RR approximates OR (222, 289). In my meta-analyses, I treated RRs equivalent to ORs. Publication bias is one type of reporting bias that occurs when the outcome of a research study influences the decision of whether or not to publish it. Systematic reviews regarding support for a hypothesis can be biased if the original individual studies are subject to publication bias (441). In my systematic review and meta-analysis, the funnel plot was used to visually assess the likelihood of publication bias. However, Egger's test, as described in Section 2.6.3 in Chapter 2, has some limitations when it is used to test for the funnel plot asymmetry. The SE of the log OR is correlated with the size of the OR because of sampling variability alone even in the absence of small-study effects. Funnel plots which were plotted using log ORs may appear asymmetric, leading to false-positive test results of the Egger's test (290). These meta-analyses included studies of different design: cross-sectional studies, case-control studies and cohort studies. However, when I used meta-regressions to explain the between-study heterogeneity, study design and level of statistical adjustment were not significantly associated with the magnitude of effect size after adjustment for multiple testing. My systematic review of the published studies on active smoking, SHS and PAD identified only two studies on the association between SHS and PAD prior to the studies in my thesis. I only included observational studies published in English. This could bring in potential selection bias in the meta-analysis. I did not include studies on other sources of tobacco other than cigarettes.

5.2.2.2 Cross-sectional Studies

As with the previous two published studies on SHS and PAD prior to this thesis, most of my studies in this thesis were cross-sectional. Cross-sectional studies are one type of observational studies used to describe the frequency of an illness or health-related characteristics, variables of interest and the relations among them as they exist in a defined population at a particular point of time.

A weakness of cross-sectional studies is that risk factor/exposure and disease/outcome are ascertained simultaneously. Therefore, a temporal relationship between exposure and outcome cannot be established. Although there is an association between exposure and outcome, cross-sectional studies cannot prove that the exposure causes the outcome. Association may in principle be due to possible reverse causation (see Appendix 5). Secondly, cross-sectional studies are often used to evaluate prevalent outcomes other than incident outcomes. There is survival bias (see Appendix 5) towards including those individuals who are less likely to die after developing the outcome and excluding individuals who develop the outcomes but die before the study. Thirdly, since there may be other confounding factors associated with both the exposure and outcome, alternative explanations need to be ruled out when trying to infer causation from a simple association (359) (Appendix 5). In my thesis, from the cross-sectional studies, SHS exposure and prevalent PAD were measured at one point in time. The observed association between high level of SHS exposure and prevalent PAD cannot demonstrate whether SHS exposure predisposes to PAD. It may be a result of those individuals exposed to high level of SHS exposure being more likely to develop PAD, or less likely to die after developing PAD, or a combination of both. However, reverse causation is very unlikely (Appendix 5). Barnoya and Glantz have suggested that the cardiovascular effects of even brief exposure to SHS could be 80% to 90% as large as that of chronic active smoking (306). Therefore, SHS exposure is very unlikely to be negatively associated with, or even a preventive factor of, PAD. If SHS exposure is associated with survival as well as incidence, survival bias may explain why the association with PAD appeared to be weaker in those over 60 years of age. If this is the case, then the magnitude of association in younger participants is likely to be a better measure of the true association.

The two studies in Chapter 4 were also cross-sectional. Ideally, measurements of tobacco exposure should be collected prior to the assay results of cardiovascular biomarkers. In the baseline data of SHeS and SFHS, these were recorded at the same time. However, the cross-sectional studies in both Chapter 3 and 4 underpin several hypotheses that might be answered in the future.

5.2.2.3 Cohort Studies

A cohort study was used in Chapter 3 to address the methodological limitations of the cross-sectional studies. I used the record linkage of SHeS 1998, 2003, 2008 and 2010 to undertake a third, retrospective cohort study to investigate SHS exposure as a risk factor for incident PAD, which was defined as the date of PAD hospitalisation or death because of PAD.

Like other record linkage, the linked datasets of SHeS have both strengths and weakness. The data are collected for other purposes and thus may not be ideally suited to test the current hypothesis. Primarily, the linked data in Scotland are overall of good quality but certain variables used to test the hypothesis may be incomplete or even unavailable (442). The baseline data of SHeS were linked to death record and hospital admission and death due to PAD. The PAD case ascertainment in my cohort study was, therefore, restricted to those participants with PAD that was sufficiently severe to warrant hospitalisation or surgery or lead to death. The observed association between SHS exposure and the incident severe PAD defined in this way could be from the result of an overall association with all incident PAD, an association with disease progression or a combination of both. Secondly, the SHeS only includes the general population living in private households and exclude others living in Scotland. This may result in sampling bias (see Appendix 5) in the baseline data and subsequently to the linked data. The linked data assumed all participants to be alive and living in Scotland all the time, but in reality, some participants might have moved out from Scotland and develop the outcome or died because of the outcome somewhere. Moreover, since some of the follow-up has been conducted on a relatively short timescale (such as SHeS 2008, 2010), the number of PAD cases is not very high and may underestimate the actual number. I did not have the periodic information on SHS exposure over time. Therefore, it was unclear whether the level of exposure measured at baseline was valid over the follow-up time period. This may be a potential bias in the cohort study in this thesis.

In the cohort study using survival analysis, SHS exposure was measured years prior to PAD or death outcome. Since the first positive association in a cohort design has been identified in my thesis, it supports the need for further investigation and replication to determine whether SHS exposure is a real cause of the disease or not.

5.2.2.4 Bias, confounding and chance

Caution should be taken whenever an inference is being made from a sample to a population. The findings of an epidemiological study may be due to alternative explanations including bias, confounding, and chance. These alternative explanations may lead to the appearance of an association between an exposure and an outcome which actually does not exist, or alternatively the absence of an association which is truly present (222). Bias is a systematic error. Some researchers consider confounding as a type of bias (361). Chance is a random error (222) (Appendix 5).

In epidemiological studies, two important considerations are internal validity and external validity. Internal validity means the rigour with which a study is designed and implemented. In relation to internal validity, all observational studies to some extent are vulnerable to built-in bias (see Appendix 5) which is generally categorised into selection bias, information bias and confounding (363).

Selection bias (Appendix 5) occurs when the method of selecting subjects into a study or their likelihood of being retained in a study distorts the exposure-outcome relationship from that present in the target population (363). If sampling is not representative of the exposure-outcome distributions in the entire target population, then the measures of association will be biased (443). There are several mechanisms that can result in selection bias, including inappropriate selection of controls in case-control studies (control selection bias), differential loss to follow-up in a cohort study (loss to follow-up bias), differences between subjects who agree to participate in studies and those who do not with regard to study outcome (volunteer bias or consent bias), nonresponse bias (missing data bias), bias attributed to selective survival among the prevalent cases (incidence-prevalence bias or selective survival bias), and healthy worker bias (361, 443). In SFHS, probands were randomly drawn from the general population from general practitioner records in Glasgow and Dundee. Over 90% of participants consented to link data with medical and related records (341). The SHeS uses multi-stage, stratified probability sampling frame and is designed to be representative of the general population living in private household nationwide in Scotland. When comparing those who had provided a saliva sample for cotinine assays with those who did not, there was no significant difference in the prevalence of IC. Therefore, my cross-sectional studies in this thesis were reliable in this respect. The participants in SFHS were adults aged ≥ 18 years. In the cross-sectional studies, in order to identify asymptomatic PAD defined by ABPI, I used a younger age cut-off of ≥ 18 years in the study using SFHS. While, the age cut-off of > 45 years was applied in the study using SHeS to include symptomatic PAD based on the IC questionnaire. This might introduce potential bias.

Cross-sectional studies and case-control studies are susceptible to survival bias. Survival bias can occur when a series of survivors are selected, if the exposure is a prognostic determinant or is related to prognostic factors, the sample of cases distorts the frequency of the exposure (444). The observed association between SHS and prevalent PAD may result from those exposed to SHS being more likely to develop PAD or less likely to die after developing PAD, or a combination of both. Over 90% of the participants in SHeS consented to passive follow-up via record linkage to routine administrative data (310). However, selection bias could also have an influence on the cohort study in this thesis. Inclusion criteria were restricted to confirmed non-smokers (self-reported non-smokers with salivary cotinine concentrations <15.0 ng/mL) free of IC at baseline and linked to hospitalisation and death record to ascertain the outcome. The extent to which those who had consented to passive follow-up differ from those who did not in terms of SHS exposure, incident PAD and some other important aspects may affect the results presented in the cohort study in thesis. Since baseline IC was defined by a claudication questionnaire, it was unknown whether or not those who were free of IC might in reality have had asymptomatic PAD at baseline. Incident PAD case ascertainment was restricted to those participants with PAD that was sufficiently severe to warrant hospitalisation or surgery or contribute to death. This can also introduce potential bias and impact the study outcome.

Information bias (Appendix 5) refers to the incorrect determination of exposure or outcome, or both (363). It is also known as observation or measurement or classification bias. In cohort studies, a concern is whether the information on the outcome is obtained in the same way for both the exposed and non-exposed group. In my cohort study, the ISD of the NHS collates and links the SHeS data to hospitalisation (SMR 01) and death certificates (collected by General Registrar Office). The outcome/disease was defined by the disease and procedure codes (ICD-9, ICD-10, OPCS). Over 90% of SHeS participants consented at each survey and had been followed up with data linkage from 1981(310). Therefore, the outcome measurement in my retrospective cohort study was reliable in this aspect. However, the case ascertainment for PAD was restricted to those participants with PAD that was sufficiently severe to warrant hospitalization or surgery or contribute to death. The observed association between SHS and incident PAD could be biased. As described in the previous section, SHS exposure was only measured at baseline. Whether these exposure measurements were valid over the latency or follow-up time at risk was unknown. This may be a potential information bias in the cohort study. In Chapter 4, information bias may occur because cholesterol concentrations were measured using a different analyser after 2010.

Reporting bias refers to selective revealing or suppression of information by subjects, that is, people's tendency to under-report the information (445). In the cross-sectional studies using SFHS, smoking status was self-reported. Due to the social undesirability of smoking, a proportion of current smokers can misclassify themselves as ex-smokers, termed "smoking deceivers" (355). Recall bias is important in retrospective case-control studies. Case may be more likely to recall past exposure, especially if the exposure is widely known to be associated with the disease being studied. Recall bias can either exaggerate or underestimate the true strength of association between the exposure and the outcome (446). In the SFHS, SHS exposure was based on self-report exposure. Recall bias is possible especially if the subjects already had some health conditions which are widely known to be associated with SHS. Then, the observed association can be exaggerated. In contrast, in the SHeS, the access of salivary cotinine measurement is a strength. In both the SFHS and the SHeS, since alcohol consumption and level of physical activity were self-reported, recall bias in these confounders is likely. Information bias from self-report of information on confounding variables can lead to either overestimate or underestimate of the association. In SFHS, SHS exposure at home was predefined and categorized into: none, a little, some and a lot exposure. It is possible that non-smokers who live with current smokers share many or most of the lifestyle factors associated with smoking. Due to this, there may be some biases which cannot be quantified. This can lead to an underestimation of the confounding effects from these lifestyle factors.

Confounding (Appendix 5) occurs when the effect of an exposure on an outcome is blurred by an extraneous factor (363). A confounding variable is a known risk factor for the outcome and is associated with the exposure but is not a result of the exposure. Confounding can be minimised by restricting, matching and randomisation at the recruitment stage, and stratifying, making multivariate statistical adjustment and doing standardised rate analysis at the analysis stage (363). In my cross-sectional studies in this thesis, I developed analytical models with multivariate statistical adjustments. I tested whether there were statistically significant interactions with covariates (age, sex and socioeconomic status). When there was a statistically significant interaction with the covariates, I stratified the analysis accordingly. For example, age is a strong confounding factor for PAD. In the cross-sectional study using SHeS in Chapter 3, the effect of age was minimised by stratifying the analysis and making multivariate adjustment. Adjustment for the demographic confounders plus BMI did not change the significant association between SHS and PAD until further adjustment for other lifestyle confounders. In my study using the SFHS on the association between SHS and PAD, I was able to adjust the well-established risk factors for PAD including demographics confounders (age, sex, deprivation quintile) and lifestyles confounders (alcohol consumption, physical activity and BMI). The association remained significant and did not change largely after adjusting for these potential confounders. In the cohort study, a high level of SHS exposure was only associated with incident PAD in male participants after adjusting for age. Further adjustment for other potential confounders, the association did not reach statistical significance. In Chapter 4, stratified analyses were also undertaken when necessary, but the overall associations between tobacco exposure and cardiovascular biomarkers persisted. As confounding bias is inherent in epidemiological studies (365), this limitation does not invalidate any of the results but underpin some hypotheses which promote further research.

External validity means the usefulness of the findings of a study with respect to other populations (447). In Chapter 3 and 4, eligible participants in each specific study were identified based on the availability of the variables of interest. These studies were conducted among the Scottish population. To complete the picture, in future, studies are needed among other populations and should be performed on more than one occasion among one population.

All observational studies are vulnerable to the effect of chance (random error) (Appendix 5). Due to random error alone, the value of the sample measurement can distort the true population value, which produces inaccurate measurement of an association between an exposure and an outcome. There are three sources of random error including sampling error, measurement error and individual biological variation (222). Different samples can produce different estimates. Random error cannot be completely eliminated but the likelihood of it occurring can be reduced. Sampling error of this type can be reduced by increasing the sample size of the study. Measurement error of this type can be minimised by using state-of-the-art methods of data collection. Individual biological variation is inevitable. Cautions must be taken whenever an inference is being made from a sample to a population (222).

Therefore, the results from my studies should ideally be replicated in a cohort study design with repeat measures of SHS exposure and objective measures of incident PAD among never smokers in different populations to reduce the play of chance in the observed association.

5.2.2.5 Estimation

A point estimate for a population parameter, which is calculated from the sample, is single-valued. CIs (defined as the point estimate±margin of error) provide the likely range of plausible values for the population mean or other population parameters including a correlation (Appendix 5). CIs also help to estimate the precision of results from a sample, compared with the true population. Therefore, it is good practice to report CIs along with the point estimate in the attempt to make an inference from a sample to a population (448). If a study is unbiased, the CI generally interprets the precision of an estimate of the association between the exposure and outcome. The wider the CI, the less convincing the estimate of the association is (297). A CI at 95% level is commonly used, which technically means if 100 different samples were taken and a 95% CI was computed for each sample, 95% of the CIs would contain the true value of parameter in the population (448). In reality, often one random sample is selected and one CI is computed. The observed interval may overestimate or underestimate the true mean value or true association (449). CI is built based on the point estimate and a margin error that incorporates the confidence level and the standard error or sampling variability (449).

In my studies, the findings may be affected by the small sample size. Because of the small number of PAD cases, some CIs were wide and therefore, the precision of the estimates of effect size was relatively low. Furthermore, since the margin error only covers the random sampling errors (448), systematic errors including nonresponse bias or loss to follow-up bias could affect the precision of an estimate (449). The results should be interpreted accordingly and should be corroborated in future large studies and meta-analyses. However, these studies add to the limited published evidence in support of the association between SHS and PAD. It is anticipated that research-based evidence will be helpful to inform policy making and clinical and public health practice. Compared to a p value, which tests whether or not there is a statistically significant difference between groups, a CI provides a method to show the strength of the effect or the association. However, there is a need to judge the clinical significance of statistically significant results. On the other hand, if the sample size is too small or the dispersion in the sample is too great, results of high clinical relevance but low statistical significance can still be meaningful (450). A decision cannot be made simply based on the p value. A very small p value and very narrow CIs generally suggest that the result is precise and is less likely to be due to chance (433). In my studies, some of the 95% CIs are wide. It is possible that the results are false-positive or the magnitude of association between SHS exposure and PAD and telomere length attrition is overestimated. However, there is suggestion of an association. Further research is needed to examine the association between SHS and PAD and cardiovascular biomarkers to reduce the possibility of false positive association or overestimation of the magnitude of association due to alternative explanations including bias, confounding and chance, and therefore better inform policy and practice.

5.2.2.6 Possible additional analyses

Very few studies conducted on the general population have collected information on both SHS and PAD. The studies in Chapter 3 and Chapter 4 were conducted using existing secondary data available in Scotland (the SHeS and SFHS). The small number of PAD cases confined the methods used. As mentioned in Chapter 3, in SheS, the definition of baseline PAD was based on the Edinburgh Claudication Questionnaire. As mentioned in Chapter 1, many PAD cases were asymptomatic. It is often challenging to include asymptomatic PAD in a retrospective cohort study based on linked data on hospitalisations and deaths. I do not have access to GP data. In the linked data of SHeS, the ascertainment of the incident PAD cases was restricted to those severe cases that lead to hospitalisation or surgery or death. The SHeS also did not collect repeat measurements of SHS exposure. In SFHS, SHS exposure was self-reported. In both datasets, some of the potential confounders were predefined. The analyses could be improved if the measurements for the SHS exposure, incident PAD, and confounders are improved.

As mentioned in Chapter 1, exposure to SHS has fallen markedly since the implementation of the smoke-free legislation in 2006 in Scotland. This may be a possible explanation that in my studies the majority of participants were either no or low exposure to SHS. In the study using SHeS, I combined the SHeS between 1998 and 2010. Since now the SHeS 2011-2014 are available, it is possible to conduct subgroup analyses split by pre-legislation period and post-legislation period if there are sufficient numbers of participants (in particular never smokers) in each exposure group. Categorising participants into: never smokers with no, low, moderate, and high SHS exposure groups, ex-smokers with no, low, moderate, and high SHS exposure groups and light, moderate and heavy current smokers will show more information on the association between cotinine concentrations and PAD, and relevant biomarkers. Furthermore, I would have included more potential confounders. The inclusion criteria of confounders would better be based on both most recent published evidence and statistical approaches including stepwise selection approach, a 10% change-in-estimate (CIE) criterion (373) and comparison of the model χ^2 on addition of additional confounders. In particular, it is important to include only never smokers and compare the absolute differences of the strength of the association by cotinine concentrations between never smokers, ex-smokers and current smokers, provided that there are sufficient numbers of participants in each survey. There will be a possible plan to corroborate the findings of this thesis using record linkage to follow-up data of telomere and other CVD-related inflammatory biomarkers measurements.

Another concern is the treatment of missing data in the analyses. There are generally three types of missing data: completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR). MCAR means there are no systematic differences between the observed values and the missing values. If missing data are all MCAR, including only participants with complete data in the analyses generally produced unbiased results but can lead to a substantial reduction of the sample size and larger standard errors (451, 452). When data are MAR or MNAR, analysing only complete data can result in biased parameter estimates and undermine the validity of the results (453). Sterne et al. suggested that multiple imputation is a useful strategy for dealing with the biases caused by missing data that are MAR. Multiple imputation replaces each missing value with a set of plausible imputed values that reflects the uncertainty around the true value. The procedure of multiple imputation involves building up multiple imputed datasets including the missing values replaced by imputed values and using standard statistical methods to fit the analytic model of interest to the imputed datasets (454). However, multiple imputation cannot deal with missing data that are MNAR (454) and it can bring in biases (455). In the secondary datasets used in my studies, it is impossible to distinguish between MAR and MNAR. In my studies, it was decided that missing data were to be coded as dummy values and included in the analyses. In future, it may be a merit to compare different techniques for dealing with missing data including multiple imputation and interpret the results accordingly.

5.3 Recommendations

5.3.1 Future research

This thesis added to the limited evidence on SHS as a risk factor for PAD. As the studies in this thesis are observational studies, they underpin several hypotheses but merit further research.

There is substantial evidence on active cigarette smoking associated with PAD. In contrast, published studies on the association between SHS and PAD are limited. Future research is needed to determine whether there is a causal link or simply an association. There are several criteria to gauge the strength of association before causality is inferred: a great magnitude of the association, consistency, a graded response to a graded dose, a temporal relationship, reversibility, a plausible mechanism (222) (Appendix 5).

The cross-sectional studies in this thesis on the association between SHS and PAD have suggested an overall association and a dose response relationship whereby the risk of PAD increased with increasing level of SHS exposure. Prior to the published studies in this thesis, only two studies had published on the association between SHS and PAD. In the cohort study, SHS exposure was measured prior to PAD or death outcome. There was a suggestion of an association between high exposure to SHS and increased risk of incident PAD events in men.

On reviewing the published evidence relating to smoking cessation on the MEDLINE and the Cochrane Library including meta-analyses of randomised controlled trials, Hobbs et al. suggested that permanent smoking cessation is probably the most clinically and cost effective intervention for PAD patients (156). Previous studies also showed a 21% reduction of admissions for ACS among never smokers during the 10 months after the smoke-free legislation in Scotland, compared with the 10 months before the legislation (116). A meta-analysis based on a systematic search for published evidence on the Science Citation Index, Google Scholar, PubMed, and Embase also demonstrated that comprehensive smoke-free legislation is associated with significantly lower rates of coronary events (RR 0.85, 95% CI 0.82-0.88), other heart disease (RR 0.61, 95% CI 0.44-0.85), and cerebrovascular accidents (RR 0.84, 95% CI 0.75-0.94) (113). In contrast, the studies on the impact of smoke-free legislation on admission of PAD are limited. However, reverse causality is unlikely.

Previous studies suggested that the mechanisms by which cigarette smoking is associated with CVD include inflammation, thrombosis, oxidation of LDL cholesterol and oxidative stress (385, 456). As mentioned in Chapter 1, sidestream smoke is often the major source of SHS (20). Sidestream smoke contains a range of chemicals similar to mainstream smoke. However, sidestream smoke contains higher concentrations of toxic gases and small (<2.5μm), respirable particles than mainstream smoke (28-31). A review based on epidemiological studies, experimental studies and clinical studies pointed out the cardiovascular effects of SHS is nearly as large as those of active smoking (306). Studies on acute effect of exposure to SHS on peripheral vascular function showed controversial results (457, 458). Studies on comparing the effect of SHS with active smoking on the conventional atherosclerosis-related biomarkers are limited.

My studies in Chapter 3 and Chapter 4 underpin several hypotheses but causality cannot be inferred. Future research is needed to address the evidentiary weakness. The findings in Chapter 3 and Chapter 4 will need to be corroborated with large cohort studies to establish temporality and intervention studies to demonstrate reversibility. Intervention studies to assess the impact of smoke-free legislation on admission of PAD will be useful. There is also a need to undertake experimental studies to explore the mechanisms by which SHS is associated with PAD. It is also useful to explore whether or not SHS carries a disproportionately higher cardiovascular risk, compared to active smoking. Future studies should divide participants into never-smokers with no exposure to SHS, never smokers with exposure to SHS, and active current smokers, and compare the changes of a number of cardiovascular biomarkers per unit change in cotinine concentration across these groups separately to avoid reverse causality.

The cohort study presented in this thesis highlighted that high exposure to SHS at baseline was associated with incident PAD in male non-smokers. However, in the record linkage of SHeS, incident PAD ascertainment was restricted to those cases which were sufficiently severe to warrant hospitalisation or surgery or lead to death, which underestimated the number of incident PAD cases in reality. A lot of the PAD cases are asymptomatic and therefore they are difficult to be included in the secondary dataset. However, in Scotland, we do not have the access to GP data. One potential option is the Clinical Practice Research Datalink (CPRD), but the CPRD does not collect data on SHS exposure. The other big dataset in the UK is the UKBiobank, but it does not collect data on PAD. In future, there may be merit in exploring data collected from primary care such as GP consultation or similar to identify early stage PAD.

The findings in this thesis suggest using ABPI to confirm PAD in future research. This is consistent with previous studies which have demonstrated the assessment of IC based on physical examination or clinical history underestimated the present of PAD (459, 460). Consequently, PAD defined by claudication questionnaires can increase the risk of weakening the actual association between SHS and all-stage PAD. Allen and colleagues have shown in their research that resting ABPI measurements correlated with 83% of PAD, defined by color Duplex ultrasound as the gold standard for PAD confirmation. When resting ABPI was combined with postexercise ABPI, the correlation increased to 85% (461). Therefore, ABPI is a reliable assessment of PAD in future research settings.

Self-report smoking status has the tendency to underestimate smoking prevalence. Cotinine is an objective measure of tobacco smoke exposure and proportionate to the amount of exposure. It is suitable for cumulative doses over short exposure periods. Salivary cotinine is non-invasive and has high sensitivity value of detecting tobacco exposure (34). In this thesis, salivary cotinine was measured only at baseline in the SHeS. No information was available on whether baseline exposure would be valid over the time period of follow-up. Therefore, in future research, in terms of revealing long-term SHS exposure conditions, repeat measures of cotinine will show more information objectively.

Also in this thesis, in the cohort study, the at-risk period of time for follow-up (till December 31, 2011) was short for linkage of SHeS 2008 and 2010 when comparing to the timescales typical for the disease development. Therefore, in future research, a longitudinal study with longer follow-up time and repeat measures of SHS exposure will provide more useful insights into whether the cumulative effect of SHS is related to incident PAD.

In order to maximise available power, in the studies using SHeS and the study using a subgroup of SFHS, I analysed never and ex-smokers together as non-smokers. The majority of the eligible participants in each study had quitted smoking for more than a year. However, since ex-smokers still carry the risk of developing PAD or other cardiovascular events, in terms of assessing the association between SHS and PAD or the effect of SHS on cardiovascular biomarkers, it would be more plausible to include only never smokers in future studies.

The prevalence and incidence of PAD are age-dependent. In this thesis, in Chapter 3, I included participants aged >45 in the cross-sectional study and cohort study using SHeS because PAD was defined as IC, whereas in the study using SFHS, I used a lower age cut-off as ≥ 18 to identify asymptomatic, early stage PAD. In future study, if ABPI or color Duplex ultrasound is the tool to identify PAD cases, a lower age cut-off is more credible not only for more complete case ascertainment but also for subgroup analysis to better understand whether the effect of SHS varies in different age group.

In this thesis, in the cohort study, high SHS exposure defined as high cotinine concentration at baseline was statistically significant with the risk of incident PAD among male participants only. It did not show significant association among non-smokers overall. The sex variation on the prevalence of PAD is controversial. However, this thesis supports the need to consider sex variation in future research on SHS as a risk for incident PAD as there are sex differences both in biology and SHS exposure conditions.

In addition, socio-economic circumstances, BMI, alcohol intake and physical activity or other unknown factors may have some confounding effects on the observed association between SHS and PAD or cardiovascular biomarkers. In this thesis, after further adjustment for some of these confounders, some of the associations became statistically non-significant. It is clear that replications of multivariate models are required in future research among different populations. In future studies, interactions with covariates should always be tested as overall results may lead to missing information and misleading conclusions.

SHS exposure is associated with PAD but the underlying mechanism is not fully understood. To understand whether SHS carries a disproportionately higher cardiovascular risk, I compared between non-smokers with high levels of SHS exposure and light/moderate active smokers using several biomarkers: CRP, HDL, TC/HDL cholesterol ratio, and fibrinogen. These results need to be replicated in a cohort study design with measures of more biomarkers relevant to different pathways of systematic atherosclerosis and with frequent measures of tobacco exposure. This thesis also included a study on the association between SHS and telomere length attrition. However, given the small sample size and its cross-sectional design, it should be corroborated in future larger studies or meta-analyses. Comparing the effect of SHS and active smoking on telomere length attrition would be helpful to establish whether the disproportionately large effect on cardiovascular biomarkers also applies to biomarkers of ageing. Also further basic science research is needed to fully understand the underlying mechanism on the cardiovascular effect of SHS exposure.

In summary, this thesis supports the evidence on SHS as an independent risk factor for PAD. There was suggestion of a dose response relationship whereby the risk of PAD increased with the increasing level of exposure. In future, observational studies especially in cohort design with long follow-up, repeat measures of SHS exposure and objective measure of PAD among never smokers in different well-defined populations will provide useful insights into answering whether there is a causality or purely an association. Randomised controlled trials might be impractical and unethical in this case. But intervention studies such as smoking cessation will be useful to demonstrate reversibility. Assessing the effectiveness of smoke-free legislation on reducing the risk of PAD related to SHS exposure will be a merit. Further investigation and replication are needed to explore the underlying mechanisms.

5.3.2 Public health and clinical implications

The global prevalence of smoking is increasing, especially in large, developing countries such as China (22). If the smoking pattern persists, cumulative tobacco-related deaths including those attributable to SHS will be over 175 million by 2030 (381). However, a 2013 WHO report indicated less than 16% of the global population are protected by comprehensive nationwide smoke-free legislation (3). In Scotland, six years after the legislation, 17% adult non-smokers reported exposure to SHS in their own home or other people's home and 11% reported exposure in public places outside buildings (27). SHS is a potential public health threat.

Globally about 202 million people were living with PAD in 2010 (119). In Scotland, from a Government report in 2011, based on the data collected in SHeS 2008 and 2010, the prevalence of Grade 1 or Grade 2 IC was 2.3% among adults overall and increased with age, from 0.7%-1.7% in those aged 16-54, to 2.7% of those aged 55-64, 4.1% of those aged 65-74, and 7.4% of those aged 75 and over (462). This estimate of prevalence is conservative, as it only includes Grade 1 and Grade 2 IC but most PAD cases are asymptomatic (122). Furthermore, the prevalence and incidence of PAD are higher in people with CVD or diabetes than those without. In this report, 20.1% of men and 16.7% of women had either CVD or diabetes. That being the case, the public health burden of PAD is remarkable. It is of public health importance that clinicians and other health care professionals assess patients and advise on the prevention, diagnosis and treatment of CVD, diabetes or hyperlipidemia. It is recommended to include objective measures of PAD such as ABPI.

My systematic review on active smoking, SHS and PAD revealed the limited research on the association between SHS and PAD. Most existing cohorts and surveys have not collected information on both SHS exposure and PAD. It is anticipated that the findings of the studies in this thesis will inform new or existing policy makers, public health physicians, clinicians and others who are dealing with PAD in the general population or in high-risk populations and in particular those who are active smokers or exposed to SHS.

In my thesis, in the cross-sectional study using SFHS, participants with PAD were more likely to be female never smokers. On the other hand, in the cohort study, a high level of SHS exposure was associated with incident PAD in male non-smokers only but not in overall non-smokers. These findings suggest that there might be a sex variation in the smoking habits and SHS exposure conditions. Future investigations may be designed accordingly.

Children who live with smokers are much more likely to start smoking themselves in adolescence or later life (463). It is critical to evaluate the cumulative health hazard related to active smoking cigarette or exposure to SHS or a combination of both since an early life. So far, a few countries or regions such as Australia, England and Wales have banned smoking in a private vehicle carrying children. Stopping smoking at home depends completely on volunteer restriction. In 2013, the theme of Faculty of Public Health in Scotland was 'Making Scotland a healthier place'. Therefore, it is essential in Scotland to take actions to protect general public and in particular children from SHS exposure.

In this thesis, variables describing SES are viewed as a potential confounder for PAD or cardiovascular biomarkers. In Chapter 4, participants with high SHS exposure were more likely to live in more socioeconomically deprived areas. This implies when high SHS exposure coexists with deprivation, the adverse effect can be worse. Studies investigating smoking cessation and SES have found that lower SES groups have higher rates of tobacco use and are less likely to successfully quit smoking (464, 465). Individuals from lower SES groups or living in deprived regions are essentially more likely to be exposed to SHS (320, 466). Moreover, PAD may not be detected in these individuals because they are less likely to engage in activities that would facilitate early diagnosis. In future, individuals from lower SES groups or living in deprived regions may be in particular need of intervention and PAD detection.

5.4 Conclusion

In summary, there is substantial evidence on the association between active cigarette smoking and PAD. This thesis adds to the limited evidence on SHS as an independent risk factor for PAD. Because of the methodological limitations of the studies, causality cannot be inferred. However, it is expected that these studies will provide a foundation for future research. This thesis also provides evidence that exposure to SHS carries a disproportionately higher cardiovascular risk than active smoking for a given level of smoke exposure. Telomere attrition per year of age may be an intermediate step between early effects of SHS and the occurrence of CVD including PAD. The association between SHS exposure and the atherosclerosis-related biomarkers and telomere attrition may contribute to the development of PAD. Nevertheless, further research is needed to better understand the underlying mechanisms. It may be possible that other confounding factors affect the observed associations in the observational studies in this thesis. Therefore, future research using a cohort design with long follow-up, repeat measures of SHS exposure and objective measure of PAD among never smokers in different well-defined large populations, will yield better insight. This thesis lends support for measures to protect the public from SHS exposure and screening PAD at an early stage.

Appendix 1: Literature search strategy

Database searched	Search terms	Date of search
Ovid Medline	peripheral arter* OR peripheral athero* OR peripheral vascular OR claudication OR ABPI OR ABI OR ankle brachial) AND (smoking OR cigarette* OR tobacco OR nicotine OR smoke* Limit to: Publication date from 1 January 1980 to 30 April 2012, humans, Journal Article, English	30 April 2012
Embase	peripheral arter* OR peripheral athero* OR peripheral vascular OR claudication OR ABPI OR ABI OR ankle brachial) AND (smoking OR cigarette* OR tobacco OR nicotine OR smoke* Limit to: Publication date from 1 January 1980 to 30 April 2012, humans, Journal Article, English	30 April 2012
PubMed	peripheral arter* OR peripheral athero* OR peripheral vascular OR claudication OR ABPI OR ABI OR ankle brachial) AND (smoking OR cigarette* OR tobacco OR nicotine OR smoke* Additional filters: Publication date from 1 January 1980 to 30 April 2012, humans, Journal Article, English	30 April 2012
ISI Web of Science	peripheral arter* OR peripheral athero* OR peripheral vascular OR claudication OR ABPI OR ABI OR ankle brachial) AND (smoking OR cigarette* OR tobacco OR nicotine OR smoke* Refined by: Publication date from 1 January 1980 to 30 April 2012, humans, Journal Article, English	30 April 2012

* a truncation symbol to retrieve plurals or varying endings

Appendix 2: Checklist for assessing the quality of quantitative studies

Criteria	**Yes (2)**	**Partial (1)**	**No (0)**	**N/A**
1.Question / objective sufficiently described?				
2. Study design evident and appropriate?				
3. Method of subject/comparison group selection or source of information/input variables described and appropriate?				
4. Subject (and comparison group, if applicable) characteristics sufficiently described?				
5. If interventional and random allocation was possible, was it described?				
6. If interventional and blinding of investigators was possible, was it reported?				
7. If interventional and blinding of investigators was possible, was it reported?				
8. Outcome and (if applicable) exposure measure(s) well defined and robust to measurement / misclassification bias? means of assessment reported?				
9. Sample size appropriate?				
10. Analytic methods described/justified and appropriate?				

11. Some estimate of variance is reported for the main results?				
12. Controlled for confounding?				
13. Results reported in sufficient detail?				
14. Conclusions supported by the results?				

The calculation of the summary score was done according to Kmet et al. **(216)**
Source: Adapted from Kmet et al. **(216)**

Appendix 3: Data extraction form template*

Reviewer: __________ Date: _____________
Author: ____________ Year: _____________
Journal: ___________ Record number: ___

Study method: Observational ☐ Other ☐

Participants:
Setting __________________
Population _______________
Sample size ______________

Intervention/exposure: _________________
Measure (s) of intervention/exposure: _______
Clinical outcome measure (s): ____________

Study characteristics

study	Year	Country	Study design	Sample size	Sex	Age	PAD definition	Referent group	Smoking status	Current smokers (N)	Non-smokers (N)	ex-smokers (N)	never smokers (N)	Effect size	CI	statistical adjustment ¶	Other disease

Year year of publication; Country country where the study was conducted; Sex sex of the participants; Age years of age of the participants; N number
¶ level of statistical adjustment in the regression models in the eligible studies

*This template was modified from the Joanna Briggs Institute (JBI) data extraction form for observational studies (221)

Authors' conclusion: ________________________________

Comments: ____

Appendix 4: PRISMA checklist*

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	56
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	57
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	58,59
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	58,59
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	59-61
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	59-61
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	59-61

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	59-61
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	61
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	61
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	61-62
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	61-62
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	61-62
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	61-62
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	61-62
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	63-65, 72
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	64-67
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	75
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	70, 71, 73, 74
Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency.	64, 65, 73, 74, 76
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	64, 65, 76

Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	64, 65, 76
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	76-83
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	77-83
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	84
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

*this template was available from (213)

Appendix 5: Epidemiological principles

In brief, the basic elements of an epidemiological study include (222, 359):

1) Formulation of the study question or hypothesis;
2) Selection of study populations and study samples;
3) Selection of indicators of exposure;
4) Measurement of exposure and disease;
5) Analysis of the relationship between exposure and disease;
6) Evaluation of the role of bias;
7) Evaluation of the role of chance.

The following table discusses chance, hypothesis vs. estimation, bias (including selection bias), confounding (including residual confounding), measurement error, causation and reverse causation.

Chance	Bias, confounding and chance can influence the quality of an epidemiological study. In reality, epidemiological studies cannot include the entire target populations and remain unchanged in time. Chance is a random error. There are three major sources of random error including sampling error, measurement error and individual biological variation. Measurement error can be minimised by using state-of-the-art methods of data collection. Sampling error can be reduced by increasing the sample size of the study. Individual variation is inevitable. Due to chance alone, a value of the sample measurement can diverge from the true population value, even if bias and confounding are absent. The evaluation of role of chance involves two components, which include hypothesis testing and the estimation of confidence interval. To determine the probability that the observed association can be explained by chance, despite being arbitrary in nature, a p value of either 0.05 or 0.01 is often used as the statistical significance value for testing the null hypothesis. If the p value is low, it is unlikely

	that the observed association would have been explained by chance alone. P value reflects both the magnitude of effect and the size of sample. If the sample size is too small, the p value can be above the level of significance. Confidence intervals (CIs) reflect the precision of the point estimate from a sample, compared with the true population and are normally presented using the 95% confidence level (467).Variations from the trues value can be minimised if the study is large in sample size and long in time (468).
Hypothesis vs. estimation	The classic hypothesis testing process includes defining the null hypothesis and the alternative hypothesis, calculating a p value, and accepting or rejecting the null hypothesis based on the p value. It is important to make a careful consideration of the statistical hypothesis to be tested, the p value associated with this test and the statistical power (1-ß) for detecting the difference of a specified magnitude between the groups being compared. If the null hypothesis is accepted, it indicates that there is no difference between the two groups to be compared. The null hypothesis is rejected because the observed study outcome was deemed to be rare under the assumption that the null hypothesis was true. Although arbitrary in nature, it has been pointed out that the cut-point for rejecting the null hypothesis is usually set when $\alpha=0.05$. The p value represents the probability of obtaining the results observed, if the null hypothesis were true. The p value and α level are related in a sense that if $\alpha=0.05$, then the null hypothesis would be rejected when $p< 0.05$. If the null hypothesis is rejected, then the alternative hypothesis is accepted as true, which indicates that there is a difference between the two groups being compared. If a p value is less than 0.01, it is very unlikely the observed results are due to chance. A p value< 0.05 indicates the observed difference is “statistically significantly” different between the two groups. However, it does not show the uncertainty around the point estimate and the likelihood of clinical significance. A point estimate for a population parameter, which is calculated from the sample, is single-valued. CIs (defined as the point estimate±margin of error) provide the likely range of plausible values for the population mean or other population parameters including a correlation. CIs reflect the precision of results from a sample, compared with the true population. If a study is unbiased, the CI generally

	interprets the precision of an estimate of the association between the exposure and outcome. The wider the CI, the less convincing the estimate of the association is. The number of subjects with the outcome, which is often influenced by the sample size, affects the width of the CI (297). Similar to the selection of 0.05 as level of significance for p value, a CI at 95% level, despite being arbitrary in nature, is usually used. It is good practice to report the point estimate alongside with the CIs wherever an inference is to be made from a sample to a population.
Bias	Bias (or systematic error) is the lack of internal validity or incorrect assessment of the association between an exposure and an outcome in the target population (361). All observational studies to some extent are vulnerable to built-in-bias generally categorised into selection bias, information bias and confounding (363). There are many specific types of bias. The principal biases are selection bias and information bias. Selection bias occurs when the method of selecting subjects into a study or their likelihood of being retained in a study distorts the exposure-outcome relationship from the true value in the target population. There are several mechanisms that can result in selection bias, including inappropriate selection of controls in case-control studies (control selection bias), differential loss to follow-up in a cohort study (loss to follow-up bias), differences between subjects who agree to participate in studies and those who do not with regard to study outcome (volunteer bias or consent bias), nonresponse bias (missing data bias), bias attributed to selective survival among the prevalent cases (incidence-prevalence bias or selective survival bias), and healthy worker bias (361, 443). Survival bias is a type of selection bias and can occur in both cross-sectional studies and case-control studies. It occurs when individuals with favourable survivorship are included in the analysis because the exposure relates to the mortality from the disease being studied. Sampling bias occurs when the selection procedure yields a non-representative sample in which the estimate of the population parameter differs from the true value in the target population (361). Information bias (or measurement bias or classification bias) occurs when the individual measurements or classifications of disease or exposure are inaccurate. Recall bias is particularly important in retrospective case-

	control studies. Reporting bias refers to selective revealing or suppression of information by subjects, that is, people's tendency to under-report the information. Publication bias is one type of reporting bias that occurs when the outcome of a research study influences the decision to whether or not to publish it. Confounding is one type of bias but it is usually considered as its own entity (361). Overadjustment bias occurs as a consequence of the control (including statistical adjustment, stratification and restriction) for an intermediate variable or a descending proxy for an intermediate variable on the causal pathway between the exposure and the outcome (298). A descending proxy for an intermediate variable is a variable that leads to imperfect measurement of intermediate variable. A descending proxy for an intermediate variable is a variable that leads to imperfect measurement of intermediate variable (469). Overadjustment would either increase net bias or decrease precision, and usually bias results towards the null (298). Bias cannot be completely eliminated in epidemiological studies. The aim, therefore, is to minimise it.
Confounding	Confounding is another major issue in epidemiological studies. It occurs when the effect of an exposure on an outcome is blurred by an extraneous factor. A confounder is an extraneous factor, which is often a determinant or known risk factor for the health outcome and is associated with the exposure but is not a result of the exposure. Confounding arises if this extraneous factor is unequally distributed among the groups being compared. Unlike a mediator, a confounder is not an intermediate step in the causal pathway between the exposure and the outcome. Confounding can be minimised by restricting, matching and randomisation at the design stage, and stratifying, making multivariate statistical adjustment and doing standardised rate analysis at the analysis stage. Residual confounding refers to the distortion that remains after controlling for confounding in the design and/or analysis of a study. It occurs when: additional confounding factors were not considered or not measured; control of confounding was not narrow enough; and there are errors in

	the measured confounders including misclassification of subjects with respect to confounding variables due to reporting or measurement errors (370, 371).
Measurement error	Measurement error can be either a source of random error or a type of systematic error (bias). If measurement error is a source of random error, it can be minimised by using state-of-the-art methods of data collection. Measurement bias refers to a type of systematic error that occurs when the measurements or classifications of exposure or outcome are inaccurate. There are different sources of measurement bias. Recall bias is particularly important in retrospective case-control studies. Recall bias occurs when there is a differential recall of information by cases and controls. It is noted that cases may be more likely to recall past exposure, especially if the exposure is widely known to be associated with the disease under study (i.e. smoking and lung cancer). Recall bias can either exaggerate or undermine the true strength of the association between an exposure and an outcome. Different laboratories and different analysers often produce different results despite measuring the same specimen or sample. Measurement error can be reduced by improving the precision of individual measurements by systematic quality control procedures. Observer bias occurs when the investigators, laboratory technicians or participants know the knowledge of the exposure status. A blind or a double-blind fashion can reduce the observer bias.
Causation	An important focus of epidemiology is to inform efforts to prevent and control disease, and to promote health and wellbeing. Therefore, there is a need to study the causation of disease or health outcome. A cause of a disease or health outcome is a condition, characteristic, event or a combination of these factors that produces the health outcome. A cause is considered as sufficient when it inevitably initiates the outcome. A cause is considered as necessary when the outcome cannot develop without it. The cause of a specific health outcome usually comprises several factors. There are almost always some environmental component causes and genetic component causes in a causation of a disease. Before an association between the exposure and the outcome is assessed for

	the possibility that it is a causal relationship, other possible explanations for the observed association have to be excluded, including the play of chance, bias and confounding. It has been pointed out that there are several criteria for judging the strength of association before making a causal inference: 1) A temporal relationship between the exposure and outcome (Does the cause precede the effect?); 2) A sufficient strength of association (Is the association between the possible cause and the effect strong, as measured by the size of relative risk?) 3) Plausibility (Is the association consistent with other knowledge i.e. laboratory experiments to explore the mechanisms?); 4) Consistency (Have other studies demonstrated similar results?); 5) A dose-response relationship (Are increased levels of exposure to a possible cause associated with the increased prevalence or incidence of the effect?) 6) Reversibility (Does the removal of a possible cause result in the reduction of disease risk?) 7) Study design (Is the study design strong in establishing causality?). Each study design has its strengths and weakness. Well-designed randomised controlled trials and cohort studies are good to assess causation. Well-designed case-control studies are viewed to provide moderate evidence. Cross-sectional and ecological studies are generally viewed as weaker evidence. As there is always unknown evidence, causal inference is often tentative and judgements have to be made on the basis of the available evidence.
Reverse causation	Reverse causation (or reverse causality) refers to a direction of cause-and-effect contrary to a common presumption or to a two-way causal relationship, as it were, a loop. It occurs when the outcome precedes and causes the exposure being studied instead of the other way around.

Appendices

Source: Adapted from Bonita R, Beaglehole R, Kjellström T. Basic epidemiology 2nd edition. WHO. 2006

Appendix 6: Logistic regression analyses of the association between secondhand smoke exposure and peripheral arterial disease, Scottish Family Health Study

		Unadjusted				Fully adjusted*			
		OR	95% CI	P value	P value for trend	OR	95% CI	P value	P value for trend
Work	None	1.00	-	-	0.395	1.00	-	-	0.542
	A little	0.38	0.14-1.05	0.061		0.45	0.16-1.23	0.121	
	Some	1.69	0.68-4.21	0.262		2.00	0.79-5.05	0.145	
	A lot	3.54	1.07-11.70	0.038		3.80	1.12-12.89	0.032	
Home	None	1.00	-	-	0.316	1.00	-	-	0.151
	A little	0.96	0.39-2.38	0.935		0.95	0.38-2.38	0.910	
	Some	1.77	0.71-4.42	0.220		1.68	0.66-4.28	0.276	
	A lot	1.34	0.42-4.30	0.622		1.13	0.34-3.71	0.841	
Other locations	None	1.00	-	-	0.635	1.00	-	-	0.346
	A little	0.74	0.48-1.14	0.176		0.76	0.48-1.20	0.240	
	some	1.20	0.55-2.62	0.652		1.38	0.62-3.09	0.435	
	A lot	3.32	1.17-9.42	0.024		3.56	1.20-10.56	0.022	
Total hours per week	0	1.00	-	-	0.214	1.00	-	-	0.208
	1-19	0.86	0.57-1.29	0.468		0.90	0.58-1.39	0.632	
	20-39	2.09	0.64-6.81	0.219		2.02	0.60-6.76	0.254	
	≥40	5.01	1.74-14.44	0.003		4.53	1.51-13.56	0.007	

OR odds ratio; CI confidence interval

*adjusted for age, sex, deprivation quintile, body mass index, physical activity and alcohol consumption

Appendix 7: Certificate of completion of Scottish Health Informatics Programme to use the Scottish Morbidity Record linked data

scriptcpd

Liya Lu

Completed a continuing professional development programme in:

SHIP: Information Governance

Via online distance learning

SHIP

Scottish Health Informatics Programme

With a 100% pass rate in the following assessments:

Legal Concepts Assessment
Legal Framework Assessment
Safe Projects Assessment
Safe Data Assessment
Safe Settings Assessment
Safe Outputs Assessment

Certificate issued: 31 August 2013

Certificate valid until: 31 August 2015

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