SEASONAL AND DIURNAL VARIATIONS IN CARDIOVASCULAR RISK FACTORS AND CARDIOVASCULAR DISEASE IN OLDER BRITISH MEN

THESIS presented for degree of DOCTOR OF PHILOSOPHY

Field of study: Epidemiology

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DECLARATION OF AUTHORSHIP

I, Claudio Sartini, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. I have used data from the British Regional Heart Study, which is an on-going prospective cohort study on cardiovascular disease that began in 1978.

ABSTRACT

Cardiovascular disease (CVD) is the leading cause of death worldwide, and its prevalence is higher among older adults. Moreover, two different temporal variations in CVD risk exist: (i) seasonal: in Europe the CVD mortality risk is higher in winter, when outdoor temperatures are lower; (ii) diurnal: CVD deaths occur more frequently in the morning. However, biological pathways of both seasonal and diurnal variations in CVD mortality have not been fully understood. In part, this may be due to lack of understanding of variations of underlying CVD risk factors in older adults, especially inflammatory markers and physical activity. Investigating physical activity variations is of special interest, as new findings could also potentially shape the development of physical activity guidelines for older people.

The aims of this thesis are twofold: (i) to investigate seasonal variations in CVD risk factors and mortality, by using outdoor temperature as the main exposure variable and seasonal factor of interest; ii) to investigate time of day variations of CVD risk factors. To achieve these objectives, data from the British Regional Heart Study of older adults were used.

Seasonal variation findings: lower outdoor temperatures were especially associated with higher blood pressure, higher LDL-Cholesterol, higher IL-6, lower physical activity levels, and with increased CVD and respiratory mortality. In conclusion, better protection against low temperatures, as well as staying active during cold weather, could help in reducing the CVD risk in older adults.

Diurnal variation findings: some CVD risk factors levels, especially blood pressure, LDL-Cholesterol and IL-6, increased linearly over the course of the daytime (in between 08:00-19:00 hours). Future studies aiming to understand the causal pathways of the diurnal variation in CVD events could focus especially on these markers' variations. Also, physical activity levels peaked in the morning, and initiatives encouraging more active behaviours in the afternoon/evening are needed.

IMPACT STATEMENT

Every year in the UK more people die in winter than summer, especially from cardiovascular disease (CVD). I started this PhD thesis in 2014 to understand why this happens. I analysed data from the British Regional Heart Study (BRHS) of older men, a suitable study to answer such question. The findings from my research demonstrated that exposure to cold temperatures, typically recorded in winter, can affect older people's health conditions (e.g. by increasing blood pressure levels) and behaviours (e.g. by increasing sedentariness) and increasing the likelihood of CVD events.

The impact of the findings from my PhD thesis are already tangible in the research community; before thesis submission, I published part of the thesis findings in 4 different research papers as first author. Such papers have been already cited 45 times in total during the years 2015-2018. This will improve my personal research profile and confirm the excellent reputation and productivity of both BRHS study team and University College London (UCL).

The findings from my PhD research will inform The National Institute for Health and Care Excellence (NICE) evidence based guideline on protecting people from cold weather. Indeed, the research findings from my thesis filled addressed gaps in knowledge mentioned in such guidelines especially providing information on how long a period of cold weather is needed before fatal cardiovascular events emerge. Moreover, other PhD findings can be included in future national, and international physical activity guidelines reports; indeed, I demonstrated that cold temperatures are a determinant of changes in physical activity levels of different intensities (e.g. light physical activity and sedentary time, less studied in the literature). Overall, my PhD findings highlighted the need of new public health strategies to address the trade-off between (i) staying active but yet limiting the exposure to cold outdoor temperatures, and (ii) staying warm but yet limiting the time spent sedentary. My research also suggested several strategies on how to address this trade-off; the dissemination of such findings could represent a further step towards engagement in more active behaviours.

Lastly, the expertise, knowledge, and data acquired for this thesis were key to generate other benefits inside Academia. When I was still conducting my PhD thesis, in 2015, I have received further funding by the National Institute of Health Research School for Primary Care Research (NIHR SPCR grant awarded to Richard W Morris for which I was named co-investigator, reference number 281) to analyse new BRHS data and investigate whether living in cold homes increased mortality, and to investigate whether primary care data could be used to predict cold-related mortality in the UK. This project was possible due to a unique collaborative effort of researchers from UCL, University of Bristol, University of Oxford and the national Centre for Sustainable Energy. The benefits of this NIHR funded research were visible outside academia; the findings were published in peer-reviewed journals and later reported by several news sites online and social media, contributing to the current public debate on how to protect people from cold weather.

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I would like to thank all my colleagues from the British Regional Heart Study for coordinating the study, collecting the data, and for giving me help and assistance in proofreading this thesis and interpreting the findings. Thanks to Dr. Sarah Barry, Prof. Gordon Lowe, Dr. Paul Welsh and Prof. Naveed Sattar from the PROSPER study for their intellectual contribution to Chapters 6 and 7.

Special thanks go to Martina for her relentless understanding and support. Thanks to my family and friends for their constant encouragement.

FREQUENTLY USED ABBREVIATIONS

Data sources

BRHS: British Regional Heart Study

Disease Outcomes

CHD: Coronary heart disease

CVD: Cardiovascular disease

ICD-9: International classification of diseases - ninth revision

Physical activity terms

CPM: Counts per minute

LIPA: Light physical activity

MVPA: Moderate to vigorous physical activity

SB: Sedentary behaviour

PA: Physical activity

Physical and blood measurements

BMI: Body mass index

BP: Blood pressure

CRP: C-reactive protein

DBP: Diastolic blood pressure

FEV_1: Forced expiratory volume in 1 second

FVC: Force vital capacity

HDL: High density lipoprotein (cholesterol)

LDL: Low density lipoprotein (cholesterol)

IL-6: Interleukin 6

PV: Plasma viscosity

SBP: Systolic blood pressure

t-PA: tissue plasminogen activator antigen

VitD: Vitamin D

vWF: von Willebrand factor

Statistical terms

CI: Confidence intervals

OR: Odds ratio

HR: Hazard ratio

RR: Rate ratio

SD: Standard deviation

Others

EWD: Excess winter deaths

NICE: National Institute for Health and Care Excellence

MET: Meteorological Office of the United Kingdom

ONS: Office for National Statistics

RH: Relative humidity

ILI: Influenza-like illness

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Chapter 1 **INTRODUCTION**

1.1 Introduction and rationale for the thesis

Cardiovascular disease (CVD) is a general term for conditions affecting the heart or blood vessels and includes all heart and circulatory diseases, particularly including Coronary Heart Disease (CHD) and Stroke. CVD is the leading cause of death worldwide, accounting for 17.9 million deaths in 2015 (1). According to the World Health Organization (WHO), the annual number of deaths from CVD worldwide is projected to increase to 20.5 million by 2020 and 24.5 million by 2030 (2). In the United Kingdom (UK) in 2016, 152,465 deaths were caused by CVD (25.5% of all deaths) according to UK official statistics (3). In addition to its impact on mortality both worldwide and in the UK, the prevalence of CVD increases with age (4). In 2011 in the UK the prevalence of CVD among men increased with age from 2% in 16-44 year olds to 34% in 75+ years olds (prevalence among men aged 45-64 and 65-74 years was 15% and 29% respectively) (5). In women the trend across age groups is similar but the prevalence is lower than in men (about 4% lower in women aged 65+ years). As CVD is also the leading cause of death in UK men (while for women it is dementia and Alzheimer's disease) (6), CVD prevention is crucial among older people and especially in men. In recent decades, there has been an increase in CVD prevalence for both men and women aged over 75; in men over 75 the CVD prevalence increased from 23% to 34% between 1988 and 2011 while in women aged over 75 increased from 27% to 30% (5). In men aged 65-74 years old the CVD prevalence also increased from 25% to 29% over this period while in women remained fairly constant around 23% (5). In the UK more people survive a heart attack than in the past (7 out 10 today against 3 out of 10 in the 1960s) which contributes to the increase in the absolute number of older people living with CVD (3). Additionally, increases in life expectancy observed in the UK raise concern about how to cope with an aging population with increasing CVD prevalence (7). At the age of 65 years, UK men and women are still expected to live 18 and 20 more years respectively (8), and the proportion of people aged 65 years and over is projected to increase from 16% in 2008 to 23% by 2033 (9). As most scientific research on CVD prevention has focused on exposures in middle-aged populations (adults less than 60 years old), more research investigating CVD variations in older adults is needed to inform future CVD prevention strategies and promotion of healthy ageing (7).

1.2 Risk factors for cardiovascular disease (CVD)

Several factors have been associated with increased CVD risk. Some factors, such as blood pressure, have been widely investigated and will be termed "established" risk factors for CVD (see paragraph 1.2.1). More recently, other risk factors (e.g. markers of inflammation and haemostasis) were linked with increased CVD risk and will be presented as "emerging" CVD risk factors (see paragraph 1.2.2).

1.2.1 Established CVD risk factors

A wide range of established risk factors for CVD mortality and all-cause mortality have been identified from epidemiological studies, including cigarette smoking, obesity, diabetes, high blood pressure, high blood LDL-cholesterol, low physical activity, and low fruit and vegetable intake increase CVD risk (2, 10); WHO estimated that such factors are responsible for more than half of the total number of cardiovascular deaths worldwide (11). Good control of both blood pressure and LDL-cholesterol, and improvements in healthy behaviour and lifestyle choices (e.g. engaging in regular physical activity) are the worldwide focus of prevention efforts for protecting people's health, promoting healthy ageing, and preventing CVD (12, 13).

Among established CVD risk factors, physical activity is of particular interest for the scope of this thesis (see also paragraphs 1.3.1.1 and 1.3.2.2). Worldwide, physical activity is an important risk factor for CVD; overall there is a graded inverse association between physical activity levels and CVD risk (14). Also, as highlighted in paragraph 1.1, CVD risk is increased in older adults at an age when physical activity levels are generally low (15-17). Therefore, to reduce CVD risk it is important to understand patterns of both physical activity and sedentary behaviour, as it has been hypothesised that are two independent risk factors for both CVD mortality and all-cause mortality (18). Understanding such patterns using conventional subjective measures (e.g. surveys) has presented substantial challenges due to the fact that study participants' may over or under report time spent in various levels of physical activity (19, 20). Wearable devices, such as hip-worn accelerometers (21), permit objective and accurate assessment of physical activity and sedentary behaviour patterns in population-based studies (17) and, of special consideration for older adults, reduce the impact of recall bias (over or under reporting), especially in view of some participants' memory loss or cognitive

impairment (19, 20, 22). Of particular relevance to this thesis are accelerometers, which can give insight into how individual activity levels vary over the course of the day (e.g. data can be averaged over 1-hour periods) and across seasons. However, to date there is very little evidence on how objectively measured physical activity and sedentary time vary by time of the day and across seasons among older people.

1.2.2 Emerging CVD risk factors

Although much of the variation in CVD risk can be explained by established risk factors, there is still uncertainty about which other factors are implicated in the aetiology of CVD (23). Emerging CVD risk factors, such as circulating proinflammatory cytokines (e.g. interleukin 6 [IL-6] (24)), are less studied in the literature and especially in older adults. Investigating IL-6 is important, as it can be potentially routinely used in blood tests to screen patients for heart problems (2), and because Mendelian randomisation studies support a causal association of Interleukin-6 with CHD (25). However, IL-6 variations and its determinants are not well understood; to know this would be important to important to CVD prevention (26, 27). Similarly to IL-6, acute phase proteins (e.g. C-reactive protein [CRP] (28)) and biomarkers of haemostasis and thrombosis (e.g. von Willebrand Factor and D-Dimer) (23) are additional emerging risk factors less studied in relation to CVD.

1.3 Variations in CVD

There are many sources of variation in CVD risk, including differences between geographical areas (between and within countries), temporal variations, and differences according to sociodemographic factors. This paragraph introduces the rationale for investigating two different types of temporal variations: (i) seasonal and (ii) diurnal variations in CVD risk. The reasons for such investigations are elucidated in two separate sections (paragraph 1.3.1 for seasonal variations, and paragraph 1.3.2 for diurnal variations).

1.3.1 Seasonal variation in CVD

By definition, the seasons are periods into which a year can be divided (e.g. winter, spring, autumn, and summer) to reflect meteorological changes resulting from the earth's changing position with regard to the sun. Seasonal variation in all-cause and CVD mortality, with a peak in winter and a nadir in summer, has been reported throughout most parts of the world,

including the UK (29-31). A common and very simple measure to estimate the seasonal variation in mortality is defined as Excess of Winter Deaths (EWDs) (32), which measures the number of deaths occurring during the winter season (December to March) compared with other specific four month periods of the year of the year (August to November preceding the winter, and April to July following the winter). As recently as 2015-2016 there were an estimated 24,300 excess winter deaths from all causes in winter in England and Wales (32) and 2,850 in Scotland (33); in relative terms, this means that 15% and 16% more people died in the winter months in England/Wales and in Scotland respectively, compared with the non-winter periods. For CVD mortality the relative differences were similar; 14% and 17% more people died from CVD in the winter months in England/Wales and in Scotland respectively in comparison with non-winter months (32). The Excess of Winter Deaths (EWDs) index contains several limitations (34); for example, it is a crude measure of seasonal variation in mortality and does not offer any insights on which seasonal factors (e.g. outdoor temperature) may be responsible for any increased risk in the winter season. The increases in all-cause and CVD mortality in winter were mainly attributed to the difference in ambient temperatures, which are generally lower in winter and higher in summer (see paragraph 1.3.1.1) (30). Outdoor temperature is widely recognised as an important determinant of seasonal variation in CVD mortality and all-cause mortality (30). The largest study of this issue and involving counts of deaths in particular locations demonstrated that worldwide the majority of the temperature-attributable deaths from any cause were associated with cold temperatures, explaining 7.3% of all-cause mortality variation (35). However, in studies where the number of deaths is aggregated by day, the unit of observation is the day rather than the individual. It cannot be assumed from such studies that relationships existing at aggregated level of analysis necessarily demonstrate the same strength as analyses at the individual level (36). Population-based cohort studies are therefore important, as they are able to investigate what happens at the individual level.

One of the objectives of this thesis is to enhance our understanding of biological pathways involved in the seasonal variation of CVD in a large population-based cohort study; this requires conducting epidemiological research in which mortality, CVD risk factors, and temperature data are collected at an individual level. Also, a further prerequisite of such research is that the linkage of (i) individual CVD risk factor measures to (ii) meteorological

factors and (iii) mortality outcomes is ascertained for these same individuals (30, 35). I am unaware of such complex linkage in previous studies of CVD to date.

1.3.1.1 Season, outdoor temperatures, CVD risk factors and CVD risk

Each season of the year is characterized by specific weather patterns. For example, in Europe one of the typical attributes of the winter season are lower ambient temperatures. In this PhD thesis, outdoor temperature is the main exposure variable of interest associated with season, because a large body of evidence has suggested that lower outdoor temperatures are associated with increased CVD risk and with all-cause mortality (29, 35, 37, 38). However, it is unclear (i) how long a period of cold temperatures is needed before CVD deaths occur, (ii) whether the association of temperature with mortality is modified by individual risk factors, and (iii) whether the temperature-mortality relationship is confounded by other seasonal trends such as the prevalence of influenza. These three major gaps in evidence explain the focus of this thesis in understanding the temperature-mortality associations.

Previous studies also hypothesized that lower outdoor temperatures, typically recorded in winter, could exert their adverse effects on CVD risk by increasing the levels of established CVD risk factors including blood pressure (39), circulating LDL-cholesterol (40), and by decreasing overall physical activity levels (41). Elucidating the biological pathways of the seasonal variations in mortality is important but filling gaps in evidence poses complex challenges (30, 32). Therefore, it is important to clarify that a special focus of my PhD thesis is to understand whether seasonal variations in outdoor temperatures are associated with (i) seasonal variations in physical activity levels of different intensities (e.g. light or moderate-to-vigorous physical activity and sedentary time) and (ii) seasonal variations in biological risk factors, because there are relatively few studies in the literature with a particular dearth concerning older adults. This thesis will place more emphasis in understanding the association of temperature with emerging CVD risk factors, as they are less studied in the literature (as specified in paragraph 1.2.2).

1.3.2 Diurnal variation in CVD

Diurnal variation refers to variation at different times of day. Marked diurnal variations in CVD mortality have been reported, with two separate peaks in early and late morning (at about 9:00 and 13:00 hours respectively) (42-44).

1.3.2.1 Diurnal variation in established and emerging CVD risk factors

Studies in middle aged adults have identified diurnal variations in established and emerging CVD risk factors. For example, for biological CVD risk factors such as Fibrinogen, blood pressure, von Willebrand Factor, Interleukin-6, C-Reactive Protein and t-PA the daily peaks are mainly concentrated in between 10:00 and 18:00 hours (45-48). However, the magnitude of the diurnal variation (e.g. the estimated percent difference between the peak and nadir calculated using a sinusoidal function) was very heterogeneous and equal to 3%, 10%, 22%, 32%, 34% and 54% for Fibrinogen, blood pressure, von Willebrand Factor, Interleukin-6, C-Reactive Protein and t-PA respectively (45-48). Overall, diurnal variations in emerging CVD risk factors, particularly for biological markers of inflammation and haemostasis measured in older adults, are much less studied (45, 46, 49-52). The reasons for investigating such variations are twofold, and include (a) understanding possible reasons for a diurnal variation in CVD events occurring in older adults, and (b) whether taking account of diurnal variations in CVD risk factors can contribute to the accuracy of CVD risk prediction (45). This thesis aims to investigate the latter reason (point b), and extend previous literature by investigating time of day variations in a comprehensive range of established and emerging biological risk factors in the same population of older adults.

1.3.2.2 Diurnal variation in physical activity

Worldwide, physical activity is an established risk factor for CVD (14). It has recently been shown that physical activity can also vary in relation to time of day; a recent UK Biobank study (over 100,000 participants aged 45-79) showed that total physical activity levels (e.g. “counts” or steps per day) were higher in the morning than in the afternoon or evening, especially in those aged 55 years or more (53). However, how physical activity levels of different intensities, such as light and moderate to vigorous physical activity (MVPA) and sedentary behaviour are structured throughout the day was not determined by the UK Biobank study or other earlier studies. To understand such diurnal variations could have important public health implications

as it has been hypothesised that MVPA and sedentary time are two independent risk factors for both CVD mortality and all-cause mortality (see also paragraph 1.1.2) (18); therefore, maintaining a regular level of activity and minimising sedentary behaviours on a daily basis is recommended to prevent CVD later in life (54). Understanding peaks and dips in activity could help in implementing effective strategies to maintain a regular level of activity (prolonging or increasing physical activity during specific parts of the day), with the prospect of reducing the overall CVD risk in older people.

1.3.3 Summary of identified research gaps and needs

From the literature reviewed (see Chapter 2 for more details), I have identified significant gaps on seasonal and diurnal variations in CVD:

- for seasonal variations, these aspects particularly include the lack of understanding of the biological pathways of the seasonal variation in CVD, the investigation of which CVD risk factors (especially physical activity levels and emerging risk factors) show a temperature-related variation, and how long a period of cold temperatures is needed before observing an increase in CVD risk factors levels and CVD mortality. Also, more studies are needed to understand whether such temperature-related associations are modified by individual characteristics.

- for diurnal variations, these particularly include investigating sedentary time and light physical activity variations. Also, conducting appropriate analyses to assess whether diurnal variations in physical activity levels of different intensities are modified by individual characteristics is needed. Lastly, it is unclear which established and emerging biological risk factors vary by time of the day and whether taking account of their diurnal variations can contribute to the accuracy of CVD risk prediction.

1.4 Thesis objectives and data

1.4.1 Aims and objectives of this PhD thesis

I will present the objectives of this thesis prioritising the importance of the research questions. Therefore I will articulate them in two parts:

Part 1: objectives from my investigation of seasonal variations in CVD risk factors, CVD mortality and all-cause mortality in older age:

(a) To demonstrate that associations between several seasonal factors (outdoor temperature, sunshine duration, relative humidity, and a proxy of influenza exposure) and objectively measured physical activity levels of different intensities exist, and to demonstrate that outdoor temperature is the most important seasonal factor affecting physical activity levels. Moreover, a further objective is to understand whether temperature-related associations with physical activity levels are modified by individual risk factors (e.g. age).

(b) To examine the associations of outdoor temperature (main seasonal factor of interest) with established and novel biological cardiovascular risk factors and to understand whether such associations (i) persist after adjustment for a proxy of influenza exposure and (ii) are modified by individual risk factors;

(c) To investigate the associations of outdoor temperature (exposure variable) with CVD mortality, respiratory mortality, and all-cause mortality (outcomes), and to understand whether such associations persist after adjusting for a proxy of influenza exposure (seasonal confounding factor). Moreover, the analysis aims to explore the role of physical activity and other CVD risk factor (blood pressure, LDL-cholesterol, and inflammation) as potential mediators of the relationship between temperature and mortality.

Part 2: objectives from my investigation of diurnal variations in CVD risk factors in older age:

(d) To examine whether objectively measured physical activity levels of different intensities (time spent in sedentary behaviours, light physical activity, and moderate-to-vigorous physical activity) vary over the course of the day in older adults, and to

examine whether physical activity levels observed in the morning, afternoon and evening, are modified by individual risk factors.

(e) To examine whether diurnal variations in a comprehensive range of established and novel biological risk factors for CVD (e.g. blood pressure and markers of inflammation among others) vary over the course of the day in older adults, and to examine whether such variations are modified by individual risk factors.

It should be noted that, to achieve the specific objectives listed above, this thesis has used data from the British Regional Heart Study (BRHS) collected at different time points and using different methods (see paragraph 1.4.2 for more details). In order to address objectives (a) and (d), the thesis used a cross-sectional analysis of data collected in BRHS men aged 70-91 years during 2010-2012. In order to achieve objectives (b) and (e), the thesis used a cross-sectional analysis of data collected during 1998-2000 (men aged 60-79 years). To achieve objective (c) the thesis used a longitudinal study design (survival analysis) with BRHS individual CVD risk factors collected twice during the periods 1998-2000 and 2010-2012 and follow-up of BRHS participants from 1998-2000 to 2014. All statistical analysis were carried out using Stata (versions 12-14, Stata Corp., College Station, Texas).

1.4.2 Data used in this PhD thesis: the British Regional Heart Study (BRHS)

The epidemiological research described in this thesis is a statistical analysis of data collected as part of the BRHS, an established and ongoing prospective study of cardiovascular disease (CVD). A more detailed description of the BRHS data and methods can be found in Chapter 3. Briefly, the BRHS is a prospective cohort of 7735 men recruited from a single representative local primary care centre in each of 24 British towns in 1978-80 (age 40-59 years) who were examined at entry to the study. Between January 1998 and March 2000, after an average of 20 years follow-up, 5522 surviving men were invited to attend a follow-up examination; 4252 men aged 60-79 years (77% of those alive and eligible) attended. Fieldwork was undertaken in the 24 towns in series between May 1998 and March 2000, ensuring that during each season towns with widely different geographical locations and CVD mortality rates were included. The measurements were taken by trained nurses in between 08:00 and 19:00 hours (anthropometry, physiological and blood samples) and men completed a detailed questionnaire.

In 2010-2012, after an average of 32 years from recruitment, the surviving cohort members (n=3137, now aged 71-91 years) were invited to attend a further follow-up examination; 1455 of 3137 men (46.4%) also participated in a study of objectively measured physical activity. Since recruitment, the men have been followed up until the present, by several postal questionnaires and through the National Health Service Central Register and reports from the general practitioners for cardiovascular mortality and morbidity.

1.4.3 Outcomes investigated in this PhD thesis

The BRHS allows an investigation of seasonal variation in mortality (deaths from all causes, from CVD and respiratory disease) and seasonal variation in CVD risk factors (physical activity levels and biological markers of CVD). This is possible because the date of death and the date of measurement for the risk factors were collected. An investigation of diurnal variation in CVD risk factors is also possible (the time of the day rounded to the nearest hour was recorded at measurement, and has been used to estimate the extent of diurnal variations in CVD risk).

1.4.4 Value of BRHS for investigation of seasonal and diurnal variation in CVD

The BRHS is a suitable cohort for investigating seasonal and diurnal variation in CVD. During the initial recruitment in 1978–1980, the order in which the towns were surveyed was chosen intentionally to avoid the confounding of regional patterns in CVD mortality with seasonal patterns (55). Representativeness of the cohort is good: for example, during nine years of follow-up (starting from 1998-2000) the excess in CVD mortality in the BRHS was of similar magnitude to that seen in official statistics (14% excess in CVD deaths in the BRHS compared with 15-20% reported in official statistics for the same age group) (55). For the scope of this thesis, the BRHS data base was supplemented by linkage with temperature data collected daily at town level since 1998, thanks to a collaboration with the UK Meteorological Office. As a result, daily outdoor temperatures for each town of residence and date are linked with the individual BRHS participant data (see paragraph 1.4.2). A detailed description of how the data were matched can be found in Chapter 3 (“Methodology”). Lastly, the BRHS is also a cohort study comprising a socio-economically and geographically representative sample of British middle-aged men in 1980 and senior citizens in 2000. Long-term co-operation from the cohort has been remarkable, with very high rates of follow-up. Measurements collected in the study

have been validated, data entry verified and record keeping maintained to an exceptionally high standard. However, the BRHS sample is made predominantly of white European men (99% Caucasian) and does not include women or ethnic minority groups. For further discussion of this point, see Chapter 9 ("Implications and conclusions").

Lastly, the BRHS data allow an investigation of diurnal variation in CVD risk factors (as specified in paragraph 1.4.3). However, the diurnal variation in CVD mortality cannot be directly studied because the time of the death within the day was not collected. This will be further discussed as a limitation in Chapters 6 and 9.

1.5 Structure of the thesis

Each of the above five objectives, from (a) to (e), represent the results Chapters of this thesis (Chapters from 4 to 9). The Chapters' titles and order are reported below:

Chapter 1 – Introduction: this Chapter provides an introduction to the importance of CVD in an aging population and an overview of the seasonal and diurnal patterns in CVD mortality, and CVD risk factors (e.g. physical activity and biological markers of CVD), outlining the importance of these patterns in older age; **Chapter 2** – Literature Review: this Chapter presents the epidemiological and etiological background of CVD, seasonal variation in CVD and diurnal variation in CVD. The Chapter also includes literature reviews of the associations of CVD risk factors which are known to vary by season and within the day (e.g. physical activity and blood pressure), and which also have been reported to be associated with CVD mortality in older age in prospective studies; **Chapter 3** – Methodology: this Chapter describes the BRHS study and methodology, the data used to achieve the thesis objectives and the methods used to analyse the data; **Chapter 4** – This Chapter reports the results of a cross-sectional study where diurnal patterns in physical activity levels of different intensities were examined using data collected in 2010-12. This work highlights where diurnal peaks and dips in physical activity occur over the course of the day, and discusses the importance of the findings especially for public health; **Chapter 5** - This Chapter reports the results of a cross-sectional study where seasonal patterns in physical activity levels of different intensities were examined using data collected in 2010-12. This work highlights which meteorological and seasonal determinants of physical activity

levels are most important; **Chapter 6** – This Chapter reports the results of a cross-sectional study where diurnal patterns of established and novel cardiovascular risk factors were examined using data collected in 1998-2000. The relevance of these diurnal patterns to CVD risk prediction, and risk stratification is also discussed; **Chapter 7** – This Chapter reports the results of a cross-sectional study where seasonal patterns (mainly temperature-related variations) of established and novel cardiovascular risk factors were examined using data collected in 1998-2000. The importance of better protection against low temperatures in reducing the levels of several CVD risk factors is discussed; **Chapter 8** - This Chapter reports the results from a time-varying survival analysis where both BRHS and outdoor temperature data collected from 1998 were used. The independent associations of outdoor temperature, established and novel cardiovascular risk factors, and physical activity levels with CVD mortality and all-cause mortality were estimated after accounting for other classic CVD risk factors, such as age, social class and smoking, as well as a measure of exposure to influenza; **Chapter 9** - Implications and conclusions: this Chapter brings together the key findings of all results Chapters, together with implications for public health and future epidemiological research.

1.6 Thesis publications

Four first-author papers (56-59) based on the material in this thesis (Chapters 4, 5, 6 and 7) have been published in peer-review journals. These publications are listed below and a full copy of each paper is included in Appendix I. The list of oral and poster presentations given at conferences based on the material in this thesis can be found in Appendix II and III.

1. **Sartini C**, Wannamethee SG, Iliffe S, Morris RW, Ash S, Lennon L, Whincup PH, Jefferis BJ: Diurnal patterns of objectively measured physical activity and sedentary behaviour in older men. BMC Public Health 2015, 15:609.

2. **Sartini C**, Morris RW, Whincup PH, Wannamethee SG, Ash S, Lennon L, Jefferis BJ: Association of Maximum Temperature With Sedentary Time in Older British Men. Journal of Physical Activity and Health 2016:1-18.

3. **Sartini C**, Barry SJ, Whincup PH, Wannamethee SG, Lowe GD, Jefferis BJ, et al. Relationship between outdoor temperature and cardiovascular disease risk factors in older people. European journal of preventive cardiology. 2017;24(4):349-56.

4. **Sartini C**, Whincup PH, Wannamethee SG, Jefferis BJ, Lennon L, Lowe GD, et al. Associations of time of day with cardiovascular disease risk factors measured in older men: results from the British Regional Heart Study. BMJ Open. 2017;7(11):e018264.

Chapter 2 LITERATURE REVIEW

2.1 Introduction

My PhD project proposal was officially submitted to University College of London (UCL) in May 2014. The literature review presented in this chapter includes original manuscripts published up to June 2015. Reports on global diseases and risk factors as well as literature reviews which made use of data collected up to the year 2015 were also included in this Chapter (1, 60). Between June 2015 and the date when this thesis was submitted in 2019, new original manuscripts were published in peer-reviewed international journals; they are not part of this literature review. Instead I have discussed them in Chapters 4, 5 and 8 within the paragraphs "comparison with other studies", and in Chapter 9 (PhD findings' discussion).

In summary, Chapter 2 presents the epidemiological and aetiological background to cardiovascular disease (CVD), and reviews existing studies of seasonal and diurnal variation in CVD risk published up to June 2015, particularly in older age. Section 2.2 describes the epidemiology and pathophysiology of CVD and the importance of CVD in older age. Section 2.3 details the literature on seasonal variation in CVD. Section 2.4 explores the seasonal variation in CVD risk factors, including established and emerging risk factors; this section includes approaches to assessment and definition of seasonal variation, and a review of the evidence for an association between outdoor temperature, the main seasonal factor investigated in this thesis, and risk of CVD and mortality in older age. Section 2.5 explores diurnal patterns of CVD risk factors and physical activity, including methods of assessing diurnal patterns and a review of the evidence for an association between time of the day and the risk factors, and whether or not this could be relevant for the overall CVD prediction.

2.2 Overview of CVD

2.2.1 Epidemiology and pathophysiology of CVD

A comprehensive review of all-cause mortality published by the Global Burden of Disease Study group showed that NCDs, such as CVDs, caused 71.3% of deaths (39.8 million) of all deaths which occurred globally from 1980 to 2015, an increase of 14.3% since 2005 (5.0 million deaths) (1). CVD is the leading cause of death worldwide, accounting for 17.9 million deaths in 2015 (1). Although death rates from CVD have been decreasing in the UK since the

early 1970s (61), 27% of deaths in the UK (155,000 in absolute number) were still caused by CVD in 2014 (62), the leading cause of death for men (63). In 2012, nearly half (46%) of all CVD deaths in the UK were from coronary heart disease (CHD) and over a quarter (26%) were from stroke (64). Overall, CHD was responsible for 16% of all male deaths and 10% of all female deaths, a total of just under 73,500 deaths. Around 41 000 deaths were from stroke, making up 6% and 9% of total deaths in men and women, respectively (64). CVD is a major contributor to morbidity and disability; the 2015 British Heart Foundation report estimated the cost to the UK economy (including premature death, disability and informal costs) to be £19 billion pounds in one year, due to health care costs (60%), productivity losses due to mortality and morbidity (23%) and informal care-related costs (17%) (65).

CHD (coronary [ischaemic] heart disease) and cerebrovascular disease (particularly stroke) are the main forms of CVD in the UK. Atherosclerosis is the common underlying disease process responsible for almost all CHD (coronary atherosclerosis) and a substantial proportion of stroke (carotid atherosclerosis). Atherosclerosis is a complex pathological process which develops over many years, characterised by chronic inflammation in the artery walls, where fatty materials and cholesterol are deposited (forming atherosclerotic plaques), narrowing the arterial lumen, obstructing blood flow and making the arteries less pliable (4, 66, 67). These plaques can eventually rupture, triggering the formation of a thrombus which, if large enough, may occlude a coronary blood vessel (causing CHD) or a cerebral blood vessel (causing a stroke) (4).

Acute myocardial infarction (MI), angina pectoris, and sudden ischaemic death are the main clinical manifestations of CHD (67-69). Acute MI, also known as ‘heart attack’, can be fatal or non-fatal and is caused by necrosis of myocardial tissue due to blockage of a coronary artery. Symptoms include chest pain, which can often radiate to the jaw, neck, arms and back, shortness of breath, dizziness, nausea and an overwhelming sense of anxiety. MI can also be silent; asymptomatic and only diagnosed retrospectively through electrocardiograms (70). The World Health Organization criteria for myocardial infarction are any two of these three conditions: prolonged chest pain, positive electrocardiogram findings and raised cardiac enzyme levels (71, 72). Angina pectoris is characterised by chest pain due to ischaemia of the myocardium and can be stable or unstable. Stable angina is likely to cause regular and

predictable symptoms. Unstable angina can cause prolonged chest pain even at rest or low levels of activity or can be previously diagnosed angina that has become more frequent, longer in duration, or lower in threshold to activity (73).

The clinical manifestations of cerebrovascular disease include transient ischaemic attacks (TIAs) and strokes (67, 68). A TIA is caused by a temporary disruption in cerebral blood flow, with symptoms (including facial weakness, arm weakness and speech problems) disappearing within 24 hours. Stroke however, is more severe with permanent symptoms lasting more than 24 hours. The current universal definition of stroke, as defined by the Stroke Council of the American Heart Association and the American Stroke Association, is: "Central nervous system infarction is brain, spinal cord, or retinal cell death attributable to ischemia, based on pathological, imaging, or other objective evidence of cerebral, spinal cord, or retinal focal ischaemic injury in a defined vascular distribution and/or clinical evidence of cerebral, spinal cord, or retinal focal ischaemic injury based on symptoms persisting ≥ 24 hours or until death, and other aetiologies excluded" (74).

2.2.2 The importance of CVD in older age

As reported in Chapter 1 (paragraph 1.1) life expectancy in the UK is increasing; the Office for National Statistics (ONS) reported in 2014 that at the age of 65 years, UK men and women are expected to live further 18 and 20 years respectively (8). The continuing increase means that the proportion of people aged 65 and over is projected to increase from 16% in 2008 to 23% by 2033 (9). This has generated increasing concern as older people are particularly at risk of non-communicable diseases, such as CVD.

Estimates from the year 2014 based on records from the Clinical Practice Research Datalink GOLD database (a sample of general practices in each of the constituent countries of the UK) reported estimates of prevalence of several CVD conditions: in men aged over 75 years, the prevalence of stroke, angina and MI was 14.9%, 17.0%, and 12.1% respectively while for men aged 65-74 years the corresponding percentages were 6.4%, 8.8%, and 7.1%. In women the trend across age group was similar but the prevalence for each of the three conditions was lower than in men (64). Moreover, a study which made of use of data obtained from two different sources during years 2004-2010, the Hospital Episode Statistics (HES) and mortality

statistics data bases in England, reported that due to increasing survival rates following MI, the population burden of CVD morbidity and disability in older people is even greater than in previous years (75).

Findings of the Global Burden of Disease Study published in 2010 showed that the CVD burden in older adults is increased in the UK in comparison to other high income countries due to higher rates of age-standardised years of lives lost from cardiovascular and circulatory disorders (76). Even a small relative risk reduction in CVD could considerably reduce absolute mortality and cardiovascular morbidity and disability risks in older adults; therefore, it is particularly important to identify and reduce exposure to risk factors for CVD in this age-group (77).

2.2.3 Established risk factors for CVD

Established risk factors for CVD and mortality are known; smoking, obesity, diabetes, high blood pressure, high blood cholesterol, physical inactivity, and low fruit and vegetable intake increase CVD risk; such risk factors may act simultaneously and it is difficult to distinguish their mutually exclusive contribution to CVD. For example, WHO estimated that worldwide the overlapping contribution of blood pressure, physical activity, obesity, saturated fat diet, and smoking to CHD mortality was 45%, 30%, 23%, 16%, 11% and 10% respectively (10). Some of those risk factors are also potentially modifiable factors; good control of both blood pressure and cholesterol, and improving healthy behaviour and lifestyle choices (e.g. engaging in regular physical activity) are the worldwide focus of prevention efforts for protecting people's health, promoting healthy ageing, and preventing CVD (12).

2.2.3.1 Blood pressure

Blood pressure is known to be causally associated with CVD (78); Evidence from a meta-analysis of individual data for one million adults in 61 prospective studies reported that throughout middle and old age (40-89 years), there is a strong and positive association between systolic blood pressure (SBP) and diastolic blood pressure (DBP) and risk of cardiovascular (and overall) mortality, seen above the usual SBP of 115 mmHg and DBP of 75 mmHg (79). Blood pressure is still an important risk factor in a public health context: a pooled analysis of 1479 studies worldwide that had measured blood pressure in 19.1 million adults from 1975 to

2015 reported that the absolute number of adults with raised blood pressure increased from 594 million in 1975 to 1.13 billion in 2015. This was partially attributed to population ageing in European countries, where blood pressure has been persistently high (especially in in central and eastern Europe) (60).

2.2.3.2 Cholesterol

In many studies, associations between high serum cholesterol levels and raised CHD risk have been reported, and there is no 'threshold' below which cholesterol levels are not associated with increased CHD risk (80). The Prospective Studies Collaboration showed that an average decrease of 1 mmol/L in total serum cholesterol was associated with about a half the risk of CHD mortality in early middle-age (40-49 years) and about a sixth of the risk in old age (70-89 years) (81). The association of elevated low density lipoprotein cholesterol (LDL) with CHD events in observational studies has long been established and the causality of this association has been also supported by randomized trials of LDL cholesterol lowering drugs (82). A meta-analysis of 27 randomised trials reported that in individuals with 5-year risk of major vascular events lower than 10%, each 1 mmol/L reduction in LDL cholesterol produced an absolute reduction in major vascular events of about 11 per 1000 over 5 years (82). Recently, mendelian randomisation findings also support a causal effect of triglycerides on CHD risk, but a causal role for HDL-C, though possible, remains less certain (83).

2.2.3.3 Physical activity and sedentary behaviour

In the past decades, numerous studies reported that physical activity reduces cardiovascular and all-cause mortality (84). For example, a systematic literature review of 33 cohort studies (883,372 participants, published between 1995 and 2007, follow-up times from 4 years to 20 years) in which physical activity levels were self-reported found that: the most active group (vs least active) had a risk reduction of 35% in CVD mortality (95% CI 30;40%) and a risk reduction of 33% in all-cause mortality (95% CI 28;37%) (84). It has been hypothesised that regular physical activity could exert its beneficial effects by reducing the levels of established and emerging risk factors, such as blood pressure, lipids, and markers of inflammation and by lessening the progression of atherosclerosis and clot rupture (85). More recently, the UK physical activity guidelines published in 2011 reported that people who do not meet the guidelines (spending 150 minutes of moderate to vigorous activity in bouts of at least 10

minutes per week) have approximately 25-35% increased mortality and morbidity (54). One BRHS study has also shown that the association between higher physical activity and lower CVD risk persists in older age (85).

More recently, contemporary researchers distinguish between time spent in doing physical activity and sedentary behaviours (86, 87) (see paragraph 2.4.4). A standard definition of sedentary behaviour has not yet been established, although it has been acknowledged that it is not simply a lack of physical activity (87). Sedentary behaviour can be defined as the time spent in activities engendering less than 1.5 Metabolic Equivalent of Task (METs) (88). In recent years, there have been an increasing number of studies which have reported associations between prolonged sedentary behaviour and health outcomes, such as mortality and cardiovascular disease, which have been independent of physical activity levels (18). In one meta-analysis published in 2015 (47 studies from 2008 to 2014, for a total of 29,917 participants) self-reported high sedentary time was associated with an 22% increase in all-cause mortality, 15% increase in CVD mortality, 14% increase in CVD incidence, 13% increase in cancer mortality, and 13% increase in cancer incidence (18).

2.2.4 Emerging risk factors for CVD

Although much of the burden of CVD can be explained by established risk factors, there is still uncertainty on which other factors are implicated in the aetiology of CVD (23). Some downstream CVD risk factors are less studied in the literature and especially in older adults; for example, circulating proinflammatory cytokines (e.g. interleukin 6 [IL-6] (24)) and other haemostatic factors are important as they are associated with arterial plaque formation, plaque rupture and thrombosis (26, 27). Prospective studies and meta-analyses have shown that markers of inflammation, particularly acute phase proteins (e.g. C-reactive protein [CRP] (28)) and circulating proinflammatory cytokines (e.g. interleukin 6 [IL-6] (24)) are related to CVD risk. Overall, inflammatory and haemostatic factors may be particularly important in older adults, in whom levels are increased (89, 90). Additionally, these risk factors are strongly related to other established cardiovascular risk factors such as smoking, physical inactivity, obesity and blood lipids (91-95).

Mendelian randomisation studies support a causal association of IL-6 with CHD (25), while such association was not found for CRP (96). To understand the role of IL-6 to CVD prevention (26, 27) is important, as in the future IL-6 could potentially be routinely used in blood tests to screen patients for heart problems; however, this will depend on future availability of accurate and relatively low-cost methods for IL-6 measurement. Haemostatic markers (e.g. fibrinogen (97), von Willebrand factor [vWF] (97, 98), and tissue plasminogen activator [t-PA] (99)) and markers of endothelial dysfunction (D-dimer) (100) are associated with an increased CVD risk in adult and older populations. However, their causal associations with CVD remain debated or not yet tested.

2.3 Seasonal variation in CVD

The purpose of the following section is to review the literature on seasonality of CVD focusing on major CVD events (MI and Stroke), and discuss the current gaps in the literature on this topic.

2.3.1 Season, mortality and CVD

The seasons are the periods of the year typically characterised by a particular kind of weather; in the Northern Hemisphere four seasons are typically identified: spring, summer, autumn, and winter (101). The passage of seasons is resulting from the earth's changing position with regard to the sun; meteorological seasons can be recognised by calculating temperature levels, one of the most important attributes (or elements) of the season. In the Northern Hemisphere summer is typically the hottest period of the year and winter the coldest.

Understanding the seasonal variation in mortality is very complex and requires understanding the seasonal variation of specific causes of death, such as CVD and respiratory disease, and their possible environmental triggers, such as outdoor temperatures (30). Evidence for associations between outdoor temperature and mortality is examined in detail in paragraph 2.4.1, being particularly relevant for the scope of this thesis. Seasonal variation in all-cause mortality has long been noted in the UK as in most European countries, and exhibits a peak in winter (December, January, February and March) and a nadir in summer (29, 30). This pattern mainly reflects the seasonal trend in number of deaths from CVD and respiratory disease (102). Overall, the increase in deaths during the winter months is generally attributed to either a

breakdown of the cardiovascular or respiratory systems (29). In England and Wales a common and simple measure to estimate the seasonal variation in mortality is defined as Excess of Winter Deaths (EWDs), which is used every year to inform the population and the media about winter mortality trends (32). The EWDs approach defines the winter season as December to March and compares the number of deaths occurring in this winter period with the average number of deaths occurring in the preceding August to November and the following April to July (non-winter season). The formula used is EWDs = winter deaths – average of non-winter deaths. By definition, this measure is very unsophisticated, but it offers an intuitive and crude estimation of the winter mortality trends of the last decades. In England and Wales from 1991/1992 to 2015/2016 the proportion of the EWDs attributable to CVD and respiratory disease was 37% and 40% respectively (32). From 1991/1992 to 2015/2016, the EWDs for CVD decreased from 47% to 26%, although the EWDs five-year moving average for CVD remained fairly constant since 2002/2003. Similarly, the EWDs five-year moving average for respiratory disease remained fairly constant around 36% from 2010/2011 to 2015/2016 (32).

In the UK, seasonal variation in mortality also varies with age. Increased mortality in the winter months particularly affects people aged 65 and over; the majority of the deaths occurred among those aged 85 and over, who are known to have higher mortality rates in winter in comparison to the rest of the year (Figure 2.1). According to England and Wales national statistics, during the period 2002/2012, the monthly average number of deaths during winter in people aged 85 and over was 18,094 (35.5%) vs 14,760 (33.5%) in summer. Conversely, younger people (<65 years of age) die less in winter (16.1%) vs summer (17.6%). To summarise, anyone vulnerable to almost any underlying medical condition, but especially older people and those with (or at risk of) respiratory and CVDs, are at increased risk of dying in winter (32).

Moreover, in the UK and in comparison to men, a higher proportion of the female population are aged 75 and over (9%, compared with 7% of males in 2013 (103)), so a higher absolute number of women than men could be exposed to cold weather. From one large UK study the winter vs non-winter mortality ratio in women compared with men was 1.11 but mainly driven by a history of respiratory illness (winter vs non winter ratio of 1.20, than cardiovascular illness (winter vs non-winter ratio of 0.97).

2.3.1.1 Main gaps in the evidence

As of today, it is not fully understood to what extent the high winter mortality rates in Europe and the UK are primarily a specific temperature-related or overall seasonal phenomenon (e.g. due to other seasonal factors, such as influenza). Plausible biological pathways linking low temperatures and specific diseases (e.g. cardiovascular disease) have been hypothesised but not fully understood (see Chapter 1 paragraph 1.3.1.1 and Chapter 2 paragraphs 2.3.2, 2.3.3, 2.4.1 and 2.4.5); enhancing our knowledge of such pathways is an objective of this PhD thesis (see Chapter 5, 7, and 8). Moreover, it is still unclear how long a period of cold temperatures is needed before CVD deaths occur (104); I tried to investigate this topic mainly in Chapter 8.

2.3.2 Seasonal variation in myocardial infarction

Seasonal variation in mortality from myocardial infarction (MI) has been well recognised for many years; a winter peak in myocardial infarction (MI) was first reported in 1937 (105). Overall, previous studies estimating the seasonal variations in MI used different methods, making the comparison of these findings very complex (37, 106-109). Overall, MI deaths occur more frequently in winter, when temperatures are typically lower; for example, a comprehensive literature review of public data sources was conducted in 2009 (e.g. publication databases, reference lists, and the websites of a number of relevant public organisations) (109); overall, 8 of the 12 studies which included relevant data from the winter season reported an increased risk of MI at lower temperatures, typically recorded in winter. An association between temperature and MI risk was found for 24 populations, including Europe: the pooled mortality RR was 1.008 per 1°C drop in the temperature averaged over the current and previous 3 days. In one study in Italy MI was slightly more frequent in winter (25.9% of the total number of MI) than in summer (22.6%) (110); in other European studies the seasonal variation (month of peak [December-February] vs nadir) in standardised mortality ratios (SMR) and hospital admissions for MI was respectively 36% (107) and 10% (106). A seasonal pattern dominated by the winter peak was found in people aged 65 and over (108); MI mortality was highest in January (RR = 1.090), and the seasonal variation in MI deaths (winter vs. summer) increased with increasing age: 5.8% for <65, 8.3% for 65 to 74, 13.4% for 75 to 84 and 15.8% for >85 years ($p<0.005$ for trend). The pathophysiologic triggers of the occurrence of MI in winter or at low temperatures may involve blood pressure and numerous haematological factors known to vary by season; this will be discussed later in paragraph 2.4.2 and paragraph 2.4.3). Evidence

of increased MI deaths (+1.1%) at very high maximum temperatures was found (a 1°C increase in temperature above the 93rd percentile, which varied from 20.9°C for the North East to 24.7°C for Greater London) during June–September in England and Wales (111). However, the association of high temperatures with respiratory mortality was stronger than the association of high temperatures with overall CVD mortality (+4.1% vs +1.8% for 1°C increase in temperature above the 93rd percentile respectively).

2.3.3 Seasonal variation in stroke

Most previous studies worldwide have reported a marked increase of both stroke mortality and stroke hospitalizations in the winter (112-117). For example, a large Finnish study collected stroke events from the FINMONICA population-based stroke register from 1982 to 1992 in 15,449 people aged 25 to 99 years (118): the rate of occurrence of stroke events was 12% and 11% greater in men and women respectively in winter than in summer. This difference was mainly due to ischaemic stroke, which is the most common type of stroke in European populations (118). A recent systematic literature review identified all population-based observational studies published before the year 2015 that investigated the association of temperature with stroke (119); in total, the authors included 21 studies with a total of 476,511 participants. The pooled results showed that lower mean temperature was associated with increased intracerebral haemorrhagic stroke (IHS) risk (for a 1 degree Celsius decrease in ambient temperature the IHS risk increased by 3%) but no association was found with ischaemic stroke.

The few previous studies which took place in the UK reported an association between season of the year and stroke incidence, with higher levels in winter (120, 121). For example, 15% more hospital admissions for stroke were registered from December to March (January was the seasonal peak) than from June to September (nadir was September) in between 1st January 1981 and 31st December 1983. Previous studies hypothesised that the occurrence of stroke may particularly be due to elevated levels of blood pressure and LDL-Cholesterol (see paragraph 2.4.2), which are known to vary by season. However, in the UK the seasonal variation in stroke remain controversial: a report from the Oxfordshire Community Stroke Project did not find a seasonal variation in the incidence of ischaemic stroke (122). The authors hypothesised that the lack of seasonal variation could be due to a higher case-fatality rate

caused by the complications of stroke, such as pneumonia, which also exhibit seasonal variation with a peak in winter.

2.4 Factors influencing the seasonal variation in CVD

The purpose of this next section is to review the literature on CVD risk factors which have two specific properties: (i) they were prospectively associated with CVD in previous studies, and (ii) they vary by season. The factors we took into account can be subdivided in three subgroups: environmental seasonal factors, established risk factors, and more recently established (or emerging) risk factors for CVD. Typically, the seasonal variation in CVD risk and CVD risk factors was estimated using outdoor temperature as the main exposure variable (see paragraph 2.4.1.1). In the last section a possible biological mechanism linking temperature and CVD will be presented.

2.4.1 Seasonal variation in meteorological factors

2.4.1.1 Outdoor temperature

In Europe and in the UK, outdoor temperatures exhibit a marked seasonal variation with lower levels in winter and higher in summer. From 1981 to 2010, the average UK outdoor maximum and minimum temperatures were approximately 6.5°C and 1°C in winter (defined as December, January, and February) and approximately 18.5°C and 10°C during summer (defined as July-August) (123). Seasonal variation in CVD is mainly attributed to seasonal variations in outdoor temperatures (e.g. sudden fall in temperature or day-to-day variations). For example, studies conducted in European cities (124-126) and countries (127-129), including Britain, have provided evidence for the association between low temperatures and increased mortality (29, 124-130). For example, in the UK day-to-day changes in outdoor temperature during winter are associated with all-cause mortality (+0.38 daily cases per million people per 1°C decrease in temperature) (130). Also, previous studies demonstrated that both extremely cold days (38, 128) and moderately cold days (35) increased mortality. Worldwide and in the UK the majority of the temperature-attributable deaths seemed to be caused by cold temperatures (7.3% of all-cause mortality) rather than by heat (0.4%) (35). Although this study included a very large number of fatal events in the analysis (about 74 million deaths), the analysis involved aggregation of the number of deaths by day (which means the unit of observation is the day rather than the individual).

2.4.1.2 Other meteorological factors

In an attempt to explain the seasonal variation in mortality and CVD mortality, previous research sought to find alternative or complementary explanatory factors to outdoor temperature, such as wind velocity, relative humidity, precipitation and sea-level pressure (30). However, none of these variables was found to exhibit a spatial distribution that resembles the year-to-year correlation map between winter mean temperature and all-cause mortality (130). However, in one study the seasonal variation in absolute humidity was associated with seasonal variations in influenza mortality (131). Overall, evidence of associations between humidity and CVD are scarce and mainly focused on the interplay between very hot temperatures and high levels of relative humidity (132), a combination of factors which is rarely seen in the UK.

2.4.2 Seasonal variation in established risk factors for CVD

2.4.2.1 Blood pressure

Previous findings consistently demonstrated a seasonal variation in blood pressure; there is also a general agreement that such variation is triggered by the seasonal variation in outdoor temperature (39, 133-137). In the BRHS, when 7735 mean aged 40-59 years were examined at baseline in 1978-1980, negative associations were found between daily maximum outdoor temperature and systolic blood pressure (-0.38 mmHg/°C; $p<0.001$) and diastolic blood pressure (-0.18 mmHg/°C) (137). Also, in one longitudinal study of repeated measures of blood pressure in more than 16,000 middle aged patients (mean age of 51 years) from the Glasgow Blood Pressure Clinic, a decrease of 10°C in outdoor temperature (a change from the highest to lowest quartile) was associated with an increase of 2.1% and 1.6% in SBP and DBP respectively (133). It is important to report that in studies of young subjects the seasonal variation in SBP was smaller (138) or absent in comparison with older subjects (137).

Overall, previous literature strongly supports the hypothesis of outdoor temperature as an environmental trigger for blood pressure. However, in the UK representativeness of the findings and precision of the estimates in older people aged 65 and over can be substantially improved by research carried out in population based studies of older adults.

2.4.2.2 Lipids

Seasonal variations in lipids have been widely studied since the mid-1920s (139). In the last three decades, an increasing number of studies on seasonal variation in lipids levels were published; the findings reported that lipids levels are generally higher during the winter months in both middle aged and older adults (although seasonal differences varied depending on which population and cholesterol component were studied (139-143)). For example, Robinson et al used data collected from 140,000 men and women from the BUPA Medical Centre (London, UK) and found a seasonal pattern, with serum total cholesterol levels being 3–5% higher in winter than in summer, and independently of body mass. Also, mean monthly cholesterol levels were negatively correlated with mean monthly air temperatures (Pearson correlation coefficient varied from -0.60 to -0.71 depending on which parameter was analysed) (139). In one study of more than 55,000 adults in Seoul (21–86 years of age), for a 10°C decrease in outdoor temperatures the levels of LDL-Cholesterol increased by 0.14 mmol/l (144). On the contrary, in one study of 478 men (mean age of 74 years) carried out in the Boston area from Halonen et al., an increase of 10°C in mean ambient temperature increased LDL-Cholesterol by 3.5% approximately, while no variation in total cholesterol was observed (40).

In one small study of 16 healthy young subjects, LDL-Cholesterol peaked in January and reached its lowest levels in July (seasonal difference of 0.29 mmol/l), while HDL cholesterol revealed an inverse pattern with a peak value in August and the lowest value in February (seasonal difference of 0.25 mmol/L) (145). Also, it is unclear whether temperature variations affect triglycerides levels as Halonen et al did not find an association (40). In other studies of middle aged German adults (mean age of 38 years) triglycerides levels were significantly greater in winter for women only (seasonal difference of 0.22 mmol/l), or did not significantly change between February and August (142). In a further study, triglycerides levels exhibited a peak in September and a nadir in April (seasonal difference of 0.19 mmol/l) (145).

Overall, there is a consensus among researchers that there is a seasonal variation in lipids, with higher levels observed during winter months and at lower temperatures. However, associations were reported as both absolute change and percent change in cholesterol levels, making the comparison of such findings difficult. Findings on total and LDL-Cholesterol were much more consistent than findings on HDL and triglycerides. In those studies statistical analysis were

mainly adjusted for age and body mass index; therefore, a common limitation of previous studies was the lack of data on physical activity, use of medication, or health behaviours that might have affected the associations. Only population-based studies using a wide and detailed range of information on life styles and behavioural factors can improve generalisability of the findings and comparison with previous studies.

2.4.3 Seasonal variation in emerging risk factors for CVD

2.4.3.1 Interleukin-6

To my knowledge, only six previous studies investigated whether seasonal variations in Interleukin-6 (IL-6) exist; four studies out of six were in older adults. The biggest European study of temperature-related variations in IL-6 in middle aged and older adults was the AIRGENE study which included 1003 participants aged 35 to 80 years (mean age of 62 years) from 6 different countries (not including the UK), who previously had MI (146). The authors found that a 10°C decrease in the 5-day-average outdoor temperature was associated with an increase of 3.3% (95%CI 0.1-6.3) in IL-6. Schauble et al analysed data from 274 participants (mean age 63) from Augsburg, Germany, with type 2 diabetes mellitus, impaired glucose tolerance or with genetic polymorphisms on the detoxification and inflammation pathways; the authors found that a 5°C decrease in the 5-day average outdoor temperature was associated with an increase of 8.0% (95% CI 0.5% to 16.2%) in IL-6 (147). Two other two studies of older adults aged between 75 and 82 years old, from the Belfast (Northern Ireland) and the Boston (US) area, did not find an association between outdoor temperature (148) or season (149) with IL-6. In a study of 154 Brazilian middle aged adults (mean age 43.5), self-reported length of light exposure was positively associated with IL-6 levels ($\beta = 0.095$ pg/ml in IL-6 per 1 hour increase in light exposure; $p<0.05$), while a further study in young Japanese adults (mean age 21) observed a seasonal variation in IL-6 with 2 peaks; one in winter and the other in summer (150).

To date, findings from studies in older adults did not provide sufficient evidence regarding the association of season or temperature with IL-6. Previous studies were limited due to lack of statistical power or reduced generalisability of the findings at national level (e.g. participants were from one location only or with previous chronic conditions). To my knowledge, seasonal

or temperature-related variation in IL-6 in older people in the general population has yet to be demonstrated in the UK.

2.4.3.2 C-Reactive Protein

Seasonal and temperature-related variations in CRP have been investigated in several previous studies. At all ages CRP levels exhibits a seasonal variation with higher levels recorded in the winter months or at lower temperatures (45, 146-148, 151-156). For example, in the AIRGENE study, a 10°C decrease in the 5-day-average of air temperature was associated with a 4% (95% CI 0.2-8.1) increase in CRP. Halonen et al conducted another study including 673 men with mean age of 74.6 years living in the Boston (US) area and found an increase of 8.0% (95% CI: 1.93, 14.8) in CRP levels per 5°C decrease in temperature (148). Rudnicka et al analysed data of 9377 men and women aged 45 years from the 1958 British Birth Cohort study and observed slightly higher levels in winter months (≈5 ng/ml between November and January vs ≈4 ng/ml from May to August). However, when seasonality was analysed fitting harmonic functions to the data (e.g. using trigonometric functions of day of the year) evidence of seasonal variation was not found (152). Moreover, a Norwegian population based study (Tromsø Study) measured CRP in 38,037 participants (mean age of 50 years) and found that CRP peaked in later winter/spring (2.54 mg/l in March).

Overall, it seems that findings from previous studies in middle aged adults are fairly consistent and excluded a peak in CRP during summer; however, the winter peak in CRP levels may be shifted and did vary by month (generally from November to March). Studies in older adults are rare; the main limitations are the reduced statistical power, due to the small number of participants recruited, and limited generalisability of the findings at national level (e.g. participants from one location only or with previous chronic conditions). To my knowledge, studies of older adults carried out in the UK have not yet demonstrated the existence of seasonal or temperature-related variation in CRP.

2.4.3.3 Other major markers of inflammation and haemostasis

Findings on seasonal variation in Fibrinogen, PV and t-PA are sparse; I found that previous studies observed higher levels of such factors in winter (45, 142, 157-161). Fibrinogen is the most studied of the three: in the Rotterdam Study, a population based study of 7983 men and

women aged 55 and over, the seasonal variation in Fibrinogen (peak in March and nadir in August) was estimated as 0.34 g/L (95%CI 0.29-0.39) and was more pronounced in subjects aged 75 years and over (160). Additional adjustments for BMI, SBP, DBP, total and LDL-Cholesterol did not substantially change the findings. Also, the seasonal variation in Fibrinogen was independent of outdoor temperature although the magnitude of the association of temperature with Fibrinogen was not provided (160). Similarly, and to the best of my knowledge, the association of temperature with t-PA and PV was not investigated in previous studies.

Seasonal variations in Vitamin D were also observed in 9377 participants (mean age=45 years) from the British 1958 Birth Cohort study. In linear regression models, the prevalence of self-reported respiratory infections (influenza, pneumonia, bronchitis or severe cold during 3 weeks prior to blood examination), was negatively correlated with Vitamin D levels; each 10 nmol/l increase in 25(OH)D was associated with a 7% lower risk of infection after adjustment for adiposity, lifestyle and socio-economic factors. Overall, it seems that findings support the hypothesis that influenza infection, a typical element of the winter season, can penetrate the epithelial cells, thus causing the reduction in vitamin D synthesis in the skin (162), and worsening health status. Vitamin D peaked in between August and September (78 nmol/L) and reached the lowest levels in between January and April (40-45 nmol/L). In a smaller study of 96 community-dwelling British men and women aged 65-74 years, Vitamin D average concentration was lowest in winter (22.7mmol/l) and highest in summer (35.4mmol/l). In one German study in older community-dwelling individuals from Southern Germany (n=1193, aged ≥65 years of which 58% men), Vitamin D levels were higher in summer (mean of 24 mg/mL) and autumn (mean of 21 mg/mL) vs winter (16 mg/mL) and spring (14 mg/mL) (163). One of major determinants of Vitamin D variation is sunlight, which is typically reduced in winter vs summer; it is known that Vitamin D levels are typically lower in subjects with reduced sunlight exposure, and the levels increase with increasing duration of sunlight exposure (164, 165).

The seasonal and temperature-related variation in vWF and D-dimer remain poorly investigated; in the British 1958 Birth Cohort study, the vWF peaked in early spring (between March and May (45)). A further study using the same data found that D-dimer showed an

unusual seasonal variation, with peaks in February/March and August/September (152). In the British 1958 Birth Cohort study there was no significant seasonal variation in FEV_1 and FVC, although descriptive statistics found lower levels of such measures in summer; for FEV_1 mean was 3322 mL in winter vs 3278 mL in summer while for FVC the mean was 4221 mL in winter vs 4142 mL in summer (166). It has been hypothesised that FEV_1 and FVC seasonal variations could be related to one of the three pollen seasons (typically tree pollen season occurs between late March and mid-May , grass pollen season from mid-May to July and weed pollen season from the end of June until September); one previous study found that in London an increase in daily total grass pollen concentrations from 2005 to 2011 were associated with increased emergency hospital admissions for asthma amongst adults, with a lag of 2 to 5 days between exposure and after accounting for outdoor temperatures (167). Also, toward the end of summer, concentrations of an airborne fungus peak in August and September and this may lead to lower levels of FEV_1 and FVC (168).

In summary, findings from studies in older adults did not provide sufficient evidence regarding the association of season or temperature especially with Fibrinogen, PV, t-PA, vWF, or D-dimer in older adults. Also, the peaks and dips in some risk factors levels (e.g. Fibrinogen, vWF, and D-Dimer levels peaked in March) was not reflecting the typical seasonal variation in outdoor temperature (nadir in January and peak in August). Further evidence is needed to support the contribution of such factors to the seasonal variation in CVD.

2.4.3.4 Seasonal variation in physical activity and sedentary behaviour

This sub-section presents the literature review on seasonal variation in physical activity (PA) and sedentary behaviour (SB) in older adults, mainly focusing on studies where physical activity was objectively measured. Sensor technology, such as accelerometery, permits objective measurements of physical activity in population-based studies such as the BRHS (see paragraph 3.4.2 for details). Accelerometers can record the exact day and time in which the activity takes place. Therefore, the use of such devices is ideal when trying to estimate specific patterns in physical activity levels, such as diurnal and seasonal variations. This should overcome concerns about questionnaire-based assessment of physical activity in older people, in whom misclassification bias and recall bias (which could be exacerbated by memory loss)

(10) are particularly likely. Accelerometers can also distinguish between time spent in physical activity levels and sedentary behaviour (see paragraph 3.4.2.2) (87).

2.4.3.5 Seasonal variation in physical activity

In 2007 a large systematic review on seasonal patterns in self-reported physical activity identified 37 relevant studies (published 1980–2006) representing a total of 291,883 participants (140,482 male and 152,085 female of different age groups, from 3 to 71 years old) from eight different countries (41). Season was categorised in different ways, for example dividing the year into 4 parts (typically winter: January–March, spring: April–June, summer: July–September, and autumn: October–December), or by using seasonal factors such as average daily temperature, length of the day (or daylight), monthly or total daily precipitation, daily barometric pressure, daily humidity, and average daylight cloud cover. The authors concluded that season and adverse weather conditions (e.g. extremely hot and cold days) are potential determinants of PA; for example, levels of physical activity in the northern hemisphere were highest in spring and summer (April– August), peaking in July–August while energy expenditure decreased in winter. Also, the decline in activity in the shorter days and adverse weather conditions was attributed to the winter season. Lastly, levels of physical activity were typically lower during cold, wet and winter months for both indoor and outdoor activity (41).

Since the use of accelerometers became more common in epidemiological studies during recent years, there have been more publications about seasonal variation in physical activity measured in participants of different age groups (169-171). However, only few studies have investigated the associations between meteorological factors and objectively measured PA in community dwelling older adults: the Nakanojo study (172-175) and PIPAOI project (176) in Japan, the Physical Activity Cohort Scotland study (177-179), a Canadian study (180) and the ActiFE study based in Germany (169). The findings suggested that older adults were more active in summer than winter and that PA levels are positively associated with higher outdoor temperature, longer day length and duration of bright sunshine (169, 172-175, 177-180). The largest study among them investigated a German population of 747 men and 577 women (mean age 74.6 years) with at least one completed day of physical activity assessment (169). In linear regression analyses, increases in global radiation (a proxy of exposure to sunlight) were

strongly associated with increases in daily walking time (16.1 minutes in men and 19.2 minutes in women) comparing an average winter day (with about 0.8 kWh/m2 radiation) vs average summer day (with about 6 kWh/m2 radiation). Also, an increase of 10.8°C in maximum daily temperature extended the walking duration by more than 7 min in both genders. When days were shorter (9 h) vs longer (16 h) the walking duration increased by 12.6 and 13.3 minutes in men and women respectively. Negative associations were seen between wind speed, daily precipitation and humidity with walking time. In mutually adjusted models for several weather parameters, daylight was no longer significant, while associations of walking time with other weather parameters was reduced, but still significant (169). Another study using activity data from community-dwelling people aged 65 and over in the Physical Activity Cohort Scotland (547 participants, mean age 78.5 years) found that for each degree increase in minimum temperature, daily activity counts increased by approximately 0.9%. Similarly for each extra hour in day length, daily activity counts increased by approximately 1.5%. This corresponds to a 16.5% difference between the longest day and shortest day at the latitude at which the study was conducted. The authors found that age, anxiety, and depression did not modify the effect of either day length or minimum temperature on daily activity. However, Witham et al. found a significant interaction between social functioning scores (measured through the SF-36 quality of life questionnaire) and day length (178): the decrease in activity observed during shorter days was especially marked among participants with lower social functioning.

All previous studies have one main limitation; they focussed exclusively on global measures of activity, particularly counts per minute (CPM), steps and time spent walking, and they did not consider time spent in different activity intensities, particularly moderate to vigorous (MVPA), light (LIPA) and sedentary behaviours (SB). Differentiating intensity of PA is important because for most health outcomes, additional benefits occur as the amount of PA increases through higher intensity, greater frequency, and/or longer duration (181). Furthermore, prolonged sedentary time is also independently associated with health outcomes (see Chapter 5 introduction, paragraph 5.2), including cardiovascular disease, regardless of PA level (18).

2.4.3.6 Seasonal variation in sedentary behaviour

Seasonal variation in sedentary behaviour (SB) has been overlooked in sedentary behaviour guidelines (182) and as a determinant of sedentary time (183, 184). Specifically, a large systematic literature review of determinants of sedentary behaviour in older adults (the DEDIPAC study) published in 2015 did not mention season, temperature or other weather parameters among the possible determinants (184). Overall, studies on seasonal variation in sedentary behaviour are less frequent than studies looking at overall activity levels or activities of different intensities (e.g. MVPA). The few published studies investigated middle aged people (185) or younger participants, such as adolescents (186) or children (187, 188). A review of seasonal variation in accelerometer-determined sedentary behaviour in children published in 2012 (16 out of 819 articles were eligible for inclusion) made no conclusions regarding the association of season with SB in children due to small sample size, lack of repeat measures, incomparable definitions of season and inconsistent accelerometer protocols. On the other hand, a small study in 46 UK adults (age 18–65 years old, 72% female) supported the concept that SB is more common in winter than summer. The percentage of time spent in SB was 68.2% in men and 70.5% in women during winter (when the average temperature was 8.1°C) vs 65.0% in men and 65.6% in women during summer (when the average temperature was 14.7°C). The authors concluded that health promotion campaigns need to encourage year-round participation in more PA, whilst limiting SB (185).

Associations between sedentary time with specific seasonal factors, such as outdoor temperature, have not been estimated in published studies of older adults up to June 2015.

2.4.4 Conceptual framework linking factors influencing the seasonal variation in CVD

The CVD seasonal variation can be explained in many ways, but this thesis especially focuses on specific possible pathways linking temperature and CVD (Figure 2.2) in older adults. Two possible putative pathways are presented below:

1) CVD deaths caused by coronary thrombosis due to exposure to cold temperatures, a typical element of the winter season. In summary, the exposure to cold temperature can trigger several changes in our body: first, cold exposure can cause a reduction or even cessation of most of the blood supply to the skin. This reduces

transfer of body heat to the skin and so reduces body heat loss, but it shifts a considerable amount of blood from the skin (even one litre or more) to the central organs of the body (189, 190). That excessive amount of blood overloads the central organs, and it is then disposed of by removal of salt and water from the blood, partly by the kidneys as urine (diuresis). This leaves most of the other components of the blood (e.g. cholesterol) more concentrated, and more prone to clot (30, 39, 138). Higher levels of cholesterol would be responsible for plaque formation on the walls of the coronary arteries. If the plaque ruptures, a platelet aggregation would occur resulting in a blood clot (via stimulation of haemostatic factors). If the blood clot persists, the heart muscle linked with the clot will start to die off due to lack of oxygen (not supplied via blood) causing a heart attack, also called myocardial infarction (31). On a macro level, vasoconstriction can increase central blood volume, which further increases blood pressure and therefore the risk of developing cardiovascular events, such as a myocardial infarction (39).

2) CVD deaths caused by changes in inflammatory status due to exposure to cold temperatures. In summary, exposure to cold temperatures can influence life-style factors; for example, during cold weather people are less active. Decreasing levels of physical activity may exert an adverse effect on cardiovascular disease in part through an inflammatory effect and by influencing haemostatic factors. For example, inflammation has been linked to CVD and lack of physical activity has been associated with an increased risk of CVD (191). The inflammatory and coagulation pathways could potentially become more active with ageing and potentially exert positive feedback on each other, increasing the risk of CVD events.

2.5 Diurnal variation in CVD

The purpose of this section is to review the literature on diurnal variation in cardiovascular disease (CVD). First, the epidemiological and aetiological background to diurnal variation in cardiovascular disease (CVD) is described. This part comprises an overview of how diurnal variation has been assessed, defined, and measured in previous studies (e.g. using hour of the day), when the majority of CVD events occur over the course of the day and which CVD risk factors may play a key role in such variation. Then, this section mainly focuses on describing diurnal variations in two subgroups of factors: (i) physical measurements and blood markers, and (ii) physical activity.

2.5.1 Epidemiology of diurnal variation in CVD

2.5.1.1 Assessing and defining diurnal variation

In epidemiological studies, diurnal variation in CVD has been assessed by comparing frequencies or absolute number of CVD events, typically myocardial infarction and stroke, by time of the day (42) over a specified period of time, generally several years (192) or decades (44). Alternatively, the CVD events are assessed by dividing the day into periods, such as day time vs night time, or by a period of 3, 4, 6 or 12 hours (44, 193). Similarly, diurnal variation in CVD risk factors levels, such as blood pressure, lipids and some well-established inflammatory and haemostatic factors, has been mainly assessed in the literature by comparing individuals' measures at various hours of the day (46, 48-50, 52, 194-201). In the last decade, wearable devices permit objective and accurate assessment of other CVD risk factors, such as physical activity (see Chapter 4 for more details). For example, accelerometers can give insight into how activity levels vary over the course of the day. However, to date there is very little evidence on how PA and SB are structured throughout the day among older people (see paragraph 2.5.3).

Less frequently, diurnal variations in CVD factors and cardiovascular events have been explored by fitting a sinusoidal model to the study data, typically using a trigonometric function of time of the day with two terms (the Fourier terms, a combination of sine and cosine) (45, 202, 203); these models can estimate a peak and a nadir for the variable of interest over 24 hours and estimate the amplitude of the diurnal variation (45, 203).

2.5.1.2 CVD events and time of the day

Literature on diurnal variation in CVD risk is extensive and focused mainly on myocardial infarctions and strokes; overall, the number of CVD events peaks in early morning (around 9:00 hours) and late morning (at about 13:00 hours) (42-44, 192, 193, 203). The most extensive data on the time of myocardial infarction were published in 1976 by the World Health Organization (WHO) in the report of the Myocardial Infarction Community Registers from 19 European centers (204). The WHO report of 8900 patients with myocardial infarction clearly demonstrated a peak incidence from 8 to 11 a.m.. More recent findings confirmed that onset of myocardial infarction (MI) exhibit a similar diurnal variation with MIs two to three times more likely to occur in the morning than in the late evening (2-8). For example, Cohen et al presented findings from 29 studies involving 66,635 patients who experienced MI for the first time in their lives. There was a 40% increase between 6 am and 11:59 am, in comparison with what would have been expected if the MI had been evenly distributed throughout the 24 hours of the day (205). Moreover, Muller et al investigated onset of MI in the Multicenter Investigation of Limitation of Infarct Size (MILIS), a United States multicentre study of acute myocardial infarction. The authors analysed the plasma creatine kinase MB (CK-MB) activity, which permitted objective assessment of the time of onset of myocardial infarction. In total, 2999 patients were admitted with myocardial infarction and screened; the results demonstrated a marked circadian variation in the time of onset of myocardial infarction with a peak incidence between 6 a.m. and noon.

Literature on diurnal variation in stroke events is also vast (44, 206-210). The major publication on stroke events was from Elliot et al (44); the authors conducted a MEDLINE search investigating diurnal variation of onset of stroke in all languages from 1966 to December 1997; they performed a search using both text word searching and the appropriate MESH headings of “circadian variation,” and “stroke, cerebrovascular accident, transient ischaemic attack, or brain attack” (44). In total, 31 publications were found and 11,816 strokes were recorded. There was a marked diurnal variation of onset of strokes, which was remarkably consistent across the various subtypes of stroke: for ischaemic stroke, haemorrhagic stroke and even transient ischaemic attacks, the excess risk during the 6 a.m. to noon time period is significantly higher than would be expected by chance: 89%, 52%, and 80% respectively, compared with the normalized risk for the other 18 hours of the day). Similarly, there is a significantly lower

risk of stroke during the night time hours (midnight to 6 AM) for each stroke subtype: 30%, 54%, and 81% respectively, compared with the normalized risk for the other 18 hours of the day. The study from Elliot et al. is the most important in this field, with similar findings reported in more recent studies published in last decade (206-208, 211).

2.5.1.3 Factors influencing diurnal variation in CVD events

Although the diurnal variation in CVD events is well documented, the biological pathways underlying such variations are not fully understood. This is partially due to lack of understanding of diurnal variations in underlying CVD risk factors (see paragraph 2.5.2) and physical activity (see paragraphs 2.5.3) especially in older adults, in whom CVD is increased. Overall, previous studies support the parallelism of diurnal variations in blood pressure and MI events (42, 48, 192) and blood pressure with stroke events (44, 206, 208); however, such studies could only speculate on the underlying pathophysiological mechanisms. For example, the diurnal variation in MIs was attenuated in patients on beta-blockers, which are known to lower blood pressure levels (42). For stroke events, it is possible that key changes in the early morning due to 'wakeup' stress and related blood pressure surge may further compromise cerebral vascular health, potentially exacerbating the possibility of stroke in the morning (206). Although different types of strokes involve different underlying pathophysiological mechanisms, there was considerable consistency in diurnal patterns of all types of strokes from the literature (44). One previous study including a population of 21,481 male physicians (mean age of 53 years, SD=9) followed up for 12 years highlighted the relevance of bouts of vigorous physical activity alternated with periods of rest or non-vigorous activity; the risk of sudden death during and up to 30 minutes after vigorous exercise was elevated and the authors hypothesised that such irregular activity could potentially trigger sudden cardiac and cerebral events (212). Therefore, it is important to study physical activity patterns per se (see paragraph 2.5.3) in studies on circadian variation in order to understand the circadian changes in circulatory control. Other risk factors that can potentially alter the diurnal patterns in CVD events are age and sex; descriptive statistics from one previous study reported an attenuated diurnal variation in patients of a younger age, and females (42). It is possible that long acting medications for primary or secondary prevention of myocardial infarction have a theoretical advantage of conferring protection in the morning hours, when the cardiovascular event rate is highest and patients are most vulnerable (205). However, a formal interaction test of time of

the day with those variables has not been performed. Among behavioural factors, habitual morning activities such as coffee drinking or cigarette smoking are unlikely to be the cause of MI events, since the MILIS findings indicated that diurnal amplitude of such events was as prominent in patients who did not smoke or drink coffee as it was in those who did (213).

Overall, literature on whether markers of inflammation and haemostasis influence time of day variation in CVD events is very limited and inconclusive (see in paragraph 2.5.2 for more details). Excluding blood pressure, the biological pathways and the role of other risk factors in the diurnal variation in CVD events is not fully understood and whether the effect of time of the day is relevant for CVD risk prediction need to be assessed. Previous studies agree it is important to investigate time of day variations beyond simple descriptive diurnal patterns (45).

2.5.2 Diurnal variations in CVD risk factors

Time of day variations in both established and emerging cardiovascular disease (CVD) risk factors in middle aged adults, such as blood pressure, lipids and some well-established inflammatory and haemostatic factors (e.g. white blood cell, red blood cell, and platelets counts) have been reported for BRHS men when aged between 40 and 59 years (200, 201) as well as in other studies (46, 48-50, 52, 194-199). The importance of assessing such diurnal variations has been repeatedly acknowledged in the literature (48, 194, 195, 200, 214, 215), as this may be relevant to CVD risk prediction and risk stratification (45). Overall, in middle-aged adults levels of blood pressure, total and LDL-cholesterol, and established markers of inflammation (e.g. white blood cell count), showed peaks in the morning, especially between 10:00 am and noon (45, 46, 194) in parallel with incidence of CVD events (see paragraph 2.5.2). However, a steady increase from early morning throughout the day time was also observed (48, 194). For example, an increase in triglycerides and total cholesterol over the course of the day was reported (194); food intake seems to be a major contributor, as triglycerides in particular can increase in response to the proportion of fat in the meal (216). Findings regarding CRP, Fibrinogen, D-dimer, and vWF reported in earlier studies of middle aged adults did not clearly confirm a diurnal pattern: for example previous studies reported that they did not find an association of time of day with CRP (196), D-dimer (214), and vWF (195). In one study, the variation in CRP, Fibrinogen, D-dimer, and vWF attributable to time of day was minimal (45). Moreover, findings from a recent meta-analysis of several small studies

which analysed IL-6 proposed a diurnal pattern, with overall IL-6 levels increased between 08:00 and 19:00 hours (47). However, in two previous very small studies of twelve (217) and five (218) participants, IL-6 peaked at night.

In summary, literature on time of day variation in CVD markers of inflammation and haemostasis in population based studies of older adults is very limited as it is mainly focused on blood pressure (48). It is important to assess effects of any diurnal variations in markers of inflammation and haemostasis on prediction of CVD risk, given the potentially wider use of such markers (e.g. IL-6) in risk stratification and their potential causal links with cardiovascular disease (219).

2.5.3 Diurnal variations in physical activity and sedentary behaviour

As reported in paragraph 2.4.4, there have been an increasing number of epidemiological studies which objectively assessed physical activity (PA) and sedentary behaviour (SB) levels using accelerometers. Due to the fact accelerometers can continuously measure PA levels (e.g. every second) or provide data aggregated by minute (see paragraph 3.4.2), they can accurately assess physical activity patterns over the course of the day. However, to date there is very little evidence on how PA and SB are structured throughout the day among older people (15, 16, 220-223). Existing studies have been small (15, 16, 220, 221, 223) or mainly have focussed on global measures of activity such as counts per minute (CPM) and moderate to vigorous PA (MVPA, see paragraph 3.4.2). Overall, they suggest that older adults were more active in the mornings than during afternoons and evening. For example, a study of 38 healthy active adults (mean age 70 years) reported fewer minutes of MVPA in the evening than in the morning or afternoon (221) and that longer bouts of activity occurred in the morning (6 am-12 pm) more often than afternoon or evening. The AGES-II study of 579 adults aged 73–98 from Iceland reported that the majority of PA occurred between 8 am and 4 pm on an average day (220); the authors also reported that sedentary time was similar across all age groups, except for the oldest age group (>85 years old) who were the most sedentary and PA levels declined with increasing age and BMI. Data from the AGES-II study (220) and from the Baltimore Longitudinal study of Ageing (223) also found that older age groups had a steeper decline in in PA levels over the course of the day. Moreover, activity levels were lower in the mornings in obese than normal weight men in a study of Canadian adults aged 20-79 years (222).

2.6 Research gaps identified from the literature

The studies reviewed in this Chapter revealed research gaps on seasonal and diurnal variations in CVD risk factors and CVD risk. This forms a basis for the investigation in this PhD thesis. I divided the main gaps in the literature in two:

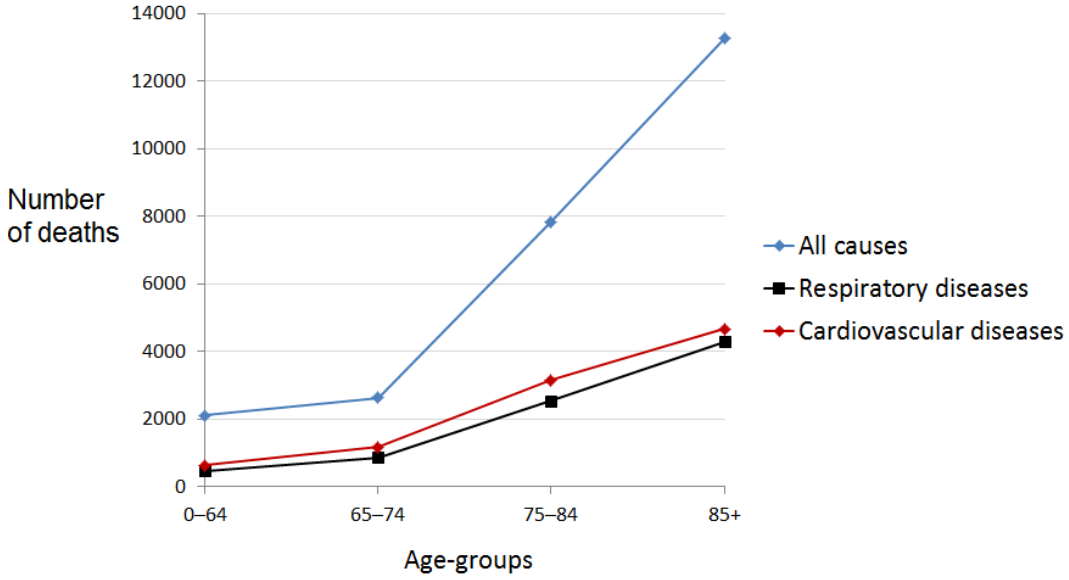
Seasonal variation:

- it is unclear (i) how long a period of cold temperatures is needed before CVD deaths occur, (ii) whether the association of temperature with mortality is modified by individual risk factors, and (iii) whether the temperature-mortality relationship is confounded by other seasonal trends such as the prevalence of influenza;
- which CVD risk factors, especially physical activity levels and emerging risk factors, show a temperature-related variation. Also, there is the need to understand whether such temperature-related associations are modified by individual characteristics.
- It is unclear whether temperature-related changes in physical activity and other emerging CVD risk factors cause an increase in CVD mortality.

Diurnal variation:

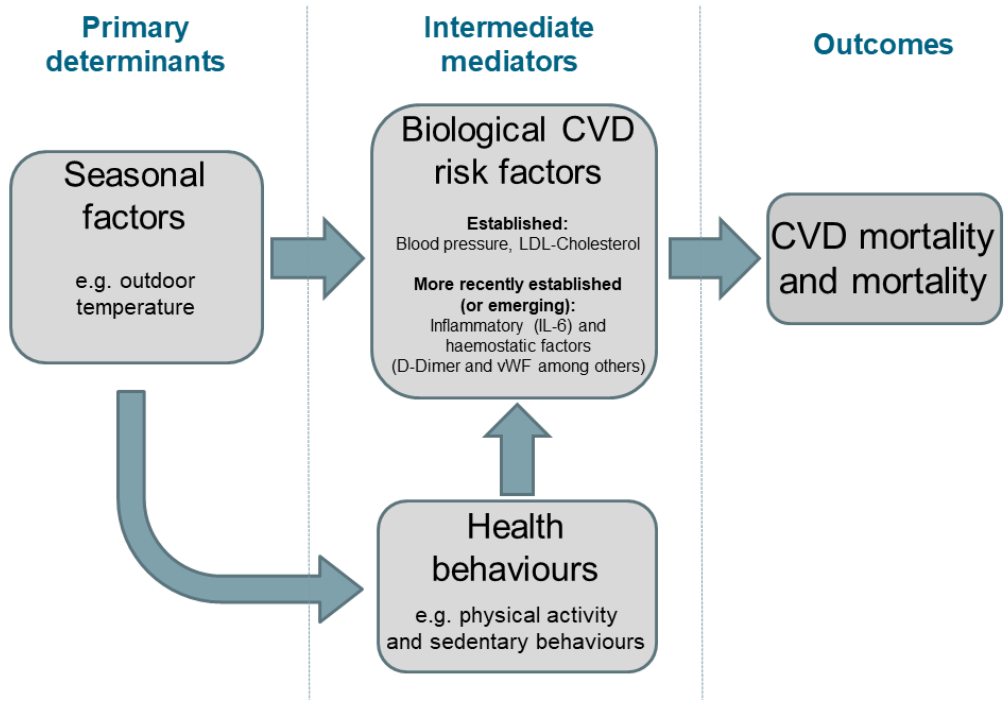
- for diurnal variations, gaps in the literature include investigating sedentary time and light physical activity variations. Also, whether diurnal variations in physical activity levels of different intensities are modified by individual characteristics is unclear.
- The need to understand which established and emerging biological risk factors exhibit a diurnal variation and whether taking account of their diurnal variations can contribute to the accuracy of CVD risk prediction.

Figure 2.1 Excess of winter deaths in England and Wales by cause and age in 2011-2012



Source: Adaptation from Office of national Statistics. 2013

Figure 2.2 Conceptual framework of the present PhD thesis



Chapter 3 **METHODOLOGY**

3.1 Introduction

This thesis comprises epidemiological analysis of data from the British Regional Heart Study (BRHS), an established prospective cohort study of cardiovascular disease. The BRHS was initiated in 1978-80 when men aged 40-59 years were selected from General Practices in 24 towns across Britain in 1978-80 (224). The cohort continues to be followed-up for morbidity through General Practice records, and for mortality through the Office of National Statistics General Register Office. Physical examination including a range of physiological measurements and blood sampling for biochemical measurements were carried out at the start of the study and at follow-up examinations 20 and 32 years later, respectively during 1998-2000 and 2010-2012. Questionnaires at regular intervals during the follow-up have been used to collect information on self-reported health and disease, lifestyle, disability, personal and socioeconomic conditions, and physical activity. From 2010 to 2015 surviving participants have been invited at yearly intervals to wear an accelerometer, a device which objectively assesses physical activity levels of different intensities.

This Chapter gives an overview of the BRHS, including the study design and methodology (paragraph 3.2), measures of meteorological variables linked to the BRHS (section 3.3), a description of the data used in this thesis, including measures of relevant cardiovascular risk factors, self-reported and objective measures of physical activity, and outcomes (paragraph 3.4), and an overview of statistical methods (section 3.5). Specific details of statistical analyses for each of the results Chapters are described in more detail in each of the relevant Chapters (Chapters 4 to 9).

3.2 The British Regional Heart Study

3.2.1 Description of the data source

The aim of the British Regional Heart Study (BRHS) was to investigate the development of cardiovascular disease and its determinants, including geographical and social variations in cardiovascular risk (225, 226). The BRHS is a prospective cohort study based on 7735 men aged 40-59 years recruited from a single local primary care centre in each of 24 British towns in 1978-80. The cohort has been continuously followed up for mortality and morbidity from

baseline until the present. Over time, as the cohort has aged, research conducted on the cohort has increasingly focused on the aetiology and prevention of cardiovascular disease in older ages. Moreover, the development and availability of small lightweight wearable devices to objectively measure physical activity of different intensities permitted an accelerometer study of physical activity levels in the BRHS. All participants provided written informed consent, obtained in accordance with the Declaration of Helsinki (227). The National Research Ethics Service (NRES) Committee for London provided ethical approval.

3.2.2 Selection procedure

The selection of towns to enter the study was based on the following criteria: (226, 228):

- All standard regions within England (North East, North West, Yorkshire and the Humber, West Midlands, East Midlands, South West, South East, and East of England), as well as Wales and Scotland had to be represented.
- Towns had to be discrete entities with populations of 50,000-100,000 at the 1971 Census. In England one larger town was included (Ipswich). In Scotland, some towns below 50,000 were considered to obtain a reasonable number of suitable towns.
- The choice of towns within regions had to reflect the variations in mortality from CVD and water hardness.
- Towns had to be representative of the region in socioeconomic terms.
- Towns with noticeable population movement or with unusual population structure were avoided.
- The study included some towns that were apparent "outliers" when CVD mortality and water hardness were plotted against each other, for example Hartlepool, Exeter, and Harrogate.
- When similar towns met the above criteria, random selection was made between the towns.

The 24 BRHS towns' locations are shown in Figure 3.1. In Table 3.1 the standardised mortality ratios for CVD in 1969-73 in men aged 35-64 years, the number of men examined in each of the 24 towns and the corresponding response rate are reported. Participants were selected from one General Practice in each town and the practices were selected based on size (practice

population >7,500), representativeness of the town's socioeconomic composition and characteristics of the town population and the willingness of the practice to participate. About 400 men aged 40-59 years were selected from the register of each General Practice randomly, stratified into equal five-year age groups (40-44, 45-49, 50-54 and 55-59 years). Men with severe mental or physical disability were excluded (between 6-10% per practice) and the remaining participants were invited to take part. Invitations were sent to almost 10,000 men, signed by their General Practitioners (GPs), encouraging them to attend the cardiovascular health check at a local venue, usually the Practice premises. The response rate for those men invited was 78%, with 18 of the 24 towns having a response rate of 75% or more (Table 3.1). This resulted in a total of 7735 men being recruited into the study, approximately 300 men from each town (225).

3.2.3 Baseline examination

Clinical measurements were made on 7735 men at baseline. Trained nurses collected anthropometric (height and weight), physiological measurements, (blood pressure, electrocardiogram, and lung function), and a blood sample. All baseline examinations were completed in 1978-1980. Men also completed a questionnaire covering date and place of birth, medical and family history, occupation, socio-economic indices, and other relevant information (225). The questionnaire is shown in Appendix IV.

3.2.4 Follow up of participants from baseline

On 01/01/1998, 5875 men were alive (76% of the original cohort). After excluding emigrations, ONS cancellations, subjects currently living overseas, subjects withdrawn from the study, 5516 men were contacted and invited to attend a follow-up clinical measurements; 4252 men (response rate 77%) aged 60–79 years attended. The examinations took place in between February 1998 and March 2000, after an average of 20 years from the baseline examination. Respondents also completed a detailed questionnaire on their lifestyle and medical history. In between May 2010 and July 2012, 3596 surviving participants aged 70-91 years were invited to attend a third reassessment of clinical measurements, after an average of 32 years from the baseline examination. 2147 men (response rate 59.7%) attended and completed a questionnaire on their lifestyle and medical history.

3.2.4.1 Follow-up questionnaires

Questionnaires at the two follow-ups in 1998-2000 and 2010-12 repeated selected baseline questions in order to detect changes in personal circumstances (e.g. marital status), medical history (e.g. cardiovascular conditions and cancer), personal risk factors (e.g. smoking status, alcohol consumption, diet, physical activity), use of medication (e.g. aspirin), self-rated health and disability (225). All standard BRHS questionnaires delivered to participants in 1998-2000 and 2010-12, are displayed in Appendix V and Appendix VI. Further details are provided in section 3.4.

3.2.4.2 Twenty year and thirty-two year examinations

At each of the two follow-up examinations (1998-2000 and 2010-2012), physical measurements were carried out by a team of specially trained nurses who rotated through different workstations to ensure that each nurse contributed an equal number of assessments to each town's data set (see Chapter 7, Table 7.2 for details of the information collected). All men were asked to provide a fasting blood sample, which was collected by using the Sarstedt Monovette system (Sarstedt, Numbrecht, Germany). Participants without diabetes were requested to fast for a minimum of 6 hours prior to their appointment time and to drink only water. Men attended a measurement session at a specified time between 08:00 and 18:00 hours. Within 6 hours of the blood sample collection time, plasma and serum samples were centrifuged, separated and frozen at –20 degrees Celsius and transferred to central laboratories for analysis. Later, the samples were transferred to a central freezer storage location at −70°C within 2 weeks of sample collection. Samples were then transferred on dry ice to a single central laboratory and were thawed immediately before new analysis. Plasma samples were used for all the analyses reported here. The analyses of biomarkers reported in this thesis were carried out after a maximum of 3 years storage for both follow-up examinations; further details of the data collected at the re-examination are described in Paragraph 3.4.

3.2.4.3 Physical activity assessment

Self-reported physical activity data were collected using the same questions approximately every two years from 1998-2000 (in 2003, 2005, and 2007), and on average every year during 2010-2016 (see Appendix V and VI for details of the information collected during 1998-2000 and 2010-2012). At each time point, a physical activity score was derived (see paragraph 3.4.1).

3.2.4.4 Mortality data

The National Health Service Central Registers (Southport for England and Wales, and in Edinburgh for Scotland) were established to collect and classify information on death (225). The Central Register sent death certificates containing identification details, date and place of death and cause of death coded using the International Classification of Diseases ninth revision (ICD-9) and subsequently the tenth edition (ICD-10), see 3.2.4.5 for more details.

3.2.4.5 Morbidity data

Information regarding non-fatal CHD and stroke events was obtained by on-going reports from General Practitioners and by regular reviews of the patients' medical records (225). At 2-yearly intervals a standard medical record review form was sent to the General Practice (see Appendix VII for details) requesting confirmation of each man's continuing registration, current address, and any new cardiovascular events (including myocardial infarction, angina, stroke, transient ischaemic attack and heart failure), or new diagnoses of cancer or diabetes or cardiovascular treatments (coronary artery bypass graft, coronary angioplasty) which had occurred within the last two years. All new non-fatal myocardial infarction and stroke events reported by the General Practices are followed up with an enquiry form to the General Practitioner or hospital consultant to obtain confirmatory evidence that case criteria have been met (225, 229). Criteria for diagnosis of non-fatal myocardial infarction are based on World Health Organization criteria for myocardial infarction, including the presence of any two of three of the following: prolonged chest pain, positive electrocardiogram findings and raised cardiac enzyme levels (71, 72). The criteria for stroke are based on an acute disturbance of cerebral function of vascular origin, producing a neurological deficit lasting for more than 24 hours (74, 225). Men who had re-registered with another General Practice were traced to the new Practice. In addition to the original 24 practices, in 1998-2000 the study included over 1400 practices nationwide (225); follow-up of participants has been maintained for 98% of surviving men throughout.

3.3 Meteorological factors

UK Meteorological data are freely downloadable to UK researchers from the Centre for Environmental Data Analysis (CEDA) web site (http://www.ceda.ac.uk). In collaboration with experts from the UK Meteorological (MET) Office (http://www.metoffice.gov.uk), 35 weather

stations were chosen to be representative of the weather for the 24 BRHS towns and their surroundings (Figure 3.2). The data covered the period between 01/01/1998 and 31/10/2014. The participants resident in each of the 24 UK towns and their surroundings were matched with the closest weather station via post code of residence (mean distance approximately 10 kilometres). Details of imputation of missing data are described in paragraph 3.3.3, while linkage with the BRHS data are described in the "Methods" sections of Chapters 5, 7 8.

3.3.1 Outdoor temperature

Mean outdoor temperature was chosen as main seasonal factor and exposure variable of interest in this PhD thesis (as reported in paragraph 1.3.1.1). Mean outdoor temperature was consistently used in the analyses conducted in Chapters 5, 7 and 8 (seasonal variations in physical activity, seasonal variations in CVD markers, and seasonal variations in CVD mortality). The Meteorological Office provided outdoor maximum and minimum temperatures in degrees Celsius (°C) during spells of 12 hours each from 9am to 9pm and from 9pm to 9am which were used only for Chapter 5 (see method section paragraph 5.4). The Meteorological Office also provided outdoor maximum and minimum temperatures in degrees Celsius (°C) during spells of 24 hours (e.g. temperatures from 9pm of 15/01/2007 to 9pm of 16/01/2007 were used for the 16/01/2007); such temperatures were used specifically for Chapters 7 and 8 (see respectively method sections in paragraph 7.4 and 8.4 for more details). Mean outdoor temperature was always calculated as the average of maximum and minimum temperatures.

3.3.2 Other meteorological variables

Sunshine duration (hours) and relative humidity (RH) % were also collected for analysis in Chapter 5 (seasonal variation in physical activity) and to investigate association of sunshine duration with Vitamin D in Chapter 7. Sunshine duration and RH % were considered of secondary importance for the scope of this thesis which focuses on outdoor temperature as the main exposure variable; moreover, and according to one previous study assessing accuracy of meteorological station network measurements, the higher spatial variability of solar radiation and RH% in comparison with temperature make them less accurate exposure variables in environmental studies (230). Rainfall and wind speed were not investigated because we considered them too prone to local fluctuations within short distances and time intervals. For analysis in Chapter 5, the percentage of relative humidity (RH%) is a single value recorded

each day at 9am ; hours of sunshine were recorded from 00:00 - 23:59 each day (see methods section in Chapter 5, paragraph 5.4.3 for more details). Snow precipitation was not explored because there was so little during the follow up periods.

3.3.3 Imputation of missing values

Because not all 35 weather stations covered the entire period (between 01/01/1998 and 31/10/2014), missing values of maximum temperature, minimum temperature, RH% and hours of sunshine were imputed similarly to previous studies (231). The overall percentage of missing data was 1.2% (between 0 and 8% depending on weather station). The imputation process began by calculating the correlations between all pairs of the 35 stations for each of the meteorological variables. To impute the missing values for a given station, the station with complete data that had the highest correlation with the station being predicted and that belonged to the same BRHS region (South, Midlands, North, Scotland) was chosen as a predictor variable in a regression model. The regression model used to predict missing temperatures included as predictors temperature in the chosen station, month and the interaction of the two variables. Imputed estimates were validated first, by comparing them to observed data values for a single year (reference) with completed data for the two stations with the highest correlation. Next, the beta coefficients from the model using that reference year was used in the imputation of the remaining years (231). The comparison of predictions to actual values returned R^2 values above 90% for all stations, and for both maximum and minimum temperatures. A similar process was used to impute missing RH% and hours of sunshine. Imputations were obtained from a regression model including humidity and maximum temperature variables from the two weather stations that showed the highest correlation with the station being predicted. The comparison of predictions to actual values returned R^2 values above 75% and 80% for RH% and hours of sunshine respectively.

3.4 Data used in this thesis

This thesis uses data from the second participants' examination (follow up at 20 years, in 1998-2000) to address the objectives reported in Chapter 6 and 7, and the third examination (follow-up at 32 years, in 2010-2012) to address the objectives of Chapters 4 and 5. To achieve the objectives of Chapter 8, the follow-up data on mortality from CVD and all-causes were collected prospectively from the second participants' examination until the 31/10/2014, and

meteorological data collected covered the same period. In addition, some socioeconomic variables have been used from questionnaires at 1978-80 (baseline). The next section will present how data on physical activity levels, established and emerging risk factors and outcomes used in this thesis were collected, processed and defined.

3.4.1 Self-reported physical activity

The self-reported physical activity data used in Chapters 6 and 7 in statistical analysis were collected during 1998-2000, while self-reported physical activity data used in statistical analysis of Chapter 8 (methods described in detail in paragraph 3.5.3) were collected in 1998-2000 (follow-up year 20) and 2010-2012 (follow-up year 32). The same self-completed questionnaire on physical activity was used at the two follow-up times (see "Methods" section of Chapters 6, 7, and 8 for more details). Men were asked questions relating to their usual patterns of physical activity under the headings of (i) regular walking and cycling related to daily journeys, (ii) recreational activity including gardening, pleasure walking, and do-it yourself jobs and (iii) Sporting activity including for example running, golf, swimming, tennis, sailing and digging. A physical activity index ranging from 0 to 46 was derived for each man based on the three domains. Scores were assigned for each type and duration of activity on the basis of the intensity and energy demands of the activities reported based on Minnesota intensity codes (41). The total score is not a measure of total time spent doing physical activity but is a relative measure of how much physical activity has been carried out. Participants were classified into a six category score based on their physical activity index: inactive (index 0-2), occasional (index 3-5; regular walking or recreational activity only), light (index 6-8; more frequent recreational activities or vigorous exercise less than once a week), moderate (index 9-12; cycling or very frequent recreational activities or sporting activity once a week), moderately-vigorous (index 13-20; sporting activity at least once a week or frequent cycling, plus frequent recreational activities or walking, or frequent sporting activity only), and vigorous index ≥ 21. The resulting 6 category score was initially validated against resting heart rate and FEV_1 from data collected when men were aged 40-59 years in 1978-80 (232). A further validation was carried out on data collected in 2010-2012 where the 6 category score was compared with objectively measured physical activity, resting heart rate, and FEV_1 when men were aged 71–93 years (233). Further support for use of self-reported physical activity comes from a more recent analysis of the BRHS physical activity questionnaire administered in 2010-

2012 which was shown to relate strongly to physical activity measured by accelerometers during the same period (233).

3.4.2 Objectively measured physical activity by using accelerometers

In Chapter 4 and 5 I used objectively measured physical activity data, collected during 2010-2012 (follow-up year 32). The BRHS men who attended the follow-up examination in 2010-2012 were asked to wear an Actigraph GT3x accelerometer (Pensacola, Florida) over the right hip on an elasticated belt for 7 days, during waking hours, removing it for bathing, swimming or showering and returning the device by post. Actigraph accelerometers record physical activity "counts" and steps, which both depend upon the frequency and intensity of the raw acceleration. The Actigraph GT3X measures accelerations in three individual orthogonal plans associated with motion [the vertical (VT), antero-posterior (AP), and medio-lateral (ML) plan], and also provides activity counts as a composite vector magnitude of these three axes (VM, which is the square root of the sum of the squares of activity counts in each axis). In this thesis we used data from the VT axis only, which is the dominant axis for walking based activities or in relation to number of steps; in the BRHS the Pearson correlation between VT and steps (by minute) was 0.92 ($p<0.001$) while the correlation between VM and steps (by minute) was 0.86 ($p<0.001$). From the VT counts is possible to derive the time spent in physical activity levels of different intensities, such as time spent in sedentary behaviour (SB), light physical activity (LIPA) and moderate to vigorous physical activity (MVPA). It must be noted that when this PhD thesis started in 2014 agreed cut-points for VM counts had not been defined in the literature.

3.4.2.1 Wear time calculation

First, to separate non-wear time from wear time, a sensitivity analysis was carried out using 3 different algorithms based on different 'non-wear time windows' of 120, 90 and 60 minutes of zero counts) in a sample of 100 randomly selected men. We compared the self-reported wear time (when the men reported putting on and taking off the accelerometer, see paragraph 3.4.2.3 "Log diary and questionnaire data") to the wear time derived from algorithms using 3 different non-wear time windows. In the sample of 100 men, the average self-reported wear time per day, over 1 week, was 863 minutes (SD=80); the difference between self-report wear time vs algorithm wear time was -8 minutes (SD=54), -1 minute (SD=57) and +28 minutes (SD=76)

for non-wear time window of 120, 90 and 60 minutes respectively. The algorithm which made use of the non-wear time window of 90 minutes performed best with the mean difference being closest to zero and the SD of differences being similar to that obtained for 120 minutes and smaller than that obtained for 60 minutes; therefore we used that option for the overall population. In detail, non-wear time was identified and excluded using the R package "Physical Activity" (234), based on (i) periods of continuous zero activity lasting more than 90 minutes or (ii) periods of zero activity lasting more than 90 minutes broken only by non-zero counts lasting up to 2 minutes, provided no activity counts were detected during both the 30 minutes before and after that interval (17), as showed in Figure 3.4. Valid wear days were defined as >=600 minutes wear time, and participants with at least 3 valid days were included in analyses (Chapters 4 and 5), a conventional requirement for estimating usual PA level (235). Overall, 98% of BRHS men who wore the accelerometer met this requirement and were included in the analysis in Chapter 4 and Chapter 5.

3.4.2.2 Definition of physical activity levels during wear time

The number of minutes per day in spent in sedentary behaviour, light physical activity (LIPA) and moderate to vigorous physical activity (MVPA) was also derived and categorised using VT count-based intensity threshold values of counts per minute (CPM) developed for older adults, as in previous studies; the cut-points used (Figure 3.3) were <100, 100-1040, >1040 CPM for sedentary time (<1.5 METs Metabolic Equivalent of Task), time spent in LIPA (1.5-2.9 METs) and MVPA (>=3 METs) respectively (221, 236). The percentage of time spent in SB, LIPA and MVPA was also calculated using the number of minutes per day spent in each of three categories of intensity divided by the wear time in minutes during the same day. Subsidiary variables were also defined, including time spent in SB, LIPA and MVPA in bouts of different length (e.g. 5, 10, 15, or 30 minutes). The number of sedentary breaks per hour was also derived (defined as at least 1 min where the accelerometer registers ≥100 counts/min following 1 minute of sedentary time). All physical activity variables were also averaged over a valid week of data and the calculation accounted for both valid days and daily wear time. The number of sedentary bouts of at least 1 hour (a period of 60 or more consecutive minutes where the accelerometer registers <100 CPM) and MVPA bouts of at least 10 minutes (a period of 10 or more consecutive minutes where the accelerometer registers more than 1040 CPM, which did not allow any "grace period" of minutes equal or less than 1040 CPM). As a subsidiary

variable to be used in sensitivity analysis, time spent in MVPA in bouts of at least 10 minutes were calculated when the accelerometer registers more than 1951 CPM was used (236).

3.4.3 Log diary and questionnaire data

For completeness of information, participants completed a log diary while wearing the accelerometer detailing when the accelerometer was put on and taken off during the seven days of wear. Participants' log diaries were checked and matched against accelerometer data to verify the date on which they started wearing the device. During the first 3 days the respondents were also asked to report the type of activity (e.g. housework, gardening, preparing meals, watching TV) that they did during each hour of the day. Participants were asked to send back the accelerometer and log-diary questionnaire by post (expenses were covered by the BRHS) as soon as possible after the 7th day of wear as the accelerometer battery lasts approximately three weeks.

3.4.4 Physical and blood measurements

3.4.4.1 Anthropometric measurements

During physical examinations in 1998-2000, height and weight were measured while the participants were standing, in light clothing and without shoes. Height was measured with a Harpenden stadiometer to the last complete 0.1 cm. Weight was measured with a Soehnle digital electronic scale to the last complete 0.1 kg. At physical examination in 2010-2012, height and weight were measured using the same procedure, but using the Tanita MA-418-BC body composition analyser (Tanita, Tokyo, Japan). Body mass index (BMI) was calculated for each man in Kg/m^2.

3.4.4.2 Blood pressure

Blood pressure (BP, in mmHg) collected at the physical examinations in 1998-2000 (follow-up year 20) was measured in duplicate in the right arm with a Dinamap 1846SX automated blood pressure monitor (Critikon Inc, Tampa, USA) with the participant seated and the arm supported (237). During 2012-2012 (follow-up year 32) BP was measured using an Omron blood pressure recorder twice in succession in the right arm, with the subject seated and the arm supported. At each examination, blood pressure was adjusted for observer variation (238), and the mean of the two blood pressure recordings was used in this thesis.

3.4.4.3 Lung function

At the physical examinations in 1998-2000, lung function measured in litres (Forced Expiratory Flow after 1 second [FEV1], and Forced vital capacity [FVC]) was measured using a Vitalograph. The final value used in all analysis was the best performance out of three readings. The lung function measurements for each participant were adjusted by height using a standard method (239): adjusted FEV1 = observed FEV1 *$(1.73/\text{height})^2$, where height is measured in metres and 1.73 is the mean height for the population.

3.4.4.4 Lipids

At the physical examinations in 1998-2000, cholesterol levels (in mmol/L) after collection were analysed at the Department of Chemical Pathology, Royal Free Hospital. Fasting serum total cholesterol and high density lipoprotein (HDL) cholesterol, and triglycerides were measured using an automated analyser Hitachi 747 (Hitachi, Tokyo, Japan) (201). Low density lipoprotein (LDL) cholesterol was calculated using the Fredrickson-Friedewald equation (240). Triglycerides and LDL cholesterol concentrations were adjusted for the period of fasting and the time of day the blood sample was taken (201). Intra- and inter-assay coefficient of variations were ≤6.5% for HDL-Cholesterol, ≤3% for total cholesterol and triglycerides.

At the physical examinations in 2010-2012, cholesterol levels and triglycerides were measured at the Department of Chemical Pathology, Royal Free Hospital (241) with enzymatic colorimetric assays using methods of Nauck et al and Wahlefeld et al, respectively (242, 243). Intra- and inter-assay coefficient of variations were ≤2% for HDL-Cholesterol, total cholesterol and triglycerides.

3.4.4.5 Inflammatory and haemostatic factors

Haemostatic and inflammatory markers were also analysed in citrated blood plasma at the Department of Medicine, University of Glasgow (244, 245). The risk factors and their units of measurement were as follows: CRP (mg/L), IL-6 (pg/mL), Fibrinogen (g/L), PV (mPa.s), t-PA (ng/mL), vWF (IU/dL), D-dimer (ng/mL), and Vitamin D (ng/mL).

During 1998-2000 examinations, plasma D-dimer, PV, and t-PA levels were measured using an enzyme-linked immunosorbent assay (ELISA; Biopool AB, Umeå, Sweden), as was vWF

antigen (Dako, High Wycombe, UK). C-reactive protein was assayed using ultrasensitive nephelometry (Dade Behring, Milton Keynes, UK). IL-6 was assayed using a high-sensitivity ELISA (R & D Sys-tems, Oxford, UK). Fibrinogen was assayed using an automated Clauss assay in a coagulometer (MDA-180, Organon Teknika, Cambridge, UK). For plasma Vitamin D, measurement of 25OHD was performed on EDTA-anticoagulated plasma via a high-throughput method for the measurement of 25OHD3 and 25OHD2 using a gold-standard liquid chromatography-tandem mass spectrometry method following an automated solid-phase extraction procedure. Our method is calibrated and controlled using reagents from Chromsystems GmbH (Manchester, United Kingdom) and is currently in routine clinical use. The lower limit of sensitivity was 4 ng/mL for both 25OHD3 and 25OHD2. Results are reported as total 25OHD (25OHD2, plus 25OHD3); virtually all participants had an undetectable 25OHD2 which is commensurate with results observed in routine National Health Service use (246). Intra- and inter-assay Coefficient of Variations (CVs) were, respectively: 4.1% and 6.6% for t-PA; 3.2% and 4.2% for vWF; 4.7% and 5.2% for D-dimer; 4.7% and 8.3% for CRP; 7.5% and 8.9% for IL-6; 2.6% and 3.7% for Fibrinogen, 6.2% and 7.9% for VitD and PV.

At the physical examinations in 2010-2012, a slightly reduced number of variables were measured (at this occasion Fibrinogen, Vitamin D, and PV were not measured). The Department of Medicine, University of Glasgow analysed fasting venous blood samples for IL-6 (pg·mL−1), CRP (mg·L−1), tPA (ng·mL−1), vWF (IU·dL−1), and D-Dimer (ng·mL−1). CRP was assayed using ultrasensitive assay on an automated clinically validated analyzer (e411; Roche, Burgess Hill, UK) using the manufacturer's calibrators and controls (coefficient of variation 6.9%). Plasma levels of high-sensitivity IL-6 (R&D Systems, Oxon, UK), tPA and D-dimer (Asserachrom assays; Stago, Theale, UK), and vWF antigen (Technozym assay; Pathway Diagnostics, Dorking, UK) were measured using enzyme-linked immunosorbent assays. Intra- and interassay coefficients of variation, respectively, were as follows: 5.9% and 11.6% (IL-6), 5.5% and 4.1% (tPA), 14.1% and 14.3% (vWF), 5.4% and 3.2% (D-dimer).

3.4.5 Socioeconomic circumstances and life-style variables

3.4.5.1 Socioeconomic position

Adult socioeconomic position was defined as the longest held occupation of participants as recorded at study entry (aged 40-59 years), via a nurse-administered questionnaire in 1978-80 (Appendix VIII). The Registrar General's Classification of Occupations (247) was used to classify subjects into six occupational social class categories: I (professional occupations e.g. barristers, physicians, engineers), II (intermediate occupations e.g. teachers, sales managers), III non-manual (skilled non-manual occupations e.g. clerks, shop assistants), III manual (skilled manual occupations e.g. bricklayers, coalminers), IV (partly skilled occupations e.g. bus conductors, postmen) and V (unskilled occupations e.g. porters, general labourers). Occupational social classes were categorised into three groups: non-manual (social classes I, II, III non-manual) and manual (social classes III manual, IV, V) and Armed Forces.

When comparing the social class status measured at baseline in 1978-1980 vs follow-up in 1998-2000, the majority of the non-manual and manual social class groups remained within the same group (86% and 83% respectively). The proportion of subjects who had changed social class status from baseline was 9%. Thus, the longest-held occupation was considered to be a stable marker of social class; therefore, it is also likely to fulfil the criterion of using a single measure of adult social class in statistical analysis. This would act as a reference point over the entire study period covered by this thesis, as it was considered to most adequately reflect the socioeconomic position of the BRHS men for most of their adult life. In conclusion, the longest-held occupation was used as the main measure of adult socioeconomic position in the thesis.

In additional to occupational social class, information was collected on other measures of socioeconomic position. The 20 year re-examination questionnaire in 1998-2000 asked participants about car and house ownership, and whether they had central heating at home (Appendix V).

3.4.5.2 Smoking status

Detailed questions on cigarette smoking habits were obtained from the self-completed questionnaire during 1998-2000 and 2010-2012. Participants were asked whether they had ever

smoked cigarettes regularly (at least 1 a day), whether they smoke cigarettes at present and, if not, at what age they gave up smoking. From the information given, men were classified into three cigarette smoking groups (never smoked; ex-smokers; current smokers) (93).

3.4.5.3 Other personal circumstances

During 1998-2000 and 2010-2012 follow-ups, the BRHS men were asked what their marital status was (single; married; widowed; divorced/separated; other); this variable was dichotomised as married vs not in statistical analysis.

During 2010-2012, BRHS men were asked "do you have any difficulties getting about outdoors?" which was grouped as "none", "slight" and "moderate/ severe/ unable to do". Men were also classified as having vision problems if they had one or more of glaucoma, macular degeneration or cataract, as in advanced age these are primary causes of visual dysfunction (248, 249). Men scoring >=2 on the 4-item Geriatric Depression score were classified as depressed (250). Participants completed the Lubben scale of social isolation which asks about interactions with family members and with friends, men scoring <12 were classed as at risk of social isolation (251). Participants reported which forms of transport they used regularly (car, public transport, dial a ride, walk or cycle); this variable was finally dichotomised: one category included those who reported walking or cycling (active transport); a second category included those who selected car, public transport and dial a ride. Information on participants' disability were collected: mobility limitation was determined by asking participants whether they had difficulty in getting about outdoors; three categories were created: none, slight, and severe mobility limitations/unable to do).

3.4.6 Prevalent disease

During 2010-2012 follow-up, BRHS men were classified according to the number of chronic conditions according to whether they recall a doctor telling them (previous heart attack, heart failure, angina, diabetes, stroke, osteoporosis, claudication, Parkinson's disease, chronic kidney disease and cancer). Participants provided a 'yes/no' response to each question. The number of chronic conditions were categorised as None, 1 or 2, and 3+ chronic conditions.

3.4.7 Incident disease

The two main outcome variables assessed in this thesis are CVD mortality and all-cause mortality. Fatal cases were ascertained through the National Health Services Central Registers (death certificates with International Classification of Diseases-Ninth Revision) as described in section 3.2.4.4). CHD fatal events were classified with ICD-9 codes 410-414. For stroke fatal events, indicating deaths with cerebrovascular disease as the underlying cause, the ICD9 codes 430–438 were used. CVD mortality was defined as all fatal CVD deaths (ICD-9 codes 390-459). All-cause mortality was defined as death from any cause. CVD mortality and mortality from all-causes were collected prospectively from the second participants' examination in 1998-2000 until 31/10/2014.

3.5 Statistical methods

All statistical analyses were carried out using Stata versions 12-14 (Stata Corp., College Station, Texas). The recurrent statistical methods used in this thesis are described below. However, statistical analyses are described in more detail in each Chapter.

3.5.1 Generalised regression models

Generalised linear models are used to analyse the relationship between an exposure variable and an outcome variable (252). Specifically, linear regression is used to relate a continuous outcome variable (y) to exposure variables (x) as follows:

$$Y = \beta_0 + \beta_1 X + \varepsilon$$

β_0 is the intercept (the value of y where x = 0) and β_1 is the regression coefficient (the increase in y for every unit increase in x), and ε is the error term. The error term ε is as assumed to be Gaussian distributed, with zero mean and variance independent of the value of x. Linear regression assumes that for any value of x, y is normally distributed and that the magnitude of the scatter of the points about the line is the same for all values of x. In this thesis the linear regression technique was used in combination with multilevel modelling (a generalization of regression methods) to assess the cross-sectional associations examined in Chapters 4, 5, 6 and 7.

3.5.2 Multilevel models

Multilevel models, also known as mixed models (252) are widely used in epidemiological studies having a hierarchical or clustered structure, and have an extended range of assumptions compared with other major general linear models (e.g., ANOVA, linear regression). The complex nature of a study design such as the BRHS necessitates the use of such models. Multilevel models recognise the existence of data hierarchies (see section "Methods" in Chapters 4, 5, 6 and 7 for details specific to those hierarchies in analyses) and have the ability to separately estimate the predictive effects of an individual predictor and its cluster-level mean (or a predictor measured just at cluster level), taking into account for residual components at each level in the hierarchy (252). Multilevel modelling can also be used to analyse repeated measures data, as when a dependent variable is measured in individuals several times over a certain period (see section "Methods" in Chapters 4 and 5); in this case the repeated measurements can be thought of clustered within individuals.

Multilevel models can be used on data with many levels (≥2), although in this thesis 2-level models were used. In the case of a two levels model with one dependent variable and one predictor, the dependent (outcome) variable is always investigated at level 1, as reported below

Equation at level 1:

$Y_{ij} = \beta_{0j} + \beta_{1j} X_{ij} + \varepsilon_{ij}$

where:

- the subscript j defines the groups (level 2) and the subscript i refers to the individual-level data (level 1)
- Y_{ij} refers to the value of the dependent variable at level 1 (subscript i refers to the individual case, subscript j refers to the group)
- X_{ij} refers to the predictor at level 1
- β_{0j} refers to the intercept of the dependent variable in group j (level 2).
- β_{1j} refers to the slope for the relationship in group j (level 2) between the predictor at level 1 and the dependent variable.
- ε_{ij} refers to the error term of prediction for the level 1 equation

In comparison with a standard regression model which does not recognise levels, the multilevel models approach assumes that each group has a different intercept β_{0j}, and different slopes coefficients β_{1j}. This is indicated in the equation by attaching a subscript j to the regression coefficients. Because the regression coefficients β_{0j} and β_{1j} vary across the groups, this variation can be modeled at the group level (or level 2):

Equation at level 2:

$\beta_{0j} = \Upsilon_{00} + \Upsilon_{01} W_j + U_{0j}$

$\beta_{1j} = \Upsilon_{10} + \Upsilon_{11} W_j + U_{1j}$

where:

- Wj refers to the predictor at the level 2;
- Υ_{00} refers to the overall intercept. This is the grand mean of the scores on the dependent variable across all the groups when all the predictors are equal to zero
- Υ_{01} is the slope of the regression coefficient used to predict β_{0j} from W_j
- U_{0j} refers to the error term in the equation for β_{0j}
- Υ_{10} refers to intercept of the regression coefficient used to predict β_{1j} from W_j
- Υ_{11} is the slope of the regression coefficient used to predict β_{1j} from W_j
- U_{1j} refers to the error term in the equation for β_{1j}

3.5.2.1 Random intercept models

This is the case of a model where intercepts are allowed to vary, and therefore, the value of the dependent variable for each single observation (level 1) is predicted by the intercept that varies across groups (level 2). This model assumes that slopes are fixed (the same across different contexts). A random intercept model was used in Chapters 5, 6, and 7 (see methods section in paragraph 5.4, paragraph 6.4, and paragraph 7.4 for further details on which explanatory variables were set at level 1 and 2).

3.5.2.2 Random intercept and slope models

This is the case of a model where both intercepts and slopes are allowed to vary across groups, meaning that they vary according to the level 2 unit. This model was used in Chapter 4 to

estimate the change in physical activity levels during the day (derived for 3 points in time: morning, afternoon, and evening) and across BRHS men (see methods section in paragraph 5.4, for further details on which explanatory variables were set at level 1 and 2).

3.5.3 Survival analysis and Cox proportional hazards regression analysis

Survival analysis is used to investigate the probability of having an event when time to a binary event is the main outcome of interest (252). Survival time for each participant is the time from a predetermined start point e.g. entry into the study, until the occurrence of the event of interest. For some participants the time to the event of interest may be censored, if the event has not occurred at the end of follow-up, they were lost to follow-up after a certain date or if they die from a cause other than the event of interest. Cox proportional hazards regression analysis is used to examine the association between an exposure variable and a time to event outcome variable, and is the most commonly used approach to the regression analysis of survival data. It assumes the ratio of the hazards comparing different exposure groups is constant over time, which is known as the proportional hazards (PH) assumption, that can be tested with the Schoenfeld's global test for the violation of proportional hazards assumption (253). In summary, the test concerns whether the slope of scaled residuals from the model against time is zero or not. If the slope of the residuals is not zero then the PH assumption has been violated. The mathematical form of the Cox proportional hazards model is:

$$h(t)=h_0(t) \times \exp(\beta_1 x_1 + \beta_2 x_2 +...+ \beta_p x_p)$$

where h(t) is the hazard at time t, $h_0(t)$ is the baseline hazard (the hazard for an individual in whom all exposure variables = 0) at time t, and x_1 to x_p are the p exposure variables.

The hazard ratio comparing exposed and unexposed individuals at time t is:

$$HR(t) = h_0(t) \times \exp(\beta_1)/h_0(t) = \exp(\beta_1)$$

where β_1 is the coefficient for the relationship between the predictor and the outcome variable associated with 1 unit increase in a single exposure variable x_1, assuming x_2, …, x_p remain constant.

Survival analysis and Cox proportional hazards regression analysis has been used to assess the prospective associations between outdoor temperature, physical activity, and CVD risk factors with the risk of cardiovascular mortality and all-cause mortality during 1998-200 to 31/10/2014 in Chapter 8. Specifically, a time-varying Cox model was used because outdoor temperature (exposure variable) has been fitted in the model as a time-varying risk factor. For this model to work it is essential to organize the data in a specific longitudinal form (see Chapter 8 for more details, paragraph 8.4). In summary, the time-varying risk factors in Cox models refers to serial measurements of that risk factor during the follow-up time. Most statistical packages such as Stata and R will easily do this analysis. However, the interpretation of the coefficient needs to be clarified; in a time-varying covariates analysis, the follow-up time for each patient is divided into different time windows (1 day, 1 month, 1 year, etc). First, for each time–window, a separate contribution to the partial likelihood function is calculated using the value of the time-dependent variable for all participants still at risk at the beginning of that specific time window. These are multiplied together for all time windows to produce a likelihood function to be maximised. The final HR, derived from a weighted average of effects observed at each time window, refers to the instantaneous relative risk for an individual with a particular covariate value (254). Alongside time dependent variables it is possible to fit non-time-dependent variables, which are fixed by definitions made. For example, social class in the BRHS was based on the longest held occupation (see paragraph 3.4.5.1); this explains why it was fitted in model as a non-time dependent variable.

This chapter has described the reasons for using BRHS data in this thesis, and a summary of such reasons have been already offered in Chapter 1 (paragraph 1.4.4), therefore this will not be repeated here. I would like to highlight here that the linkage of the BRHS data with outdoor temperature measurements collected from 1998 to 2014 was possible thanks to a collaboration with the UK Meteorological Office (see paragraph 1.4.2). This linkage allowed the statistical analysis performed in Chapter 5, Chapter 7, and Chapter 8). Lastly, the BRHS data allow an investigation of diurnal variation in CVD risk factors (as specified in paragraph 1.4.3). However, the diurnal variation in CVD mortality cannot be directly studied because the time of the death within the day was not collected. This will be further discussed in Chapters 6 and 9.

Table 3.1 Standardised mortality ratios for cardiovascular disease in men aged 35-65 years in 1969-73 in the 24 British Regional Heart Study towns

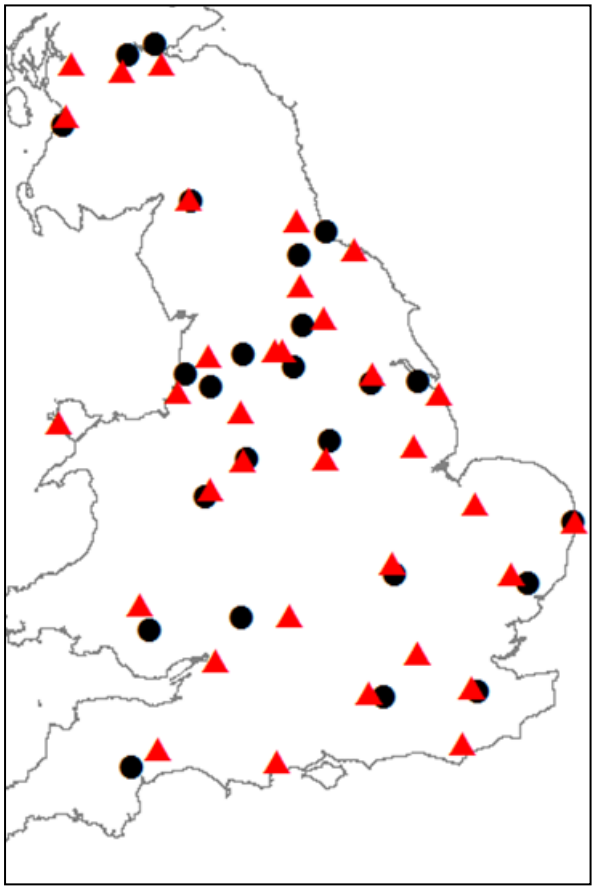
Town	Standardised mortality ratios for cardiovascular disease in men aged 35-65 years in 1969-73	Men examined (n)	Response rate (%)
Ayr	140	301	70
Bedford	80	303	73
Burnley	114	286	80
Carlisle	121	389	85
Darlington	109	382	82
Dewsbury	142	326	79
Dunfermline	118	350	80
Exeter	90	332	84
Falkirk	98	308	75
Gloucester	84	309	73
Grimbsy	96	318	71
Guildford	78	335	82
Harrogate	82	280	77
Hartlepool	101	334	70
Ipswich	92	362	85
Lowestoft	85	324	83
Maidstone	99	319	72
Mansfield	95	321	80
Merthyr Tydfil	135	282	76
Newcastle-upon-Lyme	115	293	77
Scunthorpe	109	313	76
Shrewsbury	95	310	83
Southport	114	322	80
Wigan	134	337	77

Data source: Adapted from Shaper et al (226)

Figure 3.1 British Regional Heart Study towns geographic location

Data source: Pocock et al. British Regional Heart Study: geographic variations in cardiovascular mortality, and the role of water quality. Year 1980 (228)

Figure 3.2 Geographic location of BRHS towns and UK Meteorological Office Stations



● = 24 BRHS towns
▲ = 35 MET office weather stations

Figure 3.3 Raw accelerometer data of one BRHS participant over the course of one day, and physical activity cut points (sedentary behaviours, light and moderate-to-vigorous physical activities)

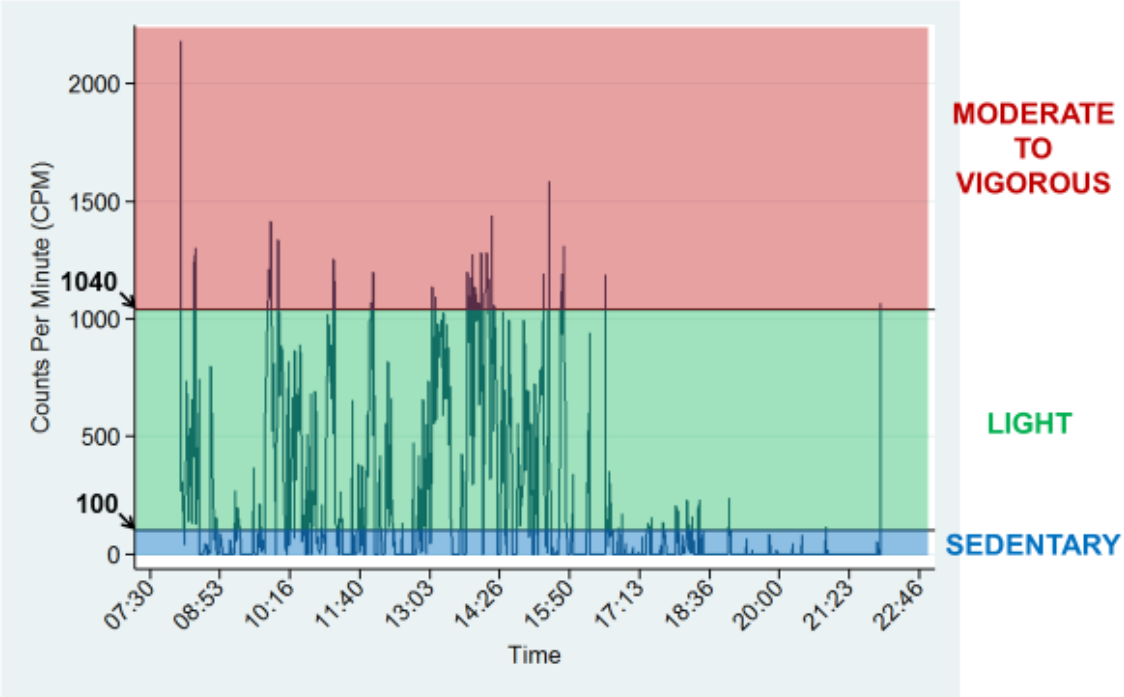
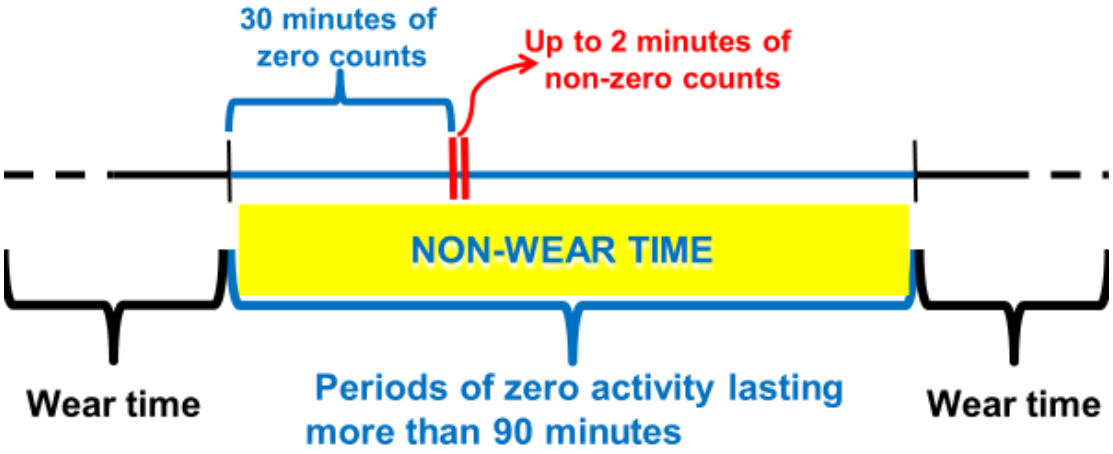


Figure 3.4 Physical activity data processing: identifying wearing time and non-wearing time in raw physical activity data (VT axis counts) by minute



Note: A period of non-wear time is a period with at least 90 minutes of consecutive zero counts, or a period of at least 90 minutes in which periods of 30 minutes of zero counts are interrupted by up to 2 minutes of non-zero counts.

Chapter 4 DIURNAL VARIATIONS IN PHYSICAL ACTIVITY LEVELS

4.1 Summary

Physical activity plays a crucial role in disease prevention particularly in older adults who are the least active and most sedentary age group. Previously published papers about physical activity in older adults, recognise that older adults can benefit greatly from increased physical activity levels. Difficulties in changing the persistently low levels of physical activity in older populations may be in part due to a lack of understanding of variations in daily patterns of activity levels in free living adults. To date, literature about how physical activity varies over the course of the day is sparse for older adults, and broadly shows that global measures of total activity are higher in the morning than in the evening. This Chapter examined how habitual physical activity varies over the course of the day among older British men. Additionally, and uniquely, the extent to which daily patterns in physical activity are modified by key individual factors (socio-demographic and health indicators) was explored. At the 32 year re-examination in 2010-2012, the surviving British Regional Heart Study (BRHS) men resident in the UK (n=3137, aged 71-91 years) were invited to attend a physical examination and were asked to wear a GT3x Actigraph accelerometer over the hip for one week. 1455 of 3137 men (46.4%) participated; 1329 men had complete data (measures of physical activity for at least 45 valid wear minutes per hour of wear and information on covariates of interest). From accelerometer raw data, percentages of time spent in sedentary behaviour (SB, <100 counts per minute [CPM]); in light (LIPA, 100-1040 CPM) and in moderate to vigorous PA (MVPA, >1040 CPM) were derived. The percentage of time spent in MVPA was highest in the morning, peaking at 10-11am (8.4%), and then declining until the evening, with the exception of a small increase at 2-3pm. LIPA followed a similar pattern. Conversely, SB levels were lowest in the morning and increased throughout the day, peaking at 9pm (88%). Multilevel models were used to assess associations between period of the day and physical activity levels; the average time spent in SB increased in the afternoon (+9 percentage points) and evening (+21 percentage points) when compared to morning.

Conversely, time spent in LIPA decreased by -6% and -16% percentage points during afternoon and evening respectively. Whether the daily patterns in activity varied by key variables related to health status was assessed: four key variables (age, presence of multiple chronic conditions, having mobility limitations and being obese) all had a disproportionate impact on the morning peak of activity, such that the oldest, obese, least healthy and least mobile men had a greater reduction in the morning peak in activity whereas reductions in afternoon and evening activity levels were less marked in these groups.

4.2 Introduction

Physical activity (PA) declines with increasing age (223, 255) and older adults, especially the oldest old, are the least active age group in the population (220, 256, 257). PA levels of older adults are generally low and levels of sedentary behaviour (SB) are high (15-17). To implement effective strategies to increase PA and reduce SB, it is important to understand patterns of both PA and SB, as they are two independent risk factors for mortality (18, 258). For example, understanding when peaks and dips in physical activity levels occur over the course of the day is important for dissemination of public health messages to older adults and health care professionals working with older adults. Accelerometers permit objective and accurate assessment of physical activity in population-based studies and, of special consideration for older adults, reduce the impact of recall bias (over or under reporting), participants memory loss or cognitive impairment (19). Accelerometers record physical activity levels at very short time intervals (e.g. 1 minute or shorter intervals), therefore they can give insight into how activity levels vary over the course of the day. However, to date there is very little evidence on how PA and SB are structured throughout the day among older people. Existing studies suggest that older adults were more active in the mornings than during afternoons and evenings (15, 16, 220, 221, 223). Moreover, and among older people (60+ years old), there has been a suggestion that age can impact the diurnal patterns of activity; for example, among free-living people from Baltimore, USA, aged 75+ years old vs 60-67 years old, the

physical activity levels appeared to be especially low in the afternoon and evening (223).

A common limitation of previous studies was the small sample size and the focus on a limited number of physical activity variables, such as global measures of activity (e.g. counts per minutes, CPM) and moderate to vigorous physical activity MVPA (15, 16, 220, 221, 223). Such studies did not consider a wider range of variables including different activity intensities as light physical activity (LIPA), SB and duration of bouts of activity (222). Moreover, it is plausible that risk factors more common in older age, such as obesity, can potentially modify the diurnal variations in physical activity; however, this was not demonstrated in previous studies. In older adults, I would expect CPM and steps to be higher in the morning, as in previous studies (220, 223), and to find a similar pattern for the time spent in LIPA and MVPA. I would also expect SB to be lower in the morning. Over the course of the day I would expect a gradual decline in the physical activity levels (223), and more time spent in sedentary behaviours especially during the evening.

4.3 Objectives

The aim of this Chapter is to investigate diurnal variations in objectively measured physical activity levels in older men from the British Regional Heart Study (BRHS). The specific aims of this Chapter are:

1) Do physical activity variables (LIPA, MVPA, and SB) vary over the course of the day in older adults?
2) Are diurnal variations in physical activity variables in older adults modified by age and other individual risk factors?

Secondary objective:

3) to describe diurnal variations of total volume in physical activity (CPM and steps) and important components of UK national PA guidelines, such as long bouts of SB (≥60 minutes) and MVPA bouts of at least 10 minutes

4.4 Methods

4.4.1 Participants

The 32-year follow-up of the BRHS took place in 2010-2012 and was previously described in Chapter 3, paragraph 3.2.4.1 and 3.2.4.2. In summary, the surviving BRHS participants resident in the UK (n=3137) were invited to attend a physical and blood examination and to wear a GT3x Actigraph accelerometer over the hip for one week. 1454 of 3137 men (46.4%) participated (see flow chart in Figure 4.1). The men were also asked to complete a questionnaire measuring socio-demographic characteristics, life style factors, and health behaviours (all variables were described in Chapter 3.4 "Data used in this thesis"). BRHS men included in the present Chapter 4 (as well as in Chapter 5) were independently mobile (not confined to wheelchair) and community dwelling (not in residential care homes).

4.4.2 Physical activity assessment

The objective physical activity assessment (e.g. accelerometer wear protocol, wear time calculation and derivation of PA measures) was described in the methods section of Chapter 3 (see paragraph 3.4.2.1 and 3.4.2.2), while the supporting role of the log diaries was described in paragraph 3.4.2.3. In summary, participants who attended the 32-year follow-up were asked to wear an Actigraph GT3x accelerometer (Pensacola, Florida) over the right hip on an elasticated belt for 7 days, during waking hours, removing it for bathing, swimming or showering and returning the device by post. For this Chapter the main PA measures of interest were the percentage of the day spent in SB, LIPA and MVPA.

4.4.3 Statistical methods

4.4.3.1 Descriptive statistics

To give a general overview of the within day variation of total PA, counts per minute and steps were plotted against hour of day (e.g. from 09:00 to 09:59, from 10:00 to 10:59, etc.). The main outcome variables were the proportions (percentages) of the day spent in (1) sedentary behaviour, (2) light PA and (3) moderate to vigorous PA. Each

outcome was calculated according to hour of the day, with the number of minutes that the accelerometer was worn in that hour used as the denominator. Due to sparse data in early morning and late evening, we examined the mean activity counts per hour between 7.00 am and 10.59 pm. Only hours with ≥ 45 valid wear minutes were included. As a descriptive analysis the percentage of time spent in SB, LIPA and MVPA was plotted against hour of day. Pearson's correlations of LIPA with MVPA, LIPA with SED, and MVPA with SED were also calculated across men and each hour of accelerometer wear over the course of 1 week.

The mean percentage of each hour of the day spent in sedentary bouts of at least 1 hour and MVPA bouts of at least 10 minutes (definitions in Chapter 3, paragraph 3.4.2.2) was also calculated and plotted against hour of the day. For example, if a bout started within 10:00 and 10:59 hours, then this would be labelled as "10am bout" (see results section - paragraph 4.5.1, and Figure 4.8).

4.4.3.2 Associations between period of the day and physical activity levels

The day was divided in 3 periods [morning (7am-12.59pm), afternoon (1pm-6.59pm) and evening (7pm-10.59pm)]. Each period had a minimum of 2 valid hours of wear time. Next, the associations between period of the day and SB, LIPA and MVPA (outcomes) were investigated.

The distributions of each outcome were investigated before carrying out statistical models: percentage of MVPA distribution was highly positively skewed as reported in previous studies (259, 260). MVPA data were highly over-dispersed with variance 5 to 6 times higher than the means within each period of the day, so a negative binomial model was used to investigate the associations between period of the day and percentage of time spent in MVPA. Linear multilevel regression models were used to investigate the associations between period of the day and percentage of time spent in LIPA and SB (normally distributed).

Two level random-intercept and random-slope models were used: in all models Level 1 was period of the day and Level 2 was the individual. At Level 2, all statistical models were additionally adjusted for age, region, mobility limitations, number of chronic conditions, BMI, depression, smoking status, social isolation, social class, use of public transport, and vision problems. These variables are worth adjusting for as their relationship with outcome is known a priori to be strong in the BRHS (17), although it is unlikely these variables are related to exposure (period of the day). Including such covariables in the model would have the scope of reducing the standard error and increasing in precision of the estimates of the effect of our exposure variable. The models were additionally adjusted for season, but the physical activity variation by season will be presented in Chapter 5, and not further investigated here. The random slope in the models allowed us to estimate variations in PA levels over the day varied between different men. The estimated slopes over the course of day were reported as mean differences between afternoon vs morning (baseline), and evening vs morning (baseline). In negative binomial models the results were reported as rate ratios (RRs) (261). A RR is as multiplicative factor: any deviation from 1 indicates a percent difference in the outcome relative to the respective reference category (baseline) in the exposure variable. Beta coefficients were reported to estimate the difference in time spent in SB and LIPA between the categories of each explanatory variable against the reference.

4.4.3.3 Interaction of period of the day with individual factors on PA and SB

From one previous study there was evidence that age can modify the diurnal patterns of activity; in the Baltimore Longitudinal Study of Aging (n = 611, 50% male, mean age 67 years), participants over 75 years of age decreased their activity over the day to a greater extent than those aged 60-67 (223). Also, it is plausible that risk factors common in older age, such as obesity among others, can potentially modify the diurnal variations in physical activity; however, this was not demonstrated in previous studies. Therefore, I explored whether the diurnal patterns in activity were modified by individual risk factors known to be strongly associated with the physical activity outcomes in the BRHS (17): (i) age group (71-75, 75-79, ≥80 years); (ii) BMI category

(<25, 25-30 and ≥30 kg/m^2); (iii) number of chronic conditions (none, 1-2, ≥3); (iv) mobility limitations (none, slight, moderate/severe/unable to do). There seemed no a priori rationale or prior scientific evidence for testing the interaction of period of the day with other risk factors such as social class, depression and smoking; therefore, I limited my investigation to those factors in relation to physical activity using descriptive plots (see paragraph 4.4.3.1); social class, depression and smoking were still included as confounders in statistical models (see paragraph 4.4.3.2).

In all interactions tests, an overall Wald test for interaction between the categories of the explanatory variables and period of the day (morning, afternoon and evening) was used.

4.5 Results

1454 of 3137 men (46.3%, see Figure 1) participated; mean age was 78.4 years (range 71-93). The characteristics of the study participants are shown in Table 4.1. Men who agreed to participate were about 2 years younger and more likely from non-manual social class; also, 10 years previously they had a lower BMI and were less likely to smoke cigarettes compared to men who did not participate. Participants took on average 4800 steps per day and spent 72.6% of the day in SB, 22.9% in light activity and 4.5% in MVPA (618, 196 and 39 minutes per day respectively). Participants had a mean of 6.7 (SD=0.8) valid days of accelerometer wear. 1329/1454 men (91.3%) had complete data [at least 2 valid hours of wear time in each period of the day (morning 7:00-12:59, afternoon 13:00-18:59, evening 19:00-22:59, and data on all covariates], and the same patterns of associations with characteristics in Table 4.1 are seen in the reduced sample. From this point forward all results refer to the 1329 men (complete case analysis).

4.5.1 Descriptive statistics

On average, the volume of PA indexed by both accelerometer CPM and the number of steps peaked around 10am and then declined until a small further increase at 2pm followed by a long decline until 9pm and then a small increase after 10pm (Figure

4.2). Figure 4.3 shows the average percentage of time spent in PA of different intensities (SB, LIPA and MVPA) plotted against hour of the day. The correlation (n=130506 observations across men and hour of the day over 1 week) of LIPA and MVPA was r=0.21, p<0.001; time spent in SB was negatively correlated with LIPA (r=-0.88, p<0.001) and with MVPA (r=-0.64, p<0.001). The pattern in daily variation of MVPA and LIPA closely followed the pattern observed for CPM. At around 10am, the proportions of each hour spent in LIPA and MVPA peaked (at approximately 30% and 8% of all activity respectively) and then declined until 1pm, followed by a slight increase in the afternoon around 2-3pm and then a long decline until around 9pm, when light activity accounted for only approximately 10% and MVPA 1% of each hour. Conversely, SB levels increased throughout the morning, with a steeper increase before 1pm and then a small dip around 3pm, followed by a slow increase to a peak of over 80% spent in SB between 8-9pm, followed by a slight decline after 9pm (Figure 4.3). Univariable descriptive plots show hourly patterns of different intensities of physical activity stratified by age, mobility limitations, number of chronic diseases, BMI categories, geriatric depression score and smoking status (Figures 4.4 and 4.5) and by social isolation, social class, active transport and vision problem, season (winter vs summer) and day of the week (Figures 4.6 and 4.7). In most cases, the patterns of mean percentage of SB, LIPA and MVPA by hour of the day followed a consistent pattern of peaks and dips at the same time of day. Descriptive plots of sedentary and MVPA bouts (Figure 4.8) showed consistent results with patterns in Figures 4.4-4.7. 49.0% of the sedentary bouts lasting ≥60 minutes over a valid week occurred in the evenings; most started between 8-9pm (13.6%) or 9-10pm (14.0 %). Conversely, most (59.5%) of MVPA bouts lasting ≥10 minutes over a valid week occurred in the morning and in particular when the peaks of MVPA were reported, at 10am (15.9%) and 11am (16.4%).

4.5.2 Associations between period of the day and physical activity levels

Associations of period of the day (evening or afternoon vs morning) with time spent in physical activity and sedentary behaviour each day were estimated using multilevel models. The magnitude and significance of the associations did not differ greatly when

adjusted for just one explanatory variable at a time or with further adjustments for all explanatory variables together, hence the fully adjusted results are reported (Table 4.2). The average time spent in SB increased in the afternoon (+9 percentage points) and evening (+21 percentage points) when compared to morning (Table 4.2, Model 1). Conversely, time spent in LIPA decreased by -6% and -16% percentage points during afternoon and evening respectively (Table 4.2, Model 2). Table 4.2 also reports associations of individual factors with the physical activity outcomes. The percentage of time spent in SB each day was significantly higher and the percentage of the day in LIPA was significantly lower in the following groups; age ≥80 years, any mobility limitations, three or more chronic diseases, and obese. Total levels of SB were higher in the men who were depressed and did not use active transport, although LIPA did not vary by these characteristics. Neither LIPA nor SB differed by social class, presence of social isolation or vision problems.

The MVPA results were reported as RRs rather than beta coefficients, due to non-normality of the outcome distribution (Table 4.3). The decline in MVPA was particularly marked over the course of the day; compared to the morning, levels of MVPA (>1040 CPM, Table 4.3 Model 1) declined substantially in the afternoon (RR=0.57, 95%CI 0.54-0.59) and in the evening (RR=0.17, 0.15-0.18). Overall, men with moderate or more severe mobility limitations compared to the reference category (no mobility limitations) had a RR of 0.50 (95%CI 0.43, 0.57) for MVPA indicating that men with mobility limitations were half as likely to spend time doing MVPA compared to people with no limitations. Moreover, men who were older, did not use active transport, were obese, depressed, had more chronic health conditions, and were smokers had lower levels of MVPA. Similar associations were seen when these analyses were repeated with a higher cut point (>1951 CPM) to define MVPA (Table 4.3, Model 2). The largest RRs (risks of having low MVPA levels) were for being over 80 compared to less than 75 years, for the category " moderate/severe limitations or unable to do" if compared with no mobility limitations and use of active transport versus car/public transport. However, when using the >1951CPM cut-point, MVPA level no longer differed by depression or smoking status.

4.5.3 Interaction of period of the day with individual factors

Four factors were significantly associated with each of SB, LIPA and MVPA levels: age, mobility limitations, chronic diseases, and BMI as observed in Table 4.2 and Table 4.3. The effects of older age, obesity, mobility limitations and chronic diseases on LIPA, MVPA and SB appeared to be more marked in the morning than in the afternoon and evening, independent of the lower overall levels of PA observed in these subgroups (as suggested in Figure 4.4 and 4.5). Interaction tests (overall Wald test) were performed to establish whether these associations differed by period of the day, see Table 4.4. The interaction tests of period of the day with age, chronic conditions, BMI and mobility limitations provided enough evidence to suggest that diurnal patterns in physical activity levels were modified by health status (Table 4.2), consistently with the trends observed in Figure 4.4 (for the variables age, chronic conditions, and mobility limitations) and Figure 4.5 (for BMI).

4.6 Discussion

4.6.1 Summary of main findings

This study investigated the diurnal variations in accelerometer-measured PA and SB levels in a large sample of older British men. In this section I address the research questions listed in the paragraph 4.3 (Objectives).

Question 1) Do physical activity variables (LIPA, MVPA, and SB) vary over the course of the day in older adults?

Yes, the analyses demonstrated that the total amount of physical activity (steps and CPM) was highest in the morning but then decreased during the day, except for a small increase at 2-3pm. LIPA and MVPA showed a similar pattern. Conversely, SB levels were lowest in the morning and increased throughout the day, peaking at 9pm (88%).

Question 2) Are diurnal variations in physical activity variables in older adults modified by age and other individual risk factors?

Yes, I found that age, mobility limitations, chronic conditions and obesity influenced LIPA, MVPA and SB levels in the morning more than in the afternoon and evening. The findings showed an attenuation of the diurnal pattern among less active subgroups (e.g. older and more infirm men). This reflects their diminished ability to maintain relatively high intensity physical activity during the morning, and this is not simply related to the generally low PA level typical of these subgroups. This information is important for policy and practice because there is scope to extend the existing activity bouts during the morning and early afternoon and also to increase activity levels later in the afternoon. Our analysis of whether or not the effects of specific health and social variables on PA levels varied by time of day offers unique new insights, as previous studies of older adults have not considered this question.

For completeness of information I also reported that overall LIPA and MVPA levels were lower (and SB higher) if BRHS men were older, had mobility limitations, more chronic health conditions and were obese. Moreover, men who did not use active transport, who were depressed and smoked cigarettes spent less time in MVPA, but those factors did not affect SB or LIPA.

Question 3) to describe diurnal variations of total volume in physical activity (CPM and steps) and important components of UK national PA guidelines, such as long bouts of SB (≥60 minutes) and MVPA bouts of at least 10 minutes

I did describe those PA variable plotting the levels by hour of the day, as secondary analysis. On average, the volume of PA indexed by both accelerometer CPM and the number of steps peaked around 10am and then declined until a small further increase at 2pm followed by a long decline until 9pm and then a small increase after 10pm. About half of the sedentary bouts lasting ≥60 minutes over a valid week occurred in the evenings; Conversely, most of MVPA bouts lasting ≥10 minutes over a valid week occurred in the morning.

4.6.2 Comparisons with other studies

To date there has been little work using hourly accelerometer data to examine diurnal physical activity patterns among older adults. Our results showing that physical activity peaks in the morning are consistent with recent studies (15, 16, 220-223), although our study extends the literature by investigating more intensities of activity (SB, light PA and MVPA) and bouts of activities. In our study the overall PA levels (measured as CPM) over the course of the day were similar to other smaller studies using the same measurement device (15, 16, 220, 221, 223). We examined PA patterns between 7.00 am and 10.59pm and a similar period (6am-10pm or 7am-9pm) was analysed in other studies due to sparse data in early morning and late evening (222). In line with our findings, a study of 38 healthy active adults (mean age 70 years) reported significantly fewer minutes of MVPA in the evening than in the morning or afternoon (221) and that longer bouts of activity occurred in the morning (6 am-12 pm) more often than afternoon or evening. The AGES-II study of 579 adults aged 73–98 from Iceland reported that the majority of PA occurred between 8 am and 4 pm on an average day (220), which fits with our findings. In line with our study, they also reported that sedentary time was similar across all age groups, except for the oldest age group (>85 years old) who were the most sedentary and PA levels declined with increasing age and BMI, but other social and health factors were not taken into account. Our findings about age modifying the daily patterns in physical activity fit with data from the AGES-II study (220) and from the Baltimore Longitudinal study of Ageing (223), which also found that older age groups had a steeper decline in in PA levels over the course of the day. Our finding that activity levels were lower in the mornings in obese than normal weight men mirrors data from a study of Canadian adults aged 20-79 years (222). To our knowledge other studies have not investigated how presence of chronic conditions and mobility limitations affect the diurnal patterns of physical activity and sedentary behaviour in older adults.

4.6.2.1 Comparison with studies published after June 2015

The literature review (see Chapter 2, Paragraph 2.1) included previous published papers published until June 2015 (one year after the proposal of this thesis was officially deposited at University College London). The findings from this Chapter

were published in June 2015 (57); later, five more papers on diurnal variation in physical activity were published from other researchers (53, 262-265). The findings were consistent with those presented in this Chapter; for example, findings from over 100,000 participants of the UK Biobank study confirmed that physical activity levels are higher in the morning (peak observed around 10-11 a.m. and 2 p.m.) followed by a decrease over the course of the day (53). A recent paper investigated daily patterns of accelerometer activity in 2967 men from the osteoporotic fractures (MrOS) study (266). Using principal component analysis, the authors identify 4 major types of diurnal patterns: the first component confirmed our overall findings (higher activity levels in the morning, followed by decrease over the course of the day). The second component was linked to those with later and earlier rise and bed times. The third component was linked to those with (i) longer biphasic (first peak in early morning, second in the afternoon) and (ii) shorter more monophasic activity patterns (one single peak in the morning). The fourth component represented morning and evening peaks in activity. Overall, having a late afternoon peak in activity was associated with a 1.4-fold higher rate of all-cause mortality (HR= 1.46, 95%CI [1.21–1.77]). Although the authors admitted that it was tempting to associate causality between specific patterns and outcome measures, their study does not directly address this hypothesis (266). A later study including patients (mean age 65.6 years) with chronic obstructive pulmonary disease (COPD) demonstrated that participants who experienced severe COPD-related symptoms (vs less severe) at the beginning of the day took significantly fewer steps especially in the morning and this also appeared to precipitate the decline in physical activity over the course of the day. The authors admitted limitations from their study; indeed, only prospective studies could prove causality between morning symptoms and physical activity during different parts of the day (264). Lastly, findings from the MRC National Survey of Health and Development tested the interaction of hour of the day with BMI categories and obesity history in older people aged 60-64 years. Despite total PA levels being especially higher in the morning among those with BMI<=25 vs >25 or >30, the authors could not support the hypothesis of an interactions between BMI or obesity history and time of day, when the overnight segment was excluded (265).

4.6.3 Strengths and limitations

This study investigates how hourly levels of objectively measured SB, LIPA and MVPA vary over the course of the day and how daily activity patterns are modified by a wide range of demographic and health characteristics. It is particularly important to investigate LIPA in population based samples of older adults, because of the high proportion of time spent in light activity. Our findings about the correlates of LIPA offer a new contribution to the ongoing debate about whether and how the PA guidelines should include recommendations on LIPA as well as MVPA (267, 268).

This study benefits from using a large scale population-based cohort of free-living older men rather than a special *at risk* population, which should increase generalizability. The response rate achieved in this study is comparable with other studies on objective measurements of daily physical activity patterns, or better than the UK Biobank study (53). Men who did not accept our invitation were about two years older and had higher BMI measured 10 years earlier; implying that overall PA (e.g. total counts or number of steps) might be lower in the general population. Our study is however limited by studying only white European men, who, based on existing literature, would be expected to have higher levels of total PA (e.g. steps), particularly MVPA, but also higher levels of SB compared with women (17). Therefore our results may not be generalizable to older women or ethnic minority populations (269). Our study did not report detailed information on mode of activity, which was self-reported only during the first three days of accelerometer wearing. The importance of this information is recognized (270) and future studies could investigate further the particular types of activities carried out during the entire week of accelerometer wear time and focusing especially on the activities which occurred during the highest and lowest peaks of activity.

4.6.3.1 Strengths and weakness of accelerometer measurements

The use of accelerometers helps to overcome problems of participants forgetting, recall bias or cognitive impairment in older people (19) and permits measurements of physical activity by hour of the day, which would be very difficult to achieve in self-reported physical activity studies. Also, accelerometers permit investigations of

specific intensities of PA, for example light intensity PA (LIPA), which is especially important because of the substantial proportion of time older men spent in LIPA activity intensity and also SB (17, 267, 271, 272). In addition, it is important to objectively measure time spent in moderate to vigorous physical activity (MVPA) because it forms the basis of current physical activity guidelines (273). In terms of understanding health benefits of PA, accelerometers are important for measuring duration in specific intensities of activity, as this is more informative than simple measures such as "time spent walking" or total daily step count which do not indicate energy expenditure.

The objectively measured PA intensities defined in this study used age-appropriate and validated cut points (221). Although the measure of sedentary time used in this work does not give any insights on posture (e.g. standing time can be potentially included), the hip-worn sedentary time measure showed strong correlation with thigh-worn sedentary time measures ($r = 0.76$) in a study of middle aged adults (274). Also, in a previous study of healthy older adults the Actigraph sedentary time cut-point of <100CPM had an estimated 93% and 58% sensitivity and specificity respectively, while 11.8% of time classified by Activpal as standing was classified as sedentary. However, in comparison, the BRHS participants are older and potentially less healthy, therefore likely to spend less time standing, which would improve classification. Accelerometers did not detect the "type" of activity; to overcome such limitation, in this study the BRHS men were asked to complete a log diary self-reporting the type of activity every hour during the first three days; however, the degree of data completeness was judged to be insufficiently consistent to study as a specific objective.

4.6.4 Implications

The marked variations in PA occurring on a within-day basis provide information which could be helpful in planning interventions to increase PA levels. Older adults do most of their MVPA and light activity during the morning. Thus, one possible strategy for interventions aiming to increase these intensities of activity would be either to focus on the morning when people are already active and when variability in

activity levels are greatest, aiming to increase the intensity or duration of existing physical activity bouts. Alternatively, interventions could focus on the afternoon period, aiming to stimulate physical activity of comparable intensity to that occurring in the morning. It is unlikely that low levels of activity in the evening can be changed, particularly in the winter months when it is dark in the late afternoons and evenings. Indeed, the combination of darkness and visual problems have been previously investigated as potential causes of falls (275). Likewise with sedentary behaviours, our findings suggest that the period in the late afternoon and early evenings are periods with high levels of SB and when bouts of SB are likely to be longest, so it may be particularly valuable to focus on efforts to break up long sedentary bouts at these times of day. Our investigation showed that age and health status affected these diurnal patterns suggesting that PA policies might be targeted by sub-groups. Among older and disabled men, lower levels of MVPA were observed in morning and afternoon than in younger healthy men, the morning peak was more reduced than the afternoon peak, suggesting that with increasing age, the higher morning peak in moderate to vigorous activity may be particularly difficult to maintain. Longitudinal analyses could offer additional insights and determine if there are independent effects on health of MVPA or SB at different times of the day.

The analysis of season and PA carried out in this Chapter was particularly relevant to the aims of Chapter 5 and the overall aim of this thesis. That fact that diurnal patterns of physical activity levels were not modified by season, as observed in this Chapter, suggested that diurnal patterns of PA are not relevant to the seasonal patterns in PA (investigated in Chapter 5), and therefore to the seasonal variation of the CVD itself. However, modifying the diurnal patterns of physical activity (e.g. increasing the levels of activity during the morning) may have beneficial effect on health in the long term; increasing PA levels on daily basis can reduce the levels of several CVD risk factors, and therefore reduce the overall risk of CVD later in life.

4.7 Conclusions

This study provides detailed data about diurnal patterns in habitual physical activity levels in free-living older men which can inform the development of effective programmes to encourage older men to be physically active. This study highlights that especially among men over 80 years old, who are obese, with multiple chronic diseases or with mobility limitations there are particular opportunities to maintain or enhance existing activity bouts during the morning and early afternoon and to reduce the duration of SB periods in the afternoon and evening hours. That fact that diurnal patterns of physical activity levels were not modified by season, as observed in this Chapter, suggested that actual diurnal patterns of PA are not relevant to the seasonal patterns in PA (see Chapter 5), and therefore to the seasonal variation of the CVD itself. However, modifying the diurnal patterns of physical activity (e.g. increasing activity levels during the morning) may benefit health in the long term; increasing PA levels on daily basis can reduce the overall risk of CVD later in life.

Table 4.1 Characteristics of men who met the inclusion criteria for the study and men who did not meet the inclusion criteria.

	Men who met inclusion criteria for the study	Men who did not meet the inclusion criteria	p-value for difference *
N	1454	1683	
Demographic and background characteristics			
Age, mean (SD)	78.4 (4.6)	80.1 (5.2)	<0.001
Region, n(%)			0.029
South	526 (36.2)	525 (31.2)	
Midlands	216 (14.9)	268 (15.2)	
North	569 (39.1)	701 (42.6)	
Scotland	143 (9.8)	189 (10.9)	
Social class, n(%)			
Manual	665 (45.8)	954 (56.7)	<0.001
Non-Manual	751 (51.6)	676 (40.2)	
Armed Forces	33 (2.3)	51 (3.0)	
Missing	5 (0.3)	2 (0.1)	
Physical Health			
BMI, mean (SD)	27.1 (3.8)		
BMI 10 years earlier, mean (SD)	26.7 (3.3)	27.2 (3.8)	<0.001
Number of Chronic conditions, n(%) [§]			
None	673 (46.3)		
1-2	668 (45.9)		
3+	108 (7.4)		
Missing	5 (0.3)		
Mobility limitations outdoors, n(%)			
None	914 (62.9)		
Slight limitations	264 (18.2)		
Moderate/severe difficulty/unable to do	237 (16.3)		
Missing	39 (2.6)		
Vision Problems, n(%)			
None	986 (68.1)		
1+ problem	463 (31.8)		
Missing	5 (0.3)		
Mental health and wellbeing			
Social isolation, (isolated), n(%)[1]			
Isolated	256 (17.6)		
Non isolated	1187 (81.6)		
Missing	11 (0.8)		
Geriatric Depression Scale, (depressed), n(%)[2]			

Depressed	316(22.1)		
Not depressed	1111 (76.4)		
Missing	27 (1.9)		
Behaviours			
Smoking status (cigarettes)			
Yes	46 (3.3)		
No	1388 (95.5)		
Missing	18 (1.2)		
Smoking status 10 years earlier (cigarettes), yes	97 (7.2)	160(12.8)	<0.001
Accelerometer data			
Number of valid days, mean (SD)	6.7(0.8)		
Wear time, mean (SD)	853(68)		
Counts/min, mean (SD)	185(110)		
Steps, mean (SD)	4800(2791)		
Minutes in SB per day [3], mean (SD)	618(84)		
Minutes in LIPA per day [4], mean (SD)	196(66)		
Minutes in MVPA (>1040 CPM) per day [5], mean (SD)	39(32)		
Minutes in MVPA (>1951 CPM) per day [5], mean (SD)	16(18)		
Percent wear time in SB per day[3], mean (SD)	72.6(9.4)		
Percent wear time in LIPA per day[4], mean (SD)	22.9(7.1)		
Percent wear time in MVPA (>1040 CPM) per day [5], mean (SD)	4.5(3.7)		
Percent wear time in MVPA (>1951 CPM) per day [5], mean (SD)	1.9(2.1)		

* Comparisons of continuous and categorical variables across groups were based on independent t tests and chi ‑ squared tests respectively. The Fisher's exact probability test was used for comparisons of sparse binary data.

§ BRHS men were classified according to the number of chronic conditions according to whether they recall a doctor telling them (list of conditions: previous heart attack, heart failure, angina, diabetes, stroke, osteoporosis, claudication, Parkinson's disease, chronic kidney disease and cancer).

Note: The correlations between steps and CPM, steps and MVPA, steps and LIPA, steps and SB were 0.93, 0.92, 0.47, and -0.46 respectively, ($p<0.001$).

[1] Lubben scale;, isolated <12

[2] Geriatric Depression Scale; depressed >2

[3] Sedentary Behaviour (SB) is at least one minute where the accelerometer registers values <100 CPM

[4]Light physical activity (LIPA) is at least one minute where the accelerometer registers values between 100-1040 CPM

[5] Moderate to vigorous physical activity (MVPA) 1+ is at least one minute where the accelerometer registers values over the specified threshold (1040 or 1951 CPM)

Table 4.2 Adjusted associations between demographic and health factors and physical activity levels: percent of time spent in SB and LIPA

	Model 1 Percent of time spent in SB (<100 CPM)	Model 2 Percent of time spent in LIPA (100-1040 CPM)
	Mean difference (95% CI) [1,2]	Mean difference (95% CI) [1,2]
Part of the day (ref: Morning)		
Afternoon	9 (9,10)	-6 (-7,-6)
Evening	21 (21,22)	-16 (-16,-15)
Age categories (ref: age < 75 years old)		
75-79 years old	0.5 (-0.4,1.5)	-0.1(-0.9,0.7)
80+ years old	3.5 (2.5,4.5)	-2.3(-3.1,-1.4)
Mobility limitation (ref: no mobility limitations)		
slight mobility limitations	1.6 (0.6,2.6)	-1.1(-2.0,-0.3)
moderate/severe limitations or unable to do	3.3 (2.1,4.6)	-2.6(-3.6,-1.5)
Chronic conditions (ref: no chronic diseases)		
1-2 chronic diseases	0.7 (-0.1,1.5)	-0.6(-1.3,0.1)
3+ chronic diseases	2.6 (1.0,4.2)	-2.1(-3.4,-0.8)
Obese (ref: non-obese, BMI<30)	1.4 (0.5,2.4)	-1.2(-2.0,-0.3)
Depressed (ref: not depressed)	0.9 (0.0,1.9)	-0.8(-1.6,0.1)
Current smoker (ref: non-smoker)	0.8 (-1.3,3.0)	-0.6(-2.5,1.2)
Use car/public transport (ref: cycle/walk)	1.0 (0.2,1.9)	-0.6(-1.3,0.1)
Social isolated (ref: not isolated)	0.2 (-0.8,1.2)	-0.2(-1.1,0.6)
Manual social class (ref: non manual)	0.2 (-0.5,1.0)	0.2(-0.5,0.9)
Vision problems (ref: none)	-0.2 (-0.6,1.1)	0.2(-0.6,0.9)
Season (ref: Winter)		
Spring	-0.9 (-2.1,0.3)	0.3 (-0.7,1.4)
Summer	-2.8 (-4.0,-1.7)	2.0 (1.0,2.9)
Autumn	0.1 (-1.0,1.2)	-0.2 (-1.1,0.8)

[1] Complete case analysis (n= 1329 in each model): men who met the inclusion criteria (Figure 1) and who had at least 2 valid hours of wear time in each period of the day (morning 7:00-12:59, afternoon 13:00-18:59, evening 19:00-22:59). A valid hour is defined as an hour with ≥45 minutes of wear time.
[2] The mean difference is the absolute difference in percent of time spent in SB (Model 1) and LIPA (Model 2) compared to the reference category of each explanatory variable. Models are multilevel linear regression models mutually adjusted for, region of residence plus all explanatory variables listed in the table.

Table 4.3 Adjusted Rate Ratios (RRs) for the percent of time spent in MVPA using two different cut offs according to demographic and health status variables

	Model 1 MVPA (> 1040 CPM)	Model 2 MVPA (> 1951 CPM)
	RR (95% CI) [1,2]	RR (95% CI) [1,2]
Part of the day (ref: Morning)		
Afternoon	0.57 (0.54,0.59)	0.50 (0.46,0.54)
Evening	0.17 (0.15,0.18)	0.11 (0.10,0.13)
Age categories (ref: age < 75 years old)		
75-79 years old	0.87 (0.79,0.96)	0.82 (0.71,0.95)
80+ years old	0.55 (0.50,0.61)	0.49 (0.42,0.57)
Mobility limitation (ref: no mobility limitations)		
slight mobility limitations	0.79 (0.71,0.89)	0.69 (0.59,0.81)
moderate/severe limitations or unable to do	0.50 (0.43,0.57)	0.33 (0.26,0.41)
Chronic conditions (ref: no chronic diseases)		
1-2 chronic diseases	0.91 (0.83,0.99)	0.91 (0.80,1.03)
3+ chronic diseases	0.66 (0.55,0.79)	0.65 (0.50,0.85)
Obese (ref: non-obese, BMI<30)	0.83 (0.75,0.93)	0.72 (0.61,0.84)
Depressed (ref: not depressed)	0.88 (0.79,0.98)	0.92 (0.79,1.08)
Current smoker (ref: non-smoker)	0.76 (0.60,0.97)	0.85 (0.60,1.21)
Use car/public transport (ref: cycle/walk)	0.75 (0.68,0.82)	0.62 (0.55,0.72)
Social isolated (ref: not isolated)	0.97 (0.87,1.08)	1.11 (0.95,1.31)
Manual social class (ref: non manual)	0.93 (0.85,1.01)	0.98 (0.86,1.11)
Vision problems (ref: none)	0.96 (0.87,1.05)	1.00 (0.88,1.15)
Season (ref: Winter)		
Spring	1.2 (1.0,1.4)	1.1 (0.9,1.3)
Summer	1.1 (1.0,1.3)	1.1 (0.9,1.3)
Autumn	1.1 (0.9,1.2)	1.0 (0.9,1.2)

[1] Complete case analysis (n= 1329 in each model): men who met the inclusion criteria (Figure 1) and who had at least 2 valid hours of wear time in each period of the day (morning 7:00-12:59, afternoon 13:00-18:59, evening 19:00-22:59). A valid hour is defined as an hour with ≥45 minutes of wear time.
[2] RR is a multiplicative factor. Compared to the reference category of each explanatory variable, any deviation from 1 indicates a relative change in percent of time spent in MVPA and a value < 1 indicates a decrease in MVPA (e.g. RR=0.91 means a decrease in MVPA by a factor of 0.91 compared to the reference, that is about 10%). Model 1 and 2 are negative binomial multilevel regression models mutually adjusted for region of residence plus all the explanatory variables in the table.

Table 4.4 Overall interaction tests (Wald test p-value) between period of the day (morning, afternoon and evening) and individual risk factors on physical activity outcomes

	Interaction of period of the day with…			
Physical activity outcomes; time spent in…	Age	Mobility limitations	Chronic conditions	BMI
SB	<0.001	<0.001	0.002	<0.001
LIPA	0.012	<0.001	0.021	<0.001
MVPA	<0.001	<0.001	0.032	<0.001

Figure 4.1 Recruitment flow chart and identification of the eligible population of men

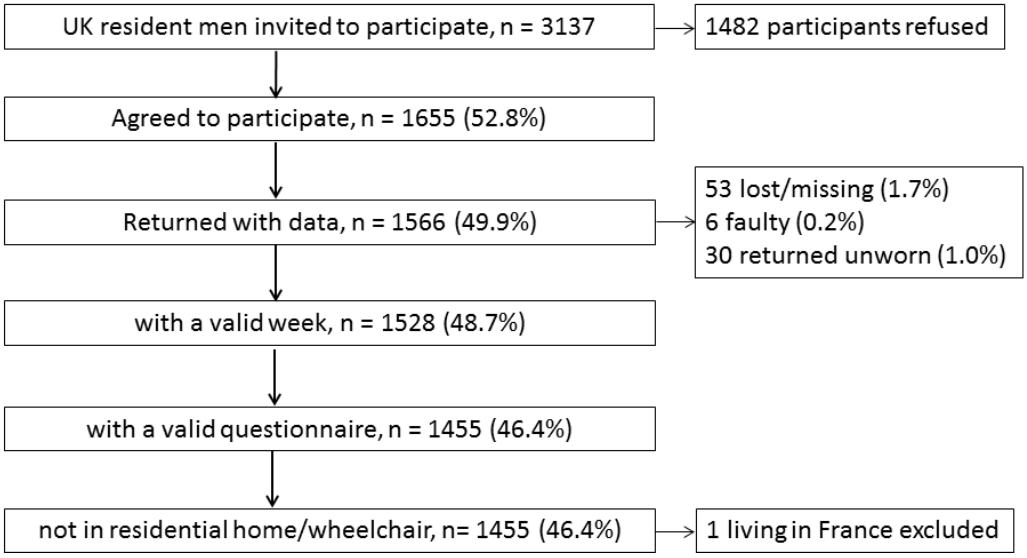


Figure 4.2 Mean accelerometer counts per minute (CPM, continuous line) and steps (dotted line) according to hour of day in 1329 men aged 71-93 years.

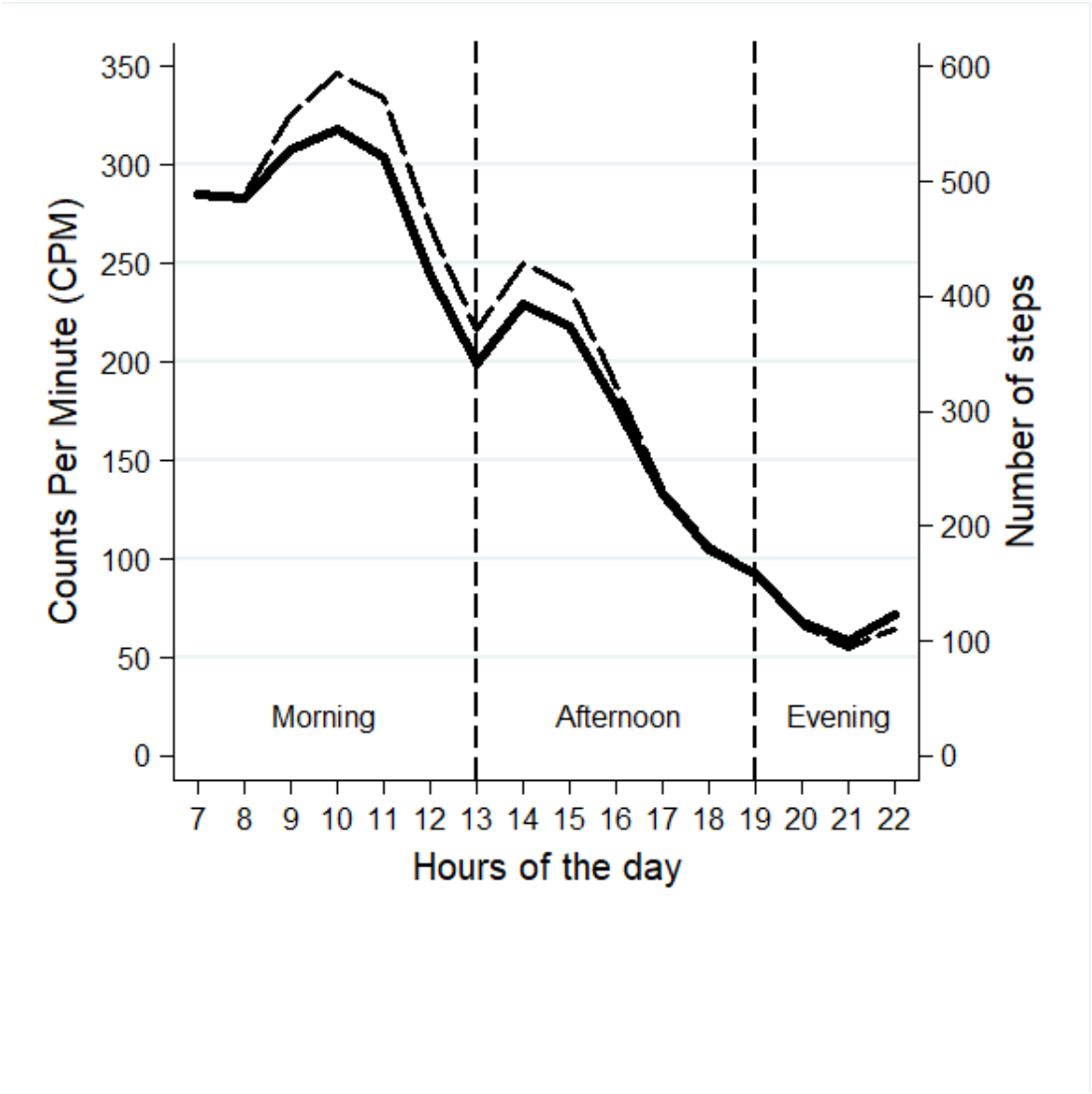


Figure 4.3 Percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day in 1329 men aged 71-93 years

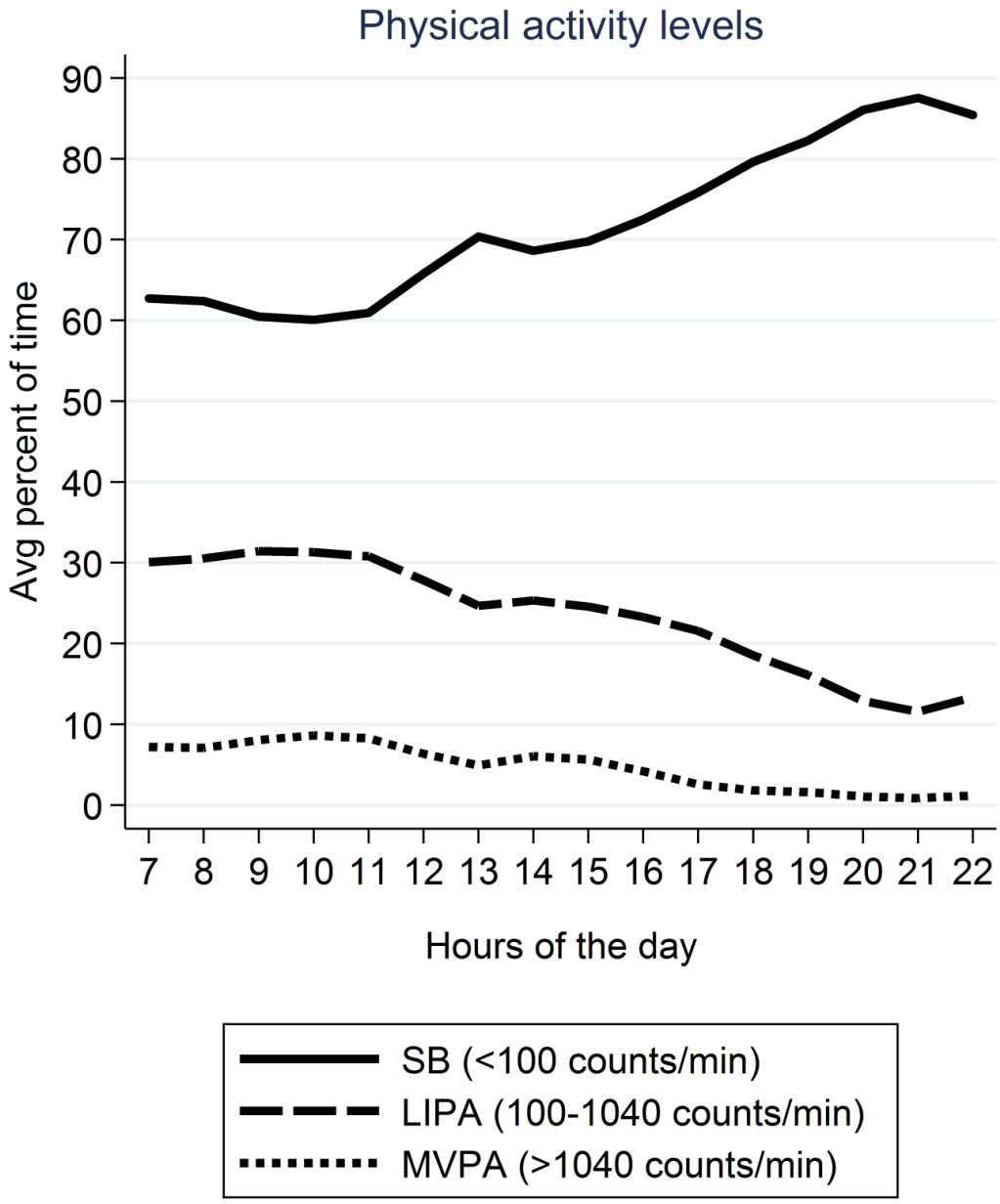


Figure 4.4 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by age group, mobility limitations and chronic conditions

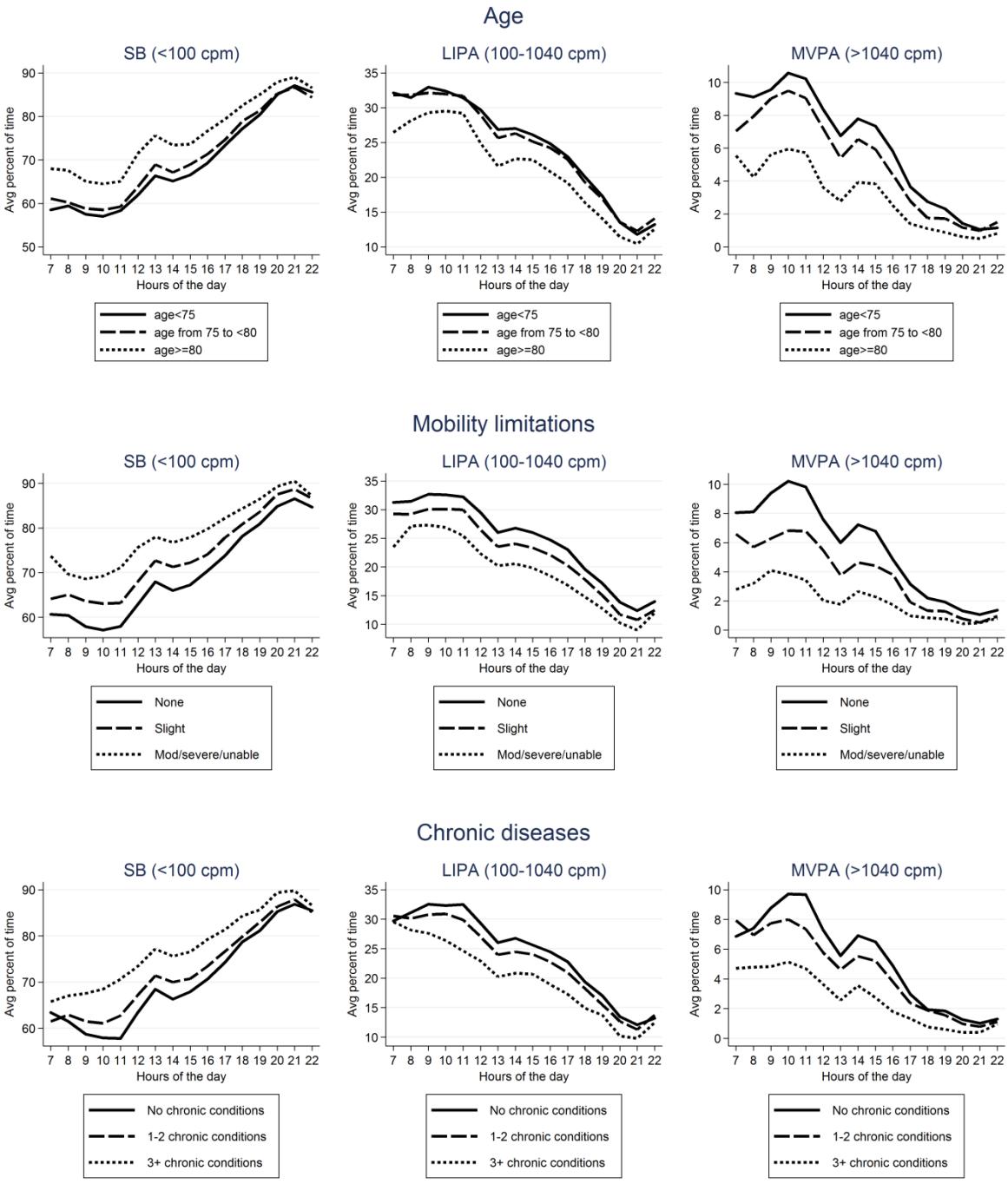


Figure 4.5 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by BMI, depression, and smoking status.

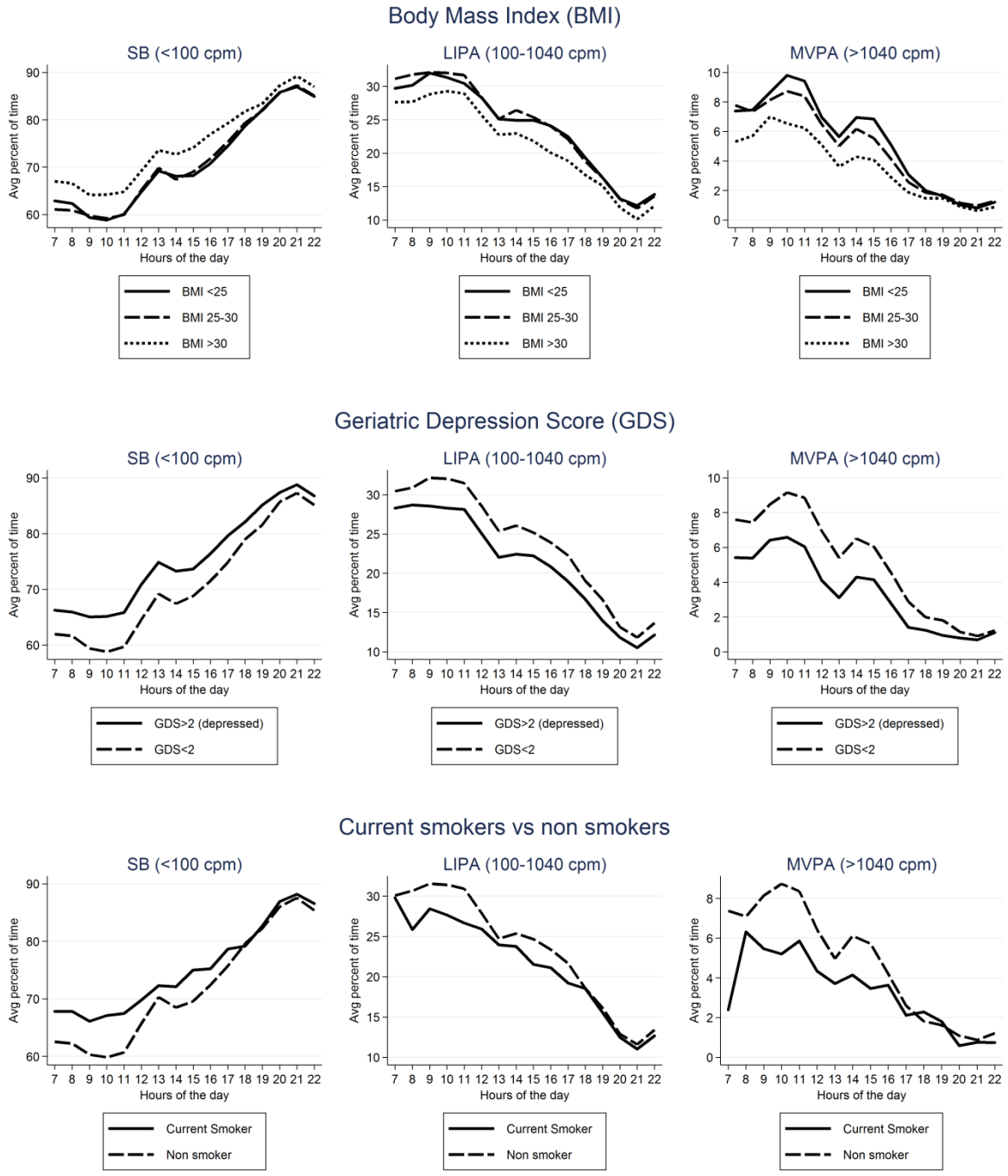
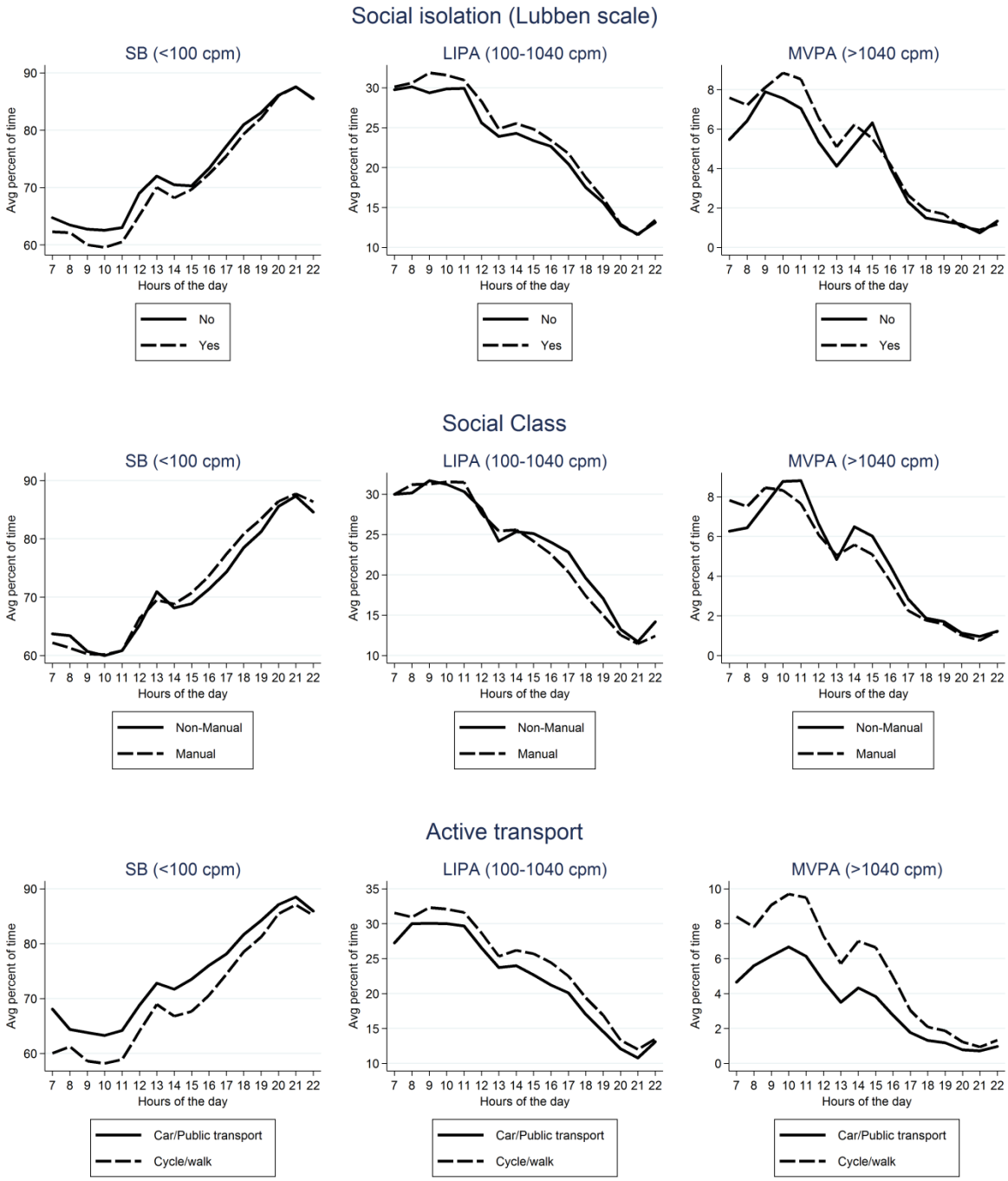


Figure 4.6 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by social isolation, social class, and use of active transport



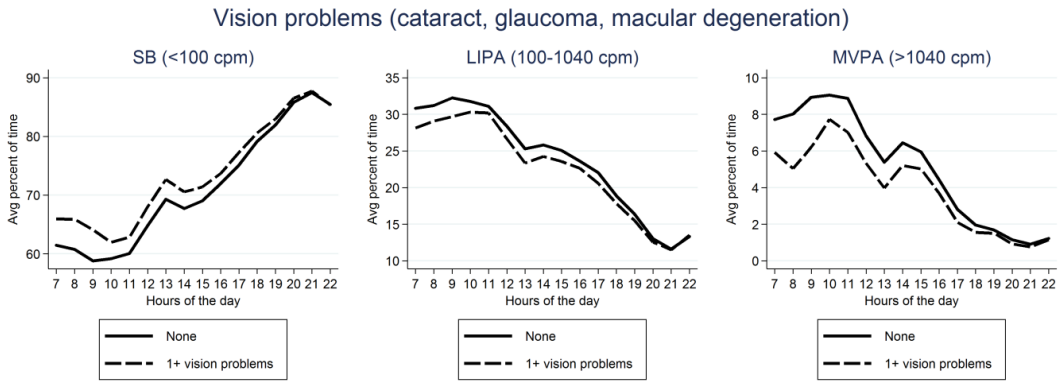


Figure 4.7 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by day of the week and season

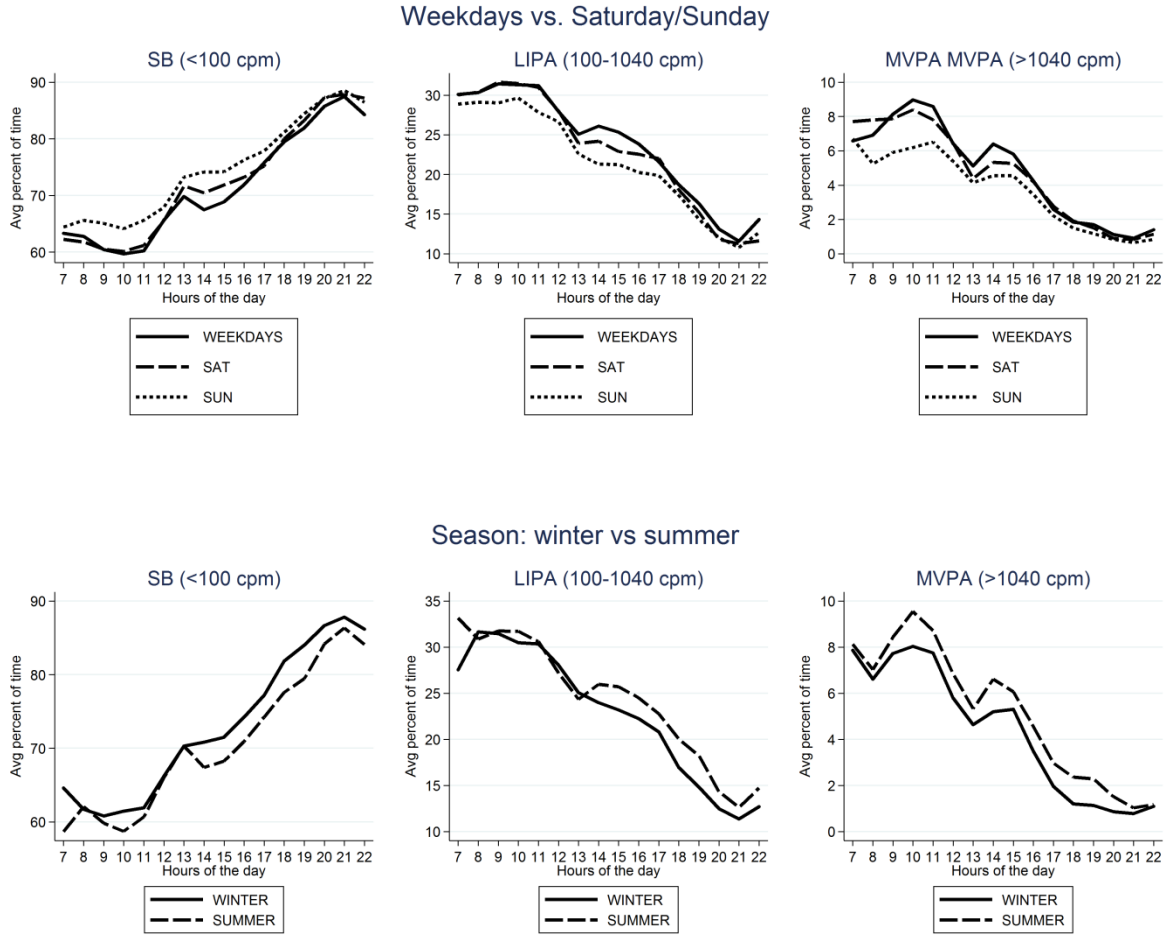
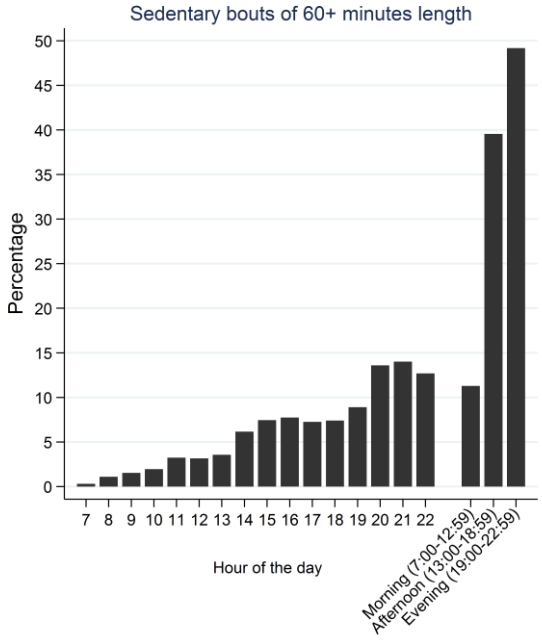
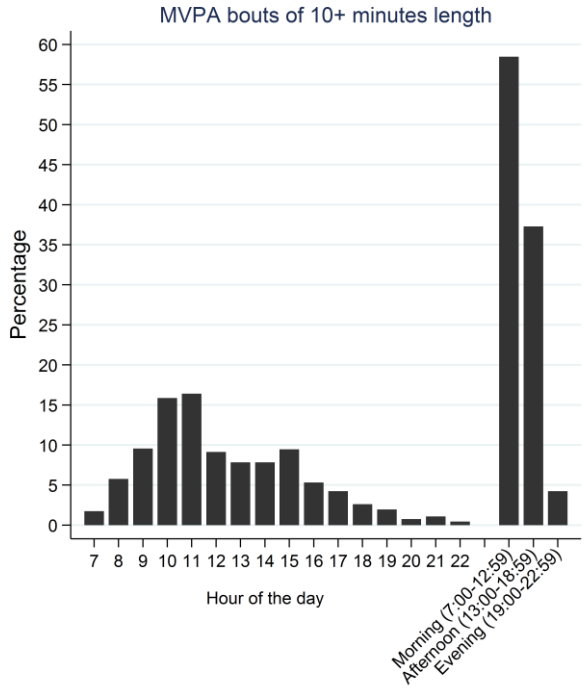


Figure 4.8 Plots from raw data: mean percentage of each hour of the day spent in sedentary bouts of 60+ minutes (top) and MVPA bouts of 10+ minutes (bottom) in 1329 men aged 71-93 years





Chapter 5 SEASONAL VARIATIONS IN PHYSICAL ACTIVITY LEVELS

5.1 Summary

Difficulties in increasing the levels of physical activity in older populations, the least active and most sedentary age group, may be in part due to a lack of understanding of seasonal patterns in physical activity levels and their determinants. Outdoor temperature is an important seasonal factor and has been acknowledged as a determinant of physical activity levels in older adults. For example, in Europe low outdoor temperatures are typically recorded in the winter season, and have been associated with less time spent walking. However, the hypothesis that outdoor temperature is a determinant of time spent in sedentary behaviours has not been explored. It is important to investigate sedentary behaviour, which is distinct from too little exercise and independently associated with cardiovascular disease and mortality, regardless of physical activity levels. Therefore, in this Chapter the main focus was to investigate the association between mean outdoor temperature (main exposure variable) and sedentary time in participants from the British Regional Heart Study (BRHS) of older men. Alongside this unique analysis on sedentary behaviour, the associations of outdoor temperature with other physical activity outcomes (number of steps, time spent in light and moderate to vigorous physical activity) were estimated and compared with findings from previous studies in older adults. Secondarily, this thesis investigated the association of other daily meteorological parameters (e.g. sunshine duration) with sedentary time and physical activity outcomes. This Chapter made use of the same data described in Chapter 4 and collected at the 32 year re-examination (3137 BRHS men aged 71-91 years who were invited to participate in a study of objectively measured physical activity). 1454 of 3137 men (46.4%) participated and wore a GT3x Actigraph accelerometer over the hip for one week in between May 2010 and July 2012. 1361 men had complete data on all covariates. Multilevel models included weekly consultation rate for influenza measured at population level; since experience of influenza like illness is another seasonally

patterned variable which may also affect ability to exercise. Additionally, associations of temperature with sedentary time and physical activity outcomes levels were adjusted for individual characteristics. When temperatures were in the lowest quintile (between -7.1°C and 6.4°C), men spent on average 19 (95%CI 12;26) minutes more per day in sedentary time in comparison with a typical summer day (temperatures in the highest quintile, between 16.2°C and 24.4°C), which corresponds to an relative increase of 2.4% (95%CI 1.6%;3.2%) in sedentary time. We also confirmed findings from previous studies in older adults which reported lower physical activity levels at lower temperatures. These findings are relevant for guidelines: interventions aimed to increase levels of activity may consider targeting older adults when temperatures are expected to be lower in winter, providing recommendations for minimising sedentariness on a daily basis, or maintaining (or enhancing) existing hours of activity.

5.2 Introduction

It has been demonstrated that both high levels of sedentary time and low levels of physical activity are independent predictors of mortality from any cause (258, 276, 277). Difficulties in modifying the low levels of physical activity observed in older populations, the least active and most sedentary age-group (17), may be in part due to a lack of understanding of the role of particular environmental influences on physical activity, especially those associated with season. As of today, the role of season has been overlooked in UK physical activity guidelines (182), and as a determinant of sedentary time (183, 184). Therefore, new research is needed to generate appropriate public health messages to older adults and professionals working with older adults (278). Until recently, previous studies used self-reported PA to investigate seasonal variations; a large meta-analysis of thirty-seven studies (published from 1980–2006) representing a total of 291,883 participants (children, adults, and older adults), reported that levels of physical activity vary by season, and that poor or extreme weather (e.g. extreme cold days) decreased participation in physical activity among various populations (41, 84), although pooled estimates of magnitudes of effect were not reported. The methods to define season and self-reported physical activity varied greatly between studies; this made a comparison of the findings complex; overall, it

was observed that study participants engaged in more active behaviours during summer (peak in June/July) (41). Much more recently, wearable devices such as hip-worn accelerometers (21) allowed objective and accurate assessment of seasonal patterns in population-based studies (17) and, of special importance for older adults, reduce the impact of recall bias (over or under reporting), participants memory loss or cognitive impairment (19, 20, 22). Until September 2018, few studies to have investigated accelerometer measured seasonal patterns in community dwelling older adults include the Nakanojo study (172-175) and PIPAOI project (176) in Japan, the Physical Activity Cohort Scotland study (177-179), a Canadian study (180) and the ActiFE study based in Germany (169). These studies used outdoor temperature to represent seasonal patterns, while other meteorological factors were not consistently investigated; the findings suggested that time spent walking (or the total number of steps) decreased at lower outdoor temperature, and shorter duration of bright sunshine (169, 172-175, 177-180). However, these studies did not consider the time spent in different activity intensities, such as light physical activity (LIPA) and sedentary behaviour (SB). Differentiating intensity of PA is very important, as prolonged sedentary time was also independently associated with health outcomes, including cardiovascular disease, regardless of PA level (18). I would intuitively expect sedentary time to be higher at lower temperatures (main proxy for season) and physical activity levels (e.g. steps) to be lower, as occur during the winter season, but this has not been yet demonstrated because temperature has been overlooked both in UK guidelines (182), and as a determinant of sedentary time (183, 184). Consistently with analysis conducted in Chapter 7 and 8, associations of temperatures with physical activity outcomes are reported after controlling for Influenza-like illness (ILI) weekly consultation rate in primary care, a proxy used in UK and other European countries to assess exposure of individuals to influenza (279-282), and which may affect the ability to engage in physical activity (283).

5.3 Objectives

The main objective of this Chapter is to investigate how physical activity levels of different intensities vary according to outdoor mean temperature in the BRHS. The main research questions (objectives) of this Chapter are:

1) Do variations in mean outdoor temperature (main seasonal factor and exposure variable analysed in this thesis) relate to variations in 4 different physical activity variables measured in the BRHS?
   Specific physical activity variables analysed:
   (i) the number of minutes per day spent in sedentary behaviour
   (ii) the number of minutes per day spent in light physical activity (LIPA),
   (iii) the number of minutes per day spent in moderate to vigorous physical activity (MVPA);
   (iv) number of steps per day

2) Do variations in mean outdoor temperature relate to variations in 4 physical activity variables after adjusting for seasonal influenza trends, and for baseline individual characteristics?
3) Is the association of mean outdoor temperature with 4 physical activity variables modified by individual risk factors?

Secondary objectives of this chapter are:

4) to conduct analyses repeating the approach described in question 1) and 2), but using other daily meteorological parameters instead of outdoor mean temperature, such as daily maximum and minimum temperatures, hours of sunshine, and relative humidity
5) to conduct sensitivity analyses repeating the approach described in question 1) and 2), but fitting all meteorological parameters as quintiles (instead of continuous variables), and one at a time, in statistical models, and investigating

their associations with the physical activity outcomes (measured in (i) minutes per day and (ii) percentage of time spent in the different activities).

5.4 Methods

5.4.1 Participants

The 32-year follow-up of the BRHS took place in between May 2010 and July 2012 and was previously described in Chapter 3 (see paragraph 3.2.4.2) and Chapter 4 (see paragraph 4.4.1). In summary, 3137 BRHS men were invited to participate in a study of objectively measured physical activity. 1454 of 3137 men (46.4%) participated and wore a GT3x Actigraph accelerometer over the hip for one week (see flow chart in Figure 4.1). All these men were independently mobile and community dwelling. Given the deliberate ordering of fieldwork in the towns to avoid having certain parts of the country done in winter and others done in summer, there should be no accidental associations between temperature and CVD risk factors

5.4.2 Physical activity assessment

The objective physical activity assessment (e.g. accelerometer data processing, wear time calculation and how PA measures were derived) was also described in the method section of Chapter 3 (see paragraph 3.4.2.1 and 3.4.2.2) and Chapter 4 (see paragraph 4.4.2), while the supporting role of the log diaries was described in paragraph 3.4.2.3.

For this Chapter the main PA variables (outcomes) of interest were (i) the number of minutes per day spent in sedentary behaviour (SB), (ii) the number of minutes per day spent in light physical activity (LIPA), (iii) the number of minutes per day spent in moderate to vigorous physical activity (MVPA); and (iv) number of steps per day.

Investigating the number of minutes, rather than the percentage of time spent in, physical activity levels was the preferred choice for the analysis (see paragraph 5.4.4), as it offered a simple and more intuitive interpretation of the results, especially regarding sedentary time (see Implications paragraph 5.6.4). However, and

consistently with Chapter 4, further sensitivity analyses were carried out using the percentage of time spent in SB, LIPA, and MVPA (as specified in paragraph 5.3).

5.4.3 Meteorological factors

Several meteorological factors were used as proxy for season; the data were provided by the UK Meteorological (MET) Office (see Methods section paragraph 3.3). For the study conducted in this chapter, the MET office provided daily maximum and minimum temperatures between 9am and 9pm, while mean temperature was calculated as the average of maximum and minimum temperatures. Mean temperature was chosen as the main seasonal factor for consistency with analysis conducted in Chapter 7 and 8 (seasonal variation in CVD markers, and seasonal variation in CVD mortality). Moreover, I decided on the use of mean temperature as the main exposure variable over maximum and minimum temperatures as it seemed the best compromise to capture PA variations over the whole daytime: maximum temperatures would more likely fall in the middle of the day, while minimum temperatures are more likely to be recorded around 9am or 9pm (the beginning and end of the time for which participants wore their accelerometer). Moreover, night-time temperatures (from 9pm to 9am) were excluded as the participants of this study had been told to remove the accelerometer during overnight sleep (see Chapter 3, paragraph 3.4.2.1 and 3.4.2.2); therefore, over-night temperatures do not relate directly to PA levels occurring in the daytime. Sunshine duration (hours) and relative humidity (RH) % were also collected but considered of secondary importance for the scope of this thesis which focuses on temperature as the main exposure variable; moreover, and according to one previous study assessing accuracy of meteorological station network measurements, the higher spatial variability of solar radiation and RH% in comparison with temperature make them less accurate exposure variables in environmental studies (see paragraph 3.3.1) (230). Rainfall and wind speed were not investigated because we considered them too prone to local fluctuations within short distances and time intervals. Snow precipitation was not explored because there was so little during the study period. The meteorological factors were linked to the accelerometer data for each day the men wore the device, as described in paragraph 5.4.2.

5.4.4 Adjusting temperature for exposure to influenza

Influenza-Like illness (ILI) weekly consultation rate (from Monday to Sunday of each week) per 100,000 population admitted to General Practice in the UK is generally used to estimate seasonal trends of influenza viruses (281, 282). In this thesis, ILI rate was consistently used in Chapter 5, 7, and 8 and included in statistical models in order to adjust temperature-related associations with the outcomes for possible seasonal confounding induced by exposure to the influenza viruses.

I decided to use ILI rate as adjustment variable in statistical models for the following reasons:

- ILI rate typically exhibits a strong peak in between December and January, when temperatures are lower: thus it relates to the main exposure variable; this explains the advantage of using ILI rates in epidemiological studies as proxy for season, as in previous studies (130, 279).
- In the UK, ILI rate includes the number of consultations for flu-like syndrome/symptoms and other illnesses including bronchitis, pneumonia, and acute respiratory infection (284), and so is associated with the outcome because such illnesses may impact the ability to exercise. I would expect the ILI rate to be higher in less active vs active individuals, as one previous study reported (283).
- I also prefer using ILI rate in comparison with generic proxies of season (e.g. dichotomic variables dividing the year in 2 parts, such as winter vs non-winter seasons) as they cannot be clearly interpreted; categorical variables or trigonometric functions of day of the year can potentially capture a seasonal trend, but this does not enhance our understanding of which biological pathways are relevant to seasonal variations in physical activity.

The limitation of using the ILI rate variable is that such a measure is typically used in studies where data are aggregated at population level. Therefore, this measure does not capture individual exposure to influenza; however, it may capture a trend in the BRHS

population: I hypothesised that the higher the ILI rate when a given individual in the BRHS was wearing their accelerometer, the more likely they were to have flu that week and thus be less active. ILI rates were collected for the study period (May 2010 to July 2012) and were freely downloadable thanks to the work carried out by the Royal College of General Practitioners in England and by Public Health Wales (281). For England and Wales together, one single value estimating the ILI weekly consultation rate per 100,000 population during the study period was used (32). ILI rates from Health Protection Scotland were not available for download; according to several on-line reports, ILI weekly consultation rates over time observed in Scottish general population were comparable to rates recorded in England and Wales, as well as the ILI rates observed in older adults aged 65+ years (281, 282). Therefore, BRHS participants residing in the 3 Scottish towns were linked to ILI weekly consultation rates recorded in England and Wales. ILI rate was linked to the accelerometer data collected during the study period and for each of the BRHS participants via week of the year and year of measurement. ILI rate is a fixed number estimated for each week (from Monday to Sunday) of the calendar year. For example, when a BRHS men started to wear the accelerometer on Wednesday [e.g. from Wednesday (day 1) to Tuesday of the following week (day 7)], the ILI rate used in statistical models (see paragraph 5.4.5.2) was the daily weighted average of 2 ILI rates [e.g. ILI rate of the first week*5 (Wednesday-Sunday) + ILI rate of the second week*2 (Monday-Tuesday, all divided by 7].

5.4.5 Statistical methods

5.4.5.1 Descriptive statistics

Several preliminary analysis were carried out to explore the data:

- Characteristics of men who met the inclusion criteria for the study (n=1454) and men who did not meet the inclusion criteria were compared
- Means and standard deviations (SD) of meteorological factors and ILI rate was compared by season, which was classified as winter (December-February),

Spring (March-May), Summer (June-August), Autumn (September-November).

- Correlations between meteorological factors and ILI rate were calculated
- Raw physical activity levels (steps, minutes spent in LIPA, MVPA, and SB) were averaged over the valid week for each participant and means (with 95% CI) were plotted against quintiles of meteorological factors and ILI rates during the study period.
- As secondary analysis, for LIPA, MVPA, and SB the results were also expressed as percentage of wear time over a valid week.

5.4.5.2 Associations between meteorological factors and physical activity levels

As it has been hypothesised that adverse weather conditions during winter, such a decrease in temperatures or cold days, are associated with a decrease in physical activity levels (41), the results of this Chapter were presented as the change in physical activity levels associated with a decrease of 1 SD (5.4°C) in mean outdoor temperature. Linear regression models were used to investigate such associations. The outcomes were: sedentary time, time spent in LIPA, time spent in MVPA and number of steps per day. Other exposure variables taken into account in the analysis were maximum and minimum temperature, sunshine duration and relative humidity (meteorological factors with SDs of 5.7°C, 5.2°C, 3.8 hours, and 12.6%)

Since data comprised repeated measures for each day of wear by individuals, multilevel models were used for regression analysis. In all multilevel models, level 1 was day order (first day of accelerometer wear, second day, etc.) and level 2 was the individual. We used a random intercept only (each individual had their own intercept) and estimated one slope for each of the meteorological factors fitted as continuous variables. Estimates from unadjusted models were compared with adjusted models, which included adjustment for:

- At level 1: several measurement variables which could vary over the week the mean wore the accelerometer (accelerometer-wear time, wear day order [first day of wear, second, etc.], day of the week).
- At level 2: the adjustment for ILI rate was made to check whether there was confounding between mean temperature and a different seasonal factor (collinearity between temperature and ILI was not observed as the Variance Inflation Factor (VIF) score was less than 1.5). Adjustment was made for age, social class, Body Mass Index (BMI), chronic conditions, mobility limitations, geriatric depression scale, vision problems, smoking status, and marital status, since the relationship of these variables with outcome is known a priori to be strong in the BRHS (17), although it is unlikely they are related to exposure (mean temperature). Including such covariables in the model would have the scope of reducing the standard error and increasing precision of the estimated effect of our exposure variable.

As a sensitivity analysis, a linear model for each of the physical activity outcomes was performed using each of meteorological parameters (fitted one at a time) divided into quintiles. The objective of this analysis was to offer a more intuitive interpretation of the findings, as meteorological parameters in the lowest quintile (e.g. mean temperature Q1, -7.1°C; 6.4°C) were representative of the typical UK winter, while meteorological parameters in highest quintile (e.g. mean temperatures Q5: 16.1°C; 24.4°C) were representative of the typical UK summer (123). This also allowed explorations of whether the relationship between physical activity and each meteorological parameter was linear.

5.4.5.3 Interaction of temperature with individual risk factors

Interaction of temperature with age and other socio-demographic characteristics and health variables (e.g. diabetes status) was not found in one previous study of Scottish older adults aged 65 and over (178). To confirm prior findings, I tested whether lower temperatures particularly affect the oldest old men in the BRHS (e.g. men aged 80+ years) and those who are obese; they can potentially perceive lower outdoor

temperatures as a more difficult barrier to overcome, opting for more sedentary behaviours in a warmer indoor environment. Therefore I tested the interaction of temperature with age categories (<75, 75-79, and 80+ years old) and temperature with BMI categories (<=24.9, 25-29.9, 30+). An overall Wald test for interaction between the categorical variables and temperature was used.

5.5 Results

5.5.1 Descriptive statistics

5.5.1.1 Participants

Among 3137 surviving men, 1454 (46.3%) provided adequate data for analysis. Comparisons between included and excluded participants were already presented in Chapter 4 (Table 4.1). In summary, participants were younger than non-participants and in a survey 10 years earlier had lower BMI and were less likely to be smokers. Participants took on average 4800 steps per day and spent 618 minutes in sedentary time, 196 minutes in LIPA and 39 minutes in MVPA. 1361/1454 men (93.6%) with complete data (accelerometer data and temperature on the same day, plus data on all covariates) had similar characteristics to those who did not have complete data on all covariates. From this point forward, all results presented here refer to the 1361 men (complete case analysis).

5.5.1.2 Meteorological parameters

Means and standard deviations of the meteorological factors and ILI rates during the study period were summarized (Tables 5.1 and 5.2). Average temperatures (mean, maximum and minimum) were lowest in winter and highest in summer. Conversely, RH% and ILI rates were highest in winter, and lowest in summer. Sunshine duration was lowest in winter and highest in spring, on those days when participants were measured. Mean temperature and sunshine were positively correlated (r=0.30, Table 5.3), while mean temperature and RH% were negatively correlated (r=-0.40, Table 5.3). Similarly, mean temperature and ILI rate was also negatively correlated (r=-0.57,

Table 5.4). A full summary of the correlations between meteorological factors and ILI rate is provided in Table 5.3 and 5.4.

5.5.1.3 Plots of physical activity levels vs seasonal factors

Over a course of a valid week, the number of steps and minutes spent in LIPA and MVPA was lower at lower temperatures (mean, maximum, and minimum), lower sunshine hours and higher level of relative humidity (Figure 5.1-5.3), and a fairly linear trend was observed. Conversely, time spent in SB was higher at lower temperatures, lower sunshine hours, and higher level of humidity (Figure 5.1-5.3). Patterns of percentage of time in LIPA, MVPA, and sedentary time vs meteorological parameters were very similar (Figure 5.4 and 5.6).

The number of steps and minutes spent in LIPA, MVPA were typically higher when ILI consultation rates were lower. Conversely, time spent in SB was higher when ILI consultation rates were also higher (Figure 5.1 and 5.4).

5.5.2 Associations between meteorological factors and physical activity levels

In an unadjusted model, a decrease of 1 SD (5.4°C) in mean temperature was associated with an increase of 7 minutes in sedentary time per day (95%CI 4;11, Table 5.5 Model 1). The additional adjustment of temperature for ILI rate and other baseline characteristics did not alter substantially the magnitude of these associations (increase of 8 minutes in sedentary time, 95%CI 5;11, Table 5.5 Model 4). Mean temperature was also strongly associated with other physical activity outcomes: a decrease of 1 SD in mean temperature was associated with a decrease of 5 minutes in LIPA per day (95%CI -7; -3), a decrease of 3 minutes in MVPA per day (95%CI -4; -2), and -234 steps per day (95%CI -341; -128). Similar associations were found for maximum temperatures, while associations with minimum temperatures were smaller (Table 5.6).

Fewer hours of sunshine and higher relative humidity were associated with higher levels of sedentary time (Table 5.6). Similarly, variations of hours of sunshine and

relative humidity were also associated with variations of time spent in LIPA, MVPA, and steps per day (Table 5.6).

An increase in ILI rate was associated with an increase in sedentary time and a decrease in physical activity levels in unadjusted models; however, after mutual adjustment with temperature (mean, maximum, or minimum) ILI rate was no longer significant (Table 5.7). ILI rate maintained its association with steps, LIPA and MVPA when adjusted for sunshine or RH% (Table 5.7).

The fully adjusted analysis of mean temperature divided into quintiles confirmed the findings of linear models when temperature was fitted as continuous variable; the relationship between temperature and the physical activity variables appeared to be linear. Men spent 19 minutes more per day (95%CI 12; 26) in sedentary time when temperatures were in the lowest compared with the highest quintile (Table 5.8). Also, men spent 13 minutes more per day (95%CI 7; 19) in LIPA and 7 minutes more per day (95%CI 3; 10) in MVPA when temperatures were in the lowest compared with the highest quintile (Table 5.8). The difference in sedentary time was similar between the bottom and top quintile of other meteorological factors (temperature maximum, minimum, and sunshine duration). In relative terms, the percentage of sedentary time increased by 2.4% (95%CI -3.2; 1.6) per day at lower mean temperatures vs higher, while percentage of time spent in LIPA and MVPA decreased by 1.6% (95%CI 1.0; 2.3) and 0.8% (95%CI 0.4; 1.2) per day respectively when comparing lower vs higher mean temperatures (Table 5.9).

5.5.3 Interaction of temperature with individual risk factors

I did find a consistent interaction between temperature and age on MVPA and steps (Table 5.10). In men aged 80+ vs <75 years old, there was an additional decrease in MVPA per a decrease in 1 SD in mean temperature of 5.6 (95%CI -8.7; -2.5, p<0.001) minutes per day (p-value for trend across the three age categories was equal to 0.001). Similar findings were found when analysing steps: in men aged 80+ vs <75 years old, there was an additional decrease in MVPA per a decrease in 1 SD in mean temperature

of 378 (95%CI -626; -124, p<0.001) steps per day (p-value for trend across the three categories was equal to 0.003). Findings do not provide enough evidence to support an interaction between temperature and age on LIPA or SB (exact p-values reported in Table 5.10).

In men with BMI of 30+ vs <25 there was an additional decrease in MVPA per a decrease in 1 SD in mean temperature of -3.9 (95%CI -7.4; -0.5, p=0.027) minutes per day (p-value for trend across the three categories = 0.070). I did not find clear evidence of interaction between temperature and BMI on steps, LIPA and sedentary time (exact p-values reported in Table 5.10).

5.6 Discussion

5.6.1 Summary of main findings

I discuss here the main findings from this Chapter according to the objective listed in paragraph 5.3.

Question 1) Do variations in mean outdoor temperature (main seasonal factor and exposure variable analysed in this thesis) relate to variations in 4 different physical activity variables measured in the BRHS?

In older British men, decreases in outdoor mean temperatures (measured during day-time in between 9am and 9pm) were associated with decreases in steps taken, LIPA and MVPA and increases in sedentary time.

Question 2) Do variations in mean outdoor temperature relate to variations in 4 physical activity variables after adjusting for seasonal influenza trends, and for baseline individual characteristics?

Results showed that associations of temperature with the outcomes remained after adjusting for a proxy of influenza severity (ILI rate) and individual characteristics. The

associations of ILI rate with the outcomes were wiped out after mutual adjustment for temperature.

Question 3) Is the association of mean outdoor temperature with 4 physical activity variables modified by individual risk factors ?

The interaction of temperature with age and BMI were found only when analysing associations with MVPA: men who were obese and older, especially those aged 80+ years or more, additionally decreased their time spent in MVPA at lower temperatures. These findings suggested that for obese and oldest old men engaging in more intense levels of activity, rather than just leisure activities, can be particularly demanding during cold days.

4) to conduct analyses repeating the approach described in question 1) and 2), but using other daily meteorological parameters instead of outdoor mean temperature, such as daily maximum and minimum temperatures, hours of sunshine, and relative humidity

Results showed that, similarly to findings of mean temperature, a decrease in maximum and minimum temperatures, a decrease in sunshine duration, and higher relative humidity were also associated with an increase in sedentary time and a decrease in steps, LIPA, and MVPA.

5) to conduct sensitivity analyses repeating the approach described in question 1) and 2), but fitting all meteorological parameters as quintiles (instead of continuous variables), and one at a time, in statistical models, and investigating their associations with the physical activity outcomes (measured in (i) minutes per day and (ii) percentage of time spent in the different activities)

These analyses confirmed the findings reported for questions 1 and 2 (temperature fitted as continuous variable in statistical models), and the relationship between

temperature divided in quintiles and the physical activity variables was linear. This analysis offered a simple and intuitive interpretation of the results, especially regarding sedentary time: during a typical winter day (temperature in the lowest quintile) older men spent 21 minutes more per day in sedentary time in comparison with a typical summer day (temperatures in the highest quintiles). In relative terms, the percentage of sedentary time increased by 2.4% per day when temperatures were in the lowest compared with the highest quintile.

5.6.2 Comparison with other studies

To our knowledge the findings on time spent in sedentary behaviour and light physical activity are novel and not previously reported. Especially for sedentary time, literature in this field is sparse; one small study of forty-six adults reported that accelerometer-measured sedentary time was higher in winter than summer, although the participants were about 40 years younger than our population (185). The majority of the studies investigated children or adolescents, who are known to have a different life-style (e.g. fixed school hours during the day) in comparison with older adults (187).

The influence of seasonal and meteorological factors on PA among older adults has been investigated in only four study settings spanning Japan, Germany, Canada and Scotland (169, 172-180). In line with these previous studies, we confirmed that PA varies according to meteorological factors, with lower PA levels at colder temperatures and lower sunshine duration. However, in our study we also examined the effect of adjusting these associations for ILI rate, an illness-specific proxy for season, which none of those previous studies has done. ILI was higher during the winter season and associated with lower level of PA, although its association with the outcomes disappeared after the adjustment for temperature (169), suggesting that daily changes in temperature may be a more important determinant of PA levels, or because ILI rates measured at national levels may not represent exposure at individual level in the BRHS. However, whether ILI rate maintains its association with physical activity if measured at individual level still has to be demonstrated. A linkage of individual data from BRHS with data recorded in primary care data (284) may help in enhancing our

understanding the relevance of ILI to seasonal variation in physical activity levels in the BRHS.

In our study the most important weather parameter in terms of effect size was maximum temperature, although the difference with mean temperature and sunshine duration effect sizes was very small (1-2 minutes difference in sedentary time, LIPA, and MVPA, and about 50-80 steps per day). Earlier studies had identified a range of different meteorological factors including global radiation (a surrogate of sunshine duration) (169); day length and diurnal minimum temperature (178); rainfall and mean temperature (173, 174) as being the most important. We would expect that, as in our results, sunshine duration is positively correlated with temperature. Our findings on temperature add new elements to the debate about which meteorological factors are most important predictors of PA; this study suggested that recommendation for increasing participation in PA should not consider minimum temperature and relative humidity among best predictors. It is possible that minimum temperature, which typically falls at the beginning and the end of the day may not capture the PA variation during the day. RH% can be seen as marker of rainfall (e.g. RH<70%, lowest quintile in this study, means the weather is typically dry when the Meteorological Office measured RH%, at 9am in the morning) and therefore suggesting that other factors than rainfall, such as temperature, may be important. However, with only one measure of RH% collected during one day, more research is needed to estimate its full contribution to PA variation.

During the study period maximum temperatures above 30°C were not recorded. It is possible that at temperatures above 30°C (more typical of warmer climate zones than the UK) physical activity may decrease, and consideration could be given to suggesting activities in adequately air conditioned indoor areas. This may have considerable implications for designing interventions for older adults, and future research can investigate this.

5.6.2.1 Comparison with studies published after June 2015

This PhD thesis started in June 2014, and the literature review of this thesis (see Chapter 2, Paragraph 2.1) included previous published papers published until June 2015. Later in 2017, results from the European Prospective Investigation into Cancer and Nutrition (EPIC) based in Norfolk, UK, (285) and a small Canadian study based in south-western Ontario (286) were published. Similarly to the findings presented in this Chapter, which were also published in the same year (57), the 4051 participants (mean age of 69 years, with a range from 49 to 92 years) from the EPIC study were less active during short day length and poor weather conditions, including high precipitation and low temperatures (285). In the Canadian study the 50 community-dwelling older adult (mean age 77 years, range 71-89 years) who wore an accelerometer between February and April of the same year were more active as the winter season transitioned to spring (286).

5.6.3 Strengths and limitations

This study benefits from using the same population of free-living older men described in Chapter 4. Therefore the strengths and limitations of this study are similar; for example, the response rate achieved is comparable or superior to other studies using objective measurements of physical activity [4]. Also, men who did not participate were slightly older and had higher BMI and lower PA score measured 10 years earlier; however, this is not a limitation for the study presented in this Chapter, as this would not be expected to affect the observed associations between season, weather and physical activity. Our results may not be generalizable to older women or ethnic minority populations (269); however, we would expect to observe a seasonal variation in women as well, as reported in the EPIC study (285). Women of the EPIC study were less active than men, but whether the seasonal variation in physical activity was greater in men vs women was not formally tested (nor was a stratified analysis by sex and season presented). As it is known that older men are generally more active than women (17), a slightly greater seasonal variation in men vs women seems plausible.

To date, this is one of the largest study to investigate in older adults the influence of season and weather on objectively measured daily activity and sedentary behaviour, how they vary by season and the influences of the most commonly used meteorological factors.

There are several advantages in using physical activity data from accelerometers, and these were already mentioned in Chapter 4. For example, the Actigraph objectively measured PA intensities used age-appropriate and validated cut points (221), as already reported in Chapter 4 paragraph 4.6.3.1. In healthy older adults the Actigraph sedentary time cut-point of <100CPM has an estimated 93% and 58% in sensitivity and specificity respectively, while 11.8% of time classified by Activpal (which also record data on posture), as standing was classified as sedentary. However, in comparison, the BRHS participants are older and potentially less healthy, therefore likely to spend less time standing, which would improve classification.

Overall, this paper did not aim to investigate the "type" of activity in relation to weather and season. Such data were not available for all men for analysis. Although men were asked to complete a log diary self-reporting type of activity every hour over three days the degree of data completeness was judged to be insufficiently consistent to study as a specific objective, as mentioned in Chapter 4 (paragraph 4.6.1).

5.6.4 Implications

The findings presented in this Chapter suggested that lower temperatures and lack of sunshine may particularly inhibit older individuals from being more active. When temperatures are lower, typically in winter, it is possible that older adults may prefer replacing some incidental light physical activity outdoors (e.g. a gentle walk for pleasure) with sedentary behaviours indoors, such as television watching (287). The results of this Chapter may have important implications for guidelines. The UK recommendations suggest that older adults should aim to minimise the time they spend being sedentary each day (183). Our findings suggested that there are opportunities for minimising sedentary behaviours particularly at low temperatures, a typical element

of the winter season. The findings reported that during a typical winter day older men spent about 20 minutes more per day in sedentary time in comparison with a typical summer day; replacing even half of that time spent in sedentary behaviours with more active behaviours every day may have beneficial effects on health over the course of the years. However, it is challenging to find ways to reduce sedentariness, as in modern life opportunities for sedentary behaviours are everywhere. On the other hand, it is likely that interventions targeting individuals' psychological and environmental barriers (beliefs, feelings, and perspectives on participations in physical activity) may be a valid alternative for replacing sedentary time with more active behaviours (288, 289). Providing recommendations for simple do-it-yourself exercises (e.g. standing up or walking while watching TV, toe rises, calf and chest stretching) could be helpful (290). In older individuals, simple targets can make the reduction in sedentary behaviour easier to achieve and relevant on a daily basis (290). Also, providing physically and economically accessible indoor opportunities in a warmed and properly heated environment (e.g. indoor exercise classes) could help in promoting more active behaviours during winter.

The temperature-related variation in sedentary time observed in this study could be relevant to the temperature-related variation in mortality risk (29). It is plausible that low temperatures in winter (primary determinant) may be a contributing factor which increases the sedentary time, as well as other risk factors levels (that are described in Chapter 7, such as inflammatory markers (148)), contributing to the excess of winter mortality (291). In this Chapter we observed an increase of about 20 minutes in sedentary time at lower versus higher temperatures. According to previous studies in older adults, replacing 30 minutes of sedentary time with light physical activity was independently associated with a significant reduction in mortality risk (HR = 0.80) (292). In relative terms, this study showed that the percentage of sedentary time increased by 2.4% at lower mean temperatures vs higher temperatures, while percentage of time spent in LIPA and MVPA decreased by 1.6% and 0.8% respectively when comparing lower vs higher mean temperatures. However, future investigations are needed to establish how temperature-related variations in sedentary time and

physical activity of different intensities may contribute to the temperature-related variations in mortality risk. For example, epidemiological studies using cheaper and more accessible consumer grade wearable devices (e.g. Fitbit) could simultaneously and continuously measure meteorological factors and physical activity levels by second, minute or daily, and could follow-up their participants for CVD. However, the precision of their device algorithms generating the data may vary over time, by device manufacturer, model and wearing position (293, 294). Also, concerns about privacy of study participants and data ownership would have to be addressed.

5.7 Conclusions

This study highlights that number of steps, and PA levels of light and moderately vigorous intensities decrease at lower temperatures, typically recorded during the winter season. These findings are relevant for guidelines: interventions may consider targeting older adults when temperatures are expected to be lower in winter, providing recommendations for minimising sedentariness on a daily basis, or maintaining (or enhancing) existing hours of activity.

Table 5.1 Meteorological factors levels during the study period, stratified by season.

The unit of observation is the unique date in each different BRHS town on which at least one BRHS participant wore the accelerometer

Variable	Number of days in Winter (Dec-Feb) n=210 [6]	Spring (Mar-May) n=301 [6]	Summer (Jun-Aug) n=425 [6]	Autumn (Sep-Nov) n=327 [6]	Total n=1263 [6]
Mean temperature (°C), mean (SD) [1]	4.6 (3.8)	12.3 (4.3)	16.0 (2.4)	11.9 (5.4)	11.9 (5.4)
Maximum temperature (°C), mean (SD) [2]	6.7 (3.8)	15.4 (4.8)	18.6 (2.9)	14.5 (5.7)	14.5 (5.7)
Minimum temperature (°C), mean (SD) [3]	2.6 (3.9)	9.2 (4.1)	13.3 (2.2)	9.2 (5.2)	9.2 (5.2)
Relative Humidity (%), mean (SD) [4]	90.4 (8.7)	79.7 (14.6)	77.8 (12.1)	82.4 (9.5)	82.6 (12.6)
Sunshine duration (hours), mean (SD) [5]	2.0 (2.4)	6.0 (4.5)	4.4 (3.7)	4.0 (3.8)	4.0 (3.8)

[1] Average of maximum and minimum air temperatures of the day (from 9am to 9pm)

[2] Highest air temperatures of the day (from 9am to 9pm)

[3] Lowest air temperatures of the day (from 9am to 9pm)

[4] Relative humidity is a single value recorded every day at 9am

[5] Duration of bright sunshine during the day, in hours (from 00:00 - 23:59)

[6] The total number of observations is calculated over every day each participant wore an accelerometer

Table 5.2 England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons (mean, SD) during the study period, stratified by season.

The unit of observation is the week of each year on which at least one BRHS participant wore the accelerometer

	Number of weeks in Winter (Dec-Feb) n= 22	Number of weeks in Spring (Mar-May) n=23	Number of weeks in Summer (Jun-Aug) n=26	Number of weeks in Autumn (Sep-Nov) n=26	Number of weeks in Total n=97
England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons, mean (SD)	19.4 (24.2)	5.4 (2.9)	2.4 (0.6)	6.9 (2.7)	8.2 (13.1)

Table 5.3 Correlations between meteorological factors during the study period.

The unit of observation is the unique date in each different BRHS town on which at least one BRHS participant wore the accelerometer (n=1263)

Meteorological factors correlations *	Mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Relative Humidity (%)	Sunshine duration (hours)
Mean temperature (°C)	1				
Maximum temperature (°C)	0.98	1			
Minimum temperature (°C)	0.98	0.93	1		
Relative Humidity (%)	-0.40	-0.42	-0.36	1	
Sunshine duration (hours)	0.30	0.40	0.19	-0.52	1

*All correlations are statistically significant ($p<0.001$)

Table 5.4 Correlations among England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons and meteorological parameters averaged over the same week (n=97) during the study period.

The unit of observation is the week of each year on which at least one BRHS participant wore the accelerometer

Correlations over number of weeks (n=97)	Mean temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Relative Humidity (%)	Sunshine duration (hours)
England/Wales Influenza-like Illness (ILI)	-0.57	-0.57	-0.57	0.31	-0.26

*All correlations are statistically significant ($p<0.001$)

Table 5.5 Adjusted associations of mean temperature with sedentary time and physical activity levels in BRHS men.

Note: All estimates are reported as mean difference in the outcome levels for a decrease in 1 standard deviation in mean temperature [1]

n=1361 in all models

Outcome	Model 1: mean temperature	Model 2: mean temperature + ILI	Model 3: mean temperature + ILI + age	Model 4: mean temperature + ILI + age + other CVD risk factors and measurement variables
Sedentary Time	7(4,11)	7(3,11)	7(4,11)	8(5,11)
Time spent in LIPA	-8(-10,-6)	-7(-9,-4)	-7(-9,-5)	-5(-7,-3)
Time spent in MVPA	-4(-5,-3)	-4(-5,-2)	-4(-5,-2)	-3(-4,-2)
Number of steps	-330(-429,-231)	-299(-412,-186)	-314(-425,-203)	-234(-341,-128)

[1] Models 1-4 are multilevel regression models (level 1=date, level 2= individual). Model 4 is additionally adjusted for social class, BMI, chronic conditions, mobility limitations, geriatric depression scale, vision problems, smoking status, marital status, daily wear time, day of the week, wear day order

Table 5.6 Adjusted associations of mean temperature with sedentary time and physical activity levels in 1361 BRHS men

All estimates are reported as mean difference in the outcome levels for a decrease in 1 standard deviation in meteorological parameter [1] (see table 5.2), n=1361 in all models

Outcome	Meteorological parameter	Model 1: unadjusted	Model 2: Model 1+ ILI	Model 3: Model 2 + age	Model 4: Model 3 + other CVD risk factors
		Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)	Mean difference (95% CI)
Sedentary time	Mean temperature (°C)	7(4,11)	7(3,11)	7(4,11)	8(5,11)
	Max temperature (°C)	9(6,12)	9(6,13)	9(6,13)	11(8,13)
	Min temperature (°C)	4(1,7)	3(-1,6)	3(-0,7)	3(-0,5)
	Sunshine duration (hours)	8(6,10)	8(6,10)	8(6,10)	10(8,11)
	Relative humidity (%)	-5(-8,-3)	-5(-7,-3)	-5(-7,-3)	-6(-8,-5)
Time spent in LIPA	Mean temperature (°C)	-8(-10,-6)	-7(-9,-4)	-7(-9,-5)	-5(-7,-3)
	Max temperature (°C)	-9(-11,-7)	-8(-11,-6)	-8(-11,-6)	-7(-9,-5)
	Min temperature (°C)	-5(-7,-3)	-3(-5,-1)	-3(-6,-1)	-2(-4,0)
	Sunshine duration(hours)	-7(-8,-5)	-6(-8,-5)	-6(-8,-5)	-6(-8,-5)
	Relative humidity (%)	5(4,7)	5(4,6)	5(4,6)	4(3,5)
Time spent in MVPA	Mean temperature (°C)	-4(-5,-3)	-4(-5,-2)	-4(-5,-2)	-3(-4,-2)
	Max temperature (°C)	-5(-6,-3)	-4(-6,-3)	-5(-6,-3)	-4(-5,-3)
	Min temperature (°C)	-2(-3,-1)	-2(-3,-0)	-2(-3,-1)	-1(-2,0)
	Sunshine duration(hours)	-3(-4,-3)	-3(-4,-2)	-3(-4,-2)	-3(-4,-3)
	Relative humidity (%)	3(2,4)	3(2,4)	3(2,3)	2(1,3)
Number of STEPS	Mean temperature (°C)	-330(-429,-231)	-299(-412,-186)	-314(-425,-203)	-234(-341,-128)
	Max temperature (°C)	-394(-489,-299)	-383(-489,-277)	-393(-498,-289)	-325(-426,-225)
	Min temperature (°C)	-194(-289,-99)	-132(-238,-26)	-149(-254,-44)	-79(-179,22)
	Sunshine duration(hours)	-289(-348,-229)	-280(-339,-220)	-278(-338,-219)	-282(-340,-225)
	Relative humidity (%)	255(193,317)	242(179,304)	236(174,298)	185(124,246)

[1] Models 1-4 are multilevel regression models (level 1=date, level 2= individual). Model 4 is additionally adjusted for social class, BMI, chronic conditions, mobility limitations, geriatric depression scale, vision problems, smoking status, marital status, daily wear time, day of the week, wear day order

Table 5.7 Adjusted associations of England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons with sedentary time and physical activity levels in BRHS men.

All estimates are reported as mean difference for an increase in 1 standard deviation in ILI (SD=13.1)

n=1361 in all models

Outcome	Unadjusted ILI
Sedentary Time	13(4,22)
Time spent in LIPA	-17(-24,-10)
Time spent in MVPA	-7(-10,-3)
Number of steps	-627(-928,-326)
	ILI adjusted for Mean temperature
Sedentary Time	3(-8,13)
Time spent in LIPA	-7(-15,1)
Time spent in MVPA	-2(-6,2)
Number of steps	-199(-540,142)
	ILI adjusted for Max temperature
Sedentary Time	-0(-11,10)
Time spent in LIPA	-5(-13,3)
Time spent in MVPA	-0(-4,4)
Number of steps	-80(-417,256)
	ILI adjusted for Min temperature
Sedentary Time	9(-2,19)
Time spent in LIPA	-12(-20,-5)
Time spent in MVPA	-4(-8,-0)
Number of steps	-441(-777,-105)
	ILI adjusted for Sunshine duration
Sedentary Time	9(-0,18)
Time spent in LIPA	-14(-21,-7)
Time spent in MVPA	-5(-9,-2)
Number of steps	-496(-798,-193)
	ILI adjusted for Relative Humidity
Sedentary Time	9(0,18)
Time spent in LIPA	-13(-21,-6)
Time spent in MVPA	-5(-8,-1)
Number of steps	-464(-767,-161)

Table 5.8 Adjusted associations between quintiles (Q) of meteorological parameters and physical activity levels in BRHS men

n=1361 in all models

Note: estimates are from multilevel regression models (level 1=date, level 2= individual) adjusted for age, social class, BMI, chronic conditions, mobility limitations, geriatric depression scale, vision problems, smoking status, marital status, daily wear time, day of the week, wear day order, and ILI

Meteorological factor	Mean difference (95%CI) in number of steps per day	Mean difference (95%CI) for time spent in LIPA (minutes per day)	Mean difference (95%CI) for time spent in MVPA (minutes per day)	Mean difference (95%CI) for time spent in SB (minutes per day)
Temperature mean				
1Q (-7.1; 6.4), reference				
2Q (6.5; 10.2)	25(-170,220)	-1(-4,3)	1(-1,3)	-0(-5,5)
3Q (10.3; 13.7)	104(-138,345)	5(-0,10)	3(-0,6)	-7(-13,-1)
4Q (13.8; 16.1)	284(10,559)	11(5,16)	4(0,7)	-14(-21,-7)
5Q (16.2; 24.4)	539(255,824)	13(7,19)	7(3,10)	-19(-26,-12)
Temperature max				
1Q (-3.5; 9.2), reference				
2Q (9.3; 13.0)	168(-32,369)	3(-1,7)	3(0,5)	-5(-10,-0)
3Q (13.1; 16.5)	296(55,536)	7(2,12)	5(2,8)	-12(-18,-6)
4Q (16.6; 19.0)	450(186,713)	14(9,20)	5(2,9)	-20(-26,-13)
5Q (19.1; 29.5)	791(511,1070)	17(11,23)	10(6,13)	-27(-34,-19)
Temperature min				
1Q (-11; -4), reference				
2Q (4.1; 8.0)	-51(-233,132)	-0(-4,3)	0(-2,2)	1(-4,5)
3Q (8.1; 11.0)	-191(-415,33)	-0(-5,4)	-2(-4,1)	3(-3,8)
4Q (11.1; 13.5)	-69(-331,194)	4(-2,9)	-0(-4,3)	-2(-9,4)
5Q (13.6; 20.0)	102(-169,373)	5(-1,10)	1(-2,5)	-5(-12,2)
Sunshine duration				
1Q (0.0; 0.3), reference				
2Q (0.4; 1.9)	63(-89,216)	2(-1,5)	1(-1,2)	-2(-6,2)
3Q (2.0; 4.5)	213(60,366)	5(2,8)	3(1,5)	-7(-11,-4)
4Q (4.6;7.4)	447(293,602)	8(5,12)	5(4,7)	-14(-18,-10)
5Q (7.5;15.4)	725(553,897)	17(14,21)	8(6,10)	-25(-29,-21)
Relative Humidity %				
1Q (43;71), reference				
2Q (72; 81)	-182(-342,-22)	-6(-9,-3)	-2(-4,-0)	8(4,12)
3Q (82; 88)	-351(-530,-171)	-9(-13,-5)	-4(-6,-2)	13(8,17)
4Q (88; 96)	-437(-608,-267)	-11(-14,-7)	-5(-7,-3)	16(12,20)
5Q (97; 100)	-404(-596,-212)	-9(-13,-5)	-4(-7,-2)	13(8,18)

Table 5.9 Adjusted associations between quintiles (Q) of meteorological parameters and percentage of time spent in physical activity levels in BRHS men

n=1361 in all models
Note: estimates are from multilevel regression models (level 1=date, level 2= individual) adjusted for age, social class, BMI, chronic conditions, mobility limitations, geriatric depression scale, vision problems, smoking status, marital status, daily wear time, day of the week, wear day order, and ILI

Meteorological factor	Mean difference (95%CI) for % of time spent in LIPA (minutes per day)	Mean difference (95%CI) for % of time spent in MVPA (minutes per day)	Mean difference (95%CI) for % of time spent in SB (minutes per day)
Temperature mean			
1Q (-7.1; 6.4), reference			
2Q (6.5; 10.2)	-0.0(-0.5,0.4)	0.1(-0.1,0.4)	-0.1(-0.6,0.5)
3Q (10.3; 13.7)	0.7(0.1,1.3)	0.3(0.0,0.7)	-1.0(-1.7,-0.3)
4Q (13.8; 16.1)	1.4(0.8,2.1)	0.5(0.1,0.9)	-1.9(-2.7,-1.1)
5Q (16.2; 24.4)	1.6(1.0,2.3)	0.8(0.4,1.2)	-2.4(-3.2,-1.6)
Temperature max			
1Q (-3.5; 9.2), reference			
2Q (9.3; 13.0)	0.4(-0.1,0.8)	0.3(0.0,0.6)	-0.7(-1.2,-0.1)
3Q (13.1; 16.5)	1.0(0.4,1.5)	0.6(0.2,0.9)	-1.5(-2.2,-0.8)
4Q (16.6; 19.0)	1.8(1.2,2.4)	0.7(0.3,1.0)	-2.4(-3.2,-1.7)
5Q (19.1; 29.5)	2.1(1.4,2.7)	1.2(0.8,1.5)	-3.2(-4.0,-2.4)
Temperature min			
1Q (-11; -4), reference			
2Q (4.1; 8.0)	0.0(-0.4,0.4)	0.0(-0.2,0.3)	0.0(-0.5,0.5)
3Q (8.1; 11.0)	0.1(-0.4,0.6)	-0.1(-0.4,0.2)	0.1(-0.5,0.8)
4Q (11.1; 13.5)	0.6(0.0,1.2)	0.1(-0.3,0.4)	-0.6(-1.4,0.2)
5Q (13.6; 20.0)	0.7(0.1,1.3)	0.2(-0.2,0.6)	-0.8(-1.6,-0.1)
Sunshine duration			
1Q (0.0; 0.3), reference			
2Q (0.4; 1.9)	0.2(-0.2,0.5)	0.1(-0.1,0.3)	-0.2(-0.7,0.2)
3Q (2.0; 4.5)	0.6(0.2,0.9)	0.3(0.1,0.5)	-0.9(-1.3,-0.5)
4Q (4.6;7.4)	1.0(0.7,1.4)	0.6(0.4,0.9)	-1.7(-2.1,-1.2)
5Q (7.5;15.4)	2.0(1.6,2.4)	0.9(0.6,1.1)	-2.9(-3.4,-2.4)
Relative Humidity %			
1Q (43;71), reference			
2Q (72; 81)	-0.7(-1.1,-0.3)	-0.2(-0.5,-0.0)	0.9(0.5,1.4)
3Q (82; 88)	-1.1(-1.5,-0.7)	-0.5(-0.7,-0.2)	1.6(1.0,2.1)
4Q (88; 96)	-1.3(-1.7,-0.9)	-0.6(-0.8,-0.4)	1.9(1.4,2.4)
5Q (97; 100)	-1.1(-1.6,-0.7)	-0.5(-0.8,-0.2)	1.6(1.1,2.2)

Table 5.10 Overall interaction tests (Wald test p-value) between outdoor temperature and individual risk factors (age and BMI) on physical activity outcomes

	Interaction of temperature with…	
Physical activity outcomes	Age	BMI
SB	0.377	0.088
LIPA	0.138	0.232
MVPA	0.001	0.070
Steps	0.003	0.264

Figure 5.1 Raw data (n=1361). Plots depicting relationship between physical activity levels (mean, 95% CI) vs (i) mean temperature and (ii) England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons

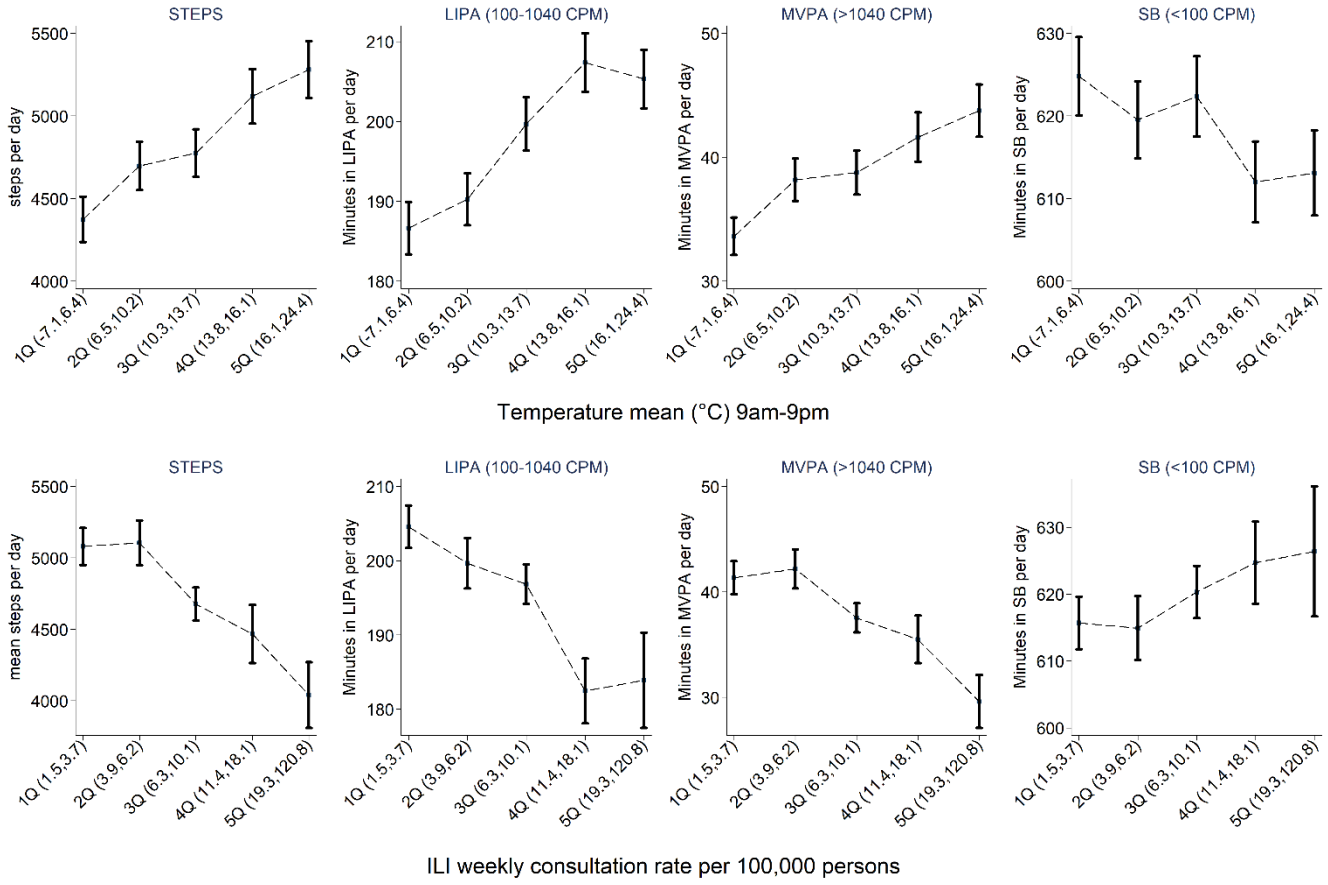


Figure 5.2 Raw data (n=1361). Plots depicting relationship between physical activity levels (mean, 95% CI), and temperature (max and min)

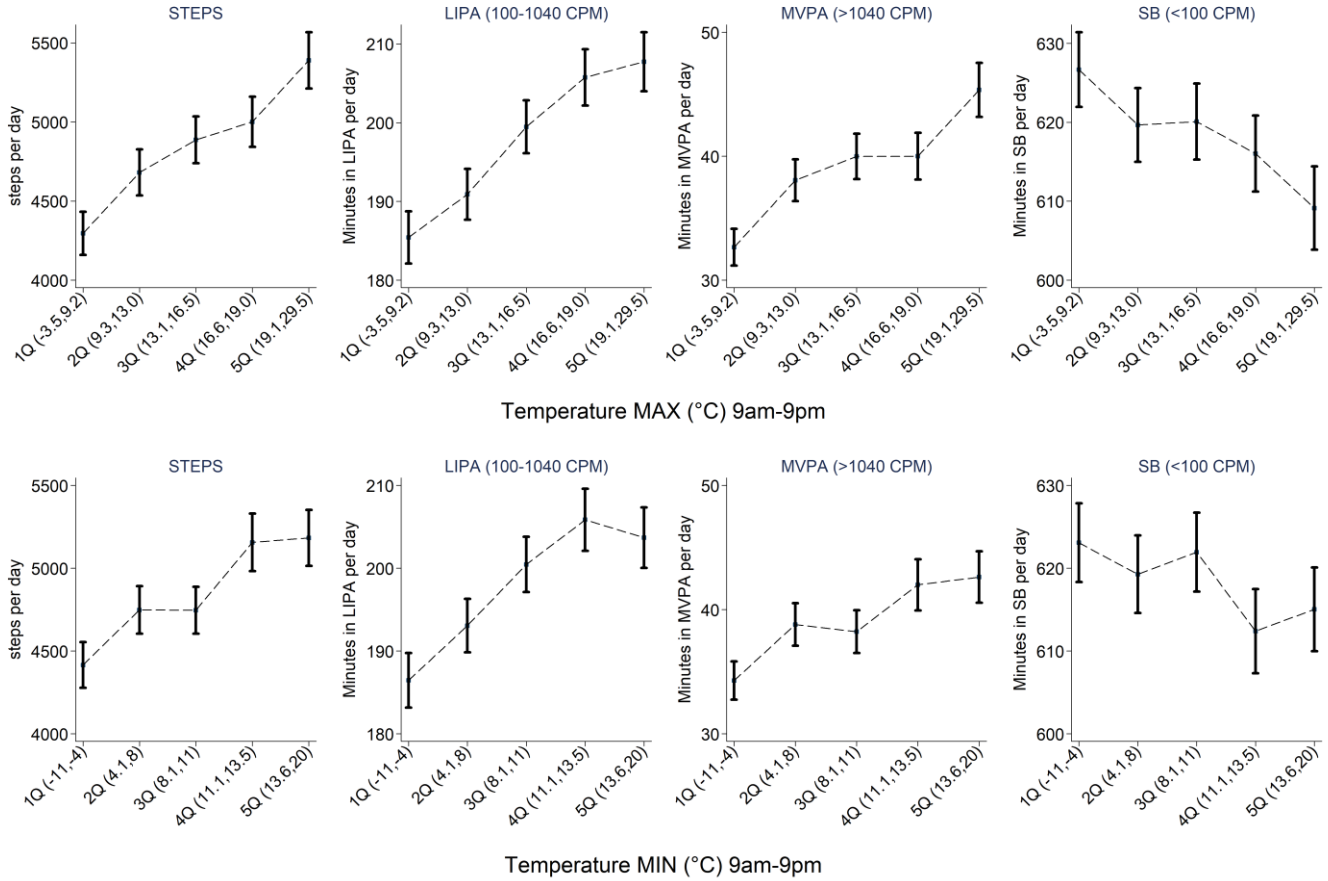


Figure 5.3 Raw data (n=1361). Plots depicting relationship between physical activity levels (mean, 95% CI), and meteorological factors

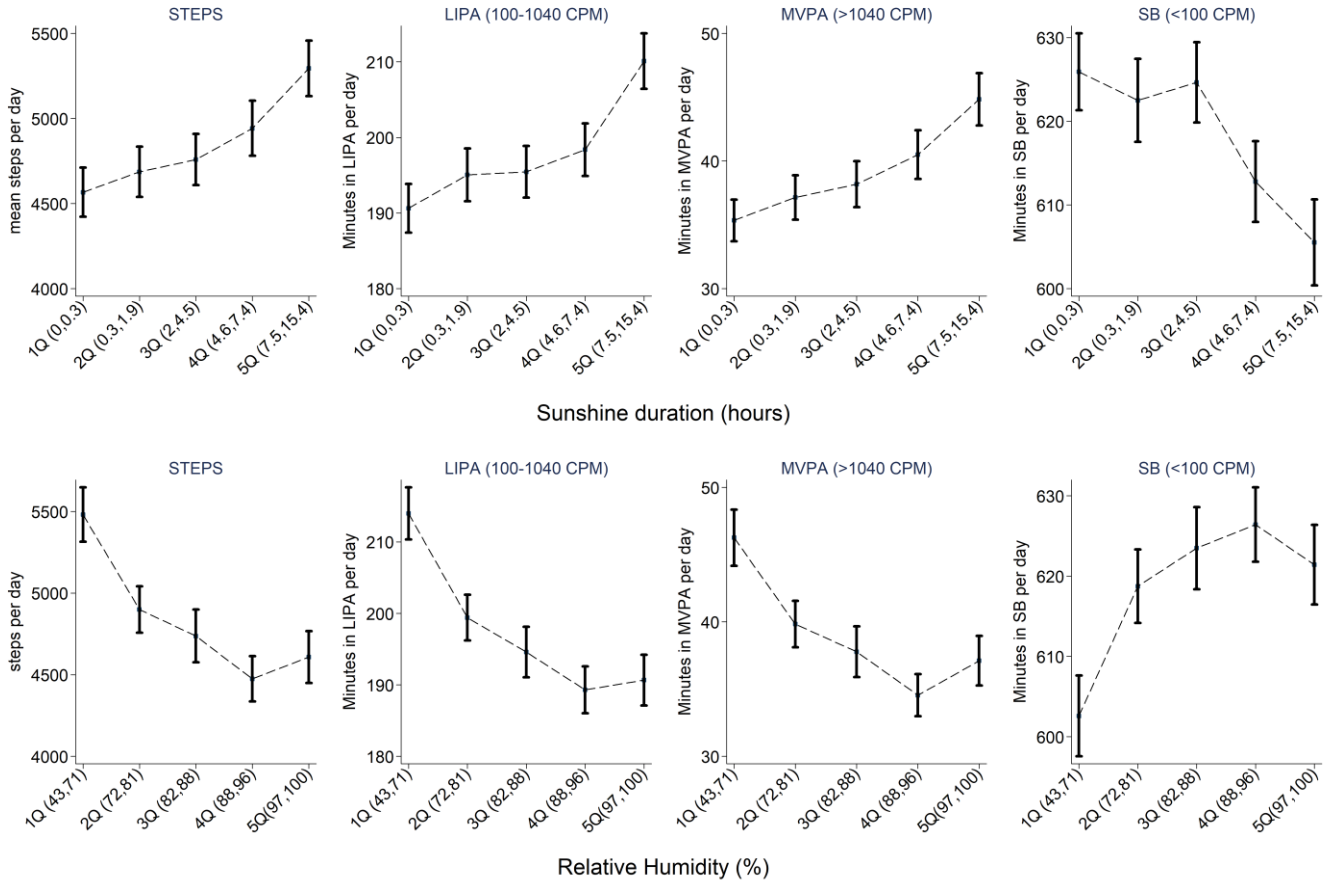


Figure 5.4 Raw data (n=1361). Plots depicting relationship between % of time spent in physical activity levels (mean, 95% CI) vs (i) mean temperature and (ii) England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons

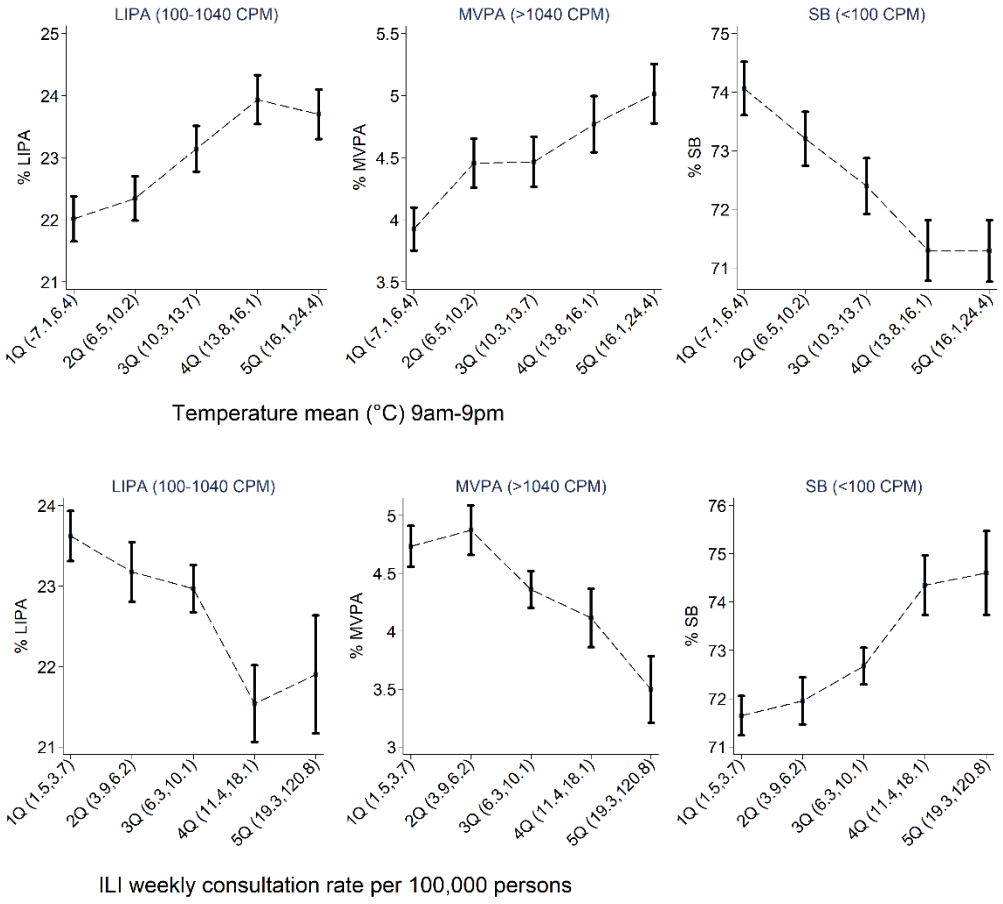


Figure 5.5 Raw data (n=1361). Plots depicting relationship between % of time spent in physical activity levels (mean, 95% CI) and temperature (max and min)

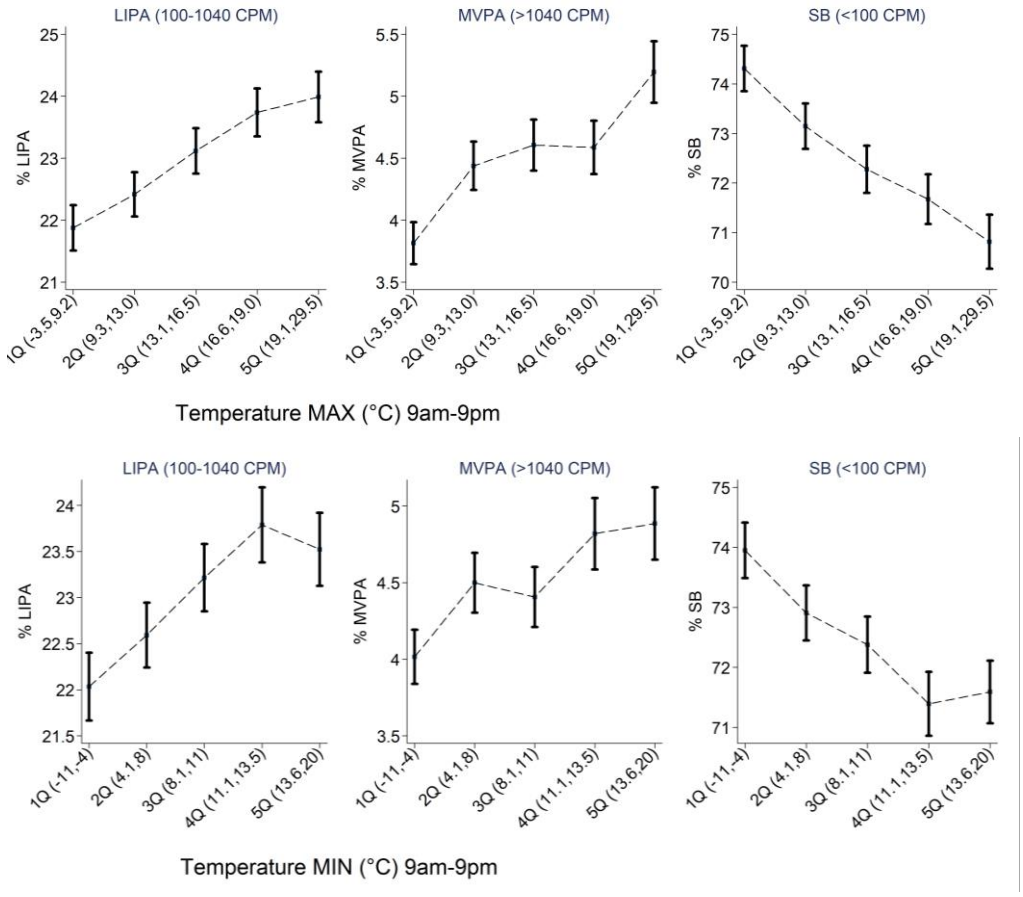
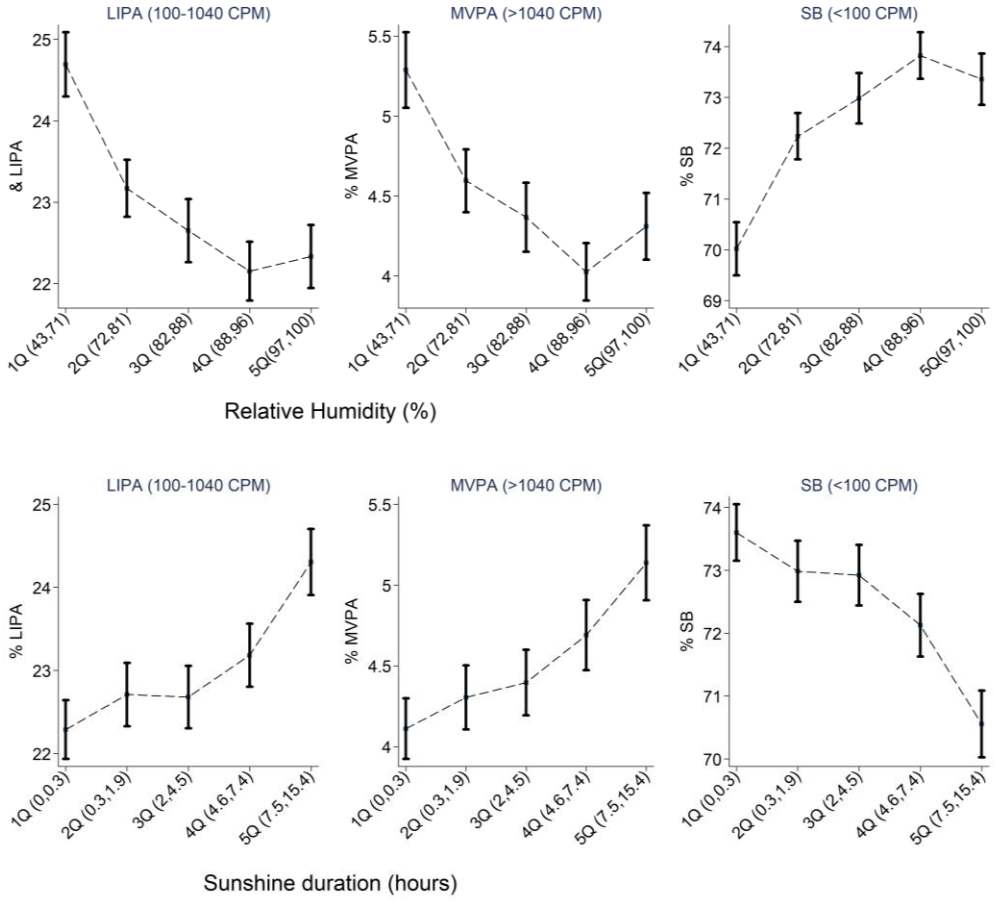


Figure 5.6 Raw data (n=1361). Plots depicting relationship between % of time spent in physical activity levels (mean, 95% CI), and meteorological factors



Chapter 6 DIURNAL VARIATIONS IN CARDIOVASCULAR DISEASE RISK FACTORS LEVELS

6.1 Summary

Previous studies have reported time of day variations in several established and novel cardiovascular disease (CVD) risk factors, such as blood pressure and biological markers of inflammation (e.g. Interleukin-6), though mainly in middle aged populations. Overall, CVD risk factors have shown peaks concentrated in between 10:00 and 15:00 hours, and it has been hypothesised that such variations may be relevant for CVD risk prediction. This Chapter addresses gaps in the literature by investigating diurnal variations in a comprehensive range of CVD risk factors measured in older adults (e.g. aged 60 years or more). To achieve such objectives, I studied variations in established and emerging CVD risk factors measured on one occasion in between 08:00 and 19:00 hours in 4252 men aged 60-79 years from the British Regional Heart Study (BRHS). The measurements were carried out in between February 1998 and March 2000 (BRHS follow-up year 20). Linear regression models were used to estimate associations between time of day and risk factors. Our findings showed that diurnal variations occurred for Interleukin-6 (IL-6), plasma viscosity, triglycerides, LDL-cholesterol, total cholesterol, and blood pressure (both systolic [SBP] and diastolic [DBP]) which increased over the course of the examination day, while tissue plasminogen activator [t-PA] antigen decreased. The associations were particularly marked for IL-6, SBP, and t-PA: over the course of the day IL-6 increased by 2.6% per hour (95% CI 1.8; 3.4%), SBP increased by 0.4 mm Hg per hour (95%CI 0.1; 0.7); conversely, t-PA decreased by 3.3% per hour (95% CI 2.9; 3.7%).

6.2 Introduction

In epidemiological studies, diurnal variation of CVD risk factors has been assessed by comparing individuals measured at various hours of the day. In this way, time of day variations in both established and emerging cardiovascular disease (CVD) risk factors in middle aged adults, such as blood pressure, lipids and some well-established inflammatory and haemostatic factors (e.g. white blood cell, red blood cell, and

platelets counts) have been reported (48, 200, 201, 295). For example, it is known that blood pressure rises after wakening in the morning (with plateau in late morning or afternoon); then, blood pressure decreases in the evening and declines even further after falling asleep, reaching the lowest peak during sleep (295). Also, during the BRHS baseline examination when men were in between 40-59 years of age, well-known risk factors such as white cell count (typical indicating infection, stress, or inflammation) and triglycerides increased their levels over the course of the day (200).

The importance of assessing such diurnal variations has been repeatedly acknowledged in the literature (48, 194, 195, 200, 214, 215), as this may be relevant to CVD risk prediction and risk stratification (45). As mentioned in Chapter 2 (paragraph 2.5.2), previous studies support the parallelism of diurnal variations in blood pressure and MI events (42, 48, 192) and blood pressure with stroke events (44, 206, 208). As such studies could only speculate on the underlying pathophysiological mechanisms, it is important to investigate time of day variations of CVD risk factors beyond simple descriptive diurnal patterns (45). Overall, the relationship between emerging CVD risk factors and time of the day has been less studied; although peaks in fibrinogen, IL-6, D-dimer, CRP, t-PA, and von Willebrand factor levels between 10:00 am and 15:00 hours have been demonstrated in middle aged or young populations (45, 46, 52). There is a need to establish whether time of day variations in emerging CVD risk factors occur in older adults, in a manner consistent with findings in younger populations. The BRHS is a population-based study of CVD in older adults and is well suited to investigate associations of time of day with CVD risk factors, due to the measurements of a comprehensive range of risk factors in between 08:00 and 19:00 hours in 4252 men aged 60-79 years. This is especially important when analysing CVD markers of inflammation (200) and blood pressure (295). I would expect to observe an increase in such risk factors over the course of the examination day.

6.3 Objectives

The aim of this study was to investigate time of day variations in established and novel biological risk factors and physical measurements in older men (60-79 years) from the British Regional Heart Study (BRHS). Considering the available literature the main research question (objective) is the following:

Do established and emerging CVD risk factors measured in older adults vary between 08:00 and 19:00 hours?

6.4 Methods

6.4.1 Participants

In both this Chapter and Chapter 7, the data collected in 1998-2000 were used. The reason to do this was previously reported in Chapter 1; in summary, at this time point I could analyse a larger sample of older adults with the most comprehensive range of relevant risk factors. For Chapter 6, this also allowed a sufficient follow-up time for testing the research question "are diurnal variations in CVD risk factors relevant to CVD risk prediction in older adults?".

The 20-year follow-up of the BRHS took place in between February 1998 and March 2000 and was previously described in Chapter 3 (see paragraph 3.2.4). In summary, 4252 surviving participants (77% response rate) aged 60-79 years who were resident in the UK attended a physical examination during which nurses took blood pressure measurements and a fasting blood sample on one occasion for each participant (see Chapter 3, paragraph 3.2.4). Participants were asked to fast for a minimum of 6 hours, during which they were instructed to drink only water, as previously reported (201). Therefore, men examined at 2pm or later may have eaten at 7am, while men examined in the morning had not eaten since the night before; overall, men with morning appointments were more likely to report longer fasting duration (>10 hours) than men with afternoon appointments (89% vs 14%) (201). Examinations and blood sampling occurred between 08:00 h and 19:00 h. Assays were carried out for a range of

biochemical and haematological markers. Participants' appointment times were non-systematically allocated. They were offered the opportunity to contact the BRHS team and change the time of examination, if unable to attend; a small proportion of participants did so.

The participants were also asked to complete a questionnaire which included questions on other established CVD risk factors, such as age, social class, smoking habits, alcohol consumption, and physical activity. Physical activity levels were self-reported, see Chapter 3 paragraph 3.4.1 (296), but the questionnaire used was recently validated using accelerometers (233). Incident CVD, including non-fatal stroke and non-fatal MI were recorded: their definitions have been reported (Chapter 3, paragraph 3.2.4.4) (291). The number of blood samples collected and included in the analyses differed according to the risk factor measurements (the number of observations varied from 3816 for Total Cholesterol to 4006 for blood pressure in complete case analyses including all covariates of interest).

6.4.2 CVD risk factors

As reported in the method section of Chapter 3 (paragraph 3.4.4), a number of established and emerging cardiovascular risk markers, including blood pressure, lipids, haemostatic and inflammatory markers, were measured. According to the existing literature, there was sufficient justification for and investigation of diurnal variation in the following risk factors:

- systolic and diastolic blood pressure (SBP and DBP) (48, 297)
- serum total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) and triglycerides (200, 201)
- circulating levels of markers of inflammation (C-reactive protein [CRP], Interleukin 6 [IL-6], fibrinogen, plasma viscosity [PV]) (45, 47, 52)
- circulating levels of markers of haemostasis (tissue plasminogen activator [t-PA] antigen, fibrin D-dimer, von Willebrand factor [vWF]) (45, 46, 195)
- Lung function variables (FEV_1, FVC, FEV_1/FVC) (298, 299)

- Vitamin D (300)

The list of the above CVD risk factors were also included in this thesis because there was further justification for investigating their seasonal variations (separately studied in Chapter 7)

6.4.3 Statistical methods

Excepting Total Cholesterol, HDL-cholesterol, LDL-cholesterol, SBP and DBP, FEV_1, FVC, and FEV_1/FVC all other outcomes were log-transformed for further analysis as their distributions were positively skewed, as reported in previous BRHS publications (55, 301). After log-transformation, data followed a normal or nearly normal distribution; this helped to meet the assumptions of inferential statistics.

6.4.3.1 Descriptive statistics

Unadjusted geometric means and 95% Confidence Intervals [CI] of the outcomes were plotted against hour of the day. For other risk factors which followed an approximately Normal distribution, arithmetic means were used.

6.4.3.2 Adjusted associations between time of day and CVD risk factors

Associations between time of day (fitted as a continuous variable, range 8-18) and the outcomes were examined using linear multilevel random intercept models (level 1 = individual, level 2 = town of residence). For outcomes which were log-transformed, the results were reported as percent difference in the outcome geometric mean per hour of sampling, and for other variables as the mean change per hour.

The town effect was important to account for, since it may have carried independent associations with the risk factors and might have been confounded with time of day if examinations were carried out on average later in the day in some towns than others. The results refer to between-person differences over the course of the examination day; specifically, the estimates from the linear model are for average difference in the

outcome levels for every hour later between 08:00 and 19:00 hours. Non linearity of association between time of day and the outcomes was investigated (see paragraph 6.4.4.3), as the literature reported that some CVD risk factor levels may increase starting from the early morning, with a possible plateau in the late morning or afternoon (46, 201, 295).

All associations between time of day and CVD risk factors were reported in:

1) Models adjusted for age
2) Models additionally adjusted for other major risk factors: social class, BMI, previous stroke or myocardial infarction (MI), physical activity, smoking status, and use of statin. These variables are worth adjusting for as their relationship with outcome is known a priori to be strong in the BRHS, although it is unlikely these variables are related to exposure (time of day). Including such covariables in the model would have the scope of reducing the standard error and increasing in precision of the estimates for our exposure variable. For lipids only, the models were always additionally adjusted for diabetes status and possible confounding of fasting time.

When the association of time of the day with the outcomes was found to be statistically significant, the proportion of variance associated with time of the day was estimated using partial R-squared.

6.4.3.3 Secondary analysis

Moreover, three further analyses were performed: (i) all models were carried out excluding men with diabetes, (ii) interactions were fitted to test whether the time of day associations were modified by age (fitted as continuous variable); (iii) a quadratic term for time of day was added to the models in order to check for non-linearity.

6.5 Results

6.5.1 Descriptive statistics

The characteristics of the study participants (mean age 68.7 years, standard deviation (SD) = 5.5) are reported in Table 6.1. Major risk factors were distributed as follows: 12.9% of were smokers, the average BMI was 26.9, 8.9% had diabetes, average blood pressure was 149 mmHg blood pressure, average total cholesterol was 6 mmol/L, and 11% were inactive. The number and percentage of BRHS men who attended the examination by time of day are displayed in Table 6.2. The majority of the men were examined between 10:00 and 12:59, and in between 14:00 and 16:59.

The relationships between time of day (by hour) and risk factors were displayed in Figures 6.1-6.4. Evidence of an increase over the course of the day was particularly noticeable for IL-6 (Figure 6.1), lipids (Figure 6.3), SBP and DBP (Figure 6.4), and FEV_1 (Figure 6.5). Conversely, levels of t-PA (Figure 6.2) were lower in the afternoon in comparison with morning; this difference was particularly marked and further investigated in a sensitivity analysis (see paragraph 6.5.2). Variations by time of day for other risk factors were not clearly observable from the plots. For completeness of information, all risk factors levels plotted in figure 6.1-6.4 are reported as mean and 95%CI in Tables 6.3-6.5.

6.5.2 Adjusted associations between time of day and CVD risk factors

The results of corresponding linear regression analyses are shown in Table 6.6: statistically significant associations between time of the day and some outcomes were found (Table 6.6, Model 2, fully adjusted). I reported here the results in order of magnitude, listing the log transformed outcomes first: over the course of the examination day IL-6 increased by 2.6% per hour (95% CI 1.8; 3.4%), Triglycerides increased by 0.8% per hour (95%CI 0.1;1.4), PV increased by 0.1% per hour (95%CI 0.0;0.1), LDL-cholesterol increased by 0.019 mmol/L per hour (95%CI 0.005; 0.033), Total Cholesterol increased by 0.027 mmol/L per hour (95%CI 0.012; 0.042), SBP increased by 0.400 mm Hg per hour (95%CI 0.112; 0.689), DBP increased by 0.191 mm Hg per hour (95%CI 0.057;0.325), FEV_1 increased by 0.009 litres (95%CI

0.002;0.016), and FEV_1/FVC increased by 0.2% (95%CI 0.0;0.3). Conversely, t-PA decreased by 3.3% per hour (95% CI 3.7; 2.9%). C-Reactive Protein, Fibrinogen, D-Dimer, von Willebrand Factor, HDL-Cholesterol, and Vitamin D showed no consistent associations with time of day (Table 6.6).

A further sensitivity analysis was performed on t-PA only (as mentioned in paragraph 6.5.1); the association between time of the day and t-PA was strongly attenuated after accounting for fasting time (fitted as continuous variable): the decrease in t-PA levels was -3.3% (95%CI -3.7; -2.9) per hour before the adjustment (Table 6.2) and -1.4% (95%CI -2.2; -0.1) after the adjustment for fasting.

6.5.3 Secondary analysis

An analysis excluding men with diabetes was performed (Table 6.2 – Model 3), but the association between time of day and the outcomes did not substantially change, except for triglycerides. For all outcomes, we also did not find evidence for an interaction between of time of day with age (results not shown). When adding a quadratic term to the model, we found a significant improvement in model fit for IL-6 only (p=0.030 for the time of day squared term). The association of time of day with IL-6 appeared to be slightly J-shaped (results not shown), with no change with time from 08:00 until 11:00, and a linear increase from 11:00 until 19:00 hours. For those risk factors associated with time of the day, the proportion of variance associated with time of the day from the fully adjusted models was <1% for IL-6, PV, lipids, BP, and FEV_1; and 2% for t-PA.

6.6 Discussion

6.6.1 Summary of the main findings

To my knowledge, this is the largest investigation of relationships between time of day and CVD risk factors in older men. I discuss below findings in relation to objectives outlined in paragraph 6.3 and the main research question:

Do established and emerging CVD risk factors measured in older adults vary between 08:00 and 19:00 hours?

Yes, some CVD risk factors levels vary by time of the day in the BRHS. After adjusting the analysis for major CVD risk factors it has been observed that some, but not all, CVD risk factors levels varied by time of day. In particular, IL-6, LDL-Cholesterol, Total Cholesterol, SBP, and lung function variables (FEV1 and FEV1/FVC) increased linearly over the course of the day and showed the strongest associations with time of the day. Conversely, a decrease in t-PA was also observed over the course of the day. C-Reactive Protein, Fibrinogen, D-Dimer, von Willebrand Factor, HDL-Cholesterol, and Vitamin D showed no consistent associations with time of day.

Also, secondary analyses which showed no evidence of interaction between time of day and age..

6.6.2 Comparison with other studies

Literature on time of day variation in CVD markers of inflammation and haemostasis in older adults is limited; to our knowledge this is the first time these findings have been reported in older adults. Findings from earlier studies of younger adults were fairly consistent with ours. For example a recent meta-analysis of several small studies which analysed IL-6 proposed a diurnal pattern, with overall IL-6 levels being higher later in the day than in the morning, similarly to our study (47). Findings from Chapter 4 showed that BRHS men were more active in the morning and in early afternoon (58) when the main activities were usually gardening and house works among others. Whether IL-6 was implicated in this daily pattern remains uncertain and can potentially be explored in future studies.

A morning surge in BP has been observed in previous studies and associated with the nocturnal fall as well as the awakening time (48). In our study only DBP increased

over the course of the examination day while average SBP remains fairly constant around 147 mm Hg. As the BRHS men were mostly examined from 9 a.m. onwards, it was not possible to assess the BP levels around the times of awakening, typically around 6-7 a.m. in the morning (58). An increase in triglycerides and total cholesterol over the course of the day was also observed elsewhere (194); food intake seems to be a major contributor, as triglycerides in particular can increase in response to the proportion of fat in the meal (216). A decrease in t-PA over the examination day was also reported in younger subjects (a UK population of 9377 men and women aged 45) (45); however, t-PA did not vary by time of the day in a previous large study of 1288 healthy 25 to 64-year-old men and women (302). Previous studies did not report the role of fasting in t-PA diurnal variations; in this Chapter, after accounting for fasting time the relationship of time of the day with t-PA was strongly attenuated. The participants were asked to fast for at least 6 hours; it is possible that t-PA levels are especially sensitive to fasting time or diet (small breakfast in the morning prior to the afternoon examination could partially explain the drop in t-PA levels observed in the afternoon vs morning).

In comparison to our study, findings regarding CRP, Fibrinogen, D-dimer, and vWF reported in earlier studies of younger adults were fairly similar. However, most of the previous studies were small, with a longitudinal study design, and representativeness at population level very reduced: they did not find an association of time of day with CRP in one study of 10 males and 3 females (age range 21–35 years) (196), with D-dimer in one study of 4 men and 5 women, mean age 51 years old (214), and with vWF in a study of 10 men with ischaemic heart disease (median age 59 years old, range 48-69) (195).

The biggest study on diurnal CVD risk factors published in middle aged populations (9377 men and women aged 45 years from the British 1958 Birth Cohort study) found that fibrinogen, D-dimer, t-PA and vWF slightly decreased over the course of the day. Moreover, the variation in CRP, Fibrinogen, D-dimer, and vWF attributable to time of day was relatively small; time of day explained less than 0.5% of variance in

fibrinogen, D-dimer, CRP, and vWF levels and approximately 6% of the variance in t-PA (45). Although this study suggested that diurnal variations in CVD risk factors could be relevant for cardiovascular risk prediction (45), the authors did not carry out survival analysis to verify this. Our findings suggested the effect of time of the day (from 08:00 h to 19:00 h) is not relevant for CVD risk assessment. With this sensitivity analysis we wanted to investigate time of day variations beyond simple descriptive diurnal patterns; to our knowledge this is the first time this finding has been reported.

6.6.3 Strengths and limitations

The BRHS cohort benefits from using a large sample and this increases statistical power and precision of estimates. The CVD risk factors measurements were carried out on one occasion only over an extended period of the day (between 08:00 and 19:00 hours), offering only a partial understanding of the variations over 24 hours (194, 297). This study did not carry out repeated measures on participants over the day: future studies involving repeated measurement over 24 hours would allow investigation of within-person circadian variations. However, with repeated measurements a possible and genuine diurnal variation may be disrupted and natural sleeping patterns altered (repeated measures are usually taken every 1-2 hours during the night) (303). Moreover, since the diurnal variation in CVD events has been reported as more marked in men than women (42), it is important to examine whether the time of day variations in CVD risk factors levels explored in this thesis are less marked in UK older women. Similarly, the BRHS is comprised of men predominantly of white European ethnic origin (see discussion in paragraph 9.4.1), and further studies are needed to examine whether these findings are different in older men of non-white-European ethnicity.

The time of death (e.g. hour of the day) of the BRHS participants was not measured in the BRHS; this is a limitation of this thesis and represent a key measurement requirement for future studies aiming to understand the causal pathways of the diurnal variation in CVD events (44, 192, 203).

6.6.4 Implications

The findings of this Chapter can be further explored by future studies assessing the same CVD risk factors levels during the 24 hours of the day to demonstrate whether a rapid increase of IL-6, LDL-Cholesterol, Total Cholesterol and SBP over the day may be relevant to the increased number of CVD events observed in early and late morning (44). Secondarily, season did not modify the time of day variations in the CVD risk factors measured in this thesis, suggesting that particular types of interventions for CVD prevention in relation to time of the day and by season are not needed.

6.7 Conclusions

Levels of Interleukin-6 (IL-6), Plasma viscosity, Triglycerides, LDL-cholesterol, total cholesterol, blood pressure (both systolic and diastolic), and lung function vary by time of the day in older men. Future studies aiming to understand the causal pathways of the diurnal variation in CVD events may focus on these markers to understand causal pathways.

Table 6.1 Individual characteristics and risk factors levels in the British Regional Heart Study (BRHS) men who attended the examinations in 1998-2000

	BRHS men (n=4252) [1]
Demographic and background characteristics	
Age (years), mean (SD)	68.7 (5.5)
Social class, n (%)	
Manual	2166 (50.9)
Non manual	1966 (46.2)
Armed Forces	112 (2.6)
Missing	8 (0.2)
Physical health	
Prevalence of stroke/myocardial infarction, n (%)	370 (8.7)
Hypertension, n (%) Missing, n (%)	2703 (63.6) 17 (0.3)
Diabetes, n (%) Missing, n (%)	380 (8.9) 220 (5.1)
BMI, mean (SD) Missing, n (%)	26.9 (3.7) 20 (0.5)
Behavioural factors	
Smoking	
Never, n (%)	1233 (29.0)
Ex-smokers, n (%)	2464 (57.9)
Smokers, n (%)	548 (12.9)
Missing, n(%)	7 (0.2)
Physical activity (PA) score	
Inactive, n (%)	471 (11.1)
Occasional, n (%)	957 (22.5)
Light, n (%)	767 (18.0)
Moderate, n (%)	591 (13.9)
Moderate vigorous, n (%)	690 (16.2)
Vigorous, n (%)	621 (14.6)
Missing, n (%)	155 (3.6)
Personal circumstances	
Lipids lowering drugs use, n (%)	327 (7.7)
Married vs not	3467 (81.5)
Biological markers, means (SD)	
CRP, mg/L Missing, n (%)	3.53 (6.86) 196 (4.6)
IL-6, pg/mL Missing, n (%)	3.18 (2.95) 202 (4.7%)
Fibrinogen, g/L Missing	3.27 (0.74) 172 (4.0)
PV, mPa.s Missing, n (%)	1.285 (0.078) 239 (5.6)
t-PA, ng/mL Missing, n (%)	11.08 (4.44) 169 (4.0%)
vWF, IU/dL	139.96 (46.19)

Missing, n (%)	169 (4.0%)
D-dimer, ng/mL	133.58 (210.74)
Missing, n (%)	173 (4.1%)
Triglycerides, mmol/L	1.86 (1.08)
Missing, n (%)	220 (5.2%)
HDL-cholesterol, mmol/L	1.32 (0.34)
Missing, n (%)	246 (5.8%)
LDL-cholesterol, mmol/L	3.89 (0.97)
Missing, n (%)	278 (6.5%)
Total cholesterol, mmol/L	6.00 (1.08)
Missing, n (%)	221 (5.2%)
Vitamin D, ng/mL	20.01 (9.24)
Missing, n (%)	453 (10.7%)
SBP sitting, mm Hg	149 (24)
Missing, n (%)	17 (0.4%)
DBP sitting, mm Hg	85 (11)
Missing, n (%)	17 (0.4%)
FEV_1, L §	2.6 (0.7)
Missing, n (%)	47 (1.1)
FVC, L §	3.4 (0.8)
Missing, n (%)	45 (1.1)
FEV_1/FVC %, mean (SD)	76.8 (11.6)
Missing, n(%)	47 (1.1)

[1] >=1 and <=15 units per week (1 unit is approximately 1 drink, such as one glass of wine)

[2] >=16 units per week (1 unit is approximately 1 drink, such as one glass of wine

Table 6.2 Number and percentage of BRHS men examined by time of the day

Time of day	Total examined = 4252 N (%)
08:00 - 08:59 h	33 (0.8)
09:00 - 09:59 h	363 (8.5)
10:00 - 10:59 h	699 (16.4)
11:00 - 11:59 h	771 (18.1)
12:00 - 12:59 h	591 (13.9)
13:00 - 13:59 h	99 (2.3)
14:00 - 14:59 h	306 (7.2)
15:00 - 15:59 h	560 (13.2)
16:00 - 16:59 h	566 (13.3)
17:00 - 17:59 h	260 (6.1)
18:00 – 19:00 h	4 (0.1)

Table 6.3 Unadjusted geometric means (95% CI) by time of the day for inflammatory and haemostatic factors measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

Time of day	CRP, mg/L	IL-6, pg/mL	Fibrinogen, g/L	PV, mPa.s	vWF, IU/dL	D-dimer, ng/mL	t-PA, ng/mL
08:00 - 09:59 h	1.77(1.57,1.99)	2.37(2.20,2.55)	3.20(3.12,3.27)	1.28(1.27,1.28)	132.56(128.16,137.12)	84.26(77.52,91.59)	11.25(10.84,11.68)
10:00 - 10:59 h	1.99(1.82,2.17)	2.45(2.33,2.59)	3.26(3.21,3.31)	1.28(1.28,1.29)	132.74(129.36,136.20)	86.10(80.80,91.74)	11.08(10.75,11.42)
11:00 - 11:59 h	1.69(1.56,1.83)	2.26(2.15,2.37)	3.18(3.13,3.23)	1.28(1.28,1.29)	135.36(132.27,138.53)	85.75(80.63,91.19)	10.89(10.60,11.19)
12:00 - 12:59 h	1.79(1.63,1.95)	2.40(2.27,2.53)	3.21(3.16,3.27)	1.29(1.29,1.30)	133.26(129.64,136.97)	85.40(79.52,91.72)	10.95(10.61,11.31)
13:00 - 13:59 h	1.62(1.34,1.95)	2.31(2.04,2.61)	3.24(3.10,3.39)	1.30(1.29,1.32)	131.65(122.91,141.00)	86.19(74.26,100.04)	11.25(10.45,12.10)
14:00 - 14:59 h	1.50(1.32,1.70)	2.39(2.22,2.58)	3.08(2.99,3.16)	1.27(1.26,1.28)	125.39(120.50,130.49)	82.91(75.94,90.51)	9.19(8.76,9.65)
15:00 - 15:59 h	1.80(1.63,1.98)	2.62(2.49,2.77)	3.19(3.13,3.25)	1.28(1.27,1.29)	134.90(131.21,138.69)	83.69(77.96,89.83)	9.23(8.91,9.55)
16:00 - 16:59 h	1.64(1.50,1.79)	2.65(2.52,2.80)	3.15(3.09,3.21)	1.29(1.28,1.29)	127.68(123.84,131.63)	82.75(76.83,89.13)	9.12(8.81,9.43)
17:00 – 19:00 h	1.65(1.44,1.89)	2.77(2.53,3.02)	3.22(3.13,3.30)	1.29(1.28,1.30)	135.12(129.36,141.12)	79.01(71.31,87.54)	8.96(8.53,9.41)

Table 6.4 Unadjusted arithmetic means (95% CI) by time of the day for lipids and blood pressure variables measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

Time of day	Triglycerides, mmol/L	Total cholesterol, mmol/L	LDL-cholesterol, mmol/L	HDL-cholesterol, mmol/L	SBP sitting, mm Hg	DBP sitting, mm Hg
08:00 - 09:59 h	1.56(1.49,1.64)	5.94(5.84,6.05)	3.85(3.75,3.95)	1.32(1.28,1.35)	148(146,150)	84(83,85)
10:00 - 10:59 h	1.59(1.53,1.65)	5.92(5.84,6.00)	3.81(3.74,3.88)	1.31(1.28,1.33)	149(147,151)	84(84,85)
11:00 - 11:59 h	1.55(1.50,1.61)	6.00(5.92,6.07)	3.89(3.82,3.96)	1.33(1.30,1.35)	148(147,150)	85(84,86)
12:00 - 12:59 h	1.59(1.53,1.66)	6.02(5.93,6.12)	3.91(3.82,3.99)	1.33(1.31,1.36)	148(146,150)	85(84,86)
13:00 - 13:59 h	1.70(1.54,1.87)	6.37(6.13,6.61)	4.17(3.97,4.37)	1.34(1.27,1.40)	151(146,156)	87(84,89)
14:00 - 14:59 h	1.65(1.55,1.74)	5.94(5.82,6.06)	3.81(3.70,3.93)	1.32(1.28,1.36)	150(147,153)	85(84,86)
15:00 - 15:59 h	1.71(1.64,1.78)	5.97(5.88,6.06)	3.81(3.73,3.89)	1.32(1.29,1.35)	150(148,153)	86(85,87)
16:00 - 16:59 h	1.68(1.62,1.75)	6.06(5.97,6.15)	3.90(3.81,3.98)	1.33(1.30,1.36)	149(147,151)	85(84,86)
17:00 – 19:00 h	1.68(1.58,1.78)	6.16(6.03,6.29)	3.98(3.86,4.11)	1.34(1.29,1.38)	150(147,153)	86(85,87)

Table 6.5 Unadjusted arithmetic means (95% CI) by time of the day for lung function variables and geometric mean for Vitamin D measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

Time of day	FEV1, L	FVC, L	FEV1/FVC %	VitD, ng/mL
08:00 - 09:59 h	2.53(2.47,2.60)	3.32(3.24,3.40)	77(75,78)	2.86(2.81,2.92)
10:00 - 10:59 h	2.55(2.50,2.60)	3.35(3.30,3.41)	76(75,77)	2.86(2.82,2.90)
11:00 - 11:59 h	2.60(2.55,2.64)	3.43(3.37,3.49)	76(75,77)	2.86(2.82,2.90)
12:00 - 12:59 h	2.58(2.52,2.64)	3.38(3.31,3.45)	77(76,78)	2.92(2.87,2.96)
13:00 - 13:59 h	2.62(2.48,2.75)	3.32(3.16,3.49)	79(77,81)	2.95(2.85,3.06)
14:00 - 14:59 h	2.64(2.57,2.71)	3.41(3.32,3.49)	78(76,79)	2.91(2.85,2.96)
15:00 - 15:59 h	2.56(2.51,2.62)	3.37(3.31,3.44)	77(76,77)	2.84(2.79,2.89)
16:00 - 16:59 h	2.65(2.59,2.70)	3.46(3.39,3.52)	77(76,78)	2.88(2.83,2.92)
17:00 – 19:00 h	2.70(2.62,2.78)	3.46(3.37,3.54)	78(77,80)	2.94(2.88,3.00)

Table 6.6 Cross-sectional adjusted associations between time of day (fitted as continuous variable) and cardiovascular disease (CVD) risk factors measured in the British Regional Heart Study (BRHS) men (aged 60-79) attending the follow-up year 20 examination in 1998-2000.

	Model 1: Age adjusted [1 3]		Model 2: Full adjustment [2 3]		Model 3: Full adjustment excluding men with diabetes [4]	
CVD risk factor	Percent difference (95%CI) in the CVD risk factor levels for each hour later of sampling	p-value	Percent difference (95%CI) in the CVD risk factor levels for each hour later of sampling	p-value	Percent difference (95%CI) in the CVD risk factor levels for each hour later of sampling	p-value
IL-6	2.3 (1.5; 3.1)	<0.001	2.6 (1.8; 3.4)	<0.001	2.4 (1.6; 3.3)	<0.001
t-PA	-3.4 (-3.9; -3.0)	<0.001	-3.3 (-3.7; -2.9)	<0.001	-3.2 (-3.6; -2.7)	<0.001
Fibrinogen	-0.2 (-0.5; 0.0)	0.082	-0.2 (-0.5; 0.1)	0.104	-0.2 (-0.5; 0.1)	0.149
CRP	-1.3 (-2.6; 0.1)	0.060	-0.9 (-2.2; 0.4)	0.175	-0.9 (-2.2; 0.5)	0.191
PV	0.1 (0.0, 0.2)	0.042	0.1 (0.0;0.1)	0.041	0.1 (0.0;0.2)	0.015
vWF	-0.2 (-0.6; 0.2)	0.274	-0.2 (-0.6; 0.2)	0.380	-0.1 (-0.5; 0.3)	0.703
D-Dimer	-0.2 (-1.2; 0.8)	0.682	-0.1 (-1.0; 0.9)	0.890	-0.1 (-1.2; 0.9)	0.801
Triglycerides	1.3 (0.7;1.9)	<0.001	0.8 (0.1;1.4)	0.020	0.5 (-0.1;1.2)	0.117
Vitamin D	0.1 (-0.6;0.7)	0.880	0.1 (-0.5;0.7)	0.717	-0.1 (-0.7;0.5)	0.736
	Absolute difference (95%CI)	p-value	Absolute difference (95%CI)	p-value	Absolute difference (95%CI)	p-value
LDL-Cholesterol, mmol/L	0.007 (-0.005;0.019)	0.274	0.019 (0.005;0.033)	0.006	0.018 (0.003;0.033)	0.016
HDL-Cholesterol, mmol/L	0.003 (-0.002;0.007)	0.235	0.004 (-0.000;0.009)	0.076	0.004 (-0.001;0.009)	0.113
Total cholesterol, mmol/L	0.018 (0.004;0.031)	0.010	0.027 (0.012;0.042)	0.001	0.026 (0.009;0.042)	0.002
Systolic Blood pressure, mm Hg	0.376 (0.084;0.668)	0.012	0.400 (0.112;0.689)	0.007	0.532 (0.228;0.836)	0.001
Diastolic Blood pressure, mm Hg	0.187 (0.051;0.323)	0.007	0.191 (0.057;0.325)	0.005	0.228 (0.086;0.369)	0.002
FEV_1	0.010 (0.002;0.017)	0.014	0.009 (0.002;0.016)	0.015	0.009 (0.001;0.017)	0.025
FVC	0.006 (-0.003;0.015)	0.175	0.006 (-0.003;0.015)	0.181	0.007 (-0.002;0.016)	0.146
FEV_1/FVC %	0.161 (0.024;0.297)	0.021	0.156 (0.021;0.290)	0.023	0.128 (-0.015;0.271)	0.079

[1] Model 1: Two level linear models (level 1 = person, level 2 = town of residence during the BRHS recruitment) adjusted for age. Model 1 used the same number of observations as Model 2 (complete case analysis). All associations are reported as difference in CVD risk factors levels per one hour of sampling over the examination day (08:00-19:00h).

[2] Model 1 additionally adjusted for social class, Body Mass Index, smoking status, alcohol consumption, physical activity, use of statin, prevalence of stroke/MI and season. Association of time of the day with lipids were additionally adjusted for fasting time and diabetes

[3] Model 1 and Model 2 used the same number of observations: 3832 for IL-6, 3863 for t-PA, 3861 for Fibrinogen, 3838 for CRP, 3863 for vWF, 3817 for Triglycerides, 3859 for D-Dimer, 3798 for PV, 3764 for LDL-Cholesterol, 3793 for HDL cholesterol, 3816 for Total Cholesterol, 4006 for SBP, 4006 for DBP

[4] Model 3 number of observations: 3398 for IL-6, 3429 for t-PA, 3427 for Fibrinogen, 3405 for CRP, 3429 for vWF, 3425 for D-Dimer, 3388 for Triglycerides, 3375 for PV, 3358 for LDL-Cholesterol, 3373 for HDL cholesterol, 3388 for Total Cholesterol, 3559for SBP, 3559 for DBP.

Figure 6.1 Unadjusted geometric means (95% CI) by time of the day for C-Reactive Protein (CRP), Interleukin-6 (IL-6), Fibrinogen, and Plasma Viscosity (PV) measured on one occasion in BRHS men aged 60-79 during the years 1998-2000.

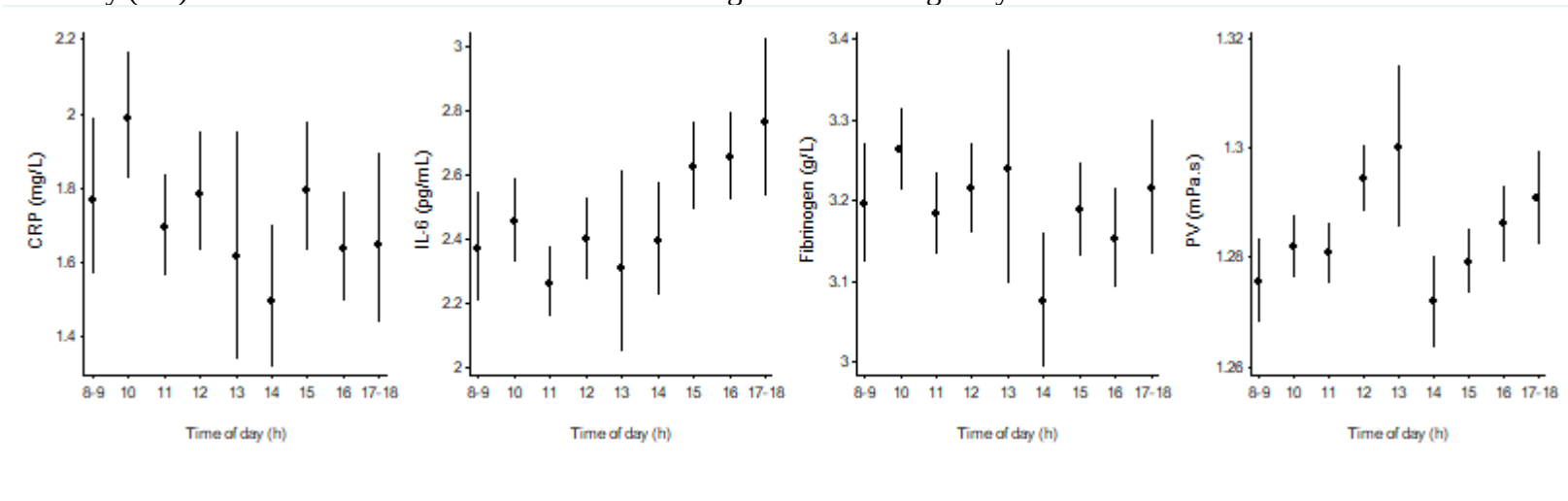


Figure 6.2 Unadjusted geometric means (95% CI) by time of the day for von Willebrand factor (vWF), fibrin D-dimer, and Tissue plasminogen activator (t-PA)

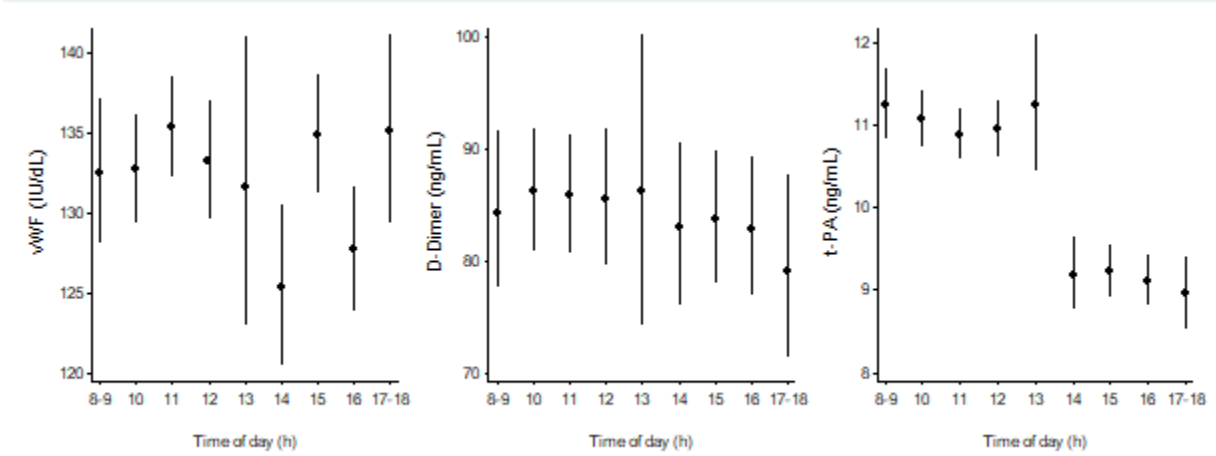


Figure 6.3 Unadjusted geometric means (95% CI) by time of the day for lipids

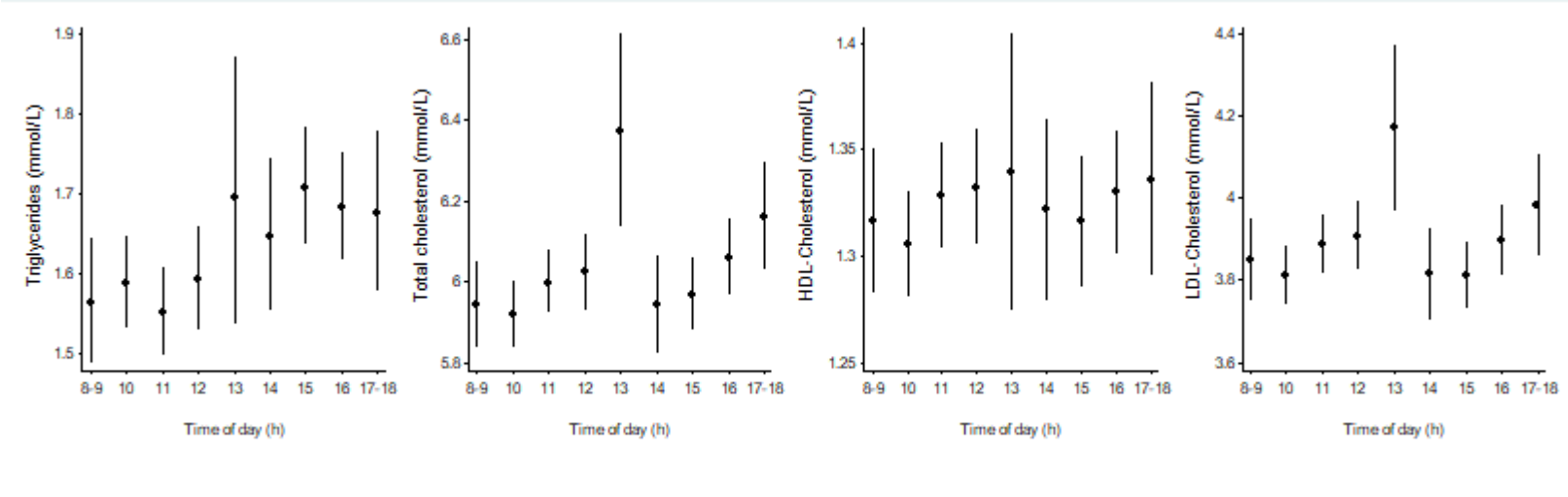


Figure 6.4 Unadjusted geometric means (95% CI) by time of the day for systolic (SBP) and diastolic (DPB) blood pressure

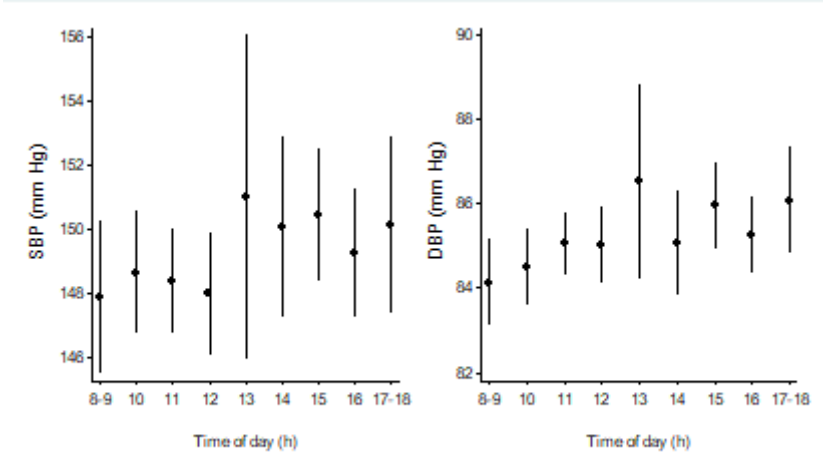
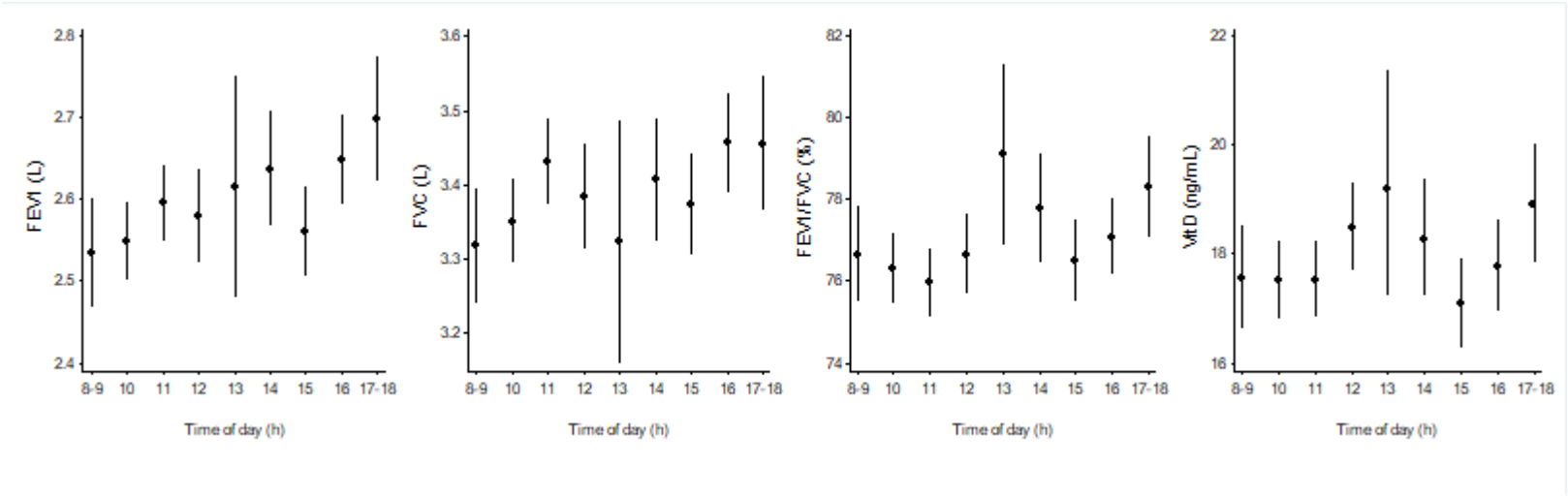


Figure 6.5 Unadjusted geometric means (95% CI) by time of day for lung function variables and Vitamin D



Chapter 7 SEASONAL VARIATIONS IN CARDIOVASCULAR DISEASE RISK FACTORS LEVELS

7.1 Summary

In most European countries cardiovascular disease (CVD) risk increases at lower temperatures, reflecting seasonal variation in all-cause and CVD mortality. Despite knowing that an association between temperature and mortality exists, the biological mechanism linking lower temperatures and higher CVD risk is not fully understood. It has been hypothesised that lower temperatures could have its adverse effect by increasing the levels of established risk factors causally associated with CVD, such as blood pressure and LDL-Cholesterol. However, little is known about associations of outdoor temperature with emerging, or recently established, causal risk factors for CVD, such as Interleukin-6 or other inflammatory and haemostatic factors. Moreover, to estimate such associations in older adults is particularly relevant as CVD is more common in older adults (60+ years old) than in younger populations. Other previous studies investigating the association of outdoor temperature with CVD risk factors in older adults were mostly small and included very few risk factors in their analysis.

The study conducted in this Chapter represents the largest investigation on temperature and CVD risk factors in older European adults. To address the aim, CVD risk factors measured on one occasion in 4252 men aged 60-79 years from the British Regional Heart Study (BRHS) were used. The measurements were carried out in between February 1998 and March 2000 (BRHS follow-up year 20). Linear models were carried out to estimate associations between outdoor mean temperature (main exposure variable) and CVD risk factors, after adjusting for exposure to influenza, measured by Influenza-like illness (ILI) weekly consultation rate in primary care at national level, and additionally adjusting for individual socio-demographic characteristics. The results showed that with a 5°C lower mean temperature measured during the examination day (lag 0), C-reactive protein was 3.6% (95%CI 0.0; 7.1%) higher, plasma viscosity was 0.4% (95%CI 0.1; 0.6%) higher, and t-PA was 2.6% (95%CI 0.7; 4.5%) higher, LDL cholesterol was 0.053 mmol/L (95%CI 0.006; 0.100) higher, and SBP was 1.220 mm Hg (95%CI 0.231; 2.210) higher. With a 5°C lower mean temperature assessed up to and including 1 week (lag 0-6) prior to the examination, IL-6 was 4.4% (95%CI 0.7; 7.9%) higher. Therefore,

public health approaches to protect the elderly against low temperatures could help in reducing CVD risk.

Please note that findings presented in this chapter have been published in 2017 as part of a larger collaboration including data from the BRHS and the study of pravastatin in elderly individuals at risk of vascular disease (PROSPER) (56).

7.2 Introduction

In the UK, Europe and worldwide, cardiovascular disease (CVD) risk increases at lower mean outdoor temperatures, and this is typically used in epidemiological studies for investigating seasonal variation in mortality (29, 35, 37, 125). It has been hypothesised that lower outdoor temperatures could exert their adverse effects by increasing the levels of well-established risk factors causally associated with CVD, such as blood pressure and lipids (78, 304). For example, in the UK effects of lower temperatures seemed to increase myocardial infarction up to 14 days after the reduction in temperature (37); this may be due to higher levels of inflammatory markers (148) and blood pressure (39) associated with lower temperatures recorded up to several days prior to CVD risk factors measurement. Moreover, in the UK and during the winter season, when temperatures are typically lower, the CVD mortality is more markedly increased in older (65+ years old) than younger adults (e.g. 18-64) (103); therefore, investigating temperature-related variations in CVD risk in older adults is of particular interest.

In older adults, higher levels in established CVD risk factors, such as total and LDL-Cholesterol, have been observed in winter (139, 141), but whether this is due to temperature is not well understood (40, 305). Moreover, recently established causal risk factors for CHD, as Interleukin-6 (25), are not widely studied in association with temperature (45). Low outdoor temperatures may increase the levels of other emerging risk factors prospectively associated with CVD (e.g. other inflammatory markers and haemostatic markers (306)), although literature supporting this hypothesis is sparse (45, 148). Overall, common limitations of previous studies investigating associations of outdoor temperature and biological markers of CVD are small sample size (142, 307), confined to a specific geographical location (148), and the investigation of clinical rather than community populations (146). Lastly, the possible

delayed effects of ambient temperature on CVD risk factors have been also overlooked in previous studies (136, 144) and findings are sparse (39, 148). In populations of older adults, whether age or the presence of other individual risk factors increase CVD risk factors levels at lower temperatures is unclear. Previous small studies of older adults which found no consistent interaction of lower temperatures with age and other risk factors, such as BMI, need to be confirmed by population-based studies to increase generalisability and precision of the findings (147, 148). The BRHS is a population-based study of CVD in older adults and is well suited to investigate associations of temperature with CVD risk factors, due to the measurements of a comprehensive range of risk factors. I would expect that higher levels of CVD risk factors (outcomes) are associated with lower mean outdoor temperatures (exposure). Especially when analysing CVD markers of inflammation and blood pressure I would expect to observe association with temperatures measured on the same day (and to some extent with temperature on previous days). Consistently with analysis conducted in Chapter 5 and 8, associations of temperatures with the outcomes are reported after adjustment for Influenza-like illness (ILI) weekly consultation rate in primary care, a proxy of exposure to seasonal influenza, a measure used for surveillance of respiratory viruses at national levels in the UK and associated especially with respiratory mortality. In this Chapter, and specifically for lung function measurements carried out in this study, I would expect to observe a decrease in lung function when ILI rates are higher.

7.3 Objectives

The aim of this study was to investigate seasonal variations in established and novel biological risk factors and physical measurements in older men (60-79 years) from the British Regional Heart Study (BRHS), with a particular focus on mean temperature-related variation in the risk factors. The main research questions (objectives) of this Chapter are:

1) Do variations in mean outdoor temperature (main seasonal factor and exposure variable analysed in this thesis) relate to variations in CVD risk factors measured in the BRHS? Mean temperature will be investigated in 5 ways:
   (i) Its level on the day of examination (lag 0)

(ii) Its level on the day of examinations, and 3 days previously (lag 0-3), representing the cumulative short-term effect over 4 days

(iii) Its level on the day of examinations, and 6 days previously (lag 0-6), representing the cumulative short-term effect over a week

(iv) Its level on the day of examinations, and 13 days previously (lag 0-13), representing the cumulative short-term effect over two weeks

(v) Its level on the day of examinations, and 27 days previously (lag 0-27), representing the cumulative short-term effect over four weeks

2) Are the temperature–risk factors relationships confounded by seasonal influenza trends? Is the measure of influenza exposure used in this study (ILI consultation rate) associated with CVD risk factors?
3) Is the association of temperature with the risk factors modified by individual socio-demographic characteristics?

7.4 Methods

7.4.1 Participants

The population for this study was the same as described in Chapter 6 (paragraph 6.4.1). In summary, participants were men who attended the 20-year follow-up which took place in between February 1998 and March 2000 (see also Chapter 3 paragraph 3.2.4, and Chapter 6, paragraph 6.4.1 for more details). 4252 surviving participants (77% response rate) aged 60-79 years who were resident in the UK attended a physical examination during which nurses took a fasting blood sample on one occasion for each participant (see Chapter 6, paragraph 6.4.2). The participants also completed a questionnaire including questions on other established CVD risk factors, such as age, social class, smoking habits, physical activity, and other behavioural and lifestyle factors.

7.4.2 CVD risk factors

Details of measurement technique and classification methods for the cardiovascular risk factors were extensively described in Chapter 3 (see paragraph 3.4.4). The measurements were carried out during 1998–2000, and the factors included (i) established risk factors, such as systolic and

diastolic blood pressure (BP, obtained sitting), and blood lipids (triglycerides, total cholesterol, high density lipoprotein [HDL] cholesterol, and low density lipoprotein [LDL] cholesterol); and (ii) emerging risk factors, such as inflammatory factors (C-reactive protein [CRP], fibrinogen, interleukin 6 [IL-6]) and plasma viscosity [PV]; haemostatic markers (tissue plasminogen activator [t-PA] antigen, fibrin D-dimer, von Willebrand factor [vWF]; and Vitamin D (VitD). According to the existing literature, there was sufficient justification for an investigation of seasonal variation in such risk factors (see paragraph 7.2). We also investigated measures of lung function (Forced Expiratory Flow after 1 second [FEV_1], and Forced vital capacity [FVC], and FEV_1/FVC %) made at the same examination, due to the fact poor lung function may be associated with exposure to influenza (see paragraph 7.2), and also a risk factor for CVD (308-310) and respiratory mortality.

7.4.3 Meteorological factors data

The UK Meteorological (MET) Office provided daily outdoor mean temperatures for the 24 towns of BRHS during the study period (see Chapter 3, paragraph 3.3). In summary, the MET office provided mean temperature data (main exposure variable), which is only available as the average of maximum and minimum temperatures collected from 9pm to 9pm of the following day of each day. For example, if a biological marker is measured on January 13th in between 8am and 6pm (as explained in Chapter 6), the mean temperature linked to such measurement is the one recorded from 9pm of January 12th to 9pm of January 13th. The mean temperature on the day of physical examination and blood sampling of each participant were linked to the CVD risk factors measured in the BRHS, as described in Chapter 3 paragraph 3.3, and this has been termed "lag 0 temperature" in this chapter. All temperature data levels up to 4 consecutive weeks prior to the day of examination were used as part of subsequent analysis (definition of temperature collected at different "lags" was offered in paragraph 7.4.5.2 alongside with description of statistical analysis fitting such temperature variables).

To the best of my knowledge, there are no specific reasons for an investigation of possible short term effects of sunshine duration in this Chapter. While it is plausible that lack of sunshine exposure is harmful to health, this would need to occur consistently over decades or the life time, rather than a few days (311). Also, although lack of sunshine is likely to lower vitamin D levels, arguments for a causal effect of Vitamin D on inflammation, or on mortality risk, has

been challenged (312). Therefore, in this chapter the findings on association of sunshine duration with Vitamin D are included only descriptively, because sunshine duration is a more plausible influence than temperature on Vitamin D levels.

Previous studies of associations of relative humidity with CVD risk factors and relative humidity with mortality are rare (130, 137, 313); the associations of humidity with blood pressure (137), and with CVD mortality worldwide (130, 137) were found not statistically significant. In one previous study of association of temperature with three CVD risk factors (IL-6, C-reactive protein and Fibrinogen), relative humidity was used only as a covariable (and estimates not reported) (146); in this study, relative humidity did not alter the association of temperature with the CVD risk factors (146). Without a strong rationale for an investigation and sparse evidence, I decided not to consider relative humidity in this Chapter.

In summary, mean temperature was used as the main exposure variable (as anticipated in paragraph 7.2), consistently with Chapter 5 and Chapter 8.

7.4.4 Adjusting outdoor temperature for influenza vs other long-term seasonal trends

Influenza-Like illness (ILI) weekly consultation rates per 100,000 population admitted to General Practice is a proxy of influenza severity; the rationale for inclusion of ILI rates in this thesis was already introduced in Chapter 5 (paragraph 5.4.4) when I investigated temperature-related variations in physical activity. Consistently with Chapters 5 and 8, ILI rate collected during the study period (February 1998 to March 2000) was included in statistical models in order to adjust temperature-related associations with the outcomes for seasonal confounding.

Please note that related findings to those presented in this chapter have been published in 2017 as part of a larger collaboration including BRHS and PROSPER data (56); in this publication I adjusted temperature with season (fitted as dichotomised variable, winter vs non-winter months) instead of ILI rates. Considering the objectives of this PhD thesis, I considered that adjusting temperature with ILI rates in statistical analysis was preferable to an adjustment for a generic (e.g. categorical, fixed or non-illness specific) proxy of season, or trigonometric functions of day of the year previously used in one earlier BRHS publication (55). While temperature and ILI associations with mortality have a plausible epidemiological link (see

paragraph 7.2), generic proxies of season cannot be clearly interpreted; they can potentially capture a seasonal trend, but this does not enhance our understanding of which biological pathways are relevant to seasonal variations in CVD.

7.4.5 Statistical methods

Excepting Total Cholesterol, HDL-cholesterol, LDL-cholesterol, SBP and DBP, FEV_1, FVC, and FEV_1/FVC all other outcomes were log-transformed for further analysis as their distributions were positively skewed, as reported in previous BRHS publications (55, 301).

7.4.5.1 Preliminary analysis (descriptive tables and plots)

Several preliminary analysis were carried out to explore the data:

- The number of participants examined from the BRHS, the total number of days and weeks when the examinations took place, and the mean (SD) of daily average outdoor temperature and weekly ILI rate during examinations were calculated;
- Unadjusted means (95% CI) of CVD risk factors levels were plotted against quintiles of mean temperature and ILI rate during the study period.

7.4.5.2 Associations of temperature with the CVD risk factors

The association of temperature with each of the CVD risk factors was estimated in (i) unadjusted linear models, (ii) ILI rate-adjusted linear models, (iii) ILI rate and age adjusted linear models, and (iv) linear models adjusted for ILI rate, age, Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day of measurement (45); for log-transformed outcomes, associations were reported as percentage difference in the geometric mean. Associations of temperature with BP variables, HDL-cholesterol, LDL-cholesterol, and Total cholesterol, were reported as linear coefficients (absolute difference).

The associations of temperature with the CVD risk factors were estimated; for log-transformed outcomes, as the percent change in the geometric mean associated with a decrease of 5°C in mean temperature, as in previous studies (39, 40), and because 5°C is also the rounded standard deviation [SD] to the nearest integer for daily mean temperature during 1998-2000 in the BRHS towns (4.8°C, see Table 7.2). Associations of temperature with BP variables, HDL-cholesterol,

LDL-cholesterol, Total cholesterol were reported as linear coefficients (absolute difference) per decrease of 5°C in mean temperature.

Associations of temperature with three measurements of lung function were investigated: (i) the forced expiratory volume in one second (FEV_1) measures amount of air a person can forcefully exhale in one second of the spirometry test; (ii) the forced vital capacity (FVC), is the amount of air which can be forcibly exhaled from the lungs after taking the deepest breath possible; (iii) the FEV_1/FVC ratio (%), a marker of Chronic Obstructive Pulmonary Disease (COPD) (168). Estimates were reported as linear coefficients (absolute difference) per decrease of 5°C in mean temperature.

Multilevel linear regression models (level 1 = individual, level 2 = town of examination) were used to take into account clustering within towns, in order to avoid possible confounding with season of measurement (314). Temperature was fitted in the model in 5 different ways:

1) Temperature at lag 0, which is the temperature measured during the examination day;
2) Cumulative short-term associations of temperature up to and including 4 days (lag 0-3) prior to the examination (= average temperature of the 4 days up to and including the day of the examination, where temperature at lag 3 is the temperature measured 3 days prior to the examination day);
3) Cumulative short-term associations of temperature up to and including 1 week (lag 0-6) prior to the examination;
4) Cumulative associations of temperature up to and including 2 weeks (lag 0-13) prior to the examination;
5) Cumulative associations of temperature up to and including to 4 weeks (lag 0-27) prior to the examination.

Overall, I would expect higher levels of CVD risk factors at lower temperatures. Especially when analysing CVD markers of inflammation (see Chapter 6, paragraph 6.6.2), blood pressure, and LDL-Cholesterol, I would expect to observe a short term association with temperatures (lag 0, lag 0-3, or lag 0-6) as in previous studies (39, 40, 148). In particular, associations of low temperatures with inflammatory risk factors levels (e.g. CRP) are expected

to be greater at lag 0 and then decreased gradually at subsequent single lags (lag 1, lag 2, lag 3 etc.) and up to lag 6, remaining fairly constant after lag 6 and up to 2 weeks prior to examination (147, 148). I would also expect Vitamin D to be lower at lower temperatures due to reduced sunshine duration

Associations between temperatures and CVD risk factors (described above in points 1) to 5) above) were adjusted for ILI rate and individual characteristics, such as age, Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day of measurement (45). The adjustment for ILI rate was made to check whether there was confounding between mean temperature and a different seasonal factor (as already explained in Chapter 5, paragraph 5.4.5.2). The adjustment for individual characteristics was performed as their relationship with CVD risk factors is known a priori to be strong, and this would reduce the standard error and increase precision of the estimated effect of the key exposure variable (see Chapter 5, paragraph 5.4.5.2), as well as adjust for confounding characteristics of those participants measured in seasons of low, as opposed to high temperature.

Outdoor temperature was also additionally adjusted for indoor temperature of examination room, which remained fairly constant over the year (peak was in June with 25°C, and nadir in May with 22°C). Moreover, the temperature of the room did not have any relationship with the outcomes (all $p>0.05$), therefore it was excluded from models presented.

The proportion of variance associated with temperature from the fully adjusted models was estimated using partial R-squared.

7.4.5.3 Interaction of temperature with individual risk factors

I also fitted an interaction between short term changes in temperature at lag 0 or lag 0-6 and age (fitted as continuous variable) to test whether the relationship of temperature with outcomes was particularly marked among the “oldest” old vs “younger” older adults; it is recognised that tolerance of sudden changes in temperature towards the extremes is more limited as people are ageing, and this could affect the ability of seniors to maintain their body temperature when exposed to cold environments (315). Also, I hypothesized that some personal circumstances such as marital status and social class (markers of fuel poverty in winter) could also potentially

interact with temperature and raise the CVD risk factors levels (316); therefore I included them in my interaction tests one at a time. Lastly, the interaction of temperature with well-established life-style factors was tested (BMI fitted as continuous variable, smoking - yes vs no, and physical activity score). An overall Wald test for interaction between the categorical variables and temperature was used.

7.4.5.4 Associations of sunshine duration and Vitamin D

As it well known that people can obtain Vitamin D from sunlight (29, 30), the analysis described at paragraph 7.4.5.2 was repeated substituting outdoor temperature with sunshine duration, and using Vitamin D as outcome.

7.5 Results

7.5.1 Descriptive statistics

The BRHS participants' characteristics and descriptive statistics were previously described and discussed in Chapter 6 as this analysis made use of the same population; therefore the results were reported only in Table 6.1 and not in this Chapter.

The number of participants examined from the BRHS, the total number of days when the examinations took place, and the mean (SD) of daily average outdoor temperature and ILI rate during examinations were also reported (Table 7.1). The total number of days when the examinations took place was 242. For some months there are more days when examinations took place than days in the month, because measurements were taken across multiple years (Table 7.1). Mean temperatures were on average equal to 5.7°C (SD=2.9) in winter (December-March) and 12.1 °C (SD=4.1) during the non-winter season (April-November). National ILI rates were on average equal to 33.9 (SD=46.4) persons per 100,000 in winter and 9 (SD=5.5) persons per 100,000 during the non-winter season. During the 242 examination days, the Pearson correlation of temperature with ILI rate was r=-0.367.

Unadjusted means (95% CI) of CVD risk factors levels were typically higher at lower temperatures (first and second quintiles of mean temperature, see Figures 7.1-7.5) and at higher ILI rate during the study period (fourth and fifth quintiles of ILI rate, see Figures 7.6-7.10).

7.5.2 Associations of mean temperature with the CVD risk factors

Adjusted associations of mean temperature at different lags with the CVD risk factors are shown in Table 7.2 – Table 7.8. A summary chart of such associations is also shown in Table 7.10; however, the following paragraphs from 7.5.2.1 to 7.5.2.3 offer a more detailed presentation of the findings, with estimates of magnitude of associations.

7.5.2.1 Associations of temperature at lag 0

In fully adjusted models (Table 7.2 – Table 7.8, Model 4, temperature lag 0), estimates showed that with a 5°C lower mean temperature measured during the examination day (lag 0), C-reactive protein was 3.6% (95%CI 0.0; 7.1%, p=0.050) higher, plasma viscosity was 0.4% (95%CI 0.1; 0.6%, p=0.002) higher, and t-PA was 2.6% (95%CI 0.7; 4.5%, p=0.009) higher, total cholesterol was 0.064 mmol/L (95% confidence Intervals (CI) 0.014; 0.113, p=0.012) higher, LDL cholesterol was 0.053 mmol/L (95%CI 0.006; 0.100, p=0.027) higher, and SBP was 1.220 mm Hg (95%CI 0.231; 2.210, p=0.016) higher. With a 5°C lower mean temperature, Vitamin D was 5% (95%CI -8.0; -2.1%, p=0.001) lower.

Associations of temperature at lag 0 with other CVD risk factors were not statistically significant.

The highest proportion of variance was observed when the outcome analysed was Vitamin D (5.2% in fully adjusted models, see Table 7.12). In each of the models, and other outcomes analysed, the proportion of variance associated with mean temperature was less than 1% (Table 7.12). Interaction effects of temperature with age on the outcomes levels were not significant (all p>0.05, data not shown).

7.5.2.2 Associations of temperature at lag 0-3 and lag 0-6

Associations of temperature up to 1 week (lag 0-3 and lag 0-6) prior to the examination day with CRP, IL-6, PV, t-PA, lung function variables and Vitamin D levels were observed (Table 7.3 – Table 7.8, Model 4). The magnitude of the associations was similar to associations from full adjusted models using temperature at lag 0: overall, the estimates showed that with a decrease in mean temperature at lag 0-3 or lag 0-6 the levels of CRP, IL-6, PV, t-PA, and FVC were higher, while Vitamin D levels were lower. Similarly to observations at lag 0, the

proportion of variance explained by temperature at lag 0-6 did not substantially change, remaining less than 1% for all outcomes apart from Vitamin D (8.4%, see Table 7.12).

7.5.2.3 Associations of temperature at lag 0-13 and lag 0-27

Associations of temperature over 2 weeks or 4 weeks (lag 0-13 and lag 0-27) prior to the examination day with CRP, IL-6, PV, t-PA, and Vitamin D were observed (Table 7.3 – Table 7.8, Model 4). The magnitude of the associations at lag 0-13 and 0-27 was similar. Overall, the estimates showed that with a decrease in mean temperature at lag 0-13 or lag 0-27 the levels of CRP, IL-6, PV, t-PA, were higher, while Vitamin D levels were lower. The proportion of variance explained by temperature at lag 0-13 and lag 0-27 did not substantially change in comparison to lag 0-6 (Table 7.12).

In comparison with lag 0 or lag 0-6, the magnitude of association at lag 0-13 was greater only for IL-6, t-PA, and Vitamin D, and similar for CRP and PV.

7.5.2.4 Associations between Influenza-like Illness weekly consultation rate and CVD risk factors

In fully adjusted models (Table 7.9, Model 3), estimates showed that with a 1 SD increase in ILI rate, FVC was 0.078 litre (95%CI 0.0034; 0.122%, p=0.001) higher, while FEV1/FVC% was 1.3% (95%CI -2.0; -0.5%, p=0.001) lower, and Vitamin D was 7.5% (95%CI -10.3; -4.9%, p<0.001) lower.

7.5.3 Interaction of temperature with individual risk factors

The interaction tests of temperature with age, social class, body mass index, smoking, physical activity, use of lipid lowering drugs medication on the outcomes levels were not significant (all p>0.05).

7.5.4 Associations of sunshine duration and Vitamin D

In fully adjusted models reported in table 7.11 (Model 4), estimates showed that with a 1 SD decrease in sunshine duration at lag 0-3 (p=0.021), lag 0-6, lag 0-13 and lag 0-27 (all p values <0.001) the levels of Vitamin D decreased. However, the association between Vitamin D and sunshine duration was weak when measured at lag 0 only (0.2%, 95%CI -1.5; 1.8, p=0.815),

but became stronger over longer lags. Overall, the magnitude of the associations per 1 SD decrease in sunshine duration was similar in comparison with models which used 1 SD decrease in outdoor temperature.

7.6 Discussion

To the best of my knowledge, this is the largest investigation of the relationships between outdoor temperature and a comprehensive range of established and emerging CVD risk factors, and in older European people. The CVD risk factors investigated here were selected for two reasons: first, there was published evidence of seasonal variation, with higher levels observed in the winter months or at lower temperatures (39, 40, 45, 55, 148); second, there was published evidence of independent associations with CVD events in meta-analyses of prospective population-based studies (79, 317-319).

7.6.1 Summary of the main findings

I discuss below findings in relation to objectives outlined in paragraph 7.3

Question 1: Do variations in mean outdoor temperature (main proxy for season) relate to variations in CVD risk factors measured in the BRHS?

Yes, the findings showed that lower temperatures were associated with higher levels of some, but not all, CVD risk factors. Specifically, temperature-related variations can be divided in 4 major groups:

1) Immediate or short-term *decrease* in temperature (at lag 0 or lag 0-3 or lag 0-6) was associated with an *increase* in CVD risk factors; in particular CRP, PV, IL-6, t-PA, SBP, Total-Cholesterol, LDL-Cholesterol, and FVC; the magnitude of associations was similar when comparing lag 0, lag 0-3 and lag 0-6.
2) Cumulative *decrease* in temperature up to 2 or 4 weeks (lag 0-13 and lag 0-27) was associated with an *increase* in a fewer CVD risk factors, in particular CRP, PV, IL-6 and t-PA; in comparison with temperature at lag 0 or lag 0-6, larger effect sizes were seen for IL-6 and t-PA but not CRP and PV.

3) *Decrease* in temperature was associated with a *decrease* in Vitamin D (temperature at any lag)
4) CVD risk factors that showed *no association* with temperature included Fibrinogen, D-Dimer, vWF, Triglycerides, HDL-Cholesterol, FEV_1 and FEV_1/FVC ratio.

Lower outdoor temperature was the major meteorological parameter investigated in this Chapter and thesis. Overall, the findings reported associations in the same direction when using the outdoor temperature at lag 0 or the average temperature of 1 week (lag 0-6), 2 weeks (lag 0-13), and 4 weeks (lag 0-27) prior to the examination date. However, the patterns at longer lags can be very different depending on the risk factor; for IL-6 the magnitude of the associations increased fairly linearly up to 2 weeks prior to the examination date, then remained fairly constant. In the case of IL-6, a decrease in 5°C in temperature was associated with an increase of 1.4% in IL-6 at lag 0, 4.4% at lag 0-6, 8.0% at lag 0-13 and 9.1% at lag 0-27. On the other hand, for risk factors such as total cholesterol and LDL-Cholesterol, associations with temperature were only seen clearly associated with temperatures on the same day, while associations with longer term temperatures were weaker and non-statistically significant..

In fully adjusted models, the proportion of variance in risk factors explained by temperature was much smaller than other risk factors, being around 1% of the total variance (except for Vitamin D, where variance explained was approximately 5%).

Question 2. Is the temperature–risk factors relationship confounded by seasonal influenza trends? Is the measure of influenza exposure used in this study (ILI consultation rate) associated with CVD risk factors?

No, the findings showed that all statistically significant associations of temperature with the CVD risk factors in unadjusted models persisted after adjustment for a proxy of exposure to influenza (ILI consultation rate). Higher ILI rates were especially associated with a decrease in Vitamin D.

Question 3. Is the association of temperature with the risk factors modified by individual socio-demographic characteristics?

There was no evidence of an interaction of temperature with age and other CVD risk factors on the wide range of CVD risk factors analysed. This finding suggested that the effect of low temperature on CVD risk may apply to the full age range of older adults

7.6.2 Comparison with other studies

In comparison to the few previous studies, the overall direction of the associations reported in this Chapter were fairly similar (39, 40, 148); in particular the findings were consistent with the suggestions from previous studies that, in addition to established risk factors such as cholesterol (40) and blood pressure (39), circulating inflammatory markers (45) and Vitamin D (166) showed strong associations with outdoor temperature and may contribute to increased incidence of CVD in winter (29). The association of temperature with Systolic Blood Pressure, LDL-cholesterol and IL-6 levels may be particularly relevant, as previous trials and Mendelian Randomization (MR) studies support their causal role in CHD risk (78, 304, 320).

7.6.2.1 Established CVD risk factors

In this study lower outdoor temperature at lag 0 was significantly associated with higher levels of SBP, consistently with previous findings (136, 138). The association of temperature with DBP was weaker and non-significant, differing from one previous study (39).

A decrease in temperature was associated with increased Total cholesterol and LDL-cholesterol, as previously reported (144). In our study a decrease of about 10°C in temperatures (approximately the difference between the coldest and warmest months, January-August) would be associated with an increase of 0.15 mmol/L in LDL-Cholesterol. According to previous studies, this absolute increase in LDL-Cholesterol was associated with a 3% increase in CVD mortality risk (304). Lastly, associations of temperature with triglycerides were not significant as observed in previous studies (40). In my analysis levels of HDL cholesterol were not associated with temperature, differing from one previous study (40); this makes the role of HDL-Cholesterol in the seasonal variation in CVD not relevant. This is consistent with HDL-Cholesterol not being causally related to CVD (321).

We found that lower temperatures were not consistently associated with a decrease in FEV_1 with a decrease in FVC and a decrease in FEV_1/FVC ratio levels, in contrast to my hypothesis.

This suggests that temperature was not a good predictor of lung function or COPD (estimated with the FEV_1/FEV ratio). There may be reasons for this; previous findings suggest a lung function decline during the non-winter months (although temperature was not investigated), as reported in two previous studies where FEV_1 and FVC levels decreased during July-September (166, 168). It is possible that lower levels of FEV_1 and FVC may indicate variations in the different seasonal respiratory symptoms experienced, such as asthmatic symptoms (168). It is plausible that in my analysis on FEV_1 and FVC, seasonal variations in temperature may reflect onset of the pollen season (with peak typically in spring, when temperatures start to increase after the winter); one previous study found that in London an increase in daily total grass pollen concentrations from 2005 to 2011 were associated with increased emergency hospital admissions for asthma amongst adults, with a lag of 2 to 5 days following exposure, and after accounting for outdoor temperatures (167). Also, toward the end of summer, concentrations of an airborne fungus peak in August and September and this may lead to lower levels of FEV_1 and FVC (168). On the other hand, we would have expected a lower FEV_1/FEV ratio in winter and at lower temperatures; although our analysis confirmed the direction of these association, the estimates were not statistically significant. In this study, a lower ILI rate (typically recorded in the non-winter months, when temperatures are higher) was associated with a decrease in FVC. Obtaining a more precise measure of ILI exposure at individual level should be performed before confirming such findings. We would have expected a lower FEV_1/FVC ratio at higher ILI rates; although our analysis confirmed the direction of these association, the decline in FEV_1/FVC ratio was mathematically driven by an increase in FVC. If this was true, the FEV_1/FVC ratio does not correspond to a good measure of COPD, where the decline is driven by a decrease in FEV_1.

7.6.2.2 Emerging CVD risk factors

A decrease in temperature was associated with increased circulating levels of markers of inflammation, such as IL-6, CRP, Fibrinogen, and plasma viscosity. This is important and support the inflammatory hypothesis of CVD previously tested in Randomized Controlled Trials (322). To date, MR studies for IL-6 suggested a causal role in coronary heart disease, in contrast to null associations in MR studies for CRP and fibrinogen (25, 96, 323) Therefore, the findings on IL-6 are particularly important: in this study, the magnitude of the associations between temperature and IL-6 increased fairly linearly up to 2 weeks prior to the examination

date, then remained fairly constant up to 4 weeks. In older people, these associations may suggest an ongoing inflammation status triggered by long-term exposure to lower temperatures up to 2 weeks followed by a slow recovery (148). A decrease of about 10°C in temperatures at lag 0-27 (similar to the monthly variation in temperature between January and August) would be associated with an average increase of 0.22 pg/mL in IL-6 levels. According to previous findings from the BRHS, this absolute difference was associated with an increase of 1.5% in CVD deaths (100). Considering that in England and Wales the excess winter deaths (EWDs) five-year moving average for CVD remained fairly constant and around 25% from 2010/2011 to 2015/2016 (32), then attributable risk to IL-6 would be approximately 1.5/25 = 5%.

It is also possible that an acute (or short-term) effect of outdoor temperature may be more marked on rapidly responding CVD risk factors, including a marker of inflammation such as CRP (324). The acute phase response indicated by increased CRP may explain why it provides closer associations and better predictions of CVD events in the short-term than other markers of inflammation, such as cytokine mediators, other acute-phase proteins such as serum amyloid A protein and albumin (324, 325). The associations of temperature with other specific markers of inflammation we studied, such as fibrinogen and plasma viscosity, were weaker than for CRP, as previously reported (146).

Findings for temperature-related variations in PV and t-PA are similar to those reported in previous studies which observed higher levels of these factors in winter (45), although the effect of temperature was not specifically tested. To our knowledge these associations with temperature are novel, and have not been previously published.

The seasonal and temperature-related variation in vWF remains poorly understood; in one previous study the peak in vWF was observed in early spring (between March and May (45)). The reasons for the lack of association with temperature are not well understood and to the best of my knowledge previous studies did not analyse such associations; therefore this findings are not supported by previous literature.

Lastly, the associations of temperature with Fibrinogen and fibrin D-Dimer were not significant; D-dimer is known to have an unusual seasonal variation, with peaks in

February/March and August/September (152). Our data suggest that Fibrinogen and D-Dimer are not the best markers of temperature-related influences.

7.6.2.3 Interaction of temperature with individual risk factors

There was no consistent evidence of an interaction of temperature with individual risk factors on CVD risk factors levels analysed; the findings presented in this Chapter were previously published as part of a meta-analysis including data from the BRHS and the PROSPER participants' age ranged from 60-82 years old) (56); these results are consistent with findings from previous studies including older adults only, where no consistent interaction of lower temperatures with increasing age and other risk factors was found (147, 148). This suggested that the effect of low temperature on CVD risk may apply to the full age range of older adults.

7.6.2.4 Vitamin D

Findings for Vitamin D showed strong associations with both temperature and sunshine duration. For Vitamin D specifically, temperature is likely to be a proxy of exposure to sun light, which is the real determinant. This investigation is included in this Chapter only with descriptive purposes; to the best of my knowledge prior studies did not reported cumulative associations of sunshine duration and Vitamin D in older adults.

7.6.3 Strengths and limitations

With the analysis conducted in this study the statistical power and precision was improved in comparison with findings reported in smaller studies of older adults (142, 307). Due to the nature of our data it was hard to distinguish between temperature-related associations and influenza like-illness associations at individual level. However, it seems that temperature variations were negatively associated with CVD risk factors of inflammation, blood pressure and cholesterol, and Vitamin D, while ILI rate was negatively associated with Vitamin D in particular. This study also excluded indoor temperature during the examination as a possible confounding factor. The indoor temperature of examination room remained fairly constant over the year and did not show any seasonal variation (peak was in June with 25°C, and nadir in May with 22°C). After mutual adjustment with outdoor temperature I excluded a relationship of indoor temperature with both outdoor temperature and the outcomes.

7.6.4 Implications

Our study provides robust evidence that outdoor temperature is related to major CVD risk factors in older adults. This study increased generalisability of existing evidence from northern European older populations and is consistent with the hypothesis that inflammation markers, on top of blood pressure and LDL-Cholesterol changes are associated with temperature. However, the causal pathway involving temperature, the risk factors, and mortality cannot be established with results from this Chapter 7, as observed associations between temperature and the risk factors were cross-sectional. To improve evidence concerning possible causality of these associations, frequent measurements of CVD risk factors over the years on the same individual followed-up for enough time (e.g. several years) may help (see Chapter 9, for broader discussion).

Our overall findings suggested that protecting older adults during cold weather is important; however, whether targeting CVD risk factors at lower temperatures would really provide opportunities for intervention remains unclear. The increase in such CVD risk factors remains a process which develops over the course of many years (e.g. increasing age is associated with narrowing of arteries and atherosclerosis, the build-up of fatty material inside your arteries); therefore, the increase in such CVD risk factors levels due to short-term exposure to daily temperatures are likely to exert only a modest effect in comparison with the increased risk that typically occurs from middle-age to older-age. Findings from this chapter showed that the proportion of variance in risk factors explained by temperature was indeed very small, being less than 1% of the total variance.

7.7 Conclusions

Our study provides robust evidence that outdoor temperature is associated with major CVD risk factors in older adults. Associations were strongest with inflammatory factors followed by associations with SBP, and cholesterol variables. In older adults a better protection against low temperatures, as well as strategies aimed to increase their physical activity levels in winter, could help in reducing the levels of several CVD risk factors.

Table 7.1 Number of participants examined from the BRHS, number of days when the examinations took place, mean (SD) of daily average outdoor temperature during examinations (1998-2000), and England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons

Month	Number of BRHS men examined	Total number of days when the examinations took place	Average daily mean outdoor temperature (SD) during examinations	Total number of weeks when the examinations took place	Average ILI weekly consultation rate during examinations
Winter season:	1450	92	5.7 (2.9)	25	33.9 (46.4)
December	209	11	4.6 (2.2)	2	34.1 (18.0)
January	268	16	3.9 (2.4)	4	103.6 (94.5)
February	559	39	5.5 (3.4)	10	22.9 (2.8)
March	414	26	7.0 (2.5)	8	13.8 (1.7)
Non-winter season:	2802	150	12.1 (4.1)	40	9.0 (5.5)
April	430	22	9.4 (2.7)	6	7.0 (0.7)
May	207	13	12.5 (2.4)	3	5.5 (0.5)
June	467	22	13.7 (2.3)	7	4.0 (0.4)
July	371	20	15.3 (2.3)	6	7.7 (2.9)
August	156	10	16.4 (2.0)	2	5.7 (0.3)
September	407	22	14.9 (2.2)	6	7.1 (3.5)
October	345	22	10.0 (2.2)	5	15.0 (5.8)
November	419	19	7.5 (2.5)	6	20.4 (9.3)
Overall study period	**4252**	**242**	**9.9 (4.8)**	**65**	**18.6 (31.2)**

[1] Note that for some months there are more days when examinations took place than days in the month, because measurements were taken across multiple years.

Table 7.2 Difference in the levels of CRP and IL-6 for 5°C decrease in outdoor mean temperature in the BRHS participants, during examinations (1998-2000)

		Model 1: Temperature		Model 2: Temperature + ILI		Model 3: Temperature + ILI + Age		Model 4: Temperature + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
Outcome	Lag for outdoor temperature	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value
CRP	Lag 0	3.3(-1.1,7.6)	0.139	2.3(-2.4,6.8)	0.330	2.8(-1.9,7.3)	0.242	3.6(0.0,7.1)	0.050
	Lag 0-3	4.1(-0.4,8.4)	0.073	3.1(-1.7,7.7)	0.195	3.4(-1.4,8.0)	0.164	3.7(0.0,7.1)	0.046
	Lag 0-6	4.4(-0.4,8.9)	0.070	3.4(-1.7,8.2)	0.188	3.6(-1.5,8.4)	0.160	3.6(-0.2,7.3)	0.061
	Lag 0-13	5.0(-0.3,10.0)	0.061	3.9(-1.7,9.2)	0.171	4.6(-1.1,9.9)	0.111	4.6(0.6,8.5)	0.023
	Lag 0-27	4.6(-1.1,10.1)	0.110	3.1(-3.1,9.0)	0.315	3.7(-2.6,9.6)	0.246	4.3(-0.1,8.5)	0.053
IL-6	Lag 0	1.3(-2.1,4.6)	0.449	1.2(-2.3,4.5)	0.507	1.8(-1.6,5.1)	0.294	1.9(-1.3,5.0)	0.244
	Lag 0-3	3.2(-0.4,6.7)	0.076	3.2(-0.5,6.8)	0.088	3.6(-0.1,7.1)	0.054	3.3(-0.1,6.5)	0.057
	Lag 0-6	4.1(0.2,7.9)	0.037	4.2(0.1,8.0)	0.043	4.4(0.5,8.2)	0.027	4.4(0.7,7.9)	0.018
	Lag 0-13	5.8(1.3,10.1)	0.012	5.9(1.3,10.3)	0.013	6.9(2.3,11.2)	0.004	6.6(2.5,10.5)	0.002
	Lag 0-27	5.7(0.7,10.4)	0.025	5.9(0.6,11.0)	0.028	6.9(1.6,11.8)	0.010	6.9(2.2,11.3)	0.004

[1] Model adjusted for age, Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.3 Difference in the levels of Fibrinogen, PV, and t-PA for 5°C decrease in outdoor mean temperature in the BRHS participants, during examinations (1998-2000)

		Model 1: Temperature		Model 2: Temperature + ILI		Model 3: Temperature + ILI + Age		Model 4: Temperature + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
Outcome	Lag for outdoor temperature	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value
Fibrinogen	Lag 0	0.2(-0.8,1.3)	0.639	0.2(-0.9,1.3)	0.715	0.3(-0.8,1.4)	0.520	0.5(-0.5,1.6)	0.302
	Lag 0-3	0.1(-1.0,1.2)	0.838	0.0(-1.1,1.2)	0.937	0.1(-1.0,1.3)	0.810	0.3(-0.9,1.4)	0.619
	Lag 0-6	-0.4(-1.6,0.8)	0.554	-0.5(-1.7,0.7)	0.448	-0.4(-1.7,0.8)	0.522	-0.2(-1.5,1.0)	0.750
	Lag 0-13	-0.3(-1.6,1.0)	0.681	-0.4(-1.9,1.0)	0.557	-0.2(-1.7,1.2)	0.759	0.1(-1.4,1.5)	0.908
	Lag 0-27	-0.6(-2.0,0.9)	0.452	-0.8(-2.4,0.7)	0.312	-0.7(-2.2,0.9)	0.424	-0.2(-1.8,1.3)	0.786
PV	Lag 0	0.4(0.1,0.6)	0.003	0.4(0.1,0.6)	0.008	0.4(0.1,0.6)	0.005	0.4(0.1,0.6)	0.002
	Lag 0-3	0.4(0.1,0.7)	0.003	0.4(0.1,0.7)	0.009	0.4(0.1,0.7)	0.007	0.4(0.1,0.6)	0.004
	Lag 0-6	0.3(0.0,0.6)	0.030	0.3(-0.1,0.6)	0.074	0.3(0.0,0.6)	0.067	0.3(0.0,0.5)	0.037
	Lag 0-13	0.3(0.0,0.7)	0.040	0.3(-0.1,0.6)	0.100	0.3(-0.1,0.6)	0.076	0.3(0.0,0.6)	0.039
	Lag 0-27	0.2(-0.1,0.6)	0.159	0.1(-0.2,0.5)	0.376	0.2(-0.2,0.5)	0.334	0.2(-0.1,0.6)	0.170
t-PA	Lag 0	1.5(-0.6,3.6)	0.155	1.7(-0.5,3.8)	0.125	1.9(-0.3,4.0)	0.085	2.6(0.7,4.5)	0.009
	Lag 0-3	2.4(0.1,4.6)	0.038	2.6(0.3,4.9)	0.026	2.7(0.4,5.0)	0.020	2.9(0.7,5.0)	0.008
	Lag 0-6	3.3(0.9,5.7)	0.007	3.6(1.1,6.1)	0.004	3.7(1.2,6.1)	0.003	4.0(1.7,6.2)	0.001
	Lag 0-13	5.3(2.5,8.0)	<0.001	5.8(3.0,8.6)	<0.001	6.1(3.2,8.8)	<0.001	5.9(3.2,8.5)	<0.001
	Lag 0-27	6.4(3.4,9.4)	<0.001	7.4(4.3,10.5)	<0.001	7.6(4.5,10.7)	<0.001	7.5(4.5,10.3)	<0.001

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.4 Difference in the levels of vWF and D-Dimer for 5°C decrease in outdoor mean temperature in the BRHS participants, during examinations (1998-2000)

		Model 1: Temperature		Model 2: Temperature + ILI		Model 3: Temperature + ILI + Age		Model 4: Temperature + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
Outcome	Lag for outdoor temperature	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value
vWF	Lag 0	-1.4(-3.2,0.3)	0.109	-1.8(-3.6,0.0)	0.052	-1.5(-3.2,0.3)	0.101	-1.3(-2.9,0.4)	0.141
	Lag 0-3	-0.3(-2.1,1.6)	0.798	-0.6(-2.5,1.3)	0.549	-0.4(-2.3,1.4)	0.673	-0.4(-2.2,1.4)	0.680
	Lag 0-6	0.2(-1.8,2.2)	0.829	-0.1(-2.2,1.9)	0.908	0.0(-2.0,2.0)	0.966	0.0(-2.0,1.9)	0.997
	Lag 0-13	0.0(-2.3,2.4)	0.965	-0.4(-2.8,2.0)	0.739	0.0(-2.3,2.4)	0.967	0.0(-2.2,2.3)	0.951
	Lag 0-27	1.0(-1.6,3.6)	0.423	0.5(-2.2,3.1)	0.699	0.9(-1.7,3.5)	0.482	0.9(-1.6,3.4)	0.461
D-Dimer	Lag 0	0.7(-2.7,4.1)	0.669	0.0(-3.6,3.5)	0.997	0.5(-2.9,3.8)	0.772	1.1(-2.2,4.2)	0.509
	Lag 0-3	0.8(-2.8,4.2)	0.667	0.0(-3.7,3.5)	0.990	0.1(-3.4,3.6)	0.930	0.6(-2.7,3.8)	0.716
	Lag 0-6	1.2(-2.6,4.8)	0.537	0.3(-3.6,4.1)	0.859	0.5(-3.2,4.1)	0.776	0.7(-2.7,4.1)	0.656
	Lag 0-13	1.0(-3.1,5.0)	0.627	0.0(-4.3,4.2)	0.994	0.8(-3.3,4.8)	0.685	1.6(-2.3,5.3)	0.416
	Lag 0-27	1.2(-3.3,5.5)	0.601	-0.1(-4.8,4.4)	0.977	0.4(-4.1,4.8)	0.838	1.4(-2.7,5.5)	0.488

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.5 Difference in the levels of Vitamin D (VitD) and Triglycerides for 5°C decrease in outdoor mean temperature in the BRHS participants, during examinations (1998-2000)

		Model 1: Temperature		Model 2: Temperature + ILI		Model 3: Temperature + ILI + Age		Model 4: Temperature + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
Outcome	Lag for outdoor temperature	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Percent difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value
VitD	Lag 0	-6.2(-9.3,-3.2)	<0.001	-5.1(-8.2,-2.1)	0.001	-5.2(-8.3,-2.3)	0.001	-5.0(-8.0,-2.1)	0.001
	Lag 0-3	-10.1(-13.6,-6.7)	<0.001	-8.7(-12.2,-5.2)	<0.001	-8.8(-12.3,-5.3)	<0.001	-7.8(-11.2,-4.5)	<0.001
	Lag 0-6	-12.0(-15.9,-8.3)	<0.001	-10.4(-14.3,-6.6)	<0.001	-10.4(-14.3,-6.7)	<0.001	-10.6(-14.5,-6.9)	<0.001
	Lag 0-13	-18.4(-23.1,-13.8)	<0.001	-16.2(-21.2,-11.5)	<0.001	-16.5(-21.3,-11.7)	<0.001	-15.6(-20.4,-11.0)	<0.001
	Lag 0-27	-22.9(-28.3,-17.8)	<0.001	-20.7(-26.4,-15.2)	<0.001	-20.9(-26.6,-15.4)	<0.001	-20.1(-25.7,-14.8)	<0.001
Triglycerides	Lag 0	1.3(-1.0,3.6)	0.249	1.2(-1.2,3.6)	0.318	1.1(-1.3,3.5)	0.366	1.4(-0.8,3.6)	0.201
	Lag 0-3	1.1(-1.4,3.5)	0.372	0.9(-1.7,3.4)	0.473	0.9(-1.7,3.4)	0.499	0.7(-1.6,3.1)	0.527
	Lag 0-6	1.2(-1.4,3.8)	0.359	1.0(-1.7,3.7)	0.457	1.0(-1.8,3.7)	0.476	0.7(-1.9,3.2)	0.589
	Lag 0-13	1.6(-1.4,4.5)	0.289	1.4(-1.8,4.5)	0.379	1.2(-1.9,4.4)	0.433	0.9(-2.1,3.7)	0.553
	Lag 0-27	0.8(-2.5,4.1)	0.627	0.4(-3.1,3.9)	0.809	0.3(-3.3,3.8)	0.863	0.0(-3.3,3.2)	0.977

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.6 Difference in the levels of total, HDL, and LDL Cholesterol for 5°C decrease in outdoor mean temperature in the BRHS participants, during examinations (1998-2000)

		Model 1: Temperature		Model 2: Temperature + ILI		Model 3: Temperature + ILI + Age		Model 4: Temperature + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
	Lag for outdoor temperature	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value
Total-C, mmol/L	Lag 0	0.063(0.016,0.111)	0.009	0.069(0.019,0.118)	0.007	0.066(0.017,0.115)	0.009	0.064(0.014,0.113)	0.012
	Lag 0-3	0.048(-0.002,0.097)	0.059	0.052(0.000,0.103)	0.050	0.051(-0.001,0.103)	0.051	0.050(-0.001,0.101)	0.058
	Lag 0-6	0.044(-0.008,0.097)	0.097	0.048(-0.006,0.103)	0.085	0.048(-0.006,0.102)	0.085	0.045(-0.009,0.100)	0.103
	Lag 0-13	0.052(-0.006,0.109)	0.080	0.057(-0.004,0.118)	0.068	0.055(-0.006,0.115)	0.077	0.053(-0.008,0.113)	0.090
	Lag 0-27	0.034(-0.030,0.098)	0.298	0.038(-0.031,0.107)	0.276	0.036(-0.032,0.104)	0.295	0.035(-0.033,0.103)	0.313
HDL-C, mmol/L	Lag 0	0.002(-0.016,0.020)	0.825	0.003(-0.016,0.021)	0.780	0.003(-0.015,0.021)	0.750	0.000(-0.017,0.017)	0.997
	Lag 0-3	0.006(-0.014,0.025)	0.565	0.006(-0.013,0.026)	0.516	0.006(-0.013,0.026)	0.503	0.007(-0.011,0.026)	0.414
	Lag 0-6	0.004(-0.017,0.025)	0.682	0.005(-0.016,0.026)	0.632	0.006(-0.016,0.026)	0.620	0.008(-0.012,0.028)	0.431
	Lag 0-13	0.008(-0.016,0.033)	0.499	0.010(-0.015,0.035)	0.443	0.010(-0.014,0.036)	0.418	0.014(-0.009,0.038)	0.226
	Lag 0-27	0.012(-0.014,0.039)	0.378	0.014(-0.014,0.043)	0.308	0.015(-0.013,0.043)	0.294	0.019(-0.008,0.045)	0.166
LDL-C, mmol/L	Lag 0	0.053(0.007,0.098)	0.023	0.057(0.010,0.105)	0.016	0.056(0.009,0.103)	0.019	0.053(0.006,0.100)	0.027
	Lag 0-3	0.036(-0.012,0.083)	0.141	0.040(-0.010,0.088)	0.117	0.039(-0.010,0.088)	0.121	0.038(-0.010,0.087)	0.126
	Lag 0-6	0.033(-0.017,0.083)	0.195	0.037(-0.016,0.089)	0.166	0.036(-0.016,0.088)	0.169	0.035(-0.017,0.087)	0.187
	Lag 0-13	0.035(-0.021,0.091)	0.223	0.040(-0.020,0.099)	0.188	0.037(-0.021,0.097)	0.207	0.036(-0.022,0.095)	0.222
	Lag 0-27	0.023(-0.039,0.084)	0.461	0.027(-0.039,0.094)	0.412	0.026(-0.039,0.091)	0.432	0.026(-0.040,0.091)	0.448

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.7 Difference in the levels of FEV_1, FVC, and FEV_1/FVC ratio for 5°C decrease in outdoor mean temperature in the BRHS participants, during examinations (1998-2000)

		Model 1: Temperature		Model 2: Temperature + ILI		Model 3: Temperature + ILI + Age		Model 4: Temperature + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
	Lag for outdoor temperature	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value
FEV_1, L	Lag 0	0.024(-0.003,0.049)	0.074	0.026(-0.001,0.053)	0.066	0.019(-0.009,0.047)	0.188	0.012(-0.014,0.036)	0.379
	Lag 0-3	0.024(-0.003,0.050)	0.074	0.026(-0.001,0.054)	0.064	0.022(-0.006,0.052)	0.126	0.017(-0.009,0.042)	0.219
	Lag 0-6	0.024(-0.004,0.052)	0.088	0.026(-0.003,0.056)	0.079	0.023(-0.007,0.053)	0.142	0.015(-0.013,0.043)	0.282
	Lag 0-13	0.023(-0.007,0.053)	0.141	0.025(-0.007,0.057)	0.133	0.016(-0.019,0.050)	0.373	0.004(-0.026,0.036)	0.775
	Lag 0-27	0.018(-0.014,0.051)	0.279	0.020(-0.016,0.056)	0.271	0.009(-0.029,0.048)	0.626	-0.004(-0.038,0.031)	0.827
FVC, L	Lag 0	0.077(0.036,0.117)	<0.001	0.065(0.023,0.107)	0.002	0.049(0.007,0.089)	0.020	0.039(-0.001,0.078)	0.058
	Lag 0-3	0.073(0.029,0.117)	0.001	0.059(0.014,0.103)	0.010	0.049(0.004,0.094)	0.032	0.043(0.001,0.087)	0.047
	Lag 0-6	0.074(0.026,0.122)	0.003	0.058(0.009,0.107)	0.019	0.049(0.001,0.098)	0.047	0.043(-0.004,0.090)	0.072
	Lag 0-13	0.085(0.029,0.141)	0.003	0.065(0.008,0.122)	0.026	0.044(-0.013,0.102)	0.128	0.034(-0.023,0.089)	0.245
	Lag 0-27	0.075(0.014,0.136)	0.015	0.048(-0.016,0.111)	0.140	0.023(-0.042,0.088)	0.485	0.006(-0.058,0.069)	0.866
FEV_1/FVC%	Lag 0	-0.6(-1.2,0.0)	0.061	-0.5(-1.1,0.1)	0.103	-0.6(-1.2,0.1)	0.072	-0.6(-1.2,0.0)	0.063
	Lag 0-3	-0.5(-1.2,0.2)	0.157	-0.4(-1.1,0.3)	0.252	-0.4(-1.1,0.3)	0.214	-0.5(-1.2,0.2)	0.171
	Lag 0-6	-0.6(-1.3,0.2)	0.133	-0.5(-1.2,0.3)	0.222	-0.5(-1.3,0.3)	0.192	-0.6(-1.3,0.2)	0.136
	Lag 0-13	-0.7(-1.6,0.2)	0.141	-0.5(-1.5,0.4)	0.263	-0.6(-1.5,0.3)	0.196	-0.7(-1.7,0.1)	0.101
	Lag 0-27	-0.6(-1.6,0.5)	0.287	-0.3(-1.4,0.7)	0.549	-0.4(-1.4,0.6)	0.452	-0.6(-1.6,0.5)	0.274

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.8 Difference in the levels of SBP and DBP for 5°C decrease in outdoor mean temperature in the BRHS participants, during examinations (1998-2000)

		Model 1: Temperature		Model 2: Temperature + ILI		Model 3: Temperature + ILI + Age		Model 4: Temperature + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
	Lag for outdoor temperature	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value	Absolute difference (95%CI) in outcome levels per 5°C decrease in outdoor temperature	p-value
SBP sitting, mm Hg	Lag 0	1.191(0.259,2.123)	0.012	1.181(0.191,2.171)	0.020	1.262(0.274,2.248)	0.012	1.220(0.231,2.210)	0.016
	Lag 0-3	0.784(-0.184,1.752)	0.112	0.717(-0.316,1.749)	0.174	0.745(-0.283,1.772)	0.155	0.692(-0.338,1.722)	0.188
	Lag 0-6	0.734(-0.296,1.764)	0.163	0.650(-0.445,1.746)	0.245	0.687(-0.402,1.776)	0.216	0.594(-0.499,1.686)	0.287
	Lag 0-13	0.394(-0.758,1.547)	0.503	0.243(-0.988,1.474)	0.699	0.389(-0.832,1.609)	0.532	0.271(-0.954,1.496)	0.665
	Lag 0-27	0.413(-0.826,1.651)	0.514	0.228(-1.121,1.579)	0.740	0.333(-1.009,1.674)	0.627	0.233(-1.113,1.580)	0.734
DBP sitting, mm Hg	Lag 0	0.499(0.060,0.938)	0.026	0.503(0.039,0.968)	0.034	0.493(0.030,0.957)	0.037	0.455(-0.001,0.911)	0.051
	Lag 0-3	0.360(-0.093,0.814)	0.119	0.346(-0.137,0.829)	0.160	0.342(-0.140,0.824)	0.164	0.303(-0.170,0.776)	0.210
	Lag 0-6	0.398(-0.081,0.877)	0.103	0.385(-0.124,0.893)	0.138	0.381(-0.127,0.887)	0.141	0.319(-0.180,0.817)	0.210
	Lag 0-13	0.303(-0.227,0.833)	0.263	0.270(-0.297,0.838)	0.351	0.253(-0.313,0.819)	0.381	0.176(-0.379,0.732)	0.533
	Lag 0-27	0.335(-0.236,0.905)	0.251	0.302(-0.321,0.925)	0.342	0.289(-0.331,0.911)	0.360	0.216(-0.394,0.825)	0.488

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.9 Associations between England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons (mean, SD) and the levels of CVD risk factors in the BRHS participants, during examinations (1998-2000).

Note: Associations are reported as percent change in the CVD risk levels per 1 SD increase in ILI

	Model 1: Unadjusted		Model 2: adjusted for mean temperature at lag 0		Model 3: adjusted for mean temperature at lag 0 + Age + other CVD risk factors (fully adjusted model) [1]	
	Percent difference (95%CI)	p-value	Percent difference (95%CI)	p-value	Percent difference (95%CI)	p-value
CRP	3.5(-0.6,7.8)	0.088	3.5(-0.6,7.8)	0.097	2.5(-0.6,5.8)	0.114
IL-6	0.9(-2.5,4.5)	0.622	0.9(-2.5,4.5)	0.629	0.3(-2.8,3.5)	0.799
Fibrinogen	0.3(-0.9,1.3)	0.670	0.3(-0.9,1.3)	0.685	0.3(-0.9,1.3)	0.698
PV	0.3(0.0,0.3)	0.119	0.3(0.0,0.3)	0.117	0.3(0.0,0.3)	0.116
t-PA	-0.6(-2.8,1.9)	0.669	-0.6(-2.8,1.9)	0.658	-0.6(-2.8,1.3)	0.505
vWF	1.3(-0.6,2.8)	0.202	0.9(-0.6,2.8)	0.218	1.3(-0.3,2.8)	0.163
D-dimer	2.2(-0.9,5.1)	0.179	1.9(-0.9,4.8)	0.188	2.2(-0.6,4.8)	0.135
VitD	-7.8(-10.9,-4.9)	<0.001	-7.8(-10.6,-4.9)	<0.001	-7.5(-10.3,-4.9)	<0.001
Triglycerides	0.9(-1.5,3.2)	0.472	0.9(-1.5,3.2)	0.469	0.6(-1.5,2.8)	0.556
	Absolute difference (95%CI)	p-value	Absolute difference (95%CI)	p-value	Absolute difference (95%CI)	p-value
Total cholesterol, mmol/L	0.003(-0.044,0.047)	0.926	0.003(-0.044,0.047)	0.921	0.000(-0.044,0.047)	0.977
HDL-cholesterol, mmol/L	-0.003(-0.019,0.016)	0.820	-0.003(-0.019,0.016)	0.820	0.000(-0.019,0.016)	0.966
LDL-cholesterol, mmol/L	0.000(-0.044,0.044)	0.955	0.000(-0.044,0.041)	0.956	-0.003(-0.047,0.041)	0.897
FEV_1, L	0.003(-0.022,0.031)	0.771	0.006(-0.022,0.034)	0.714	0.003(-0.019,0.028)	0.716
FVC, L	0.084(0.041,0.128)	<0.001	0.081(0.037,0.125)	<0.001	0.078(0.034,0.122)	0.001
FEV_1/FVC, %	-1.2(-2.0,-0.4)	0.004	-1.2(-2.0,-0.4)	0.003	-1.3(-2.0,-0.5)	0.001
SBP sitting, mm Hg	0.393(-0.487,1.270)	0.382	0.349(-0.521,1.223)	0.432	0.284(-0.587,1.158)	0.522
DBP sitting, mm Hg	0.144(-0.265,0.555)	0.487	0.150(-0.259,0.558)	0.472	0.125(-0.271,0.524)	0.536

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.10 Summary table of associations between mean temperature at different lags and CVD risk factors, and between ILI and CVD risk factors

The summary was derived from Table 7.4 to Table 7.10 previously reported (see Model 4, fully adjusted). The legend is reported below:
The arrow ↑ indicates an increase in CVD risk factor levels associated with a decrease in mean temperature (or increase in ILI)
The arrow ↓ indicates a decrease in CVD risk factor levels associated with a decrease in mean temperature (or increase in ILI)
The blank cell indicates a non-significant association between the CVD risk factor and mean temperature (or ILI)

	Decrease in temperature at:				
	Lag 0	Lag 0-3	Lag 0-6	Lag 0-13	Lag 0-27
CRP	↑	↑		↑	↑
IL6			↑	↑	↑
Fibrinogen					
PV	↑	↑	↑	↑	
t-PA	↑	↑	↑	↑	↑
vWF					
D-Dimer					
Triglycerides					
Total-C	↑				
LDL-C	↑				
HDL-C					
FEV1					
FVC		↑			
FEV1/FVC %					
SBP	↑				
DBP					
VitD	↓	↓	↓	↓	↓

Increase in ILI	
CRP	
IL6	
Fibrinogen	
PV	
t-PA	
vWF	
D-Dimer	
Triglycerides	
Total-C	
LDL-C	
HDL-C	
FEV1	
FVC	↑
FEV1/FVC %	↓
SBP	
DBP	
VitD	↓

Table 7.11 The change in the levels of Vitamin D for 1 standard deviation (3.5 hours) decrease in sunshine duration in the BRHS participants, during examinations (1998-2000)

		Model 1: sunshine duration		Model 2: sunshine duration + ILI		Model 3: sunshine duration + ILI + Age		Model 4: sunshine duration + ILI + Age + other CVD risk factors (fully adjusted model) [1]	
	Lag for outdoor temperature	Absolute difference (95%CI) in outcome levels	p-value	Absolute difference (95%CI) in outcome levels	p-value	Absolute difference (95%CI) in outcome levels	p-value	Absolute difference (95%CI) in outcome levels	p-value
Vitamin D	Lag 0	-0.2(-1.9,1.5)	0.789	0.2(-1.4,1.9)	0.765	0.2(-1.5,1.9)	0.7903	0.2(-1.5,1.8)	0.815
	Lag 0-3	-4.1(-7.2,-1.1)	0.007	-3.1(-6.2,-0.1)	0.043	-3.1(-6.2,-0.1)	0.0430	-3.4(-6.4,-0.5)	0.021
	Lag 0-6	-9.2(-13.7,-4.9)	<0.001	-7.8(-12.2,-3.5)	<0.001	-7.9(-12.2,-3.6)	<0.001	-8.0(-12.2,-3.9)	<0.001
	Lag 0-13	-15.3(-22.6,-8.6)	<0.001	-12.0(-19.2,-5.3)	<0.001	-12.3(-19.5,-5.6)	<0.001	-12.4(-19.4,-5.8)	<0.001
	Lag 0-27	-26.2(-35.9,-17.2)	<0.001	-22.0(-31.7,-13.0)	<0.001	-22.3(-32.1,-13.3)	<0.001	-22.2(-31.7,-13.3)	<0.001

[1] Model additionally adjusted for Body Mass Index (BMI), social class, smoking, marital status, physical activity score, and time of day

Table 7.12 Total variance explained (Full adjusted models), and variance explained by temperature at different lags in CVD risk factors in the BRHS, during the study period (1998-2000)

Included in this table are variance explained when an association of temperature with CVD risk factors was found significant in Model 4, Tables 7.3-7.9)

BRHS	Total variance explained al lag 0 (%)	Variance explained by temperature at lag 0 (%)	Variance explained by temperature at lag 0-6 (%)	Variance explained by temperature at lag 0-13 (%)	Variance explained by temperature at lag 0-27 (%)
CRP	14.14	0.16	0.14	0.25	0.25
IL-6	17.70	0.03	0.11	0.25	0.26
t-PA	24.47	0.08	0.11	0.25	0.31
PV	8.54	0.31	0.12	0.18	0.11
Vitamin D	21.00	5.21	8.38	10.07	11.06
LDL-cholesterol	3.23	0.09	0.22	0.10	0.01
Total cholesterol	3.30	0.13	0.21	0.13	0.01
SBP sitting	6.09	0.27	0.10	0.00	0.00

Figure 7.1 Unadjusted geometric means (95% CI) plotted on log scale, by quintiles (Q) of mean temperature, of measurement for C-Reactive Protein (CRP), Interleukin-6 (IL-6), Fibrinogen, and Plasma Viscosity (PV) measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

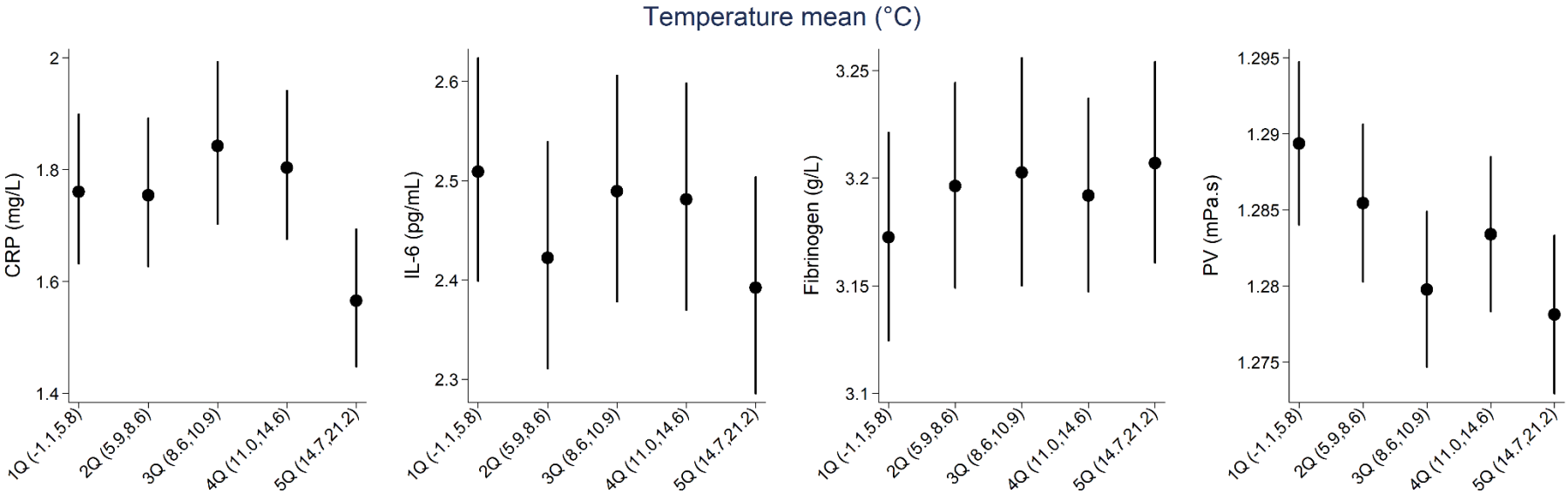


Figure 7.2 Unadjusted geometric means (95% CI) plotted on log scale, by quintiles (Q) of mean temperature, in von Willebrand Factor (vWF), D-Dimer, Tissue Plasminogen Activator (t-PA), and Vitamin D levels measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

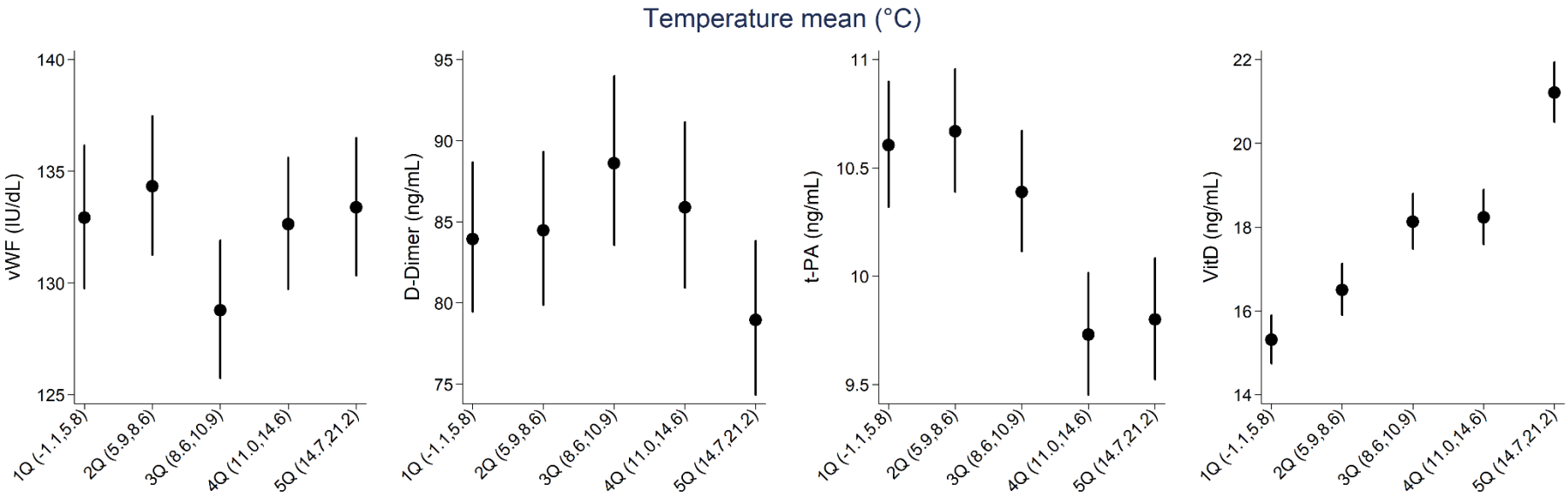


Figure 7.3 Unadjusted geometric means (95% CI), by quintiles (Q) of mean temperature, of lipids levels measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

Note: Triglycerides levels are plotted on log scale

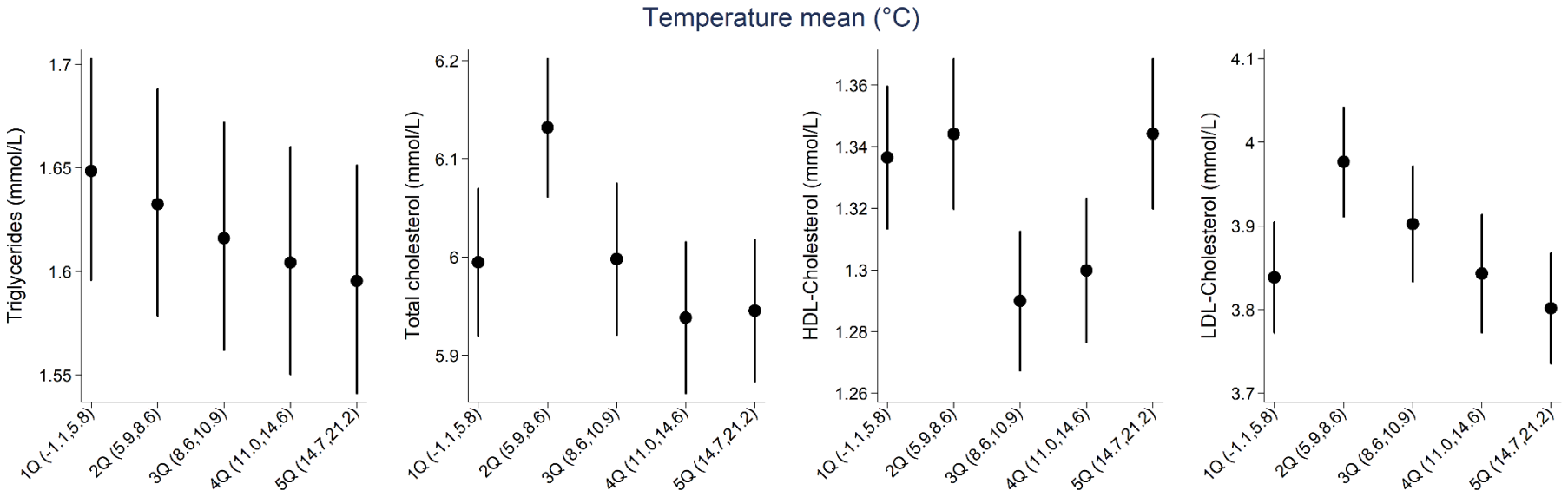


Figure 7.4 Unadjusted geometric means (95% CI), by quintiles (Q) of mean temperature, of measurement for lung function variables measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

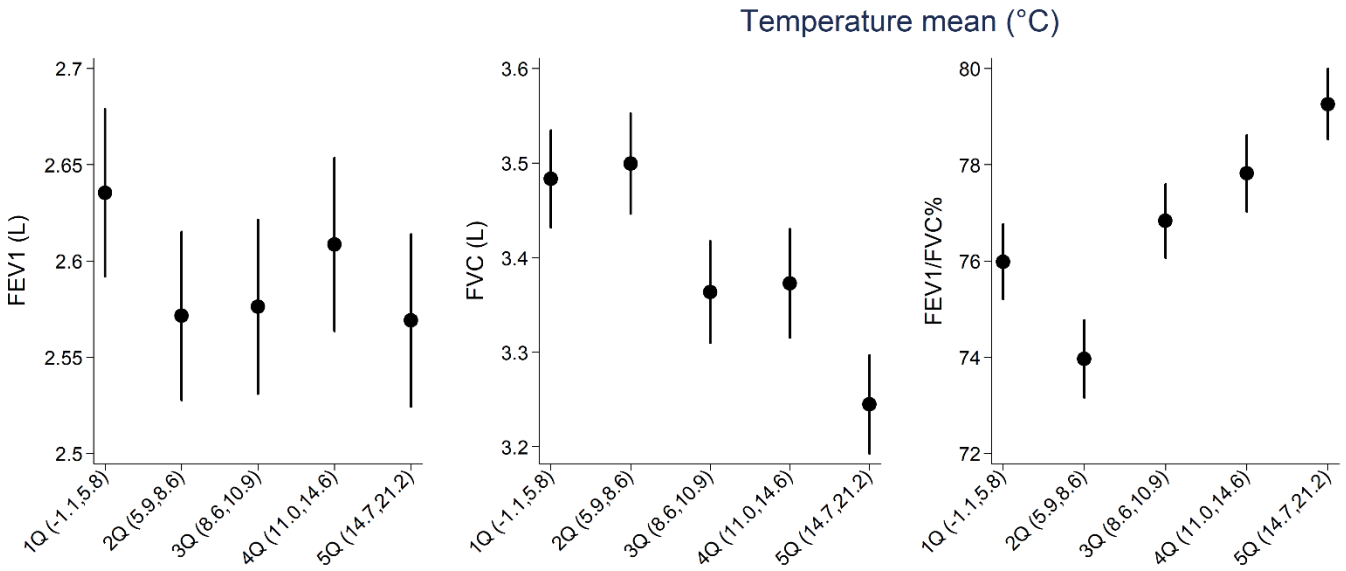


Figure 7.5 Unadjusted geometric means (95% CI), by quintiles (Q) of mean temperature, of measurement for blood pressure variables measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

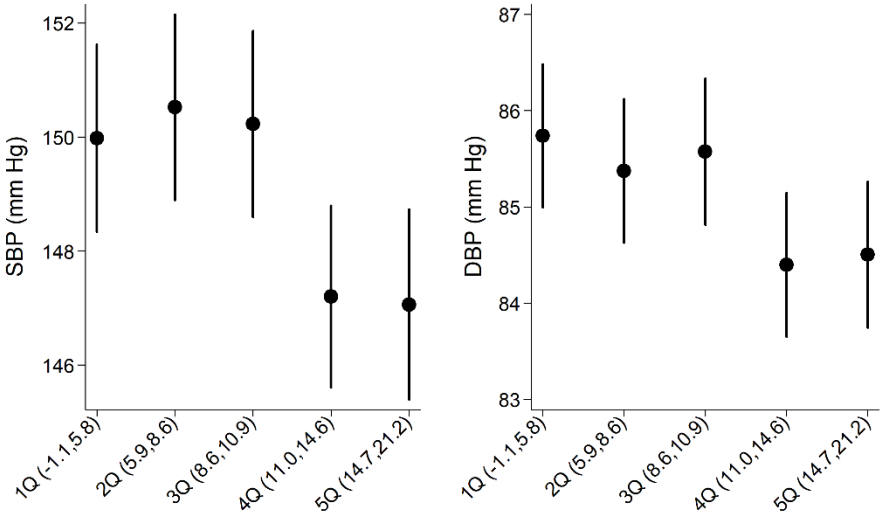


Figure 7.6 Unadjusted geometric means (95% CI) plotted on a log scale, by quintiles (Q) of England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons, of measurement for C-Reactive Protein (CRP), Interleukin-6 (IL-6), Fibrinogen, and Plasma Viscosity (PV) measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

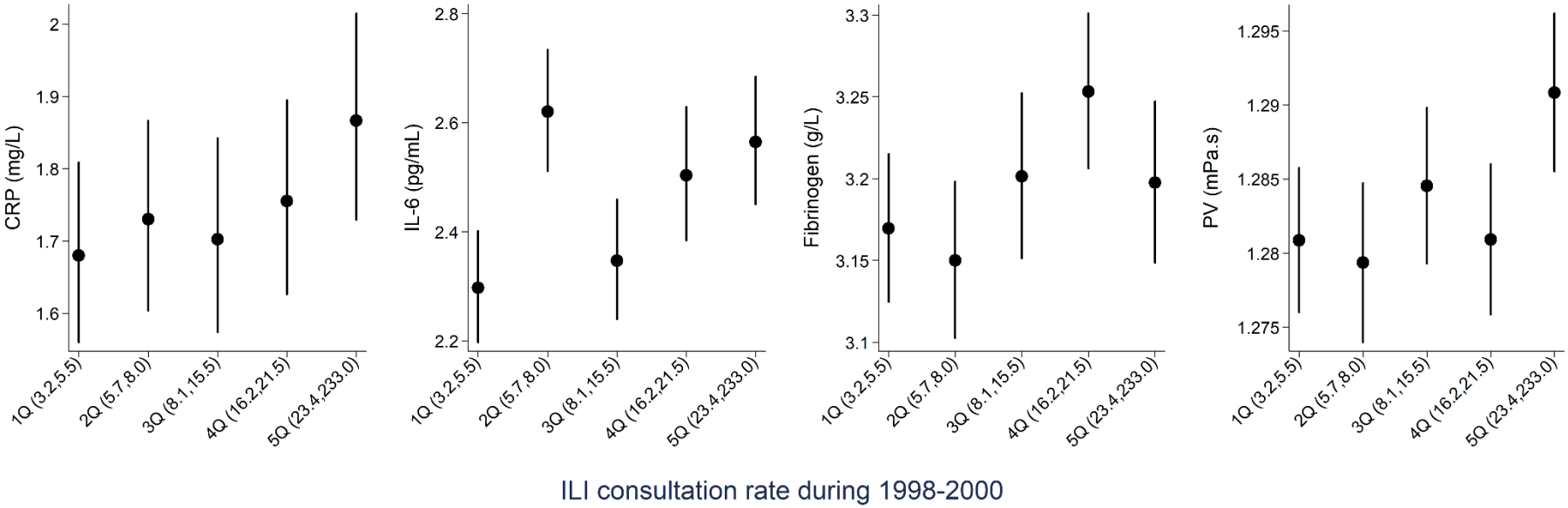


Figure 7.7 Unadjusted geometric means (95% CI) plotted on a log scale, by quintiles (Q) of England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons, in von Willebrand Factor (vWF), D-Dimer, Tissue Plasminogen Activator (t-PA), and Vitamin D levels measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

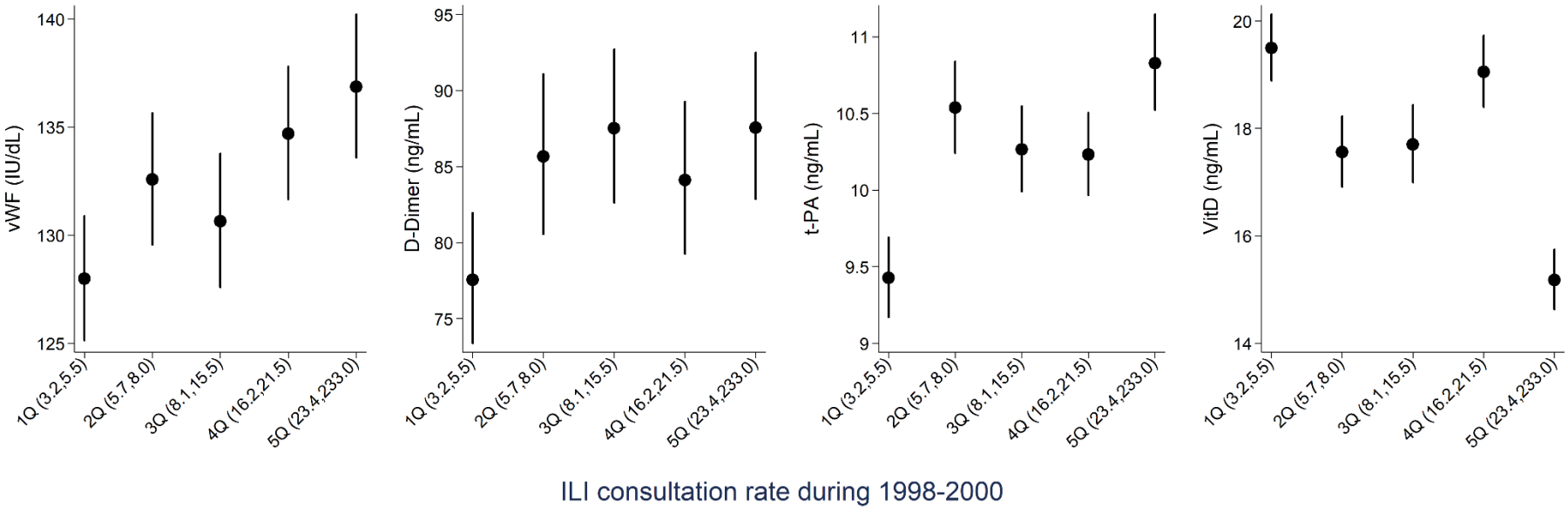


Figure 7.8 Unadjusted geometric means (95% CI), by quintiles (Q) of England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons, of lipids levels measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

Note: Triglycerides levels are plotted on log scale

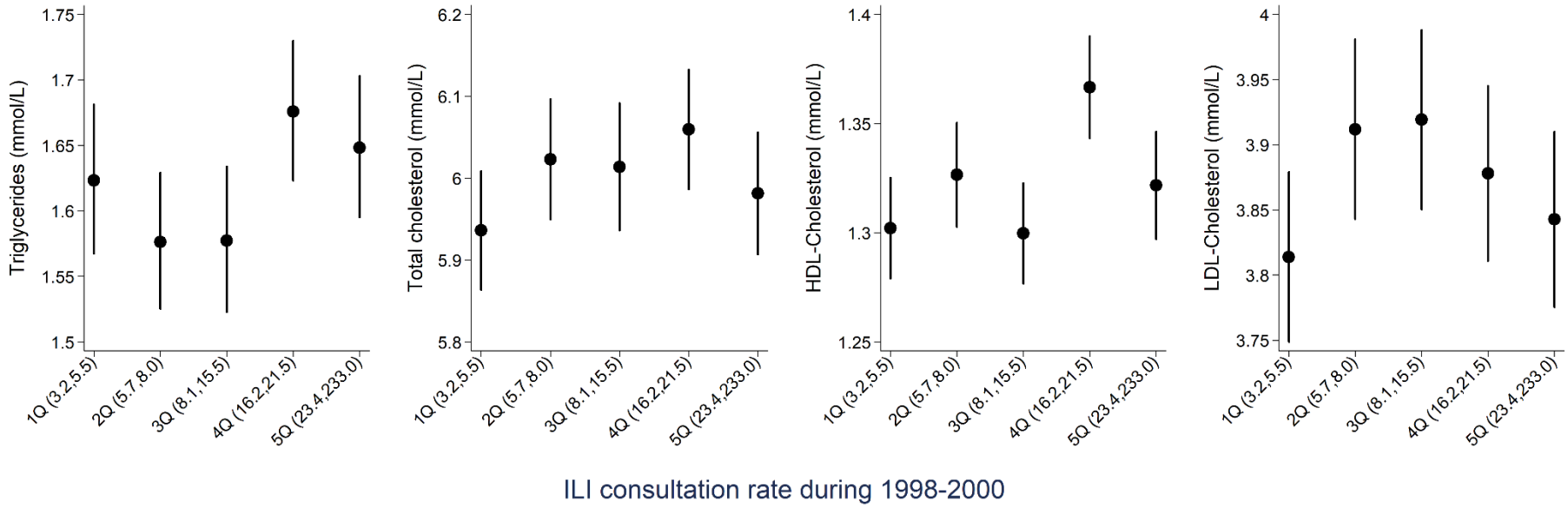


Figure 7.9 Unadjusted geometric means (95% CI), by quintiles (Q) of England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons, of measurement for lung function measured on one occasion in BRHS men aged 60-79 during the years 1998-2000

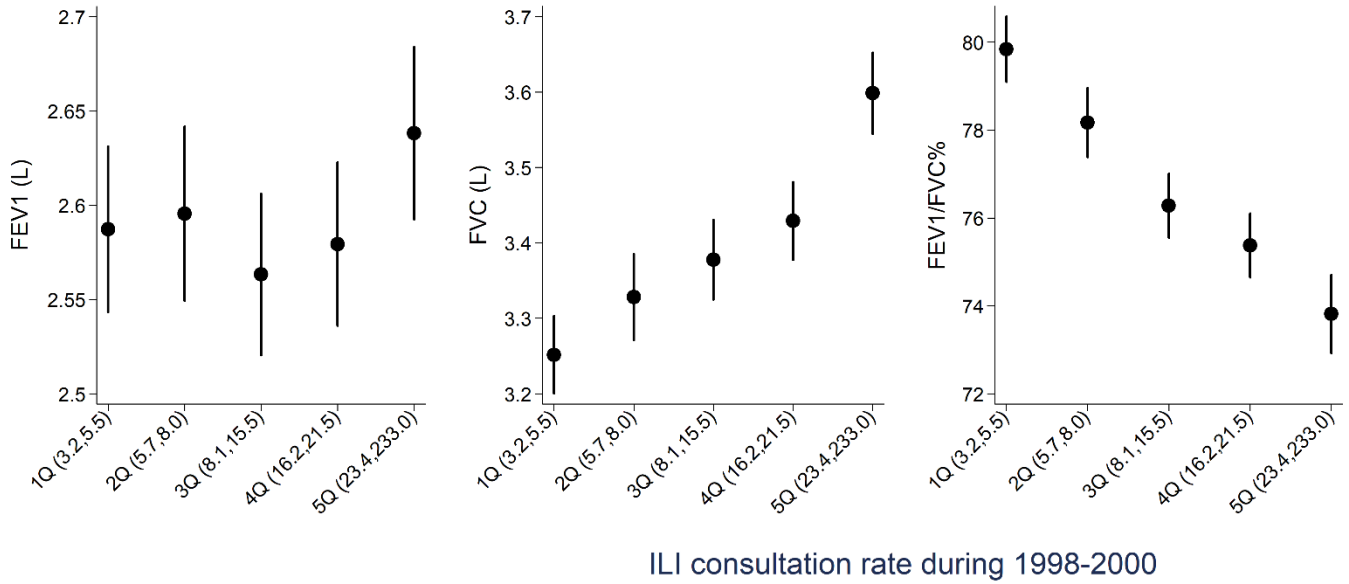
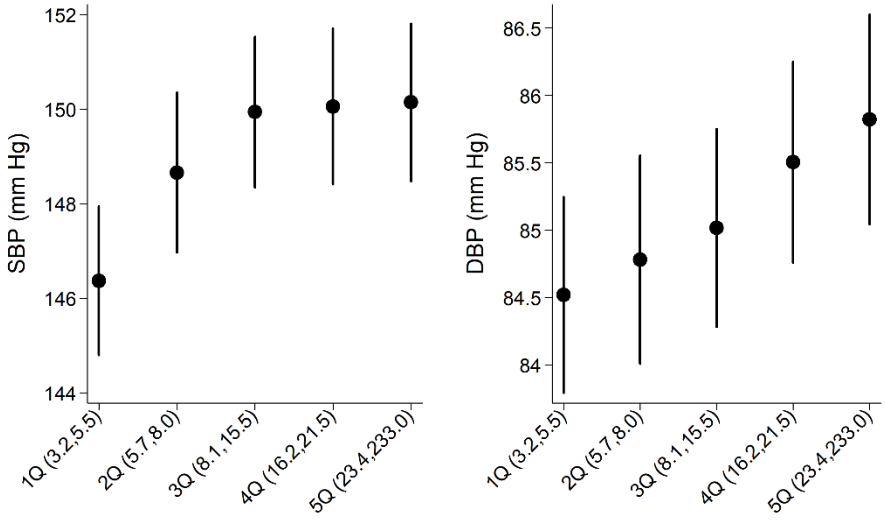


Figure 7.10 Unadjusted geometric means (95% CI), by quintiles (Q) of England/Wales Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons, of measurement for blood pressure variables measured on one occasion in BRHS men aged 60-79 during the years 1998-2000



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Chapter 8 ASSOCIATIONS OF OUTDOOR TEMPERATURE WITH MORTALITY

8.1 Summary

Outdoor temperature is widely recognised as an important determinant of seasonal variation in all-cause mortality. Worldwide, most of the temperature-related mortality burden has been attributable to low temperatures. Mean outdoor temperature is the most common proxy of temperature exposure used in epidemiological studies; previous studies in 15 European cities and 3 countries including Britain, reported estimates of associations between low temperatures and increased mortality. The vast majority of the previous studies are not conducted using individual level data; in such studies, the number of deaths is aggregated by day, and it cannot be assumed that relationships existing at aggregated level of analysis necessarily demonstrate the same strength at the individual level. Therefore population-based cohort studies such as the BRHS are needed to understand how the effect of temperature might operate at the individual level. In this Chapter, I hypothesised that lower outdoor temperatures (main exposure variable and reflective of season) increase mortality risks from all-causes, CVD mortality and respiratory mortality (outcomes) in the British Regional Heart Study (BRHS). To test this hypothesis, a time-varying covariates survival analysis was performed during a follow-up period of 14.9 years; over this period, outdoor mean temperatures were collected on daily basis and linked to the BRHS men's individual data via date and post code of residence. This analysis included 4252 men from the BRHS, aged 60-79 years at baseline (1998-2000). The men attended two examinations (at baseline and in 2010-2012) which included blood sampling and completion of a general lifestyle survey. Data from both blood measurements and surveys were included in the time-varying covariates survival analysis. The BRHS men were followed up from February 1998 to October 2014 for all-cause, cardiovascular (CVD), coronary heart disease (CHD), stroke, and respiratory mortality.

Overall, lower temperatures were associated with increased mortality risk. This peak at lower temperatures was driven by increased CHD and respiratory mortality. The additional adjustment for individual risk factors (potential mediators) fitted in the model one at a time (physical activity, SBP, IL-6, LDL-Cholesterol, and lung function) slightly reduced the

magnitude of the association of temperature with mortality by at most 1%. After adjustment for a potential confounding seasonal factor (a proxy of exposure to influenza measured by Influenza-like illness weekly consultation rate in primary care at national level) and potential effect modifiers of the temperature-mortality relationship such as age, social class, body mass index, smoking, marital status, and use of medication, a decrease of 5°C in outdoor temperatures increased CHD mortality by 12.8% (Hazard Ratio (HR) = 1.128; 95% CI 1.041-1.207; p=0.005), respiratory mortality by 11.0% (HR=1.110, 95%CI 1.002-1.207, p=0.047), and all-cause mortality by 5.9% (HR=1.059; 95%CI 1.016-1.100; p=0.008). Lower temperatures also increased CVD mortality by 10.4% (HR=1.104; 95%CI 1.035-1.168, p=0.004), although a specific association of temperature with stroke mortality was not found in this study (HR=1.047; 95%CI 0.886; 1.199, p=0.585). Our findings also suggested a non-linear relationship of temperature with respiratory mortality only: a decrease in temperature of 5°C below 19.3°C (97.5^{th} percentile of temperature) increased mortality (HR=1.135; 95%CI 1.032-1.228, p=0.011), while an increase in temperature of 5°C above 19.3°C also increased mortality (HR=6.720; 95%CI 2.592-21.553; p=0.008). Overall, a better protection against low temperatures, typically recorded in winter, could help in reducing mortality risks, especially for CVD and respiratory mortality.

8.2 Introduction

Outdoor temperature is widely recognised as an important determinant of seasonal variation in all-cause mortality (30). Mean outdoor temperature is the most common measure of temperature exposure used in epidemiological studies (35, 130). The largest study to date, which measured daily number of deaths in 384 cities worldwide, demonstrated that most of the temperature-attributable deaths were associated with cold temperatures rather than with heat. This study estimated that 7.29% of total mortality was attributable to cold temperatures, while only 0.42% to heat, although there were substantial differences between regions and countries (35). It is also known that the association of low temperature with increased mortality have been reported to last up to 2, 3 or 4 weeks both in Europe (125) and the US (326). Overall, studies conducted in European cities (124-126) and countries (127-129), including Britain, have provided evidence for the association between low temperatures and increased mortality (29, 124-130). For example, in the UK day-to-day changes in outdoor temperature during winter are associated with all-cause mortality (+0.38 daily cases per million people per 1°C

decrease in temperature) (130). Typically, the higher death rate at lower temperatures is generally attributed to either a breakdown of the cardiovascular or respiratory systems (29). Previous studies used different definitions of risk measures and used various designs and analytical methods, which made the comparison of temperature-related mortality risks between studies difficult (29, 35, 38, 124-129, 231, 327). Despite such studies including a very large number of fatal events in their analysis (e.g. about 74 million deaths in the largest study published to date (35)), the major feature is the aggregation of the number of deaths by day (which means the unit of observation is the day rather than the individual). However, it cannot necessarily be assumed that relationships existing at the aggregated level of analysis necessarily demonstrate the same strength at the individual level (36). Population-based cohort studies, such as the BRHS are able to investigate relationships at the individual level. Also, it is not well understood which people are more susceptible to cold temperatures, because findings on factors associated with modification of the temperature-related risk of death are sparse, inconsistent, and have not tested the possible interaction of temperature with a comprehensive range of risk factors in statistical analysis (37, 109, 129, 328-330). Routine data can usually be disaggregated by sex and age groups, but studies with individual data permit the investigation of a much wider range of effect modifying variables; to understand this is important as it may inform CVD prevention strategies aiming to protect more susceptible individuals. Only epidemiological studies which make use of data collected at an individual level can test such hypothesis; a further prerequisite of such studies is that the linkage of (i) individual CVD risk factor measures to (ii) meteorological factors and (iii) mortality outcomes is ascertained for these same individuals. As of today, such a complex linkage has not been reported in previous studies of CVD.

The main objective of this chapter is therefore to estimate associations between mean outdoor temperature (main variable to represent seasonal effects) and mortality (outcome) at individual level in the BRHS, and whether such associations differ according to different categories of individual risk factors. I would expect lower temperature to increase all-cause mortality, CVD mortality and respiratory mortality. As seasonal factors (e.g. influenza activity) other than weather may also contribute to increased mortality in winter (327), associations with mortality outcomes are reported after mutual adjustment of temperature and Influenza-like illness (ILI) weekly consultation rate in primary care..

8.3 Objectives

To examine the associations of outdoor mean temperature with the risk of CVD and all-cause mortality in older age (60-79 years). The main research questions of this Chapter are:

1) Do variations in mean outdoor temperature relate to variations in coronary heart disease (CHD), stroke, CVD, respiratory, and all-cause mortality in older British men from the BRHS?
2) Is the temperature–mortality relationship confounded by seasonal influenza trends?
3) Is the proxy for seasonal influenza used in this study (ILI consultation rate) associated with mortality?
4) Is the association of temperature with mortality non-linear? If so, for which causes of death is non-linearity found?
5) Is the magnitude of association of temperature-mortality different after adjustment for potential mediators such as physical activity, blood pressure, LDL-cholesterol and IL-6?
6) Is the association of temperature with mortality modified by different levels of individual risk factors (e.g. age) ?

8.4 Methods and data collection

8.4.1 Participants

Data used in this chapter are based on individual risk factors collected over time and starting from the 20 year re-examination of the British Regional Heart Study participants in 1998-2000 (aged 60-79 years) and in 2010-12 (see also Chapter 3). In 1998-2000, 4252 men (77% of survivors) completed a questionnaire answering questions on their lifestyle and medical history, attended a physical examination and provided a fasting blood sample. In 2010-12, 1722 surviving participants repeated the examination and completed the same questionnaire.

8.4.2 Risk factors

As accelerometers were used in the BRHS starting from 2010, we do not have physical activity objectively measured in 1998-2000. Therefore, using only self-reported physical activity in this chapter seemed a sensitive approach for two reasons: (i) consistency in physical activity

assessment methods at both follow-ups, and (ii) good accuracy of the BRHS physical activity questionnaire in 2010-12 when compared against accelerometer measured physical activity (233). Paragraph 8.5.5 will explain how such data were fitted in time varying covariate models. Self-reported physical activity was classified into six groups based on intensity and frequency of exercise (inactive; occasional; light; moderate; moderately vigorous and vigorous).

Alcohol intake was classified into three groups based on the number and frequency of alcoholic drinks consumed per week (none; occasional/light; moderate/heavy). Marital status was categorised as married vs not; and smoking status as current vs not. Lipid-regulating drug use (331), was classified by using the British National Formulary (BNF) medication (code 2.12, Cardiovascular System, Lipid-Regulating Drugs). At the 20 year examination in 1998-2000,, men also reported if their house was centrally heated vs not. As described in detail in Chapter 3 (paragraph 3.2.4.2), at follow-up year 20 and 32 measures of body mass index (BMI), systolic blood pressure (SBP), and lung function were assessed by physical examination, while plasma concentrations of LDL-cholesterol levels, and interleukin 6 [IL-6]) were measured from fasting blood samples. As described in Chapter 3, (paragraph 3.4.5), social class was measured using the baseline questionnaire in 1978-80. In summary, social class was based on the longest held occupation coded using the Registrar General's occupational classification and participants were classified as manual, non-manual or armed forces (HMF).

8.4.3 Follow-up and mortality

Information on the date and cause of death was collected through the National Health Service Central Register (death certificates coded using International Classification of Diseases, ninth revision [ICD-9]). Five outcomes were examined in this chapter were: Major CHD/MI deaths (ICD9 codes 410-414.9), Stroke deaths (ICD9 codes 430 – 438.9), CVD mortality (ICD-9 codes 390-459), respiratory mortality (ICD9 codes 460-519.9) and all-cause mortality. In the time-varying survival analysis (see methods section of this Chapter), participants were prospectively followed for mortality from baseline (date of examination at follow-up year 20) to date of death, or to the censoring date (31/10/2014) if still alive. For this chapter, 8 out of 4252 men attending the physical and blood examination in 1998-2000 and who later died outside the UK were excluded, as outdoor temperature data were collected in the UK only.

8.4.4 Outdoor temperature

As specified in Chapter 3, paragraph 3.3.1, daily mean outdoor temperature was provided by the UK Meteorological Office and calculated as the average of maximum and minimum temperatures during the study period. In this Chapter, we focused our investigation on mean temperature as primary determinant as it has been associated with mortality in previous studies (see introduction of Chapter 3 paragraph 3.3.1). For the entire follow-up period, exposure to daily outdoor mean temperature from the closest weather station to the town of residence of BRHS men was collected and linked to the BRHS men via postcode of residence registered at follow-up year 20. I did this because men's geographical mobility was very limited: for example, a very high percentage (78.7%, 1689 men out of 2147) did not change postcode within town between follow-up year 20 and 32. Also, most surviving men who did change post code of residence continued to live in the same town, so the proportion remaining in the same town was 95.7% (2055 out of 2147). The remaining 4.3% (92 out of 2147) of men who changed postcode and town of residence over time, the majority (76 out of 92) remained in the original town up to 2005, which is about half of the time of the follow-up period of this study.

8.4.5 Seasonal influenza

Seasonal factors (e.g. influenza virus infection) other than weather may also contribute to increase CVD mortality (332) and all-cause mortality in winter (327). Influenza-Like illness (ILI) weekly consultation rate per 100,000 population admitted to General Practice is generally used in ecological studies as proxy of seasonal trends of influenza viruses (32, 279, 280, 333); ILI was extensively described and already used in in Chapter 5 (paragraph 5.4.4) and Chapter 7 (paragraph 7.4.4) to assess whether temperature-related associations with the outcomes were confounded by ILI. The inclusion of ILI in statistical analysis is preferred to the use of generic (e.g. fixed or non-illness specific) proxy of season, such as month or trigonometric functions of day of the year (see subsequent paragraph 8.5.6 entitled "Controlling for influenza and long-term seasonal trends") (202, 279). While temperature and ILI associations with mortality have a plausible epidemiological link (see Introduction section of this Chapter), generic proxies of season cannot be clearly interpreted; they can potentially capture a seasonal trend, but this does not enhance our understanding of which biological pathways are relevant to seasonal variations in CVD, and risk over-adjusting the effect of the exposure of interest (temperature).

In this Chapter, ILI data for England and Wales were used. ILI rate was not available prior to 1999, while BRHS data collection in this study started from 1998. Therefore, in this Chapter ILI weekly consultation rates during the year 1998 were estimated using the weekly average of the period 1999-2014.

8.5 Statistical methods

8.5.1 Participants

BRHS participants' characteristics collected during 1998-2000 and 2010-2012 were summarised using mean and SD for continuous variables and frequencies (number and percentages) for categorical variables, using two different approaches: (i) Comparing participants' characteristics of the 4252 men who attended the examination in 1998-2000 with 1593 who attended in 2010-2, (ii) comparing characteristics between examinations only for those present at both follow-up examinations. This would help in understanding how such individual factors vary over time.

8.5.2 Follow-up and mortality

The median follow-up period and interquartile range for all-cause mortality were calculated in years. The total number of deaths during follow-up was calculated and also subdivided by cause of death [CHD, stroke, and total CVD deaths (= CHD + stroke + other CVD deaths)], and deaths from respiratory causes.

8.5.3 Outdoor temperature

Daily temperature data were plotted against day of the year to offer an overview of temperature distribution during the study period. Also, quintiles of outdoor temperatures were calculated using all temperature data during the follow-up period (daily outdoor temperatures recorded in the 35 weather stations used in this study). Then, numbers and percentage of deaths (total and by cause of death) were summarised by quintiles of outdoor temperature.

8.5.4 Seasonal influenza

Over the average year, the mean and SD of weekly ILI consultation rate per 100,000 persons during the study period were calculated. Weekly ILI rates were also plotted against outdoor temperatures during the study period to offer an overview of the two different seasonal trends.

8.5.5 Time varying covariate survival models

The Cox proportional hazards model is widely used to estimate the probability of having an event when time to a binary event is the main outcome of interest (252), as described in Chapter 3 (paragraph 3.5.3). In the Cox model the survival time for each participant is calculated; this is the time from a predetermined start point - e.g. entry into the study - until the occurrence of the event of interest. In event-time analysis, continuous time-dependent covariates can be used; in this study the main time-dependent covariate of interest is outdoor mean temperature recorded every day over the follow-up period and linked with each of the BRHS participants by date and post-code of residence recorded at baseline (1998-2000). Therefore, the data base used in this study assumed a longitudinal form: for each day prior to study exit the BRHS men were exposed to outdoor temperature for that day, and thus one record was generated in the data base.

Differently from temperature, other time-dependent covariates (see paragraph 8.4.1) were not collected on a daily basis. To understand how those covariates were stored in the data base a figure has been presented (Figure 8.1). For example, in Figure 8.1 and for participant n.1 and n.3, individual covariates' initially assumed the value recorded in 1998-2000; that value was considered constant for each day until subsequent examination (2010-12). At that point, individual covariates then assumed the value recorded in 2010-12 until the study exit (death date or date of censoring). This would allow a more accurate assessment of individual risk factors levels, rather than using one single measurement collected at baseline.

For each of the five outcomes, we fitted a time-varying Cox proportional hazards model estimating the adjusted risk of death for 5°C (≈1 SD) variation in outdoor temperature, after controlling for a proxy of seasonal influenza (a time varying covariate, see paragraph 8.5.6) and adjusting for individual risk factors. Non-linearity of associations of temperature with the outcomes were investigated (see paragraph 8.5.7).

To standardise as much as possible the analytical approach used in Chapter 5 and 7, and the conceptual framework of the present PhD thesis (see Chapter 2, Figure 2.2), several statistical analyses were carried out in the following order:

- a model with temperature only (unadjusted)
- a model additionally adjusted for physical activity as potential mediator of the temperature-mortality relationship
- a model additionally adjusted for potential modifiers of the temperature-mortality relationship (age, social class, BMI, smoking, and marital status, and lipids lowering drugs), and adjusted for ILI (potential seasonal confounder/modifier); this was the complete case analysis (n=4196). The adjustment for ILI was also carried out separately (temperature + ILI → mortality outcomes, see paragraphs 8.5.6 and 8.6.6)
- a separate and additional adjustment for four major risk factors that showed associations with temperature in Chapter 7: SBP, IL-6, LDL-Cholesterol and FEV1/FVC (fitted one at a time). When performing such separate models the number of observation in the complete case analysis was 4185, 4037, 3972, and 4167 respectively.

As a sensitivity analysis, I also reported estimates of association of temperature at lag 0-13 with mortality, similarly to previous studies which observed associations with temperature to last up to 2, 3 or 4 weeks (125, 326). This approach and choice were also supported by Chapter 7 findings; although most of the CVD risk factors were more strongly associated with temperature recorded at lag 0 and lag 0-6 (greater effect size at shorter vs longer lags), a fewer number of CVD risk factors were associated with temperature at lag 0-13 and lag 0-27. In this chapter, investigating associations only at lag 0-13 appeared to be a sensible strategy; in Chapter 7 the magnitude of the temperature-CVD factors associations did not substantially change nor increase at lag 0-27 vs 0-13.

Stata version 14.0 was used to implement this analysis, specifying the presence of clustered observation within each individual over time from the date of entry to date of exit from the study (death date or censoring date at 31/10/2014). Considering the data base structure described in this paragraph, the functions *stset* and *stcox* with the options *cluster* (for estimated standard errors) are used as standard methods to take into account repeated observations nested within individuals. The test of proportional-hazards assumption (PH chi-square test) was performed for the full model (global PH test) and for every single predictor in fully adjusted models.

8.5.6 Adjusting for influenza vs other long-term seasonal trends

One of the objectives of this Chapter is to enhance our understanding of biological pathways involved in the seasonal variation of CVD; therefore, whether outdoor temperature was still associated with mortality after adjusting for illness-specific seasonal trends (which are more common in cold seasons) was investigated. The main approach used in this study was to control temperature for ILI weekly consultation rate, a proxy of seasonal influenza exposure used for surveillance of respiratory viruses at national levels in the UK (281). ILI rates are more informative by definition in comparison with generic seasonal components that mimic fixed seasonal patterns, such as (i) a trigonometric function of day of the year used in previous studies to predict mortality [sine wave, see Figure 8.2] (128); (ii) a trigonometric function of day of the year with two terms used to estimate seasonal variation in mortality [Fourier terms, see Figure 8.3] (202).

Some other considerations were made before choosing the adjustment for ILI as the most appropriate strategy in this Chapter. A model including temperature and ILI was preferred also for statistical reasons: there was a lower absolute Pearson correlation of temperature with ILI (r=-0.36) in comparison with correlation of temperature with a seasonal term calculated using a sine wave function (r=0.70) or of temperature with Fourier terms (cosine component r=-0.78, sine component r=-0.34).

8.5.7 Non-linear association of temperature with mortality

Non-linear associations of temperature with mortality were explored by using two piecewise linear splines (e.g. temperature split in two parts) separated by one knot. The knot was placed at the 97.5th percentile (equal to 19.3°C) of the outdoor mean temperature time series, as suggested in one previous study (35). Although in the UK temperature can be low even during the summer time, the two piecewise linear splines would estimate the cold component (daily temperature ≤97.5th percentile respectively) and the non-cold component (temperature above 97.5th percentile respectively) related to mortality, to increase comparability with previous studies (35, 231). This approach would broadly estimate the non-linear (typically a U, J or V shape) relationship of temperature with mortality suggested in previous studies (326). Despite

being simple, this approach was preferred to a model fitting several flexible cubic splines (202), as the interpretation of the coefficients is more intuitive

Overall, as the linear model performed better than the two piecewise linear splines model (lower AIC score) for CVD and all-cause mortality it was retained as best model to fit the data. However, it was decided to report also results from models using two piecewise linear splines, as they added useful insights (see results 8.6.7); these results were presented alongside the ones from the linear model.

8.5.8 Interaction of temperature with individual risk factors

Several major CVD risk factors known for being associated with mortality, such as increased SBP, can potentially interact with temperature and increase the risk of mortality. In this Chapter I investigated such hypothesis focusing mainly on specific CVD risk factors (LDL-cholesterol, IL-6, SBP, and physical activity) because they were also associated with temperature in Chapter 7 and Chapter 5. Also, I hypothesized that some personal circumstances such as marital status and social class (markers of fuel poverty in winter) could also potentially interact with temperature (316); therefore I included them in my interaction tests.

BRHS population is a population of older adults of 60+ years old; the interaction of outdoor temperature with age fitted as continuous variable was also performed, to investigate whether or not the temperature-related mortality risks are increased in the oldest old who are in this age group. Lastly, the interaction of temperature with well-established life-style factors was tested (BMI fitted as continuous variable, and current smoking - yes vs no).

Some considerations were made before testing an interaction with other variables: for example, there was no evidence in the BRHS for an association of alcohol consumption with mortality in my analysis (results not shown, but I compared non-drinker vs occasional/light drinkers or moderate/heavy vs occasional/light drinkers as suggested in one previous BRHS paper (334)). Overall, the relationship of average or occasional alcohol consumption and mortality is still not fully understood (335, 336), and it seemed reasonable not to consider alcohol in relation to temperature and mortality in this PhD thesis.

8.6 Results

8.6.1 Participants

Participants' characteristics and individual factors measured at baseline are shown in Table 8.1. For completeness of information, participants' characteristics for those with measures collected at baseline and follow-up were also reported in Table 8.1. In participants with 2 measures (collected at follow-ups 1998-2000 and 2010-2012) the levels of the risk factors changed over time: in 2010-2012 vs 1998-2000 the proportion of active smokers was lower; conversely the BMI, the proportion of those who were inactive and who were using lipids lowering drug medication was higher (Table 8.1). In 2010-2012 vs 1998-2000 the average levels of LDL-cholesterol were lower, the average levels of blood pressure and inflammation were higher, and the FEV_1/FVC ratio was lower.

8.6.2 Follow-up and mortality

The median follow-up period was 14.9 years (interquartile range 8.7-15.7 years). 2017 deaths were registered: 764 died from CVD (446 CHD deaths, 154 stroke deaths, and 164 from other CVD), 256 from respiratory causes.

8.6.3 Outdoor temperature

All mean outdoor temperatures recorded during the study period were plotted against day of the year in Figure 8.4. The average was 9.9°C (SD=5.2°C, Minimum=-13.1°C, Maximum=27.0°C) as reported in Table 8.2. Lower temperatures were recorded in December and January. All deaths registered were summarised by quintiles of outdoor temperature; a graded decrease in number of deaths from lower to higher quintiles occurred especially for CHD, CVD and all-cause mortality (Table 8.3).

8.6.4 Seasonal influenza

Over the average year, the weekly national ILI consultation rate during the BRHS study period was 13.5 (SD=19.2) per 100,000 persons. The typical profile of a seasonal outbreak of influenza each winter included a high peak regularly detected in January and December (281), as shown in Figure 8.5. The weekly average consultation rate in December/January was approximately 20 (min=5; max=233) vs 5 recorded during the rest of the year (min=0;

max=153), therefore, the weekly variation within both season and month was strong. The Pearson correlation of ILI with temperature during the study period was r=-0.36.

8.6.5 Time varying covariate survival models

Overall, mortality risks were higher at lower temperatures (Table 8.4). Mortality risks were particularly high at lower temperatures for CHD and respiratory mortality (Table 8.4, Model 1). The additional adjustment for physical activity reduced the magnitude of the association of temperature with mortality outcomes by at most 0.5% (see Table 8.4, Model 2). For example, the association of decrease in temperature at lag 0 with CHD deaths changed from HR = 1.145 (95% CI 1.067; 1.217) to HR = 1.141 (95% CI 1.063,1.213) before and after adjustment for physical activity respectively. After additional adjusting for seasonal trends and individual risk factors (Table 8.4, Model 3) a decrease of 5°C in outdoor temperatures was associated with an increase in CHD mortality of 12.8% (Hazard Ratio (HR) = 1.128; 95% CI 1.041-1.208; p=0.005), an increase in respiratory mortality of 11.0% (HR=1.110, 95%CI 1.002-1.207, p=0.046), and an increase in all-cause mortality of 5.9% (HR=1.059; 95%CI 1.016-1.100; p=0.008). Lower temperatures were associated with an increase in CVD mortality of 10.4% (HR=1.104; 95%CI 1.035-1.168, p=0.004), although a specific association of temperature with stroke mortality was not found in this study (HR=1.047; 95%CI 0.886; 1.199, p=0.586). Associations of temperature up to 2 weeks (lag 0-13) prior to date of death were observed; for CHD and CVD mortality associations of temperature at lag 0 were slightly greater than those observed at lag 0-13. Conversely, for respiratory and all-cause mortality associations of temperature at lag 0 were slightly smaller than those observed at lag 0-13. The additional adjustment for individual risk factors fitted in model one at a time (SBP, IL-6, LDL-Cholesterol, FEV_1) further reduced the magnitude of the association of temperature with mortality outcomes by at most 1% (Tables 8.5-8.9).

Overall, associations of some individual risk factors with at least one mortality outcome were found (Tables 8.5-8.9): increasing age, smoking, higher SBP, higher IL-6, lower physical activity levels, and lower FEV_1/FVC rate were all associated with an increase in mortality. Higher LDL-cholesterol, and manual social class were associated particularly with CHD mortality.

8.6.6 Adjusting for influenza vs long-term seasonal trends

Lower temperature, higher ILI consultation rate, and trigonometric functions of day of the year (sine wave only and Fourier terms) predicted mortality (CHD, CVD, respiratory and all-cause mortality) in unadjusted models (Table 8.10).

The unadjusted seasonal variation in mortality calculated using Fourier terms was 0.342 for CHD, 0.323 for CVD, 0.519 for respiratory causes, and 0.224 for all-cause mortality, Table 8.10). As specified in Table 8.10, the number 0.342 for CHD was calculated from β coefficients of Fourier terms (sine and cosine terms); this number represent an estimate of the sinusoidal seasonal variation in mortality over the average year (e.g. the number 0.342 for CHD means 34% variation in CHD mortality when comparing the peak vs nadir of the sinusoidal function, where the peak is in the winter months and nadir in summer months). If there was no seasonal variation the sinusoidal variation would have been equal to zero. Higher Influenza-like illness (ILI) weekly consultation rate was associated with increased CHD, respiratory and all-cause mortality in unadjusted models (Table 8.10).

In sensitivity analysis, the magnitude of the association of temperature with mortality all outcomes before and after adjusting for ILI rate remained fairly consistent (Table 8.10, see coefficient of outdoor temperature in Model 1 vs Model 5). Also, after the mutual adjustment of temperature with ILI, the latter seemed to lose its association with CHD mortality, but retained a weak positive association with respiratory mortality (p=0.063). Applying models including mutual adjustment of temperature with Fourier terms led to null estimates: I found that all three variables (temperature, cosine, and sine terms) were no longer associated with mortality; when I observed Figure 8.3 (yearly cosine term) and Figure 8.4 (yearly temperature trends) it appeared that the cosine component has a similar (inverse) trend if compared with outdoor temperature (the variable of interest). The two variables may compete when used to explain the variation in mortality, widening standard errors of risk estimates for the three seasonal factors.

8.6.7 Non-linear association of temperature with mortality

Our findings also suggested a V-shaped relationship of temperature with respiratory mortality: a decrease in temperature of 5°C below 19.3°C [97.5th percentile] increased mortality

(HR=1.135; 95%CI 1.032-1.228, p=0.011), while an increase in temperature of 5°C above 19.3°C also increased mortality (HR=6.720; 95%CI 2.592-21.553; p=0.008).

8.6.8 Interaction of temperature with individual risk factors

Overall, there was no clear evidence of interaction between temperature and age, social class, body mass index, smoking, physical activity, use of lipids lowering drugs medication, SBP, IL-6, LDL-Cholesterol and lung function were not significant. One single interaction of marital status with temperature was found: men who were not married (e.g. single, divorced or widowed) versus not were at increased risk of CHD mortality when temperatures decreased (p=0.027).

8.7 Discussion

8.7.1 Summary of the main findings

I discuss below findings in relation to objectives outlined in paragraph 8.3.

Question 1. Do variations in temperature relate to variations in coronary heart disease (CHD), Stroke, CVD, respiratory, and all-cause mortality in older British men from the BRHS?
Yes, I found that mean outdoor temperature related to mortality in the BRHS. Overall, mortality incidence was associated with lower temperatures. This peak at lower temperatures was driven by increased CHD and respiratory mortality, and findings were consistent even after adjusting for a proxy of seasonal influenza. A decrease of 5°C in outdoor temperatures increased CHD mortality by 12.8%, respiratory mortality by 11.0%, and all-cause mortality by 5.9%. Lower temperatures also increased CVD mortality by 10.4%, although evidence was lacking for specific association of temperature with stroke mortality in this study. The additional adjustment for individual risk factors slightly reduced the magnitude of the association of temperature with mortality by at most 1%. Associations of temperature up to 2 weeks (lag 0-13) prior to date of death were observed; for CHD and CVD mortality associations of temperature at lag 0 were slightly greater than those observed at lag 0-13. Conversely, for respiratory and all-cause mortality associations of temperature at lag 0 were slightly smaller than those observed at lag 0-13.

Question 2. Is the temperature–mortality relationship confounded by seasonal influenza trends?

No. The findings showed that the magnitude of the association of temperature with mortality before and after adjusting for ILI rate remained fairly consistent

Question 3 Is the proxy for seasonal influenza used in this study (ILI consultation rate) associated with mortality?

The findings presented in this Chapter did not offer a fully comprehensive answer to this question; the association of ILI with mortality disappeared after adjustment for temperature (similarly to what happen in Chapters 5 with physical activity outcomes and in Chapter 7 with CVD risk factors). This finding suggested temperature carries the strongest association with mortality. However, the role of ILI as confounder or mediator of the relationship between temperature and mortality should not be excluded, and could be better clarified in future prospective studies using a more accurate assessment of influenza exposure (e.g. at regional, GP or individual-level, and counting accesses to primary care; see the paragraph 8.7.3 "*Strengths and limitations*"). Overall, and after adjustment for temperature, ILI rate seemed to lose its association with CVD and all-cause mortality, but retained a weak positive association with respiratory mortality.

Question 4. Is the association of temperature with mortality non-linear? If so, for which causes of death a non-linearity is found?

Evidence of non-linearity was found for the temperature-respiratory mortality relationship: both a decrease in temperature below 19.3°C [97.5th percentile], and an increase in temperature above 19.3°C increased mortality. There was no clear evidence of non-linearity of associations with other outcomes.

Question 5. Is the association of temperature with mortality modified by individual risk factors?

Overall, there was no evidence to support that. Interactions tests of temperature with major risk factors were performed; in one case I found that men who were not married (single, divorced or widowed) are at particularly increased risk of CHD mortality at lower temperatures.

However, considering this is the only significant interaction test, results should be interpreted with caution as it may be due by chance.

8.7.2 Comparison with other studies

Temperature-related mortality variations have been widely studied in the past four decades and their findings were consistent with those observed here (29, 35, 37, 124, 125, 127, 128, 130, 337). As in previous studies, it was necessary in this study to control temperature for confounding of long-term seasonal patterns in order to account for both associations of daily outdoor temperature with mortality (short-term patterns) and associations of other seasonal variables with mortality (influenza or long-term seasonal patterns). However, in the literature there is no single agreed method to account for seasonality (30, 202); some previous ecological and observational studies used ILI rates (130, 284, 338), or fixed trigonometric functions of day of the year, such as Fourier terms (339, 340). The simplest solution was to adjust for ILI rate only; with ILI we would have hoped to account for illness-specific seasonal trends in mortality, reducing noise associated with daily mortality variations in the data, and reducing risk of collinearity with temperature (128). However, the analysis as it stands cannot distinguish between the association of temperature with mortality vs association of ILI with mortality because the ILI measure was not very refined (e.g. it was not collected at individual level, nor were regional variations assessed). Also, the ILI measure did not distinguish between subtypes of influenza such as the A(H3N2) type (more common in January and February), which was associated with increased mortality in Europe during the 2016/2017 winter season, even after adjusting for temperature (341). Future observational studies could collect a more accurate measure of ILI (e.g. with daily frequency, at GP practice or individual level), and test it in new analysis aimed to assess its role as confounder or mediator between temperature and mortality (342). Alternatively, BRHS data could be linked with routinely recorded primary care data in the future and retrospectively assess a more accurate consultation rate in primary care due to influenza.

In this study we used a different methodological approach in comparison with previous studies. To the best of my knowledge, the use of time-varying covariates survival models using individual level data is unprecedented for population-based studies of temperature and mortality; such an approach went beyond the use of simple survival models collecting

information at baseline only, as it combined the use of individual risk factors, weather and seasonal variables repeatedly collected over time at different intervals. Also, the findings from survival models were presented as HR, a relative risk measure, which can be broadly translated in absolute terms if considering the daily number of deaths from CVD estimated from the BHF (343). It is known that every day 305 people die from CVD in the UK among those aged 75+ years; in this Chapter I found +10.4% increase in daily CVD deaths (12.5% in CHD deaths) per a decrease in 5 degrees Celsius in mean outdoor temperature recorded on the same day; this should correspond to an increase of approximately 32 CVD deaths for that specific day, of which approximately 22 are attributable to CHD.

Differently from many ecological studies worldwide, this study collected information at individual level; this allowed testing for interaction of temperature with a comprehensive range of individual risk factors. For example, an interaction of temperature with age was tested: findings could only show that within a group of BRHS older men (60+ years old) each individual has same relative risk of dying at lower temperatures, as in one previous UK study of older adults aged 75+ years (129). The BRHS did not enrol younger participants (e.g. 15-64 years old), so we cannot assess how the exposure at lower temperatures affect older (60+ years old) vs younger men. Overall, our interaction tests confirmed prior findings indicating no consistent evidence that a temperature-related (or seasonal-related) variation in mortality is modified by lifestyle and socio-demographic risk factors measured at macro area level across Europe (37, 125, 129, 344). For example, one previous study, found no evidence that the association of cold temperature with CVD mortality was modified by obesity, smoking habit, alcohol intake, and hypertension (37). My finding on the effect modification of marital status is plausible, as being not married is a known determinant of fuel poverty in winter (316), a factor which may increase the chances of living in cold homes and therefore worsening the CVD profile. However, further studies should increase our understanding of which population groups experience increasing death rates at lower temperatures, as an excess of winter mortality in the UK still persists (29). To the best of my knowledge, an interaction of temperature and physical activity levels (inactive vs active men) on mortality was not tested in previous studies; therefore the lack of interaction found in this chapter cannot be confirmed by previous literature. This requires further epidemiological research (see discussion, Chapter 9).

Overall, we confirmed that lower temperatures, rather than heat, were more strongly associated with mortality (35). In this study there was no evidence of increased risk of CVD mortality and all-cause mortality from the warmest temperatures, in contrast with some previous studies carried out in England and Wales (345), and London (35). It is plausible that my analysis did not have enough statistical power to detect an association, as very hot days were not very common particularly in Scotland, as confirmed by the UK meteorological office time series (123). It is also possible that in the BRHS population the non-linearity of the association could be cause-specific, as we found evidence of non-linearity only when we investigated the temperature-respiratory mortality relationship. A previous study investigated the heat‐related deaths in England and Wales between 1993 and 2003 and found a small increase in deaths associated with high temperatures (in between 20-25°C); the association was strongest for respiratory mortality (RR ≈ 1.0-1.4 when temperatures were in between 20-25°C) in comparison with other causes (RR ≈ 1.0-1.2 when temperatures were in between 20-25°C) (346). In Spain, where temperatures are generally higher and hot days more common, at higher temperatures the association with respiratory mortality was much stronger than the association with CVD mortality. For example, at mean temperatures of 32°C (very uncommon in the UK) the authors found a RR≈3 for respiratory mortality vs RR≈1.8 for CVD mortality in comparison with RR≈1 observed in between 18-20°C. The authors concluded that the effect of hot days on mortality largely varied by cause of death, affecting especially respiratory mortality (347).

8.7.3 Strengths and limitations

This study benefits from using a large scale population-based cohort of free-living older men rather than a special *at risk* population, which should increase generalizability. Also, the BRHS towns were chosen to be socioeconomically representative of all major geographic regions in Great Britain (England, Scotland and Wales) (123, 124). The response rate achieved in this study during 1998-2000 (the baseline population for this specific work) was high and equal to 77% (225). The cohort has been successfully followed until the present (achieving a very high follow-up rates, of about 98%, for clinical endpoints (224)), which means that objective measurements of CVD incidence and mortality, and other causes of death were available and usable in survival analysis. This is one of the largest studies to simultaneously investigate the influence of meteorological and seasonal factors and measures of a comprehensive range of individual risk factors, and to test the interaction of temperature with those factors. The main

limitation is the inclusion of only male participants; in the UK and in comparison to men, a higher proportion of the female population are aged 75 and over (9%, compared with 7% of males in 2013 (103)), so we would expect a higher absolute number of women exposed to cold weather than men (129). Our results may not be generalizable to older women or ethnic minority populations (269).

Not all fatal events in the BRHS represented sudden deaths and for some individuals, a non-fatal event may still have occurred up to 28 days previously (296, 348, 349). The exact date of such events was not known and thus our analysis refers to mean temperature recorded the same day of the death rather than at the event which preceded it. Mortality may be more sensitive to temperature than non-fatal events. This might have led to an underestimation of the true association between temperature and mortality, due to random misclassification of temperature (exposure) The daily meteorological data used for this study was collected from local weather stations on average 10 kilometres distant from each study town, so temperature information should be very accurate, or at least more accurate than other weather variables (e.g. humidity or rainfall) due to its lower spatial variability (230).

We could not assess whether specific individual risk factors, such as elevated blood pressure and IL-6, acted as mediators of the relationship between temperature and mortality, as specified in the main hypothesis of this thesis (see Chapter 1, paragraph 1.1.2). Ideally, to test this hypothesis the individual factors should have been collected at least twice per year (once in winter and once in summer, therefore closer to the date of death) to offer new insights on their role as mediators. In this study, physical and biological CVD risk factors were collected on two occasions during 1998-2000 and 2010-2012. Operational end economic costs of such data collections over time are very high. It is not surprising that neither the BRHS nor other ongoing population-based studies worldwide collected physical and blood markers on a yearly basis over decades.

8.7.4 Implications

The findings presented in this chapter confirm that in older adults higher mortality rates at lower temperatures remains an important public health problem in the UK and elsewhere (327). However, how to prevent the rise of CVD during winter, when temperatures are typically

lower, remains not fully understood. Clear suggestions on which interventions are best to prevent this cannot be derived from this work, and the reasons are twofold:

1) the causal pathways linking temperature variations with mortality were not established from this work. For example, I could not assess whether specific risk factors, such as elevated blood pressure and IL-6, acted as mediators of the relationship between temperature and mortality, as specified in the main hypothesis of this thesis (Chapter 1, paragraph 1.3.1.1). Ideally, to test this hypothesis the individual factors should have been collected more frequently over the study period (see limitations highlighted in paragraph 8.7.3) This is a key challenge in future epidemiological studies; for example, future prospective studies could simultaneously measure meteorological factors, blood pressure and physical activity by second, minute or daily by using consumer grade wearable devices.

2) I did not find clear evidence on which sub-groups of the BRHS population are particularly affected by low temperatures; for example, there was no evidence of interaction between temperature and major risk factors, such as blood pressure, markers of inflammation and physical activity. Therefore, how to plan interventions targeting older adults at higher risk cannot be suggested from my findings. A recent review on health burdens associated with cold weather, suggested that intervention measures intended to fight fuel poverty will likely play a key role in defining future health burdens associated with cold weather (327). Although fuel poverty was not measured in this PhD thesis, very recent findings from the BRHS demonstrated that living in a cold home was associated with increased mortality risks (350) (please note this work was not part of the present PhD research); therefore, an improvement of current strategies at national level tackling cold homes in winter is needed, and may help in reducing the seasonal variation in mortality

Lastly, a further important strategy to prevent mortality at lower temperature may be providing more effective recommendations and communication at national and local level: several people interviewed in a recent qualitative study were unaware of the cardiovascular risk associated with low temperatures (351). The authors concluded that a key challenge for health agencies

still remains identifying people who are potentially vulnerable during cold weather but who are not known to local health services (351).

8.8 Conclusions

Overall, in the BRHS mortality incidence was associated consistently with lower temperatures after controlling for a proxy of influenza exposure. This mortality peak at lower temperatures was driven by increased CHD and respiratory mortality. The additional adjustment for physical activity, as well as blood pressure, LDL-cholesterol and IL-6, slightly reduced the magnitude of the association of temperature with mortality outcomes. There was no evidence of interaction between temperature and major risk factors, such as age. A better protection of all older adults against low temperatures, typically recorded in winter, could help in reducing mortality risks.

Table 8.1 BRHS participants' characteristics collected during 1998-2000 and 2010-2012.

	4252 BRHS men who attended the examination in 1998-2000	1593 BRHS men with data at both follow-up examinations		
		Examination in 1998-2000	Examination in 2010-2012	
Demographic and background characteristics				
Age (years), mean (SD)	68.7 (5.5)	66.3 (4.7)	78.6 (4.7)	
Social class, n (%) Manual Non-Manual Armed forces Missing	2166 (50.9) 1966 (46.2) 112 (2.6) 8 (0.2)	840 (52.7) 709 (44.5) 39 (2.5) 5 (0.3)	same as 1998-2000	
Physical health				
BMI, mean (SD) Missing, n (%)	26.9 (3.7) 20 (0.5)	26.8 (3.3) 4 (0.3)	27.1 (3.8) 17 (1.0)	
Behavioural factors				
Smoking				
Smokers vs not, n (%)	548 (12.9)	115 (7.2)	50 (3.1)	
Missing, n(%)	7 (0.2)	0 (0)	0 (0)	
Alcohol consumption				
None, n (%)	431 (10.3)	117 (7.4)	190 (11.9)	
Occasional/light, n (%)[1]	2949 (70.5)	1152 (73.0)	1122 (70.4)	
Moderate/Heavy, n (%) [2]	779 (18.6)	305 (19.1)	207 (13.0)	
Unclassified, n (%)	26 (0.6)	4 (0.3)	14 (0.9)	
Missing, n (%)	67 (1.6)	16 (1.0)	60 (3.8)	
Physical activity (PA) score				
Inactive, n (%)	471 (11.1)	89 (5.6)	261 (16.4)	
Occasional, n (%)	957 (22.5)	291 (18.3)	351 (22.0)	

Light, n (%)	767 (18.0)	272 (17.1)	339 (21.3)	
Moderate, n (%)	591 (13.9)	273 (17.1)	228 (14.3)	
Moderate vigorous, n (%)	690 (16.2)	318 (20.0)	197 (12.4)	
Vigorous, n (%)	621 (14.6)	308 (19.3)	131 (8.2)	
Missing, n (%)	155 (3.6)	42 (2.7)	86 (5.4)	
Personal circumstances				
Married vs single/widowed/divorced/separated, n(%)	3456 (81.6)	1392 (89.8)	1204 (76.7)	
Lipids lowering drugs use, n (%)	327 (7.7)	116 (7.3)	788 (49.5)	
Biological markers and physical measurements				Correlation (r) [3]
LDL-Cholesterol, mmol/L, mean (SD) Missing, n (%)	3.90 (1.00) 278 (5.2)	3.94 (0.95) 83 (5.2)	2.61 (0.94) 79 (5.0)	0.30
Systolic Blood pressure, mm Hg, mean (SD) Missing, n(%)	149 (24) 17 (0.4)	146 (22) 6 (0.4)	147 (19) 3 (0.2)	0.28
Lung function, FEV_1/FVC % [§] Missing, n (%)	76.8 (11.6) 47 (1.1)	77.8 (10.6) 5 (0.3)	74.1 (8.9) 131 (8.2)	0.37
IL-6, pg/mL Missing, n (%)	3.1 (2.9) 202 (4.7)	2.62 (2.44) 69 (4.3)	4.32 (4.61) 92 (5.7)	0.34

[1] >=1 and <=15 units per week (1 unit is approximately 1 drink, such as one glass of wine)

[2] >=16 units per week (1 unit is approximately 1 drink, such as one glass of wine

[3] All correlations significant at level 0.001

[§] adjusted for height

Table 8.2 Distribution of daily mean outdoor temperature in the BRHS towns in between 1998 and 2014 by quintiles (Q)

	mean (SD) [min, max]	Q1 mean (min, max)	Q2 mean (min, max)	Q3 mean (min, max)	Q4 mean (min, max)	Q5 mean (min, max)
Distribution of mean outdoor temperature	9.9 (5.2) [-13.1, 27.0]	2.5 (-13.1,5.2)	6.8 (5.3, 8.5)	10.2 (8.6, 11.6)	13.3 (11.7, 14.8)	17.0 (14.9, 27.0)

Table 8.3 Percentage and number of deaths registered in the BRHS towns by quintiles (Q) of mean temperature during the study period (1998-2014)

Number of deaths in the 24 BRHS towns during study period	Mean Temperature Q1, n (%)	Mean Temperature Q2, n (%)	Mean Temperature Q3, (n) %	Mean Temperature Q4, n (%)	Mean Temperature Q5, n (%)
All (n=2017)	467 (23.2)	419 (20.8)	376 (18.6)	366 (18.1)	389 (19.3)
CHD deaths (n=446)	114 (25.6)	93 (20.9)	84 (18.8)	85 (19.1)	70 (15.7)
Stroke deaths (n=154)	37 (24.0)	31 (20.1)	26 (16.9)	28 (18.2)	32 (20.8)
CVD deaths (n=764)	196 (25.7)	149 (19.5)	144 (18.8)	138 (18.1)	137 (17.9)
Respiratory deaths (n=256)	68 (26.6)	54 (21.1)	50 (19.5)	40 (15.6)	44 (17.2)

Note: Quintiles of outdoor temperatures were calculated using all temperature data during the follow-up period (daily outdoor temperatures recorded in the 35 weather stations during the study period).

Table 8.4 Results from time-varying covariates survival models: associations of outdoor mean temperature at lag 0 and lag 0-13 with mortality in the BRHS during 1998-2000 and 31/10/2014.

Note: Estimates are reported as Hazard Ratios (HR) with 95% Confidence Intervals per a decrease of 5°C (≈1 Standard Deviation) in outdoor temperatures. For each model there was no evidence that the proportional-hazards assumption has been violated (p>0.05)

Outcome	Number of observations (complete case analysis)	Exposure	Model 1		Model 2		Model 3	
			HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
CHD death	n= 4196, deaths=437	Mean temperature Lag 0	**1.145 (1.067; 1.217)**	**<0.001**	**1.141 (1.063,1.213)**	**<0.001**	**1.128 (1.041; 1.208)**	**0.005**
		Mean temperature Lag 0-13	**1.129 (1.039; 1.210)**	**0.006**	**1.123 (1.033,1.205)**	**0.008**	**1.103 (1.002; 1.194)**	**0.045**
Stroke death	n= 4196, deaths=152	Mean temperature Lag 0	1.072 (0.910; 1.211)	0.362	1.074 (0.897, 1.223)	0.388	1.047 (0.866; 1.199)	0.586
		Mean temperature Lag 0-13	1.067 (0.885; 1.219)	0.447	1.082 (0.903, 1,123)	0.343	1.035 (0.828; 1.206)	0.718
CVD death	n= 4196, deaths=749	Mean temperature Lag 0	**1.114 (1.051; 1.172)**	**0.001**	**1.114 (1.051, 1.173)**	**<0.001**	**1.104 (1.035; 1.168)**	**0.004**
		Mean temperature Lag 0-13	**1.108 (1.037; 1.173)**	**0.003**	**1.107 (1.038, 1.173)**	**0.003**	**1.096 (1.017; 1.168)**	**0.018**
Respiratory death	n= 4196, deaths=256	Mean temperature Lag 0	**1.140 (1.034; 1.234)**	**0.011**	**1.137 (1.031, 1.231)**	**0.013**	**1.110 (1.002; 1.207)**	**0.046**
		Mean temperature Lag 0-13	**1.160 (1.042; 1.263)**	**0.009**	**1.155 (1.037, 1.259)**	**0.011**	**1.126 (1.004; 1.233)**	**0.043**
All causes	n= 4196, deaths=1991	Mean temperature Lag 0	**1.069 (1.030; 1.108)**	**0.001**	**1.067 (1.028, 1.106)**	**0.001**	**1.059 (1.016; 1.100)**	**0.008**
		Mean temperature Lag 0-13	**1.078 (1.034; 1.121)**	**0.001**	**1.075 (1.031, 1.118)**	**<0.001**	**1.067 (1.019; 1.112)**	**0.007**

Model 1: Unadjusted (temperature only)
Model 2: Model 1 additionally adjusted for physical activity
Model 3: Model 2 additionally adjusted for physical activity, age, social class, BMI, smoking, marital status, lipids lowering drugs, and ILI

Table 8.5 Results from time-varying covariates survival models: associations of outdoor mean temperature and other risk factors with CHD mortality in the BRHS during 1998-2000 and 31/10/2014

Note: Estimates are reported as Hazard Ratios (HR) with 95% Confidence Intervals. Blank cells for SBP, IL-6, LDL and FEV_1/FVC % mean they were not used in the model.

	Model 1, n= 4196, deaths=437		Model 2, n=4185, deaths=435		Model 3, n=4037 , deaths=407		Model 4, n=3972 , deaths=393		Model 5, n=4167 , deaths=434	
Covariables	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
Mean Temperature [1]	**1.128 (1.041; 1.207)**	**0.005**	**1.129 (1.042; 1.209)**	**0.005**	**1.106 (1.013; 1.189)**	**0.025**	1.082 (0.985; 1.169)	0.093	**1.122 (1.034; 1.202)**	**0.008**
ILI [2]	1.055 (0.959; 1.162)	0.275	1.058 (0.959; 1.164)	0.265	1.075 (0.978; 1.183)	0.134	1.068 (0.963; 1.183)	0.214	1.058 (0.961; 1.166)	0.245
Age, years	**1.135 (1.115; 1.155)**	**<0.001**	**1.129 (1.109; 1.150)**	**<0.001**	**1.129 (1.108; 1.150)**	**<0.001**	**1.140 (1.119; 1.162)**	**<0.001**	**1.123 (1.102; 1.144)**	**<0.001**
Social class (ref: non-manual)										
Manual	**1.241 (1.022; 1.509)**	**0.030**	**1.274 (1.047; 1.549)**	**0.015**	1.218 (0.996; 1.490)	0.054	**1.233 (1.005; 1.513)**	**0.045**	1.181 (0.971; 1.437)	0.097
Armed forces	1.004 (0.524; 1.925)	0.990	1.090 (0.572; 2.078)	0.793	0.963 (0.480; 1.931)	0.916	0.879 (0.426; 1.812)	0.726	0.940 (0.494; 1.789)	0.850
BMI	1.015 (0.988; 1.041)	0.278	1.011 (0.985; 1.037)	0.426	1.005 (0.978; 1.034)	0.711	1.007 (0.978; 1.036)	0.644	1.013 (0.988; 1.039)	0.311
Smokers vs not	**1.611 (1.233; 2.105)**	**0.001**	**1.622 (1.240; 2.122)**	**<0.001**	**1.521 (1.151; 2.010)**	**0.003**	**1.686 (1.272; 2.236)**	**<0.001**	**1.467 (1.122; 1.918)**	**0.005**
Married vs not	1.237 (0.994; 1.540)	0.057	**1.252 (1.005; 1.559)**	**0.045**	1.227 (0.979; 1.540)	0.076	**1.298 (1.032; 1.634)**	**0.026**	1.238 (0.994; 1.541)	0.056
Lipid-regulating drugs use, yes vs not	**1.772 (1.349; 2.328)**	**<0.001**	**1.831 (1.393; 2.406)**	**<0.001**	**1.790 (1.343; 2.385)**	**<0.001**	**2.102 (1.556; 2.840)**	**<0.001**	**1.744 (1.325; 2.294)**	**<0.001**
Physical activity (ref: Inactive)										
Occasional	**0.607 (0.459; 0.802)**	**0.001**	**0.579 (0.438; 0.766)**	**<0.001**	**0.684 (0.509; 0.919)**	**0.012**	**0.662 (0.490; 0.894)**	**0.007**	**0.629 (0.474; 0.835)**	**0.001**
Light	**0.527 (0.390; 0.713)**	**<0.001**	**0.496 (0.366; 0.672)**	**<0.001**	**0.568 (0.411; 0.784)**	**0.001**	**0.535 (0.386; 0.742)**	**<0.001**	**0.563 (0.415; 0.763)**	**<0.001**
Moderate	**0.512 (0.364; 0.720)**	**<0.001**	**0.499 (0.355; 0.700)**	**<0.001**	**0.548 (0.381; 0.789)**	**0.001**	**0.515 (0.355; 0.745)**	**<0.001**	**0.564 (0.399; 0.796)**	**0.001**
Moderately vigorous	**0.533 (0.380; 0.747)**	**<0.001**	**0.499 (0.355; 0.701)**	**<0.001**	**0.633 (0.441; 0.909)**	**0.013**	**0.578 (0.403; 0.828)**	**0.003**	**0.594 (0.421; 0.837)**	**0.003**
Vigorous	**0.350 (0.237; 0.518)**	**<0.001**	**0.338 (0.229; 0.499)**	**<0.001**	**0.413 (0.274; 0.621)**	**<0.001**	**0.365 (0.241; 0.553)**	**<0.001**	**0.394 (0.265; 0.586)**	**<0.001**
SBP, mm Hg			**1.007 (1.003; 1.012)**	**0.001**						
Log(IL-6), pg/mL					**1.403 (1.229; 1.602)**	**<0.001**				
LDL, mmol/L							**1.136 (1.034; 1.247)**	**0.008**		
FEV_1/FVC %									0.999 (0.991; 1.001)	0.959

[1] Hazard ratios estimated per a decrease of 5°C (≈1 Standard Deviation) in outdoor temperatures

[2] Hazard ratios estimated per an increase of 20 consultations per week for influenza-like illness in primary care

Table 8.6 Results from time-varying covariates survival models: associations of outdoor mean temperature and other risk factors with Stroke mortality in the BRHS during 1998-2000 and 31/10/2014

Note: Estimates are reported as Hazard Ratios (HR) with 95% Confidence Intervals. Blank cells for SBP, IL-6, LDL and FEV1/FVC % mean they were not used in the model.

	Model 1, n= 4196, deaths=152		Model 2, n=4185, deaths=151		Model 3, n=4037 , deaths=144		Model 4, n=3972 , deaths=140		Model 5, n=4167 , deaths=149	
Covariables	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
Mean Temperature [1]	1.047 (0.866; 1.199)	0.586	1.054 (0.873; 1.205)	0.538	1.051 (0.863; 1.208)	0.568	1.034 (0.844; 1.193)	0.708	1.063 (0.884; 1.213)	0.468
ILI [2]	1.073 (0.907; 1.269)	0.416	1.077 (0.910; 1.274)	0.392	1.058 (0.869; 1.289)	0.573	1.077 (0.901; 1.287)	0.417	1.068 (0.894; 1.279)	0.462
Age, years	**1.164 (1.128; 1.201)**	**<0.001**	**1.160 (1.123; 1.197)**	**<0.001**	**1.161 (1.124; 1.199)**	**<0.001**	**1.169 (1.131; 1.207)**	**<0.001**	**1.161 (1.124; 1.199)**	**<0.001**
Social class (ref: non-manual)										
Manual	1.077 (0.772; 1.503)	0.663	1.083 (0.775; 1.515)	0.64	1.109 (0.788; 1.562)	0.552	1.127 (0.797; 1.595)	0.499	1.078 (0.768; 1.513)	0.665
Armed forces	**2.423 (1.183; 4.966)**	**0.016**	**2.347 (1.108; 4.972)**	**0.026**	**2.346 (1.102; 4.998)**	**0.027**	**2.331 (1.101; 4.932)**	**0.027**	**2.423 (1.181; 4.971)**	**0.016**
BMI	0.966 (0.923; 1.010)	0.132	0.963 (0.920; 1.007)	0.100	0.956 (0.913; 1.001)	0.056	0.959 (0.913; 1.007)	0.095	0.968 (0.925; 1.012)	0.152
Smokers vs not	**1.642 (1.036; 2.602)**	**0.035**	1.390 (0.853; 2.267)	0.187	1.514 (0.927; 2.471)	0.097	**1.575 (0.974; 2.545)**	**0.064**	**1.600 (1.011; 2.532)**	**0.045**
Married vs not	1.009 (0.676; 1.507)	0.965	0.944 (0.623; 1.431)	0.787	0.952 (0.622; 1.460)	0.823	0.938 (0.621; 1.416)	0.76	0.995 (0.667; 1.486)	0.982
Lipid-regulating drugs use, yes vs not	0.903 (0.495; 1.647)	0.740	0.765 (0.399; 1.465)	0.419	0.914 (0.466; 1.793)	0.794	0.897 (0.492; 1.637)	0.723	0.886 (0.487; 1.612)	0.692
Physical activity (ref: Inactive)										
Occasional	**0.582 (0.356; 0.953)**	**0.031**	**0.556 (0.339; 0.911)**	**0.020**	**0.552 (0.336; 0.908)**	**0.019**	**0.485 (0.292; 0.805)**	**0.005**	**0.578 (0.352; 0.950)**	**0.031**
Light	**0.449 (0.264; 0.766)**	**0.003**	**0.429 (0.251; 0.732)**	**0.002**	**0.415 (0.243; 0.710)**	**0.001**	**0.362 (0.209; 0.627)**	**<0.001**	**0.429 (0.250; 0.736)**	**0.002**
Moderate	**0.497 (0.273; 0.906)**	**0.022**	**0.483 (0.264; 0.882)**	**0.018**	**0.454 (0.245; 0.844)**	**0.013**	**0.419 (0.227; 0.776)**	**0.006**	**0.517 (0.284; 0.942)**	**0.031**
Moderately vigorous	**0.467 (0.260; 0.839)**	**0.011**	**0.448 (0.251; 0.802)**	**0.007**	**0.480 (0.262; 0.877)**	**0.017**	**0.419 (0.232; 0.757)**	**0.004**	**0.491 (0.273; 0.885)**	**0.018**
Vigorous	**0.479 (0.266; 0.864)**	**0.014**	**0.443 (0.243; 0.809)**	**0.008**	**0.484 (0.263; 0.890)**	**0.02**	**0.453 (0.251; 0.815)**	**0.008**	**0.505 (0.280; 0.911)**	**0.023**
SBP, mm Hg			1.006 (0.999; 1.012)	0.102						
Log(IL-6), pg/mL					**1.430 (1.150; 1.777)**	**0.001**				
LDL, mmol/L							1.075 (0.902; 1.281)	0.420		
FEV_1/FVC%									1.001 (0.994; 1.024)	0.224

[1] Hazard ratios estimated per a decrease of 5°C (≈1 Standard Deviation) in outdoor temperatures

[2] Hazard ratios estimated per an increase of 20 consultations per week for influenza-like illness in primary care

Table 8.7 Results from time-varying covariates survival models: associations of outdoor mean temperature and other risk factors with CVD mortality in the BRHS during 1998-2000 and 31/10/2014

Note: Estimates are reported as Hazard Ratios (HR) with 95% Confidence Intervals. Blank cells for SBP, IL-6, LDL and FEV1/FVC % mean they were not used in the model.

	Model 1, n= 4196, deaths=749		Model 2, n=4185, deaths=746		Model 3, n=4037 , deaths=706		Model 4, n=3972 , deaths=686		Model 5, n=4167 , deaths=740	
Covariables	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
Mean Temperature [1]	**1.104 (1.035; 1.168)**	**0.004**	**1.106 (1.037; 1.171)**	**0.003**	**1.094 (1.022; 1.161)**	**0.012**	**1.078 (1.003; 1.147)**	**0.042**	**1.104 (1.034; 1.168)**	**0.004**
ILI [2]	1.026 (0.946; 1.114)	0.525	1.028 (0.947; 1.116)	0.508	1.037 (0.953; 1.127)	0.410	1.033 (0.946; 1.125)	0.481	1.030 (0.949; 1.119)	0.480
Age, years	**1.146 (1.131; 1.162)**	**<0.001**	**1.142 (1.126; 1.158)**	**<0.001**	**1.142 (1.126; 1.159)**	**<0.001**	**1.152 (1.136; 1.169)**	**<0.001**	**1.139 (1.122; 1.155)**	**<0.001**
Social class (ref: non-manual)										
Manual	**1.115 (0.962; 1.293)**	0.149	1.134 (0.977; 1.315)	0.098	1.110 (0.953; 1.294)	0.179	1.134 (0.971; 1.324)	0.112	1.069 (0.921; 1.241)	0.380
Armed forces	1.160 (0.733; 1.835)	0.526	1.201 (0.760; 1.897)	0.432	1.123 (0.691; 1.826)	0.639	1.078 (0.662; 1.756)	0.761	1.109 (0.704; 1.747)	0.656
BMI	1.020 (1.000; 1.040)	0.055	1.016 (0.996; 1.037)	0.116	1.012 (0.991; 1.033)	0.277	1.016 (0.994; 1.038)	0.149	1.020 (1.000; 1.040)	0.046
Smokers vs not	**1.539 (1.243; 1.906)**	**<0.001**	**1.558 (1.258; 1.928)**	**<0.001**	**1.416 (1.131; 1.773)**	**0.002**	**1.584 (1.267; 1.979)**	**<0.001**	**1.452 (1.169; 1.803)**	**0.001**
Married vs not	1.176 (0.992; 1.394)	0.062	**1.189 (1.003; 1.410)**	**0.046**	1.148 (0.962; 1.368)	0.126	**1.212 (1.015; 1.447)**	**0.034**	1.151 (0.970; 1.367)	0.108
Lipid-regulating drugs use, yes vs not	**1.352 (1.082; 1.689)**	**0.008**	**1.387 (1.109; 1.735)**	**0.004**	**1.309 (1.036; 1.654)**	**0.024**	**1.536 (1.199; 1.968)**	**0.001**	**1.346 (1.078; 1.681)**	**0.009**
Physical activity (ref: Inactive)										
Occasional	**0.631 (0.509; 0.782)**	**<0.001**	**0.606 (0.488; 0.751)**	**<0.001**	**0.680 (0.544; 0.851)**	**0.001**	**0.646 (0.514; 0.812)**	**<0.001**	**0.653 (0.526; 0.811)**	**<0.001**
Light	**0.508 (0.402; 0.643)**	**<0.001**	**0.484 (0.382; 0.613)**	**<0.001**	**0.525 (0.410; 0.671)**	**<0.001**	**0.491 (0.383; 0.630)**	**<0.001**	**0.535 (0.422; 0.678)**	**<0.001**
Moderate	**0.512 (0.393; 0.665)**	**<0.001**	**0.499 (0.384; 0.649)**	**<0.001**	**0.526 (0.399; 0.692)**	**<0.001**	**0.495 (0.374; 0.654)**	**<0.001**	**0.559 (0.429; 0.728)**	**<0.001**
Moderately vigorous	**0.539 (0.416; 0.699)**	**<0.001**	**0.513 (0.396; 0.664)**	**<0.001**	**0.611 (0.466; 0.801)**	**<0.001**	**0.553 (0.422; 0.725)**	**<0.001**	**0.595 (0.459; 0.773)**	**<0.001**
Vigorous	**0.396 (0.297; 0.527)**	**<0.001**	**0.379 (0.284; 0.506)**	**<0.001**	**0.444 (0.329; 0.600)**	**<0.001**	**0.406 (0.302; 0.547)**	**<0.001**	**0.440 (0.328; 0.589)**	**<0.001**
SBP, mm Hg			**1.006 (1.003; 1.009)**	**<0.001**						
Log(IL-6), pg/mL					**1.410 (1.273; 1.563)**	**<0.001**				
LDL, mmol/L							**1.115 (1.035; 1.202)**	**0.004**		
FEV_1/FVC %									1.003 (0.997; 1.009)	0.360

[1] Hazard ratios estimated per a decrease of 5°C (≈1 Standard Deviation) in outdoor temperatures

[2] Hazard ratios estimated per an increase of 20 consultations per week for influenza-like illness in primary care

Table 8.8 Results from time-varying covariates survival models: associations of outdoor mean temperature and other risk factors with respiratory mortality in the BRHS during 1998-2000 and 31/10/2014

Note: Estimates are reported as Hazard Ratios (HR) with 95% Confidence Intervals. Blank cells for SBP, IL-6, LDL and FEV1/FVC % mean they were not used in the model.

	Model 1, n= 4196, deaths=256		Model 2, n=4185, deaths=254		Model 3, n=4037 , deaths=245		Model 4, n=3972 , deaths=237		Model 5, n=4167 , deaths=250	
Covariables	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
Mean Temperature [1]	**1.110 (1.002; 1.207)**	**0.047**	1.108 (0.998; 1.206)	0.053	**1.125 (1.016; 1.221)**	**0.026**	**1.115 (1.003; 1.213)**	**0.043**	1.108 (0.998; 1.206)	0.054
ILI [2]	1.107 (0.998; 1.229)	0.055	**1.112 (1.002; 1.231)**	**0.045**	**1.114 (1.004; 1.236)**	**0.041**	1.110 (0.992; 1.241)	0.066	**1.121 (1.014; 1.236)**	**0.024**
Age, years	**1.178 (1.150; 1.206)**	**<0.001**	**1.179 (1.150; 1.208)**	**<0.001**	**1.169 (1.140; 1.200)**	**<0.001**	**1.170 (1.141; 1.200)**	**<0.001**	**1.155 (1.126; 1.185)**	**<0.001**
Social class (ref: non-manual)										
Manual	1.118 (0.864; 1.446)	0.398	1.106 (0.854; 1.432)	0.447	1.160 (0.892; 1.509)	0.268	1.175 (0.901; 1.533)	0.234	0.948 (0.728; 1.235)	0.693
Armed forces	1.677 (0.893; 3.150)	0.108	1.612 (0.811; 3.203)	0.173	1.767 (0.903; 3.457)	0.097	1.776 (0.932; 3.384)	0.081	1.311 (0.682; 2.518)	0.416
BMI	0.970 (0.930; 1.012)	0.156	0.971 (0.930; 1.014)	0.179	0.964 (0.924; 1.005)	0.086	0.966 (0.925; 1.009)	0.116	0.984 (0.946; 1.023)	0.415
Smokers vs not	**2.335 (1.678; 3.249)**	**<0.001**	**2.313 (1.656; 3.231)**	**<0.001**	**2.028 (1.427; 2.883)**	**<0.001**	**2.268 (1.607; 3.202)**	**<0.001**	**1.766 (1.246; 2.503)**	**0.001**
Married vs not	1.076 (0.797; 1.454)	0.631	1.074 (0.794; 1.453)	0.642	1.071 (0.786; 1.460)	0.665	1.055 (0.766; 1.451)	0.744	1.022 (0.754; 1.385)	0.889
Lipid-regulating drugs use, yes vs not	0.814 (0.511; 1.295)	0.385	0.803 (0.505; 1.278)	0.355	0.803 (0.493; 1.306)	0.376	0.689 (0.411; 1.157)	0.159	0.783 (0.491; 1.250)	0.305
Physical activity (ref: Inactive)										
Occasional	**0.633 (0.444; 0.903)**	**0.012**	**0.645 (0.451; 0.924)**	**0.017**	**0.643 (0.443; 0.933)**	**0.020**	**0.633 (0.436; 0.917)**	**0.016**	**0.736 (0.514; 1.054)**	**0.094**
Light	**0.461 (0.310; 0.684)**	**<0.001**	**0.474 (0.318; 0.707)**	**<0.001**	**0.488 (0.324; 0.735)**	**0.001**	**0.477 (0.316; 0.720)**	**<0.001**	**0.611 (0.409; 0.914)**	**0.017**
Moderate	**0.349 (0.218; 0.560)**	**<0.001**	**0.355 (0.221; 0.570)**	**<0.001**	**0.383 (0.237; 0.619)**	**<0.001**	**0.347 (0.213; 0.566)**	**<0.001**	**0.507 (0.314; 0.818)**	**0.005**
Moderately vigorous	**0.205 (0.116; 0.361)**	**<0.001**	**0.209 (0.118; 0.369)**	**<0.001**	**0.240 (0.135; 0.427)**	**<0.001**	**0.223 (0.126; 0.395)**	**<0.001**	**0.301 (0.169; 0.536)**	**<0.001**
Vigorous	**0.252 (0.148; 0.429)**	**<0.001**	**0.243 (0.141; 0.418)**	**<0.001**	**0.271 (0.157; 0.468)**	**<0.001**	**0.259 (0.150; 0.449)**	**<0.001**	**0.393 (0.233; 0.662)**	**0.001**
SBP, mm Hg			0.997 (0.991; 1.002)	0.270						
Log(IL-6), pg/mL					**1.442 (1.197; 1.738)**	**<0.001**				
LDL, mmol/L							**0.837 (0.725; 0.967)**	**0.016**		
FEV_1/FVC %									**0.972 (0.962; 0.983)**	**<0.001**

[1] Hazard ratios estimated per a decrease of 5°C (≈1 Standard Deviation) in outdoor temperatures
[2] Hazard ratios estimated per an increase of 20 consultations per week for influenza-like illness in primary care

Table 8.9 Results from time-varying covariates survival models: associations of outdoor mean temperature and other risk factors with all-cause mortality in the BRHS during 1998-2000 and 31/10/2014

Note: Estimates are reported as Hazard Ratios (HR) with 95% Confidence Intervals. Blank cells for SBP, IL-6, LDL and FEV1/FVC % mean they were not used in the model.

	Model 1, n= 4196, deaths=1991		Model 2, n=4185, deaths=1983		Model 3, n=4037 , deaths=1891		Model 4, n=3972 , deaths=1839		Model 5, n=4167 , deaths=1969	
Covariables	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
Mean Temperature [1]	**1.059 (1.016; 1.100)**	**0.008**	**1.058 (1.015; 1.099)**	**0.008**	**1.054 (1.010; 1.097)**	**0.016**	1.042 (0.997; 1.086)	0.068	**1.056 (1.013; 1.097)**	**0.012**
ILI [2]	1.033 (0.984; 1.081)	0.196	1.033 (0.986; 1.083)	0.177	1.039 (0.990; 1.090)	0.122	1.035 (0.986; 1.088)	0.170	1.039 (0.990; 1.088)	0.119
Age, years	**1.122 (1.112; 1.131)**	**<0.001**	**1.120 (1.111; 1.130)**	**<0.001**	**1.115 (1.105; 1.125)**	**<0.001**	**1.122 (1.113; 1.132)**	**<0.001**	**1.111 (1.102; 1.121)**	**<0.001**
Social class (ref: non-manual)										
Manual	**1.097 (1.001; 1.202)**	**0.047**	**1.101 (1.005; 1.206)**	**0.040**	1.081 (0.983; 1.188)	0.107	**1.100 (1.000; 1.210)**	**0.050**	1.048 (0.957; 1.149)	0.313
Armed forces	1.271 (0.984; 1.641)	0.066	**1.301 (1.005; 1.686)**	**0.046**	1.272 (0.968; 1.670)	0.084	1.237 (0.949; 1.612)	0.115	1.196 (0.927; 1.544)	0.167
BMI	0.999 (0.987; 1.012)	0.879	0.998 (0.986; 1.011)	0.791	0.994 (0.981; 1.007)	0.371	0.997 (0.984; 1.011)	0.686	1.001 (0.988; 1.013)	0.927
Smokers vs not	**1.686 (1.485; 1.914)**	**<0.001**	**1.694 (1.493; 1.921)**	**<0.001**	**1.534 (1.341; 1.756)**	**<0.001**	**1.674 (1.465; 1.913)**	**<0.001**	**1.535 (1.350; 1.746)**	**<0.001**
Married vs not	**1.135 (1.023; 1.260)**	**0.017**	**1.135 (1.023; 1.260)**	**0.017**	**1.116 (1.003; 1.243)**	**0.045**	**1.144 (1.026; 1.275)**	**0.016**	1.106 (0.996; 1.229)	0.059
Lipid-regulating drugs use, yes vs not	0.891 (0.766; 1.036)	0.134	0.896 (0.771; 1.043)	0.157	0.854 (0.729; 1.000)	0.051	0.889 (0.751; 1.053)	0.173	0.873 (0.751; 1.014)	0.075
Physical activity (ref: Inactive)										
Occasional	**0.649 (0.566; 0.743)**	**<0.001**	**0.639 (0.558; 0.733)**	**<0.001**	**0.666 (0.579; 0.767)**	**<0.001**	**0.650 (0.564; 0.749)**	**<0.001**	**0.680 (0.594; 0.779)**	**<0.001**
Light	**0.563 (0.487; 0.651)**	**<0.001**	**0.556 (0.480; 0.643)**	**<0.001**	**0.572 (0.492; 0.665)**	**<0.001**	**0.551 (0.473; 0.641)**	**<0.001**	**0.608 (0.526; 0.703)**	**<0.001**
Moderate	**0.503 (0.428; 0.590)**	**<0.001**	**0.500 (0.425; 0.587)**	**<0.001**	**0.523 (0.443; 0.617)**	**<0.001**	**0.500 (0.422; 0.592)**	**<0.001**	**0.560 (0.475; 0.659)**	**<0.001**
Moderately vigorous	**0.542 (0.461; 0.638)**	**<0.001**	**0.536 (0.455; 0.631)**	**<0.001**	**0.581 (0.491; 0.688)**	**<0.001**	**0.538 (0.454; 0.639)**	**<0.001**	**0.613 (0.520; 0.723)**	**<0.001**
Vigorous	**0.457 (0.387; 0.540)**	**<0.001**	**0.450 (0.381; 0.533)**	**<0.001**	**0.495 (0.416; 0.588)**	**<0.001**	**0.467 (0.393; 0.555)**	**<0.001**	**0.523 (0.441; 0.620)**	**<0.001**
SBP, mm Hg			1.002 (1.000; 1.004)	0.080						
Log(IL-6), pg/mL					**1.330 (1.242; 1.424)**	**<0.001**				
LDL, mmol/L							1.001 (0.954; 1.050)	0.974		
FEV_1/FVC %									**0.995 (0.991; 0.998)**	**0.012**

[1] Hazard ratios estimated per a decrease of 5°C (≈1 Standard Deviation) in outdoor temperatures
[2] Hazard ratios estimated per an increase of 20 consultations per week for influenza-like illness in primary care

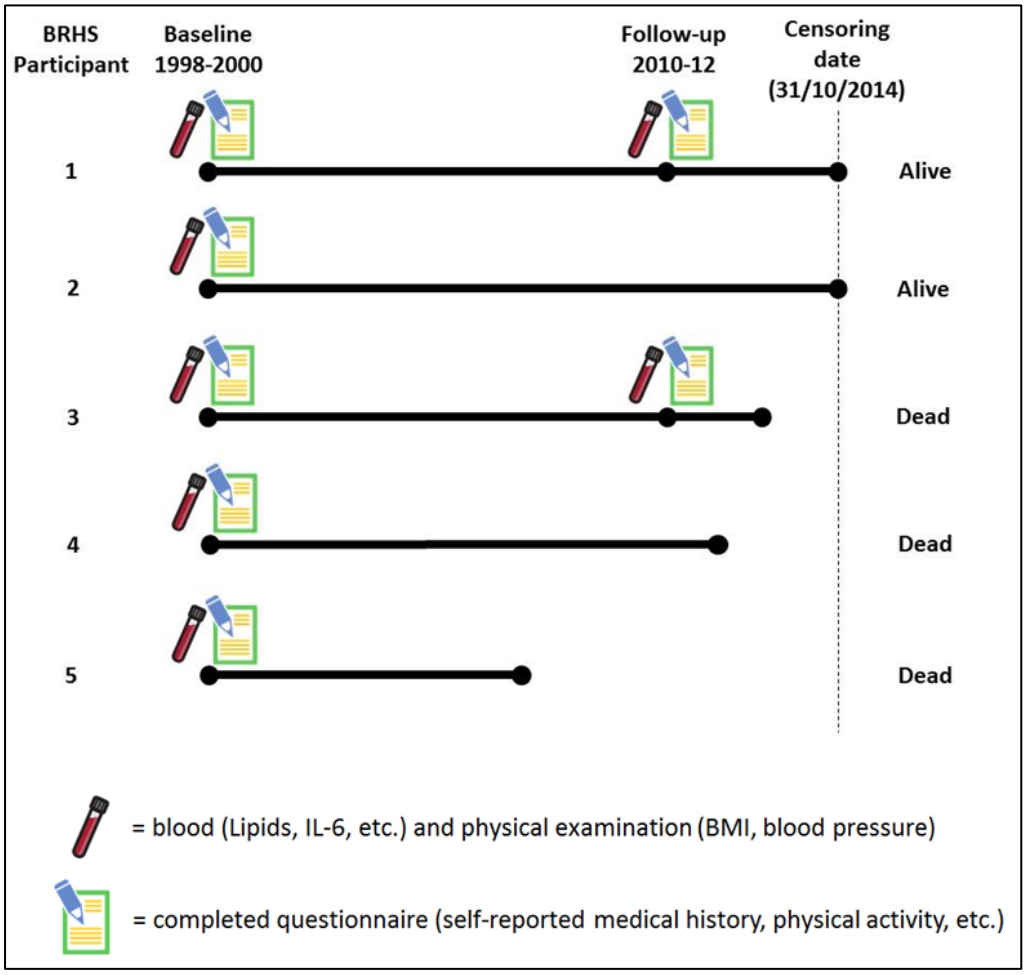
Table 8.10 Results from time-varying covariates survival models: unadjusted and mutually adjusted estimates (β coefficients with p-values) for mean outdoor temperature, England and Wales Influenza-like illness (ILI) consultation rate per 100,000 people, and long terms seasonal trends fitted using a sine wave function of day of the year and by using Fourier terms (sine + cosine functions).

	CHD death		Stroke death		CVD death		Respiratory death		All causes	
Unadjusted Models	β coeff	p-value	β coeff	p-value	β coeff	p-value	β coeff	p-value	β coeff	p-value
Model 1: Temperature	-0.029	0.001	-0.020	0.229	-0.025	0.000	-0.030	0.011	-0.015	0.001
Model 2: ILI	0.004	0.025	0.004	0.222	0.003	0.076	0.007	0.005	0.003	0.017
Model 3: Sine wave component [1]	-0.169	0.013	-0.151	0.199	-0.150	0.004	-0.247	0.009	-0.103	0.001
Model 4: Seasonal variation (from cosinor function) [2]	0.342	0.042	0.370	0.311	0.323	0.008	0.519	0.022	0.224	0.002
Mutually adjusted models										
Model 5: Temperature + ILI										
Temperature	-0.027	0.0051	-0.016	0.366	-0.024	0.002	-0.024	0.045	-0.013	0.004
ILI coefficient	0.0021	0.3927	0.003	0.498	0.001	0.727	0.005	0.063	0.001	0.301
Model 6: Temperature + Sine wave component										
Temperature	-0.0279	0.0213	-0.011	0.617	-0.021	0.030	-0.014	0.400	-0.010	0.099
Sine wave component [1]	-0.0228	0.8057	-0.091	0.568	-0.041	0.559	-0.172	0.192	-0.051	0.253
Model 7: Temperature + Cosinor function										
Temperature	-0.0415	0.010	0.002	0.937	-0.023	0.074	-0.007	0.727	-0.008	0.331
Seasonal variation (from cosinor function) [2]	0.248	0.438	0.399	0.645	0.069	0.815	0.430	0.376	0.131	0.475
Piecewise linear splines models Model 8: Temperature (piecewise linear splines)										
Cold component (temperature ≤97.5 pct)	-0.0301	0.0012	-0.022	0.200	-0.026	0.000	-0.036	0.002	-0.016	0.000
Heat component (temperature >97.5 pct)	-0.0199	0.9465	0.125	0.658	0.054	0.753	0.347	0.007	0.097	0.227

[1] The sine wave with a period of 365 days, with fixed minimum to occur on day 1 (January 1) and maximum at day 182 (July 1).The formula for the sine wave with modified period and phase was $y(t)=\sin((2*\pi/365)*(x-81.75))$, where x varied between 1 and 365

[2] Beta coefficient (β coeff)of seasonal variation were calculated by using the betas coefficient from the Fourier terms (*s* for sine and *c* for cosine term). Statistical significance of the sinusoidal parameters was determined by the F-test. If the hypothesis that both coefficients are different from zero is rejected, there is no sinusoidal seasonal variation. Otherwise, the seasonal variation is 2x season amplitude, where seasonal amplitude is the square root of ($s^2 + c^2$). β coeff = 0.342 for CHD means 34% variation in CHD mortality when comparing the peak vs nadir of the sinusoidal function, where the peak is in the winter months.

Figure 8.1 Data used in the time-varying covariates survival model in the BRHS



Note: For the entire follow-up period and for each of the BRHS men, exposure to outdoor mean temperature was collected daily and assigned to the men by using the closest weather station to their town of residence.. Individual factors were collected once or twice over the time period (1998-2000 and 2010-12), depending on the men follow-up exit date and participants' attendance to each of the examinations. Participants were followed-up for mortality up to 31/10/2014.

Figure 8.2 Sine wave function of day of the year describing a period of 365 days, where fixed minimum to occur on day 1 (January 1) and maximum at day 182 (July 1).

The formula is: function(DOY)=sine((2*π/365)*(DOY − 81.75)), where DOY (X axis) is a value between 1 and 365.

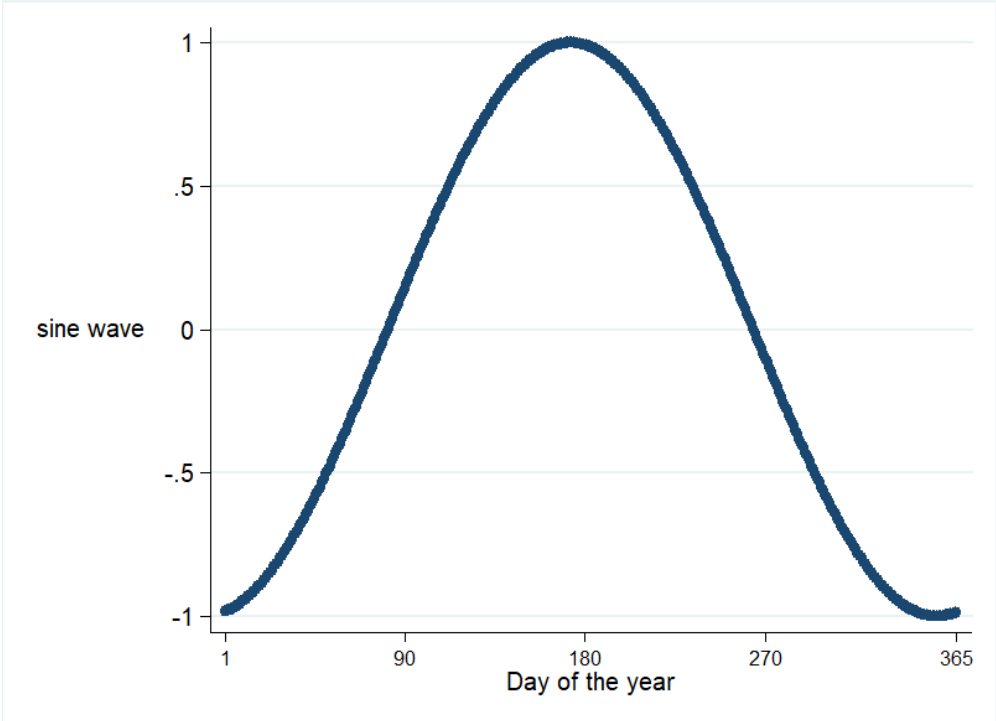


Figure 8.3 The Fourier terms representing pairs of sine and cosine functions of day of the year (x axis), with an underlying period reflecting the full seasonal cycle.

The formulas are: (i) sine function(DOY) = sine((2*π*DOY/365); (ii) cosine function(DOY)=cosine((2*π*DOY/365), where DOY is the day of the year (X axis, a value between 1 and 365).

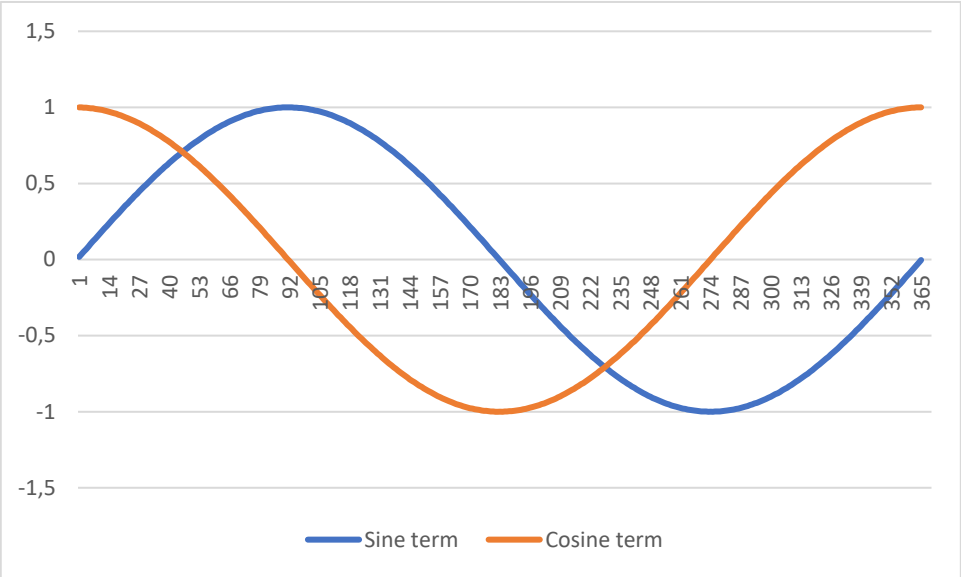


Figure 8.4 Mean temperature (°C) vs day of the year during 1998-2014. Temperatures data were measured daily by the UK Meteorological Office in 35 UK towns

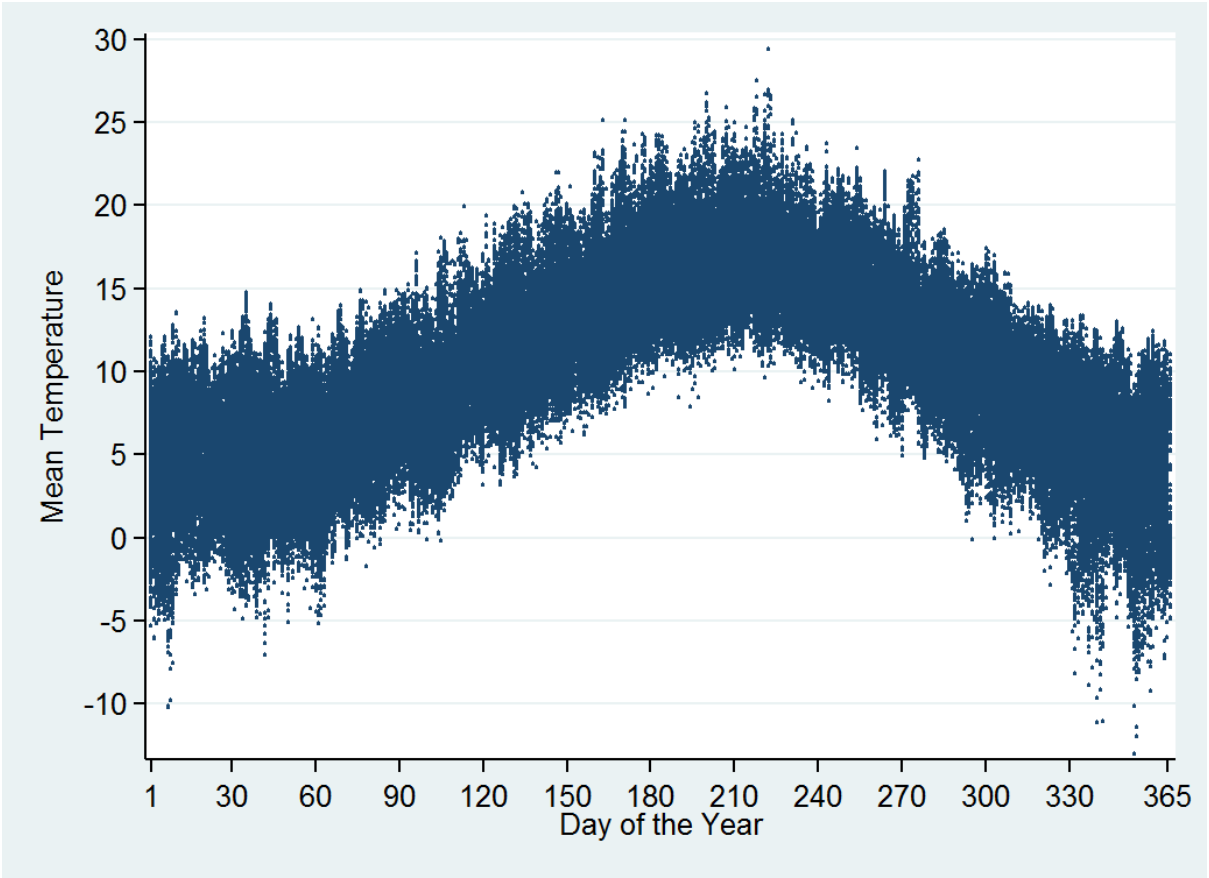


Figure 8.5 Influenza-like Illness (ILI) weekly consultation rate per 100,000 persons in England and Wales vs average outdoor mean temperature measured the same week during 1999-2014

ILI data were collected by the Royal College of General Practitioners in England and by Public Health Wales, while temperature data were collected by the UK Meteorological Office from 35 UK weather stations

Chapter 9 **IMPLICATIONS AND CONCLUSIONS**

9.1 Summary

This chapter reviews the key findings of this thesis and their implications in relation to public health and future epidemiological research. The findings can be separated according to the importance of the research questions for this thesis in "seasonal variation findings" and "diurnal variation findings".

Seasonal variation findings: in older British men 1) the CVD mortality risks are higher at lower temperatures (recorded on the day of death); cumulative associations of temperature with CVD mortality up to and including 2 weeks prior to the date of death were also observed; 2) major risk factors measured at study baseline did not modify the temperature-mortality relationship; 3) the observed increase in CVD mortality at lower temperatures was mainly driven by increase in CHD mortality; 4) a decrease of outdoor temperatures was associated with an increase of established and emerging CVD risk factors levels (e.g. blood pressure and IL-6) and with a decrease in physical activity. In addition, it was also found that lower outdoor temperatures were associated with increased respiratory and all-cause mortality in older men, and up to 2 weeks prior to date of death. Respiratory mortality risks also increased at higher temperatures.

Diurnal variation findings: several biological CVD risk factors levels varied by time of the day; for example, blood pressure, LDL-cholesterol and IL-6 increased over the course of the day. Also, physical activity levels of different intensities (e.g. time spent in light physical activity and sedentary behaviours) varied over the course of the day. For example, the time spent in sedentary behaviours was lower in the morning, and gradually increased over the course of the day.

Findings of potential public health importance are: i) that it is important for policy-makers, epidemiologists and health economists to consider in future policy and research the impact of seasonal variations in outdoor temperatures on CVD, respiratory, and all-cause mortality in older adults, especially the impact of lower

temperatures during winter; ii) that efforts are needed to maintain regular physical activity levels across seasons in older adults; iii) that further efforts are needed to encourage older men to be more physically active throughout the day. In summary, the key general public health messages are the need to protect all older people against low temperatures, especially in winter, and to promote more active behaviours across seasons and on a daily basis. This would help to reduce CVD in older age.

The implications for future epidemiological research emerging from findings of this thesis can be separated in two main areas:

Main implications for research investigating seasonal variation in CVD mortality: 1) the need to carry out larger observational studies investigating the seasonal variation in CVD and all-cause mortality in both males and females of all ages, in people of different ethnic groups, and in countries with different climates and at different latitudes; 2) to further explore the causal pathways of seasonal variations in CVD mortality, with a particular focus on the role of outdoor temperature, influenza, and modifiable CVD risk factors in such pathways (especially physical activity and blood pressure); 3) in interventional studies, to demonstrate that engaging in regular physical activity across different temperature ranges and across the year yet limiting the exposure to cold outdoor temperatures is possible 4) in cohort studies, exploring whether incidence of CVD events is lower among older adults who are able to maintain the same level of activity across the seasons in comparison to those who become less active in winter 5) improving existing studies (see point 2) by increasing the frequency and accuracy of CVD risk factors measurements, such as continuous recording of individual temperature exposure (e.g. at home, or indoor/outdoor environments), physical activity levels, and blood pressure by using wearable devices and smartphones.

Main implications for research investigating diurnal variation in CVD mortality: 1) to further explore the causal pathways of diurnal variations in CVD mortality, with a particular focus on the role of blood pressure and physical activity in such pathways;

2) in physical activity studies, exploring whether incidence of CVD events is lower among older adults who are typically more active in the morning vs older adults who have different patterns; 3) in physical activity studies, exploring whether the occurrence of CVD events in the morning, afternoon or evening is more frequent among older adults who are typically more active in the morning than in older adults who are not.

9.2 Introduction

9.2.1 The studies carried out in this thesis

The research in this thesis has addressed several important questions on seasonal and diurnal variations in CVD risk factors and seasonal variations in CVD mortality, respiratory mortality and all-cause mortality in older men. To answer the research questions of this thesis, data collected from an ongoing UK population based study of CVD in older men, the British Regional Heart Study (BRHS), were used. In total, five studies were conducted and their findings were presented in Chapters 4, 5, 6, 7, and 8.

The BRHS data collection spanned a period of almost 40 years (from 1978-80 to the present) and the five studies carried out in this thesis did not all use data from the same time points over this period. The reasons are as follows:

- the two studies on diurnal and seasonal patterns in objectively measured physical activity (Chapter 4 and 5) used data collected during 2010-12, the first time point at which accelerometers were used and for which there is a wide range of other data available.
- the two studies on diurnal and seasonal patterns in established and emerging CVD risk factors (Chapter 6 and 7) used data collected in 1998-2000 as at this time point I could analyse a larger sample of older adults, with the most comprehensive range of relevant factors (e.g. Vitamin D has not been measured for blood samples taken at the 2010-12 follow-up)
- the time-varying survival analysis of associations between temperature and mortality was carried out using follow-up data from 1998-2000 to 2014

(Chapter 8); by study design, this approach exploited all information available and relevant to this thesis' hypothesis (risk factors and individual characteristics from the two follow-ups plus outdoor temperature data collected from 1998 to 2014). This approach also gave sufficient time for large numbers of events to occur.

9.2.2 How the key findings from the studies are presented

I will present in the next paragraph (9.3 – key findings) the results from the 5 studies mentioned in the introduction prioritising the importance of the research questions for this thesis.

1) Seasonal variations findings: results from the survival analysis estimating the association between outdoor temperature (main seasonal factor and exposure variable) and variations in CVD mortality, respiratory mortality and all-cause mortality in BRHS men (Chapter 8); next, results from the cross-sectional studies estimating the seasonal variations in objectively measured physical activity (Chapters 5) and seasonal variations in established and emerging risk factors for CVD (Chapter 7) in BRHS men;

2) Diurnal variations findings: results from the cross-sectional studies on diurnal variations in objectively measured physical activity (Chapter 4), and diurnal variations in established and emerging risk factors for CVD (Chapter 6) in BRHS men.

9.3 Key findings

I will summarise in paragraphs 9.3.1 and 9.3.2 the main findings from this PhD thesis; the public health implications of such findings will be discussed later in Chapter 9.5.

9.3.1 Findings on seasonal variations in CVD, respiratory and all-cause mortality and seasonal variation in CVD risk factors

In Chapter 8 I used an appropriate method to exploit the BRHS data and enhance our understanding of the association between outdoor mean temperatures (main seasonal factor and exposure variable analysed in this thesis) and mortality. Specifically, the survival analysis conducted in Chapter 8 fitted outdoor temperature and influenza rates as time-varying exposure variables, while allowing adjustment for confounding of temperature with influenza over the follow-up time. Moreover, due to data collection at individual level in the BRHS, this approach allowed testing the long-term interaction of temperature with baseline participants' socio-demographic characteristics (age, social class, marital status) or behavioural factors (e.g. smoking, or degree of physical activity). The findings demonstrated that lower outdoor temperature was associated with an increase in CVD, respiratory, and all-cause mortality. Also, associations of temperature up to 2 weeks prior to date of death were observed. The adjustment of temperature for individual risk factors slightly reduced the magnitude of the association of temperature with mortality by at most 1%. Overall, there was no evidence to suggest that the association of temperature with mortality was modified by individual characteristics.

Findings from Chapter 5 showed that seasonal variations in outdoor temperature influenced the levels of physical activity; for example during a typical winter day (mean temperature in between - between -7.1°C and 6.4°C) older men spent about 20 minutes more per day in sedentary time in comparison with a typical summer day (mean temperatures in between 16.2°C and 24.4°C). Chapter 7 showed that lower temperatures were associated with increased levels of several risk factors for CVD, such as Systolic Blood Pressure (SBP), LDL-Cholesterol, and with emerging risk factors as Interleukin-6 (IL-6).

Overall, findings from Chapter 5, 7, and 8 showed that while the association of temperature with mortality and the association of temperature with some CVD risk

factors are strong, the causal pathways involving all these factors leading to increased CVD mortality cannot be ascertained by these findings.

9.3.2 Findings on diurnal variations in CVD risk factors

Findings from Chapter 4 and Chapter 6 showed that physical activity and some established and emerging CVD risk factors levels exhibited a diurnal variation in older men. Chapter 4 findings showed that men do most of their physical activity during the morning, with a peak in between 10:00-11:00 hours and a second small peak around 14:00. This suggested that there are particular opportunities to prolong or enhance existing activity bouts (e.g. in light physical activity) during the morning or alternatively reducing sedentary time in the afternoon and evening hours. Chapter 6 showed that time of day variations of some established and emerging CVD factors exist. For example, blood pressure, IL-6 and cholesterol levels increased while t-PA levels decreased.

9.4 Novelty of the present findings

9.4.1 Novel findings on seasonal variations in CVD risk

The results presented in Chapter 5 and 7 provided new insights on temperature-related patterns in risk factors which have been less studied in the literature. Chapter 5 extends the literature by investigating objectively measured physical activity levels of different intensities (e.g. time spent in sedentary behaviours and light physical activity), rather than overall measures of physical activity, such as number of steps. The novel part of Chapter 7 was the inclusion of markers of inflammation, which were less studied (e.g. IL-6, CRP, and Vitamin D) or previously not studied (PV and t-PA) among older adults. Moreover, a side-by-side comparison of temperature-related variations of such a comprehensive list of markers was not performed in previous studies (17 in total). This allowed me to (i) distinguish between those markers associated with temperatures vs not in the same population, and (ii) identify which markers are related to short term vs long-term variations in temperature, or both. The main novelties of Chapter 8 (association of temperature with mortality) are two: 1) the use of a method applied to data collected over time and at individual level (time-varying survival analysis); and

2) testing whether the association of temperature with mortality was modified by individual risk factors. Only data collected at individual level allowed such hypotheses to be tested.

9.4.2 Novel findings on diurnal variations in CVD risk factors

Chapter 4 extends the literature regarding diurnal variations in objectively measured physical activity levels in older adults analysing levels of intensity not investigated in earlier studies (time spent in sedentary behaviours, light, and moderate-to-vigorous physical activity), and by identifying a clear peak in activity in the morning hours. Moreover, this work demonstrated how diurnal variations in activity differed by individual characteristics; four key variables (age, presence of multiple chronic conditions, having mobility limitations and being obese) had a disproportionate impact on the morning peak of activity, such that the oldest, obese, least healthy and least mobile men had a greater reduction in the morning peak in activity than in the afternoon and evening. Chapter 6 enhances our understanding of time of day variations in markers of inflammation and haemostasis extending the list of markers analysed in comparison with previous studies, and reports analysis in older adults rather than middle aged populations. Similarly to Chapter 7, a side-by-side comparison of the diurnal patterns of 17 markers was performed and this was not done in earlier studies.

9.5 Public health implications of findings

9.5.1 Implications of findings for CVD and all-cause mortality risks reduction at lower temperatures

The main discussion point emerging from the overall findings of Chapter 5, 7, and 8 is to understand whether the association temperature-CVD mortality is likely to be causal, and what are the possible implications of understating this. I concluded that it is unlikely that exposure to lower outdoor temperature is the direct cause of death; other CVD risk factors (e.g. blood pressure) are likely to be on the causal pathway leading to CVD death because they are influenced by changes in outdoor temperature (and this was demonstrated in Chapter 5 and 7). However, the causality of such

pathways could not be ascertained in this thesis (e.g. whether old people die from CVD because of cold temperature-related increase in high blood pressure, or because of cold temperature-induced inactivity). Despite not fully understanding the causal pathways of seasonal variation in CVD mortality (but also respiratory mortality and all-cause mortality), some general public health implications can be suggested. First, limiting exposure to lower temperatures in older adults should be highly recommended for mortality risk prevention. Second, recommendations to protect against cold temperatures should be extended to the UK older population as a whole; providing special recommendations to protect sub-groups of the older population do not find enough justification based on my findings from Chapter 8. other findings from the same Chapter suggested that protecting old people from colder - rather than warmer - temperatures remains the priority for CVD prevention. On the other hand, warmer temperatures were associated with respiratory mortality; therefore, prevention strategies for reducing respiratory mortality should be extended to both lower and higher temperatures. Specifically for CVD, findings from Chapter 5 and 7 cannot be used to inform ad-hoc preventive measures to decrease the levels of some specific CVD risk factors at lower temperatures. To do so, it would be necessary to know whether:

(i) some risk factors (e.g. blood pressure) increase rapidly due to sudden drops in temperature, and this leads immediately to CVD events;
(ii) some risk factors increase at lower temperature (e.g. cholesterol levels or inactivity) but this just accumulates risk, and CVD events can then happen at any time;
(iii) chronic cardiovascular and respiratory diseases that are generally more common in older age interact with seasonal factors occurring in winter, such as drops in temperatures or influenza, leading immediately to CVD events in winter.

The findings from Chapter 5 are relevant for UK physical activity guidelines and for overall CVD prevention: engaging in more active behaviours across different temperature ranges and across the year yet limiting the exposure to cold outdoor

temperatures (as well as staying warm yet limiting the time spent sedentary) can be certainly beneficial for improving people's health. However, how to do this has been not yet demonstrated (see paragraph 9.6 for further discussion).

Overall, findings from Chapter 7 and 8 do not provide enough justification for the preventive use of medication for specific biological modifiable risk factor control (e.g. medications to lower blood pressure, lipids levels, or inflammation) during exposure to low temperature. For example, while adults who are taking aspirin vs not seemed less vulnerable to the effects of cold (lower hospital admissions for myocardial infarction) (37), but whether this is due to changes in platelet function, and for hypertensive patients only, is unknown: more findings from interventional studies are needed to recommend such approach for CVD prevention (see paragraph 9.6 for further discussion).

9.5.2 Implications of physical activity findings for overall CVD and all-cause mortality risks reduction

The main public health implication of findings from Chapter 4 and 5 (diurnal and seasonal variation in physical activity levels) is that understanding when peaks and dips in physical activity levels occur is useful for promoting regular physical activity; this is important because engaging in regular exercise could reduce the overall CVD and mortality risks and improving life expectancy. Successful intervention strategies aiming to increase physical activity levels or changing older adults' daily routine from less active to more active behaviours depend on evidence-based findings like those shown in Chapter 4 and 5. Findings from Chapter 4 showed an attenuation of the diurnal morning peak of activity among less active subgroups (e.g. older and more infirm men). This reflects their diminished ability to maintain relatively high intensity physical activity during the morning, and this is not simply related to the generally low physical activity level typical of these subgroups. This information is important for policy and practice because there is scope to extend the tendency for existing activity bouts especially in the morning. Indeed, it is unlikely that low levels of activity in the evening can be changed, since the combination of darkness and visual problems have been previously investigated as potential causes of falls. Likewise with sedentary

behaviours, findings from Chapter 4 suggest that the period in the late afternoon and early evenings are periods with high sedentary time and when sedentary bouts are likely to be longest; therefore, it may be particularly valuable to focus on efforts to break up long sedentary bouts at these times of day. Examples of potential strategies on how to do this are discussed in paragraph 9.5.4. Findings from Chapter 5 showed that older men are less active at lower temperatures and the public health implication of this was discussed in paragraph 9.5.1.

9.5.3 Implications of diurnal variations in biological risk factors findings for overall CVD and all-cause mortality risks prediction

Although the findings from Chapter 6 did not demonstrate that variations within the day in CVD risk factors immediately cause CVD death, important considerations should be made. As of today, the biological mechanism of the circadian variation in CVD events remains not fully understood but the circadian variation in blood pressure seems to be involved (352). Previous evidence supported a possible relationship between the morning surge in blood pressure and cardiovascular events connected with the upright posture after the awakening (48). However, how to regulate blood pressure over 24 hours (e.g. via medical treatment), and whether this can reduce CVD events in the morning is still unclear. On the other hand, it is unlikely that changes in LDL-cholesterol levels within the day immediately cause CVD events, as the accumulation of cholesterol in plaques usually occurs over several years before becoming life-threatening. However, it is plausible that dietary changes in the short period (a few days or months) can explain changes in LDL-cholesterol levels, but this would just accumulate risk, and CVD events can then happen at any time. For IL-6, there is still uncertainty surrounding its diurnal variation patterns, as findings from previous studies showed different peaks and dips of IL-6 during the day; despite the fact high IL-6 levels are associated with high CVD risk in the long term, as of today the evidence to support that diurnal variation in IL-6 explains the diurnal variation in CVD events is weak.

9.5.4 Potential strategies for CVD and all-cause mortality risk reduction at lower temperatures

I identified six strategies that could help in reducing the mortality risks due to exposure to lower temperatures.

1) Improvements of national communication platforms predicting adverse seasonal events (e.g. cold spells) and raising awareness of their consequences;

2) strategies addressing rapid heat loss from the body's core; it is known that low temperatures in winter could exert their adverse effects on individuals by causing the body to lose heat faster than it can make heat, lowering the body's temperature. Therefore, preventive strategies may focus on primary care or social care teams helping older adults during winter; recommendations on wearing proper winter clothes (dressing in several layers of loose-fitting wool, silk, polypropylene clothing, or mittens and a hat), eating well-balanced meals, or reducing alcohol and caffeine are important as they mitigate the heat loss from the body's core (353);

3) energy efficiency housing interventions addressing heat loss from the house; a recent BRHS study demonstrated that living in a cold home was associated with increased mortality risks (350). Comfortable room temperatures at home (higher than 18 degrees Celsius) can address specific risk factors such as high blood pressure, and worse lung conditions (354). This would improve people's living conditions and potentially reduce mortality risks;

4) strategies addressing lower levels of activity in winter among older adults, by providing physically and economically accessible indoor opportunities to engage in physical activities. Staying regularly active could mediate the adverse effect of low outdoor temperatures, for example by addressing higher levels of inflammation (355). Every strategy would need to maintain a trade-

off in between staying active yet limiting the exposure to cold outdoor temperatures, and staying warm yet limiting the time spent sedentary;

5) specific recommendations for improving dietary patterns during cold weather aimed to lower cholesterol levels. Cold itself does not cause the cholesterol levels to increase, but some behavioural patterns may affect them. For example, higher cholesterol levels can be induced by changes in diet during the cold season, such as eating more comfort food, which is often higher in fat. Such diet choices can be accompanied with more time spent indoors (e.g. at home), and more time spent sedentary. Improving such health behaviours can potentially reduce mortality risks.

6) Strategies aimed to communicate with older people to help them monitor and manage cold-related health symptoms in winter, by using novel ad-hoc strategies such as creating apps for smartphones.

9.5.5 Potential strategies to maintain regular physical activity levels in older age and prevent CVD and all-cause mortality

Regardless of seasonal and diurnal patterns in physical activity, targeting older adults' psychological barriers (beliefs, feelings, and perspectives on participation in physical activity) may be a valid strategy for replacing sedentary time with more active behaviours (288, 289), and therefore reducing mortality risks. For example, providing recommendations for simple do-it-yourself exercises (e.g. standing up or walking while watching TV, toe rises, calf and chest stretching) could be helpful. In older individuals, simple targets can make the reduction in sedentary behaviour easier to achieve and relevant on a daily basis (290). All of the above recommendations for exercising can help to increase the intensity or duration of bouts of existing physical activities in the morning, when older adults are more active. Alternatively, ad-hoc interventions could focus on the afternoon period, aiming to stimulate physical activity of comparable intensity to that occurring in the morning. Special recommendations should be given for more infirm men, such those with mobility limitations: workouts

programs focusing on the upper part of the body alternate with office-type exercises (using chairs and tables as support) can be implemented easily even in indoor environments.

9.6 Implications for future epidemiological research

Although the findings from this thesis enhance our understanding of seasonal and diurnal patterns in CVD, there are still some considerations which call for a need to extend investigations on these variations in CVD and mortality in older age and in other populations. Future studies investigating variations in CVD can be divided in two main areas: Implications of the findings for future epidemiological research investigating (i) seasonal variation (see paragraph 9.6.1), and (ii) diurnal variation (see paragraph 9.6.2) in CVD risk and CVD risk factors.

9.6.1 Implications of the findings for future studies investigating seasonal variation in CVD risk and CVD risk factors

9.6.1.1 CVD seasonal variation studies in men and women

Future UK epidemiological studies on CVD seasonal variation should include older women, because they are an important segment of the older UK population (103), and because CVD risk prevalence is now higher in women than in previous decades (356). The advantage of including men and women in the same study (and measuring the same CVD risk factors and outcomes) is the increased generalisability of the findings, but I would not expect the magnitude of the temperature-mortality association to be substantially different in men vs women. The exposure to cold temperatures and the consequences of it for the human body, should be more an intrinsic physiological phenomenon, regardless of sex. This consideration is supported by previous findings: seasonal variations in established CVD risk factors levels measured in a large study (over 450,000 repeated measurements of risk factors in 149,650 individuals between 1985 and 1999) and found that such variations were not modified by sex (357). However, this study is old and outdoor temperature as well as other CVD risk factors (e.g. physical activity, see paragraph 9.6.1.4 for further discussion) were not measured.

Similarly, in one British study the seasonal patterns in emerging CVD risk factors did not differ by sex (45).

9.6.1.2 CVD seasonal variation studies including participants of ethnic origin, and in countries with different climate and latitudes

The BRHS towns were selected in the late 1970s because they did not have appreciable population movement (compared with cities) but they hence had a small proportion of non-white ethnic minority groups. Therefore, future studies including people from other ethnic groups are needed, to increase generalisability of the findings. There is evidence of considerable variation in CVD mortality rates by ethnic groups; for example, people of a South Asian heritage are at a greater risk of having a stroke (358). According to the 2011 Census, Scotland, the North East and Wales were the regions with the highest percentages of the population from the White British group (96%, 93% and 93% respectively), with the lowest being the West Midlands (at 79.2%), and the London area (at 44.9%) (359, 360). Additionally, the size of the foreign-born population in the UK increased from about 5.3 million in 2004 to just under 9.4 million in 2017 (361); whether newly arrived migrant older people from warmer climates will adapt rapidly to the UK's colder climate could be investigated in future studies; these trends and differences should be taken into account in future population based studies of temperature and mortality in the UK.

Worldwide, the attributable overall mortality risks due to cold appeared to be different in major cities located in different continents but without a clear pattern by ethnic heritage, climate or latitude (about 10% of overall mortality is explained by cold in China, while this percentage is 9% in Italy, 8% in the UK, 7% in South Korea, 5% in the US, 4% in Sweden, 3% in Thailand and Brazil) (35). Overall, whether ethnicity modifies the temperature-mortality relationship is unclear. In the case of older adults with South Asian heritage, it is plausible that risk factors that are generally more common in Asian groups increased their levels at lower temperatures. However, this was not yet demonstrated in the literature. Future studies could also investigate what proportion of cold-related mortality by country is explained by different national health

systems (e.g. public vs private, or systems adopting seasonal influenza vaccination programs) and house quality (e.g. living in cold homes vs not).

9.6.1.3 Studies investigating the mediation role of individual risk factors between temperature and mortality

As specified in paragraph 9.3.1, causal pathways linking temperature variations with CVD risk factors changes which subsequently lead to increased mortality could not be established in this thesis. Ideally, to test this hypothesis, future studies should improve what has been done in the BRHS: they should collect individual factors much more frequently over the study period; then, the same time-varying covariate survival analysis carried out in Chapter 8 (time varying factors: temperature and CVD risk factors; outcome: mortality) could have been carried out, but the time varying nature of the risk factors would then be more genuinely represented. In the BRHS two physical and blood examinations were planned (once during 1998-2000 and once during 2010-2012) as operational end economic costs of such data collections over time are very high. It is not surprising that neither the BRHS nor other ongoing population-based studies worldwide have collected such data on a yearly basis over decades. This is a key challenge in future epidemiological studies; to evidence the biological pathways linking temperature variations with mortality data on outdoor temperature, risk factors levels and mortality will need to be collected in a narrow time window (e.g. once a month for the risk factors, daily for temperature and mortality). Other studies could also simultaneously measure meteorological factors, blood pressure and physical activity by second, minute or daily by using wearable devices. The simultaneous measurements could allow a pathway (mediation) analysis where direct and indirect estimates of associations of temperature, blood pressure, and physical activity on mortality could be estimated. Several consumer grade wearable device manufacturers, such as Fitbit or Apple, have already requested the United States Food and Drug Administration (FDA) approval for their wearables to be used in health technology studies (362). However, the precision of their device algorithms generating the data must be validated and concerns about privacy of study participants will have to be addressed. Alternatively, future studies using sensors measuring vital signs and

body temperature could demonstrate causal pathways leading to CVD events investigating whether CVD risk factors variations due to sudden (or immediate) changes in outdoor temperatures within the same day, lead to organ damage and the likelihood of subsequent cardiovascular events (363).

9.6.1.4 Studies investigating seasonal variations in physical activity and sedentary behaviour

Previous research showed that older women are generally less active than men (17, 271). Moreover, in the UK people from the Asian ethnic group are less active than the overall average (364). Therefore, future studies can investigate whether differences in physical activity levels are especially marked at lower vs higher temperatures when comparing women vs men, or when comparing people from different ethnic groups. These questions were not answered by previous research and can increase our knowledge in physical activity patterns in populations which are different from the BRHS.

The findings from Chapter 5 (paragraph 9.3.1) provided enough justification for further interventional studies in older populations examining (i) whether engaging in regular physical activity across different temperature ranges and across the year yet limiting the exposure to cold outdoor temperatures is possible; (ii) whether replacing sedentary time with physical activity in winter on a daily basis is possible; (iii) whether incidence of CVD events is lower among older adults who are able to maintain the same level of activity across the seasons in comparison to those who become less active in winter. These questions were not answered by previous research.

9.6.1.5 Studies investigating the role of influenza in future studies investigating the CVD seasonal variation

The analysis performed in Chapter 8 (estimating associations of temperature with mortality, adjusted for a proxy of influenza exposure) could not isolate the seasonal mortality patterns due to outdoor temperature and seasonal mortality patterns due to influenza, for example seasonal flu and seasonal respiratory infections. It is important

to continue to investigate this topic in future studies and enhance our understanding of the biological pathways linking temperature, influenza and mortality (see paragraph 9.6.2). To improve current studies estimating the mortality burden attributable to both temperature and influenza, it would be ideal to collect all data at individual level. Also, future studies could investigate the association of temperature with mortality in non-flu periods during winter only or the whole year.

9.6.1.6 Studies investigating the association of indoor house temperature with CVD

There is a need for further epidemiological studies to improve the accuracy of individual exposure to temperature in the house, where older adults are likely to spend most of their time. This is important because comfortable temperatures indoor (e.g. a regular room temperature at home around 18 degrees Celsius) can potentially address specific risk factors such as high blood pressure and worse lung conditions. Future studies can investigate whether lower indoor temperatures at home in winter are associated with increased mortality, even after accounting for outdoor temperature and other individual risk factors. To demonstrate this can offer evidence-based justifications for implementing public health strategies aiming to modify home indoor temperatures and preventing CVD (see paragraph 9.5.1). As of today, the excess winter mortality in the United Kingdom (UK) has been partially attributed to cold housing (316, 365), with an extra 5500 more deaths occurring annually in the coldest homes than would occur if those homes were warm (366); however, this was a broad estimation generated from a UK study using information at household level rather than individual level.

9.6.2 Implications of the findings for future studies investigating diurnal variation in CVD risk and CVD risk factors

Time of death of the BRHS participants was not measured; this is a limitation of this thesis and represents a key measurement requirement for future studies aiming to understand the causal pathways of the diurnal variation in CVD events (44, 192, 203). For example, if the time of death is measured, further studies could investigate whether

the rapid increase of blood pressure over the day (see paragraph 9.5.3) continuously measured via wearable devices or patches, could explain the increased number of CVD events observed in early and late morning.

In Chapter 6 the variations of biological risk factors by time of day were explored using between-participant variation only, as the measurements were carried out on one occasion for each participant in between 08:00 and 19:00 hours. This offered only a partial understanding of the variations of biological risk factors over the 24 hours (194). In future studies, carrying out blood samples measurement of the same factors analysed in Chapter 6 over the 24 hours to investigate within-person circadian variations would be possible, although this is inherently difficult given the likely disruption of natural sleeping patterns when carrying out measurements overnight (303). Moreover, since diurnal variation in CVD events has been reported to be more marked in men than women (42), it would be interesting to investigate whether the time of day variations in CVD risk factors levels explored in this thesis are less marked in UK older women.

Findings from Chapter 4 (see paragraph 9.3.2) provided enough justification to (1) explore whether incidence of CVD events is lower among older adults who are typically more active in the morning vs older adults who have different patterns; (2) explore whether the occurrence of CVD events in the morning, afternoon or evening is more frequent among older adults who are typically more active in the morning than in older adults who are not; (3) identify other physical activity patterns that can explain the occurrence of CVD events over the 24 hours of the day, such as the total amount of physical activity during the most active 30 minutes of the day.

9.7 Concluding statement

In recent decades, there has been an increase in CVD prevalence in older people from the UK. The population in the UK is also ageing due to a steady increase in life expectancy over time. Since the 1930s, the number of people aged over 65 years in the UK has more than doubled. CVD remains the main cause of mortality in UK men,

accounting for nearly a quarter of all deaths in both men and women, and is a major contributor to morbidity and disability. Also, the seasonal variation in CVD deaths remains one of the main causes of the seasonal variation in overall mortality and such variation is particularly marked in older people compared with middle aged and younger populations. Results from this thesis have demonstrated that seasonal variations in outdoor temperature, the main seasonal factor and exposure variable used in this thesis, is an important determinant of the seasonal variation in CVD, respiratory and all-cause mortality, and suggested that possible biological pathways may involve temperature-related changes in both established (especially blood pressure and physical activity) and emerging CVD risk factors (e.g. markers of inflammation). These findings emphasize the need for policy-makers, epidemiologists and health economists to consider in future policy and research the impact of seasonal variations in outdoor temperatures on CVD, respiratory, and all-cause mortality in older adults. The findings related to my investigation on physical activity variations emphasize that further efforts are needed to maintain regular physical activity levels across seasons and throughout the day in older adults; this would help in reducing CVD disease in older age. Lastly, although diurnal variations in several biological CVD risk factors were observed, future research could focus more on blood pressure, LDL-Cholesterol and IL-6 diurnal variations. Overall, the main implications for epidemiological research are for future studies to demonstrate the causal pathways involved in the (i) seasonal and (ii) diurnal variation in CVD mortality.

APPENDIX I THESIS PUBLICATIONS

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RESEARCH ARTICLE Open Access

Diurnal patterns of objectively measured physical activity and sedentary behaviour in older men

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Abstract

Background: Physical activity (PA) levels among older adults are generally low and sedentary behaviour (SB) very common; increasing PA and reducing SB levels could have appreciable health benefits. Quantifying PA and SB patterns through the day could help in defining strategies for change. We examined within day variations in PA and SB and whether these varied by demographic factors and health status.

Methods: Men aged 71–91 years participating in an established UK population-based cohort study were invited to wear a GT3x Actigraph accelerometer over the hip for one week in 2010-12. Percentages of time spent in sedentary (SB, <100 counts per minute [CPM]); in light (LIPA, 100-1040 CPM) and in moderate to vigorous PA (MVPA, >1040 CPM) were derived. Multilevel models were used to estimate the associations between demographic factors and health status and SB, LIPA and MVPA.

Results: 1455 of 3137 men invited (46.4 %) participated and provided adequate data. Men spent 73 % of the day in SB, 23 % in LIPA and 4.5 % in MVPA (619, 197 and 39 min per day respectively). The percentage of time spent in MVPA was highest in the morning, peaking at 10-11 am (8.4 %), and then declining until the evening, with the exception of a small increase at 2-3 pm. LIPA followed a similar pattern. Conversely, SB levels were lowest in the morning and increased throughout the day, peaking at 9 pm (88 %). Men who were older, did not use active transport, had mobility limitations, were obese, depressed, had more chronic health conditions, and were smokers had lower levels of MVPA. The impacts of older age, obesity, mobility limitations and chronic diseases on LIPA, MVPA and SB were more marked in the morning than in the afternoon and evening.

Conclusions: Levels of MVPA and LIPA are highest in the morning (peak at 10-11 am) and decrease during the day. SB increases through the course of the day to peak in the evening. Interventions to encourage older men to be physically active may need to take account of current PA patterns, aiming to prolong active morning bouts of PA and/or reducing SB in the afternoon and evening hours.

Keywords: Physical activity, Light activity, Sedentary behaviour, Cohort study, Older adults, Accelerometer, Health conditions, Within day variations

Background

Physical activity (PA) declines with increasing age [1–3] and older adults, especially the oldest old, are the least active age group in the population [4–6]. PA levels of older adults are generally low and levels of sedentary behaviour (SB) are high [7–9]. To implement effective strategies to increase PA and reduce SB, it is important to understand usual PA and SB patterns. Accelerometers permit objective and accurate assessment of these patterns in population-based studies and, of special consideration for older adults, reduce the impact of recall bias (over or under reporting), participants memory loss or cognitive impairment [10]. Accelerometers can give insight into how activity levels vary over the course of the day. However, to date there is very little evidence on how PA and SB are structured throughout the day among older people. Existing studies reporting on how activity varies throughout the course of the day have been small [4, 6–8, 11] or mainly have focussed on global measures of activity as CPM and MVPA without considering the wider range of outcomes including different activity intensities as LIPA, SB and duration of bouts of activity [2]. Existing studies suggest that that older adults were more active in the mornings than during afternoons and evenings [2, 4, 6–8, 11], and this may have implications for strategies aim to increase PA on daily basis.

The aim of this study is to investigate diurnal variations in objectively measured LIPA, MVPA and SB in older men, in a much larger study population than previously investigated. We use data from the British Regional Heart Study, an established population-based cohort of community-dwelling older men. The second aim is to determine the extent to which the diurnal variation in PA and SB is modified by key demographic and health status variables including age, body mass index, social class, health conditions (depression, vision problems, and chronic diseases), mobility limitations and social isolation. In a subsidiary descriptive analysis we explore diurnal variation in long bouts of SB (≥60 min) and MVPA bouts of at least 10 min, because these are an important component of UK national PA guidelines [12].

Methods

Participants

The British Regional Heart Study (BRHS) is a prospective cohort of 7735 men recruited from a single local primary care centre in 24 British towns in 1978-80 (age 40-59 years). In 2010-2012, all surviving cohort members resident in the UK (n = 3137) were invited to attend a further physical examination including measurements of weight and height and to participate in a study of objectively measured physical activity. The National Research Ethics Service (NRES) Committee for London provided ethical approval. Participants provided informed written consent to the investigation, which was performed in accordance with the Declaration of Helsinki [13].

Objective physical activity assessment

Procedures for distribution and wearing

Participants were invited to attend an assessment by study nurses at their local primary care centre. All men who attended were asked to wear an Actigraph GT3x accelerometer (Pensacola, Florida) over the right hip on an elasticated belt for 7 days, during waking hours, removing it for bathing, swimming or showering and returning the device by post.

Data processing

Actigraph accelerometers record "counts" and steps, which both depend upon the frequency and intensity of the raw acceleration [14]. Accelerometer data were processed using standard methods, as described previously [9]. In brief, raw data from movements registering on the vertical axis were integrated into 60 s epochs; therefore counts per minute (CPM) were derived. Non-wear time was identified and excluded using the R package "Physical Activity" [15], based on (i) periods of continuous zero activity lasting more than 90 min or (ii) periods of zero activity lasting more than 90 min broken only by non-zero counts lasting up to 2 min, provided no activity counts were detected during both the 30 min before and after that interval [9]. Valid wear days were defined as ≥600 min wear time, and participants with at least 3 valid days were included in analyses, a conventional requirement for estimating usual PA level [16].

Derived variables

The number of minutes per day spent in SB, LIPA and MVPA was categorised using count-based intensity threshold values of counts per minute developed for older adults [9, 11, 17]: <100 CPM for sedentary behaviour (<1.5 Metabolic Equivalent of Task, MET), 100-1040 for light activity (1.5-3 MET) and >1040 for MVPA,(≥3 MET). The cut-point of 1040 CPM was calibrated to identify moderate intensity activities (≥3 MET) in a sample of older adults [11], but we also investigated the more widely used cut-point of 1952 CPM which was calibrated to identify moderate intensity activities (≥3 MET) in middle-aged adults [17]. Two further summary measures of SB and MVPA were calculated: number of sedentary bouts of at least 1 h (a period of 60 or more consecutive minutes where the accelerometer registers <100 CPM) and MVPA bouts of at least 10 min (a period of 10 or more consecutive minutes where the accelerometer registers more than 1040 CPM).

Log diary and questionnaire data

Participants completed a log diary, detailing when the accelerometer was put on and taken off during the seven days of wear. During the first 3 days the men were also asked to report the type of activity (*e.g.* housework, gardening, preparing meals, watching TV) that they did during each hour of the day. Participants' log diaries were checked and matched against accelerometer data to verify the date on which they started wearing the accelerometer. Age and season were derived from the first wear day. Season was categorised as summer (Jun-Aug), autumn (Sep-Oct), winter (Nov-Feb), and spring (Mar-May). Men were asked "do you have any difficulties getting about outdoors?" which was grouped as "none", "slight" and "moderate, severe and unable to do". Men reported a medical diagnosis of any of the following chronic conditions; heart attack, heart failure, angina, diabetes, stroke, osteoporosis, claudication, Parkinson's disease and chronic kidney disease. Men were classified as having vision problems if they had one or more of glaucoma, macular degeneration or cataract, as in advanced age these are primary causes of visual dysfunction [18, 19]. Men scoring >=2 on the 4-item Geriatric Depression score were classified as depressed [20]. Cigarette smoking was self-reported. Participants completed the Lubben scale of social isolation which asks about interactions with family members and with friends, men scoring <12 were classed as at risk of social isolation [21]. Participants reported which forms of transport they used regularly (car, public transport, dial a ride, walk or cycle), those who reported regular walking or cycling were classified as using active transport. The social class classification was based on the longest-held occupation of subjects reported at study entry in 1978–1980. Participants' occupations were grouped as non-manual or manual.

Statistical methods

Analyses were carried out using STATA/SE 13 [22] and MLwiN Version 2.02 [23]. To give a general overview of the within day variation of total PA, counts per minute and steps were plotted against hour of day. The main outcome variables were the proportions (percentages) of the day spent in (1) sedentary behaviour, (2) light PA and (3) moderate to vigorous PA. Each outcome was calculated according to hour of the day, with the number of minutes that the accelerometer was worn in that hour used as the denominator. Due to sparse data in early morning and late evening, we examined the mean activity counts per hour between 7.00 am and 10.59 pm. Only hours with ≥ 45 valid wear minutes were included. A first descriptive analysis was undertaken: for 1329 men with complete data, the percentage of time spent in SB, LIPA and MVPA was plotted against hour of day. In order to explore whether the diurnal patterns were modified by selected variables, the data were stratified by (i) age group (<75, 75-79, ≥80 years) (ii) mobility limitations (none, slight, moderate/severe/unable to do) (iii) number of chronic conditions (none, 1-2, ≥3) (iv) BMI category (<25, 25-30 and ≥30 kg/m^2) (v) depression (depressed vs not) (vi) smoking status (current smoker vs not) (vii) social isolation (at risk of isolation vs not at risk) (viii) social class (manual vs non-manual) (ix) use of public transport (walk/cycle vs car/public transport) (x) vision problems (yes vs no) (xi) season (winter vs summer) and (xii) weekend vs weekday.

The distributions of each outcome were investigated: percentage of MVPA distribution was highly positively skewed as reported in previous studies [24, 25]. MVPA data were highly over-dispersed with variance 5 to 6 times higher than the means within each period of the day, so negative binomial model were used to investigate which factors were related to the percentage of time spent in MVPA and the results were reported as rate ratios (RRs) [26]. A RR is as multiplicative factor: any deviation from 1 indicates a percent difference in the outcome relative to the respective reference category (baseline) in the exposure variable. Linear multilevel regression models were used to investigate which factors were related to the percentage of time spent in LIPA and SB (normally distributed). Beta coefficients were reported to estimate the difference in time spent in SB and LIPA between the categories of each explanatory variable against the reference. In all multilevel models Level 1 was period of the day [morning (7 am-12.59 pm), afternoon (1 pm-6.59 pm) and evening (7 pm-10.59 pm)] and Level 2 was the individual. Each period (morning, afternoon and evening) had a minimum of 2 valid hours of wear time.

Two level random-intercept and random-slope models were used with adjustments for age, season, region and part of the day, and one additional covariable at a time (mobility limitations, number of chronic conditions, BMI, depression, smoking status, social isolation, social class, use of public transport, and vision problems). Next, fully adjusted models were run using all explanatory variables together. The random slope in the models allowed us to test the hypothesis that the changes in PA levels over the day varied between different men. The estimated slopes over the course of day were reported as mean differences between afternoon and evening vs morning (baseline).

For variables which were significantly associated with sedentary behaviour, light and MVPA levels, interactions were fitted to test whether the associations differed according to period of the day (morning, afternoon, evening). An overall Wald test for interaction between the categories of those explanatory variables and period of the day (morning, afternoon and evening) was used.

Results

The recruitment flow chart and the inclusion criteria used are presented in Fig. 1. Among 3137 surviving men, 1655 (52.8 % response) agreed to participate and 1455 men (46.4 % response) with a mean age of 78.5 years (range 71-93) provided both physical activity and questionnaire information. All these men were independently mobile and community dwelling. The characteristics of the study participants are shown in Table 1. Men who agreed to participate were younger and 10 years previously had a lower BMI compared to men who did not participate. Participants took on average 4827 steps per day and spent 72.6 % of the day in SB, 22.9 % in light activity and 4.5 % in MVPA (619, 197 and 39 min per day respectively). Participants had a mean of 6.5 (SD = 1.2) valid days of accelerometer wear. 1329/1455 men (91.3 %) had complete data on all covariates, and the same patterns of associations with characteristics in Table 1 are seen in the reduced sample. From this point forward all results refer to the 1329 men (complete case analysis).

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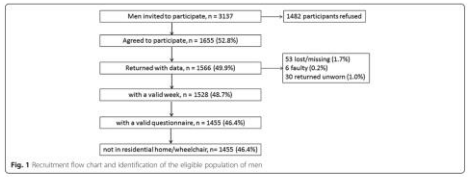

Fig. 1 Recruitment flow chart and identification of the eligible population of men

Diurnal patterns of PA and SB

The intensity of PA indexed by both accelerometer CPM and the number of steps peaked around 10 am and then declined until a small further increase at 2 pm followed by a long decline until 9 pm and then a small increase after 10 pm (Fig. 2). Figure 3 shows the average percentage of time spent in PA of different intensities (SB, LIPA and MVPA) for each hour of the day. LIPA and MVPA were positively correlated within each hour: $r = 0.21$ ($p < 0.001$), and conversely, hourly SB was negatively correlated with LIPA ($r = -0.88$, $p < 0.001$) and with MVPA ($r = -0.64$, $p < 0.001$).

The pattern in daily variation of MVPA and LIPA closely followed the pattern observed for CPM. At around 10 am, the proportions of each hour spent in LIPA and MVPA peaked (at approximately 30 % and 8 % of all activity respectively) and then declined until 1 pm, followed by a slight increase in the afternoon around 2–3 pm and then a long decline until around 9 pm, when light activity accounted for only approximately 10 % and MVPA 1 % of each hour. Conversely, SB levels increased throughout the morning, with a steeper increase before 1 pm and then a small dip around 3 pm, followed by a slow increase to a peak of over 80 % spent in SB between 8-9 pm, followed by a slight decline after 9 pm (Fig. 3). Among men who completed log diaries, commonly reported activities around 11 am were gardening, shopping, moderate housework and do-it-yourself (DIY). Similar activities were reported around 3 pm, although more men reported gardening and fewer men reported housework in the afternoon.

Univariable descriptive plots show hourly patterns of different intensities of physical activity stratified by age, mobility limitations, chronic diseases, BMI, geriatric depression score and smoking status (Figs. 4 and 5) and by social isolation, social class, active transport and vision problems, season (winter vs summer) and day of the week (Figs. 6 and 7). In most cases, the patterns of mean percentage of SB, LIPA and MVPA by hour of the day followed a consistent pattern of peaks and dips at the same time of day. Descriptive statistics on sedentary and MVPA bouts (plots not presented) showed consistent results with patterns in Figs. 4, 6, 7. 49.0 % of the sedentary bouts lasting ≥60 min over a valid week occurred in the evenings; most started between 8-9 pm (13.6 %) or 9-10 pm (14.0 %). Conversely, most (59.5 %) of MVPA bouts lasting ≥10 min over a valid week occurred in the morning and in particular when the peaks of MVPA were reported, at 10 am (15.9 %) and 11 am (16.4 %).

Associations of social and demographic factors with PA and SB

Associations (main effects) of social and demographic factors with total time spent in physical activity and sedentary behaviour each day were estimated using multilevel models. The magnitude and significance of the associations did not differ greatly when adjusted for just one explanatory variable at a time or with further adjustments for all explanatory variables together (Table 2 for SB and LIPA and Table 3 for MVPA), hence the fully adjusted results are reported. The diurnal patterns estimated from the model were that average time spent in SB increased in the afternoon (+9 %) and evening (+21 %) when compared to morning (Table 2, Model 1). Conversely, time spent in LIPA decreased by -6 % and -16 % during afternoon and evening respectively (Table 2, Model 2). Additionally, percentage of time spent in SB each day was significantly higher and the percentage of the day in LIPA was significantly lower in the following groups; age ≥80 years, any mobility limitations, three or more chronic diseases, and obese. Total levels of SB were higher in the men who were depressed and did not use active transport, although LIPA did not vary by these characteristics. Neither LIPA nor SB differed by social class, presence of social isolation and vision problems.

Table 1 Characteristics of men who met the inclusion criteria for the study and men who did not accept the invitation to participate

	Men who met inclusion criteria for the study	Men who did not meet the inclusion criteria	*p*-value
N	1455	1682	
Demographic and background characteristics			
Age, mean (SD)	78.5(4.6)	80.1 (5.2)	<0.001
Region, n(%)			0.031
South	526(36.2)	525(31.2)	
Midlands	217(14.9)	267(15.2)	
North	569(39.1)	701(42.6)	
Scotland	143(9.8)	189(10.9)	
Social class (manual), n(%)	666(45.7)	953 (56.7)	<0.001
Physical Health			
BMI, mean (SD)	27.1(3.8)		
BMI 10 years earlier, mean (SD)	26.7(3.3)	27.2(3.8)	<0.001
Number of Chronic conditions, n(%)			
None	674(46.5)		
1-2	668(46.0)		
3+	108(7.5)		
Mobility limitations outdoors, n(%)			
None	915(64.6)		
Slight limitations	264(18.6)		
Moderate/severe difficulty/unable to do	237(16.7)		
Vision Problems (none), n(%)	992(68.2)		
Mental health and wellbeing			
Social Isolation, (isolated), n(%)[a]	257(17.8)		
Geriatric Depression Scale, (depressed), n(%)[b]	316(22.1)		
Behaviours			
Mode of transport used regularly (cycle/walk), n(%)	923(63.4)		
Smoking status (cigarettes), smoker	46(3.2)		
Smoking status 10 years earlier (cigarettes), smoker	97(7.2)	160(12.9)	<0.001
PA levels/day, mean (IC 95 %)			
Counts/min (CPM)[c]	186(180,191)		
Steps[c]	4827(4698,4956)		
Percent wear time in SB per day[c,d]	72.6(72.1,73.0)		
Percent wear time in LIPA per day[c,e]	22.9(22.6,23.3)		
Percent wear time in MVPA 1+ per day[c,f]	4.5(4.3,4.7)		
Minutes in SB per day[c,d]	619(615,623)		
Minutes in LIPA per day[c,e]	197(194,200)		
Minutes in MVPA 1+ per day[c,f]	39(37,41)		
Wear time[g]	853(850,857)		
Number of valid days, mean (SD)	6.7(0.8)		

[a]Lubben scale, isolated <12
[b]Geriatric Depression Scale, depressed >2.
[c]Means adjusted for age, day order, wear time, season of accelerometer wear and region of residence
[d]Sedentary Behaviour (SB) is at least one minute where the accelerometer registers values <100 CPM
[e]Light physical activity (LIPA) is at least one minute where the accelerometer registers values between 100-1040 CPM
[f]Moderate to vigorous physical activity (MVPA) 1+ is at least one minute where the accelerometer registers values over 1040 CPM
[g]Means are adjusted for age, day order, season of accelerometer wear and region of residence

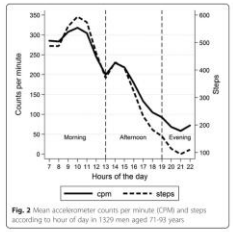

Fig. 2 Mean accelerometer counts per minute (CPM) and steps according to hour of day in 1329 men aged 71-99 years

The MVPA results were reported as RRs rather than beta coefficients, due to non-normality of the outcome distribution. The decline in MVPA was particularly marked over the course of the day; compared to the morning, levels of MVPA (>1040 CPM, Table 3 Model 1) declined substantially in the afternoon (RR = 0.75, 95 % CI 0.54-0.59) and in the evening (RR = 0.17, 0.15-0.18). Additionally, men with moderate or more severe mobility limitations

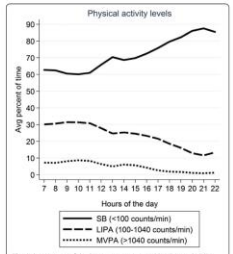

Fig. 3 Percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day in 1329 men aged 71-99 years

compared to the reference category (no mobility limitations) had a RR of 0.50 (95 % CI 0.43, 0.57) for MVPA indicating that men with mobility limitations were half as likely to spend time doing MVPA compared to people with no limitations. Moreover, men who were older, did not use active transport, were obese, depressed, had more chronic health conditions, and were smokers had lower levels of MVPA.

Similar associations were seen when these analyses were repeated with a higher cut point (1952 CPM) to define MVPA (Table 3, Model 2). The largest RRs (risks of having low MVPA levels) were for being over 80 compared to less than 75 years, for the category "moderate/severe limitations or unable to do" if compared with no mobility limitations and use of active transport versus car/public transport. However, when using the 1952 CPM cut-point, MVPA level no longer differed by depression or smoking status.

Factors associated with modified diurnal patterns of PA and SB

Four factors were significantly associated with each of SB, LIPA and MVPA levels: age, mobility limitations, chronic diseases, and BMI. The effects of older age, obesity, mobility limitations and chronic diseases on LIPA, MVPA and SB appeared to be more marked in the morning than in the afternoon and evening, independent of the lower overall levels of PA observed in these subgroups. Interaction tests were performed to establish whether these associations differed by period of the day. The tests for interaction (Wald tests) were all statistically significant ($p < 0.05$ for age, chronic conditions and BMI and $p < 0.001$ for mobility limitations).

Discussion

This study investigated the diurnal variations in accelerometer-measured PA and SB levels in a large sample of older British men. The analyses demonstrated that the total amount of physical activity (steps and CPM) was highest in the morning but then decreased during the day, except for a small increase at 2-3 pm. LIPA and MVPA showed a similar pattern. Conversely, SB levels were lowest in the morning and increased throughout the day, peaking at 9 pm (88 %). Commonly reported activities in the morning were shopping, gardening, housework and DIY.

We also examined diurnal variations of SB and PA by demographic factors and health status. Men who were older, had mobility limitations, more chronic health conditions and were obese tended to spend more time in SB and were less physically active. Moreover, men who did not use active transport, who were depressed and smoked cigarettes spent less time in MVPA, but those factors did not affect SB or LIPA. Age, mobility limitations, chronic conditions and obesity influenced LIPA, MVPA and SB

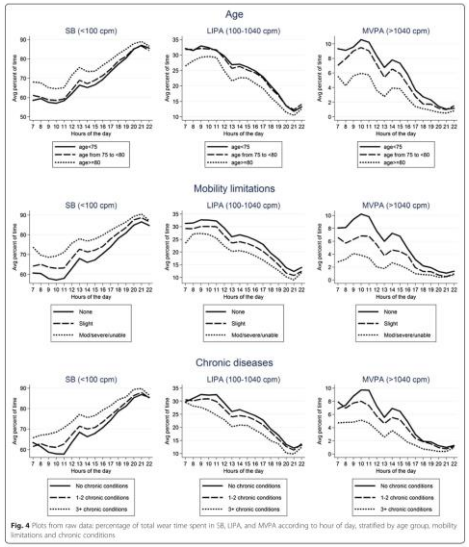

Fig. 4 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by age group, mobility limitations and chronic conditions

levels in the morning more than in the afternoon and evening. The findings showed an attenuation of the diurnal pattern among less active subgroups (*e.g.* older and more infirm men). This reflects their diminished ability to maintain relatively high intensity physical activity during the morning, and this is not simply related to the generally low PA level typical of these subgroups. This information is important for policy and practice because there is scope to extend the tendency for existing activity bouts during the morning and early afternoon and also to increase

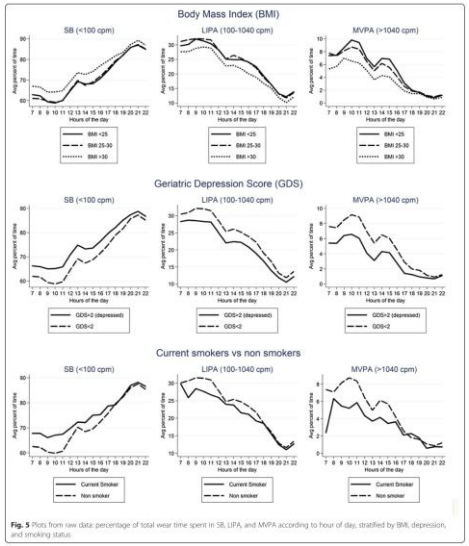

Fig. 5 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by BMI, depression, and smoking status

activity levels later in the afternoon. Our analysis of whether or not the effects of specific health and social variables on PA levels varied by time of day offers unique new insights, as previous studies of older adults have not considered this question.

Comparisons with other studies

To date there has been little work using hourly accelerometer data to examine diurnal physical activity patterns among older adults. Our results showing that physical activity peaks in the morning are consistent with recent

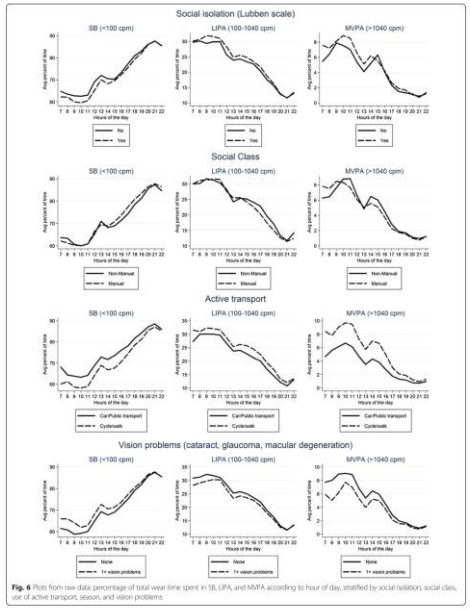

Fig. 6 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by social isolation, social class, use of active transport, season, and vision problems

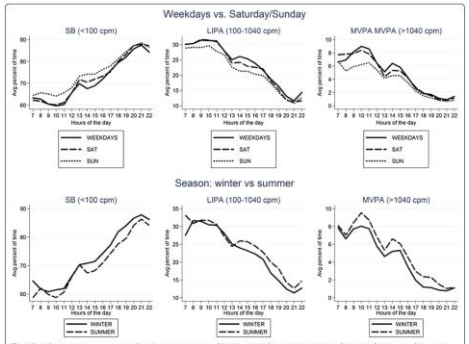

Fig. 7 Plots from raw data: percentage of total wear time spent in SB, LIPA, and MVPA according to hour of day, stratified by day of the week and season

studies [2, 4, 6–8, 11], although our study extends the literature by investigating more intensities of activity (SB, light PA and MVPA) and bouts of activities. In our study the overall PA levels (measured as CPM) over the course of the day were similar to other smaller studies using the same measurement device [4, 6–8, 11]. We examined PA patterns between 7.00 am and 10.59 pm and a similar period (6 am-10 pm or 7 am-9 pm) was analysed in other studies due to sparse data in early morning and late evening [2]. In line with our findings, a study of 38 healthy active adults (mean age 70 years) reported significantly fewer minutes of MVPA in the evening than in the morning or afternoon [11] and that longer bouts of activity occurred in the morning (6 am-12 pm) more often than afternoon or evening. The AGES-II study of 579 adults aged 73–98 from Iceland reported that the majority of PA occurred between 8 am and 4 pm on an average day [4], which fits with our findings. In line with our study, they also reported that sedentary time was similar across all age groups, except for the oldest age group (>85 years old) who were the most sedentary and PA levels declined with increasing age and BMI, but other social and health factors were not taken into account. Our findings about age modifying the daily patterns in physical activity fit with data from the AGES-II study [4] and from the Baltimore Longitudinal study of Ageing [6], which also found that older age groups had a steeper decline in in PA levels over the course of the day. Our finding that activity levels were lower in the mornings in obese than normal weight men mirrors data from a study of Canadian adults aged 20-79 years [2]. To our knowledge other studies have not investigated how presence of chronic conditions and mobility limitations affect the diurnal patterns of physical activity and sedentary behaviour in older adults.

Strengths and limitations

This study investigates how hourly levels of objectively measured SB, LIPA and MVPA vary over the course of the day and how daily activity patterns are modified by a wide range of demographic and health characteristics. It

Table 2 Adjusted associations between demographic and health factors and physical activity levels: percent of time spent in SB and LIPA[a]

	Model 1	Model 2
	Percent of time spent in SB (<100 CPM)	Percent of time spent in LIPA (100-1040 CPM)
	β (95 % CI)[b]	β (95 % CI)[b]
Part of the day (ref: Morning)		
Afternoon	9 (9,10)	-6 (-7,-6)
Evening	21 (21,22)	-16 (-16,-15)
Age categories (ref: age < 75 years old)		
75-79 years old	0.5 (-0.4,1.5)	-0.1(-0.9;0.7)
80+ years old	3.5 (2.5,4.5)	-2.3(-3.1;-1.4)
Mobility limitation (ref: no mobility limitations)		
slight mobility limitations	1.6 (0.6,2.6)	-1.1(-2.0;-0.3)
moderate/severe limitations or unable to do	3.3 (2.1,4.6)	-2.6(-3.6;-1.5)
Chronic conditions (ref: no chronic diseases)		
1-2 chronic diseases	0.7 (-0.1,1.5)	-0.6(-1.3;0.1)
3+ chronic diseases	2.6 (1.0,4.2)	-2.1(-3.4;-0.8)
Obese (ref: non-obese, BMI < 30)	1.4 (0.5,2.4)	-1.2(-2.0;-0.3)
Depressed (ref: not depressed)	0.9 (0.0,1.9)	-0.8(-1.6;0.1)
Current smoker (ref: non-smoker)	0.8 (-1.3,3.0)	-0.6(-2.5,1.2)
Use car/public transport (ref: cycle/walk)	1.0 (0.2,1.9)	-0.6(-1.3;0.1)
Social isolated (ref: not isolated)	0.2 (-0.8,1.2)	-0.2(-1.1;0.6)
Manual social class (ref: non manual)	0.2 (-0.5,1.0)	0.2(-0.5;0.9)
Vision problems (ref: none)	-0.2 (-0.6,1.1)	0.2(-0.6;0.9)

[a]Complete case analysis (n = 1329 in each model): men who met the inclusion criteria (Fig. 1) and who had at least two valid hours of wear time in each period of the day (morning 7:00-12:59, afternoon 13:00-18:59, evening 19:00-22:59). A valid hour is defined as an hour with ≥45 min of wear time
[b]β coefficient represents the difference in percent of time spent in SB (Model 1) and LIPA (Model 2) compared to the reference category of each explanatory variable. Models are multilevel linear regression models mutually adjusted for season of accelerometer wear, region of residence plus all explanatory variables listed in the table

is particularly important to investigate LIPA in population based samples of older adults, because of the high proportion of time spent in light activity. Our findings about the correlates of LIPA offer a new contribution to the ongoing debate about whether and how the PA guidelines should include recommendations on LIPA as well as MVPA [27, 28].

This study benefits from using a large scale population-based cohort of free-living older men rather than a special *at risk* population, which should increase generalizability. The response rate achieved in this study is comparable with other studies on objective measurements of daily physical activity patterns [4]. Men who did not accept our invitation were about two years older and had higher BMI measured 10 years earlier; implying that overall PA (e.g. total counts or number of steps) might be lower in the general population. Our study is however limited by studying only white European men, who, based on existing literature, would be expected to have higher levels of PA, particularly MVPA, compared with women [9]. Therefore our results may not be generalizable to older women or ethnic minority populations [29]. Our study did not report detailed information on mode of activity, which was self-reported only during the first three days of accelerometer wearing. However, the importance of this information is recognized and future studies could investigate further the particular types of activities carried out during the highest and lowest peaks of activity [30]. A further area for future study would be the assessment of seasonal patterns of PA and SB (although these were not an objective of this study). Future analysis is needed to determine whether or not seasonal variations in PA and SB are observed even after adjustment for confounding of weather variables (e.g. temperature or sunshine duration). We investigated whether diurnal patterns in PA and SB were modified by season (winter vs. summer) and day of the week (Sunday vs Saturday or Monday-Friday). Whilst PA levels are generally lower (and SB levels higher) in winter and on Sundays, but we did not find evidence of effect modification on diurnal patterns.

Implications

The marked variations in PA occurring on a within-day basis provide information which could be helpful in

Table 3 Adjusted Rate Ratios (RRs) for the percent of time spent in MVPA using two different cut offs (>1040 and >1951 counts per minute) according to demographic and health status variables[a]

	Model 1	Model 2
	Percent of time spent in MVPA (>1040 CPM)	Percent of time spent in MVPA (>1951 CPM)
	RR (95 % CI)[b]	RR (95 % CI)[b]
Part of the day (ref: Morning)		
Afternoon	0.57 (0.54,0.59)	0.50 (0.46,0.54)
Evening	0.17 (0.15,0.18)	0.11 (0.10,0.13)
Age categories (ref: age < 75 years old)		
75-79 years old	0.87 (0.79;0.96)	0.82 (0.71,0.95)
80+ years old	0.55 (0.50,0.61)	0.49 (0.42;0.57)
Mobility limitation (ref: no mobility limitations)		
slight mobility limitations	0.79 (0.71;0.89)	0.69 (0.59;0.81)
moderate/severe limitations or unable to do	0.50 (0.43;0.57)	0.33 (0.26;0.41)
Chronic conditions (ref: no chronic diseases)		
1-2 chronic diseases	0.91 (0.83;0.99)	0.91 (0.80;1.03)
3+ chronic diseases	0.66 (0.55;0.79)	0.65 (0.50;0.85)
Obese (ref: non-obese, BMI < 30)	0.83 (0.75;0.93)	0.72 (0.61;0.84)
Depressed (ref: not depressed)	0.88 (0.79;0.98)	0.92 (0.79,1.08)
Current smoker (ref: non-smoker)	0.76 (0.60;0.97)	0.85 (0.60,1.21)
Use car/public transport (ref: cycle/walk)	0.75 (0.68;0.82)	0.62 (0.55;0.72)
Social isolated (ref: not isolated)	0.97 (0.87,1.08)	1.11 (0.95,1.31)
Manual social class (ref: non manual)	0.93 (0.85,1.01)	0.98 (0.86,1.11)
Vision problems (ref: none)	0.96 (0.87,1.05)	1.00 (0.88,1.15)

[a]Complete case analysis (n = 1329 in each model): men who met the inclusion criteria (Fig. 1) and who had at least two valid hours of wear time in each period of the day (morning 7:00-12:59, afternoon 13:00-18:59, evening 19:00-22:59). A valid hour is defined as an hour with ≥45 min of wear time
[b]A Rate Ratio (RR) is a multiplicative factor. Compared to the reference category of each explanatory variable, any deviation from 1 indicates a change in percent of time spent in MVPA and a value < 1 indicates a decrease in MVPA (e.g. RR = 0.91 means a decrease in MVPA by a factor of 0.91 compared to the reference, that is about 10 %). Model 1 and 2 are negative binomial multilevel regression models mutually adjusted for season of accelerometer wear, region of residence plus all the explanatory variables in the table

planning interventions to increase PA levels. Older adults do most of their MVPA and light activity during the morning. Thus, one possible strategy for interventions aiming to increase these intensities of activity would be either to focus on the morning when people are already active and when variability in activity levels are greatest, aiming to increase the intensity or duration of existing physical activity bouts. Alternatively, interventions could focus on the afternoon period, aiming to stimulate physical activity of comparable intensity to that occurring in the morning. It is unlikely that low levels of activity in the evening can be changed, particularly in the winter months if it is dark in the late afternoons and evenings. Indeed, the combination of darkness and visual problems have been previously investigated as potential causes of falls [31]. Likewise with sedentary behaviours, our findings suggest that the period in the late afternoon and early evenings are periods with high levels of SB and when bouts of SB are likely to be longest, so it may be particularly valuable to focus on efforts to break up long sedentary bouts at these times of day. Our investigation showed that age and health status affected these diurnal patterns suggesting that PA policies might be targeted by sub-groups. Among older and disabled men, lower levels of MVPA were observed in morning and afternoon than in younger healthy men, the morning peak was more reduced than the afternoon peak, suggesting that with increasing age, the higher morning peak in moderate to vigorous activity may be particularly difficult to maintain. Longitudinal analyses could offer additional insights and determine if there are independent effects on health of MVPA or SB at different times of the day.

Conclusions

This study provides detailed data about diurnal patterns in habitual physical activity levels in free-living older men which can inform the development of effective programmes to encourage older men to be physically active. This study highlights that especially among men over 80 years old, who are obese, with multiple chronic diseases or with

mobility limitations there are particular opportunities to maintain or enhance existing activity bouts during the morning and early afternoon and to reduce the duration of SB periods in the afternoon and evening hours.

Abbreviations
CPM: Counts per minute; PA: Physical activity; LIPA: Light physical activity; MVPA: Moderate to vigorous physical activity; SB: Sedentary behaviour; MET: Metabolic equivalent of task; RR: Rate ratio.

Competing interests
The authors have no competing interests to report. Ethics: The National Research Ethics Service (NRES) Committee for London provided ethical approval. Participants provided informed written consent to the investigation, which was performed in accordance with the Declaration of Helsinki.

Authors' contributions
CS contributed to making the databases, designing statistical analyses, interpretation of data and drafted the manuscript for publication; SGW conceived the study, and participated in its design and coordination helped to interpret the data and revise the manuscript critically for important intellectual content, SI and RWM interpreted the data and revised the manuscript critically. SA and LL collected, downloaded and helped to interpret the data. PHM conceived and raised funding for the study, and participated in its design and coordination, acquisition of data and helped to interpret the data and to draft the manuscript. BJ contributed to conception and design of the study, acquisition of data, analysis and interpretation of data and drafted the manuscript. All authors read and approved the final manuscript.

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Human Kinetics
ORIGINAL RESEARCH

Association of Maximum Temperature With Sedentary Time in Older British Men

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Background: Sedentary behavior is very common in older adults and a risk factor for mortality. Understanding determinants of sedentary behavior may help in defining strategies aimed to reduce the time spent sedentary. The degree of difference in sedentary time attributable to varying temperatures has not been yet estimated in older men. **Methods:** Men aged 71 to 91 years participating in an established UK population-based cohort study were invited to wear an Actigraph GT3X accelerometer for 1 week in 2010–12. Outcome was sedentary time (<1.5 Metabolic Equivalent of Task) in minutes per day. Associations between daily outdoor maximum temperature and accelerometer-measured sedentary time were estimated using multilevel models. **Results:** 43% (1361/3137) of invited men participated in the study and provided adequate data. Men spent on average 615 minutes in sedentary time per day (72% of the total accelerometer-wear time). After adjusting for covariates, men spent 26 minutes more per day ($P < .001$) in sedentary time when temperatures were in the lowest (–3.5; 9.2°C) versus highest quintile (19.1; 29.5°C). **Conclusions:** Sedentary time in older adults is highest at lowest temperatures, typically recorded in winter. Findings are relevant for guidelines: interventions may consider targeting older men in winter providing recommendations for minimizing sedentariness on daily basis.

Keywords: sedentary behavior, older adults, weather, epidemiology, accelerometry

A standard definition of sedentary behavior has not yet been established, although contemporary researchers agree that sedentary behavior is not simply a lack of physical activity.[1] Sedentary behavior can be defined as the time spent in activities engendering less than 1.5 Metabolic Equivalent of Task (METs).[2] In recent years, there have been an increasing number of studies which have reported associations between prolonged sedentary behavior and health outcomes, such as mortality and cardiovascular disease, which have been independent of physical activity levels.[3] Therefore, understanding determinants of sedentary behavior may help in defining strategies aimed to reduce the time spent sedentary. This is particularly important in older adults, who are known as the most sedentary of all age-groups.[4]

A few previous studies in older adults have demonstrated that low outdoor temperatures were associated with less time spent in physical activity,[5,6] although an association with sedentary time was not investigated. We would intuitively expect sedentary time to be higher at lower temperatures, as occur during the winter season. However, the degree of difference in sedentary time attributable to varying outdoor temperatures has not been estimated in previous studies of older adults. Outdoor temperature has been overlooked in sedentary behavior guidelines,[7] and as determinant of sedentary time.[8,9] To our knowledge an association of temperature with sedentary time in older adults has not been previously documented.

Considering the gaps in knowledge of previous research, we have therefore investigated how sedentary time (<1.5 METs) varies according to outdoor maximum temperature in a large UK population based cohort study of community-dwelling older men.

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Methods

Participants

The British Regional Heart Study (BRHS) is a prospective cohort of men recruited from a single local primary care center in 24 British towns in 1978–80.[10] In 2010–2012, the surviving participants resident in the United Kingdom (UK), then aged 71 to 91, were invited to attend a further physical examination and to participate in a study of objectively measured physical activity, on which the analyses presented here are based. Men who met the inclusion criteria (not living in a residential home and not being on wheelchair) were included. Participants completed a log diary (detailing when the accelerometer was worn) and a comprehensive health status questionnaire. The participants' individual characteristics and questionnaire data were already described elsewhere.[11] The National Research Ethics Service Committee for London provided

ethical approval. Participants provided informed written consent to the investigation, which was performed in accordance with the Declaration of Helsinki.

Measurements and Data Analysis

Repeated measures of physical activity levels per each participant were recorded over the course of 1 week by using accelerometers. Methods for accelerometer-data extraction and processing were previously described in detail,[11] and added here as supplementary material (see Online Supplementary Material, Appendix S1). In brief, the number of minutes per day in spent in sedentary behavior, light physical activity (LIPA) and moderate-to-vigorous physical activity (MVPA) was derived and categorized using count-based intensity threshold values of counts per minute (CPM) developed for older adults, as in previous studies; the cut-points used were <100, 100 to 1040, >1040 CPM for sedentary time (<1.5 METs), time spent in LIPA (<1.5 to 2.9 METs) and MVPA (≥3 METs) respectively.[4,12,13] Number of steps per day was also recorded as a measure of overall physical activity. Then, maximum temperatures were linked to the accelerometer data for each day the men wore the device. Daily temperatures (maximum and minimum), hours of sunshine, and relative humidity were provided by the UK Meteorological (MET) Office (see Online Supplementary Material, Appendix S1). Maximum temperature was used as the main exposure variable and divided into quintiles. Quintiles were chosen as temperatures in the lowest quintile (1Q, –3.5°C; 9.2°C) were representative of the typical UK winter, while temperatures in highest quintile (5Q: 19.1°C; 29.5°C) were representative of the typical UK summer.[14] The main outcome investigated was sedentary time measured in minutes per day. In preliminary analysis, the correlations between sedentary time and other PA variables (steps, LIPA, and MVPA) were calculated. Linear multilevel models (level 1 was the date of wear and level 2 was the individual) with random intercept only were used to estimate associations between quintiles of maximum temperature and sedentary time. Quintiles of maximum temperature were derived counting every day each participant wore an accelerometer. The highest quintile of maximum temperature (5th quintile, 5Q) was chosen as reference quintile, and the results were reported as mean difference in sedentary time between the reference vs lower quintiles. As in one previous study,[11] the model was adjusted for measurement variables [accelerometer-wear time, wear day order (first day of wear, second, etc), day of the week, age, social class, Body Mass Index (BMI), chronic conditions, mobility limitations, geriatric depression scale, vision problems, smoking status, and day length (a proxy for season)]. The adjustment for day length was made to check whether there was confounding between temperature and a different seasonal term (collinearity was not observed as the Variance Inflation Factor (VIF) score was less than 1.5). As sensitivity analysis, a linear model was performed using maximum temperature as a continuous variable instead of the quintiles.

Subsidiary Analyses

For completeness of information, we investigated associations of maximum temperature with different outcomes related to sedentary behavior: total number of sedentary breaks per day, daily number of sedentary bouts of <30 minutes, and daily number of sedentary bouts of ≥30 minutes.

Associations of minimum temperature, hours of sunshine, and relative humidity (continuous variables) with sedentary time were also estimated.

A further investigation was performed to corroborate findings from previous studies which made use of different physical activity outcomes, rather than sedentary time. Therefore, associations of temperatures (maximum and minimum), hours of sunshine, and relative humidity with daily (i) number of steps, (ii) minutes spent in LIPA, and (iii) minutes spent in MVPA were estimated.

We also performed stratified analysis by excluding men who were depressed or/and with mobility limitations. All analyses were carried out using STATA/SE 13[15] and MLwiN Version 2.30.[16]

Results

1455 (46%) surviving men participated and met the inclusion criteria. 1361 men (43.4%) had data on all covariates (complete case analysis) and they had same mean age (78.5 years, SD = 4.6) and BMI (26.7, SD = 3.3) in comparison with 1455 men who met the inclusion criteria. The 1361 men with complete data were used in the final analysis: men had a mean of 6.5 (SD = 1.2) valid days of accelerometer wear; they wore the accelerometer for 855 minutes per day (SD = 93) and took on average 4872 steps per day (SD = 2767). The average sedentary time per day was 615 minutes (SD = 83), corresponding to 72% of the total accelerometer wear time; time spent in LIPA and MVPA was 198 minutes (SD = 65) and 39 minutes (SD = 32) respectively. The correlations between daily sedentary time with steps, LIPA, and MVPA were –0.46, –0.54, and –0.47 respectively (all P-values < 0.001).

Descriptive Statistics

The median for maximum temperature in the lowest quintile was 6.3°C (between -3.5°C and 9.2°C) and in highest quintiles was 20.8°C (between 19.1°C and 29.5°C). In descriptive plots, unadjusted sedentary time was highest when temperatures were in the lowest quintiles, and then decreased at higher temperatures (Figure 1).

Associations Between Maximum Temperatures and Sedentary Time

The adjusted associations from multilevel models between quintiles of maximum temperature and sedentary time are shown in Table 1. In summary, lower temperatures were associated with more time spent in sedentary behavior ($P < .001$). In particular, men spent 26 minutes more per day (95% CI 19–33) in sedentary time when temperatures were in the lowest compared with the highest quintile (Table 1). When analyzing maximum temperature as continuous variable, a negative linear association with sedentary time was observed: a decrease in 1 SD (5.8°C) in maximum temperature was associated with an increase of 11 minutes per day (95% CI 8–13) in sedentary time ($P < .001$). The adjustment for day length did not alter the magnitude of these associations; day length was not significantly associated with sedentary time ($P = .212$).

Subsidiary Analyses

A decrease of 1 SD (5.8°C) in maximum temperature was associated with a decrease of 2 (95% CI 1–3) breaks in sedentary time per day, and an increase of 0.2 (95% CI 0.1–0.3) daily number of longer sedentary bouts (≥30 minutes). No association was found between maximum temperature and daily number of shorter sedentary bouts (<30 minutes).

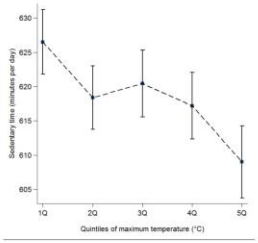

Figure 1 — Raw data (n = 1361). Plots depicting relationship between sedentary time (mean, 95% CI), and quintiles (Q) of maximum temperature. *Note.* Quintiles of maximum temperature were derived counting every day each participant wore an accelerometer (median, minimum and maximum): 1Q: 6.3 (–3.5, 9.2); 2Q: 11.0 (9.3, 13.0); 3Q: 15.3 (13.1, 16.5); 4Q: 17.9 (16.6, 19.0); 5Q: 20.8 (19.1, 29.5). P-value for the difference between the quintiles was $P < .001$.

Table 1 Adjusted Associations Between Quintiles (Q) of Maximum Temperature and Sedentary Time (n = 1361)[a]

Quintiles of maximum temperature (°C)	Mean difference (95% CI) in sedentary time (minutes per day)
5Q (19.1; 29.5)	Reference
4Q (16.6; 19.0)	+7 (3; 11)
3Q (13.1; 16.5)	+14 (10; 19)
2Q (9.3; 13.0)	+21 (15; 27)
1Q (–3.5; 9.2)	+26 (19; 33)

[a] Multilevel regression models (level 1 = date, level 2= individual) adjusted for age, social class, BMI, chronic conditions, mobility limitations, geriatric depression scale, vision problems, smoking status, daily wear time, day of the week, wear day order, and day length.

Note. P-value for trend < 0.001.

Variations in hours of sunshine and relative humidity were associated with variations in sedentary time. On the other hand, association of minimum temperature with sedentary time was not significant (Online Supplementary Material, Appendix S1, Table 1).

Maximum temperature was also strongly associated with other physical activity outcomes: a decrease of 1 SD in maximum temperature was associated with –7 minutes in LIPA per day (95% CI –9 to –5), –4 minutes in MVPA per day (95% CI –5 to –2), and –323 steps per day (95% CI –428 to –218). Similarly, variations of hours of sunshine and relative humidity were also associated with variations of time spent in LIPA, MVPA, and steps per day, although the magnitude of associations was smaller. Association of minimum temperature with physical activity was not significant (Online Supplementary Material, Appendix S1, Table 1).

In stratified analysis, the magnitude of associations between temperature and sedentary time were not materially affected by excluding men who were depressed or/and with mobility limitations (Online Supplementary Material, Appendix S1, Table 2).

Discussion

In this large study of older British men, outdoor maximum temperature was associated with accelerometer-measured sedentary time: a decrease in maximum temperatures was associated with an increase in sedentary time after controlling for potential confounding variables (measurement variables, individual characteristics, and day length).

Overall Findings

The analysis of maximum temperature subdivided in quintiles offered a simple and intuitive interpretation of the results: during a typical winter day (temperature in the lowest quintile) older men spent 26 minutes more per day in sedentary time in comparison with a typical summer day (temperatures in the highest quintiles). Perception of cold may particularly inhibit older individuals from spending time outdoors. Apart from the discomfort and need to wear suitable clothing, there may be a fear of falling due to ice. Consequently, older adults may prefer replacing some incidental light physical activity outdoors (eg, a gentle walk for pleasure) with sedentary behaviors indoors, such as television watching.[17]

We focused our investigation on maximum temperature as primary determinant as it is more accurate than other meteorological factors due to a lower spatial variability.[18] However, in subsidiary analysis we also demonstrated that less hours of sunshine and higher relative humidity, typical elements of the winter season in UK, were also associated with an increase in sedentary time. To our knowledge these findings are novel and not previously reported. Literature in this field is sparse; 1 small study of 46 adults demonstrated that accelerometer-measured sedentary time is higher in winter than summer, although the participants were about 40 years younger than our population.[19] The majority of the studies investigated children or adolescents, which are known to have a different life-style in comparison with older adults.[20]

Strengths and Limitation

This study used data from the BRHS, which is a large scale population-based cohort of older men, rather than an institutionalized older population. The magnitude of associations between temperature and sedentary time were not materially affected by excluding men who were depressed or/and with mobility limitations. Thanks to accelerometers it was possible to overcome problems of recall error, which is known to be more common in older individuals.[21] Therefore, an objective measure is more accurate and recommended, considering the proportion of time older adults spent in sedentary behaviors.[22] Moreover, we corroborated previous findings which have investigated accelerometer-measured physical activity outcomes: as in earlier studies we showed that low maximum temperatures, fewer hours of sunshine, and higher relative humidity were associated with fewer steps per day, and less time spent in LIPA and MVPA.[5,6] We also demonstrated that the association of maximum temperature with

physical activity was strongest in comparison with associations of sunshine duration and humidity with physical activity. Our findings suggested that maximum temperature is the most important predictor of physical activity in the UK. However, earlier studies which took place in Germany, Scotland and Japan had identified a range of different meteorological factors as being the most important, such as global radiation,[21] day length and diurnal minimum temperature,[2] rainfall and mean temperature.[24,25] However, we would expect that, as in our results, radiation and other temperature variables are positively correlated with maximum temperature.

The study have some limitations: men who did not accept our invitation to participate in the study were about 2 years older and had higher BMI measured 10 years earlier; implying that overall physical activity (eg, total number of steps) might be lower in the general population. Our study is also limited by studying almost exclusively white European older men, who would be expected to spend more time in sedentary behavior, compared with younger individuals.[4] Moreover, our results may not be generalizable to women, or to other ethnic groups.[26]

We defined sedentary behavior based solely on intensity, rather than intensity and posture (more widely used), as this study did not aim to investigate the "type" of sedentary behaviors (eg, sitting at a computer, lying on the couch, driving, etc). However, the activity monitors we used provide useful estimates of sedentary time, as they have minimal bias in comparison with other devices able to detect intensity, position and posture.[27] The importance of position and posture is widely recognized and future studies could further investigate the particular types of sedentary behaviors (eg, watching TV) carried out during the lowest peaks of activity.

Also, during the study period maximum temperatures never reached levels above 30°C. At those high temperatures, more typical of warmer climate zones than the UK, sedentary time may start to increase. During heat waves local authorities tend to alert older individuals, who are usually asked to remain indoors in the heat of the day, to get some rest and sit when necessary, and not engage in strenuous activities.

Implications

The results may have important implications for guidelines. The UK recommendations suggest that older adults should aim to minimize the time they spend being sedentary each day.[8] Our findings provided more justification for minimizing sedentary behaviors particularly at low temperatures, a typical element of the winter season. Replacing some of the time spent in sedentary behaviors into more active behaviors may have beneficial effects on health. However, to find ways to reduce sedentariness is challenging, as in modern life opportunities for sedentary behaviors are everywhere. To date, findings from the ProActive65+ trial suggested that older adults with poor self-rated health, higher BMI and history of smoking are more likely to reduce the sedentary time from an exercise intervention.[28] On the other hand, it is likely that interventions targeting individuals' psychological and environmental barriers (beliefs, feelings, and perspectives on participations in physical activity) may be a valid alternative for replacing sedentary time with more active behaviors.[29,30] Providing recommendations for simple do-it-yourself exercises (eg, standing up or walking while watching TV, toe rises, calf and chest stretching) could be helpful. In older individuals, simple targets can make the reduction in sedentary behavior easier to achieve and relevant on a daily basis.[31] Also, providing physically and economically accessible indoor opportunities for promoting more active behaviors during winter should be encouraged.

The temperature-related variation in sedentary time observed in this study could be relevant to the temperature-related variation in mortality risk.[32] It is plausible that persisting low temperatures in winter (primary determinant) may be a contributing factor which increases the sedentary time, as well as other risk factors levels (eg, inflammatory markers, such as C-Reactive Protein and Interleukin-6[33]) contributing to the excess of winter mortality.[34] We estimated an increase of 26 minutes in sedentary time at lower versus higher temperatures. According to previous studies in older adults, replacing 30 minutes of sedentary time with light physical activity was independently associated with a significant reduction in mortality risk (HR = 0.80).[35] However, future investigations are needed to establish how temperature-related variations in sedentary time may contribute to the temperature-related variations in mortality risk.

Conclusions

In this study of older adults, we demonstrated that sedentary time increased at lower maximum temperatures. These findings are relevant for guidelines: interventions may consider targeting older adults in winter, when temperatures are lower, providing recommendations for minimizing sedentariness on a daily basis.

Acknowledgments

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Associations of time of day with cardiovascular disease risk factors measured in older men: results from the British Regional Heart Study

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ABSTRACT

Objective We estimated associations of time of day with cardiovascular disease (CVD) risk factors measured in older men.

Methods CVD risk factors (markers of inflammation and haemostasis, and cardiac markers) were measured on one occasion between 08:00 and 19:00 hours in 4252 men aged 60–79 years from the British Regional Heart Study. Linear models were used to estimate associations between time of day and risk factors. When an association was found, we examined whether the relationship between risk factors and cardiovascular mortality was affected by the adjustment for time of day using survival analyses.

Results N-terminal pro-brain natriuretic peptide (NT-proBNP) levels increased by 3.3% per hour (95% CI 1.9% to 4.8%), interleukin-6 (IL-6) increased by 2.6% per hour (95% CI 1.8% to 3.4%), while tissue plasminogen activator (t-PA) decreased by 3.3% per hour (95% CI 3.7% to 2.9%); these associations were unaffected by adjustment for possible confounding factors. The percentages of variation in these risk factors attributable to time of day were less than 2%. In survival analyses, the association of IL-6, NT-proBNP and t-PA with cardiovascular mortality was not affected by the adjustment for time of day. C reactive protein, fibrinogen, D-dimer, von Willebrand factor and cardiac troponin T showed no associations with time of day.

Conclusions In older men, markers of inflammation (IL-6), haemostasis (t-PA) and a cardiac marker (NT-proBNP) varied by time of day. The contribution of time of day to variations in these markers was small and did not appear to be relevant for the CVD risk prediction.

Strengths and limitations of this study

- ► To our knowledge, this is the largest investigation of relationships between time of day and cardiovascular disease (CVD) risk factors in older men.
- ► This study assessed the contribution of time of the day to the overall variation of CVD risk factors, and established its relevance for the CVD prediction.
- ► The relationship of the CVD risk factors to time of day was explored using between-participant variation only.

BACKGROUND

Previous studies have reported time of day variation in both established and emerging cardiovascular disease (CVD) risk factors in middle-aged adults, such as blood pressure, lipids and some well-established inflammatory and haemostatic factors (eg, white cell, red blood cell and platelet counts).[1–3] However, the extent to which some emerging CVD risk factors such as interleukin (IL)-6 , a marker of inflammation causally associated with CHD in a recent study,[4] and N-terminal pro-brain natriuretic peptide (NT-proBNP), a marker of heart failure,[5] vary by time of day have been less studied. Moreover, very little is known on time of day variations in other emerging risk factors prospectively associated with CVD (eg, tissue plasminogen activator (t-PA), D-dimer, von Willebrand factor (vWF) and cardiac troponin T (cTnT)), although their causal association with CVD remain debated or not yet tested.

We would expect that time of day variations in some emerging CVD risk factors measured in older adults may occur, consistent with findings in younger populations.[6] However, in older adults, the degree of difference attributable to time of day has not been yet estimated; establishing its importance and its effects on prediction of CVD risk is important given the potentially wider use of NT-proBNP in risk stratification (as shown in a recent major meta-analysis in the general population[7]) and potential causal link between IL-6 and CVD.[4] Therefore, the aim of this study was to investigate how emerging CVD risk factors, including markers of inflammation, haemostasis and myocardial function, varied by time of day in older British men.

METHODS

Participants

The British Regional Heart Study (BRHS) is a prospective cohort study of CVD involving 7735 middle-aged men (40–59 years) selected in 1978–1980 from the age–sex registers of one local primary care centre in 24 British towns.[8] The 24 towns were selected to represent the variation in CVD across the UK.[9] Participants provided informed written consent to the investigation, which was performed in accordance with the Declaration of Helsinki.[10]

Follow-up examination

In 1998–2000, an average of 20 years after the initial recruitment, 4252 surviving participants (77% response rate) aged 60–79 years who were resident in the UK attended a physical examination during which nurses took a fasting blood sample on one occasion for each participant. The men were asked to fast for a minimum of 6 hours, during which they were instructed to drink only water, as previously reported.[2] The blood samples were collected between 08:00 and 19:00 hours and then assayed for a range of biochemical and haematological markers. Participants' appointment times were non-systematically allocated. They were offered the opportunity to contact the BRHS team and change the time of examination if unable to attend; a small proportion of participants did so.

The participants were also asked to complete a questionnaire that included questions on other established CVD risk factors, such as age, social class, smoking habits, alcohol consumption and physical activity. Specifically, physical activity levels were self-reported[11] and recently validated using accelerometers.[12] Incident CVDs, including non-fatal stroke and non-fatal myocardial infarction (MI), were recorded: their definitions have been reported elsewhere.[13] Men were also asked whether a doctor had ever told them that they had heart failure.[5] The number of blood samples collected and included in the analyses differ according to the risk factor measurements (the number of observations varied from 3580 for NT-proBNP to 3863 for von Willebrand Factor in complete case analyses including all covariates of interest).

CVD risk factors

Circulating levels of markers of inflammation (C reactive protein (CRP), IL-6 and fibrinogen), cardiac markers (NT-proBNP and cTnT) and markers of haemostasis (t-PA antigen, fibrin D-dimer and vWF) were measured.

D-dimer and t-PA levels were measured using an ELISA (Biopool AB, Umeå, Sweden), as was vWF antigen (Dako, High Wycombe, UK). CRP was assayed using ultrasensitive nephelometry (Dade Behring, Milton Keynes, UK). IL-6 was assayed using a high-sensitivity ELISA (R&D Systems, Oxford, UK). Fibrinogen was assayed using an automated Clauss assay in a coagulometer (MDA-180, Organon Teknika, Cambridge, UK). NT-proBNP and cardiac troponin T (cTnT) were measured in plasma samples on an automated clinically validated immunoassay analyser (e411, Roche Diagnostics, Burgess Hill, UK) using the manufacturers' calibrators and quality control reagents. Intra-assay and interassay coefficient of variations were respectively: 4.1% and 6.6% for t-PA; 3.2% and 4.2% for vWF; 4.7% and 5.2% for D-dimer; 4.7% and 8.3% for CRP; 7.5% and 8.9% for IL-6; 2.6% and 3.7% for fibrinogen and 4.4% and 7.7% for NT-proBNP and cTnT.

The samples were centrifuged and separated on the morning or afternoon of collection and stored on site at −20°C until they were transferred to a central freezer storage location at −70°C within 2 weeks of sample collection. Samples were then transferred on dry ice to a single central laboratory and were thawed immediately before analysis. Plasma samples were used for all the analyses reported here. The original sample collection took place between January 1998 and March 2000. Most of the analyses described here were carried out during 2000, after a maximum of 3 years storage; NT-proBNP and cTNT were analysed in 2009.

Statistical methods

First, the distributions of the outcomes were examined; the outcomes were log-transformed as the distributions were positively skewed. Therefore, analysis was carried out on their log-transformed values throughout. Unadjusted geometric means and 95% CIs of the outcomes were plotted against hour of the day.

Adjusted associations between time of day and the outcomes

Associations between time of day (fitted as a continuous variable, range 8–18) and the outcomes were examined using linear multilevel random intercept models (level 1=individual, level 2=town of residence). The results can be interpreted as between-person variations over the course of the examination day; the estimates from the linear model were reported as the difference in the outcome levels per hour of sampling over the examination day. As the outcomes were log-transformed, the results were reported as per cent difference in the outcome geometric mean per hour of sampling. All models were initially adjusted for age only. Next, the models were adjusted for age and other possible confounding factors: social class, body mass index, previous stroke or MI, physical activity, smoking status, alcohol consumption, use of statin and a sexasonal term (fitted using a cosinor function, as in previous studies).[14] As NT-proBNP and cTnT are principally markers of heart failure, the association with time of day was adjusted for previous heart failure.

When the association of time of the day with the outcomes was found to be statistically significant, the proportion of variance associated with time of the day was estimated using partial R^2.

Sensitivity analyses

Six sensitivity analyses were performed: (1) all models were additionally adjusted for fasting time and diabetes; (2) all models were carried out excluding men with diabetes; (3) interactions were fitted to test whether the time of day associations were modified by age (fitted as continuous variable); (4) as NT-proBNP and cTnT were acknowledged as specific cardiac markers,[5] interactions were fitted to test whether the time of day associations were modified by previous heart failure (yes/no); (5) to explore the potential of undiagnosed heart failure or cardiac damage influencing findings for NT-proBNP and cTnT, we repeated regression models after excluding men with NT-proBNP >400 pg/mL; and (6) a quadratic term for time of day was added to the models in order to check for non-linearity.

As IL-6 has been causally associated with cardiovascular risk,[4] and prospectively associated with CVD mortality in the BRHS sample used here,[15] we investigated the relevance of time of day to the cardiovascular risk prediction by performing two survival analyses: in the first analysis, we used Cox models where unadjusted log IL-6 was used as the predictor and CVD mortality as the clinical outcome, then we repeated the same analysis using log IL-6 standardised by the time of day rather than unadjusted log IL-6. For completeness of information, we repeated this sensitivity analysis for NT-proBNP and t-PA.

RESULTS

The characteristics of the study participants (mean age 68.7 years, SD=5.5) are reported in table 1. The associations between time of day (by hour) and risk factors are shown in figure 1. Evidence of an increase over the course of the day was particularly noticeable for IL-6 and for NT-proBNP (figure 1). Also, levels of t-PA were lower in the afternoon in comparison with morning, while variations by time of day for other risk factors were not clearly observable from the plots (figure 1). The results of corresponding linear regression analyses are shown in table 2; statistically significant associations between time of the day and some outcomes were found (table 2); over the course of the examination day, NT-proBNP levels increased by 3.3% per hour (95% CI 1.9% to 4.8%) and IL-6 increased by 2.6% per hour (95% CI 1.8% to 3.4%). Conversely, t-PA decreased by 3.3% per hour (95% CI 3.7% to 2.9%). The proportion of variance associated with time of the day from the fully adjusted models was 0.5%, 1%, and 2% for NT-proBNP, IL-6 and t-PA, respectively.

Sensitivity analyses

Overall, we found that fasting time did not alter the magnitude of associations between time of the day and the outcomes reported in table 2. Only the association between time of the day and t-PA was strongly attenuated after accounting for fasting time (fitted as continuous variable): the decrease in t-PA levels was −3.3% (95% CI −3.7 to −2.9) per hour before the adjustment (table 2) and −1.4% (95% CI −2.2% to −0.1%) after the adjustment for fasting. An additional adjustment for diabetes status did not alter the magnitude of the association between hour of the day and the outcomes. We also performed the analysis excluding men with diabetes completely (table 2, model 3), but the association between time of day and the outcomes did not substantially change.

For all outcomes, we also did not find evidence for an interaction between of time of day with age (results not shown).

Table 1 Individual characteristics and risk factor levels in the British Regional Heart Study of men who have attended the examination in 1998–2000

Demographic and background characteristics	
Age (years), mean (SD)	68.7 (5.5)
Social class (manual)	
Manual, n (%)	2166 (51.1)
Non-manual, n (%)	1966 (46.3)
Armed Forces, n (%)	112 (2.6)
Physical health	
Body mass index, mean (SD)	26.9 (3.7)
Prevalence of stroke/myocardial infarction, n (%)	153 (3.6)
Prevalence of heart failure, n (%)	390 (9.2)
Diabetes, n (%)	478 (11.2)
Behavioural factors	
Smoking	
Never, n (%)	1233 (29.1)
Ex-smokers, n (%)	2464 (58.0)
Smokers, n (%)	548 (12.9)
Alcohol consumption	
None, n (%)	431 (10.3)
Occasional/light, n (%)*	2949 (70.5)
Moderate/heavy, n (%)†	779 (18.6)
Physical activity level	
Inactive, n (%)	471 (11.5)
Occasionally, n (%)	957 (23.4)
Light, n (%)	767 (18.7)
Moderate, n (%)	591 (14.4)
Moderate vigorous, n (%)	690 (16.8)
Vigorous, n (%)	621 (15.1)
CVD risk factor, geometric mean (SD)	
CRP, mg/L	1.74 (3.03)
IL-6, pg/mL	2.46 (1.94)
Fibrinogen, g/L	3.19 (1.25)
t-PA, ng/mL	10.23 (1.50)
vWF, IU/dL	132.41 (1.40)
D-dimer, ng/mL	84.32 (2.32)
NT-proBNP, pg/mL	101.50 (3.32)
cTnT, pg/mL	12.07 (1.64)

*≥1 and ≤15 units per week (1 unit is approximately one drink, such as one glass of wine).
†≥16 units per week (1 unit is approximately one drink, such as one glass of wine).
CRP, C reactive protein; cTnT, cardiac troponin T; IL-6, interleukin-6; NT-proBNP, N-terminal pro-brain natriuretic peptide; t-PA, tissue plasminogen activator; vWF, von Willebrand factor.

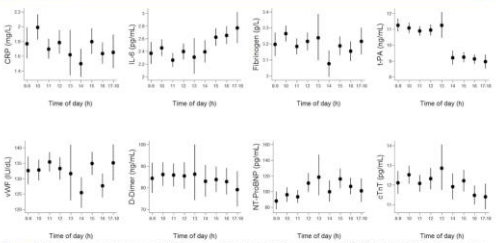

Figure 1 Unadjsted geometric means (95% CI) by time of the day* for C reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, tissue plasminogen activator (t-PA), von Willebrand factor (vWF), fibrin D-dimer, N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponin T (cTnT) measured on one occasion in BRHS men aged 60–79 during the years 1998–2000. *Total number of men examined per hour was 33 (0.7%) at 08:00–08:59, 363 (8.5%) at 8:00–9:59hours, 699 (16%) at 10:00–10:59hours, 771 (18%) at 11:00–11:59hours, 591 (14%) at 12:00–12:59hours, 99 (2%) at 13:00–13:59hours, 306 (7%) at 14:00–14:59, 560 (13%) at 15:00–15:59hours, 566 (13%) at 16:00–16:59, 260 (6%) at 17:00–17:59 and 3 (<0.1%) at 18:00–18:59.

In stratified analysis, NT-proBNP levels increased by 3.4% (95% CI 1.9% to 4.8%, p<0.001) per hour in older men without heart failure. Although men who previously had heart failure had increased NT-proBNP levels, there was no evidence for an interaction between previous heart failure with time of the day (p=0.954). After excluding 466 men with NT-proBNP level of >400 pg/mL (12% of the sample), associations between time of the day measures and NT-proBNP remained statistically significant and slightly increased in magnitude (3.9% (95% CI 2.7% to 5.1%), p<0.001). As reported in the main analysis, no significant associations were found between time of the day and cTnT in stratified analysis.

When adding a quadratic term to the model, we found a significant improvement in model fit for IL-6 only (p=0.030 for the time of day squared term). The association of time of day with IL-6 appeared to be slightly J-shaped, with a linear increase starting from 11:00 to 19:00 hours.

We examined whether adjustment for hour of day affected the associations between risk factors and CVD mortality. In survival analysis, higher levels of log IL-6 were associated with increased CVD mortality (HR=1.70, 95% CI 1.54 to 1.87). Standardising IL-6 by time of the day did not change the relationship (HR=1.71, 95% CI 1.55 to 1.88). Also, standardising NT-proBNP levels by time of the day did not alter the magnitude of the effect on CVD mortality (HR=1.92, 95% CI 1.81 to 2.04). Finally, associations of t-PA levels with increased CVD mortality did not change substantially before (HR=1.74, 95% CI 1.45 to 2.09) and after standardising (HR=1.77, 95% CI 1.47 to 2.14) by time of the day.

DISCUSSION

To our knowledge, this is the largest investigation of relationships between time of day and CVD risk factors in older men. After adjusting our analysis for potential confounding factors, we demonstrated that some, but not all, CVD risk factors levels varied by time of day. In particular, NT-proBNP and IL-6 increased linearly over the course of the day. Conversely, a decrease in t-PA was also observed; however, after accounting for fasting time, the relationship with time of the day was strongly attenuated (therefore fasting time could partially explain the drop in t-PA levels observed in the afternoon vs morning). Our analyses showed that the contribution of time of the day to the overall variation of NT-proBNP, IL-6 and t-PA was small and without clinical importance; we observed that time of day did not have a sufficiently strong effect to be taken into account when assessing the impact of IL-6, NT-proBNP and t-PA on CVD mortality. Lastly, an association of time of day with other risk factors was not observed.

Literature on time of day variation in emerging CVD markers of inflammation and haemostasis in older adults is limited; to our knowledge, this is the first time these findings have been reported in older adults. Findings from earlier studies of younger adults were fairly consistent with ours. For example, a recent meta-analysis of several small studies that analysed IL-6 proposed a diurnal pattern, with

Table 2 Cross-sectional adjusted associations between time of day (fitted as continuous variable) and cardiovascular disease (CVD) risk factors measured in the British Regional Heart Study (BRHS) men (aged 60–79) attending the follow-up year 20 examination in 1998–2000

CVD risk factor‡	Model 1: age adjusted* Per cent difference (95% CI) in the CVD risk factor levels per hour of sampling§	p Value	Model 2: fully adjusted† Per cent difference (95% CI) in the CVD risk factor levels per hour of sampling§	p Value	Model 3: fully adjusted† excluding men with diabetes Per cent difference (95% CI) in the CVD risk factor levels per hour of sampling	p Value
NT-proBNP	**3.5 (2.0 to 5.0)**	**<0.001**	**3.3 (1.9 to 4.8)**	**<0.001**	**3.5 (2.0 to 5.0)**	**<0.001**
IL-6	**2.6 (1.7 to 3.4)**	**<0.001**	**2.6 (1.8 to 3.4)**	**<0.001**	**2.4 (1.6 to 3.3)**	**<0.001**
t-PA	**−3.3 (-3.8 to −2.9)**	**<0.001**	**−3.3 (−3.7 to −2.9)**	**<0.001**	**−3.2 (−3.6 to −2.7)**	**<0.001**
Fibrinogen	−0.2 (−0.5 to 0.0)	0.088	−0.2 (−0.5 to 0.1)	0.104	−0.2 (−0.5 to 0.1)	0.149
cTnT	−0.4 (−0.9 to 0.2)	0.194	−0.4 (−1.0 to 0.2)	0.174	−0.4 (−1.0 to 0.2)	0.165
CRP	−1.0 (−2.3 to 0.4)	0.151	−0.9 (−2.2 to 0.4)	0.175	−0.9 (−2.2 to 0.5)	0.191
vWF	−0.2 (−0.6 to 0.2)	0.374	−0.2 (−0.6 to 0.2)	0.380	−0.1 (−0.5 to 0.3)	0.703
D-dimer	−0.0 (−1.0 to 1.0)	0.929	−0.1 (−1.0 to 0.9)	0.890	−0.1 (−1.2 to 0.9)	0.801

Associations are reported as per cent difference in CVD risk factors levels per 1 hour of sampling over the examination day (08:00–19:00 hours). The statistically significant associations are marked in bold
*Model 1: two level linear models (level 1=person and level 2=town of residence during the BRHS recruitment) adjusted for age. Model 1 used the same number of observations of Model 2 (complete case analysis).
†Model 1 additionally adjusted for social class, body mass index, smoking status, alcohol consumption, physical activity, use of statin and season. Associations with IL-6, t-PA, fibrinogen, CRP, vWF and D-dimer were additionally adjusted for prevalence of stroke/MI, while association of time of the day with NT-proBNP and cTnT models were additionally adjusted for prevalence of heart failure.
‡Model 1 and Model 2 used the same number of observations: 3582 for NT-proBNP, 3832 for IL-6, 3863 for t-PA, 3861 for fibrinogen, 3827 for cTnT, 3838 for CRP, 3863 for vWF, 3859 for D-dimer.
§Model 3 number of observations: 3176 for NT-proBNP, 3398 for IL-6, 3429 for t-PA, 3427 for fibrinogen, 3398 for cTnT, 3405 for CRP, 3429 for vWF, 3425 for D-dimer.
CRP, C reactive protein; cTnT, cardiac troponin T; IL-6, interleukin-6; MI, myocardial infarction; NT-proBNP, N-terminal pro-brain natriuretic peptide; t-PA, tissue plasminogen activator; vWF, von Willebrand factor.

overall IL-6 levels increased between 08:00 and 19:00 hours as in our study.[16] However, in two earlier very small studies of 12[17] and 5[18] participants, IL-6 peaked in the night-time. It is possible that peaks in IL-6 levels may be associated with cognitive symptoms of depression[19] and daily activities, although in the BRHS population, this has not yet been investigated. One previous study found that BRHS men were more active in the morning and in early afternoon[20] when the main activities were usually gardening, house works, shopping or leisure walking. Whether IL-6 was implicated in this daily pattern remains uncertain and can potentially be explored in future studies.

Moreover, one previous study reported increased levels of NT-ProBNP over the course of day[21] as we observed in our study. A decrease in t-PA over the examination day was also reported in younger subjects (a 45-year-old UK population of 9377 men and women)[6]; however, t-PA did not vary by time of the day in a previous large study of 1288 healthy men and women aged 25–64 years.[22]

In comparison with our study, findings regarding CRP, fibrinogen, D-dimer, vWF and cTnT reported in earlier studies of younger adults were similar; a few previous studies reported that they did not find an association of time of day with CRP,[23] D-dimer[24] and vWF.[25] In one study, the variation in CRP, fibrinogen, D-dimer and vWF attributable to time of day was minimal.[6] Literature on cTnT is scarce; one small previous study of repeated measures in seven participants with type 2 diabetes reported a decrease in cTnT between 08:00 and 20:00.[26]

Although one previous study suggested that diurnal variations in CVD risk factors could be relevant for cardiovascular risk prediction,[6] a prediction model like the one described in our survival analysis was not performed. Our findings suggested the effect of time of the day (from 08:00 hours to 19:00 hours) is not relevant for the CVD risk assessment. With this sensitivity analysis, we wanted to investigate time of day variations beyond simple descriptive diurnal patterns; to our knowledge, this is the first time this finding has been reported.

Strengths and limitations

The BRHS cohort benefits from using a large-scale population-based sample of free-living older men, and this increases statistical power and precision of estimates. However, the BRHS comprises male participants, predominantly of white European ethnic origin, so findings may not be generalisable to women and non-white ethnic groups. The CVD risk factors measurements were carried out on one occasion over an extended period of the day (between 08:00 and 19:00 hours), offering only a partial understanding of the variations over the 24 hours.[27 28] Therefore, in this study, the relationship of the CVD risk

factors to time of day was explored using between-participant variation only. In future studies, it may be advantageous to carry out repeated measurement of the risk factors over the 24 hours in order to investigate within-person circadian variations. However, with repeated measurements, a possible and genuine diurnal variation may be disrupted and natural sleeping patterns altered (repeated measures are usually taken every 1–2 hours during the night).[29]

Implications

Variations of some CVD factors (in particular IL-6 and NT-proBNP) over the course of the day were observed, suggesting the role of time of the day as potential confounder during the measurements. However, standardising these biological markers by time of day was not particularly relevant for the cardiovascular risk prediction. Also, other sensitivity analyses (stratified analysis and interaction tests) did not add relevant insights suggesting that time of day variations may be not important for clinical risk stratification in general. Further studies assessing both CVD risk factors levels and clinical outcomes (eg, fatal or non-fatal CVD events) during 24 hours are required to demonstrate whether a rapid increase of IL-6 over the day may be relevant to the increased number of CVD events observed in early and late morning,[30] and whether the increased levels of NT-proBNP over the day are related to the afternoon peak in sudden death following heart failure.[31]

CONCLUSIONS

Variations in time of day were associated with variations of some, but not all, CVD risk factors measured in older adults. The contribution of time of the day to the markers' overall variation was small and unlikely to affect the CVD risk prediction or clinical risk stratification.

Contributors CS processed the data, performed statistical analyses, drafted and revised the manuscript and incorporated revisions of coauthors. PHW, SGW, BJJ and RWM contributed to the design of the study and revised the manuscript. LL enrolled participants and collected data. PHW, RWM and GSW raised grant funding. All authors gave an intellectual contribution to the manuscript and approved the final version.

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Relationship between outdoor temperature and cardiovascular disease risk factors in older people

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Abstract

Background: Previous studies demonstrated that lower outdoor temperatures increase the levels of established cardiovascular disease risk factors, such as blood pressure and lipids. Whether or not low temperatures increase novel cardiovascular disease risk factors levels is not well studied. The aim was to investigate associations of outdoor temperature with a comprehensive range of established and novel cardiovascular disease risk factors in two large Northern European studies of older adults, in whom cardiovascular disease risk is increased.

Design and methods: Data came from the British Regional Heart Study (4252 men aged 60–79 years) and the Prospective Study of Pravastatin in the Elderly at Risk (5804 men and women aged 70–82 years). Associations between outdoor temperature and cardiovascular disease risk factors were quantified in each study and then pooled using a random effects model.

Results: With a 5˚C lower mean temperature, total cholesterol was 0.04 mmol/l (95% confidence interval (CI) 0.02–0.07) higher, low density lipoprotein cholesterol was 0.02 mmol/l (95% CI 0.01–0.05) higher and SBP was 1.12 mm Hg (95% CI 0.60–1.64) higher. Among novel cardiovascular disease risk factors, C-reactive protein was 3.3% (95% CI 1.0–5.6%) higher, interleukin-6 was 2.7% (95% CI 1.1–4.3%) higher, and vitamin D was 11.2% (95% CI 1.0–20.4%) lower.

Conclusions: Lower outdoor temperature was associated with adverse effects on cholesterol, blood pressure, circulating inflammatory markers, and vitamin D in two older populations. Public health approaches to protect the elderly against low temperatures could help in reducing the levels of several cardiovascular disease risk factors.

Keywords

Biomarkers, outdoor temperature, older adults, cardiovascular disease risk factors

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Introduction

In the UK and most European countries, cardiovascular disease (CVD) risk increases at lower temperatures, a typical element of the cold season.[1,2] As CVD risk during the cold season is more markedly increased in older rather than younger adults,[3] investigating temperature-related variations in CVD risk factors in older adults is of particular interest.

It has been hypothesised that lower outdoor temperatures could exert their adverse effects by increasing the levels of well-established risk factors causally associated with coronary heart disease (CHD),[4,5] such as blood pressure[6] and lipids.[7] However, associations of temperature with recently established causal risk factors for CHD, such as interleukin-6,[8] are not well studied.[9] Also, low outdoor temperatures may increase the levels of other novel risk factors prospectively associated with CVD (e.g. inflammatory markers, haemostatic markers),[10] although the literature supporting this hypothesis is sparse.[9,11] Higher outdoor temperature is also a proxy measure for sunlight exposure, and hence potentially related to the level of vitamin D which has consistently been associated with chronic disease incidence although its causal association remains hotly debated.[12]

Common limitations of previous studies investigating associations of outdoor temperature and CVD risk factors are small sample size,[13,14] the specific geographical location,[11] and the investigation of clinical populations.[15] Therefore, large population-based studies which explore associations of outdoor temperature with a comprehensive range of CVD risk factors are required to improve statistical power and estimate precision.

Considering the gaps in knowledge from previous research, the aim of this study was to investigate the strength of relationship between established and novel biological risk factors and outdoor temperature in two large Northern European studies of older adults.

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Methods and participants

Participants from the British Regional Heart Study (BRHS) and the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) provided informed written consent, which was performed in accordance with the principles of the Declaration of Helsinki. The designs of BRHS and PROSPER, both prospective studies of cardiovascular disease comprising several thousand participants, have been previously described.[16]

Cardiovascular risk factors measurement (outcomes)

For both BRHS and PROSPER, details of measurement values and classification methods for the cardiovascular risk factors were extensively described[16] and are briefly reported here in the Supplementary Material, Cardiovascular Risk Factors Measurements. The measurements were carried out during 1997–2000, and the factors included (a) established risk factors, such as systolic and diastolic blood pressure (BP) obtained sitting, and blood lipids (triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol); and (b) novel risk factors, such as inflammatory factors (C-reactive protein (CRP), fibrinogen, interleukin 6 (IL-6)) and plasma viscosity (PV); haemostatic markers (tissue plasminogen activator (t-PA) antigen, fibrin D-dimer, von Willebrand factor (vWF); and vitamin D (VitD).

Temperature data

National meteorological offices provided daily outdoor mean temperatures for the 24 towns of BRHS and three locations of PROSPER during the study period. Definition of outdoor mean temperature on the examination day (lag 0) has been extensively described elsewhere.[16]

Statistical methods

Descriptive statistics

Temperature and the unadjusted outcomes' levels were examined by month of measurement. Then, excepting total cholesterol, *p*, HDL-cholesterol, LDL-cholesterol, systolic BP (SBP) and diastolic BP (DBP), all other outcomes were log-transformed for further analysis as their distributions were positively skewed.

Associations (main effects) of temperature with the CVD risk factors

For log-transformed outcomes, associations were reported as the percentage change in the geometric mean associated with a decrease of 5˚C in mean temperature (5˚C being the standard deviation of daily mean temperature for the years 1997–2000 in the BRHS and PROSPER towns). Associations of temperature with BP variables, HDL-cholesterol, LDL-cholesterol, and total cholesterol, were reported as linear coefficients (absolute change) per decrease of 5˚C in mean temperature.

Before being considered for pooling, data from the BRHS and PROSPER were analysed separately due to differences in study design, inclusion criteria and measurement protocols. In BRHS, multilevel linear regression models (level 1 = individual, level 2 = town of examination) were used to take into account clustering within towns.[17] Associations were adjusted for established CVD risk factors and possible confounders, such as age, body mass index (BMI), social class, smoking, alcohol consumption, physical activity score and time of day (measurement variable).[9] In PROSPER, linear regression models were used to estimate the associations of temperature with the outcomes. Associations were adjusted for the same variables as for BRHS except for physical activity, social class, and time of day which were not ascertained, but for sex and location. In BRHS and PROSPER separately, the proportion of variance associated with temperature from the fully adjusted models was estimated using partial R-squared. We also fitted an interaction between temperature and age (both fitted as continuous variables) to test whether the relationship of temperature with outcomes was particularly marked among older participants.

Pooled analysis

Regression coefficients from fully adjusted models of BRHS and PROSPER were pooled using a random effects model, to take account of heterogeneity between the two studies where it occurred, for each of the outcomes separately.

Sensitivity analysis

The cumulative short-term effect of temperature on the CVD risk factors was also investigated, using the temperature moving average of seven days which included lag days from 0–6 prior to the examination day (lag 0–6).

An additional adjustment of outdoor temperature (at lag 0) with a seasonal term, such as day length or sine and cosine terms,[18] was evaluated. However, since the variance inflation factor scores were between 9–13 for these seasonal terms when included with temperature, collinearity would have been induced and therefore the adjustment was not recommended in this case. Alternatively, we tested an adjustment of temperature with season fitted as binary variable (winter (December–March) vs summer (April–November)).

In BRHS, outdoor temperature was also additionally adjusted for indoor temperature (not available in PROSPER). As indoor temperature did not have any effect on the outcomes (all $p > 0.05$), and did not alter the magnitude of the associations of outdoor temperature with outcomes, it was not considered further.

As lung function measurement (forced expiratory volume in one second, or FEV_1) was also available in the BRHS, a further sensitivity analysis was carried out adding FEV_1 as covariate in models predicting CRP levels, to take into account the possible temperature-related variation in CRP due to poor respiratory health in winter.

Results

Participants

The BRHS and PROSPER participants' characteristics are shown in Table 1. In BRHS, 4252 men out of 5516 survivors (77%) were examined during the study period. In PROSPER, 5804 participants out of 23,770 (24%) screened individuals participated in the clinical

Table 1. The British Regional Heart Study (BRHS) and Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) participant characteristics during examinations (1997–2000).

	BRHS men ($n = 4252$)[1]	PROSPER participants ($n = 5804$)[2]
Demographic and background characteristics		
Sex (male), *n* (%)	4252 (100)	2806 (48.0)
Age (years), mean (SD)	68.7 (5.5)	75.3 (3.3)
Social class (manual), *n* (%)	2166 (51.0)	–
Physical health		
Prevalence of stroke/myocardial infarction, *n* (%)	370 (8.7)	979 (16.9)
Hypertension, *n* (%)	2703 (63.8)	3592 (61.9)
Diabetes, *n* (%)	380 (9.4)	623 (10.7)
BMI, mean (SD)	26.9 (3.7)	26.8 (4.1)
Behavioural factors		
Smoking		
Never, *n* (%)	1233 (29.1)	1969 (33.9)
Ex-smokers, *n* (%)	2464 (58.0)	2277 (39.2)
Smokers, *n* (%)	548 (12.9)	1558 (26.8)
Alcohol consumption		
None, *n* (%)	431 (10.3)	2576 (44.4)
Occasional/light, *n* (%) [3]	2949 (70.5)	2698 (46.5)
Moderate/heavy, *n* (%) [4]	779 (18.6)	530 (9.1)
Unclassified, *n* (%)	26 (0.6)	–
Physical activity (PA) score		
Inactive, *n* (%)	471 (11.5)	–
Occasional, *n* (%)	957 (23.4)	–
Light, *n* (%)	767 (18.7)	–
Moderate, *n* (%)	591 (14.4)	–
Moderate vigorous, *n* (%)	690 (16.8)	–
Vigorous, *n* (%)	621 (15.2)	–
Biological markers, means (SD)		
CRP, mg/l	3.53 (6.86)	5.94 (11.07)
IL-6, pg/ml	3.18 (2.95)	3.40 (3.08)
Fibrinogen, g/l	3.27 (0.74)	3.39 (0.74)
PV, mPa.s	1.285 (0.078)	1.296 (0.077)
t-PA, ng/ml	11.08 (4.44)	11.02 (4.04)
vWF, IU/dl	139.96 (46.19)	140.62 (45.98)
D-dimer, ng/ml	133.58 (210.74)	316.85 (189.48)
Triglycerides, mmol/l	1.86 (1.08)	1.54 (0.74)
HDL-cholesterol, mmol/l	1.32 (0.34)	1.28 (0.36)
LDL-cholesterol, mmol/l	3.89 (0.97)	3.78 (0.83)
Total cholesterol, mmol/l	6.00 (1.08)	5.66 (0.94)
Vitamin D, ng/ml	20.01 (9.24)	16.57 (9.94)
SBP sitting, mm Hg	149 (24)	155 (22)
DBP sitting, mm Hg	85 (11)	84 (11)

BMI: body mass index; CRP: C-reactive protein; DBP: diastolic blood pressure; HDL: high density lipoprotein; IL-6: interleukin 6; LDL: low density lipoprotein; PV: plasma viscosity; SBP: systolic blood pressure; SD: standard deviation; t-PA: tissue plasminogen activator; vWF: von Willebrand factor.
[1]BRHS men from England and Wales: $n = 3804$ (89.5%); from Scotland: $n = 448$ (10.5%).
[2]Participants from Glasgow: $n = 2520$ (43.4%); from Cork: $n = 2184$ (37.6%), and from Leiden: $n = 1100$ (19.0%).
[3]≥1 and ≤15 units per week (one unit is approximately one drink, such as one glass of wine).
[4]>16 units per week (one unit is approximately one drink, such as one glass of wine).

trial of pravastatin vs placebo. PROSPER participants were on average about seven years older than BRHS participants, with a higher percentage of never-smokers, and were less likely to drink alcohol (Table 1).

Outdoor temperature of the day of examination by month

In both studies, daily mean temperatures on the day of examination were usually between 4–9°C from November–April and between 10–16°C from May–October (see Supplementary Material, eTable 1).

CVD risk factors descriptive statistics by month

Highest levels of the CVD risk factors analysed were observed from November–April (see Supplementary Material, eTables 2–5). This variation was particularly marked for SBP, DBP, total cholesterol, CRP, IL-6, t-PA, vWF and PV. Conversely, VitD levels were lowest in colder months.

Associations of temperature with the CVD risk factors

Adjusted associations of mean temperature on day of measurement with the CVD risk factors are shown in Table 2 for each study separately, and pooled.

Pooled estimates showed that with a 5°C lower mean temperature, total cholesterol was 0.04 mmol/l (95% confidence interval (CI) 0.02–0.07) higher, LDL cholesterol was 0.02 mmol/l (95% CI 0.01–0.05) higher, and SBP was 1.12 mm Hg (95% CI 0.60–1.64) higher. Among novel CVD risk factors, CRP was 3.3% (95% CI 1.0–5.6%) higher, IL-6 was 2.7% (95% CI 1.1–4.3%) higher, t-PA was 1.9% (95% CI 1.0–2.9%) higher, fibrinogen was 0.7% (95% CI 0.2–1.3%) higher, and plasma viscosity was 0.4% (95% CI 0.3–0.5%) higher. There was no evidence of heterogeneity between studies (p-values > 0.05).

With a 5°C lower mean temperature, VitD was 11.2% (95% CI 1.0–20.4%) lower. In this case, there was evidence of heterogeneity between studies ($I^2 = 97.3\%$; p-value < 0.001), though the effect was in the same direction and statistically significant for both studies.

Associations of temperature with DBP, vWF and D-dimer, triglycerides, and HDL-cholesterol were not statistically significant. Results for HDL-cholesterol suggested heterogeneity ($I^2 = 90.6\%$; p-value = 0.001) with association of a decrease in temperature significant for the PROSPER study only.

Proportion of variance in risk factors explained by temperature

The highest proportion of variance was observed when the outcome analysed was VitD (5.1% and 5.6% in the BRHS and PROSPER fully adjusted models respectively). In each of the models, and other outcomes analysed, the proportion of variance associated with mean temperature was less than 1% (Supplementary Material, eTable 6).

Interactions between temperature and age

Interaction effects of temperature with age on the outcomes levels were mainly not significant (data not shown). However, interactions were found in PROSPER alone for VitD and HDL-cholesterol. A 5°C decrease in mean temperature was associated with an additional decrease of –0.8% per year of age (95% CI –1.4– –0.3%) for Vitamin D, and +0.003 mmol/l per year of age (95% CI 0–0.006) for HDL-cholesterol. No interactions were found in BRHS.

Sensitivity analysis

Cumulative short-term associations of temperature up to one week (lag 0–6) prior to the examination day with the CVD risk factors levels were observed (not shown). As the magnitude of the associations was very similar to associations using temperature at lag 0 (primary analysis), only associations at lag 0 were presented.

An additional adjustment for season fitted as binary variable (winter vs summer) barely changed the magnitude of the associations of outdoor temperature (results were not shown).

In BRHS, an additional adjustment for lung function (FEV_1) did not substantially change the effect of CRP: the percentage increase in CRP due to a decrease in temperature was 4.1% (0.7–7.3%) and 4.6% (1.4–7.8%) for models without and with lung function.

Discussion

To our knowledge, the pooled analysis of the BRHS and PROSPER is the largest investigation of the relationships between outdoor temperature and an extensive range of CVD risk factors, both established and novel in older European people. The CVD risk factors investigated here were selected for two reasons: first, there was published evidence of seasonal variation, with higher levels observed in the cold season (November–April); second, there was published evidence of independent association with CVD events in meta-analyses of prospective population-based studies.[19–22]

Table 2. The change in the levels of cardiovascular disease (CVD) risk factors for a single standard deviation (5°C) decrease in outdoor mean temperature in the British Regional Heart Study (BRHS) and Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) participants, during examinations (1997–2000).

	BRHS[a]		PROSPER[b]		POOLED (BRHS + PROSPER)[c]			
								Test of heterogeneity
	Percentage change (95% CI)	p-Value	Percentage change (95% CI)	p-Value	Percentage change (95% CI)	I^2 (%)	p-Value	
CRP, mg/l	4.1 (0.7–7.3)	0.017	2.4 (–0.8–5.7)	0.075	3.3 (1.0–5.6)	0.0	0.468	
IL-6, pg/ml	1.8 (–1.3–4.8)	0.246	3.0 (1.1–4.9)	0.001	2.7 (1.1–4.3)	0.0	0.525	
Fibrinogen, g/l	0.5 (–0.5–1.6)	0.286	0.8 (0.2–1.4)	0.007	0.7 (0.2–1.3)	0.0	0.677	
t-PA, ng/ml	2.5 (0.6–4.4)	0.010	1.7 (0.6–2.8)	<0.001	1.9 (1.0–2.9)	40.6	0.461	
PV, mPa.s	0.4 (0.2–0.6)	<0.001	0.4 (0.3–0.6)	<0.001	0.4 (0.3–0.5)	0.0	1.000	
vWF, IU/dl	–1.0 (–2.6–0.7)	0.268	1.0 (0.0–2.0)	0.029	0.1 (–1.7–2.1)	74.0	0.050	
D-dimer, ng/ml	1.6 (–1.5–4.6)	0.292	–0.6 (–2.1–0.9)	0.482	0.1 (–1.9–2.2)	0.0	0.516	
Vitamin D, ng/ml	–6.1 (–9.1– –3.2)	<0.001	–16.0 (–17.5– –14.5)	<0.001	–11.2 (–20.4– –1.0)	97.3	<0.001	
Triglycerides, mmol/l	1.5 (–0.7–3.6)	0.175	–0.1 (–1.3–1.1)	0.442	0.4 (–1.0–1.9)	39.3	0.199	
	Absolute change (95% CI)	p-Value	Absolute change (95% CI)	p-Value	Absolute change (95% CI)	I^2 (%)	p-Value	
HDL-cholesterol, mmol/l	0.00 (–0.01–0.02)	0.844	0.03 (0.02–0.04)	<0.001	0.02 (–0.01–0.05)	90.6	0.001	
LDL-cholesterol, mmol/l	0.05 (0.00–0.09)	0.039	0.02 (0.00–0.05)	0.038	0.03 (0.01–0.05)	0.0	0.346	
Total cholesterol, mmol/l	0.06 (0.01–0.11)	0.015	0.04 (0.01–0.06)	0.004	0.04 (0.02–0.07)	0.0	0.464	
SBP sitting, mm Hg	1.22 (0.29–2.16)	0.010	1.08 (0.45–1.71)	<0.001	1.12 (0.60–1.64)	0.0	0.796	
DBP sitting, mm Hg	0.47 (0.04–0.90)	0.032	0.13 (–0.20–0.46)	0.270	0.27 (–0.06–0.60)	34.5	0.217	

BMI: body mass index; CI: confidence interval; CRP: C-reactive protein; DBP: diastolic blood pressure; HDL: high density lipoprotein; IL-6: interleukin 6; LDL: low density lipoprotein; PV: plasma viscosity; SBP: systolic blood pressure; t-PA: tissue plasminogen activator; vWF: von Willebrand factor.
[a]Multilevel linear regression models (level 1 = individual, level 2 = town of examination) were used. The models were adjusted for age, social class, BMI, smoking, alcohol consumption, physical activity, and time of measurement. Complete case analysis ($n = 3832$)
[b]Linear regression models were used. The models were adjusted for town, age, BMI, smoking, alcohol consumption, and sex. Complete case analysis ($n = 5804$)
[c]Results from the two studies were pooled using a random effects model. The command metan with the option random available in Stata/SE 14. The percentage of variation across studies that is due to heterogeneity was reported using the I^2 statistic.

Overall findings

Lower outdoor temperature, measured on the day of clinical examination, was associated with higher levels of most CVD risk factors analysed. Conversely, lower outdoor temperature was associated with a lower VitD. The direction and magnitude of these associations were similar in comparison with other studies,[6,7,11] and persisted after adjustment for classic risk factors such as age, BMI, smoking, alcohol consumption, and physical activity. The findings were similar when using the outdoor temperature moving average of seven days, which included lag days from 0–6 prior to the examination day (lag 0–6). In fully adjusted models, the proportion of variance in risk factors explained by temperature was much smaller than other risk factors, being around 1% of the total variance (except for VitD, where variance explained was approximately 5%). There was no

consistent evidence of an interaction of temperature with age on the wide range of CVD risk factors analysed.

These findings would be consistent with the suggestions from previous studies that, in addition to established risk factors such as cholesterol[7] and BP,[8] circulating inflammatory markers,[9] and VitD[23] showed strong associations with outdoor temperature and may contribute to increased incidence of CVD in winter.[1] The association of temperature with SBP, LDL-cholesterol and IL-6 levels may be particularly relevant, as previous trials and Mendelian randomization (MR) studies support their causal role in CHD risk.[4,5,24]

Established CVD risk factors

In this study lower outdoor temperatures were significantly associated with higher levels of SBP consistently with previous findings.[25] The association with DBP was weaker and non-significant. Seasonal variation in SBP was previously shown to be greater in older than in younger subjects (while DBP was similar), and highly significantly related to outdoor temperature.[26]

We found decrease in temperature was associated with increased total cholesterol and LDL-cholesterol, as previously reported.[27] In our study, a decrease of about 10°C in temperatures would be associated with an increase of 0.06 mmol/l in LDL-cholesterol. According to previous studies, this absolute increase in LDL-cholesterol leads to an increase of approximately 1% in CVD mortality risk.[4] The importance of HDL-cholesterol as a marker of CHD risk has been emphasised through its inclusion in the Framingham Risk Score.[28] When pooling results from the two studies, we found no clear association between temperature and HDL-cholesterol although a positive association was seen in PROSPER. Lastly, associations of temperature with triglycerides were not significant as observed in previous studies.[7]

Novel CVD risk factors

A decrease in temperature was associated with increased circulating levels of markers of inflammation, such as IL-6, CRP, fibrinogen and plasma viscosity. The inflammatory hypothesis of CVD is currently being formally tested in randomized controlled trials (RCTs).[24] To date, MR studies for IL-6 suggested a causal role in CHD, in contrast to null associations in MR studies for CRP and fibrinogen.[8] Therefore, the findings on IL-6 are particularly important: in this study a decrease of about 10°C in temperatures (difference between the coldest and warmest month, January–August) would be associated with an increase of 0.06 pg/ml in IL-6 levels. According to previous IL-6 observational data this absolute difference was associated to an increase of 4.5% in CVD deaths.[29] This broad estimation is in line with previous studies which took place in the same years (1998–2007) and attributed to temperatures 7% of the winter mortality in England and Wales.[30]

It is also possible that an acute (or short-term) effect of outdoor temperature may be more marked on rapidly responding CVD risk factors, such as CRP.[31] The CRP behaviour may explain why it provides closer associations and better predictions of CVD events in the short-term than other markers of inflammation. The associations of temperature with other specific markers of inflammation we studied, such as fibrinogen and plasma viscosity, were smaller in comparison with CRP, as previously reported.[15]

Findings for PV and t-PA are similar in comparison with previous studies which observed higher levels of these factors in winter,[9] although the effect of temperature was not specifically tested. To our knowledge these associations with temperature are novel, and have not been previously published. On the other hand, the association of temperature with vWF and fibrin D-dimer was not significant. The seasonal variation in temperature did not show a good agreement with variations observed in vWF and D-dimer: vWF's seasonal peak was previously observed in early spring (between March–May)[9] when outdoor temperature already started its annual average increase from February; D-dimer seemed to have an unusual seasonal variation, with peaks in February/March and August/September.[32]

VitD

Findings for VitD showed strong associations with temperature in pooled analysis, though this varied between the studies. However, for VitD specifically, temperature is likely to be a proxy of exposure to sunlight, which is the real determinant. In our study a decrease of about 10°C in temperatures would be associated with a decrease of approximately 4 ng/ml (=10 nmol/l) in VitD levels. According to previous observational studies, this absolute decrease in VitD is associated with an increase of approximately 4% in CVD deaths and events[12] although any causal role remains contentious.

Strengths and limitations

By pooling PROSPER and BRHS we substantially improved statistical power and precision in comparison with findings reported in other studies of older adults. The participants lived mostly in the UK but also in Ireland and the Netherlands. The PROSPER study

included both women and men. Moreover, novel CVD risk factors measurements in both studies were performed over the same time period, in the same Glasgow University laboratories using the same assays. However, the two study designs are different and this may partially explain the heterogeneity of the findings for HDL cholesterol, as well as the interactions of temperature with age; the PROSPER participants in comparison with the BRHS participants were about seven years older on average, with a higher percentage of never-smokers, and less likely to drink alcohol. They were also at elevated CVD risk and around half had prevalent CVD. Due to the nature of our data and risk of collinearity between temperature and other seasonal terms, it was not possible to distinguish between temperature-related effects and effects due to other factors which are known to vary by season: for example, in winter higher prevalence of influenza or other respiratory viruses or diseases, such as rheumatic disorders, may be relevant to the CRP seasonal variation. Despite this limitation, we took into account of season as binary variable (winter vs summer), and alternatively fitting respiratory health in sensitivity analysis: an additional adjustment for lung function was performed and specifically when using CRP as outcome. The results still showed that lower outdoor temperatures were significantly associated with an increase in the outcome levels. Moreover, although indoor temperature was not available in the PROSPER, we added this variable in the BRHS models and we showed the effect of outdoor temperatures was not confounded by indoor temperatures.

Implications

Our study provides robust evidence that outdoor temperature is associated with variations in the major CVD risk factors in older adults. This study increased generalisability of existing evidence from northern European older populations and is consistent with the hypothesis that inflammation markers, on top of BP and LDL-cholesterol changes, could play a key role in intermediate processes leading to the cold-related CVD mortality. Also, there was no consistent evidence of an interaction of temperature with age (participants in the two studies ranged from 60–82 years old) on the wide range of CVD risk factors analysed; this finding suggested that the effect of low temperature on CVD risk may apply to the full age range of older adults. Public health approaches to protect elderly populations against low temperatures could help in reducing levels of several CVD risk factors, and thus CVD risk itself, in winter.

Conclusions

Variations of outdoor temperature in the short-term were associated with variations in the majority of CVD risk factors analysed. Associations were strongest with inflammatory factors (particularly CRP, and its major cytokine driver, IL-6) and VitD, followed by associations with SBP, and cholesterol variables. Better protection against low temperatures could help in reducing the levels of several CVD risk factors.

Author contribution

Study, concept, design, and acquisition of data: PHW, SGW, RWM; data collection: LL; laboratory analysis: PW; analysis and interpretation of data: CS, SJEB, RWM; drafting the article: CS, RWM; revising the article critically for important intellectual content: CS, SJEB, PHW, SGW, GDOL, BJJ, IF, NS, RWM; final approval of the version to be published: CS, SJEB, PHW, SGW, GDOL, BJJ, LL, PW, IF, NS, RWM.

Declaration of conflicting interests

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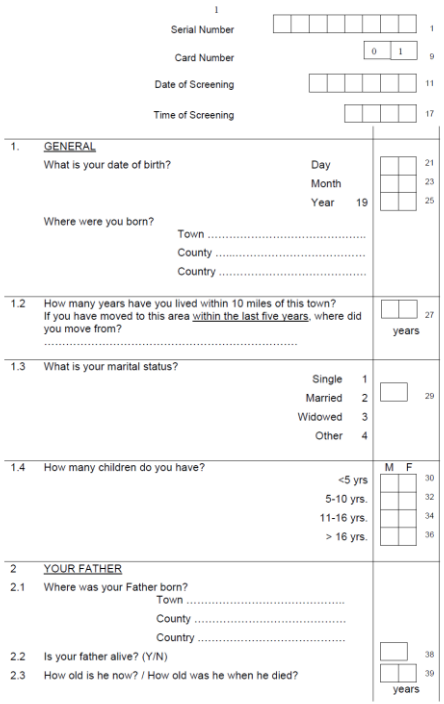
APPENDIX II CONFERENCE ORAL PRESENTATIONS

1) The 9th UK & Ireland Conference on Occupational and Environmental Epidemiology (OEEC). 2015. Hazards of cold spells for incidence of cardiovascular disease in older British men. Claudio Sartini, Goya Wannamethee, Lucy Lennon, Peter Whincup, and Richard Morris
2) Society for Social Medicine (SSM) Conference. 2015. Objectively measured physical activity and sedentary behaviour in older adults: diurnal patterns and their determinants. Claudio Sartini, S Goya Wannamethee, Steve Iliffe, Richard W Morris, Sarah Ash, Lucy Lennon, Peter H Whincup, and Barbara J Jefferis
3) International Conference on Ambulatory Monitoring of Physical Activity and Movement (ICAMPAM). 2015. Influence of season and meteorological factors on objectively measured physical activity and sedentary behaviour patterns among older UK men. Claudio Sartini, S Goya Wannamethee, Steve Iliffe, Richard W Morris, Sarah Ash, Lucy Lennon, Peter H Whincup, and Barbara J Jefferis

APPENDIX III CONFERENCE POSTER PRESENTATIONS

1) International Conference on Ambulatory Monitoring of Physical Activity and Movement (ICAMPAM). 2015. Objectively measured physical activity and sedentary behaviour in older adults: diurnal patterns and their determinants. Claudio Sartini, S Goya Wannamethee, Steve Iliffe, Richard W Morris, Sarah Ash, Lucy Lennon, Peter H Whincup, and Barbara J Jefferis
2) The Royal Society of Medicine (RSM) Conference 2015. Influence of season and weather on objectively measured physical activity and sedentary behaviour patterns. Claudio Sartini, S Goya Wannamethee, Steve Iliffe, Richard W Morris, Sarah Ash, Lucy Lennon, Peter H Whincup, and Barbara J Jefferis
3) Society for Social Medicine (SSM) Conference 2016. Associations of outdoor temperature and cardiovascular disease risk factors in the elderly: evidence from two Northern European prospective studies. Claudio Sartini, Sarah JE Barry, Peter H Whincup, S Goya Wannamethee, Gordon DO Lowe, Barbara J Jefferis, Lucy Lennon, Paul Welsh, Ian Ford, and Richard W Morris.
4) International Society for Environmental Epidemiology (ISEE). 2016. Associations of outdoor temperature and cardiovascular disease risk factors in the elderly: evidence from two Northern European prospective studies. Claudio Sartini, Sarah JE Barry, Peter H Whincup, S Goya Wannamethee, Gordon DO Lowe, Barbara J Jefferis, Lucy Lennon, Paul Welsh, Ian Ford, and Richard W Morris

APPENDIX IV BASELINE QUESTIONNAIRE IN 1978-80



1

Serial Number 1

Card Number 0 1 9

Date of Screening 11

Time of Screening 17

1. GENERAL

What is your date of birth? Day 21 Month 23 Year 19 25

Where were you born?

Town .....

County .....

Country .....

1.2 How many years have you lived within 10 miles of this town? If you have moved to this area within the last five years, where did you move from? ..... 27 years

1.3 What is your marital status?

Single 1
Married 2
Widowed 3
Other 4 29

1.4 How many children do you have?

	M	F	
<5 yrs			30
5-10 yrs.			32
11-16 yrs.			34
> 16 yrs.			36

2 YOUR FATHER

2.1 Where was your Father born?

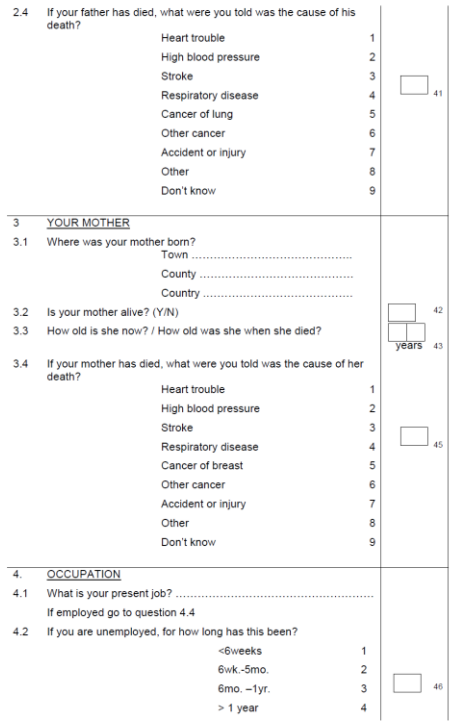
Town .....

County .....

Country .....

2.2 Is your father alive? (Y/N) 38

2.3 How old is he now? / How old was he when he died? 39 years



2.4 If your father has died, what were you told was the cause of his death?

Heart trouble 1
High blood pressure 2
Stroke 3
Respiratory disease 4
Cancer of lung 5
Other cancer 6
Accident or injury 7
Other 8
Don't know 9 41

3 YOUR MOTHER

3.1 Where was your mother born?

Town .....

County .....

Country .....

3.2 Is your mother alive? (Y/N) 42

3.3 How old is she now? / How old was she when she died? 43 years

3.4 If your mother has died, what were you told was the cause of her death?

Heart trouble 1
High blood pressure 2
Stroke 3
Respiratory disease 4
Cancer of breast 5
Other cancer 6
Accident or injury 7
Other 8
Don't know 9 45

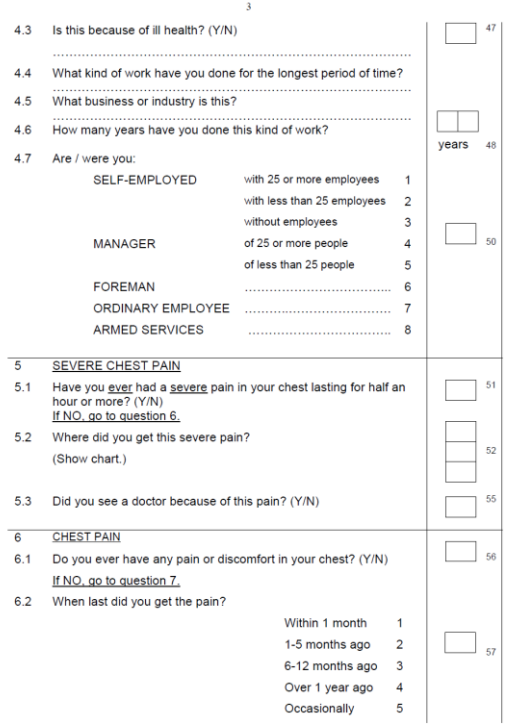
4. OCCUPATION

4.1 What is your present job? .....

If employed go to question 4.4

4.2 If you are unemployed, for how long has this been?

<6weeks 1
6wk.-5mo. 2
6mo. –1yr. 3
> 1 year 4 46



3

4.3 Is this because of ill health? (Y/N) 47

4.4 What kind of work have you done for the longest period of time? .....

4.5 What business or industry is this? .....

4.6 How many years have you done this kind of work? 48 years

4.7 Are / were you:

SELF-EMPLOYED with 25 or more employees 1
with less than 25 employees 2
without employees 3
MANAGER of 25 or more people 4
of less than 25 people 5
FOREMAN 6
ORDINARY EMPLOYEE 7
ARMED SERVICES 8 50

5 SEVERE CHEST PAIN

5.1 Have you ever had a severe pain in your chest lasting for half an hour or more? (Y/N) 51
If NO, go to question 6.

5.2 Where did you get this severe pain? 52
(Show chart.)

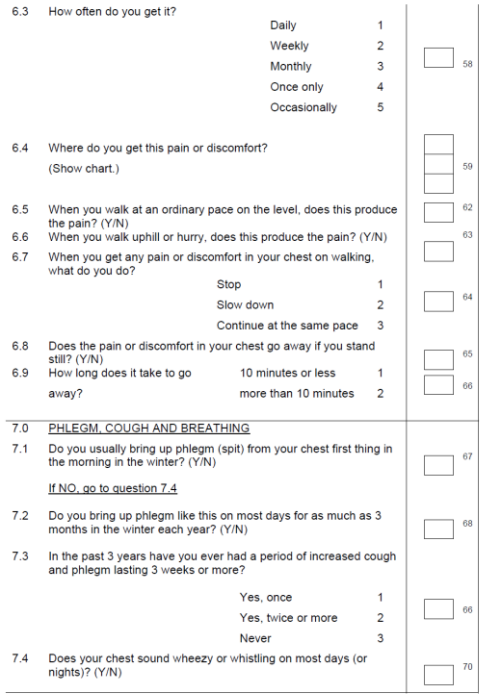
5.3 Did you see a doctor because of this pain? (Y/N) 55

6 CHEST PAIN

6.1 Do you ever have any pain or discomfort in your chest? (Y/N) 56
If NO, go to question 7.

6.2 When last did you get the pain?

Within 1 month 1
1-5 months ago 2
6-12 months ago 3
Over 1 year ago 4
Occasionally 5 57



6.3 How often do you get it?

Daily	1	58
Weekly	2	
Monthly	3	
Once only	4	
Occasionally	5	

6.4 Where do you get this pain or discomfort? (Show chart.) 59

6.5 When you walk at an ordinary pace on the level, does this produce the pain? (Y/N) 62

6.6 When you walk uphill or hurry, does this produce the pain? (Y/N) 63

6.7 When you get any pain or discomfort in your chest on walking, what do you do?

Stop	1	64
Slow down	2	
Continue at the same pace	3	

6.8 Does the pain or discomfort in your chest go away if you stand still? (Y/N) 65

6.9 How long does it take to go away?

10 minutes or less	1	66
more than 10 minutes	2	

7.0 PHLEGM, COUGH AND BREATHING

7.1 Do you usually bring up phlegm (spit) from your chest first thing in the morning in the winter? (Y/N) 67

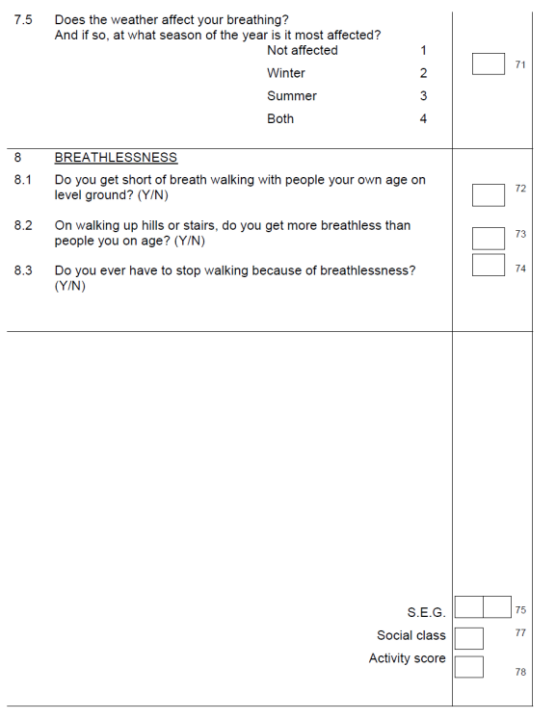
If NO, go to question 7.4

7.2 Do you bring up phlegm like this on most days for as much as 3 months in the winter each year? (Y/N) 68

7.3 In the past 3 years have you ever had a period of increased cough and phlegm lasting 3 weeks or more?

Yes, once	1	66
Yes, twice or more	2	
Never	3	

7.4 Does your chest sound wheezy or whistling on most days (or nights)? (Y/N) 70



7.5 Does the weather affect your breathing? And if so, at what season of the year is it most affected?

Not affected	1	71
Winter	2	
Summer	3	
Both	4	

8 BREATHLESSNESS

8.1 Do you get short of breath walking with people your own age on level ground? (Y/N) 72

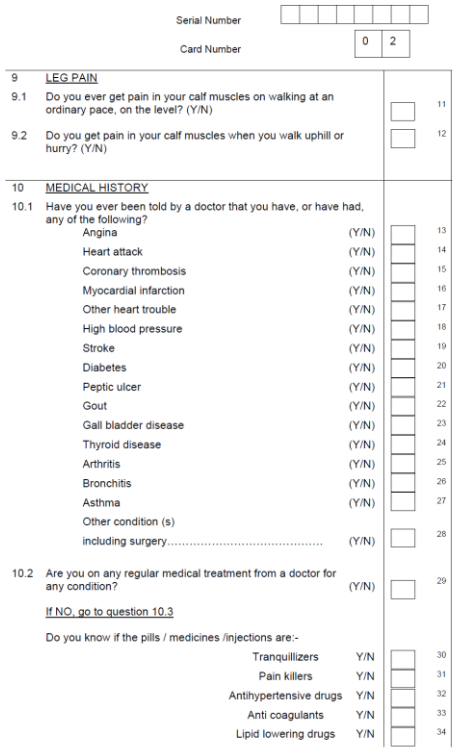
8.2 On walking up hills or stairs, do you get more breathless than people you on age? (Y/N) 73

8.3 Do you ever have to stop walking because of breathlessness? (Y/N) 74

S.E.G. 75

Social class 77

Activity score 78



Serial Number

Card Number 0 2

9 LEG PAIN

9.1 Do you ever get pain in your calf muscles on walking at an ordinary pace, on the level? (Y/N) 11

9.2 Do you get pain in your calf muscles when you walk uphill or hurry? (Y/N) 12

10 MEDICAL HISTORY

10.1 Have you ever been told by a doctor that you have, or have had, any of the following?

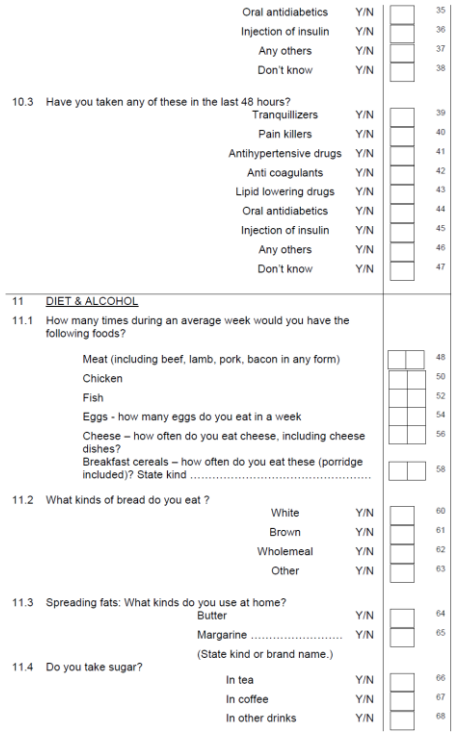
Angina	(Y/N)	13
Heart attack	(Y/N)	14
Coronary thrombosis	(Y/N)	15
Myocardial infarction	(Y/N)	16
Other heart trouble	(Y/N)	17
High blood pressure	(Y/N)	18
Stroke	(Y/N)	19
Diabetes	(Y/N)	20
Peptic ulcer	(Y/N)	21
Gout	(Y/N)	22
Gall bladder disease	(Y/N)	23
Thyroid disease	(Y/N)	24
Arthritis	(Y/N)	25
Bronchitis	(Y/N)	26
Asthma	(Y/N)	27
Other condition (s) including surgery.....	(Y/N)	28

10.2 Are you on any regular medical treatment from a doctor for any condition? (Y/N) 29

If NO, go to question 10.3

Do you know if the pills / medicines /injections are:-

Tranquillizers	Y/N	30
Pain killers	Y/N	31
Antihypertensive drugs	Y/N	32
Anti coagulants	Y/N	33
Lipid lowering drugs	Y/N	34



Oral antidiabetics Y/N 35
Injection of insulin Y/N 36
Any others Y/N 37
Don't know Y/N 38

10.3 Have you taken any of these in the last 48 hours?
Tranquillizers Y/N 39
Pain killers Y/N 40
Antihypertensive drugs Y/N 41
Anti coagulants Y/N 42
Lipid lowering drugs Y/N 43
Oral antidiabetics Y/N 44
Injection of insulin Y/N 45
Any others Y/N 46
Don't know Y/N 47

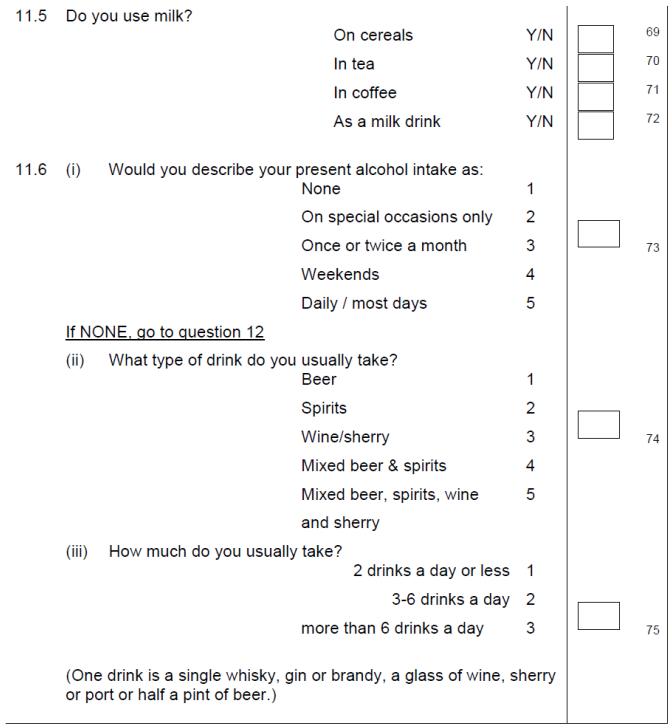
11 DIET & ALCOHOL

11.1 How many times during an average week would you have the following foods?
Meat (including beef, lamb, pork, bacon in any form) 48
Chicken 50
Fish 52
Eggs - how many eggs do you eat in a week 54
Cheese – how often do you eat cheese, including cheese dishes? 56
Breakfast cereals – how often do you eat these (porridge included)? State kind 58

11.2 What kinds of bread do you eat ?
White Y/N 60
Brown Y/N 61
Wholemeal Y/N 62
Other Y/N 63

11.3 Spreading fats: What kinds do you use at home?
Butter Y/N 64
Margarine ..... Y/N 65
(State kind or brand name.)

11.4 Do you take sugar?
In tea Y/N 66
In coffee Y/N 67
In other drinks Y/N 68



11.5 Do you use milk?
On cereals Y/N 69
In tea Y/N 70
In coffee Y/N 71
As a milk drink Y/N 72

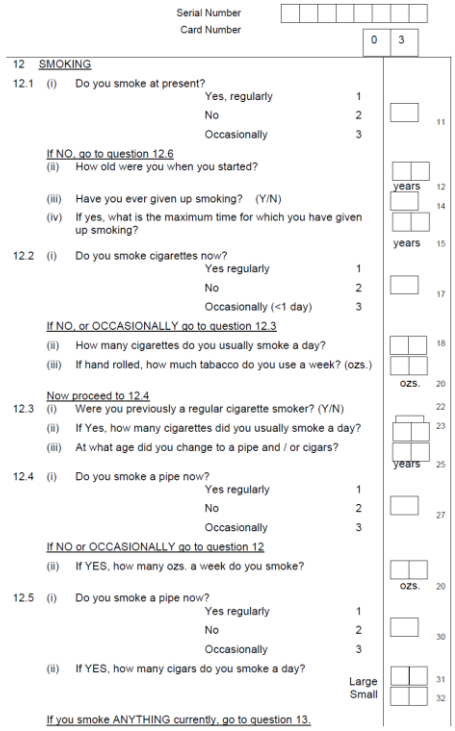
11.6 (i) Would you describe your present alcohol intake as:
None 1
On special occasions only 2
Once or twice a month 3 73
Weekends 4
Daily / most days 5

If NONE, go to question 12

(ii) What type of drink do you usually take?
Beer 1
Spirits 2
Wine/sherry 3 74
Mixed beer & spirits 4
Mixed beer, spirits, wine and sherry 5

(iii) How much do you usually take?
2 drinks a day or less 1
3-6 drinks a day 2
more than 6 drinks a day 3 75

(One drink is a single whisky, gin or brandy, a glass of wine, sherry or port or half a pint of beer.)



Serial Number
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12 SMOKING

12.1 (i) Do you smoke at present?
Yes, regularly 1
No 2 11
Occasionally 3

If NO, go to question 12.6
(ii) How old were you when you started? years 12
(iii) Have you ever given up smoking? (Y/N) 14
(iv) If yes, what is the maximum time for which you have given up smoking? years 15

12.2 (i) Do you smoke cigarettes now?
Yes regularly 1
No 2 17
Occasionally (<1 day) 3

If NO, or OCCASIONALLY go to question 12.3
(ii) How many cigarettes do you usually smoke a day? 18
(iii) If hand rolled, how much tabacco do you use a week? (ozs.) ozs. 20

Now proceed to 12.4
12.3 (i) Were you previously a regular cigarette smoker? (Y/N) 22
(ii) If Yes, how many cigarettes did you usually smoke a day? 23
(iii) At what age did you change to a pipe and / or cigars? years 25

12.4 (i) Do you smoke a pipe now?
Yes regularly 1
No 2 27
Occasionally 3

If NO or OCCASIONALLY go to question 12
(ii) If YES, how many ozs. a week do you smoke? ozs. 20

12.5 (i) Do you smoke a pipe now?
Yes regularly 1
No 2 30
Occasionally 3

(ii) If YES, how many cigars do you smoke a day?
Large 31
Small 32

If you smoke ANYTHING currently, go to question 13.

12.6 (i) Have you ever smoked for a more than 1 month ? (Y/N) ☐ 35

How much did you usually smoke

Cigarettes (per day) ☐☐ 36

Pipe (ozs) (per week) ☐☐ 38

Cigars (per day) Large ☐☐ 40

Small ☐☐ 42

If NO, go to question 13.

(ii) At what age did you start smoking? ☐☐ years 44

(iii) At what age did you finally stop smoking? ☐☐ years 46

(iv) What was the maximum time between these two ages for which you gave up smoking? ☐☐ years 48

13 EXERCISE

13.1 (i) Do you usually walk or cycle in the course of your journeys to or from work each day?

No 1

Walk 2

Cycle 3 ☐ 50

If YES, how many minutes do these journeys take? ☐☐ mins 51

(ii) Apart from your journeys to or from work, do you usually walk or cycle on weekdays?

No 1

Walk 2

Cycle 3 ☐ 50

If YES, how many minutes do you walk/cycle each day? ☐☐ mins 51

(iii) Would you say that in your occupation you are physically :

Very active 1

Fairly active 2

Average 3

Fairly inactive 4

Very inactive 5 ☐ 56

13.2 On average, a man of your age spends 4 hours on most weekends on some of the following activities: walking, gardening, household chores, DIY projects. Compared to such a man, how physically active do you consider yourself?

Very active 1

Fairly active 2

Average 3

Fairly inactive 4

Very inactive 5 ☐ 57

13.3 Apart from these activities, do you take active physical exercise, e.g. running, digging, swimming, tennis, golf, sailing, etc.

No 1

Occassionally 2

Frequently 3 ☐ 58

If NO or Occasionally – stop here.

13.4 Please state type of activity.....

13.5 How many years have you been involved in this activity? ☐☐ years 59

13.6 How many times a month (on average) do you undertake these activities?

Winter ☐☐ 61

Summer ☐☐ 63

Administrator ☐ 65

Coder ☐ 66

APPENDIX V FOLLOW-UP QUESTIONNAIRE IN 1998-2000

Study Number : ☐☐☐☐☐☐☐☐

BRITISH REGIONAL HEART STUDY

20 YEAR FOLLOW-UP SURVEY

Thank you for attending this follow-up survey. It would be very helpful if you could complete this questionnaire, which will bring us up to date with your health and lifestyle.

Most questions can be answered simply by ticking the correct box ☑

All information will be treated as **strictly confidential**.

The Research Nurse will help you with any problems.

Thank you for your help.

Conditions affecting the heart or circulation

1.0 Have you **ever** been told by a doctor that you have or have had any of the following conditions ?

		Yes	No	If after 1996, please give year
(a)	Heart attack (coronary thrombosis or myocardial infarction)	☐	☐	19_____
(b)	Heart failure	☐	☐	19_____
(c)	Angina	☐	☐	19_____
(d)	Other heart trouble	☐	☐	19_____
(e)	High blood pressure	☐	☐	19_____
(f)	Aortic Aneurysm	☐	☐	19_____
(g)	Narrowing or hardening of the leg arteries (including claudication)	☐	☐	19_____
(h)	Deep Vein Thrombosis (clot in the deep leg vein)	☐	☐	19_____
(i)	Pulmonary Embolism (clot on the lung)	☐	☐	19_____

Treatment for heart trouble

2.0 Have you **ever** had any of the following **TREATMENTS** for chest pain or heart disease ?

		Yes	No	If **Yes,** please give year of treatment	
(a)	Angioplasty of coronary arteries ('balloon treatment')	☐	☐	19_____	19_____
(b)	Coronary artery bypass graft (CABG) operation	☐	☐	19_____	19_____

Stroke

		Yes	No	Year of first diagnosis
3.0	Have you **ever** been told by a doctor that you have had a stroke ?	☐	☐	19_____
(a)	**If Yes,** did the symptoms last for more than 24 hours ?	☐	☐	

Cancer

		Yes	No
4.0	Have you **ever** been told by a doctor that you have or have had Cancer ?	☐	☐

If Yes, please give the following information:-

OFFICE USE

(a) Cancer Site_____ ☐☐☐ Year first diagnosed 19_____

Diabetes

Please answer all the questions

5.0 Have any of your close 'blood' relatives (your parents, brothers or sisters) **ever** had diabetes ? Yes ☐ No ☐

If Yes, please list any of these relatives who have had diabetes and if possible their age when they were first diagnosed: OFFICE USE

(a) Mother _____ ☐☐

(b) Father _____ ☐☐

(c) Brothers _____ ☐ ☐☐

(d) Sisters _____ ☐ ☐☐

5.1 Have you **ever** been told by a doctor that you have (or have had) diabetes? Yes ☐ No ☐

(a) **If Yes,** in what year was your diabetes first diagnosed ? 19_____

Chest pain

6.0 Do you ever have any pain or discomfort in your chest ?

Yes ☐

No ☐☐ If **No**, go to Question 7.0 on the next page

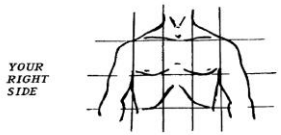
6.1 Do you know the cause of the pain ? Yes ☐ No ☐

(a) **If Yes**, please state: OFFICE USE ☐

_____

(b) Where do you get this pain or discomfort ?

Please mark **X** on the appropriate places



OFFICE USE ☐☐☐

(c) When you walk at an ordinary pace on the level does this produce the chest pain ?

Yes ☐1
No ☐2
Unable to walk on level ☐3

(d) When you walk uphill or hurry does this produce the chest pain ?

Yes ☐1
No ☐2
Unable to walk on level ☐3

Chest pain continued

(e) When you get any pain or discomfort in your chest on walking, what do you do?

Stop ☐1
Slow down ☐2
Continue at the same pace ☐3

(f) Does the pain or discomfort in your chest go away if you stand still ? Yes ☐ No ☐

(g) How long does it take to go away ?
10 minutes or less ☐1
More than 10 minutes ☐2

(h) Overall is the chest pain
Becoming more frequent ☐1
Staying about the same ☐2
Becoming less frequent ☐3

Previous Chest Pain

7.0 Have you previously had chest pain, which has stopped because of an operation? Yes ☐ No ☐

(a) If **Yes,** please give details: OFFICE USE ☐

_____

Severe chest pain

Leg pain

8.0 Have you **ever** had a **severe** pain across the front of your chest lasting for half an hour or more ?

9.0 Do you get pain or discomfort in your leg (or legs) when you walk?

Yes ☐ No ☐ ☐ If **No**, go to question 9.0 on the next page

Yes ☐1

(a) If **Yes**, what year did this happen ? 19____ ☐2 ☐ If **No** or **Unable to walk**, go to question 10.0, on the next page

(b) Did you seek doctor because of this pain? ☐3

Unable to walk

Yes ☐ No ☐ OFFICE USE

9.1 Do you know the cause of the pain ? ☐ ☐

(a) If Yes, what was/were you told was the cause _____

Yes ☐ No ☐

(b) Does this pain ever begin when you are standing still or sitting ?

(c) Do you get the pain if you walk uphill or hurry?

Yes ☐1

No ☐2

Unable to walk ☐3

(d) Do you get the pain walking at an ordinary pace on the level?

Yes ☐1

No ☐2

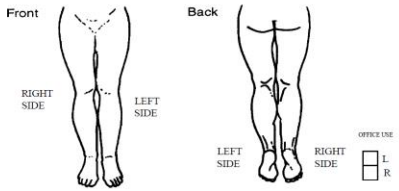
Unable to walk ☐3

(e) What happens to the pain if you stand still?

Usually continues more than 10 minutes ☐1

Usually disappears in 10 minutes or less ☐2

(f) Please mark on the diagram below where you get the pain.



Smoking

10.0 Have you **ever** smoked cigarettes regularly (at least 1 a day) ?

Yes ☐1

No ☐2 ☐ If **No**, go to question 10.3 below

10.1 Do you smoke cigarettes at present?

Yes ☐1

No ☐2

(a) **If Yes**, how many cigarettes do you smoke a day at present ? ☐☐

(If hand-rolled, how much tobacco do you use a week ? ☐☐ oz / ☐☐☐ grams)

(b) **If No**, at what age did you give up ? ☐☐ years

10.2 Have you changed your cigarette smoking habits over the last three years ?

No ☐1

Yes, increased ☐2

Yes, decreased ☐3

Yes, given up ☐4

Pipe & Cigar Smoking

10.3 Have you **ever** regularly smoked a pipe ? Yes ☐ No ☐

(a) **If Yes**, do you currently smoke a pipe ? Yes ☐ No ☐

(b) **If Yes,** how much tobacco do you smoke per week? ☐☐ oz / ☐☐☐ grams

10.4 Have you **ever** regularly smoked cigars ?

Yes ☐1

No ☐2 ☐ If **No**, go to question 10.5 below

(a) **If Yes**, do you currently smoke cigars ? Yes ☐ No ☐

(b) **If Yes,** how many cigars do you smoke per week ? ☐☐

Other exposure to Cigarette smoke

10.5 Does your wife / partner smoke cigarettes ?

Yes ☐1 ☐ Number per day ☐☐

Ex -Smoker ☐2

No ☐3

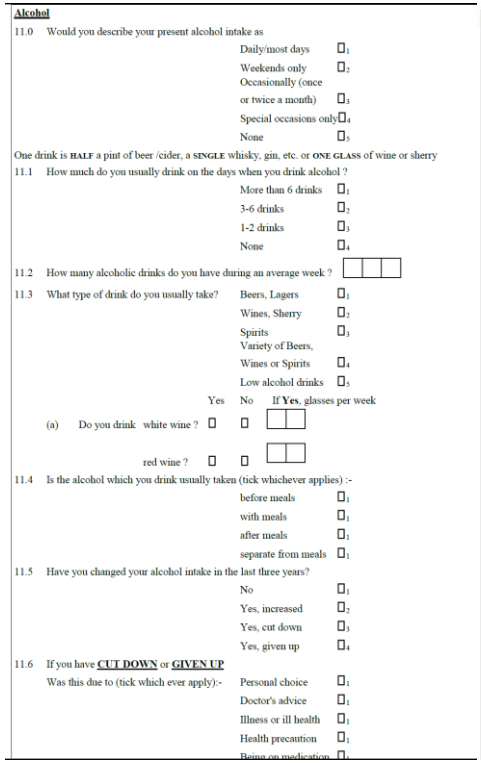
Does not apply ☐4

10.6 For about how many hours each day are you exposed to other people's cigarette smoke ?

(a) at home ☐☐ (hours)

(b) outside the home ☐☐ (hours)

(c) Tick here if rarely exposed to cigarette smoke ☐1



Alcohol

11.0 Would you describe your present alcohol intake as

- Daily/most days ☐1
- Weekends only ☐2
- Occasionally (once or twice a month) ☐3
- Special occasions only ☐4
- None ☐5

One drink is **HALF** a pint of beer /cider, a **SINGLE** whisky, gin, etc. or **ONE GLASS** of wine or sherry

11.1 How much do you usually drink on the days when you drink alcohol ?

- More than 6 drinks ☐1
- 3-6 drinks ☐2
- 1-2 drinks ☐3
- None ☐4

11.2 How many alcoholic drinks do you have during an average week ? ☐☐☐

11.3 What type of drink do you usually take?

- Beers, Lagers ☐1
- Wines, Sherry ☐2
- Spirits ☐3
- Variety of Beers, Wines or Spirits ☐4
- Low alcohol drinks ☐5

	Yes	No	If **Yes**, glasses per week
(a) Do you drink white wine ?	☐	☐	☐☐
red wine ?	☐	☐	☐☐

11.4 Is the alcohol which you drink usually taken (tick whichever applies) :-

- before meals ☐1
- with meals ☐1
- after meals ☐1
- separate from meals ☐1

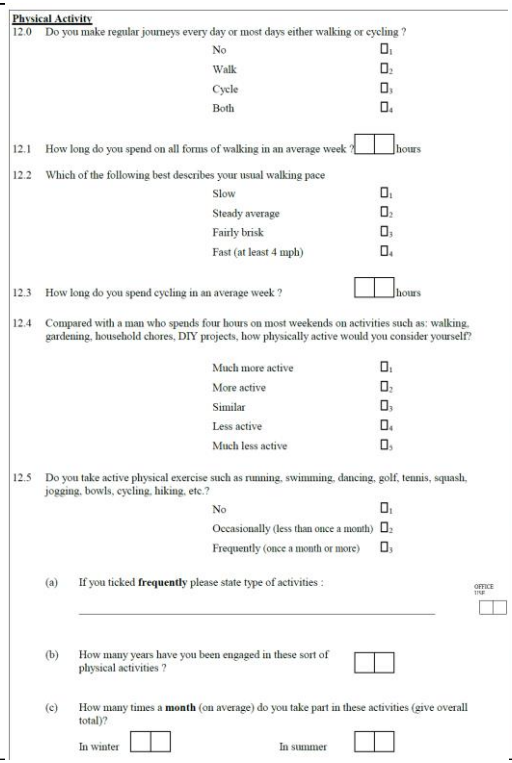
11.5 Have you changed your alcohol intake in the last three years?

- No ☐1
- Yes, increased ☐2
- Yes, cut down ☐3
- Yes, given up ☐4

11.6 If you have **CUT DOWN** or **GIVEN UP**

Was this due to (tick which ever apply):-

- Personal choice ☐1
- Doctor's advice ☐1
- Illness or ill health ☐1
- Health precaution ☐1
- Being on medication ☐1



Physical Activity

12.0 Do you make regular journeys every day or most days either walking or cycling ?

- No ☐1
- Walk ☐2
- Cycle ☐3
- Both ☐4

12.1 How long do you spend on all forms of walking in an average week ? ☐☐ hours

12.2 Which of the following best describes your usual walking pace

- Slow ☐1
- Steady average ☐2
- Fairly brisk ☐3
- Fast (at least 4 mph) ☐4

12.3 How long do you spend cycling in an average week ? ☐☐ hours

12.4 Compared with a man who spends four hours on most weekends on activities such as: walking, gardening, household chores, DIY projects, how physically active would you consider yourself?

- Much more active ☐1
- More active ☐2
- Similar ☐3
- Less active ☐4
- Much less active ☐5

12.5 Do you take active physical exercise such as running, swimming, dancing, golf, tennis, squash, jogging, bowls, cycling, hiking, etc.?

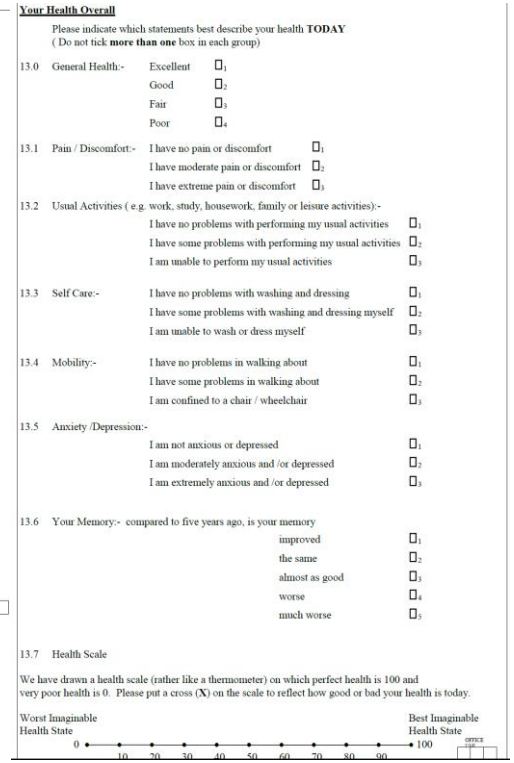
- No ☐1
- Occasionally (less than once a month) ☐2
- Frequently (once a month or more) ☐3

(a) If you ticked **frequently** please state type of activities : _____ OFFICE USE ☐☐

(b) How many years have you been engaged in these sort of physical activities ? ☐☐

(c) How many times a **month** (on average) do you take part in these activities (give overall total)?

In winter ☐☐ In summer ☐☐



Your Health Overall

Please indicate which statements best describe your health **TODAY**
(Do not tick **more than one** box in each group)

13.0 General Health:-

- Excellent ☐1
- Good ☐2
- Fair ☐3
- Poor ☐4

13.1 Pain / Discomfort:-

- I have no pain or discomfort ☐1
- I have moderate pain or discomfort ☐2
- I have extreme pain or discomfort ☐3

13.2 Usual Activities (e.g. work, study, housework, family or leisure activities):-

- I have no problems with performing my usual activities ☐1
- I have some problems with performing my usual activities ☐2
- I am unable to perform my usual activities ☐3

13.3 Self Care:-

- I have no problems with washing and dressing ☐1
- I have some problems with washing and dressing myself ☐2
- I am unable to wash or dress myself ☐3

13.4 Mobility:-

- I have no problems in walking about ☐1
- I have some problems in walking about ☐2
- I am confined to a chair / wheelchair ☐3

13.5 Anxiety /Depression:-

- I am not anxious or depressed ☐1
- I am moderately anxious and /or depressed ☐2
- I am extremely anxious and /or depressed ☐3

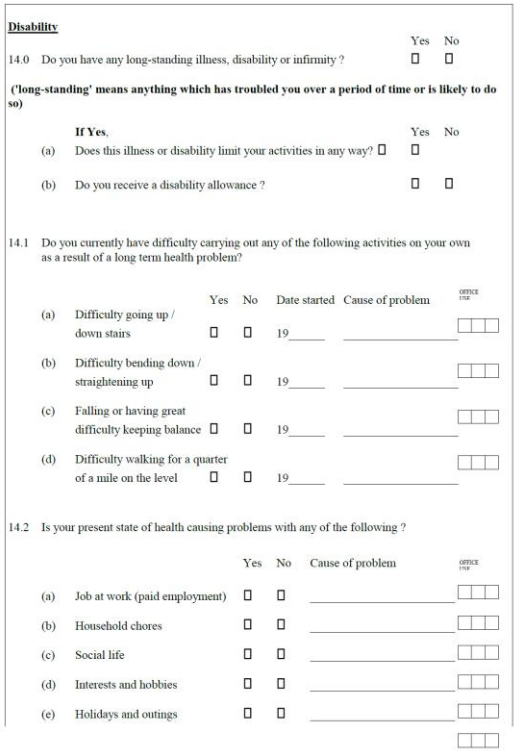
13.6 Your Memory:- compared to five years ago, is your memory

- improved ☐1
- the same ☐2
- almost as good ☐3
- worse ☐4
- much worse ☐5

13.7 Health Scale

We have drawn a health scale (rather like a thermometer) on which perfect health is 100 and very poor health is 0. Please put a cross (**X**) on the scale to reflect how good or bad your health is today.

Worst Imaginable Health State 0 — 10 — 20 — 30 — 40 — 50 — 60 — 70 — 80 — 90 — 100 Best Imaginable Health State OFFICE USE ☐☐



Disability

14.0 Do you have any long-standing illness, disability or infirmity ? Yes ☐ No ☐

('long-standing' means anything which has troubled you over a period of time or is likely to do so)

If Yes,

(a) Does this illness or disability limit your activities in any way? Yes ☐ No ☐

(b) Do you receive a disability allowance ? Yes ☐ No ☐

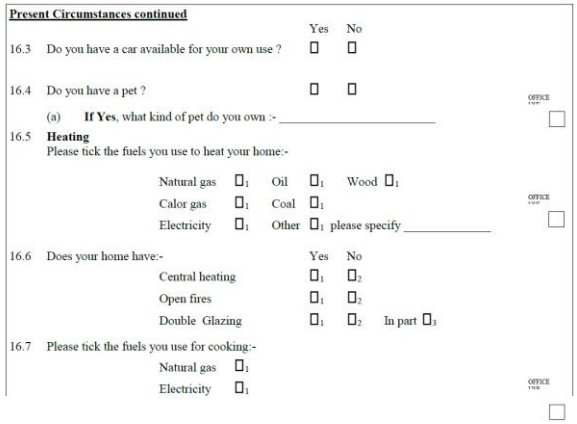
14.1 Do you currently have difficulty carrying out any of the following activities on your own as a result of a long term health problem?

		Yes	No	Date started	Cause of problem	OFFICE USE
(a)	Difficulty going up / down stairs	☐	☐	19____	_____	☐☐
(b)	Difficulty bending down / straightening up	☐	☐	19____	_____	☐☐
(c)	Falling or having great difficulty keeping balance	☐	☐	19____	_____	☐☐
(d)	Difficulty walking for a quarter of a mile on the level	☐	☐	19____	_____	☐☐

14.2 Is your present state of health causing problems with any of the following ?

		Yes	No	Cause of problem	OFFICE USE
(a)	Job at work (paid employment)	☐	☐	_____	☐☐
(b)	Household chores	☐	☐	_____	☐☐
(c)	Social life	☐	☐	_____	☐☐
(d)	Interests and hobbies	☐	☐	_____	☐☐
(e)	Holidays and outings	☐	☐	_____	☐☐

☐☐



Present Circumstances continued

16.3 Do you have a car available for your own use ? Yes ☐ No ☐

16.4 Do you have a pet ? Yes ☐ No ☐

(a) **If Yes**, what kind of pet do you own :- _____ OFFICE USE ☐

16.5 **Heating**

Please tick the fuels you use to heat your home:-

Natural gas ☐1 Oil ☐1 Wood ☐1

Calor gas ☐1 Coal ☐1 OFFICE USE ☐

Electricity ☐1 Other ☐1 please specify _____

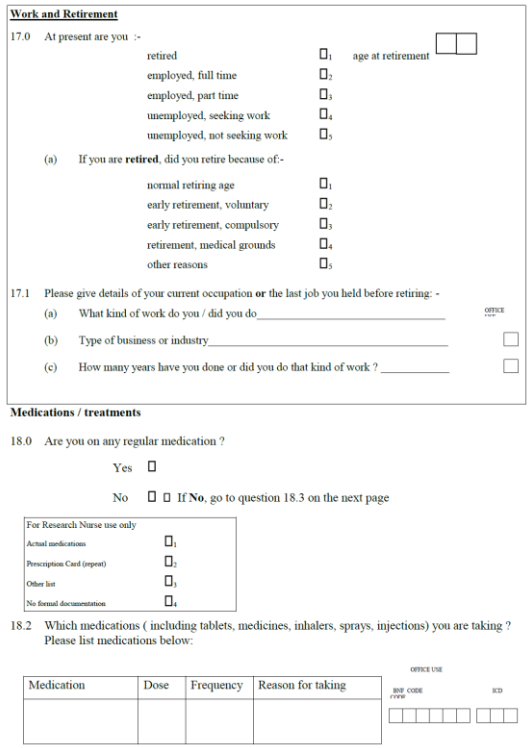
16.6 Does your home have:-

	Yes	No	
Central heating	☐1	☐2	
Open fires	☐1	☐2	
Double Glazing	☐1	☐2	In part ☐3

16.7 Please tick the fuels you use for cooking:-

Natural gas ☐1

Electricity ☐1 OFFICE USE ☐



Work and Retirement

17.0 At present are you :-

retired	☐1	age at retirement ☐☐
employed, full time	☐2	
employed, part time	☐3	
unemployed, seeking work	☐4	
unemployed, not seeking work	☐5	

(a) If you are **retired**, did you retire because of:-

normal retiring age	☐1
early retirement, voluntary	☐2
early retirement, compulsory	☐3
retirement, medical grounds	☐4
other reasons	☐5

17.1 Please give details of your current occupation **or** the last job you held before retiring: -

(a) What kind of work do you / did you do_____ OFFICE USE

(b) Type of business or industry_____ ☐

(c) How many years have you done or did you do that kind of work ? _____ ☐

Medications / treatments

18.0 Are you on any regular medication ?

Yes ☐

No ☐ ☐ If **No**, go to question 18.3 on the next page

For Research Nurse use only	
Actual medications	☐1
Prescription Card (repeat)	☐2
Other list	☐3
No formal documentation	☐4

18.2 Which medications (including tablets, medicines, inhalers, sprays, injections) you are taking ? Please list medications below:

Medication	Dose	Frequency	Reason for taking

OFFICE USE

INF CODE / OPR ☐☐☐☐☐☐ ICD ☐☐☐

Aspirin

		Yes	No	
18.3	Do you take aspirin regularly ?	☐	☐	If **No**, go to question 18.3(b) below
(a)	If Yes , year started 19 ☐☐			
	Dose ☐☐☐ mg			
	Frequency / week ☐			
	Reason for use _____			
	On Prescription Yes ☐ No ☐			

		Yes	No
18.3 **(b)**	If **No**, have you taken aspirin regularly in the past ?	☐	☐

☐☐

If Yes , year started 19

year stopped 19 ☐☐

Reason for taking _____

On Prescription Yes ☐ No ☐

Warfarin

		Yes	No
18.4	Have you taken warfarin regularly at any time ?	☐	☐

If Yes , year started 19 ☐☐

Duration in months ☐☐

Reason for taking _____

GTN

18.5 Have you ever taken GTN tablets under the tongue (or spray) to relieve pain in the chest ?

Yes ☐ No ☐

(a) **If Yes,** when was the last time you used them ? ☐☐☐ mths ago

Vitamins & Minerals

		Yes	No
18.6	Do you regularly take any vitamin or mineral tablets?	☐	☐

(a) **If Yes**, please give details :-

Name of vitamin / mineral ☐☐	Daily Dose ☐☐☐	Year Started ☐☐
		19_____
		19_____
		19_____
		19_____

Blood Cholesterol Test

			Yes	No
19.0	Have you ever had your blood cholesterol measured ?		☐	☐
(a)	**If Yes**, were you told that the result was	High	☐1	
		Normal	☐2	
		Low	☐3	
		Not told	☐4	

(b) **If High**, have you been advised to take any particular action ? (please give details)

Diet ☐1

Drugs ☐1

Eating and drinking

20.0 What time did you last have something to eat or drink other than water ?

☐☐ . ☐☐ hours If yesterday please tick ☐1

21.0 **Consent to follow up studies**

An important part of this study is to observe the future health of the people taking part. We are therefore seeking your permission to receive specific information related to heart disease and stroke, particularly from the records held by your general practitioner. All these details would be treated in **absolute confidence** by the Research Team.

Do you agree to us following your future health through your health records ?

□1 Agreed □2 Not Agreed

We will arrange to have your blood sample checked for cholesterol and other factors which are important for heart disease risk. The results of these tests will be sent back to your doctor in the next four to five weeks. If any of the results give cause for concern, you will be asked to make an appointment with your doctor.

Do you agree to us passing the test results to your doctor ?

□1 Agreed □2 Not Agreed

Part of your blood sample will be frozen and kept for special scientific studies of factors affecting heart disease risk, which may help us to understand how to prevent heart disease in the future. Among the factors we may need to study will be the way in which genetic factors affect heart disease risk.

Would you allow us to use your sample in this way ?

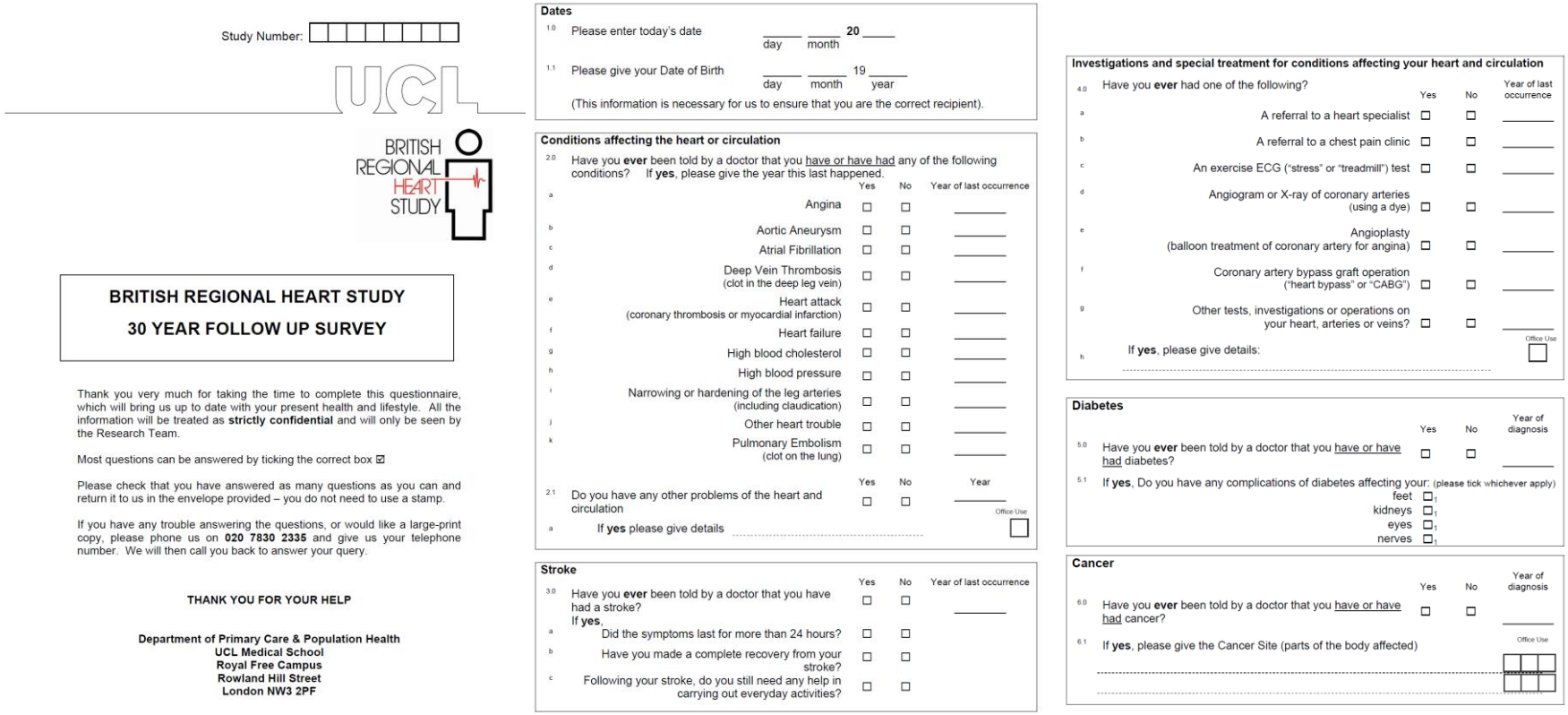
□1 Agreed □2 Not Agreed

I agree to allow the Research Team to continue to study my health in accordance with the criteria above. I understand that any details recorded will be treated in complete confidence.

Signed: _____

Date:

APPENDIX VI FOLLOW-UP QUESTIONNAIRE IN 2010-2012



Study Number: ☐☐☐☐☐☐☐☐

UCL

BRITISH REGIONAL HEART STUDY

BRITISH REGIONAL HEART STUDY

30 YEAR FOLLOW UP SURVEY

Thank you very much for taking the time to complete this questionnaire, which will bring us up to date with your present health and lifestyle. All the information will be treated as **strictly confidential** and will only be seen by the Research Team.

Most questions can be answered by ticking the correct box ☑

Please check that you have answered as many questions as you can and return it to us in the envelope provided – you do not need to use a stamp.

If you have any trouble answering the questions, or would like a large-print copy, please phone us on **020 7830 2335** and give us your telephone number. We will then call you back to answer your query.

THANK YOU FOR YOUR HELP

Department of Primary Care & Population Health
UCL Medical School
Royal Free Campus
Rowland Hill Street
London NW3 2PF

Dates

1.0 Please enter today's date _____ _____ **20** ____ (day / month)

1.1 Please give your Date of Birth _____ _____ 19 ____ (day / month / year)

(This information is necessary for us to ensure that you are the correct recipient).

Conditions affecting the heart or circulation

2.0 Have you **ever** been told by a doctor that you have or have had any of the following conditions? If **yes**, please give the year this last happened.

		Yes	No	Year of last occurrence
a	Angina	☐	☐	_____
b	Aortic Aneurysm	☐	☐	_____
c	Atrial Fibrillation	☐	☐	_____
d	Deep Vein Thrombosis (clot in the deep leg vein)	☐	☐	_____
e	Heart attack (coronary thrombosis or myocardial infarction)	☐	☐	_____
f	Heart failure	☐	☐	_____
g	High blood cholesterol	☐	☐	_____
h	High blood pressure	☐	☐	_____
i	Narrowing or hardening of the leg arteries (including claudication)	☐	☐	_____
j	Other heart trouble	☐	☐	_____
k	Pulmonary Embolism (clot on the lung)	☐	☐	_____

		Yes	No	Year
2.1	Do you have any other problems of the heart and circulation	☐	☐	_____
a	If **yes** please give details			Office Use ☐

Stroke

		Yes	No	Year of last occurrence
3.0	Have you **ever** been told by a doctor that you have had a stroke?	☐	☐	_____
	If **yes**,			
a	Did the symptoms last for more than 24 hours?	☐	☐	
b	Have you made a complete recovery from your stroke?	☐	☐	
c	Following your stroke, do you still need any help in carrying out everyday activities?	☐	☐	

Investigations and special treatment for conditions affecting your heart and circulation

		Yes	No	Year of last occurrence
4.0	Have you **ever** had one of the following?			
a	A referral to a heart specialist	☐	☐	_____
b	A referral to a chest pain clinic	☐	☐	_____
c	An exercise ECG ("stress" or "treadmill") test	☐	☐	_____
d	Angiogram or X-ray of coronary arteries (using a dye)	☐	☐	_____
e	Angioplasty (balloon treatment of coronary artery for angina)	☐	☐	_____
f	Coronary artery bypass graft operation ("heart bypass" or "CABG")	☐	☐	_____
g	Other tests, investigations or operations on your heart, arteries or veins?	☐	☐	_____
h	If **yes**, please give details:			Office Use ☐

Diabetes

		Yes	No	Year of diagnosis
5.0	Have you **ever** been told by a doctor that you have or have had diabetes?	☐	☐	_____

5.1 If **yes**, Do you have any complications of diabetes affecting your: (please tick whichever apply)
feet ☐1
kidneys ☐2
eyes ☐3
nerves ☐4

Cancer

		Yes	No	Year of diagnosis
6.0	Have you **ever** been told by a doctor that you have or have had cancer?	☐	☐	_____
6.1	If **yes**, please give the Cancer Site (parts of the body affected)			Office Use ☐☐☐ ☐☐☐

Other medical conditions

7.0 Have you **ever** been told by a doctor that you have or have had any of the following conditions?

	Yes	No
a Anaemia	☐	☐
b Asthma	☐	☐
c Bronchitis	☐	☐
d Cataract	☐	☐
e Chronic Kidney disease	☐	☐
f Crohn's disease	☐	☐
g Depression	☐	☐
h Emphysema	☐	☐
i Gall bladder disease	☐	☐
j Gastric, peptic or duodenal ulcer	☐	☐
k Glaucoma	☐	☐
l Gout	☐	☐
m Liver disease, cirrhosis or hepatitis	☐	☐
n Macular degeneration	☐	☐
o Osteoporosis	☐	☐
p Parkinson's disease	☐	☐
q Pneumonia	☐	☐
r Prostate trouble	☐	☐
s Shingles	☐	☐
t Ulcerative colitis	☐	☐

u Other conditions, please give details

Office Use ☐☐☐

Arthritis

8.0 Have you **ever** been told by a doctor that you have or had arthritis? Yes ☐ No ☐ Year of diagnosis _____

8.1 If **yes**, please give the type of arthritis if known,:

Osteoarthritis ☐1
Rheumatoid arthritis ☐2
Other (please give details) ☐3

Office Use ☐

8.2 Which joints are affected: (Please tick whichever apply)

Knees ☐1 Back ☐1
Hips ☐1 Neck ☐1
Feet ☐1 Shoulders ☐1
Hands and / or wrists ☐1 Other (please specify) ☐1

Office Use ☐

Joint pain, swelling or stiffness

9.0 During **the past year** have you had pain, aching, stiffness or swelling on most days for at least one month, in your: (Please tick whichever apply)

Knees ☐1 Back ☐1
Hips ☐1 Neck ☐1
Feet ☐1 Shoulders ☐1
Hands and / or wrists ☐1 Other (please specify) .....

Office Use ☐

Lower back pain

	Yes	No
10.0 Have you **ever** had pain in your lower back on most days for at least one month?	☐	☐
10.1 If **yes**, have you had this in the **last year**?	☐	☐

Fractures and falls

	Yes	No	Please give year
11.0 Have you had spells of dizziness, loss of balance or a sensation of spinning in the last year?	☐	☐	
11.1 Have you **ever** fractured your hip?	☐	☐	_____
11.2 Have you **ever** fractured your wrist?	☐	☐	_____
11.3 Have you had a fall in the **last year**?	☐	☐	
11.4 If **yes**, how many times _____			
	Yes	No	
11.5 Did you receive medical attention for any of these falls?	☐	☐	

Operations

	Yes	No
12.0 Have you had any major operations since 2007?	☐	☐
12.1 If **yes**, please give details:		

Office Use ☐

Chest Pain

	Yes	No	Unable to walk on level
13.0 Do you **ever** have any pain or discomfort in your chest?	☐	☐	
If **yes**,	Yes	No	Unable to walk on level
13.1 When you walk at an ordinary pace on the level, does this produce the pain?	☐1	☐2	☐3
	Yes	No	Unable to walk uphill
13.2 When you walk uphill or hurry, does this produce the pain?	☐1	☐2	☐3

Breathlessness

	Yes	No	Unable to walk
14.0 Do you **ever** get short of breath walking with other people of your own age on level ground?	☐1	☐2	☐3
14.1 On walking uphill or upstairs, do you get more breathless than people of your own age?	☐1	☐2	☐3
14.2 Do you **ever** have to stop walking because of breathlessness?	☐	☐	
14.3 In the **past year** have you at any time been awoken at night by an attack of shortness of breath?	☐	☐	

Cough and Wheeze

	Yes	No
15.0 Do you usually bring up phlegm (or spit) from your chest first thing in the morning in the winter?	☐	☐
15.1 Do you bring up phlegm like this on most days for as much as three months in the winter each year?	☐	☐

15.2 In the **past four years** have you had a period of increased cough and phlegm lasting for 3 weeks or more?

Yes, once ☐1
Yes, twice or more ☐2
Never ☐3

	Yes	No
15.3 Does your chest ever sound wheezy or whistling?	☐	☐
15.4 If **yes**, does this happen on most days or nights?	☐	☐

15.5 How many times in the past year have you had a chest infection requiring antibiotic treatment from your doctor?

None ☐1
Once ☐2
More than once ☐3

Eyesight

	Yes	No
16.0 Using glasses or corrective lenses if needed, can you see well enough to recognise a friend at a distance of 12 feet/ four yards (**across a road**)?	☐	☐
16.1 If **no**, can you see well enough to recognise a friend at a distance of one yard?	☐	☐

16.2 In the **past four years** has your sight:

deteriorated ☐1
improved ☐2
stayed the same ☐3

Hearing

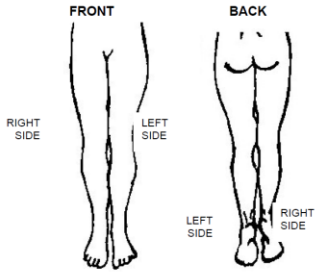
		Yes	No	
17.0	Is your hearing good enough to follow a TV programme at a volume others find acceptable (using a hearing aid if needed)?	☐	☐	
17.1	If **no**, can you follow a TV programme with the volume turned up?	☐	☐	
17.2	In the **past four years** has your hearing: deteriorated ☐1 / improved ☐2 / stayed the same ☐3			
		Yes	No	Occasionally
17.3	Do you use a hearing aid?	☐1	☐2	☐3

Leg Pain

		Yes	No	Unable to walk
18.0	Do you get pain or discomfort in your leg or legs when you walk?	☐	☐	☐3
	If **yes**,			
a	Do you know the cause of the pain?	☐	☐	Office Use
b	If **yes**, please state cause			☐
		Yes	No	
18.2	Does this pain ever begin when you are standing still or sitting?	☐	☐	
		Yes	No	Unable to walk
18.3	Do you get the pain if you walk uphill or hurry?	☐1	☐2	☐3
18.4	Do you get the pain walking at an ordinary pace on the level?	☐1	☐2	☐3

18.5 What happens to the pain if you stand still?
Usually continues more than 10 minutes ☐1
Usually disappears in 10 minutes or less ☐2

18.6 Please mark on the diagram below where you get the pain.



Office Use L ☐ R ☐

Weight

19.0 What is your present weight (indoor clothes, without shoes)?
___ ___ Stones ___ ___ Pounds **or** ___ ___ ___ Kilograms

19.1 If you have no scales and have made an estimate please tick here ☐

		Yes	No
19.2	Have you tried to lose weight in the **last four years**?	☐	☐

19.3 If **yes**, did you: (Please tick whichever apply)
Change your diet? ☐1
Take more exercise? ☐1

19.4 Has your weight changed in the **last four years**?
Not changed ☐1
Increased ☐2
Decreased ☐3
Both increased and decreased ☐4
Don't know ☐5

If your weight has changed in the last four years:

		Yes	No
19.5	was this change intentional?	☐	☐

19.6 was it the result of (Please tick whichever apply)
Personal choice ☐1
Medical advice ☐1
Illness or ill health ☐1

Smoking

Cigarette smoking

		Yes	No
20.0	Do you smoke cigarettes at present?	☐	☐

20.1 If **yes** , How many cigarettes a day do you smoke at present ____ ____

20.2 Have you changed your cigarette smoking habits during the past four years?
No ☐1
Yes, increased ☐2
Yes, cut down ☐3
Yes, given up ☐4

Pipe and cigar smoking

		Yes	No
20.3	Do you currently smoke a pipe?	☐	☐
20.4	Do you currently smoke cigars?	☐	☐

Alcohol Intake

21.0 Would you describe your present alcohol intake as
Daily/most days ☐1
Weekends only ☐2
Occasionally once or twice a month ☐3
Special occasions only ☐4
None ☐5

One drink is **HALF A PINT** of beer/lager/cider, a **SINGLE** whisky, gin, etc. or **ONE GLASS** of wine or sherry

21.1 How much do you usually drink on the days when you drink alcohol?
More than 6 drinks ☐1
5-6 drinks ☐2
3-4 drinks ☐3
1-2 drinks ☐4

21.2 How many alcoholic drinks do you have during an average week? ___ ___

21.3 What type of drink do you usually take?
Beers, Lagers ☐1
Wines, Sherry ☐1
Spirits ☐1
Combination of Beers, Wines or Spirits ☐1
Low alcohol drinks ☐1

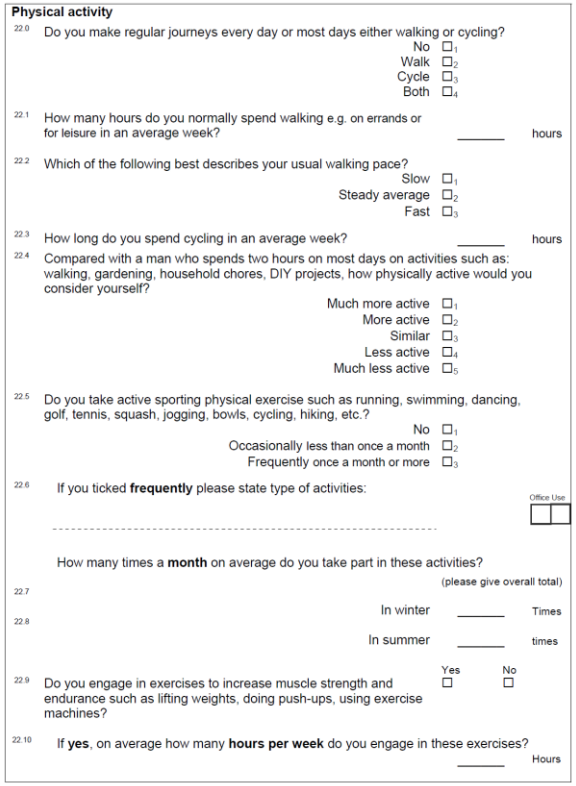
			Yes	No	If **yes**, number of glasses per week
21.4	Do you drink	white wine	☐	☐	_____
21.5		red wine	☐	☐	_____

21.6 Is the alcohol which you drink usually taken..... (Please tick whichever apply)
before meals ☐1
with meals ☐1
after meals ☐1
separate from meals ☐1

		Yes	No
21.7	Have you reduced your alcohol intake in the last four years?	☐	☐

21.8 If **yes**, was this due to: (please tick whichever apply)
Personal choice ☐1
Doctor's advice ☐1
Illness or ill-health ☐1
Other reasons ☐1

		Yes	No
21.9	Have you ever felt you ought to cut down on your drinking?	☐	☐
21.10	Have people annoyed you by criticizing your drinking?	☐	☐
21.11	Have you ever felt bad or guilty about your drinking?	☐	☐
21.12	Have you had a drink first thing in the morning (eye-opener) to steady your nerves or get rid of a hangover?	☐	☐



Physical activity

22.0 Do you make regular journeys every day or most days either walking or cycling?
No ☐1
Walk ☐2
Cycle ☐3
Both ☐4

22.1 How many hours do you normally spend walking e.g. on errands or for leisure in an average week? _____ hours

22.2 Which of the following best describes your usual walking pace?
Slow ☐1
Steady average ☐2
Fast ☐3

22.3 How long do you spend cycling in an average week? _____ hours

22.4 Compared with a man who spends two hours on most days on activities such as: walking, gardening, household chores, DIY projects, how physically active would you consider yourself?
Much more active ☐1
More active ☐2
Similar ☐3
Less active ☐4
Much less active ☐5

22.5 Do you take active sporting physical exercise such as running, swimming, dancing, golf, tennis, squash, jogging, bowls, cycling, hiking, etc.?
No ☐1
Occasionally less than once a month ☐2
Frequently once a month or more ☐3

22.6 If you ticked **frequently** please state type of activities:

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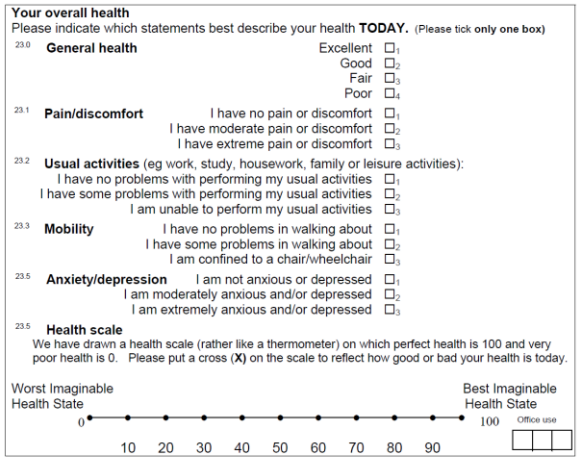
How many times a **month** on average do you take part in these activities?
(please give overall total)

22.7 In winter _____ Times

22.8 In summer _____ times

22.9 Do you engage in exercises to increase muscle strength and endurance such as lifting weights, doing push-ups, using exercise machines? Yes ☐ No ☐

22.10 If **yes**, on average how many **hours per week** do you engage in these exercises? _____ Hours



Your overall health

Please indicate which statements best describe your health **TODAY.** (Please tick **only one box**)

23.0 **General health**
Excellent ☐1
Good ☐2
Fair ☐3
Poor ☐4

23.1 **Pain/discomfort**
I have no pain or discomfort ☐1
I have moderate pain or discomfort ☐2
I have extreme pain or discomfort ☐3

23.2 **Usual activities** (eg work, study, housework, family or leisure activities):
I have no problems with performing my usual activities ☐1
I have some problems with performing my usual activities ☐2
I am unable to perform my usual activities ☐3

23.3 **Mobility**
I have no problems in walking about ☐1
I have some problems in walking about ☐2
I am confined to a chair/wheelchair ☐3

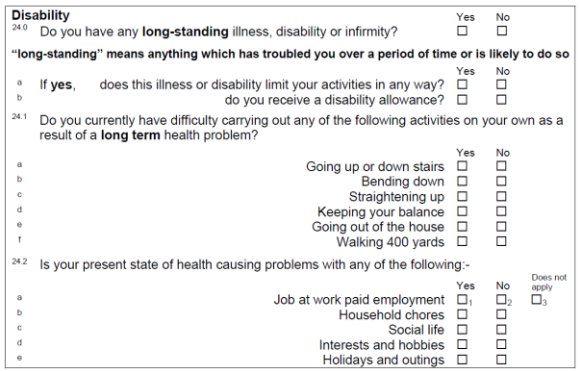
23.5 **Anxiety/depression**
I am not anxious or depressed ☐1
I am moderately anxious and/or depressed ☐2
I am extremely anxious and/or depressed ☐3

23.5 **Health scale**
We have drawn a health scale (rather like a thermometer) on which perfect health is 100 and very poor health is 0. Please put a cross (**X**) on the scale to reflect how good or bad your health is today.

Worst Imaginable Health State 0 — Best Imaginable Health State 100

10 20 30 40 50 60 70 80 90

Office use



Disability

24.0 Do you have any **long-standing** illness, disability or infirmity? Yes ☐ No ☐

"long-standing" means anything which has troubled you over a period of time or is likely to do so

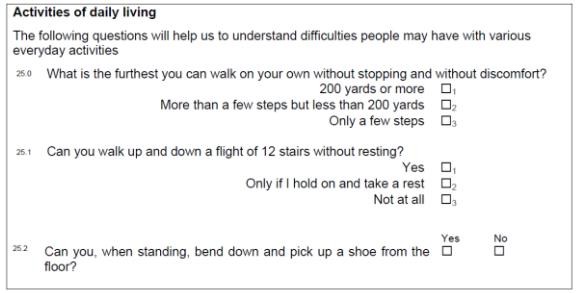
a If **yes**, does this illness or disability limit your activities in any way? Yes ☐ No ☐
b do you receive a disability allowance? Yes ☐ No ☐

24.1 Do you currently have difficulty carrying out any of the following activities on your own as a result of a **long term** health problem?

		Yes	No
a	Going up or down stairs	☐	☐
b	Bending down	☐	☐
c	Straightening up	☐	☐
d	Keeping your balance	☐	☐
e	Going out of the house	☐	☐
f	Walking 400 yards	☐	☐

24.2 Is your present state of health causing problems with any of the following:-

		Yes	No	Does not apply
a	Job at work paid employment	☐1	☐2	☐3
b	Household chores	☐	☐	
c	Social life	☐	☐	
d	Interests and hobbies	☐	☐	
e	Holidays and outings	☐	☐	



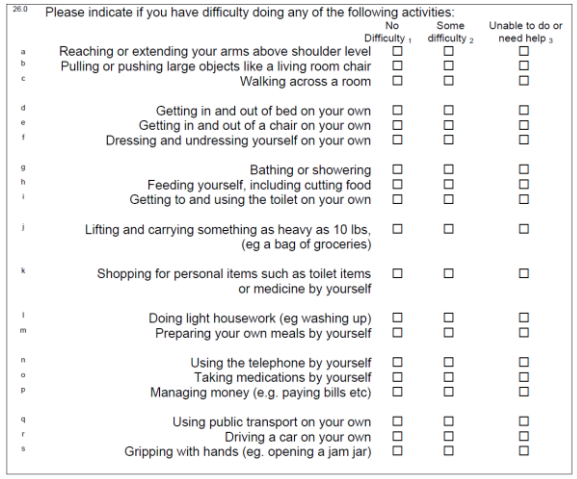
Activities of daily living

The following questions will help us to understand difficulties people may have with various everyday activities

25.0 What is the furthest you can walk on your own without stopping and without discomfort?
200 yards or more ☐1
More than a few steps but less than 200 yards ☐2
Only a few steps ☐3

25.1 Can you walk up and down a flight of 12 stairs without resting?
Yes ☐1
Only if I hold on and take a rest ☐2
Not at all ☐3

		Yes	No
25.2	Can you, when standing, bend down and pick up a shoe from the floor?	☐	☐



26.0 Please indicate if you have difficulty doing any of the following activities:

		No Difficulty 1	Some difficulty 2	Unable to do or need help 3
a	Reaching or extending your arms above shoulder level	☐	☐	☐
b	Pulling or pushing large objects like a living room chair	☐	☐	☐
c	Walking across a room	☐	☐	☐
d	Getting in and out of bed on your own	☐	☐	☐
e	Getting in and out of a chair on your own	☐	☐	☐
f	Dressing and undressing yourself on your own	☐	☐	☐
g	Bathing or showering	☐	☐	☐
h	Feeding yourself, including cutting food	☐	☐	☐
i	Getting to and using the toilet on your own	☐	☐	☐
j	Lifting and carrying something as heavy as 10 lbs, (eg a bag of groceries)	☐	☐	☐
k	Shopping for personal items such as toilet items or medicine by yourself	☐	☐	☐
l	Doing light housework (eg washing up)	☐	☐	☐
m	Preparing your own meals by yourself	☐	☐	☐
n	Using the telephone by yourself	☐	☐	☐
o	Taking medications by yourself	☐	☐	☐
p	Managing money (e.g. paying bills etc)	☐	☐	☐
q	Using public transport on your own	☐	☐	☐
r	Driving a car on your own	☐	☐	☐
s	Gripping with hands (eg. opening a jam jar)	☐	☐	☐

General Fitness

Can you do any of the following activities:

		Yes	No
27.0	run a short distance?	☐	☐
27.1	do heavy work around the house (eg lifting & moving heavy furniture)	☐	☐
27.2	do gardening (eg raking leaves, weeding & pushing the lawn mower)	☐	☐
27.3	participate in moderate activities like golf, bowling, dancing or doubles tennis?	☐	☐
27.4	participate in strenuous sports like swimming or singles tennis?	☐	☐
27.5	have sexual relations?	☐	☐

Mobility Aids

		Yes	No
28.0	Do you use any mobility aids?	☐	☐

If **yes,** which aids or appliances do you use to help with day to day activities?:

Walking stick ☐1
Walking frame ☐1
Wheelchair ☐1

Sleeping Patterns

29.0 On most nights, how would you rate the quality of your sleep?

Excellent ☐1
Good ☐2
Fair ☐3
Poor ☐4

29.1 On average: how many hours of sleep do you have each night? _____ hours

29.2 how much sleep (if any) do you have during the daytime? _____ hours

29.3 During **the last month**, did you have difficulties falling asleep?

rarely ☐1
sometimes ☐2
often ☐3

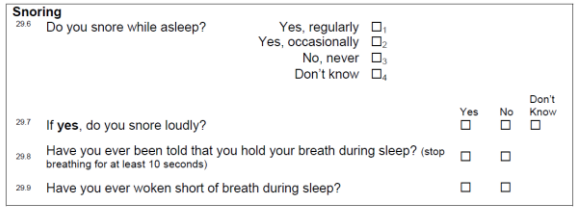
29.4 how often did you wake up during the night?

rarely ☐1
sometimes ☐2
often ☐3

29.5 What are the most frequent reasons for waking? (Please tick all that apply)

To go to the bathroom ☐1
Coughing ☐1
Arthritis pain ☐1
Leg cramps ☐1
Thirsty, need a drink of water ☐1
General worrying ☐1
Other please specify ☐1

Office use ☐

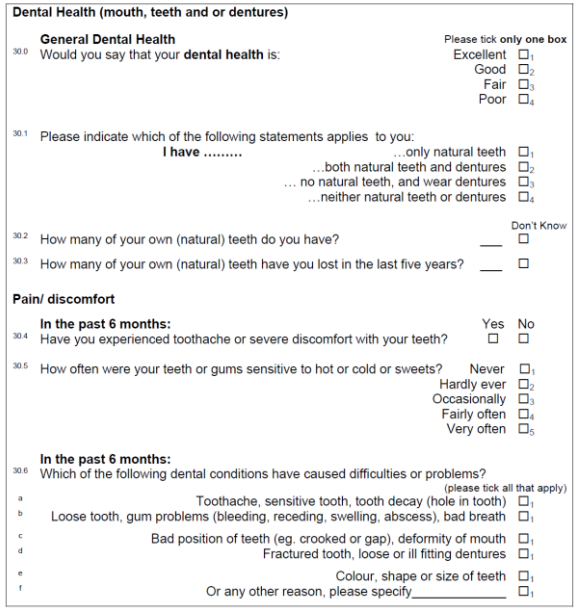


Snoring

29.6 Do you snore while asleep?

Yes, regularly ☐1
Yes, occasionally ☐2
No, never ☐3
Don't know ☐4

		Yes	No	Don't Know
29.7	If **yes**, do you snore loudly?	☐	☐	☐
29.8	Have you ever been told that you hold your breath during sleep? (stop breathing for at least 10 seconds)	☐	☐	
29.9	Have you ever woken short of breath during sleep?	☐	☐	



Dental Health (mouth, teeth and or dentures)

General Dental Health

30.0 Would you say that your **dental health** is: Please tick **only one box**

Excellent ☐1
Good ☐2
Fair ☐3
Poor ☐4

30.1 Please indicate which of the following statements applies to you:

I have .....

…only natural teeth ☐1
…both natural teeth and dentures ☐2
… no natural teeth, and wear dentures ☐3
…neither natural teeth or dentures ☐4

		Don't Know
30.2	How many of your own (natural) teeth do you have? ___	☐
30.3	How many of your own (natural) teeth have you lost in the last five years? ___	☐

Pain/ discomfort

In the past 6 months:

		Yes	No
30.4	Have you experienced toothache or severe discomfort with your teeth?	☐	☐

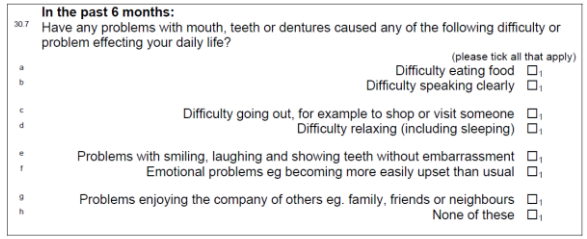
30.5 How often were your teeth or gums sensitive to hot or cold or sweets?

Never ☐1
Hardly ever ☐2
Occasionally ☐3
Fairly often ☐4
Very often ☐5

In the past 6 months:

30.6 Which of the following dental conditions have caused difficulties or problems? (please tick all that apply)

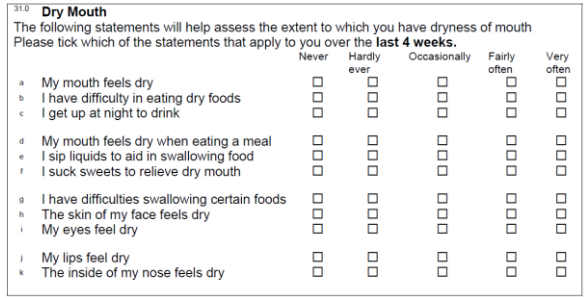
a Toothache, sensitive tooth, tooth decay (hole in tooth) ☐1
b Loose tooth, gum problems (bleeding, receding, swelling, abscess), bad breath ☐1
c Bad position of teeth (eg. crooked or gap), deformity of mouth ☐1
d Fractured tooth, loose or ill fitting dentures ☐1
e Colour, shape or size of teeth ☐1
f Or any other reason, please specify_____ ☐1



In the past 6 months:

30.7 Have any problems with mouth, teeth or dentures caused any of the following difficulty or problem effecting your daily life? (please tick all that apply)

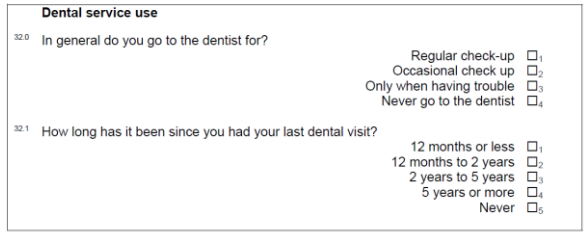
a Difficulty eating food ☐1
b Difficulty speaking clearly ☐1
c Difficulty going out, for example to shop or visit someone ☐1
d Difficulty relaxing (including sleeping) ☐1
e Problems with smiling, laughing and showing teeth without embarrassment ☐1
f Emotional problems eg becoming more easily upset than usual ☐1
g Problems enjoying the company of others eg. family, friends or neighbours ☐1
h None of these ☐1



Dry Mouth

31.0 The following statements will help assess the extent to which you have dryness of mouth
Please tick which of the statements that apply to you over the **last 4 weeks.**

		Never	Hardly ever	Occasionally	Fairly often	Very often
a	My mouth feels dry	☐	☐	☐	☐	☐
b	I have difficulty in eating dry foods	☐	☐	☐	☐	☐
c	I get up at night to drink	☐	☐	☐	☐	☐
d	My mouth feels dry when eating a meal	☐	☐	☐	☐	☐
e	I sip liquids to aid in swallowing food	☐	☐	☐	☐	☐
f	I suck sweets to relieve dry mouth	☐	☐	☐	☐	☐
g	I have difficulties swallowing certain foods	☐	☐	☐	☐	☐
h	The skin of my face feels dry	☐	☐	☐	☐	☐
i	My eyes feel dry	☐	☐	☐	☐	☐
j	My lips feel dry	☐	☐	☐	☐	☐
k	The inside of my nose feels dry	☐	☐	☐	☐	☐



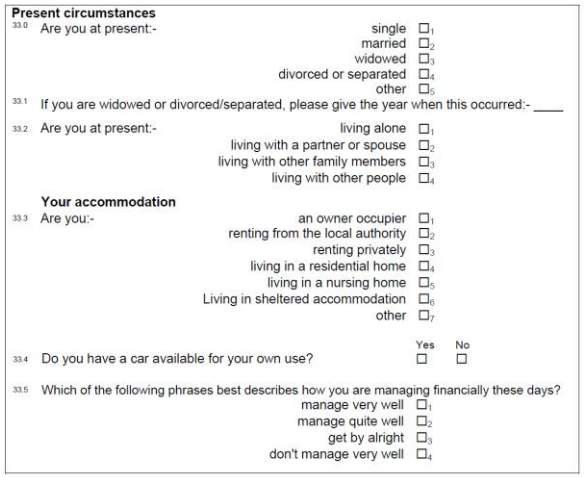
Dental service use

32.0 In general do you go to the dentist for?

Regular check-up ☐1
Occasional check up ☐2
Only when having trouble ☐3
Never go to the dentist ☐4

32.1 How long has it been since you had your last dental visit?

12 months or less ☐1
12 months to 2 years ☐2
2 years to 5 years ☐3
5 years or more ☐4
Never ☐5



Present circumstances

33.0 Are you at present:-
- single ☐1
- married ☐2
- widowed ☐3
- divorced or separated ☐4
- other ☐5

33.1 If you are widowed or divorced/separated, please give the year when this occurred:- ____

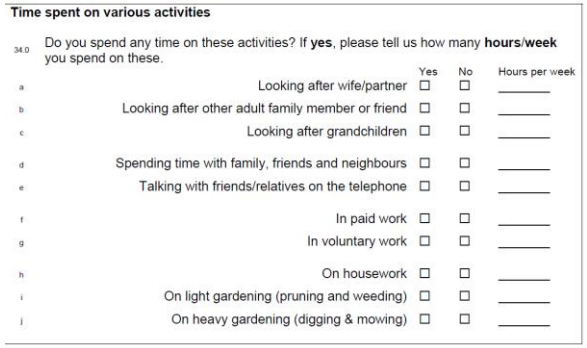
33.2 Are you at present:-
- living alone ☐1
- living with a partner or spouse ☐2
- living with other family members ☐3
- living with other people ☐4

Your accommodation

33.3 Are you:-
- an owner occupier ☐1
- renting from the local authority ☐2
- renting privately ☐3
- living in a residential home ☐4
- living in a nursing home ☐5
- Living in sheltered accommodation ☐6
- other ☐7

		Yes	No
33.4	Do you have a car available for your own use?	☐	☐

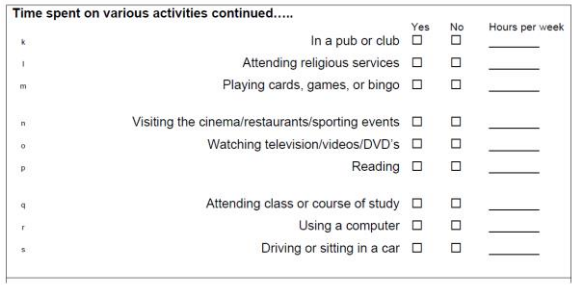
33.5 Which of the following phrases best describes how you are managing financially these days?
- manage very well ☐1
- manage quite well ☐2
- get by alright ☐3
- don't manage very well ☐4



Time spent on various activities

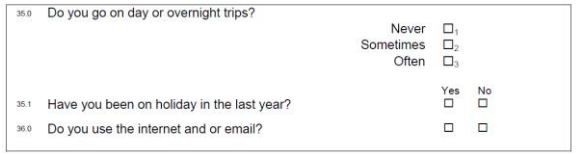
34.0 Do you spend any time on these activities? If **yes**, please tell us how many **hours/week** you spend on these.

		Yes	No	Hours per week
a	Looking after wife/partner	☐	☐	_____
b	Looking after other adult family member or friend	☐	☐	_____
c	Looking after grandchildren	☐	☐	_____
d	Spending time with family, friends and neighbours	☐	☐	_____
e	Talking with friends/relatives on the telephone	☐	☐	_____
f	In paid work	☐	☐	_____
g	In voluntary work	☐	☐	_____
h	On housework	☐	☐	_____
i	On light gardening (pruning and weeding)	☐	☐	_____
j	On heavy gardening (digging & mowing)	☐	☐	_____



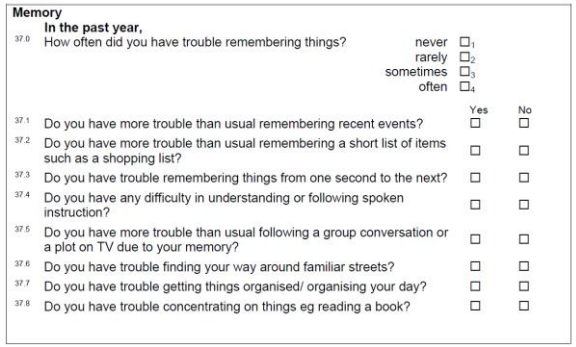
Time spent on various activities continued.....

		Yes	No	Hours per week
k	In a pub or club	☐	☐	_____
l	Attending religious services	☐	☐	_____
m	Playing cards, games, or bingo	☐	☐	_____
n	Visiting the cinema/restaurants/sporting events	☐	☐	_____
o	Watching television/videos/DVD's	☐	☐	_____
p	Reading	☐	☐	_____
q	Attending class or course of study	☐	☐	_____
r	Using a computer	☐	☐	_____
s	Driving or sitting in a car	☐	☐	_____



35.0 Do you go on day or overnight trips?
- Never ☐1
- Sometimes ☐2
- Often ☐3

		Yes	No
35.1	Have you been on holiday in the last year?	☐	☐
36.0	Do you use the internet and or email?	☐	☐

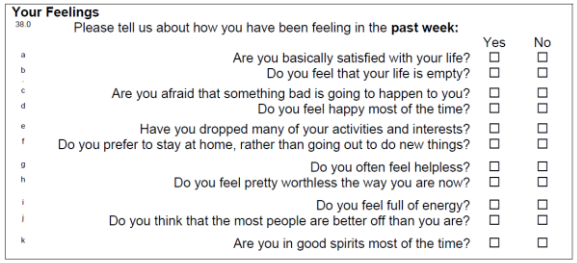


Memory

In the past year,

37.0 How often did you have trouble remembering things?
- never ☐1
- rarely ☐2
- sometimes ☐3
- often ☐4

		Yes	No
37.1	Do you have more trouble than usual remembering recent events?	☐	☐
37.2	Do you have more trouble than usual remembering a short list of items such as a shopping list?	☐	☐
37.3	Do you have trouble remembering things from one second to the next?	☐	☐
37.4	Do you have any difficulty in understanding or following spoken instruction?	☐	☐
37.5	Do you have more trouble than usual following a group conversation or a plot on TV due to your memory?	☐	☐
37.6	Do you have trouble finding your way around familiar streets?	☐	☐
37.7	Do you have trouble getting things organised/ organising your day?	☐	☐
37.8	Do you have trouble concentrating on things eg reading a book?	☐	☐



Your Feelings

38.0 Please tell us about how you have been feeling in the **past week:**

		Yes	No
a	Are you basically satisfied with your life?	☐	☐
b	Do you feel that your life is empty?	☐	☐
c	Are you afraid that something bad is going to happen to you?	☐	☐
d	Do you feel happy most of the time?	☐	☐
e	Have you dropped many of your activities and interests?	☐	☐
f	Do you prefer to stay at home, rather than going out to do new things?	☐	☐
g	Do you often feel helpless?	☐	☐
h	Do you feel pretty worthless the way you are now?	☐	☐
i	Do you feel full of energy?	☐	☐
j	Do you think that the most people are better off than you are?	☐	☐
k	Are you in good spirits most of the time?	☐	☐

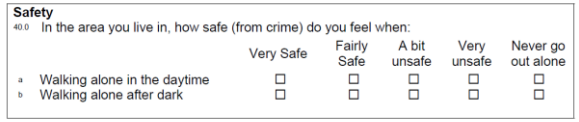
Your local area

Services

		Yes	No
39.0	Would you say that this is an area in which you enjoy living?	☐	☐

Please rate the following things in your local area and neighbourhood:
(tick one box on each line)

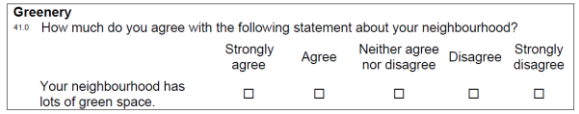
		Very Good	Good	Average	Poor	Very Poor
a	Social and leisure activities for people like yourself	☐	☐	☐	☐	☐
b	Facilities for people of your age	☐	☐	☐	☐	☐
c	The quality & frequency of rubbish collection	☐	☐	☐	☐	☐
d	Your local health service (e.g. your GP or the local hospital)	☐	☐	☐	☐	☐
e	Local transport to where you want to go	☐	☐	☐	☐	☐
f	Your area for having somewhere nice to go for a walk	☐	☐	☐	☐	☐



Safety

40.0 In the area you live in, how safe (from crime) do you feel when:

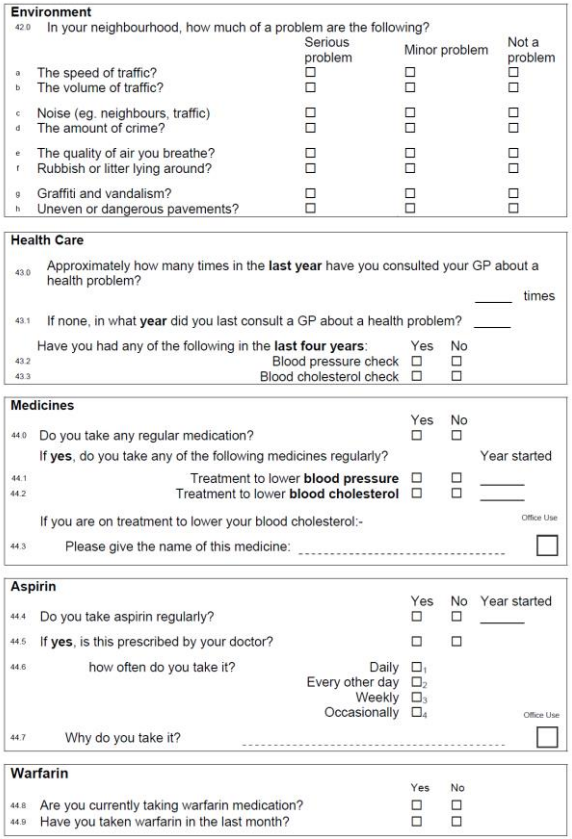
		Very Safe	Fairly Safe	A bit unsafe	Very unsafe	Never go out alone
a	Walking alone in the daytime	☐	☐	☐	☐	☐
b	Walking alone after dark	☐	☐	☐	☐	☐



Greenery

41.0 How much do you agree with the following statement about your neighbourhood?

	Strongly agree	Agree	Neither agree nor disagree	Disagree	Strongly disagree
Your neighbourhood has lots of green space.	☐	☐	☐	☐	☐



Environment

42.0 In your neighbourhood, how much of a problem are the following?

		Serious problem	Minor problem	Not a problem
a	The speed of traffic?	☐	☐	☐
b	The volume of traffic?	☐	☐	☐
c	Noise (eg. neighbours, traffic)	☐	☐	☐
d	The amount of crime?	☐	☐	☐
e	The quality of air you breathe?	☐	☐	☐
f	Rubbish or litter lying around?	☐	☐	☐
g	Graffiti and vandalism?	☐	☐	☐
h	Uneven or dangerous pavements?	☐	☐	☐

Health Care

43.0 Approximately how many times in the **last year** have you consulted your GP about a health problem? ____ times

43.1 If none, in what **year** did you last consult a GP about a health problem? ____

Have you had any of the following in the **last four years**:

		Yes	No
43.2	Blood pressure check	☐	☐
43.3	Blood cholesterol check	☐	☐

Medicines

		Yes	No	Year started
44.0	Do you take any regular medication?	☐	☐	
	If **yes**, do you take any of the following medicines regularly?			
44.1	Treatment to lower **blood pressure**	☐	☐	____
44.2	Treatment to lower **blood cholesterol**	☐	☐	____

If you are on treatment to lower your blood cholesterol:- Office Use

44.3 Please give the name of this medicine: ---------- ☐

Aspirin

		Yes	No	Year started
44.4	Do you take aspirin regularly?	☐	☐	____
44.5	If **yes**, is this prescribed by your doctor?	☐	☐	

44.6 how often do you take it? Daily ☐1 Every other day ☐2 Weekly ☐3 Occasionally ☐4

Office Use

44.7 Why do you take it? ---------- ☐

Warfarin

		Yes	No
44.8	Are you currently taking warfarin medication?	☐	☐
44.9	Have you taken warfarin in the last month?	☐	☐

Medications

Details of ALL medicines

45.0 Please write down details of all medicines– including tablets, injections, inhalers, eye-drops etc – which you take regularly. Please also include any medications which you buy for yourself.

	Name of medicine	Reason for taking (if known)	Year started	Is this prescribed? Yes	Is this prescribed? No	Office Use
1				☐	☐	
2				☐	☐	
3				☐	☐	
4				☐	☐	
5				☐	☐	
6				☐	☐	
7				☐	☐	
8				☐	☐	
9				☐	☐	
10				☐	☐	

Please use the back of the questionnaire if more space is needed to record this information.

Vitamins, minerals and complementary therapies

		Yes	No
46.0	Do you regularly (at least once a week or more) take any vitamins, minerals and complementary therapies?	☐	☐
46.1	Do you take any **multi vitamin & minerals**?	☐	☐

46.2 If **yes**, how often to you take them?

Daily ☐
1-6 times per week ☐
Less than once per week ☐

46.3 How long have you been taking them?

Less than one year ☐
Between 1-5 years ☐
More than 5 years ☐

Office Use

46.4 Please give the brand name/ preparation: ---------- ☐

46.5 Not counting multi vitamins, do you take any of the following vitamin/ minerals?

	Name of vitamin/ mineral	Yes	How **often** do you take them? Daily	1-6 times per week	Less than once per week	How **long** have you been taking them? Less than one year	Between 1-5 years	More than 5 years
a	Vitamin A	☐	☐	☐	☐	☐	☐	☐
b	Vitamin B	☐	☐	☐	☐	☐	☐	☐
c	Vitamin C	☐	☐	☐	☐	☐	☐	☐
d	Vitamin D	☐	☐	☐	☐	☐	☐	☐
e	Vitamin E	☐	☐	☐	☐	☐	☐	☐
f	Calcium	☐	☐	☐	☐	☐	☐	☐
g	Cod liver Oil	☐	☐	☐	☐	☐	☐	☐
h	Fish oil	☐	☐	☐	☐	☐	☐	☐
i	Garlic	☐	☐	☐	☐	☐	☐	☐
j	Glucosamine	☐	☐	☐	☐	☐	☐	☐
k	Magnesium	☐	☐	☐	☐	☐	☐	☐
l	Selenium	☐	☐	☐	☐	☐	☐	☐

46.6 Other, please give details: (please include homeopathic and herbal treatments)

Name of vitamin/ mineral	How **often** do you take them? Daily	1-6 times per week	Less than once per week	How **long** have you been taking them? Less than one year	Between 1-5 years	More than 5 years
	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐

PART II : YOUR DIET

How to fill in the diet questionnaire

The following questions are mostly about how often you **USUALLY** eat different sorts of food each week.
If you usually eat a food **every day**, ring **7** days a week.
If you usually eat a food on **three days a week**, ring **3**, and so on.

For foods which you eat **less than once a week:-**
Ring **M** if you eat it **at least** once a month.
Ring **R** if you eat it **less than** once a month, or if you **never** eat it at all.

Please ring **one** answer for each of the foods listed. Remember to circle **R** if you never eat a food.

EXAMPLE

	Number of days each week	Monthly	Rarely / Never
Food eaten every day 7 days a week	(7) 6 5 4 3 2 1	M	R
Food eaten on three days a week	7 6 5 4 3 (2) 1	M	R
Food eaten less often than once a week but at least once a month	7 6 5 4 3 2 1	(M)	R
Food eaten never or less than once a month	7 6 5 4 3 2 1	M	(R)

Diet

		Yes	No	Office Use
D1.0	Are you on any special diet eg vegetarian, low fat, diabetic?	☐	☐	
D1.1	If **yes**, please give details:			☐

Meat	Number of days each week	Monthly	Rarely / Never
D2.0 Beef including minced beef, beef burgers	7 6 5 4 3 2 1	M	R
D2.1 Lamb	7 6 5 4 3 2 1	M	R
D2.2 Pork, bacon, ham, salami	7 6 5 4 3 2 1	M	R
D2.3 Chicken, turkey, other poultry	7 6 5 4 3 2 1	M	R
D2.4 Tinned meat all types, corned beef, etc	7 6 5 4 3 2 1	M	R
D2.5 Pork Sausages	7 6 5 4 3 2 1	M	R
D2.6 Beef Sausages	7 6 5 4 3 2 1	M	R
D2.7 Meat Pie, Pasties	7 6 5 4 3 2 1	M	R
D2.8 Liver, kidney, heart	7 6 5 4 3 2 1	M	R

Fish	Number of days each week	Monthly	Rarely / Never
D3.0 White fish cod, haddock, hake, plaice, fish fingers, etc	7 6 5 4 3 2 1	M	R
D3.1 Kippers, herrings, pilchards, tuna, sardines, salmon, mackerel including tinned	7 6 5 4 3 2 1	M	R
D3.2 Shellfish	7 6 5 4 3 2 1	M	R

Please remember to circle ® if you never eat a food

Please remember to circle ® if you never eat a food

Vegetables fresh, tinned, dried, frozen	Number of days each week	Monthly	Rarely / Never
D4.0 **Potatoes:** boiled, baked, mashed	7 6 5 4 3 2 1	M	R
D4.1 chips or fried from shop	7 6 5 4 3 2 1	M	R
D4.2 chips or fried cooked at home	7 6 5 4 3 2 1	M	R
D4.3 roast potatoes	7 6 5 4 3 2 1	M	R
D4.4 Green vegetables, salads	7 6 5 4 3 2 1	M	R
D4.5 Carrots	7 6 5 4 3 2 1	M	R
D4.6 Parsnips, swedes, turnips, beetroot, And other root vegetables	7 6 5 4 3 2 1	M	R
D4.7 Baked or butter beans, lentils, peas, chickpeas, sweetcorn	7 6 5 4 3 2 1	M	R
D4.8 Onions cooked, raw, pickled	7 6 5 4 3 2 1	M	R
D4.9 Garlic	7 6 5 4 3 2 1	M	R
D4.10 Spaghetti and other pasta	7 6 5 4 3 2 1	M	R
D4.11 Rice all types except pudding rice	7 6 5 4 3 2 1	M	R
D4.12 Tomatoes fresh, tinned, pureed	7 6 5 4 3 2 1	M	R
How often do you eat fresh vegetables in:			
D4.13 summer	7 6 5 4 3 2 1	M	R
D4.14 winter	7 6 5 4 3 2 1	M	R

Fresh Fruit	Number of days each week	Monthly	Rarely / Never
How often do you eat fresh fruit in :			
D5.0 summer	7 6 5 4 3 2 1	M	R
D5.1 winter	7 6 5 4 3 2 1	M	R

D5.2 Number of apples eaten a week ____ ____

D5.3 Number of pears eaten a week ____ ____

D5.4 Number of oranges or grapefruit eaten a week ____ ____

D5.5 Number of bananas eaten a week ____ ____

D5.6 Number of other fruits eaten a week (please give name and quantity)

NAME OF FRUIT	QUANTITY	Office Use
		☐☐
		☐☐
		☐☐
		☐☐
		☐☐

Please remember to circle ® if you never eat a food

Please remember to circle ® if you never eat a food

Cheese	Number of days each week	Monthly	Rarely / Never
D6.0 Full- fat cheese eg Cheddar, Leicester, Stilton, Brie, soft cheeses	7 6 5 4 3 2 1	M	R
D6.1 Low-fat cheese eg Edam, Cottage cheese, reduced fat cheeses	7 6 5 4 3 2 1	M	R

Bread	Number of days each week	Monthly	Rarely / Never
D7.0 White bread	7 6 5 4 3 2 1	M	R
D7.1 Brown bread	7 6 5 4 3 2 1	M	R
D7.3 Wholemeal	7 6 5 4 3 2 1	M	R
D7.4 Bread rolls	7 6 5 4 3 2 1	M	R
D7.5 Crispbread Ryvita, cream crackers, etc	7 6 5 4 3 2 1	M	R

D7.6 please give name of crispbread etc: .....

Further details about your bread

	How many slices/ Rolls per day?	Are the slices thick, medium or thin? Please circle your answer.		
D7.7 White Bread	___ ___	$THICK_1$	$MEDIUM_2$	$THIN_3$
D7.8 Brown Bread	___ ___	$THICK_1$	$MEDIUM_2$	$THIN_3$
D7.9 Wholemeal Bread	___ ___	$THICK_1$	$MEDIUM_2$	$THIN_3$
D7.10 Bread Rolls	___ ___	$LARGE_1$	$MEDIUM_2$	$SMALL_3$

Breakfast Cereals	Number of days each week	Monthly	Rarely / Never
D8.0 Grapenuts, Porridge, Ready Brek, Special K, Sugar Puffs, Rice Crispies	7 6 5 4 3 2 1	M	R
D8.1 Cornflakes, Muesli, Shredded Wheat, Sultana Bran, Weetabix	7 6 5 4 3 2 1	M	R
D8.2 Bran Flakes, Puffed wheat	7 6 5 4 3 2 1	M	R
D8.3 All Bran, Wheat Bran	7 6 5 4 3 2 1	M	R
D8.4 Another Cereal please give name:	7 6 5 4 3 2 1	M	R

Biscuits, puddings and sweets	Number of days each week	Monthly	Rarely / Never
D9.0 Digestive biscuits, plain biscuits	7 6 5 4 3 2 1	M	R
D9.1 Sweet biscuits, sponge cakes, scones, buns	7 6 5 4 3 2 1	M	R
D9.2 Ice cream, sweet yoghurts, trifle	7 6 5 4 3 2 1	M	R
D9.3 Fruit cake, fruit bread, plum pudding	7 6 5 4 3 2 1	M	R
D9.4 Fruit tart, jam tart, fruit crumble	7 6 5 4 3 2 1	M	R
D9.5 Milk puddings rice, tapioca	7 6 5 4 3 2 1	M	R
D9.6 Tinned fruit, jellies	7 6 5 4 3 2 1	M	R
D9.7 Sweet sauces, chocolate, custard	7 6 5 4 3 2 1	M	R
D9.8 Chocolate, chocolate bars, sweets all types	7 6 5 4 3 2 1	M	R

Please remember to circle ® if you never eat a food

Please remember to circle ® if you never eat a food

Eggs	Number of days each week	Monthly	Rarely / Never
D10.0 Eggs boiled, poached, fried, scrambled	7 6 5 4 3 2 1	M	R
D10.1 Eggs in baked dishes eg flans, quiches, soufflés, egg custard, etc	7 6 5 4 3 2 1	M	R

Other foods	Number of days each week	Monthly	Rarely / Never
D11.0 Soups all kinds, home-made, tinned, packet	7 6 5 4 3 2 1	M	R
D11.1 Nuts, nut butter eg salted or unsalted peanuts	7 6 5 4 3 2 1	M	R
D11.2 Savoury snacks eg potato crisps, corn chips, crackers	7 6 5 4 3 2 1	M	R
D11.3 Chutney, brown sauce, tomato sauce	7 6 5 4 3 2 1	M	R
D11.4 Sweet spreads eg jam, honey, marmalade chocolate spread	7 6 5 4 3 2 1	M	R

Drinks and Juices non-alcoholic	Number of days each week	Monthly	Rarely / Never
D12.0 Natural fruit juices including tomato juice	7 6 5 4 3 2 1	M	R
D12.1 Fizzy drinks and Non-diet squashes	7 6 5 4 3 2 1	M	R
D12.2 **Low calorie** (diet) squashes and fizzy drinks	7 6 5 4 3 2 1	M	R

Milk

D13.0 What type of milk do you usually drink?

Cow's Milk ☐1
Soya Milk ☐2
Other, please give details ..... Office Use ☐

D13.1 Roughly how much milk do you drink a day in tea, coffee, milky drinks or cereals?

none at all ☐1
half pint or less ☐2
between half and one pint ☐3
more than one pint ☐4

D13.2 What kind of milk do you usually use?

full fat milk, fresh or dried ☐1
semi-skimmed milk, fresh or dried ☐2
fully skimmed milk, fresh or dried ☐3
other kinds of milk, eg condensed, evaporated ☐4

Salt

D14.0 How much salt is added to your food in cooking?

a lot ☐1
a little ☐2
none ☐3

D14.1 How much salt is added to your food on your plate?

a lot ☐1
a little ☐2
none ☐3

Fats

D15.0 What do you usually spread on bread? Give brand name Office Use

butter ☐1 .....
full-fat soft margarine ☐1 ..... ☐
low-fat soft margarine ☐1 .....
hard margarine ☐1 ..... ☐

D15.1 How do you normally spread the fat? thinly ☐1 / average ☐2 / thickly ☐3

How often do you eat home-fried food including chips, cooked with :-

	Number of days each week	Monthly	Rarely / Never
D15.2 Lard, dripping, solid vegetable oil	7 6 5 4 3 2 1	M	R

Give brand name and type ..... Office Use ☐

D15.3 Liquid vegetable oil 7 6 5 4 3 2 1 M R

Give brand name and type ..... Office Use ☐

Your household

D16.0 How many people normally eat in your household?

Number of adults including yourself ____ Number of children 1 to 4 years old ____

Number of children 5 to 16 years old ____ Number of babies under 1 year old ____

How much of the following foods does **your household** use on average **each week** including cooking and baking? If you live on your own, please give the amounts which you yourself eat a week.

D16.1	Butter	___ ___ lbs	___ ___ozs or	___ ___ ___ grams
D16.2	Margarine	___ ___ lbs	___ ___ozs or	___ ___ ___ grams
D16.3	Lard and solid vegetable oil	___ ___ lbs	___ ___ozs or	___ ___ ___ grams
D16.4	Liquid vegetable oil eg Sunflower, Corn, Groundnut oil		___ ___ozs or	___ ___ ___ ml
D16.5	Olive Oil		___ ___ozs or	___ ___ ___ ml
D16.6	Cream		___ ___ozs or	___ ___ ___ ml
D16.7	Full- fat cheese eg Cheddar, Leicester, Stilton, Brie, & soft cheeses	___ ___ lbs	___ ___ozs or	___ ___ ___ grams
D16.8	Low-fat cheese eg reduced fat cheddar, reduced fat soft cheeses, Edam	___ ___ lbs	___ ___ozs or	___ ___ ___ grams
D16.9	Sugar	___ ___ lbs	___ ___ozs or	___ ___ ___ grams

Hot drinks

Coffee

D17.0 How many cups of **coffee** do you have a day? _____ Cups per day

D17.1 Is this: Ground coffee ☐1 Instant coffee ☐2

D17.2 Is it decaffeinated: Yes ☐1 No ☐2

D17.3 How many teaspoons of **sugar** do you take in each cup? _____ Teaspoons
Do not count artificial sweeteners

Tea

D17.4 How many cups of **tea** do you have a day? _____ Cups per day

D17.5 How many teaspoons of **sugar** do you take in each cup? _____ Teaspoons
Do not count artificial sweeteners

Other Hot Drinks

D17.7 How many cups of other hot drinks (e.g. hot chocolate, malted milk, Horlicks) do you have a day? _____ Cups per day

Alcoholic Drinks

	Yes	No	Seldom
D18.0 Have you ever consumed alcoholic drinks?	☐	☐	☐
D18.1 Do you take alcoholic drinks at present?	☐1	☐2	☐3

Think back carefully over the last seven days. Please write the number of alcoholic drinks you have consumed on each day during the past week. It may help if you try to remember where you were and who you were with on each day. For each day, write in how much you have drunk:

(i) the **number** of **half pints** of non-alcoholic beer, lager, etc
(ii) the **number** of **half pints** of low-alcohol beer, lager, etc
(iii) the **number** of **half pints** of beer, lager, shandy, cider, stout, etc
(iv) the **number** of **single glasses** of whisky, vodka, gin, rum, etc
(v) the **number** of **single glasses** of wine, sherry, martini, port, etc

	Half-pints of **non-alcoholic** beer	Half-pints of **low-alcohol** beer	Half-pints of beer, lager, shandy	Single glasses of Spirits	Single glasses of wine
Monday					
Tuesday					
Wednesday					
Thursday					
Friday					
Saturday					
Sunday					

	Yes	No
D18.2 Would you say last week was fairly typical of what you usually have to drink in one week?	☐1	☐2

	More	Less
D18.3 If last week was not typical, would you normally drink more or less in a week?	☐1	☐2

Thank you very much for completing the questionnaire.
Please return it to us with the appointment card in the envelope provided.
No stamp is needed.

APPENDIX VII GENERAL PRACTICE MEDICAL RECORD REVIEW FORM USED FOR BIANNUAL MORBIDITY FOLLOW-UP

Serial No: SERNO

Name: MR FIRST NAME SECOND NAME SURNAME
Address ADDR1
ADDR2
ADDR3
ADDR4 POSTCODE
DOB: DOB
NHS No: NHS NO

Please tick if address is correct ☐

New address:

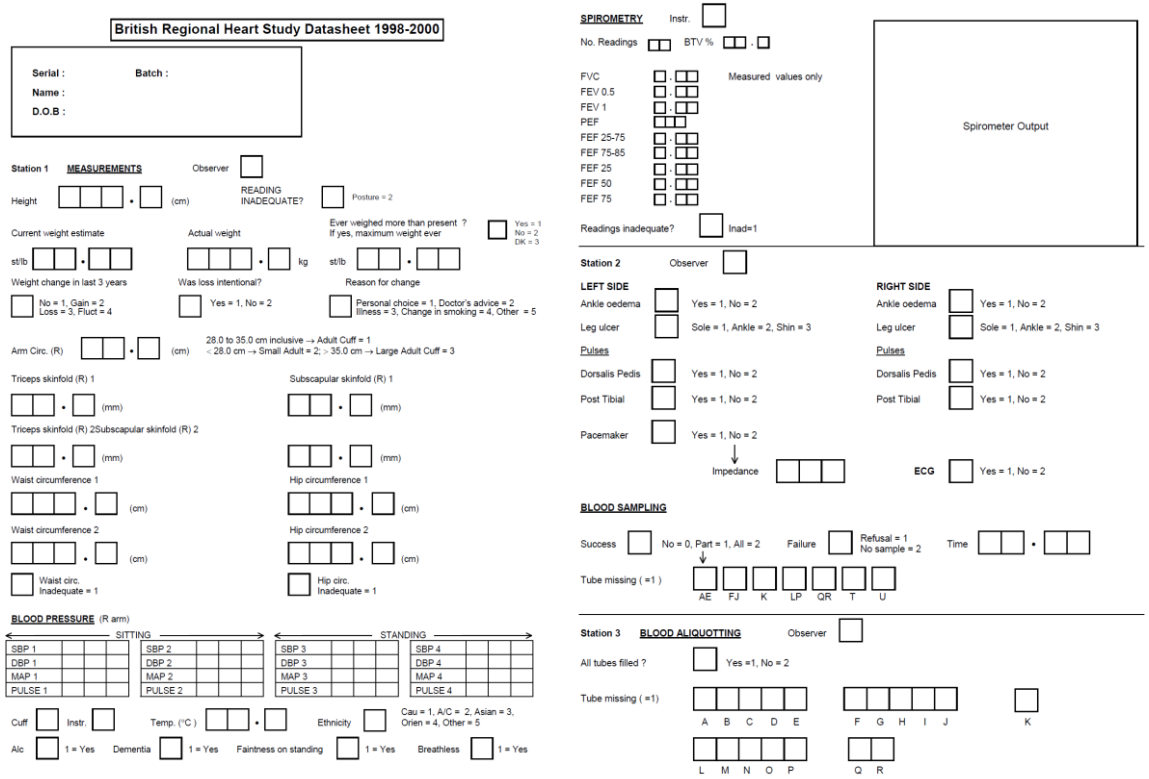
THE QUESTIONS ON THIS PAGE (1-6) RELATE TO THE PERIOD FROM **1ST JANUARY 2010** TO DATE

		YES	NO	
1	Is the above patient still registered with you ?	☐	☐	
2	Has he **consulted** you since 1st January 2010?	☐	☐	
3	Was any consultation for a **new episode** of:	**YES**	**NO**	(day, month, year)
	***Myocardial Infarction** (MI) Heart attack, Coronary thrombosis	☐	☐	Date: ……*
	***Acute Coronary Syndrome**	☐	☐	Date: ……*
	Angina Exertional or stress related chest pain	☐	☐	Date: ……
	***Stroke** Cerebrovascular accident (CVA), cerebral thrombosis, haemorrhage, embolism	☐	☐	Date: ……*
	Transient Ischaemic Attack (TIA) Cerebrovascular disturbance (<24 hours); leaving no residual damage	☐	☐	Date: ……
	Diabetes (NIDDM Type 2 / IDDM Type 1)	☐	☐	Date: ……
	***Heart Failure** Congestive Cardiac Failure - (CCF) or Left Ventricular Failure - (LVF)	☐	☐	Date: ……*
	Other Cardiovascular disease:			
	Peripheral Arterial Disease (PAD,PVD) Intermittent claudication, lower limb ischaemia	☐	☐	Date: ……
	Aortic Aneurysm rupture, dissection	☐	☐	Date: ……
	***Deep Vein Thrombosis** (DVT) blood clot in the leg	☐	☐	Date: ……*
	***Pulmonary Embolism** (PE) blood clot in the lung	☐	☐	Date: ……*

*** If Yes, please send a copy of the hospital letter or discharge summary**

		YES	NO	
4	Has he been referred to a Consultant for any new cardiovascular condition?	☐	☐	Date: ……
	Diagnosis : ……			
5	Have any of the following procedures taken place:	**YES**	**NO**	
	Coronary Artery Bypass Graft (CABG)	☐	☐	Date: ……
	Coronary Angioplasty (PTCA) Percutaneous coronary angioplasty, balloon treatment. Insertion of stents ☐	☐	☐	Date: ……
6	Is there a **READ code** entry for a G3, G6 or C10 code, for this time period? If yes, please complete the full code below	**YES**	**NO**	Date of entry
	CHD G3……	☐	☐	……
	Stroke G6……	☐	☐	……
	Diabetes C10……	☐	☐	……
		YES	**NO**	
7	Has he had a Cancer diagnosis?	☐	☐	Date: ……
	Site: ……			

APPENDIX VIII PHYSICAL EXAMINATION DATA SHEET USED IN 1998-2000 AND 2010-12



British Regional Heart Study Datasheet 1998-2000

Serial : Batch :
Name :
D.O.B :

Station 1 **MEASUREMENTS** Observer

Height (cm) READING INADEQUATE? Posture = 2

Current weight estimate st/lb Actual weight kg st/lb Ever weighed more than present ? If yes, maximum weight ever Yes = 1 No = 2 DK = 3

Weight change in last 3 years No = 1, Gain = 2 Loss = 3, Fluct = 4 Was loss intentional? Yes = 1, No = 2 Reason for change Personal choice = 1, Doctor's advice = 2 Illness = 3, Change in smoking = 4, Other = 5

Arm Circ. (R) (cm) 28.0 to 35.0 cm inclusive → Adult Cuff = 1 < 28.0 cm → Small Adult = 2; > 35.0 cm → Large Adult Cuff = 3

Triceps skinfold (R) 1 (mm) Subscapular skinfold (R) 1 (mm)

Triceps skinfold (R) 2Subscapular skinfold (R) 2 (mm) (mm)

Waist circumference 1 (cm) Hip circumference 1 (cm)

Waist circumference 2 (cm) Hip circumference 2 (cm)

Waist circ. Inadequate = 1 Hip circ. Inadequate = 1

BLOOD PRESSURE (R arm)

SITTING		STANDING	
SBP 1	SBP 2	SBP 3	SBP 4
DBP 1	DBP 2	DBP 3	DBP 4
MAP 1	MAP 2	MAP 3	MAP 4
PULSE 1	PULSE 2	PULSE 3	PULSE 4

Cuff Instr. Temp. (°C) Ethnicity Cau = 1, A/C = 2, Asian = 3, Orien = 4, Other = 5

Alc 1 = Yes Dementia 1 = Yes Faintness on standing 1 = Yes Breathless 1 = Yes

SPIROMETRY Instr.

No. Readings BTV %

FVC
FEV 0.5
FEV 1
PEF
FEF 25-75
FEF 75-85
FEF 25
FEF 50
FEF 75

Measured values only

Readings inadequate? Inad=1

Spirometer Output

Station 2 Observer

LEFT SIDE
Ankle oedema Yes = 1, No = 2
Leg ulcer Sole = 1, Ankle = 2, Shin = 3
Pulses
Dorsalis Pedis Yes = 1, No = 2
Post Tibial Yes = 1, No = 2
Pacemaker Yes = 1, No = 2
Impedance

RIGHT SIDE
Ankle oedema Yes = 1, No = 2
Leg ulcer Sole = 1, Ankle = 2, Shin = 3
Pulses
Dorsalis Pedis Yes = 1, No = 2
Post Tibial Yes = 1, No = 2

ECG Yes = 1, No = 2

BLOOD SAMPLING

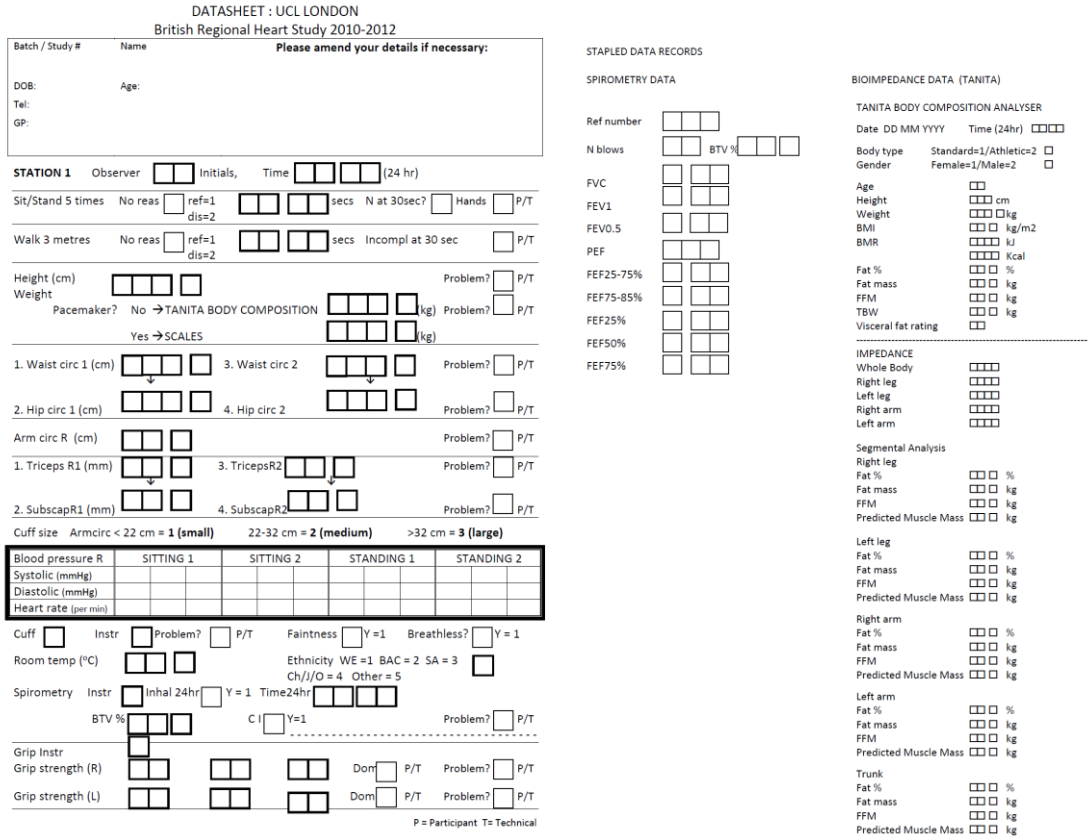
Success No = 0, Part = 1, All = 2 Failure Refusal = 1 No sample = 2 Time

Tube missing (=1) AE FJ K LP QR T U

Station 3 **BLOOD ALIQUOTTING** Observer

All tubes filled ? Yes =1, No = 2

Tube missing (=1) A B C D E F G H I J K L M N O P Q R



DATASHEET : UCL LONDON
British Regional Heart Study 2010-2012

Batch / Study # | Name | **Please amend your details if necessary:**

DOB: | Age:
Tel:
GP:

STATION 1 Observer ☐☐ Initials, Time ☐☐ ☐☐ (24 hr)

Sit/Stand 5 times — No reas ☐ ref=1 dis=2 ☐☐ ☐☐ secs N at 30sec? ☐ Hands ☐ P/T

Walk 3 metres — No reas ☐ ref=1 dis=2 ☐☐ ☐☐ secs Incompl at 30 sec ☐ P/T

Height (cm) ☐☐☐ ☐ Problem? ☐ P/T
Weight
Pacemaker? No →TANITA BODY COMPOSITION ☐☐☐ ☐ (kg) Problem? ☐ P/T
Yes →SCALES ☐☐☐ ☐ (kg)

1. Waist circ 1 (cm) ☐☐☐ ☐ 3. Waist circ 2 ☐☐☐ ☐ Problem? ☐ P/T
2. Hip circ 1 (cm) ☐☐☐ ☐ 4. Hip circ 2 ☐☐☐ ☐ Problem? ☐ P/T

Arm circ R (cm) ☐☐ ☐ Problem? ☐ P/T

1. Triceps R1 (mm) ☐☐ ☐ 3. TricepsR2 ☐☐ ☐ Problem? ☐ P/T
2. SubscapR1 (mm) ☐☐ ☐ 4. SubscapR2 ☐☐ ☐ Problem? ☐ P/T

Cuff size Armcirc < 22 cm = **1 (small)** 22-32 cm = **2 (medium)** >32 cm = **3 (large)**

Blood pressure R	SITTING 1	SITTING 2	STANDING 1	STANDING 2
Systolic (mmHg)				
Diastolic (mmHg)				
Heart rate (per min)				

Cuff ☐ Instr ☐ Problem? ☐ P/T Faintness ☐ Y =1 Breathless? ☐ Y = 1
Room temp (°C) ☐☐ ☐ Ethnicity WE =1 BAC = 2 SA = 3 ☐
Ch/J/O = 4 Other = 5
Spirometry Instr ☐ Inhal 24hr ☐ Y = 1 Time24hr ☐☐☐☐
BTV % ☐☐☐ C I ☐ Y=1 Problem? ☐ P/T
Grip Instr
Grip strength (R) ☐☐ ☐☐ ☐☐ Dom ☐ P/T Problem? ☐ P/T
Grip strength (L) ☐☐ ☐☐ ☐☐ Dom ☐ P/T Problem? ☐ P/T

P = Participant T= Technical

STAPLED DATA RECORDS

SPIROMETRY DATA

Ref number ☐☐☐
N blows ☐☐ BTV % ☐☐☐

FVC	☐	☐☐
FEV1	☐	☐☐
FEV0.5	☐	☐☐
PEF	☐☐☐	
FEF25-75%	☐	☐☐
FEF75-85%	☐	☐☐
FEF25%	☐	☐☐
FEF50%	☐	☐☐
FEF75%	☐	☐☐

BIOIMPEDANCE DATA (TANITA)

TANITA BODY COMPOSITION ANALYSER

Date DD MM YYYY Time (24hr) ☐☐☐☐
Body type Standard=1/Athletic=2 ☐
Gender Female=1/Male=2 ☐

Age ☐☐
Height ☐☐☐ cm
Weight ☐☐☐ ☐kg
BMI ☐☐ ☐ kg/m2
BMR ☐☐☐☐ kJ
☐☐☐☐ Kcal
Fat % ☐☐ ☐ %
Fat mass ☐☐ ☐ kg
FFM ☐☐ ☐ kg
TBW ☐☐ ☐ kg
Visceral fat rating ☐☐

IMPEDANCE
Whole Body ☐☐☐☐
Right leg ☐☐☐☐
Left leg ☐☐☐☐
Right arm ☐☐☐☐
Left arm ☐☐☐☐

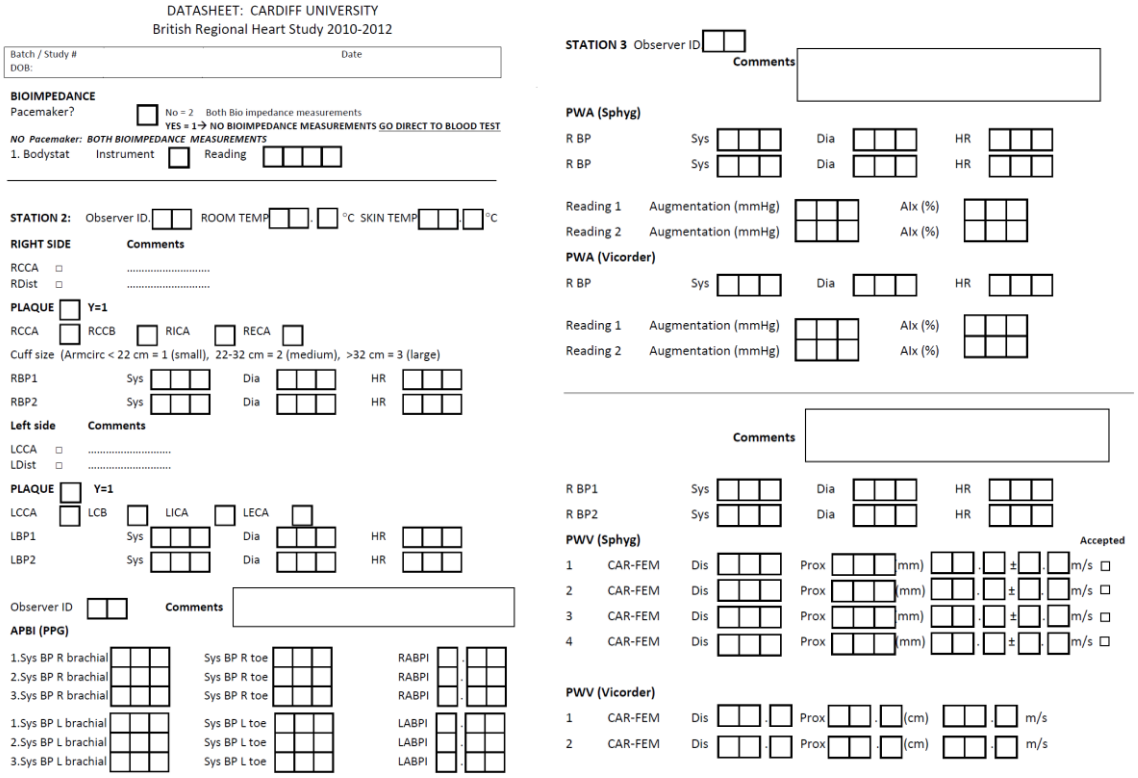
Segmental Analysis
Right leg
Fat % ☐☐ ☐ %
Fat mass ☐☐ ☐ kg
FFM ☐☐ ☐ kg
Predicted Muscle Mass ☐☐ ☐ kg

Left leg
Fat % ☐☐ ☐ %
Fat mass ☐☐ ☐ kg
FFM ☐☐ ☐ kg
Predicted Muscle Mass ☐☐ ☐ kg

Right arm
Fat % ☐☐ ☐ %
Fat mass ☐☐ ☐ kg
FFM ☐☐ ☐ kg
Predicted Muscle Mass ☐☐ ☐ kg

Left arm
Fat % ☐☐ ☐ %
Fat mass ☐☐ ☐ kg
FFM ☐☐ ☐ kg
Predicted Muscle Mass ☐☐ ☐ kg

Trunk
Fat % ☐☐ ☐ %
Fat mass ☐☐ ☐ kg
FFM ☐☐ ☐ kg
Predicted Muscle Mass ☐☐ ☐ kg



DATASHEET: CARDIFF UNIVERSITY
British Regional Heart Study 2010-2012

Batch / Study # Date
DOB:

BIOIMPEDANCE
Pacemaker? No = 2 Both Bio impedance measurements
YES = 1→ NO BIOIMPEDANCE MEASUREMENTS GO DIRECT TO BLOOD TEST
NO Pacemaker: BOTH BIOIMPEDANCE MEASUREMENTS
1. Bodystat Instrument Reading

STATION 2: Observer ID. ROOM TEMP . °C SKIN TEMP . °C

RIGHT SIDE **Comments**
RCCA □ .....
RDist □ .....

PLAQUE **Y=1**
RCCA RCCB RICA RECA

Cuff size (Armcirc < 22 cm = 1 (small), 22-32 cm = 2 (medium), >32 cm = 3 (large)

RBP1 Sys Dia HR
RBP2 Sys Dia HR

Left side **Comments**
LCCA □ .....
LDist □ .....

PLAQUE **Y=1**
LCCA LCB LICA LECA
LBP1 Sys Dia HR
LBP2 Sys Dia HR

Observer ID **Comments**

APBI (PPG)
1.Sys BP R brachial Sys BP R toe RABPI .
2.Sys BP R brachial Sys BP R toe RABPI .
3.Sys BP R brachial Sys BP R toe RABPI .
1.Sys BP L brachial Sys BP L toe LABPI .
2.Sys BP L brachial Sys BP L toe LABPI .
3.Sys BP L brachial Sys BP L toe LABPI .

STATION 3 Observer ID
Comments

PWA (Sphyg)
R BP Sys Dia HR
R BP Sys Dia HR
Reading 1 Augmentation (mmHg) AIx (%)
Reading 2 Augmentation (mmHg) AIx (%)

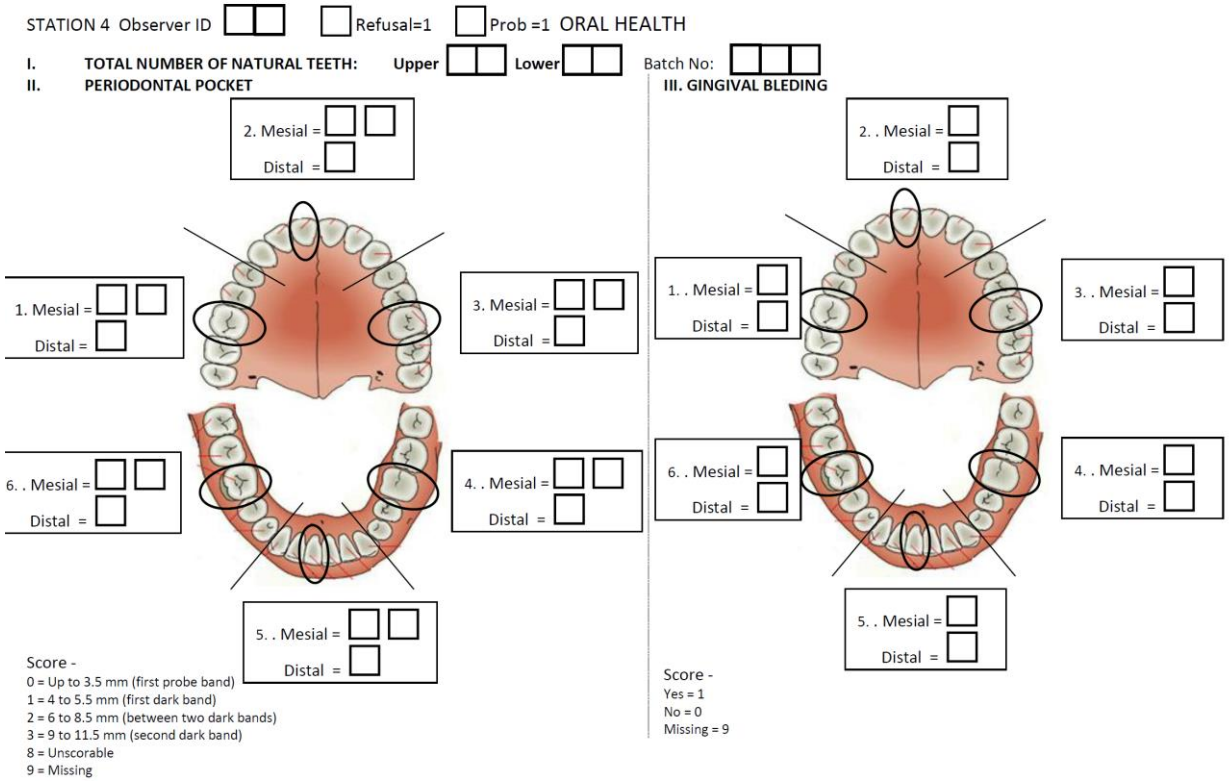
PWA (Vicorder)
R BP Sys Dia HR
Reading 1 Augmentation (mmHg) AIx (%)
Reading 2 Augmentation (mmHg) AIx (%)

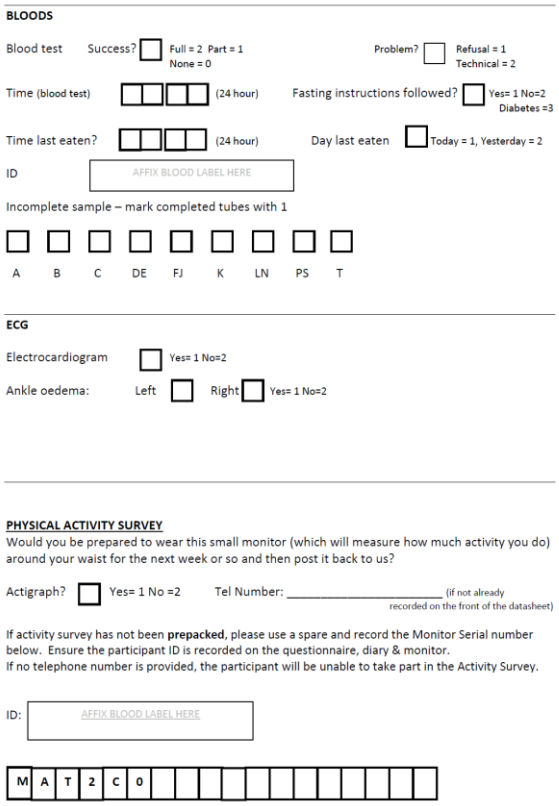
Comments

R BP1 Sys Dia HR
R BP2 Sys Dia HR

PWV (Sphyg) **Accepted**
1 CAR-FEM Dis Prox (mm) . ± . m/s □
2 CAR-FEM Dis Prox (mm) . ± . m/s □
3 CAR-FEM Dis Prox (mm) . ± . m/s □
4 CAR-FEM Dis Prox (mm) . ± . m/s □

PWV (Vicorder)
1 CAR-FEM Dis . Prox . (cm) . m/s
2 CAR-FEM Dis . Prox . (cm) . m/s


STATION 4 Observer ID
Refusal=1
Prob =1 ORAL HEALTH
I. TOTAL NUMBER OF NATURAL TEETH: Upper Lower
Batch No:
II. PERIODONTAL POCKET
III. GINGIVAL BLEDING
2. Mesial =
Distal =
1. Mesial =
Distal =
3. Mesial =
Distal =
6. . Mesial =
Distal =
4. . Mesial =
Distal =
5. . Mesial =
Distal =
2. . Mesial =
Distal =
1. . Mesial =
Distal =
3. . Mesial =
Distal =
6. . Mesial =
Distal =
4. . Mesial =
Distal =
5. . Mesial =
Distal =
Score -
0 = Up to 3.5 mm (first probe band)
1 = 4 to 5.5 mm (first dark band)
2 = 6 to 8.5 mm (between two dark bands)
3 = 9 to 11.5 mm (second dark band)
8 = Unscorable
9 = Missing
Score -
Yes = 1
No = 0
Missing = 9



BLOODS

Blood test Success? ☐ Full = 2 Part = 1 None = 0 Problem? ☐ Refusal = 1 Technical = 2

Time (blood test) ☐☐☐☐ (24 hour) Fasting instructions followed? ☐ Yes= 1 No=2 Diabetes =3

Time last eaten? ☐☐☐☐ (24 hour) Day last eaten ☐ Today = 1, Yesterday = 2

ID AFFIX BLOOD LABEL HERE

Incomplete sample – mark completed tubes with 1

☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐ ☐

A B C DE FJ K LN PS T

ECG

Electrocardiogram ☐ Yes= 1 No=2

Ankle oedema: Left ☐ Right ☐ Yes= 1 No=2

PHYSICAL ACTIVITY SURVEY

Would you be prepared to wear this small monitor (which will measure how much activity you do) around your waist for the next week or so and then post it back to us?

Actigraph? ☐ Yes= 1 No =2 Tel Number: _____ (if not already recorded on the front of the datasheet)

If activity survey has not been **prepacked**, please use a spare and record the Monitor Serial number below. Ensure the participant ID is recorded on the questionnaire, diary & monitor.
If no telephone number is provided, the participant will be unable to take part in the Activity Survey.

ID: AFFIX BLOOD LABEL HERE

M A T 2 C 0 ☐☐☐☐☐☐☐☐☐☐☐☐☐

CONSENT

We will arrange to have your blood sample checked for cholesterol and other factors which are important for heart disease risk. The results of the blood tests and other measurements will be sent back to your doctor in the next four to five weeks. If any of the results give cause for concern, you will be asked to make an appointment with your doctor.

1. Do you agree to us passing the test results to your doctor?

☐1 Agreed ☐2 Not Agreed

Part of your blood sample will be frozen and kept for special scientific studies of factors affecting heart disease risk, which may help us to understand how to prevent heart disease in the future. Among the factors we may need to study will be the way in which genetic factors affect heart disease risk.

2. Would you allow us to use your sample in this way?

☐1 Agreed ☐2 Not Agreed

Following the future health of all the men taking part remains a very important part of the study. However, because of new data protection laws, we are only able to continue to do this if you give us **specific written permission**.

In order to update your health record effectively, we need to obtain routine information from your family doctor and, where appropriate, from hospitals and several National Health Service agencies listed below*. We are particularly concerned to know about illnesses of the heart and circulation, diabetes, cancer and other disabling conditions. Even if you do not have any of these conditions, the review of your medical records is of very great importance to us. The information we obtain is kept securely and is only seen by members of our small research team.

3. Do you agree to us following your future health through your health records?

☐1 Agreed ☐2 Not Agreed

I agree to allow the Research Team to continue to study my health in accordance with the criteria above. I understand that any details recorded will be treated in complete confidence.

Signed: _____

Print name: _____

Date: _____

*The agencies related to the National Health Service are:-
-the NHS Information Centre
-the General Register Office
-the National Cancer Intelligence Centre
-the Primary Care Patient Registration Service

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