Arterial stiffness, cardiovascular risk and physical functioning in the Whitehall II study

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Declaration of authorship

I, Carlos Valencia, 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.

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Contents

Index of tables

Index of figures

ABSTRACT

Background: Arterial stiffness measured by carotid-femoral Pulse Wave Velocity (cf-PWV) is a predictor of cardiovascular events, incident hypertension and a cross-sectional marker of low physical functioning. This thesis aims to expand the knowledge on the bidirectional relationship between cf-PWV and incident hypertension, investigate the predictive value of a second cf-PWV measurement and study its prospective effects on physical function.

Methods: Data from 5236 participants of the Whitehall II study from 2008 to 2019 were used to examine the relationship between incident hypertension and baseline arterial stiffness, as well as arterial stiffness progression among different blood pressure status subgroups. Linear mixed models were used to assess the progression in cf-PWV between subgroups and logistic regression models were used to estimate the odds of incident hypertension. The risk of clinical events was validated through hospital health records and analysed using survival models with a mean follow-up of 11.2 years. The prospective relationship between arterial stiffness and change in standardised measures of physical functioning 8 years later was assessed using linear mixed models.

Results: A bidirectional relationship between arterial stiffness and hypertension was observed. Participants in the highest tertile of cf-PWV at baseline had three times higher odds of incident hypertension than participants in the first tertile. Participants with uncontrolled blood pressure at baseline had the highest increase in cf-PWV compared to normotensives. Change between two measurements of a-PWV did not improve the C-statistic but adding a single measurement to the 10-year atherosclerotic cardiovascular disease score improved both the C-statistic and the

net reclassification index. Baseline and prospective change of cf-PWV were associated to decline in the scores of the physical component of the SF-36 questionnaire and the instrumental activities of daily living.

Conclusions:

The bidirectional relationship between arterial stiffness and hypertension shown in some studies was replicated in the Whitehall II cohort. The sample size allowed for subgroup comparisons that were previously unpowered in previous studies. A second compared to a single measurement of cf-PWV did not seem to improve the predictive ability of cardiovascular risk models. Higher C-statistic and net reclassification index for prediction models using the components of the 10-year atherosclerotic cardiovascular disease score were seen after including cf-PWV. Finally, cf-PWV is a prospective marker of decrease in standardised measures of physical functioning.

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Glossary of units and abbreviations

95% Cl:	95% confidence limits
PWV:	Pulse wave velocity
Cf-PWV:	Carotid-femoral pulse wave velocity
Ba-PWV:	Brachial-ankle pulse wave velocity
a-PWV:	Aortic pulse wave velocity
AS:	Arterial stiffness
CVD:	Cardiovascular disease
MRI:	Magnetic resonance imaging
DALY:	Disability adjusted life year
RAS:	Renin-angiotensin system
ROS:	Reactive oxygen species
CCB:	Calcium channel blockers
BB:	Beta blockers
ACEI:	Angiotensin converter enzyme inhibitors
ARB:	Angiotensin receptor blockers
OR:	Odds ratio
m/s:	Metres per second

Chapter 1 Introduction

1 Introduction

Age-related transformations take place in all body systems, including the circulatory, respiratory and musculoskeletal system. Among these age-related transformations, there are structural and functional changes in conduit arteries, particularly the aorta, which may lead to progressive stiffening. Arterial stiffness (AS) has been linked with raised cardiovascular disease (CVD) risk, [1] poor age-related physical function [2] and end-organ damage. [3] It may be that pulsatility of the blood supply to the brain, as a consequence of a rigid aorta, contributes to cognitive decline and dementia. [4]

The pathological processes underlying aortic stiffening are being studied. Rigidity appears to be the consequence of arteriosclerotic changes in the arterial wall, particularly collagen cross-linking, [5] elastin structural degradation, [6] and vascular inflammation. [7] There may be a feedback cycle between increasing blood pressure and arterial stiffening, which contributes to the progressive changes in arterial morphology and physiology that define AS. The interaction between risk factors, health behaviours and altered biological mechanisms can increase or delay the process of arterial stiffening. [8]

There are some key epidemiological aspects in the epidemiology of CVD, age and AS. Firstly, the global population is becoming older: the proportion of individuals older than 60 years is estimated to increase from 10% in 2015 to over 20% by 2050. [9] Secondly, CVD incidence and mortality is declining in high-income countries, leading to an increasing proportion of older people who may be living with mild or moderate CVD morbidity, including aortic stiffness, from midlife onwards.[9]

Cardiovascular mortality, stroke, myocardial infarction, and ischaemic heart disease among other major cardiovascular events are predicted by arterial stiffness (AS). [10,11]

AS is associated with some cardiovascular risk factors. Low physical activity,

adiposity and overweight are cardiovascular risk factors among them.[12] AS is a correlate of unhealthy ageing, frailty, and higher frequency of disability as well. [13] The fracture of elastin fibres and their progressive collagen replacement provides some of the biological plausibility of the association between CVD and AS. (Yoon et al., 2016) These are shared arterial stiffening mechanisms in CVD and ageing found in different cells and tissues.

A variety of methods for assessing AS have been developed over time. Pulse pressure and Osler's manoeuvre were among the first clinical demonstrations of arterial stiffness. Device-based techniques based on ultrasonography, magnetic resonance imaging (MRI) or arterial tonometry have been used mostly in research, with their potential clinical application depending on the cost, technical requirements, time or questions addressed. Carotid-femoral pulse wave velocity (cf-PWV) is a technique measuring the travel speed of the pressure pulse wave along an arterial segment of a known length.[15,16]

Studying the interaction between arterial stiffness, cardiovascular risk factors and major cardiovascular events could improve the understanding of how delaying arterial stiffening would contribute to reducing the frequency CVD major outcomes.[17]

This thesis aims to examine the association of PWV with blood pressure, major cardiovascular outcomes, and physical functioning. The background chapter is divided in three subsections. The first subsection summarises the evidence in the relationship between arterial stiffness and hypertension; the second subsection describes the most recent research studying the link between arterial stiffness and major adverse cardiovascular events; and the third subsection describes the literature studying the association arterial stiffness and different measures of physical functioning. The methods chapter describes the data structure, exposures,

outcomes, and covariates used in the thesis. The first research chapter (chapter 5) shows the influence of blood pressure and antihypertensive medication status on arterial stiffness and the process of arterial stiffening. Also shows the likelihood of incident hypertension over time according to arterial stiffness at baseline. The second research chapter (chapter 6) shows the likelihood of incident Major Adverse Cardiovascular Events from measurements of arterial stiffness while the last research chapter (chapter 7) studies both the cross-sectional and the prospective associations of arterial stiffness with objective and subjective measurements of physical functioning. The discussion summarises the findings from the analyses, describes, weights potential biases and limitations of the results and outlines the implications of this thesis for future research.

Chapter 2 Background

2 Background

2.1 Healthy ageing, a definition with multiple elements

Although there are multiple definitions of healthy ageing, a universal and standardised concept is still lacking. The World Health Organisation includes various elements within the definition of “developing and maintaining the functional ability that enables wellbeing in older age”, while underlying that this process might not necessarily be free of disease.[18] Other authors consider the ageing process as the arrival to an advanced chronological age with high physical and mental function while being free of disease and disability. Even if there are different definitions, there are some common aspects between them. [19] The concepts that usually convey the different definitions of ageing are the abilities of individuals to successfully carry out physical and mental tasks at an advanced chronological age, while maintaining a good social interaction with other individuals around them.

There are multiple dimensions within the concept of healthy ageing. These not only include cardiovascular health, but also other objective, biomedical dimensions such as cognitive function and physical function. In addition, subjective factors might be reflected on the interaction between the individual and their social environment. This chapter aims to describe the different elements within the definition of healthy ageing and how they are related to cardiovascular health and arterial ageing.

2.2 Cognitive healthy ageing

Within the biomedical aspects of healthy ageing, cognitive function is one of the most studied topics related to healthy ageing, as the loss of cognitive function and the progression to dementia have been traditionally associated with an advanced chronological age.

The first step is to define cognitive function. This can usually be defined in three different ways. First, psychometric testing; second, through cohort standards or cohort differences; third, the neurocognitive approach.[20]

Psychometric testing is the ability to attain a desired score on tests designed to measure the ability of an individual to solve problems in arithmetic, math, logical reasoning, verbal ability, memory, attention, and decision making, among other areas.

The psychometric approach differentiates two groups of cognitive skills. The first is the group of fluid cognitive abilities that are related to the way individuals process information, such as the speed of retrieving information, the way of solving new problems recall time. The second group of cognitive abilities is crystallised cognitive abilities and is related to the accumulation of information through the lifespan, acculturation, and general experience of events.[21]

The cohort approach refers to the lack of comparability of different birth cohorts, or groups of individuals born in the same year. This lack of comparability seems to arise from the fact that more recent cohorts tend to achieve higher baseline levels of fluid cognitive abilities and higher scores in tests of general intelligence, compared to older birth cohorts.

The third approach is known as the neurocognitive method, which consists in a more ‘global’ measurement of cognitive function. This means that although the more ‘classic’ functions of cognitive ability, such as information processing, problem solving, attention, memory and language are considered, there are also other functions such as visual perception, social and emotional processing, and assessing the function of the limbic area that fall within the definition of neurocognition.

The notion of cognitive ageing can be defined differently according to these three approaches.

The psychometric approach to cognitive ageing uses data from different psychometric tests that have been applied in different countries along the XXth century. Results have shown that the fluid intelligence tends to peak along the start of adult life and tend to decline steadily as individuals enter adulthood and old age, while the general and crystallised intelligence features increase steadily until midlife and then tend to remain steady.

Moreover, for a given individual belonging to a certain population and age, a score below the average for his age, population and education level would be a potential sign of cognitive ageing.

The cohort approach for cognitive ageing compares scores only between individuals that belong to a particular group born each year. The specification of a birth cohort is key to appropriately establishing thresholds of cognitive ageing, as more recent cohorts tend to have higher scores and have less decrease in cognitive function over the lifespan than the earlier ones. [22]

Finally, the neurocognitive approach to ageing tends to be based on the decline in the performance in psychometric tests; including an additional arrange of neurocognitive tests and executive functions, while also studying the expression of genes and neurological patterns consistent with an ageing nervous system, such as diminishing density of brain cortex of some areas of the brain.[23]

2.3 Physical function

A high score of physical functioning is another hallmark of successful ageing. Physical function in older age has different components, such as the density and

quantity of muscle mass and muscle strength. Body composition is another characteristic that is associated with healthy ageing. Individuals with healthy body composition tend to have higher muscle density, lean body mass and a higher bone mass density.

Physical functioning tests are alternative parameters to body composition in the measurement of healthy ageing.

The concept of healthy ageing in physical functioning refers to the maintenance of a mean score or set of scores in the performance of physical and social tasks that are key to the survival of the individual.

2.4 Evidence and theories of ageing: from cells to individuals

2.4.1 Ageing: Morbidity, programming or just a consequence of living?

Chronological age is defined as the amount of time units spent from the birth of an individual to a given point on their life trajectory and ultimately to death. Biological age is a related but different concept, referring to the structural and metabolic integrity of the tissues and organs of an individual, their fertility and their ability to adapt to the challenges brought by the environment, while maintaining its structural and metabolic integrity.[24]

There are phenotypic or structural characteristics that are synonyms of biological ageing and can be highlighted on cells, tissues, and organisms as a whole.

On a biochemical level, ageing can be defined as the damage of the structural macromolecules, especially DNA degradation. On a cell level, ageing is considered as the accumulation of macrophages, waste products, and changes on cell division processes leading to either senescence, when cell there is an arrest in cell

reproduction while metabolic status is conserved, or into apoptosis, a process of programmed cellular death.[25]

A lower activity or total impairment of the cancer preventing mechanisms stopping uncontrolled cell reproduction is also a hint of an aged cell.20 On a broader scope, biologically aged tissues possess lower efficiency in fulfilling their function, less resilience to environmental challenges and lower ability to repair themselves. Aged organisms are mainly composed of biologically aged tissues, constituting a barrier to the performance of necessary activities to the survival of individuals.21

Biological age and chronological age have been regarded as synonyms of reduced overall health since times of ancient Greece.[28] The terms ‘age-related diseases’, and ‘geriatric syndromes’ are explained by this proximity. This cluster of diseases includes cardiovascular conditions, such as heart failure, atherosclerosis and arterial stiffness, metabolic and inflammatory diseases as diabetes, chronic obstructive pulmonary disease, or musculoskeletal conditions. Neurodegenerative diseases as Alzheimer’s or other types of dementia are in this group as well. A common feature of geriatric syndromes is that they tend to be chronic, reduce the ability of individuals to be independent in fulfilling the necessary tasks for survival and occur simultaneously with failures in other organs and systems.[29]

The prevalence of the cluster of geriatric syndromes tends to increase with age, and almost one third of the individuals with one of such conditions is above 65 years old.[30–32] In the UK and other high-income countries, the age-specific incidence is either stabilised or in decline, although there is an increase in the absolute number of individuals with these conditions as a result of higher life expectancy.[33,34]

The shortening and delayed onset of the more symptomatic and disability-producing stages, independently of the time lived with the disease, is defined as compression

of morbidity. With most chronic diseases, it is correlated with a higher average age of onset and diagnosis, especially in patients with dementia, and is not necessarily explained by the existence of better disease treatments. In the second half of the 20th century and start of the 21st, survival time after dementia diagnosis has been constant or decreased.[35]

A traditional view of ageing is that disability and disease are the final steps in ageing[36] in a process that is consequence of the normal decay secondary to repeated bodily function over time, leading to tissue damage, also known as the 'wear and tear' theory of ageing, widely used by gerontologists.[37]

Cohort studies show evidence of the effects of disease and biological ageing in associations between cardiovascular disease in middle age and disability in later life.[38] When individuals are exposed to recommended levels of cardio metabolic markers and less frequent unhealthy behaviours suggested by primary prevention guidelines, they are less likely to suffer from disability in later life.[39]

While the disease-accelerated pattern of ageing offers a clearer explanation on the specific causes of disability and lack of functioning in later life, it does not explain why individuals without chronic disease also age biologically, declining in physical functioning before dying.

An alternative explanation would be the 'wear and tear' theory, showing that despite the absence of overt disease, the constant use of body systems is going progressively towards functional decline and loss of structural integrity. This hypothesis does not seem supported by the widely found association between active lifestyles and lower risk of CVD or some types of cancer,[40,41] a higher number of years without disability [42] and a longer life expectancy.[43]

Given that there are around 300 descriptions of ageing patterns,[44] this section does not pursue to exhaustively describe them. It rather intends to highlight some of the ageing theories from cells to humans and the pertinence of the application of delayed ageing strategies to age-related diseases and specifically to cardiovascular ageing.

2.4.2 Cellular ageing as a genetically programmed event

If the absence of overt disease or the exposure to a healthy lifestyle does not explain the ageing process, there could be predetermined factors which promote and accelerate ageing. Genetically induced ageing is a topic to explore in this regard and the study of stem cells are in the spotlight of research about this topic.

Cells have different phases of reproduction that follow one another on a cyclic way and can partially stop and resume according to the specific needs of the tissue or reproductive age of the organism. Towards the end of the lifespan of an individual, an increasing number of cells goes into a stationary phase in which there is no cell reproduction, called senescence. Other cells go into a phase called apoptosis, which is a genetically programmed cellular death.[45] Both functions are involved in stopping uncontrolled reproduction of cells with defective genetic material, which potentially leads to cancer. If all the processes seem to be genetically predictable, this could be the evidence of the existence of a predetermined end of life, irrespective of the diseases of the organism. Research has focused on stem cells, a particular cellular type, to understand more about this process.

Stem cells are pluripotent, undifferentiated cells that can differentiate to any type of cell of an organism and are able to produce an entire new tissue from a single cell. These cells are currently studied and regarded as one of the keys to understand longevity.[46] Stem cells were initially associated with biological ageing and lower body

regeneration ability, as early experimental reports showed higher numbers of stem cells in tissues and organs of young individuals, compared to tissues of old individuals.[47]

Although stem cell replication in laboratory conditions suggests that reproduction without the effects of ageing is possible, and that any stem cell can produce any differentiated tissue, not all stem cells act equally, as there is influence by the organs and surrounding environment in which they are.[48] This has been shown experimentally when seeing that senescent stem cells extracted from tissues of elderly individuals can start to replicate and produce entire tissues out from a single cell if they are placed on a different environment.[49] Finally, there is no correlation between the number or quality of stem cells in an organism and its longevity and for now, a master program managing stem cells is not seen as the key of stopping ageing.[50]

2.4.3 Energetic costs of body maintenance and reproduction

If there is no such thing as a master program that sets the chronological point at which cells, tissues and organisms must decay, perhaps the key fact in ageing has to do with a balance between energy consumption and body maintenance, as proposed by Kirkwood in the 'Disposable Soma Theory of Ageing'.[51] His theory states that young individuals are kept in their state of fitness due to repair mechanisms of DNA and proteins. As these mechanisms are supposed to be high in metabolic energy consumption, and the repair of an old individual is metabolically more expensive than creating a new one, reproduction repair tasks are left behind in the metabolic priorities during reproductive periods.

This accumulation of damages and the progressive degradation of the organism, leads to biological ageing and death. The Disposable Soma theory also links

longevity to energy consumption, reproduction, and energy intake, proposing that there is a critical threshold of food and calorie availability linked to reproductive ability. Below that threshold, the organisms are in a stage of controlled senescence, as the lack of energy does not enable them to progress to a reproductive state. The extension of the lifespan would be an evolutionary trait to allow these organisms to survive until there is a more favourable environment that provides energy not only for them but for the metabolically demanding process of reproduction and for feeding the offspring once they are born. Once the threshold of reproduction is met, the lack of action in the protection mechanisms unfolds the ageing effects, as already described.

Some flaws of this theory rely on the claim of energetic efficiency, as the body is equipped with a set of repair mechanisms able to restore it to a previous state without a high consumption in energy, while the metabolic demands of creating new cells, tissues, and organisms as parts of reproduction, are almost twice the demand of a normal organism. Longevity and fertility have not yet been linked in evolutionary studies,[52] as this theoretical model still fails to explain why females live longer than males in many species. Contradicting the notion of an energy surplus leading to decreased longevity.[53]

2.4.4 Dietary restriction in ageing

Beyond the Disposable Soma, caloric restriction and food intake restriction have been analysed on their own, being among the most consistent hypotheses on the extension of lifespan across invertebrates and small mammals.

Experiments on how diets and caloric restriction can impact ageing and longevity have been carried out mainly in bacteria, insects and nematodes, given their availability, ease of culture in laboratory conditions and their relatively short lifespan.

Seminal works with the fly *Drosophila melanogaster* show that altering the diet of the insects can increase the lifespan up to 53% with a maximal dietary restriction. Further dietary restrictions decrease the gains in lifespan.[54]

A multicellular invertebrate organism named *Caenorhabditis elegans*, has been kept alive experimentally with increases in its chronological age of 40% compared to its original lifespan.[55]

Lifespan extensions are not limited to invertebrate organisms, with extensions also being reported in mice after hypocaloric or isocaloric intermittent fasting.[56] The most extensive meta-analysis on life extension on rodents included mice and rats studies from 1934 to 2012, showing maximum lifespan increases of 20% in mice and 32% in rats.[57]

All these associations found in animal models need to be validated in humans.

As with many mechanisms involved in ageing, dietary restriction does not have one monotone, deterministic association to lifespan increase, and even can take to reduction in lifespan, or a prolonged lifespan with drastically lower offspring production. Although the impact of dietary restriction on the lifespan could be just the result of a total lower calorie count, other experimental evidence suggests that protein-carbohydrate ratio in the diet could be even more determinant of lifespan. [58,59] Introducing a reduction in the metabolic rate through genetic mechanisms could be a contributing phenomenon involved in the same metabolic pathway.

This leads to the study of the interplay of genetic models into this relationship and poses the question whether metabolic or caloric restriction is just a mechanism activating a genetic pathway slowing down the ageing process.

2.4.5 Modification of genetic pathways on ageing

Whether genetic pathways control ageing on their own or due to metabolic restrictions, has been the topic of an entire discipline. Genetic targets have been studied as candidates for controlling ageing. To improve the way these are studied, genetic pathways have been grouped into 'hallmarks of ageing', that are pathways common pathways leading to a specific mechanism producing specific physical features of ageing, including nutrient-sensing abilities of the cells, epigenetic alterations, instability of the genome and altered cell communication, amongst others.[60]

Silencing the expression of ageing-related genes involved in one or more of these hallmarks of ageing has increased the lifespan of mice between a median of 7% and 68% in mice.[61]

A mechanism linking caloric and metabolic restrictions with genetic pathways is the genetic expression of the Growth Hormone (GH) and the Insulin Growth Factor-1 (IGF-1) decrease with age, and are involved on metabolic management, as well as proliferation and maintenance of cardiovascular and neurologic cells, being involved on delayed ageing. Despite the hints on this, the evidence on GH/IGF-1 signalling is not uniform and there are also findings leading to age acceleration.[62,63] This route is also involved in promoting biological ageing through other ´hallmark features´ beyond the nutrient pathway, such as inflammation or cellular senescence.58

Another mechanism highlighted as a 'hallmark feature of ageing' involves the ability of an organism to break down faulty proteins. This is one of the functions of the mechanistic target of rapamycin (mTOR), a protein that maintains the support structure of the cells, or cytoskeleton. The activity of mTOR decreases with biological

ageing and is correlated with a lower metabolic and regenerative performance of an organism.[65]

Features of ageing beyond the modification of DNA are also important to be discussed. Epigenetic factors are another of the central 'hallmark features of ageing'.[66] These promote the alteration and expression of a variety of genes that are involved in the lifespan of unicellular and multicellular models.61 A link between epigenetic modifications has been also described with a reduced activity and a total halt of function as the organisms increase their chronological age.[68]

Another molecular and genetic mechanism that contributes to ageing is the decrease and deletion of mitochondrial DNA (mtDNA). Mitochondria are organelles in charge of energy production for each cell and ultimately for the organism. Their function is core in all cellular process, and that seems to be the reason why they possess an independent DNA machinery from the main cell DNA.[69] A correlation has been found between the density and quality of mitochondria and alterations in the lifespan of *C. elegans.*64 Whether this correlation is the result of a role of an activation of biological ageing by the depletion of mitochondria or if the lack of mitochondria is just a result of biological ageing is still matter of debate.[71,72]

There are some drawbacks relative to genetic studies of lifespan extension. The murine models are the opening gate for studying ageing on humans, with some of the lifespan extensions lacking validated pathways and modifications, not only in the experimentation procedures, but also in the murine subjects used. These conditions difficult replication of the experiments, not allowing for the original hypotheses to be further tested.[61]

Finally, the achievement of an increase in the lifespan of bacteria, nematodes, and small mammals, does not imply providing them with eternal life as these individuals

continue their loss of functionality and ultimately die after the extension of the lifespan. This fact could be interpreted in two different ways. It either could be a hint of the existence of a robust master program managing ageing that is not overruled by changing just a group of genes under its control, or the prove that there is no such program and that the mechanisms prolonging life are not entirely interrelated.[73]

2.4.6 Integration of ageing mechanisms and chronic diseases

The most known effort to integrate all the mechanistic theories on biological ageing is known as the Network Theory of Ageing. It uses mathematical models testing the effects of diet restriction, free radicals, and mitochondrial alterations. The central hypothesis of the model suggests that an initial imbalance in the cellular environment releases free radicals that attack mitochondria, leading to a less functional cell phenotype.[74]

An integrative theory on ageing is important, as the experiments testing various ageing pathways are every time more numerous and diverse, but not always integrated and parallel experiments being frequently published without using evidence from each other.69 The integration not only should be done within mechanistic and physiologic research. It should also be done with observational evidence in humans, behavioural determinants of ageing, as well as epidemiologic evidence of risk factors for diseases of ageing and strategies for healthy ageing.[76]

To conclude, I would like to highlight two main approaches extrapolating mechanistic theories from invertebrates to humans and applying them to ageing and cardiovascular ageing. The first one would be to examine how strategies of delayed ageing and compression of morbidity would relate to mechanistic approaches found in studies of invertebrate and small mammals. The second one would be examining

the amount of available evidence on ageing populations of non-human animals, studying the similarities with ageing on human populations.[77]

Given that the evidence about the existence of a general program of ageing, either in humans or in other species is not conclusive, the next step has been to target specific mechanisms involved in biological ageing.[78] Gene and cell therapy have been proposed as tools in this process, aiming to preserve stem cells that usually decay during development of metabolic syndrome, frailty, neurologic diseases, cancer and cardiovascular diseases.[79–81] More specifically, these genetic interventions would rely in the action of in-vivo or ex-vivo mechanisms that involve the insertion of modified genes in the body via biologic carriers or the extraction and reintroduction of body cells after treating them genetically outside the body.

The therapeutic uses of these alternatives are still in early development. As an example, stimulation of nerve growth factor for lowering the rate of cognitive decline in Alzheimer's is still in phase 1 trials.[82] In other neurologic diseases as Parkinson's, using gene therapy to stimulate the codification of dopamine stimulating genes had successful results in avoiding or slowing the rate of death of neurons in the *substantia nigra* of the brain. Despite the good results in phase 1 trials, phase 2 trials did not show its therapeutic value so far, and similar cases have been seen in early clinical trials with key genetic targets of metabolic, cardiovascular, and musculoskeletal diseases.[83]

Beyond efficacy, tolerability, and adverse effects currently difficult delivering these therapeutic solutions in most diseases,[84] although stem cell transplants have been successfully used in other conditions such as amyloidosis.[85] Finally, another challenge for the application of these mechanistic approaches in the most common

diseases of ageing is their polygenic nature, as treating only one gene would not achieve the desired therapeutic effects.[86]

An intermediate step between gene therapy and ageing delaying strategies, is the search of second uses of conventional therapeutic solutions. Metformin, an oral antidiabetic drug, has been used for around 35 years in patients with type 2 diabetes mellitus, given that improves metabolic syndrome, insulin sensitivity and lipid metabolism. Metabolic and genetic studies in the early 21st century have been revealing that metformin is a genetic modifier, down-regulating genes that promote biological ageing in diabetes, a disease that promotes cardiovascular, neurologic, and musculoskeletal ageing.[87]

Metformin has been acting as a disabler of mechanisms that promote ageing, being one of the first 'gene therapies' in an age-related disease. The mechanisms are mainly modifiers of protein kinases and TOR like protein signal messengers, involved in cell energy management. The drug profile and action of metformin promoted the idea of mixed solutions between modification of genetic pathways and delayed ageing strategies.[88]

2.4.7 Ageing phenotype in chronic diseases and delayed ageing strategies

Biological ageing implies the existence of a set of characteristics compatible with low physical performance at increasing chronological age. Some authors have called this as the ageing phenotype.[89] These characteristics include reduced tissue integrity. Examples are a heart with enlarged and stiffer walls, larger chambers, and lower arterial compliance.[81] On a neurologic level, axons and support tissue start to degrade, and kidney volume tend to decrease. The musculoskeletal system diminishes the density and strength of bones and the flexibility of cartilage, among other functions.[90] The presence of the ageing phenotype usually goes unnoticed until

there is a critical impairment in function, that impairment threshold leads the person to changes in functionality and a severe impairment in function leading to frailty and death.

The postponing of the time of arrival of the ageing phenotype, a phenomenon called compression of morbidity", was observed besides the increases in life expectancy at birth.[91] These increases were not only the result of the result of improved medical treatments, but also the consequence of changes in diet, social networks and patterns of physical activity. [92,93] The latter are at the core of delayed ageing strategies, combining primary and secondary prevention. In other words, alternating treatments of geriatric syndromes such as hypertension, diabetes, or atherosclerosis, with a lifestyle promoting physical activity and dietary components that allow to keep healthy levels of biomarkers such as blood cholesterol, blood pressure or blood glucose and preventing an organism to arrive to a state of ageing phenotype.[94]

The importance of the age delaying strategies in reducing the incidence of the main causes of morbidity and mortality in the elderly, is also suggested by the presence of some of the aforementioned deleterious ageing mechanisms in the main groups of diseases chronic diseases, but also their activation in the presence of presence of risk factors for these diseases.[95] This is also at the core of the strategies of delayed vascular ageing that are going to be discussed in this thesis.

2.5 Arterial stiffness

Arteries are the vessels that carry blood from the heart to the rest of the body to supply oxygen to organs and tissues. There is a blood pressure gradient between the two movements of contraction of the cardiac cycle, with the arteries needing to adapt to these pressure differences. One of the adaptation mechanisms is the

degree of compliance and resistance to the blood flow in each part of the cardiac cycle. The adaptive function not only guarantees the continuous blood flow to the rest of the body. It also has a protective effect on the blood vessels located in the peripheral circulation as these are not suited for pulsatile but steady blood flow. The adaptive function is partly explained by the anatomic characteristics of arteries; more elastic and predominantly collagen-filled aorta and central arteries, and with a lower proportion of elastic fibres in peripheral arteries. The study of arterial stiffness has increased along the last years, as the rate of stiffening is not uniform among human populations, it started to be classified as a marker of accelerated ageing. The following section will explain the arterial physiology, its relationship with ageing, the molecular mechanisms behind it, and the bidirectional relationship between hypertension and arterial stiffness.

2.5.1 Arterial stiffness: a definition

The term ‘stiffness’ refers to a physical property of solid materials, either elastic or non-elastic, defined as the force needed to deform their structure. More specifically, in biomechanics stiffness can be defined as the ratio between a physical pressure or ‘load’ and the change in the shape of the structure as follows:

$$Stiffness = \frac{Load}{Deformation}$$

Applying this generic definition to the arterial structure, one definition of arterial stiffness could be how much resistance to deformation is exerted by the wall of an artery in opposing a physical force or load applied by an external source, such as the force applied by blood flow along different stages of the cardiac cycle.

Although useful for teaching purposes, this concept needs to be refined. When defining arterial stiffness in a biomechanical way, other concepts need to be considered to measure it appropriately. First, the structure of arteries is not uniform,

as there are predominantly elastic blood vessels such as the aorta, carotid, or femoral arteries while there are other arteries that are predominantly muscular. Secondly, the application of the load is different if it is applied within different structures, especially the anatomic ones, presenting a variety of anatomic details, such as bifurcations, valves, or tortuosity, among others.[96] Given this, when describing arterial stiffness, particular attention must be given to the specific load, the segments measured and the type of deformation that is being measured. [97] The load on the arterial wall can be applied in multiple ways, not only force, but also tensile stress or momentum. The deformation can be of different types as well, such as displacement, or strain.

The study of stiffness in arteries, which are elongated and cylinder-like structures, was better addressed using a formula that allowed to account for load and deformation in a more accurate way. The scientist Thomas Young addressed this problem defining the modulus of elasticity *E* (or Young's modulus) using the ratio between tensile stress σ and strain ε as measures of load and deformation, respectively:

$$E = \frac{\sigma}{\varepsilon}$$

Despite this improvement in explaining stiffness in arteries, there are more methodological challenges. First, the linearity of the ratio between strain and stress has been a subject of discussion. Hooke's law states that this ratio is linear. This theoretical relationship is not always fulfilled in the arteries. This is explained by the heterogeneous composition of the arterial wall, with layers composed of different proteins with a variety of elasticity levels. Apart from this, the degradation secondary to the ageing process adds more variation to this originally linear response.

All these challenges to accurately measuring arterial stiffness have evolved into the development of a variety of arterial stiffness indexes. A detailed explanation of the most used arterial stiffness indexes can be found in the section 2.2.6.

2.5.2 Bramwell-Hill distensibility equation, relationship with arterial properties and blood pressure

The physiologist Thomas Young calculated the speed of blood flow in the arteries after each heartbeat, by using Newtonian mechanics of fluid equations where the speed of the fluid would be the squared ratio of the bulk modulus, or how compressible a fluid is in response to pressure, and the density (ρ) of the fluid as follows:

$$\sqrt{\frac{Bulk\ modulus}{\rho}}$$

As blood is a non-compressible fluid, physiologists John Bramwell and Archibald Hill define the velocity of the pulse wave produced by a heartbeat (Pulse Wave Velocity) as the squared ratio of the percentage increase of volume (V) per increase of pressure (p) in mm Hg multiplied by a constant.[98]

$$PWV = \frac{0.357}{\sqrt{\frac{V}{\frac{dV}{dp}}}}$$

The equation can also be expressed in other ways such as:

$$PWV = \sqrt{\frac{V}{\rho} \cdot \frac{dV}{dp}}$$

The main takeaway message from the Bramwell-Hill equation [99] is that the pulse wave velocity should be understood as the result of the change in volume according to a given change in pressure. If arteries are to accommodate a higher deformation

for the same increase in blood pressure, the arteries are less stiff hence the PWV would be lower. In other words, between the same arteries in two different individuals (i.e. two aortic segments of two different individuals), the more efficient artery should be the one with lower PWV, keeping all the other values constant.
All these changes explained in the Bramwell-Hill and derived equations, emphasise the relevance of the mechanical properties of the arteries in PWV.

2.5.3 Ageing and the onset of arterial stiffness

The primary function of blood vessels is to transport nutrients to all the tissues of the body and to collect waste products.[100] As the rate of blood flow varies in response to the change in the metabolic requirements from different tissues; arteries adapt to it by changing their diameter. In addition, blood pressure increases during the systolic phase and decreases during the diastolic phase of the cardiac cycle. The arterial property of distensibility allows the additional blood flow within the systolic phase and ensures that it is constant along the smaller arterial vessels that are almost free of pulsations. [101] The difference between the systolic and diastolic blood pressure is called the “pulse pressure” and is determined mainly by the effect of stroke volume and the total distensibility of the arterial tree.[100]
Systolic blood pressure increases with chronological age while diastolic blood pressure is steady, [102,103] in an age-related increment in aortic stiffness, that is mostly seen in the proximal arteries with a wider diameter, contrasting with the small changes in the stiffness of peripheral arteries. [104] The changes are observed in the arterial structure on a microscopic level, where elastic fibres of the aorta are increasingly replaced by collagen. [105]
The structural changes of the cardiovascular system progressively produce physiological changes beyond the homeostatic capabilities of the circulatory system.

The triggered pathologic mechanisms intensify and increase the rate of vascular damage, ending in a vicious cycle where increased chronological age and pathologic processes intensify each other reciprocally. [106,107]

The presence of some life course risk factors can predict arterial stiffness. The trajectories of health behaviours such as salt and sugar intake, [108] smoking, [109] alcohol drinking, [110] sedentary behaviour, 101 markers of glycaemic control such as HbA1c [112] and inflammatory markers like C-reactive protein [113] have been associated with higher arterial stiffness. Hereditary characteristics related to race or ethnicity [114] or parental history of hypertension have also been associated with stiffer arteries. [115] Positive associations between PWV and early-life characteristics such as low birth weight have been shown using life course study approaches.[116]

Biochemical and mechanical transformations occurring in the vascular structure could relate to chronological age, pathological ageing, or a combination between these two factors. Chronological age is associated with transformation in vascular structure. Involving segmentation and replacement of elastic fibres with collagen fibres. Physiological function is the first explanation for this. The longer a human being lives, the higher number of heartbeats, and necessarily higher number of contraction and expansion cycles, providing physical strain to the elastic fibres that confer compliance to the arterial structure.[117–119] Pathological processes can alter arterial function or arterial structure at an earlier age than the only presence of physiological cardiovascular ageing would do. Alterations such as arterial calcification, or vascular inflammation could accelerate the rate of arterial stiffening, increasing the expected levels of PWV for a given chronological age. [120] Finally, a synergistic action between physiological vascular ageing and pathological arterial stiffness has been also described. [120] After a threshold of damage in arterial

structures as a consequence of the normal process of vascular ageing, there is further activation of degenerative processes promoting arterial stiffness and impairment of its delaying mechanisms, combining pathological and physiological vascular ageing. An example of could be feedback mechanism of the dysregulation of the renin-angiotensin system (RAS), by the presence of reactive oxygen species (ROS), unstable molecules which can oxidise and damage cellular components. [119] Dysregulation of RAS has been described as an ageing-related process, as its activation increases progressively with chronological age, along with a decrease in its antagonist systems.[121] In the elderly, the increased activation of this system promotes increased vasoconstriction, excessive proliferation of vascular smooth muscle cells, building an excessive extracellular matrix with increased collagen, all compatible processes with arterial ageing.[122] An increased action of the RAS system produces a decoupling of the nitric oxide production system linked with the Angiotensin II. Angiotensin II has capabilities of producing ROS, that are usually counter regulated physiologically, but with ageing these mechanisms are impaired ant the production of ROS by Angiotensin II increases.[123] These ROS have the immediate effect of producing alteration in the mitochondria, that produce even more ROS. Once ROS have affected the RAS system, the combined physiological effects of high blood pressure, and arterial stiffness could feedback each other on a snowball effect.[124]

2.5.4 Lifestyle factors and delayed ageing strategies.

Lifestyle factors including carbohydrate and fat intake, alcohol intake, smoking, and physical activity, are contributors to the acceleration of ageing and cardiovascular ageing. Reduction of calorie intake in a diet containing fibre, proteins with reduced carbohydrates and fats has been linked to a reduction in frequency of population health outcomes. The clearest data of population effects of calorie restriction without undernourishment have been during war

times in Scandinavian countries, where controlled diets including all the macro nutrients were associated to fewer hospitalisations and deaths secondary to cardiovascular events.[125] The diet modulation has been associated with inhibition of well-documented markers of inflammation, ageing acceleration and vascular ageing, such as NFK-B factor, Insulin Growth Factor, or glycation-end products as well as promoting increased presence of oxidoreductases protecting mitochondrial function.[126]

A body mass index in the overweight and obesity categories are linked to faster ageing and maintaining a BMI below these thresholds is linked with food intake and physical activity. Being overweight is associated with an endocrine environment with has accelerated telomere shortening, epigenetic mechanisms like DNA methylation, production of interleukins and suppression of multiple genetic repair mechanisms.[127]

Regarding physical activity, examples of ROS-productive biomarkers are C-reactive protein and interleukin-6. These are involved in decoupling cellular processes leading to chronic diseases like kidney disease and diabetes.[128] Trajectories of individuals in the Whitehall II study have shown that sustained moderate and high levels of physical activity over the life course, are associated with lower levels of these biomarkers.[129] Other prospective cohorts have shown that individuals with these levels of physical activity tend to live a higher number of years free of disease and have higher functioning in elder age.[130]

Alcohol and smoking are promoters of cardiovascular ageing. Although on a population level, low and moderate alcohol consumption are associated with improved cardiovascular outcomes, and in some cases healthy ageing, [131,132] on a physiological level, alcohol promotes ROS production and DNA degradation, also being associated with decreased density in brain structures associated with emotions and cognitive function.[133]

Epidemiological evidence from the Whitehall II study has shown that alcohol intake also promotes vascular ageing.[110]

Cigarette smoke contains at least 3.000 molecules with documented abilities for the promotion of reactive oxygen species, plus the tar itself, which contains free radicals.[124] This is part of the explanation of the links of smoking to ageing and age-related diseases such as

osteoporosis, dementia and diabetes.[134] Telomere shortening is also a consequence of smoking.[135] These molecules are also linked both to vascular ageing through arteriosclerosis and atherosclerosis. Smoking promotes the first by increasing the vascular tone in the peripheral circulation, and the latter by increasing inflammatory mechanisms and platelet aggregation in the vascular matrix, leading to atherosclerotic plaque formation.[136]

2.5.5 Atherosclerotic mechanisms of arterial stiffness

Atherosclerosis is the name given to the inflammatory process of deposition of macrophages and other circulating cells in the arterial wall, progressively modifying the extracellular matrix and collecting circulating Low-Density Lipoproteins (LDL). This progressive collection further increases inflammation and alters the endothelium and other layers of the arteries, altering also its physical properties, a factor that could explain a relationship between atherosclerosis and arterial stiffness.[137] Despite this, the links in molecular studies are not entirely reflected in pathological and epidemiological studies. Reports from 304 autopsies in elderly Japanese individuals showed a low correlation between PWV and the formation of atherosclerotic plaques.129 Despite well described pathophysiological mechanisms, the epidemiological evidence is contradictory. The lack of association in some studies could be attributed to small sample sizes,[139] although sample size is not the only methodological issue. The Rotterdam Study, a large population-based cohort, showed associations between carotid plaques and cf-PWV, independent of most commonly reported risk factors, but not of smoking.[140] A systematic review of the association between risk cf-PWV and common cardiovascular risk factors, many of them also relevant to atherosclerosis, were not independently associated to cf-PWV after adjustment for hemodynamic variables, such blood pressure and heart rate. This dependent association between risk factors for atherosclerosis and arterial

stiffness could indicate that atherosclerotic processes occur after stiffening ones [141,142]

2.5.6 Molecular mechanisms on arterial ageing

Several molecular mechanisms play a role in the onset and progression of arterial stiffness. Historically, vascular stiffening was attributed exclusively to a dysregulation of the extracellular matrix. [143] New evidence suggests that additional molecular and cellular determinants, such as new regulator proteins of vascular smooth muscle cell tone and changes in the interactions between cells and extracellular matrix, may explain some structural changes on all the layers of the arterial structure, i.e. the endothelium, intima or adventitia. [144] Table 1 shows some of these mechanisms, and Figure 1 (adapted from Zieman[144]) depicts the specific layer on which they act. Understanding the multifactorial nature of arterial stiffness can also guide clinical and population research.

Table 1. Molecular mechanisms involved in arterial stiffness

Layer/Mechanism	Reference
Endothelium	
Endothelial dysfunction	145
Increased endothelial permeability	146
Intima	
Proliferation of cellular adhesion molecules (I-CAM)	147
Proinflammatory cytokines (TGF-ß)	148
Increase in leukocytes	149
Media	
Fracture and decrease in elastin	149
Deregulation of Renin Angiotensin Aldosterone system	150
Reduction of regulatory T-lymphocytes	151
Adventitia	
Extracellular matrix cellular proliferation (Fibroblasts)	152
Intima/media	
Calcification of the arterial wall	153
Accumulation of advanced glycation products (AGE's)	154
Increase in Vascular Smooth Muscle Cell adhesion	155
Media/Adventitia	

Excessive collagen production	[149]
Extrinsic influences	
Altered secretion of angiotensin II	[156]
Sodium	[157]
Lipids	[158]
Reduced binding of nitric oxide	[159,160]

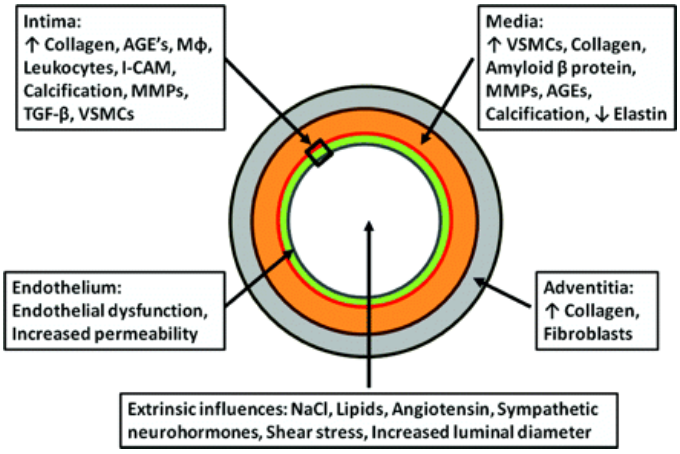


Figure 1. Arterial ageing mechanisms by arterial layers

2.5.7 Measurement of arterial stiffness through Pulse Wave Velocity

Throughout the cardiac cycle, the heart expels blood in the systole or contraction phase. The expelled blood travels from the left ventricle to the peripheral circulation, generating a wave called the 'sphygmic wave' that propagates along the arterial tree. [161] The speed at which the wave propagates along the arterial tree is known as pulse wave velocity (PWV). As seen, PWV is quantified as the distance travelled by the wave (Δx) divided by the time required by the wave to travel that distance (Δt).

$$\mathrm{PWV} = \frac{\triangle x}{\triangle \mathrm{t}}$$

Studies assessing the stiffness of the arterial segment between the carotid, aorta and iliac arteries have a larger amount of evidence in cardiovascular risk prediction compared to other methods for assessing arterial stiffness, hence the gold-standard method for the assessment of PWV is carotid-femoral PWV (cf-PWV).[162] Cf-PWV is

defined as the time that the pulse wave takes to travel from the carotid to the femoral artery, and it is measured in metres per second. [163,164] When it is performed by applanation tonometry, pressure transducers (tonometers) are placed on the skin surrounding carotid and femoral arteries in order to detect the pulse wave. Blood pressure and an electrocardiogram are taken simultaneously because they are correlated with PWV. Pulse wave detection using cuffs is an alternative method to applanation tonometry. Figure 2 (adapted from Marieb and Hoehn)[165] shows the anatomic points where the tonometers are placed in cf-PWV assessment, as well as the formula for its calculation.

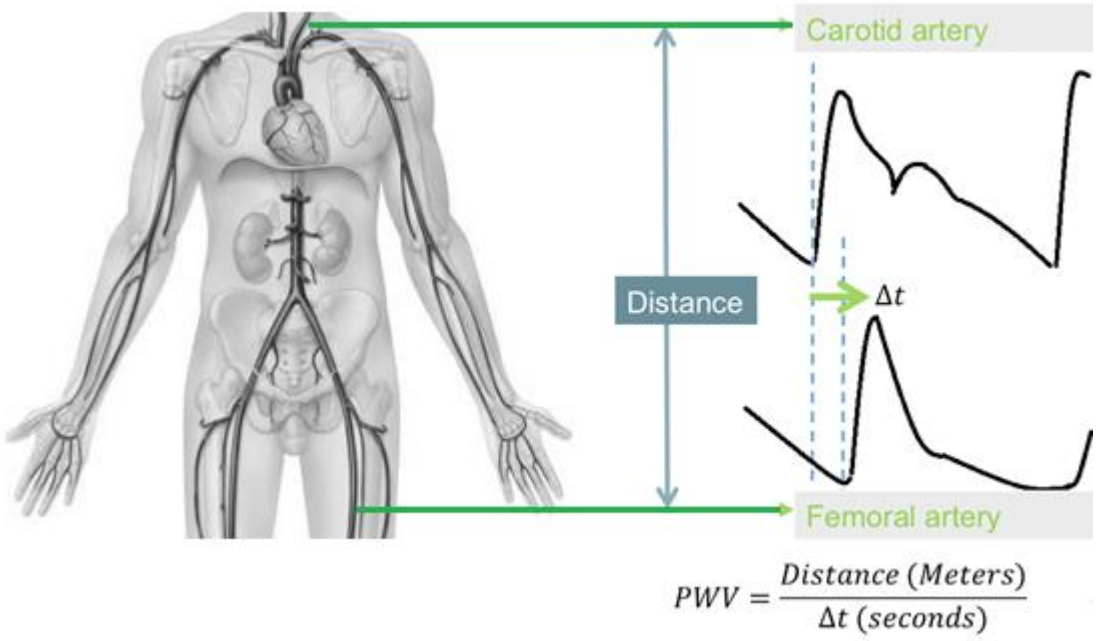


Figure 2. PWV measurement in a carotid-femoral PWV measuring device

2.5.8 Assessment of arterial stiffness Types of techniques for non-invasive measurement of arterial stiffness

Different non-invasive methods for the assessment of arterial stiffness have been developed over time, including clinical methods, imaging-based methods and techniques based on pulse wave analysis. The main techniques for measurement of

arterial stiffness are carotid-femoral cf-(PWV) and brachial-ankle pulse wave velocity (ba-PWV).

Pulse pressure (PP) is defined as the difference between systolic and diastolic blood pressure. Arterial stiffness results in an increase in systolic blood pressure (SBP) and no further increase or decrease in diastolic blood pressure (DBP). As such, an increase in PP can be used as a surrogate measure for arterial stiffness. Measuring brachial PP is a relatively simple, low-cost procedure. However, PP measured in peripheral arteries is different from PP in central arteries. Given that, using brachial PP as a surrogate of pulse pressure in the aorta or the carotid artery is less accurate. [166–168]

Imaging-based techniques are another group of methods for assessing arterial stiffness. The general principle of ultrasound or MRI techniques is to observe the diameter of a particular artery along the cardiac cycle and simultaneously observe the pressure. By performing these techniques, researchers can observe a change in diameter given certain change in pressure. Methods such as elastic modulus, arterial compliance or the arterial distensibility coefficient are methods based in imaging techniques. Most ultrasound methods can assess the characteristics of superficial arteries, and some methods using MRI can estimate different properties and characteristics of the aorta. Imaging-based techniques have the advantage of being completely non-invasive.[169] Also, these are the only kinds of methods that allow measurement of the elasticity of the arterial wall. A disadvantage of ultrasound techniques is that the quality of the measurement can depend on the ability of the operator. Moreover, the time required to perform the procedure is longer than the required time for other techniques.[170]

The remaining group of techniques is characterised by analysing pressure waveforms generated from the contraction of the left ventricle and the reflection of the waves in the arterial system. The pressure augmentation is defined as the difference between the peak generated by the left ventricle and the peak generated by its reflection. The ratio of these two quantities is known as the peripheral Augmentation Index (AI). [171]

Another index, known as the capacitive compliance model, analyses the shape of the waveform in order to estimate the systemic compliance of the arterial system. The potential advantage of the capacitive compliance model is that it can estimate the stiffness of the arterial system as a whole. The calculation of the arterial capacitance model uses the ratio of the area under the systolic blood pressure curve and the product of total peripheral resistance and the difference of blood pressure at the end of the systole and diastole. [172] One of its disadvantages is that is based on a model of electrical capacitance, getting to the final formula of stiffness as a ratio of the stroke volume and the pulse pressure, assumption that might be inaccurate for its clinical use.

2.5.8.1 Carotid femoral pulse wave velocity as gold standard for measuring arterial stiffness

The gold standard for measurement of arterial stiffness is carotid-femoral pulse wave velocity, which is also based on the analysis of the pulse waveform. Such denomination of cf-PWV is due to its independent prediction of cardiovascular events and mortality derived both from cardiovascular and non-cardiovascular causes.[173] Also, it has a superior predictive power when it was compared to alternative measurements of arterial stiffness in the Framingham study, which also measured predictive power of pulse pressure, augmentation index and other surrogate non-

invasive measurements[174] An additional advantage for its designation as the gold standard is that this predictive ability was replicated in other subgroups of patients such as end-stage renal disease.[175] Also, physiologically, other measurements of stiffness as pulse pressure are also dependant of aortic geometry, making cf-PWV a better option.[176]

Table 2 summarizes the main methods for the assessment of arterial stiffness and their definition. More detail about PWV will be given in the following section.

Table 2. Methods for the assessment of arterial stiffness

Method	Definition	Assessment method
Pulse pressure	Pressure differential between systolic and diastolic blood pressure	Blood pressure
Elastic modulus	Pressure change required for stretching 100% from resting diameter	Ultrasound or MRI^{+}
Arterial compliance	Absolute diameter (or area) change for a given blood pressure	Ultrasound or MRI^{+}
Arterial distensibility	Relative diameter (or area) change for a given blood pressure	Ultrasound or MRI^{+}
Stiffness index	Ratio of ln(systolic/diastolic pressure) to relative change in diameter*	Ultrasound
PWV	Velocity of travel of the pulse wave along an arterial segment	Blood pressure waveform
Augmentation index	Difference between systolic peak and percentage	Blood pressure waveform
Capacitive/oscillatory compliance	Obtained by the exponential component of diastolic pressure decay	Blood pressure waveform

***ln: Natural logarithm, $^{+}$MRI: Magnetic Resonance Imaging,**

The availability of a variety of techniques which measure different arterial segments has potential implications regarding the comparability of results, given that the compliance of one artery of the human anatomy is not directly comparable to the characteristics of the arterial tree as a whole. [163] For example, the conduit, elastic arteries are more compliant than the distal, muscular ones. Aortic PWV approximates to 5 metres per second (m/s) compared to femoral artery PWV, that is around 8 m/s. [177]

As there are multiple techniques, anatomical sites and external characteristics affecting the measurement, an expert consensus which issued some recommendations to improve the standardization of the measurements and reduce heterogeneity and improve comparability of the studies. [178,179]

The two most widely used techniques are brachial-ankle PWV (ba-PWV), more commonly used in Japan as well as other Asian countries;[180] and carotid-femoral PWV (cf-PWV), which is currently the gold-standard technique. [181]

Brachial-ankle PWV (ba-PWV), an alternative method to cf-PWV, was developed as a less invasive alternative for daily use in clinical settings.[182,183] Accordingly, ba-PWV is measured through a device that using cuffs. These cuffs are placed on the arm and ankle, detecting the pulse wave in the radial and tibial artery, respectively. [162]

2.5.9 Blood pressure measurement during pulse wave velocity assessment.

As PWV varies with blood pressure and heart rate, it is recommended that their measurement is done simultaneously. During PWV measurement, blood pressure is measured using an oscillometric sphygmomanometer. A cuff inflates and deflates to detect the arterial pressure, while calculates the systolic and diastolic blood pressure according to an algorithm set up by the manufacturer. [184]

2.5.10 Arterial stiffness as risk predictor and associations with cardiovascular health outcomes and biomarkers

Arterial stiffness is a marker of vascular ageing and a risk predictor of cardiovascular and non-cardiovascular outcomes.[185] Carotid-femoral pulse wave velocity (cf-PWV) is a surrogate measure of arterial stiffness, which has predictive value for cardiovascular events.[186] One standard deviation (SD) increase in cf-PWV has been associated with increased risk in 48% for a major cardiovascular event (HR, 1.48; 95% CI, 1.16, 1.91).(Mitchell et al., 2010b) It also predicts left ventricular hypertrophy,

stroke, heart failure and renal failure.[188] It has been estimated that the hazard ratios for coronary heart disease and cardiovascular disease per standard deviation increase in PWV are 1.35 (95% CI 1.22,1.50), and 1.45 (95% CI 1.30,1.61), respectively.[189] The association between arterial stiffness and all-cause and cardiovascular (CVD) mortality has been reported both in individuals with classic cardiovascular risk factors, such as hypertension, obesity, diabetes, smoking, hypercholesterolemia, and coronary heart disease.[190]

Increased PWV measurements independently predict conditions such as congestive heart failure, [191] end-stage renal disease[192] and myocardial infarction.[193] Other PWV correlates include chronological age,[13] systolic blood pressure,[194] mean arterial pressure,[195] and adiposity.[196–198]

Adding PWV to prediction models built with classic cardiovascular risk factors improved the prediction of cardiovascular events in the general population attending primary care settings. [199] The improved prediction of cardiovascular risk by the use of PWV has also been observed in different sub populations. It has shown improvements in receiver-operating characteristics curves (0.70 to 0.72; p=0.001) after the addition of cf-PWV) in some population cohorts.[200] The same has been reported in other subpopulations such as of the increased cardiovascular risk in older individuals,[201] diabetic individuals,[199] hospitalised patients with stable angina [202] as well as those with hypertension, dyslipidaemia, and chronic kidney disease. [203]

Arterial stiffness has also been associated with markers of target organ damage such as albuminuria, [204] faster decline in kidney function, [205] aortic aneurysms, [202] insulin resistance,[202] fatty liver disease, [206] emphysema severity, [207] heart failure and lower grey matter density. [208]

2.6 Burden of hypertension and its link to arterial stiffness

2.6.1 The global burden of hypertension and contributing factors

Hypertension is one of the main risk factors for cardiovascular disease (CVD), besides cardiac failure or coronary heart disease. It is also a risk factor for brain and kidney damage, [209,210] explaining 45% of deaths due to heart disease, 51% of deaths due to stroke, and 41% of the global disability adjusted life years (DALYs) which increased from 95 million in 1990 to 140 million in 2015. [211] In addition, it is predicted that the worldwide prevalence of hypertension will increase from 25% in 2000 to 29% in 2025.[212] The absolute number of hypertensive individuals in the world increased from 594 million in 1975 to 1.13 billion in 2015 and is expected to reach 1.56 billion by 2025.[212]

Some of the following phenomena could explain the increasing global burden of hypertension: increasing blood pressure with age, global population ageing, lack of hypertension awareness and treatment, as well as unsuccessful control of blood pressure.[213]

Although there are no objective comparisons of which factor contributes more to the burden of hypertension, population ageing seems to be the most relevant phenomenon from an epidemiological point of view, as the prevalence of hypertension necessarily increases with age and the absolute number of elderly individuals is increasing. Even in some of the healthiest elderly populations that are past the peak of the epidemic of cardiovascular disease of the 20th century, the prevalence of hypertension in middle-aged and elderly men ranges between 40% in the UK and 59% in Finland.[214] When the world population is stratified by country income, middle-income countries have a high but steady burden of hypertension and low-income countries are the biggest contributors to the burden of hypertension, with

this difference being partly explained by the declining awareness and control of hypertension according to country income.[215]

Blood pressure is constant until about 20 years of age when it starts to increase with age. Systolic blood pressure keeps rising until age 80, whereas diastolic blood pressure shows an increasing pattern until age 50 when it starts to decrease.[216] Consequently, the estimated worldwide mean systolic blood pressure by 2015 was 119.6 mm Hg (95% CI 118.3, 120.7) in males aged from 25 to 29 years and 140.8 mm Hg (95% CI 139.7, 142.1) in those over 80 years of age. An even larger difference was seen in females, being 114.3 mm Hg (95% CI 113.1, 115.4) in females aged from 25 to 29, and 142.3 mm Hg in those over 80 years of age.[217]

An important contributor to the higher occurrence of hypertension worldwide is population ageing. The global life expectancy at birth has increased, going from 65.3 years in 1990 to 71.5 in 2013. [218,219] Life expectancy at age 60 has also increased, especially in high-income countries like the UK, where the gain in life expectancy over the same period was 6.2 years.[220] Given the growing proportion of older adults in the general population and the larger incidence and prevalence of hypertension within them, an increase in the global occurrence of hypertension is a likely consequence.[211] Higher peripheral vascular resistance, impaired neurohormonal mechanisms and declined kidney function are frequent physiological impairments that explain increased blood pressure in elderly individuals.[221]

Low rates of diagnosis and treatment for hypertension contributes to the burden of morbidity and mortality associated with hypertension. However, a low rate of diagnosis and treatment for hypertension contributes to the burden of morbidity and mortality associated with hypertension. A large proportion of individuals with hypertension are not aware of their disease, which goes along the proportion of

worldwide awareness, estimated at only 46.5%.[222] Low awareness is partly because hypertensive individuals are rarely symptomatic, or only experience non-specific symptoms such as a mild headache or fatigue.[223] Consequently, many hypertensive individuals suffer steady, chronic damage and only become aware of their condition after a heart attack or a catastrophic consequence of hypertension.[224] However, in high-income countries like England, Canada and the US, overall hypertension awareness is higher, at 65.3%, 83.4% and 81%, respectively.[225] The presence of mass screening programs and improved blood pressure control schemes in general practitioner practices like the Quality and Outcomes Framework in the UK, may have played a role in the improved awareness in adults, which was below 50% in 1994.[226] Beyond the age-dependent increase, older global population and hypertension awareness, uncontrolled and resistant hypertension are additional contributing factors to the burden of hypertension.[227] The proportion of hypertensive individuals who receive treatment varies among country income categories. In high-income countries such as the UK, the US or Canada, the proportion of treated hypertensives in the total population reaches 51.3%, 79.9% and 74.0% respectively. In the same countries, the proportion of treated individuals who did not reach blood pressure control was 23.9%, 14.0% and 21.2%.[225] The proportion of treated patients is different across country income categories. In middle-high income countries like China, 46.8% of hypertensive individuals treated and 20% of the treated individuals achieved blood pressure control.[228] Also, a proportion of uncontrolled hypertensive individuals have the condition of resistant hypertension, which is defined as not reaching blood pressure control despite having the adequate doses of three different antihypertensive drugs, including a diuretic.[229] In the UK, the prevalence of resistant

hypertension increased from 1.75% in 1995 to 7.76% in 2008 and declined to 6.46% in 2015.[230]

As described above, population ageing phenomena, physiological changes over time and different aspects of hypertension diagnosis, as well as adherence and adequacy of treatment, are contributing factors for the onset and continuation of hypertension over the life course.

2.6.2 Impact of blood pressure control on arterial stiffness, evidence from short-term, clinical trials: a literature review.

One of the most relevant findings from the clinical trials assessing the effect of antihypertensive drugs on arterial stiffness is the suggestion that arterial stiffness is attenuated by antihypertensive treatment, independently of the magnitude of the reduction in blood pressure.[231] This reduction beyond hemodynamic factors is hypothesised to be the result of counteracting other stiffening mechanisms such as inflammatory mediators, intermediate metabolites of glucose promoting glycated-end products, and ROS liberation.[232] In addition, clinical trials have not reported statistically significant differences in the progression of arterial stiffness between types of antihypertensive therapy. [233] Some of the meta-analyses summarizing short-term evidence will be described below.

A meta-analysis of 15 small scale, short-term (4 weeks to 6 months) randomised controlled clinical trials, carried out between 1987 and 1994, compared the effect of angiotensin converter enzyme inhibitors (ACEI), placebo, calcium channel blockers (CCB), beta-blockers (BB) and diuretics on reducing carotid-femoral pulse wave velocity. The study included 294 patients with untreated essential hypertension. Participants taking ACEI and diuretics showed reductions of 1.8 m/s and 1.4 m/s

respectively, compared to participants receiving placebo, who showed a reduction of 0.5 m/s. [231]

Another meta-analysis included information from 53 clinical trials comparing changes in PWV between 1650 hypertensive patients receiving Angiotensin Receptor Blockers (ARB) and 1659 subjects in control groups during a mean follow-up of 19 months. [234] The weighted mean difference in cf-PWV was -0.4 m/s (95% CI -81.8, -3.2) for patients using ARB compared to those on placebo. There were no significant differences between patients allocated to ARB compared to those receiving other antihypertensive drugs.

Another meta-analysis of randomised controlled trials pooled data from 17 trials comparing the change in cf-PWV between patients receiving BB alone compared with patients receiving placebo, ACEIs or ARBs.[235] The duration of the trials varied between 4 weeks and 12 months. Authors did not find significant differences when compared BB with other antihypertensive drugs but found a reduction in cf-PWV of -1.1 m/s (95% CI: -1.6, -0.7 p <0.001), when compared BB with placebo.

2.6.3 Longitudinal studies assessing the relationship between hypertension and PWV: a literature review

A narrative literature review was performed to answer the following questions regarding the association between arterial stiffness and blood pressure:

1) What is the existing evidence for a bidirectional relationship between blood pressure, hypertension and arterial stiffness in longitudinal studies?

   Partitioning the bidirectional relationship into its components gives two separate questions to be assessed. Namely:

   a) What is the existing evidence on the association of baseline arterial stiffness leading to hypertension?

b) What is the existing evidence on the association of baseline hypertension leading to arterial stiffness?

2) What is the effect of blood pressure control on the progression of arterial stiffness measured by PWV in longitudinal studies?
3) What is the relationship in clinical studies?

After assessing the evidence for the above questions, it is important to examine the gaps in the evidence in the association between hypertension and arterial stiffness.

2.6.3.1 Search strategy

A search was performed in Medline, with the terms specified in Table 3, where titles and abstracts were screened looking for longitudinal studies that investigated the association between arterial stiffness and blood pressure control. After title screening, 462 abstracts and 36 full-text articles were selected. In total, 16 articles were finally included.

For question 1b, four longitudinal studies selected and included in a meta-analysis, where the pooled effect of baseline arterial stiffness on incident hypertension was assessed with a random-effect model meta-analysis.

Table 3. Search strategy for articles on the association between PWV and Hypertension (14/03/18)

	Terms	Items found
1	Hypertension [Mesh]	237696
2	High blood pressure	560022
3	Blood Pressure [Mesh]	290213
4	Systolic blood pressure	550367
5	Diastolic blood pressure	550367
6	((("Hypertension"[Mesh]) OR high blood pressure) OR Blood Pressure [Mesh]) OR systolic blood pressure) OR diastolic blood pressure	**854096**
7	(("Pulse Wave Analysis"[Mesh]) OR pulse wave velocity)) OR (("Vascular Stiffness"[Mesh]) OR arterial stiffness) OR aortic stiffness)	**19422**
10	Cohort Studies [Mesh]	1718779
13	Longitudinal studies	175058
14	((Cohort Studies [Mesh]) OR longitudinal studies)	1756062
15	**#6 AND #7 AND #14**	**1933**
	Filters: Humans	**1897**
	Approved by title screening	**462**
	Selected by abstract	**36**
	Full text included	**16**

2.6.3.2 Overview of included studies

After the literature search was performed, longitudinal studies with the aim of studying the longitudinal relationship between arterial stiffness and hypertension were selected. Studies assessing change in arterial stiffness according to baseline blood pressure or risk of incident hypertension according to baseline arterial stiffness were included. Table 4 describes the main findings of the reviewed studies.

The participants were mainly from North America, Europe and Japan. Sample sizes ranged from to 316 to 6692. Most studies reported data from both genders and the age groups included at the baseline ranged from 38 to 60 at baseline. The date of publication ranged from 1999 to 2018. Although the source of evidence was mainly focused on studies that measured arterial stiffness using cf-PWV (10 studies) the gold standard, studies which measured arterial stiffness with ultra-sonographic techniques (3 studies) or with ba-PWV (3 studies) were also included as they used odds ratios (OR) or regression coefficients for reporting the force of the evidence.

Although some studies found bidirectional relationships, some others only reported one way of the association. Additional descriptions are included in the subsections for each question.

2.6.3.3 PWV assessment methods and statistical treatment in the selected studies

10 out of the 16 measured arterial stiffness trough cf-PWV, 3 used ba-PWV and 3 used imaging-based procedures. The statistical techniques used varied between the studies. Four studies (i) tested differences between means, [236–238] (ii) used multiple linear regression, (iii) used linear mixed models, [239–241] (iv) used logistic regression models [242–247] and (v) mixed models plus cox-proportional hazards models.[248] Ten out of the 16 studies measured arterial stiffness trough cf-PWV, 3 used ba-PWV and 3 used imaging-based procedures.

2.6.3.4 What is the existent evidence on the association of baseline hypertension leading to arterial stiffness?

Eleven longitudinal studies which investigated the association between baseline hypertension and arterial stiffness at follow-up were identified. [236–241,245,248–251] Six of the studies were population-based cohort studies located in the United States. [241,248–250,252] and Italy.[239] The remaining studies recruited ambulatory patients from clinics in France,[236] Italy,[238] Japan[237,246] and Brazil.[240] Mean age at baseline in the included studies varies from 43 to 68 years. Sample sizes varied from 240 to 4358 and participants were from Europe, United States and Brazil. With the exception of the Framingham study, all the 11 studies found a higher rate of increase in arterial stiffness in individuals with sustained hypertension in more follow-up visits, compared with individuals who remained normotensive in more visits individuals. Five studies reported changes in stiffening rate using PWV units (m/s). Variations ranged from 0.03 m/s to 1.5 m/s and can be seen in Table 5. 6 studies reported the differences in stiffening rate as ß coefficients. [236–240,248–251,253] The report from the

SardiNIA study, a cohort studying ageing in elderly individuals from the Mediterranean, showed that the progression of arterial stiffness in hypertensives depended on the age of onset of hypertension. [239] The Baltimore Longitudinal Study of Ageing (BLSA), found a sex difference in arterial stiffening. The rate of stiffening changed according to blood pressure tertiles in men but not in women. [253] Most studies selected demographic characteristics and cardiovascular risk factors as adjusting covariates (Table 4).

Some gaps in the evidence assessing the relationship between blood pressure and arterial stiffness were identified. One of the studies that found a bi-directional relationship had the smallest sample size of the included studies (n=775), which allowed to test the main hypotheses of the study but with a lack of statistical power when testing the association in subgroups.[253] Most of the studies present results from predominantly of white European or Asian origin. Only the BLSA, the Multi Ethnic Study of Ageing (MESA) and a Brazilian study included mixed populations. [240,251,253] Given that individuals from black ethnicity tend to have more severe forms of hypertension and greater PWV values, more studies with including black populations are needed. Regarding the use of antihypertensive drugs, although most of the studies adjusted for medication use, none of them accounted for the number of medications taken by the participants included in the study, thus not accounting for resistant hypertension. A Brazilian study with a sample of resistant hypertensives assessed the relationship with arterial stiffness.[240] Studying the role of AS and resistant hypertension is important given that patients with uncontrolled hypertension could be either receiving suboptimal treatments, or being truly resistant hypertensives, on which additional inflammatory or anatomic mechanisms could be the drivers of higher blood pressure and arterial stiffness. The influence of sex on the

association between arterial stiffness and blood pressure has diverging results. BLSA reported a relationship existing only in men,[253] the Study of Women's Health Across the Nation (SWAN) heart study, a cohort of female individuals reported the same relationship. [249] The diverging evidence underscores the importance of both the overall sample size and the representativity of the included subgroups.

2.6.3.5 What is the existent evidence on the association of baseline arterial stiffness leading to hypertension?

Eight longitudinal studies evaluating association between arterial stiffness and blood pressure with samples of normotensive untreated individuals at baseline were identified. Two of the study populations consisted of male Japanese construction workers, [246,254] the remaining studies included male and female individuals from non-occupational cohorts in the United States. [243,248,252,255] and Finland. [242] The remaining study included outpatients from a hypertension clinic in Greece. [256] All the studies were identified as potentially eligible for inclusion in the meta-analysis, but three were not included. Two of them did not report a combined OR for their total population [248,254] and the third one assessed arterial stiffness with a different standard (carotid ultrasonography [256]). Table 4 shows that all the studies described above found increased odds of hypertension at follow-up, per each additional standard deviation of cf-PWV or ba-PWV at baseline. Figure 3 shows a pooled OR of 1.3 (95% CI 1.01, 1.44) for incident hypertension according to baseline arterial stiffness.

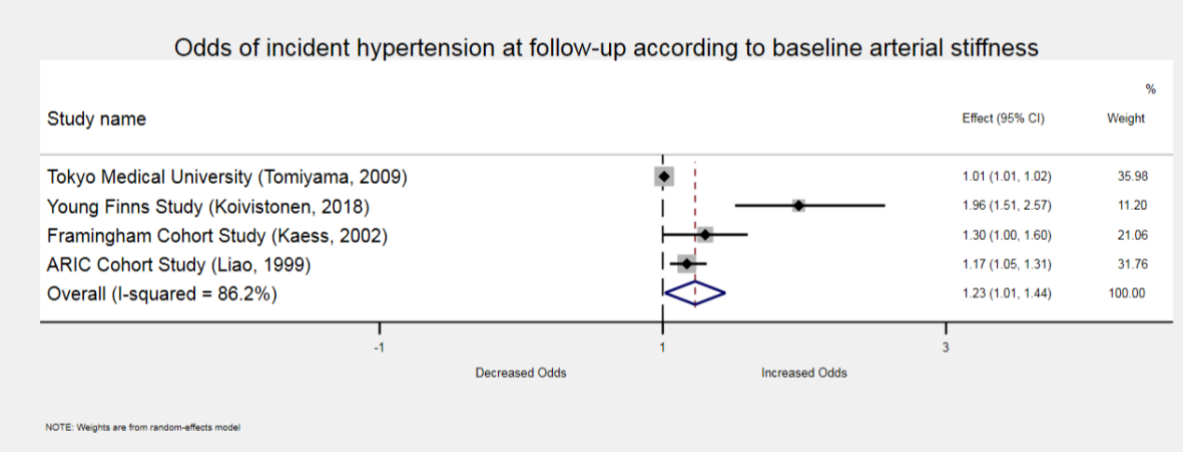


Figure 3. Pooled odds ratios for incident hypertension according to baseline arterial stiffness in longitudinal studies

After aggregating overall estimates from 4 longitudinal studies including 10677 participants investigating the association between arterial stiffness and incident hypertension, the pooled OR for developing hypertension at the next follow-up within populations of normotensive individuals was 1.23 (95% CI 1.01, 1.44). The results showed statistically significant heterogeneity (I^2 = 86.2% p<0.001) and a sensitivity analysis showed that after removing the ARIC Cohort study,[243] the pooled results became statistically non-significant (OR: 1.35; 95% CI 0.91, 1.79).

Some limitations of the current evidence of baseline arterial stiffness and incident hypertension were identified. Although the ARIC study includes one of the largest samples for studies of arterial stiffness, its sample was drawn from a clinical setting, and excluded 10% of normotensives at follow-up, potentially limiting the generalisability of the findings to clinical populations. Finally, as most of the studies estimated the effect for the development of hypertension from baseline arterial stiffness by using a 1 SD change as point of reference, the OR could not be comparable if the distribution of the independent variables is not similar. The risk of misclassification could be present in some studies given that the measurement of blood pressure was done at a singular occasion without any measures taken for

reducing white coat hypertension. Also, ascertainment bias in the measurement could not be ruled out in the assessment of arterial stiffness given that the measurements were taken with a conventional sphygmomanometer. Other studies that took blood pressure measurements in a hospital setting, could overestimate the blood pressure level thus overestimating the size of the effect of arterial stiffness on incident hypertension.

In addition, there are some limitations to the meta-analysed results. The search was conducted exclusively in Medline and there is a possibility of missing some studies published in other databases. heterogeneity of the included results, which could be explained by the sociodemographic differences in the populations and the sensitivity analysis showed that this finding could be subject to publication bias. Also, the pooling conducted was a pooling of overall estimates from the selected studies and not from individual participant estimates. An additional disadvantage was that studies with the two most popular methods used were mixed in order to have a larger sample.

Finally, the excluded studies, although with results in the same direction and a similar size effect, could have an impact on the overall estimate as well.

2.6.3.6 What is the effect of blood pressure control on the progression of arterial stiffness measured by PWV in longitudinal studies?

Six studies included in the literature review found different trajectories of increase in arterial stiffness measured by cf-PWV according to the baseline category of blood pressure. The SWAN study is a study designed for examining biological changes across the menopause in 554 women with a follow-up ranging from 1.1 to 4.3 years.[249] In a fully adjusted model, including a subsample of 316 participants, a mean

change of 0.68 m/s or 0.29 m/s/year at follow-up per each mm Hg of systolic blood pressure was found (ß=3.46; SE =1.3; p<0.001).

In the ERA-JUMP study, progression of cf-PWV was assessed in middle-aged men who were free of CVD at recruitment. After follow-up, the average increase in PWV was 0.3% (0.25 m/s/year), whereas per each mm Hg increased in SBP, there was an increase 0.03 of increase in cf-PWV per year. The increase in SD units in PWV was 0.15 per year.

The SardiNIA study did not find any impact of antihypertensive medication on PWV trajectories. Regression coefficients remained unchanged when compared regression coefficients before and after adjusting for antihypertensive medications (ß= 0.05 for men; 0.06 for women) The same effect was observed when compared models before and after including participants taking medications in the linear mixed models. [239] Four of the six studies found an association between the increase in cf-PWV and the status of blood pressure control. Benetos et al. found that the rate of annual arterial stiffening was 1.5 times higher in treated hypertensive than in normotensive individuals (0.014 m/s/; 0.008 m/s), although the models did not show statistically significant association between baseline SBP and cf-PWV. [236] Meani et al.,[238] reported that arterial stiffness was 2 times greater in controlled hypertensives than in normotensives (1.46 m/s vs 0.62; p<0.05). Tomiyama[237] reported an arterial stiffening rate in uncontrolled hypertensive individuals as being twice the rate of controlled ones. Tedla and colleagues reported a linear trend of arterial stiffening with the number of visits with controlled BP with a distensibility coefficient 4 times greater for the participants with normal blood pressure in all the follow-up visits (ADC 2.4 vs 2.4 p for trend=0.008). In the BLSA,[241] researchers did not find differences in

the arterial stiffness trajectory when they accounted for antihypertensive treatment in the mixed models (ß=0.03; p=0.8 for men; ß=0.02; p=0.9 for women).

Some limitations on the evidence reporting PWV progression and blood pressure control were identified. As other studies previously discussed, using a linear regression model could have limitations in the analysis of longitudinal trajectories, given that it fails to account for intra-individual differences. Also, it does not accommodate the unbalanced number of observations between baseline and follow-up. Given that the patients included in the study published by Benetos *et al.,*[236] were selected from a clinical setting, the generalisability to hypertensive individuals in the general population might be reduced. Also, the fact that the baseline SBP was not associated to change in cf-PWV in the regression models, despite finding that the rate of stiffening was higher in treated hypertensive patients compared to normotensive ones could be the result of reverse causality, as the treatment could equalise the blood pressure at baseline, supressing the association. Both Benetos *et al.,*[236] and Meani *et al*.,[238] studies used linear regression techniques for assessing the longitudinal change in arterial stiffness, assuming independence between individual observations.

2.6.4 Summary of the literature review on hypertension and arterial stiffness

The first question of the review aimed to gather the current evidence of a bidirectional relationship between hypertension and the progression of arterial stiffness. Most of the selected studies found a relationship between hypertension at baseline and the progression of arterial stiffness. Only one report, the Framingham study, could not find an influence of hypertension on the progression of arterial stiffness.

Regarding the influence of arterial stiffness on hypertension, five studies found that the odds for becoming hypertensive were between 1.01 and 1.96 times higher in the individuals with PWV at 1 standard deviation higher at baseline. Only one study in one of the cohorts with the longest follow-up on arterial stiffness, found a bidirectional relationship, finding that higher baseline cf-PWV was associated with larger increases in blood pressure, and simultaneously finding that higher SBP at baseline was associated with larger increases in arterial stiffness over time with dose-dependent associations.

The third question of the review was about the effect of blood pressure control on the progression of arterial stiffness in longitudinal studies. Half of the studies that assessed the progress in arterial stiffness according to blood pressure trajectories found a modification of the trajectory by antihypertensive medication. [236,257,258] The magnitude varied from almost twice the effect for treated hypertensives to a difference of 1.5 times with longer time of controlled hypertension. It is possible that the age distribution could have a potential effect on finding an effect modification due to antihypertensive drugs, given that it has been reported in both elder and middle adults, but the studies that failed to see a modification of the trajectory by age group present a wider range.

The fourth question tried to investigate the relationship between blood pressure control and change in PWV in clinical studies. Compiled evidence shows that patients receiving anti-hypertensive medication show reduction in arterial stiffness, even when treated for short time periods.[231] Although PWV is correlated with blood pressure, the reported reduction in arterial stiffness in clinical trials is independent of changes in arterial pressure.[231] Finally, the summarized evidence showed that any

class of blood pressure lowering medication is better than placebo for reducing arterial stiffness, but no class of antihypertensive seem to be superior to another. [259]

2.6.5 Gaps in the evidence in the association between hypertension and arterial stiffness

The gaps on the evidence could be summarized on: i) the strength of epidemiological association between AS and PWV, ii) lack of adjustment for key variables during statistical modelling, iii) the lack of statistical power to test the variation within different subgroups and the differences between treatment and prevention of arterial stiffness.

The lack of reproducibility between studies in finding the bidirectional association between arterial stiffness and hypertension could be the result of either its small effect size or the presence of varying degrees of exposure. [260]

The type of parameterization in some studies has the potential effect of changing the interpretation of the results. Some reports investigating longitudinal PWV associations have not accounted for some baseline variables and some others have concluded that there is no effect modification after adjusting for a covariate but have not included interactions with time or have performed subgroup analysis.[260,261]

Although it has been previously described that age is strengthens the association between arterial stiffness and hypertension,[262] more scientific knowledge is needed to understand whether hypertensive individuals have similar rates of arterial stiffening at all ages, or what could be the effect modification of age in the stiffening rate. The same questions apply to diabetic participants. New studies in already existing prospective cohorts with an appropriate sample size could help in solving these questions.

Is arterial stiffness reversible? Although this question is more suitable to a clinical trial,[170] observational studies could add some evidence to that field of research. As the risk of major cardiovascular outcomes such as stroke or cardiovascular mortality is reduced by restoring blood pressure levels to a pre-established goal, is the prevention of stiffening via the early treatment of hypertension equivalent to treat stiffness with antihypertensive therapy or with improvement in lifestyle factors? [263] More evidence is needed to understand the bidirectional relationship between hypertension and arterial stiffness. Assessing the effect of arterial stiffness on the development of hypertension in populations transitioning to older ages, where the change from normal blood pressure towards hypertension is likely to be observed, is one of the starting points for better understanding of this association.

Table 4. Selected studies on the association between PWV and blood pressure

Year	1st author, (reference)	Name of study	N	Age at baseline, mean (SD)	Mean follow-up; years (SD)	Assessment of arterial stiffness	Covariates	Change in trajectory by AHM	BP effect on Arterial stiffening	Arterial stiffness effect on incident hypertension	Statistical method	Comments
1999	Liao, [243]	ARIC Cohort Study	6692	56.7 (5.7)	3.3 (0.9)	ADC	DBP, PP, CAD, HR, Age, Eth, Sex, Sm, Ed, BMI	NSNP	NSNP	+	LogR	OR for developing hypertension =1.17 (95% CI 1.05,1.31)
2002	Benetos, [236]	INSERM	675	47.6 (0.6)	5	Cf-PWV	Age, HR, SC	+	+ (Not an independent predictor)	NSNP	Student *t*	Normotensive individuals = 0.008 m/s/year (p = 0.02) Treated hypertensive individuals = 0.014 m/s/year
2005	Dernellis, [247]	Vostanion Hospital, Mytilini, Greece	2512	Range: 35-94	4	ASI	Age, HR, DM, PA, alcohol, smoking	NSNP	NSNP	+	LogR	OR for women = 1.19 per ASI (95% CI 1.09, 1.25) unit OR for men = 1.22 (95% CI 1.11, 1.36)
2006	Tomiyama, [237]	Tokyo Medical University	538	45 (8)	3	Ba-PWV	TC, TG, HDL, FC, SC, BMI	+	+	NSNSNP	Student *t*	Annual increase in PWV for HBP = 0.19 m/s
2007	Yambe, [244]	Tokyo Medical University	100	39.3 (8)	3	Ba-PWV	Age, BMI, SBP, DBP, HDL, SC, HR, TG, FPG, TC	NSNP	NSNP	+	LogR	Adjusted OR for highest quartile of PWV = 1.5 (95% CI 0.8, 3.0)

2008	Najjar, [248]	Baltimore Longitudinal Study of Ageing	449	53 (17)	Median 4.3 (Range: 2-12)	Cf-PWV	Age, BMI, MAP,	NSNP	+	+	Cox/LLMM	HR: 1.10 (95% CI 1.00,1.30) p=0.03 for incident hypertension
2009	Tomiyama, [246]	Tokyo Medical University	777	42 (8)	3	Ba-PWV	BMI, DBP, SBP, HR, MetS, TG	NSNP	NSNP	+	LogR	OR for incident hypertension = 1.01 (95% CI 1.01, 1.02)
2011	Birru, [249]	SWAN study	316	50.1 (2.6)	2.3	Cf-PWV	LDL-C, DBP SBP HDL-C, TG, FG, Eth	NSNP	+	NSNP	LR	β = 3.46 (1.25) for baseline hypertension category
2012	El Khoudary, [250]	ERA-JUMP	240	45 (2.9)	4.6 (0.2)	Cf-PWV	TG, fibrinogen, adiponectin, HR, antihypertensive use, lipid medication	NSNP	+	NSNP	LR	β = 0.07 (S.E.= 0.03) β = 0.3 m/s/year per mmHg in SBP (p-value =0.04)
2012	Kaess, [245]	Framingham Heart Study, USA	1759	60 (9)	6.5 (0.7)	Cf-PWV	Age, sex, BMI, height, TG	NSNP	-	+	LLMM/LogR	OR for incident hypertension = 1.3 (1.0, 1.6)
2013	Alghatrif, [241]	Baltimore Longitudinal Study of Ageing	775	59.0 (15.7)	25	Cf-PWV	Age at entry, time, HR, SBP, WC, Eth, Glucose, TG	-	+	+	LLMM	β =0.17 for increase in systolic blood pressure β =0.039 for hypertensive men; 0.53 for hypertensive
2014	Scuteri, [239]	SardiNIA, Italy	4358	43.7 (17.6)	5.4 (2.0)	Cf-PWV	SBP, HR, BMI, age group.	-	+	NSNP	LLMM	Progression in PWV differs according to the age of onset of BP
2014	Roderjan, [240]	UFRJ, Brazil	442	68.5 (10.8)	5	Cf-PWV	Age, HR, age, sex, diabetes, CVD	NSNP	+	NSNP	LLMM	0.11 m/s/year in resistant hypertensives

2017	Tedla, [251]	MESA, USA	2051	63.7		ADC	Smoking, PA, BMI, SBP, DBP, AHM, diabetes, HDL-C, TC, GFR	+	+	NSNP	LR	β = 3.6 for controlled BP at 3 visits; 2.4 for controlled BP at 4 visits
2018	Meani, [238]	St. Gerardo Hospital, Monza	333	54.5 (12.6)	3.7	Cf-PWV	Age, sex, HR, BMI, LDL-C, TG, FG, SC	-	+	NSNP	Student *t*	PWV = 1.5 m/s in uncontrolled hypertensives vs. 0.6 m/s in controlled follow-up
2018	Koivistoinen, [242]	CRYFS, Finland	1449	38 (5.0)	4	Cf-PWV	Age, sex, LDL-C, HDL-C, BMI, FG, insulin, smoking, CRP, HR, DBP	NSNP	NSNP	+	LogR	OR for incident hypertension = 1.96 (95% CI 1.51, 2.57) per 1-SD increase

Legend: **+**:Associated **-**:Not associated **NSNP**: Not shown/presented; **SBP**: Systolic Blood Pressure; **DBP**: Diastolic Blood Pressure; **PWV**: Pulse Wave Velocity **ADC**: Arterial Distensibility Coefficient; **af**:aortic-femoral; **AHM**: Anti-hypertensive medication; **ASI**: Aortic Stiffness Index; **B**: Baseline **ba**: Brachial-ankle **BLSA**: Baltimore Longitudinal Study of Ageing; **cf**: Carotid-femoral; **Cox**: Cox proportional hazards model **CRYFS**: Cardiovascular Risk in Young Finns Study; **ERA-JUMP**: Electron-Beam Tomography Among Japanese and US men in the Post-World War II Birth Cohort; **Eth**: Ethnicity; **F**: Follow-up; **FG**: Fasting glucose **HDL-C**: High-Density Lipoprotein Cholesterol; **HR:** Heart rate **LDL-C**: Low Density Lipoprotein Cholesterol; **LogR**: Logistic regression **LR**: Linear regression **MAP**: Mean arterial pressure **MESA**: Multi Ethnic Study of Atherosclerosis; **MetS**: Metabolic syndrome, **PA**: Physical activity; **SC**: Serum creatinine **SD**: Standard deviation **TC**: Total cholesterol; **TG**: Triglycerides **UFRJ**: Universidad Federal Rio de Janeiro,

2.7 Major adverse cardiovascular events and Cardiovascular Disease

2.7.1 The burden of CVD and major adverse cardiovascular events

A global estimated of 18 million deaths occurred during 2017 having cardiovascular disease (CVD) as the underlying cause, [264] corresponding to one third of all deaths in people older than 35 years old worldwide.[265] In that year, two major subtypes of CVD, ischaemic heart disease and stroke were the first two global causes of mortality, being responsible for 8 and 6 million global deaths, respectively. [266] CVD risk screening and targeting individuals at high risk of cardiovascular outcomes in prevention strategies is a useful tool against CVD, although risk prevention with population strategies promoting lifestyle changes such as reduction in serum cholesterol, smoking and lower blood pressure, prior to the development of their associated health risks is even a more powerful tool.[267,268] Secondary prevention, such as acute treatment of major CVD events, more efficient healthcare networks, and more effective medications; and finally, improved tertiary prevention aimed to reduce or reverse long-term disability, both linked to higher economic development. [269]

Despite the declining global trend in CVD, attention must be drawn to some relevant aspects of its epidemiology. Firstly, the declining trend is not uniform across all world regions and nearly 80% of the global mortality derived from CVD occurs in low and middle-income countries. [270] These countries have increasing proportions of urban populations living in environments that promote the development of CVD, as well as health inequalities with varying access levels to material conditions and health infrastructure, that promote CVD risk factors and

contribute to the onset and progression of CVD. [269] Secondly, even as the prevalence of CVD and its related disability and morbidity are showing decaying trends, CVD will remain as the most common disability between the populations aged 65 to 84 years old by 2025. [271] Third, despite the declining prevalence, the absolute number of cases will increase as a consequence of increased life expectancy, requiring adequate treatment and follow-up of their conditions. [271]

2.7.2 CVD events and the need for risk prediction scores

These two contrasting scenarios of CVD burden: high-income countries with decreasing burden of CVD morbidity and related disability, or low and middle-income countries with steady or increasing CVD burden, require equally refined and efficient prediction models that receive input from affordable, easy to use clinical tools.[265] Many CVD event prediction tools have been developed and calibrated to identify at-risk individuals in specific settings or populations. Applying these tools to very different populations than the ones they were validated in could lead to biased estimations, which make validation in other types of populations more valuable.[272] Although individuals at high risk of CVD should be the priority of prevention strategies, an important number of major cardiovascular events occur in low and intermediate risk populations. This is a justification for research in tools that could improve event prediction in these groups.[273]

2.7.3 Major Adverse Cardiovascular Events

Some studies validating risk prediction scores for CVD events usually group different subtypes of them under the term Major Adverse Cardiovascular Events (MACE) although there is no standard definition of what specific events are

included within this umbrella term.[274] These usually include, but are not limited to fatal and non-fatal events of stroke, myocardial infarction, coronary heart disease and coronary death.[275]

Pooling different types of cardiovascular events under the MACE term brings the additional advantage of an integrated outcome of cardiovascular morbidity and mortality and increasing statistical power.

Although a potential disadvantage of a combined endpoint could be the comparability between the outcomes of different studies, given that the type of events included can be heterogeneous, sensitivity analyses modifying the types of events included in the definition could improve comparability and address potential biases arising from this practice.[276]

2.7.4 Physiological relationship between types of MACE and arterial stiffness

This chapter will focus mostly on stroke and coronary heart disease subtypes of MACE, and their relationship with arterial stiffness.

The association between arterial stiffness and stroke, could have more than one physiological explanation as the definition of stroke includes ischaemic, haemorrhagic, and other subtypes. [277] It has been suggested that arterial stiffness tends to be more associated with lacunar and haemorrhagic strokes compared to ischaemic stroke subtypes.[278] The main physiological mechanism acting within the haemorrhagic stroke category could be the presence of a pulsatile component of blood pressure acting on blood vessels of the peripheral circulation that are more suited to withstand steady blood flow. A fact that can be furthered by the low

vascular resistance of the cerebral blood vessels.[279] Other studies studying specific associations between high arterial stiffness and haemorrhagic stroke secondary to small vessel disease support this hypothesis.[280]

The physiological background for the association between arterial stiffness and coronary heart disease involves the function of the aorta and its interaction with the carotid arteries during the cardiac cycle. Arterial stiffness has an influence on reducing the diastolic blood pressure as the systolic blood pressure increases, resulting in pulse pressure widening.[281] The increase in diastolic blood pressure retards left ventricular filling, which is the part of the cardiac cycle where the coronary arteries provide blood flow to the cardiac myocytes. The influence of stiffness also leads to the early return of pulse waves, which increases ventricular afterload, increasing the energy expenditure from the heart and thus progressively leading to further myocardial ischaemia, increasing the likelihood of a myocardial infarction.[282]

2.7.5 Prospective epidemiologic studies on the relationship between arterial stiffness and MACE

The relationship between arterial stiffness and MACE has been observed both cross-sectionally [283] and longitudinally. [11,284] As it might be difficult to rule out causality and temporality of effects using cross-sectional analyses, the focus of this review will be on longitudinal research studying the relationship between arterial stiffness and cardiovascular events.

A systematic review and meta-analysis published in 2010 compiled 14 studies on this topic with results from prospective cohorts.[285] This study was the first that

aimed to extract a compiled estimator of cardiovascular risk comparing low and high-risk populations defined previously as individuals with low and high arterial stiffness, independently of the method used to assess it. Although a difference in prediction between high-risk and low-risk subpopulations was not found, the authors found that the group of individuals with high arterial stiffness had a RR for CVD events two times higher than the group with low arterial stiffness and that there is a 1.14 higher risk of cardiovascular events per m/s of PWV. They also found that arterial stiffness is a predictor of both cardiovascular and all-cause mortality in the overall population studied.[286] The meta-analysed studies varied in their measurement techniques of arterial stiffness, which allowed to include studies using either tonometry or imaging techniques as ultrasound doppler flow.

The latest systematic review and meta-analysis on risk of major CVD outcomes and arterial stiffness, was published by Ben-Shlomo and colleagues in 2014. [287] The authors only included 17 cohorts from 16 studies published until 2012, and which assessed aortic stiffness using carotid-femoral pulse wave velocity. This work included. As in the systematic review published by Vlachopolous and colleagues,[285] this work also included a mix of low and high-risk populations. However, its aims differed slightly compared to the first systematic review and meta-analysis, as it intended to both have a compiled estimator with the best measuring tools available and to estimate what was the effect of adding aortic PWV to the risk prediction profiles, measured by the C-statistic.

After compiling the information from 17,365 participants, adjusted pooled estimates of CVD events have a Hazard Ratio of 1.30 (95%CI: 1.18, 1.43) per log PWV units, and an increase in the Harrel's C-statistic for all-cause mortality models from 0.70

to 0.71 models with a difference of 0.0046 (95% CI: 0.0010, 0.0082; p: 0.013) and an increase from 0.6780 to 0.6832 with a difference of 0.0053 (95% CI: 0.0013, 0.0093; p=0.010). The authors did not find a difference in prediction ability measured by C-statistics after adding cf-PWV to models estimating time to CVD mortality and stroke events.[287]

To assess whether further evidence on the risk of MACE according to AS was published from January 2013 until July 2020, a search was performed in Medline including medical subject headings (MeSH) for the events included in the definition of MACE as stroke, myocardial infarction and coronary heart disease. Also, alternative terms for indexing arterial stiffness and cohort studies were included in the search. After selecting the relevant evidence by title, abstract and full-text screening, 8 studies were included in the search. All the search and selection process can be seen in

Table 5.

Table 5. Search strategy for studies assessing the relationship between MACE and arterial stiffness

Number	Terms	Items found
1	Stroke [Mesh]	136,264
2	Myocardial Infarction [Mesh]	176,109
3	Coronary Disease [Mesh]	218,502
4	Cardiovascular Diseases [Mesh]	2,397,878
5	**1 OR 2 OR 3 OR 4**	2,397,878
6	Pulse Wave Analysis [Mesh]	4,266
7	Pulse Wave Velocity	14,283
8	Carotid-Femoral Pulse Wave Velocity [Mesh]	25
9	Vascular Stiffness [Mesh]	6,151
10	Arterial stiffness	15,817
11	**6 OR 7 OR 8 OR 9 OR 10**	**23,104**
12	Cohort studies	2,036,231
13	Longitudinal Studies [Mesh]	137,911
14	**7 OR 8**	2,036,231
15	**5 AND 11 AND 14**	**2,424**
16	Filters: Observational Studies, humans	**181**
	Approved by title screening	**138**
	Approved for final text after abstract screening	**13**
	Final inclusion	**8**

The evidence from the studies included in this review is summarised in Table 6. In total, eight studies including a total of 1899 individuals were reviewed. The mean age at baseline varied from 51 to 63 years and 7 out the 8 studies included special subpopulations such as patients with chronic kidney disease, renal transplant recipients or individuals from hospital registries. Five studies used measured aortic PWV using either cf-PWV or ba-PWV. The remainder used imaging techniques combined with tonometry to assess PWV.

The two most important details about this review are the definition of MACE and the probability of events predicted by arterial stiffness. The definition of MACE is not uniform across all the studies, where two of the 8 studies use definitions of

Table 6. Cohort studies assessing the relationship between arterial stiffness and MACE between 2013 and 2020.

Year	1st author	Name of study	N	Male (%)	Age at baseline, mean (SD)	Mean follow-up; years (SD)	Assessment of arterial stiffness	Covariates	Mean PWV	MACE outcome	Events	HR (95%CI)	Comments
2013	Claes[288]	Incident renal transplant recipients	115	65.0	51.0	3.0 (1.33)	cf-PWV (Sphygmocor)	Bivariate models: Model 1: PWV+CVD history Model 2: PWV+HbA1c Model 3: PWV+Age Model 4: PWV + AC	7.6 (2.0)	Myocardial infarction Stroke Peripheral Artery Disease Claudication Vascular intervention Sudden death	13	Model 1: 1.3 (1.02, 1.69) Model 2: 1.47 (1.20, 1.23) Model 3: 1.15 (1.04, 1.26) Model 4: 1.24 (0.97, 1.60)	Hazard Ratios only from bivariate models
2014	Avramovski[289]	Chronic Hemodialysis Patients	80	66.3	59.3 (11.8)	2.6 (0.8)	Doppler-Ultrasound	Age, sex, ethnicity	12.5 (2.01) Range: 8.2, 18.2	CVD mortality (stroke, peripheral vascular disease, arrhythmia, congestive heart failure, MI)	23	1.43 (1.17, 1.75)	Hemodialysis patients
2016	Wijkman[290]	CARDIPP study	565	64.3	60.5 (58.0, 63.0)	7.9 Range: (38, 3537) IQR: (2506-3172)	cf-PWV (Sphygmocor)	Age, sex, diabetes duration, systolic blood pressure, heart rate, total cholesterol, HbA1C, eGFR, smoking status	10.4 IQR (9.0, 11.5)	CVD mortality Hospitalisation for MI Hospitalisation for stroke	88	Model 1: 1.232 (1.098, 1.383) Model 2: 1.142 (1.003, 1.301)	
2017	Feistritzer[291]	Hospital Cohort STEMI	160	73	58.0 (12.0)	1.8 (1.3)	CMR Distensibility coefficient	Model 1: Unadjusted Model 2: PWV + Age Model 3: PWV + Sex Model 4: PWV + NT-proBNP Model 5: PWV + Multivessel disease Model 6: PWV + LVSV	6.8 (5.9, 8.3)	Death Nonfatal myocardial reinfarction New congestive heart failure Stroke	19	Model 1: 4.1 (1.5-11.5) Model 2: 4.1 (1.5-11.5) Model 3: 4.1 (1.5-11.5) Model 4: 4.0 (1.4-11.5) Model 5: 4.3 (1.4-13.3) Model 6: 4.1 (1.5-11.3) Model 7: 3.5 (1.2-9.6)	(PWV >Median: 7.3 m/s)

2017	Park [292]	GNUH-AMI registry	411	75.2	63.8 (13.5)	0.98	ba-PWV	NS	16.39 (4.97)	Cardiac death re-AMI Revascularisation by repeat PCI Admission to congestive heart failure Stroke	27	Univariate: 4.32 (1.94, 9.61) Multivariate: 3.38 (1.44, 7.93)	
2017	Sulemane [293]	Chronic Kidney Disease	106	Non MACE: 48.7 MACE: 54.3	Non MACE: 54.5 (13) MACE: 60.3 (12)	Median: 4.1 IQR (0.92, 5.25)	cf-PWV (Sphygmocor)	NS	Non-MACE: 8.6 (1.7) MACE: 11.5 (2.41)	All-cause mortality Acute coronary syndrome Stable angina requiring revascularisation (PCI or CABG) Heart failure hospitalisation Stroke hospitalisation	26	Univariate: 1.33 (1.08, 1.69) Multivariate: 1.31 (1.05, 1.41)	
2017	Ferreira[294]	F.H. Manhes Hospital, Fleury-Mérogis	278	61.2	53 (16)	6.17 (3.42, 9.58)	Doppler-Ultrasound + tonometry (Complior method)	Model 1: Unadjusted Model 2: Age, gender, smoking history, dialysis, diabetic status, calcium and phosphate product, pre-existing CVD, LVM, Early transmitral /Atrial contraction ratio	11.0	Coronary Artery Disease, Mesenteric Infarction, sudden death, congestive heart failure, stroke	91	Model 1: 1.35 (1.27, 1.44) Model 2: 2.03 (1.35, 3.04)	
2018	Maruhashi [295]	Flow-Mediated Dilation Japan Study A. FMD-J A	462	85.7	63.8 (8.7)	Median: 49.2 (IQR: 43.2, 56.1)	ba-PWV	Model 1: Hypertension, dyslipidaemia, diabetes mellitus, smoking Model 2: Model 1 + SBP, antihypertensive drugs, statin use, glucose level	16.4 (0.29)	Myocardial infarction Coronary restenosis New coronary artery stenosis Stroke Heart failure Sudden death	66	Unadjusted: 1.79 (1.05, 3.03) Model 1: 1.86 (1.01, 3.44) Model 2: 1.99 (1.04, 3.82)	Using ba-PWV

Legend: AC: Aortic calcification ba-PWV: brachial-ankle Pulse Wave Velocity BP: Blood Pressure CABG: Coronary Artery Bypass Graft cf-PWV: carotid-femoral Pulse Wave Velocity CMR: Cardiac Magnetic Resonance DBP: Diastolic Blood Pressure eGFR: Estimated glomerular filtration rate HbA1c Glycated haemoglobin. HR: Hazard Ratio IQR: Interquartile range LVSV: Left Ventricular Stroke Volume MACE: Major Adverse Cardiovascular Events MI: Myocardial Infarction NS: Not shown PCI: Percutaneous Coronary Intervention re-AMI: Recurrent Acute Myocardial Infarction SD: Standard Deviation SBP: Systolic Blood Pressure

cardiovascular events using only fatal and non-fatal cases of stroke, myocardial infarction or coronary heart disease, while other studies also included clinical procedures for cardiovascular events such as angina. The hazard ratios for cardiovascular events varied from 1.5 to 4.3 per unit of cf-PWV, being higher usually on the studies predicting mortality events from CVD and lower in the studies pooling a variety of fatal and non-fatal events.

The variety of endpoints included under the umbrella term MACE, has the benefit of increasing the sample size, leading to a higher statistical power. This might attenuate the magnitude of the final estimate if the outcomes differ widely in magnitude. A potential solution for the heterogeneity of the events is to show the magnitude and size of the associations including separate types of events, showing if there are any significant heterogeneities within the types of cases included.

Among the three studies that used cf-PWV to measure aortic stiffness, the results from the study of Incident Renal Transplant Recipients[288] showed Hazard Ratio (HR) estimates for MACE between 1.15 and 1.47 per m/s of cf-PWV, although the number of events is small (13) and these events are from bivariate models as further analyses would be underpowered.

The Cardiovascular Risk Factors in Patients with Diabetes (CARDIPP) study[290] showed a similar magnitude, with an effect of 1.14 per m/s after adjustment for common cardiovascular risk factors. The third study[293], using a cohort of 106 chronic kidney disease patients, showed a HR of 1.31 in the final model.

The two included studies using ba-PWV were hospital composed of Asian participants. The Gyeongsang National University Hospital-AMI registry (GNUH-

AMI)[61] is based in South Korea and compared participants with high and low PWV, so the estimate after adjustment (HR= 3.38), does not account for units of PWV.

The Flow-Mediated Dilation Japan study, (FMD-JA) [295] included middle-aged population from Japan, the groups were also defined using a threshold of median ba-PWV. After adjustments, the group of individuals above the median, had a 1.99 higher hazard of CVD events compared to the group below the median.

Finally, three included studies used imaging techniques to measure arterial stiffness. Avramovski[296] and colleagues used a doppler-ultrasound technique showed a Hazard Ratio of 1.43 for CVD mortality for participants above the cut-off point of 12 m/s compared with those below.

The hospital cohort of inpatients with ST elevation myocardial infarction[291] included 73 patients with CVD events, and defining the models including participants below and above the median. Given the small number of events (E=19), the authors only presented bivariate models. The highest attenuation was seen after adjusting for Left Ventricular Stroke Volume. (HR =3.5).

Finally, a cohort from the Manhes hospital in France[294], using doppler-ultrasound techniques coupled with arterial tonometry, reported an adjusted HR of 2.03 per m/s of PWV.

2.7.6 Summary of the literature review and gaps in the evidence

Meta-analyses from literature published before 2009 have shown that aortic stiffness is a predictor of major CVD events, not only measured with cf-PWV, the gold-standard technique, but also with ba-PWV. Although not regarded as gold-

standard method for the assessment of arterial stiffness, imaging techniques also predicted the incidence of MACE.

The magnitude of the effect size per unit of PWV is similar to that reported in the literature in studies from 2014 and before, but the strength of the associations tends to be smaller, with lower sample sizes being one potential explanation for the lack of evidence.

The populations of all the studies included in this narrative review published after 2014, tend to come from hospital settings and patient registries with prevalent CVD, being likely that there was an overestimation of the effect compared to population-based or occupational cohorts.

There are three different questions resulting from the gaps in the reviewed literature:

1) What is the ability of cf-PWV to predict incident MACE in an occupational cohort?
2) Is the predictive power of two measurements of arterial stiffness superior to the predictive power of a single measurement of arterial stiffness?
3) How different is the prediction of incident CVD events by arterial stiffness in populations with and without prevalent CVD?
4) Is cf-PWV also a predictor of CVD mortality and non-CVD mortality events?

2.8 Physical functioning

Physical functioning is defined as the ability of an individual to perform tasks required to live independently and to guarantee their own survival.[297] Commonly measured activities are included within categories of personal hygiene, feeding, dressing, moving and continence. Being able to feed oneself, having a shower, walking and using the toilet without help are some subjective markers of physical function.[298]

While some authors use the terms physical function and disability interchangeably, [299] a distinction must be made between the two. While the former addresses the previously described activities in the context of the person, the latter refers to the loss of capacity to interact normally. In other words, a disability can be defined as an individual not being able to perform an activity within the boundaries of time and quality standards of the individuals' society. The effect of that gap between the individual activity threshold and the societal standards of social disadvantage and lost opportunities and income is defined as handicap.[300]

Besides the importance of using measurements of physical functioning as tools for tracking the ability of an individual to work and perform necessary tasks for daily life and to be part of the society, a variety of these tools have been identified as independent predictors of decline in other body systems (e.g. cardiovascular, neurologic) and also as predictors of decline in overall health and increased mortality risk.[301]

2.8.1 Epidemiology of disability and low physical functioning

It was estimated that in 2017, 24% of the world population reported having at least one form of disability.[302] The prevalence is not uniform across countries and can be explained by multiple factors, being country income level one of them. It varies from 9% in European countries, 10% in Latin America and 15% in some African countries.[302] Regardless of the trends on a country level, the prevalence of disability increases with age. Forecasting until 2025 for individuals aged 65 and beyond in England and Wales projected 20% of disabilities for men and 23% for women.[271] Thus, countries with older populations are more likely to have a higher prevalence of individuals with a disability in elderly age groups and a higher absolute number of individuals with disabilities in the overall population.

Different standards in the definition of disability can make cross-country comparisons more difficult. Walking speed is one of the most used assessments of physical functioning as standardised measurements have higher comparability. Nationally representative surveys such as the NHANES in the United States reported a prevalence of 26% in women aged 65 and more, with an increasing prevalence with age when analysing smaller age subgroups.[303] Data from community-based European cohorts with a mean age of 70 show a prevalence of 56.4%[304] and smaller studies in French samples aged 80 years and above in showed a prevalence of slow walking speed of 84%.[305]

Representative samples from studies in middle-income countries such as Mexico, Colombia and South Africa, with mean ages of 68.7, 70.9 and 80 years,

respectively, reported a prevalence of low walking speed of 50.4% and 23.2% and 34.2%.[306,307]

Finally, reports from community surveys in low-income countries such as Ghana showed a prevalence of slow walking speed of 40.9% in adults aged 65-69, and 23.2% in adults aged 80 years and more.[308]

When observing the frequency of low physical functioning using other objective measures in ethnically diverse samples as the UK biobank, low grip strength was reported to be 52% in white, 49% in black and 81% in South Asian participants.[309]

2.8.2 Predictors of decline in physical functioning

Age is the strongest predictor of decline in physical functioning and disability. The life expectancy at birth of the world population by 1990 was 64.2, with the number of people with 65 years old and higher being 328 million, a 6.5% of the total world population. By 2020, the group of adults aged more than 65 rose to 727 million, equivalent to a 10% of the total world population. With a projected increase to 12% by 2030.[310]

According to country income level, the proportion of individuals older than 65 years old in 2019 was 18%, 8.7 and 3.0% in high, middle, and low-income countries, respectively. The expected proportion for 2030 in the former group of countries will be 22%.[310]

Apart from ageing, demographic factors as marital status, sex or low socioeconomic position, chronic musculoskeletal conditions such as osteoarthritis, osteoporosis or sarcopenia, traumatic injuries, and their subsequent chronic

pain,[311] and vascular conditions as heart failure, myocardial infarction or stroke are associated with low physical functioning or a steeper decline into disability.[312]

2.8.3 Cardiovascular disease, arterial stiffness, and physical functioning: literature review.

The literature review described that the trend in CVD and the impairments in physical functioning are not occurring with a uniform pattern around the world. The disability secondary to CVD is presenting a stable pattern in most high-income countries from Western Europe and North America, while its prevalence in middle-income and low-income countries is increasing.[265]

The main link between CVD and decrease in physical functioning relates to the acute impairment of neurologic and musculoskeletal functions related to major acute cardiovascular events such haemorrhagic stroke or myocardial infarction. This usually affects extensive areas of the brain cortex, deriving in speech or mobility impairments, reduced muscular strength.[313]

The decrease in physical functioning secondary to chronic cardiovascular conditions has a lower impact in motor abilities compared with acute cardiovascular events. Individuals with chronic conditions such as congestive heart failure, or venous insufficiency tend to live over longer periods with a lower physical functioning ability than the average population but staying above a threshold that allow certain independence, until the onset of the more severe stages of disease before death.[314–317]

Cross-sectional evidence from the Whitehall II and other studies, assessing the relationship between arterial stiffness and low physical functioning could show associations and size effects that are the result of reverse causation. Low-physical activity tend to produce high arterial stiffness, or high arterial stiffness could lead to cardiovascular events or subclinical damage, producing an impairment in physical activity.[13]

The nature of the available evidence leads to hypothesise that subclinical vascular disease is a potential marker of low physical functioning, and leaves gaps in the evidence that might be addressed answering the following questions:

1. What is the existing literature on the cross-sectional association between arterial stiffness and measures of physical functioning?
2. What is the existing literature in the prospective association between arterial stiffness and trajectories of physical functioning?

2.8.3.1 Search strategy

To answer these questions, a review was performed using a search strategy in Medline. The search strategy can be seen in Table 7. After title, abstract and full-text screening, looking for both cross-sectional and prospective observational studies, 12 studies were included in the final revision.

Table 7. Search strategy for identifying studies on the relationship between Arterial Stiffness and physical functioning (05/02/2020)

Search	Terms	Items found
1	"Vascular Stiffness"[Mesh]	5,706
2	"Pulse Wave Analysis"[Mesh]	3,912
3	"Carotid-Femoral Pulse Wave Velocity"[Mesh]	4
4	Arterial stiffness	9,031
5	**((("Vascular Stiffness"[Mesh]) OR ("Pulse Wave Analysis"[Mesh] OR "Carotid-Femoral Pulse Wave Velocity"[Mesh] OR "arterial stiffness")))**	**12,296**
6	Activities of Daily Living[Mesh]	99,589
7	"Age Factors"[Mesh]	497,034
8	"Motor Activity"[Mesh]	284,208
10	"Frailty"[Mesh]	2,150
11	"Physical Fitness"[Mesh]	29,080
12	"Walking Speed"[Mesh]	1,146
13	"Hand Strength"[Mesh]	14,589
14	"Quality of Life"[Mesh]	188,877
15	"finger tapping"	2,195
16	"chair rises"	70
17	(((((((("Activities of Daily Living"[Mesh]) OR "Age Factors"[Mesh]) OR "Motor Activity"[Mesh])) OR "Frailty"[Mesh]) OR "Physical Fitness"[Mesh]) OR "Walking Speed"[Mesh]) OR "Hand Strength"[Mesh]) OR "Quality of Life"[Mesh] OR "finger tapping" OR "chair rises")	**1,045,736**
18	**#5 AND #17**	**1,574**
	Selected for abstract screening	**160**
	Selected for full-text screening	**25**
	Included in the final review	**12**

2.8.4 Summary of the included studies

The main characteristics of the studies, including the name of the study, sample size and association measurements can be seen in Table 8.

Ten out of 12 of the selected studies were checking cross-sectional associations between arterial stiffness and measurements of physical functioning. Apart from a study carried out in China, the rest of them included predominantly populations of Caucasian origin from Europe and the United States with mean ages varying from 43 to 69 years. Most of the studies measured arterial stiffness using cf-PWV, with

two using the Arterial Index X (Aix) and the Cardio Ankle vascular index (CAVI). Only one study did not find a relationship between arterial stiffness measured by ba-PWV and the scores of the PCS-12, although there was a trend towards significance with the Aix.[318] The rest of the studies showed a negative cross-sectional relationship, where the increase in stiffness meant a decrease in the ability to function or decrease in functional reserve marked by a frailty state.[319]

In the two longitudinal studies assessing the prospective relationship, one assessed muscular mass as an indirect measure of functioning, finding sarcopenia developed at a higher rate with higher cf-PWV. The second study did find a relationship with overall physical ability but there was no relationship between the standardized score of cf-PWV and the scale of activities of daily living. [320]

Although the heterogeneity of the measurements of physical functioning used in the different studies did not allow for pooling of the estimates, there was a replication of the association between arterial stiffness and physical functioning limitation between cross-sectional studies, both in subjective scales as the Instrumental Activities of Daily Living (IADL) or in objective measurements as walking speed.

The two longitudinal studies included in the final review showed associations between arterial stiffening and objective measures of physical functioning such as muscular strength and the physical ability battery, while not finding links with the Activities of Daily Living (ADL) scale.

Table 8. Studies assessing the relationship between arterial stiffness and indicators of physical functioning

1st author, year of publication	Name of study	Aim(s)	N	Age at baseline, mean (SD)	Mean follow-up; years (SD)	Assessment of arterial stiffness	Outcome	Covariates	Statistical method used	Comments	Association
Cross-sectional studies											
Brewer, 2007[321]	Mayo Clinic	Arterial stiffness and walking distance on treadmill	106	69.0 (10.5)	NA	Aix, T_r	Walking distance	Age, sex, BMI, diabetes, smoking history, MAP, BP medication use, lipid-lowering medication use, and ABI	Survival analysis/linear regression	Lower walking distance per Aix unit (-0.032; SD= 0.010 p=0.001)	(-)
Brunner, 2011 [322]	Whitehall II study	PWV and physical function	5392	65.4 (5.8)	NA	cf-PWV	Walking speed, SF-36, lung function	Age, sex, ethnicity, PP, MAP, HR, AHT, CHD	Linear mixed model regression	OR= ADL1.20 (p<0.001) per m/s - 0.67 (<0.001) per m/s.	(-)
Gonzales, 2013 [323]	Texas Tech University	Influences of PWV in gait performance	21	68.0 (5.0)	NA	cf-PWV	Walking speed	Age, BMI, waist circumference, SBP	Partial correlation analysis	Correlation PWV, Gait speed = -0.48; corr 2-min walk distance, PWV = -0.51	(-)
Lane, 2013 [324]	University of Illinois	Arterial stiffness and walking ability in end-stage renal disease patients	42	44.0 (5.0)	NA	cf-PWV	Walking speed	Age	Spearman Correlation	Correlation: -0.39, p= 0.015; Regression: B=-11.9 p= 0.036 per m/s	(-)
Gonzales, 2015 [325]	Texas Tech University	Test fatigability between according to PWV	45	65.3 (3.9)	NA	cf-PWV	Walking fatigability	Model 1: Age, sex Model 2: Age, sex, BMI, FBG, SBP, average steps per day, and 30-min peak stepping cadence	ANCOVA	cf-PWV 2.0 m/s higher in the group with higher fatigability	(-)
Garcia, 2016 [318]	MARK study	Vascular structure and Health Related Quality of Life	303	60.5 (8.5)	NA	ba-PWV	Health Related Quality of Life	Smoking, alcohol, PA, BMI, BP and diet	Linear regression	PWV: 0.01 (95%CI: -0.53, 0.52) Mental component: -0.08 (95% CI: -0.65, 0.50)	NF
Fahs, 2018 [326]	Lindenwood University	Arterial stiffness, strength and lean body mass.	71	43	NA	cf-PWV	Correlation between Arterial Stiffness, strength, and lean body mass	Age, sex, body fat percentage, Mean Arterial Pressure	ANOVA partial correlation	Corr = -0.23 (Absolute strength) - 0.48 (Relative strength)	(-)

Joseph, 2019 [327]	MUST study	Compare 6-minute walking distance in patients with osteoarthritis and arterial stiffness	352	63.2 (8.8)	NA	cf-PWV	6 Minute Walking Distance Test	HR, MAP, age, and sex	Linear regression	−0.003 metres/second [95% CI −0.005, −0.001], P = 0.007).	(-)
Xue, 2019 [328]	Beijing Tongren Hospital	Association between frailty and CAVI	171	Non-frail: 72.7 (8.5) Pre-frail: 82.4 (5.6) Frail: 87.7 (5.7)	NA	CAVI	Frailty	Age, BMI, ADL, CAVI, ABI, haemoglobin, albumin, eGFR, hs-CRP and LDL-C	Correlation analysis and ordinal logistic regression	OR (Frail vs. Non-Frail): 2.01 (95% CI= 1.49, 2.70)	(-)
Orkaby, 2019 [319]	Framingham Heart Study	Association between frailty and cf-PWV	2171	Non-frail 68.1 (6.1); Prefrail = 70.6 (7.0); 75.5 (7.2)	NA	cf-PWV	Arterial stiffness	Demographics, including age, sex, cohort (Offspring or Omni), self-report of education, employment, retirement status, and marital status were examined. Smoking was assessed as never, former, or current. CVD events were adjudicated.	Weighted Kappa, generalized linear regression	Agreement between both definitions of frailty. Higher cf-PWV for higher frailty categories: Non-frail 10.0 Prefrail 10.3 Frail 10.5 (p <0.001)	(-)
Longitudinal studies											
Abbatecola, 2012 [329]	Health ABC study	PWV and mass decline	2405	65.1	6	cf-PWV	Skeletal muscle decline	Age, height, weight, BMI, race, site, diabetes, ankle-brachial index, IL-6, PAD, SBP, smoking, CHD	Linear mixed models	Per 1 m/s of PWV: Men: Sarcopenia Index -0.0990 kg/m2 (p= 0.01) Follow-up: SI: -0.10 per m/s (p=<0.001) SI -0.1043 (p =<0.001) Lean arm: -0.099 (p =<0.001) Leg mass: -0.22 (p =<0.001)	(-)
den Ouden, 2014 [320]	Utrecht University	Change in PWV and change in ADL disability in middle-age	490	61.0	9.6 (2.4)	cf-PWV	Decline of physical ability and activities of daily living	Adjusted for age, gender, education, fat mass, mini mental-state examination, diabetes, number of chronic diseases, systolic blood pressure and diastolic blood pressure	Generalized Estimation Equation	Δ physical ability per Δ Z-score PWV: 0.042 (95%CI= 0.017, 0.067). Δ ADL per Δ Z-score PWV: 0.002 (95%CI= -0.032; 0.028)	(-/NF)

ABI: Ankle-brachial index; ADL: Activities of Daily Living; AHT: Antihypertensive medication; AIX: Arterial Index X; ANCOVA: Analysis of Covariance; ba-PWV: brachial-ankle Pulse Wave Velocity; BMI: Body Mass Index; BP: Blood Pressure; corr: Correlation; cf-PWV: carotid-femoral Pulse Wave Velocity; CHD: Coronary Heart Disease; eGFR: effective Glomerular Filtration Rate; HR: Heart Rate; IADL: Instrumental Activities of Daily Living; MAP: Mean Arterial Pressure; NA: Not applies; OR: Odds Ratio; PA: Physical Activity; SD: Standard Deviation; SBP: Systolic Blood Pressure; T_r = Reflected wave arrival time; (+) Positive association found (-) Negative association found;

The main gaps in the evidence that remained after performing the systematic review were:

1) Do objective measurements and subjective measurements of physical functioning have a different relationship with arterial stiffness?
2) Is there a relationship between arterial stiffening and subjective scales of physical activity such as ADL and IADL?
3) Are these potential relationships present also in subgroups of individuals with history of target-organ dysfunction such as coronary heart disease or chronic kidney disease?
4) Does age modify the associations?

All these questions are input for modelling the analysis of the potential prospective association between physical functioning and AS in the Whitehall II study.

Chapter 3 Methods

3 Methods

The methods chapter describes the datasets used in this thesis: the Whitehall II study and the Hospital Episode Statistics. It also describes the main exposures and outcomes, the study waves (phases) with relevant data that was used in this thesis. Further details on the parameterisation of variables and details of the modelling will be provided in each research chapter.

3.1 Data structure

The Whitehall II cohort is a prospective study of civil servants. Recruitment phase started in 1985, with participants being included from different branches of the British civil service based in London. Out of the 14,121 civil servants invited to participate in the study, 73% agreed to be provide information via the self-administered questionnaire and clinical examinations that made part of the phase (wave) 1 and took until 1988. New phases of data collection were carried out every two or three years, phase 3 (1991-93), phase 5 (1997-99), phase 7 (2003-04), phase 9 (2008-09), phase 11 (2012-13) and phase 12 (2015-16). The phase 13 has been under way from 2019 onwards. The mean age at phase 1 was 44.4 SD (6.05).

Most variables used for the analyses of this thesis were selected from phases 9 and 11, although some variables from phase 1 until phase 9 were used in other analyses. The exposures, outcomes and covariates used in the thesis are shown in Table 9

Table 9. Availability and usage of the selected variables for the thesis

Phase	1	3	5	7	9	11	12
	1985-88	1991-93	1997-99	2002-04	2008-09	2012-13	2015-16
Median age	44	49	55	60	65	69	71
Exposure variables							
Baseline cf-PWV							
Blood pressure status (BP + BP Treatment)							
Δ cf-PWV							
Outcome variables							
MACE							
Time-adjusted **Δ** cf-PWV							
Frailty							
SF-36 physical component score							
SF-36 mental component score							
Walking speed							
Forced Expiratory Volume							
Finger tapping							
Grip strength							
Chair rises							
Activities of Daily Living							
Instrumental Activities Daily Living							
Covariates							
Age							
Sex							
Ethnicity							
Systolic Blood Pressure							
Diastolic Blood Pressure							
Mean arterial pressure							
Heart rate							
Body Mass Index							
Waist Circumference							
Total cholesterol							
Type II diabetes							
History of CVD (stroke, MI, CHD)							
Marital status							
Mini-mental score							
Prevalent cancer							
Smoking							
Alcohol drinking							
Physical activity							

Legend: Shaded background: used in the analysis; cf-PWV: carotid-femoral Pulse Wave Velocity; CHD: Coronary Heart Disease; MI: Myocardial Infarction; NRI: Net Reclassification Improvement

3.2 Attrition and representativity of the cohort

A total of 10,308 civil servants attended the baseline clinical screening and completed a self-administered questionnaire. Out of these, 65.6% attended at the clinical screening of phase 9. Up to phase 12, carried out in 2015-16, the proportion of responders was 55%. Non-responders to the next clinical screening were almost two times more likely to die from cardiovascular disease compared to responders.[330] All the response rates by clinical screening can be seen in Table 10

Table 10. Response rates per phase of clinical screening.

Phase	N	Response rate*
1	10,308	-
3	8,815	85.5
5	7,870	76.3
7	6,967	67.6
9	6,761	65.6
11	6,308	61.2
12	5,632	54.6

*Percentage of target population or of initial responders

Despite the attrition rates shown, response rate has been maintained relatively high along the development of the study and within desirable levels for an observational study.[331]

With regard to representativity, the members of the Whitehall II cohort are members of the civil service, which in general have access to better living conditions compared to the general population. The potential influence of the 'healthy worker effect' on the external validity of the results was studied elsewhere, [332] showing that results from the Whitehall II cohort are still applicable to the wider population.

3.3 Exposures

The exposure variables used in the main research chapters of the thesis will be explained in this section.

3.3.1 Carotid-femoral Pulse Wave Velocity

3.3.1.1 Baseline measurement

Carotid femoral Pulse Wave Velocity (cf-PWV) is the gold-standard for the assessment of arterial stiffness[333] and was introduced to the Whitehall II study at the research clinic from phase 9 onwards, with additional measurements at phases 11 and 12. The same device was used at phases 9 and 11 with a different device used at phase 12. To reduce the potential lack of comparability between measurements made with different devices, only measurements at phases 9 and 11 of data collection were included in the analyses of chapter 4.

In order to test the hypothesis of the bidirectional relationship between hypertension and arterial stiffness in chapter 4, tertiles of baseline cf-PWV at phase 9 were used to estimate the risk of progression to hypertension in the next phase, in participants with normal or high-normal blood pressure.

The change between measurements of cf-PWV was parameterized in two different ways according to different chapters of the thesis. In chapter 5, I tested whether the change between measurements improved predictive ability of Cox proportional hazards models assessing time to cardiovascular events. The change between measurements was calculated using regression residuals using the measurements at phases 9 and 11.

3.3.2 Blood pressure status

Blood pressure status is a composite variable that was parameterised using history of high blood pressure, prescriptions of blood pressure medication or clinical measurements of high blood pressure from the research clinics between phase 1 and phase 9, the baseline of the study for the analyses in of chapter 4.

3.4 Outcome variables

3.4.1 Major Adverse Cardiovascular Events

Major Adverse Cardiovascular Events are a composite outcome including coronary heart disease, myocardial infarction and stroke. The data source for the events are the Hospital Episode Statistics coming from the NHS central register. Information about all the clinical events from phase 5 until March 2019 was available. Events occurring between Phase 9 up to March 2019 were used in the analyses.

3.4.2 Time-adjusted Δ cf-PWV

In the analysis of chapter 4, the rate of arterial stiffening or change of stiffness between measurements was assessed by fitting an interaction with time in the models using measurements at phase 9 and 11.

3.4.3 SF-36 Scale

The SF-36 scale was first measured in the Whitehall II study through the self-administered questionnaire of study phase 3, during 1991-93. It consisted of a 36-item form that assessed physical and mental health indicators. Results of the assessments of both mental and physical dimensions were used for analyses of this thesis at phase 9 and onwards.

3.4.4 Physical functioning battery

Objective measurements of physical functioning were introduced at different phases of the study. Walking speed, and lung function measurements were first measured during the research clinic of phase 7, (2002-04). A timed walk was assessed using a standardised protocol and using an 8-feet walking platform. Lung function was assessed through forced expiratory volume.

Measurements of grip strength, chair rises, and finger tapping were included into the physical functioning battery of the study at the clinic of phase 9 (2011-12). The objective and subjective physical functioning measurements from phase 9 onwards were used in the analyses of this thesis. Data from phase 9 was used to test the cross-sectional association with arterial stiffness and data from phases 9, 11 and 12 to test the prospective associations that can be seen in chapter 6.

3.4.5 Sociodemographic factors

3.4.5.1 Sex, age, ethnicity

Sociodemographic information is routinely collected in the study questionnaire. The study population includes males and females, age is calculated by the research team at the date of questionnaire completion and at each date of screening, rather than collected directly from asking participants. Information about ethnicity was assessed at baseline in 1985-88 and then reassessed at phase 3.

3.4.5.2 Physiological, body composition measurements and comorbidities

Physiological measurements related to arterial stiffness were relevant adjustments for some research hypotheses, especially in chapter 4. Systolic and diastolic blood pressure were measured at all the research clinics since recruitment. Mean arterial

pressure and heart rate were used for adjustment in models using cf-PWV as an outcome.

Body composition measurements at phases 9 and 11 were also used. Body Mass Index (kg/m^2) was calculated using the formula of (weight/height^2). Waist circumference was measured with a tape measure according to the study protocols.

Levels of total cholesterol measured in mmol/l of participants at the clinics of phase 9 and 11 were used in the adjustments of the models in chapter 4. For the calculation of the 10-year ASCVD score, the analyses of MACE events in chapter 5 and estimating the improvement in predictive ability provided by cf-PWV, total cholesterol was transformed to mg/dl.

The history of CVD (myocardial infarction, coronary heart disease, stroke), coded as a categorical variable (yes/no), before baseline (phase 9) was used in chapter 5 to assess the improvement in prediction brought by cf-PWV in low and intermediate risk participants.

Marital status, a categorical variable (yes/no), and mini-mental score were used as adjustments for models assessing the risk of non-cardiovascular and all-cause mortality in chapter 5.

3.4.5.3 Health behaviours

Health behaviours were used as adjustments in chapters 4 and 5. Smoking was included as a categorical variable (current smoker, never smoker, ex-smoker), alcohol drinking was tested both as a categorical variable (yes, no) and as a continuous measure of number of units consumed during the last week.

Chapter 4 Aims and objectives

4 Aims and objectives

The results from the review chapter showed that there are inconsistencies in the evidence assessing the bidirectional relationship between the progression of arterial stiffness and hypertension, with few studies with the sample size of Whitehall II being able to show differences within key subgroups.

In addition, the review updated the summary of evidence in arterial stiffness and cardiovascular risk, showing a lack of studies assessing the improvements in cardiovascular risk models using repeated measurements of arterial stiffness compared to individual measurements. Finally, the literature review also showed a lack of evidence in the prospective association between vascular ageing and markers of physical functioning. Taking these knowledge gaps into account, the overall aim of this thesis is to investigate the reciprocal association of arterial stiffness and blood pressure, and to test arterial stiffness as a potential predictor for stroke, mortality and physical function in the Whitehall II study. The individual aims and objectives for each chapter are as follows:

Chapter 5. Bidirectional relationship between blood pressure and arterial stiffness

Aim: To study the bidirectional association between blood pressure and arterial stiffness progression in participants of the Whitehall II study.

Objective 5.1: To assess the association between incident hypertension and arterial stiffness categories

Objective 5.2: To investigate the longitudinal relationships between blood pressure control categories and arterial stiffness progression.

Chapter 6. Arterial stiffness, major adverse cardiovascular events and mortality in the Whitehall II study.

Aim: To study the association between arterial stiffness and the risk of Major Adverse Cardiovascular Events in the Whitehall II study.

Objective 6.1. Determine the risk of Major Adverse Cardiovascular Events (MACE)according to carotid-femoral pulse wave velocity in the Whitehall II study.

Objective 6.2. To test whether adding a second measure of pulse wave velocity improves prediction models for MACE.

Objective 6.3. Study the predictive value of pulse wave velocity on non-cardiovascular mortality and all-cause mortality in participants of the Whitehall II study.

Objective 6.4. To investigate the role of antihypertensive medication as an effect modifier on the relationship between cf-PWV and MACE.

Chapter 7. Arterial stiffness and change in physical functioning markers in the Whitehall II study.

Aim: To reproduce the cross-sectional association between arterial stiffness and physical functioning and to test the longitudinal associations between arterial stiffness, as measured by carotid-femoral Pulse Wave Velocity, and physical functioning related outcomes.

Objectives

Objective 7.1: Assess the cross-sectional relationship between cf-PWV and markers of physical functioning.

Objective 7.2: Assess the relationship between cf-PWV at baseline, change in cf-PWV and change in markers of physical functioning.

Objective 7.3. To estimate the relationship between cf-PWV and physical functioning in subgroups with target organ damage (chronic kidney disease, history of major adverse cardiovascular events, stroke).

Chapter 5 Bidirectional relationship between arterial stiffness and blood pressure in the Whitehall II study

5

5.1 Introduction

Age-related transformations take place in all body systems, including the circulatory, respiratory, and musculoskeletal system. Among these age-related transformations, there are structural and functional changes in conduit arteries, particularly the aorta, which may lead to progressive stiffening. Arterial stiffness (AS) has been linked with raised cardiovascular disease (CVD) risk, [1] poor age-related physical function [2] and end-organ damage. [3] Higher pulsatility in brain vessels, as consequence of a rigid aorta, could be a contributing factor to cognitive decline and dementia. [4]

The pathological processes underlying aortic stiffening are being studied. Rigidity appears to be the consequence of arteriosclerotic changes in the arterial wall, particularly collagen cross-linking, [5] elastin structural degradation [6] and vascular inflammation. [7] There may be a vicious cycle between increasing blood pressure and arterial stiffening, which contributes to the progressive changes in arterial morphology and physiology that define AS. The interaction between risk factors, health behaviours and altered biological mechanisms can increase or delay the process of arterial stiffening. [8]

There are some key epidemiological aspects in the epidemiology of CVD, age and AS. Firstly, the global population is becoming older: the proportion of individuals older than 60 years is estimated to increase from 10% in 2015 to over 20% by 2050. [9] Secondly, CVD incidence and mortality is declining in developed countries with result that an increasing proportion of older people may in future live with a mild or moderate degrees of cardiovascular damage, including aortic stiffness, for many years.[9]

The incidence of major cardiovascular outcomes and the mortality risk from

cardiovascular causes is well predicted by arterial stiffness. [10] This has been described for cardiovascular outcomes such as stroke, myocardial infarction and ischaemic heart disease. [11] AS is also associated with cardiovascular risk factors, such as low physical activity [12], adiposity and overweight, in addition to being a correlate of unhealthy ageing, frailty, and higher rates of disability. [13] The CVD mortality risk and the incidence of major CVD outcomes such as stroke, myocardial infarction and ischaemic heart disease is well predicted by arterial stiffness.[10,11] AS is also associated with cardiovascular risk factors, such as low physical activity, [12] adiposity and overweight, in addition to being a correlate of unhealthy ageing, frailty, and higher rates of disability. [334] The biological plausibility of the association between CVD and AS is explained by mechanisms such as the progressive fracture of elastin fibres, successive collagen replacement and telomere shortening. [14] These are shared arterial stiffening mechanisms in CVD and ageing found in different cells and tissues.

Different methods for assessing AS have been developed over time. Pulse pressure and Osler's manoeuvre were among the first clinical demonstrations of arterial stiffness. In addition to clinical methods, a variety of techniques based on ultrasonography, magnetic resonance imaging (MRI) or arterial tonometry have been used in research and clinical practice. Carotid-femoral pulse wave velocity (cf-PWV) is a technique based on arterial tonometry. [15] This method measures the travel speed of the pressure pulse wave along an arterial segment of known length. [16] Cf-PWV is a non-invasive method and is currently used as the gold standard for assessment of AS. Characteristics like cost, technical requirements, time, or the questions being addressed, restrict different techniques to clinical or research settings.

Studying the role that PWV plays as a mediator of cardiovascular risk factors and major cardiovascular events can eventually lead to a better understanding of different ways to delay arterial stiffening, ultimately reducing the incidence of CVD and its major outcomes. [17]

This thesis aims to examine the association of PWV with blood pressure, major cardiovascular outcomes, and physical functioning. The scope of this chapter is to cover the first of those aspects, with a special focus on the influence of blood pressure control in the progression of arterial stiffness.

5.2 Objectives

The objectives of this chapter are to:

1. To assess the association between incident hypertension and arterial stiffness categories
2. To investigate the longitudinal relationships between blood pressure control categories and arterial stiffness progression.

5.3 Rationale:

The bidirectional relationship between arterial stiffness and hypertension has not been consistently described in the published literature. This study will show an association between arterial stiffness at baseline and incident hypertension, the higher the arterial stiffness in a subgroup of normotensive individuals, the more likely that this group of individuals is going to develop hypertension at the next follow-up. On the opposite side of the association, the group of participants with established hypertension is going to develop arterial stiffness at a higher rate. The status of treatment of hypertension, as well as the groups of antihypertensive medication, will modify this association.

5.4 Methods

This section will show the methods used for objectives 5.1 and 5.2. Specific details about the categorization of blood pressure and cf-PWV when used as outcomes or exposures for these objectives are explained in sections 5.6 and 5.7.

5.5 Population

Data from participants of the Whitehall II study were used to address the aims and objectives covered in this chapter. The Whitehall II study is an ongoing cohort that was set up to investigate the socioeconomic gradients in mortality, as well as mental and physical disease. Recruitment for the study took place between 1985 and 1988, with civil servants from twenty departments and a target population aged between 35 and 55. The response rate was 73% (74% among men, 71% among women), and after exclusion of those individuals who were ineligible, a final sample size of 10,308 men (67%) and women (33%), was achieved. Participants were asked to complete self-administered questionnaires every 2-3 years and attend clinical assessments every 4-5 years. Clinical data are available from screening examinations completed in 1991/93, 1997/99, 2003/04, 2008/09, 2012/13 and 2015/16.

5.6 Blood pressure measurement

Blood pressure measurements were administered at every clinic visit and followed the study protocols. With the patient in supine position, after resting for 10 minutes and with the upper right arm exposed, systolic blood pressure and diastolic blood pressure readings in millimetres of mercury were obtained using the oscillometric sphygmomanometer Omron HEM 907. Different cuffs were used according to the size needed by the patient. Two different readings were taken with an interval of 1 minute between them. In the case of a difference greater than 10 mm Hg between the two measurements, a third measurement was obtained. Mean arterial pressure in millimetres was calculated using the formula: ((SBP +2(DBP))/3 The adopted

hypertension definition was from the European Guidelines of Hypertension, defined by either a SBP ≥140 mm Hg, a DBP ≥90 mm Hg or the current use of an antihypertensive medication. [335]

Information about history of hypertension was obtained using the Whitehall II questionnaire. Information regarding antihypertensive medication was gathered through the study questionnaire, where details of medicines taken during the last fourteen days were obtained. To be classified as an antihypertensive drug, the prescription should be done by a doctor and the reason for taking the medication should be written as hypertension. The class of hypertensive agent was also recorded.

Recruited civil servants gave consent to participate and the University College London ethics committee approved this protocol.

5.7 Carotid-femoral Pulse wave velocity (cf-PWV)

The measurement of arterial stiffness was introduced in the Whitehall II study during the clinical examination at the phase 9 clinic in 2008/09 (n=4347) and was re-measured in the clinics at phase 11 in 2012-13 (n=4485). Carotid femoral PWV measures were taken using the SphygmoCor (AtCor) device. As explained in section 3.3.2, this model measures carotid femoral Pulse Wave Velocity using two tonometers, one placed in the carotid artery and other placed in the femoral artery. According to study protocols, the distance of the segment between the carotid and femoral site (path length), was assessed using a tape measure. After this measure, study personnel registered the PWV results, taking at least two different measurements for verifying repeatability. In the case where a difference ≥ 0.5 m/s was found between the first two measurements, a third reading was taken. The mean of the total of measurements taken was used as the final PWV figure. Study

personnel performed the measurements with standardized methods for carotid-femoral PWV (AtCor, Australia [336]).

5.8 Study population for objective 5.1

This objective intends to investigate the association between arterial stiffness category and incident hypertension. Participants were included if they participated in the PWV assessment at phase 9 and, had systolic blood pressure lower than 140 mm Hg and systolic blood pressure lower than 90 mm Hg at the phase 9 clinic and had no history of hypertension or antihypertensive medication from phases 1 to 7. Figure 4 shows the selection process of the eligible sample according to history of antihypertensive medication or blood pressure in phases 1 to 9.

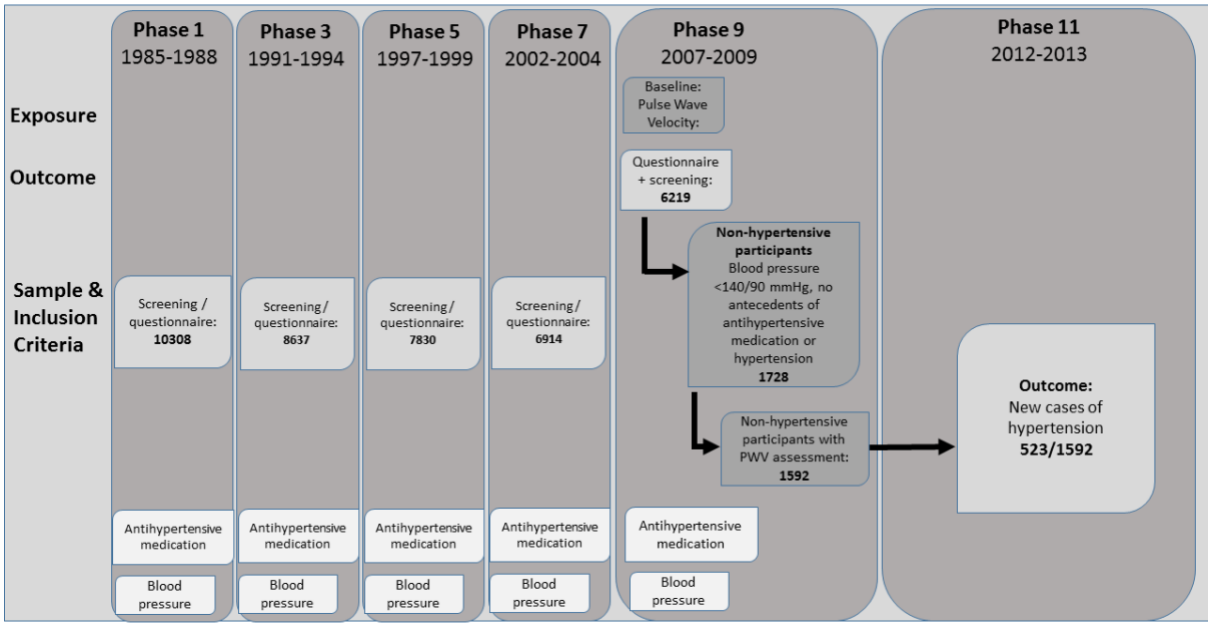


Figure 4. Sample selection for objective 5.1

5.9 Study population for objective 5.2 and definition of blood pressure control categories

This objective investigates the association between baseline blood pressure control category and 5-year change in cf-PVW in the Whitehall II study. Blood pressure control categories at phase 9 were created according to blood pressure measurements at the clinic for this phase, history of hypertension and history of antihypertensive treatment since the start of the study. Four blood pressure control categories were created. First, participants with no record of antihypertensive medication use across study phases 1 to 9 and who have no record of systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg were categorised as normotensive untreated. Participants who have had any hypertension diagnosis, received antihypertensive medication, or been measured as having raised blood pressure (≥140/90mm Hg) between study phases 1 to 8 and had blood pressure ≤140/90mm Hg at phase 9 were categorised as controlled hypertensives. Participants with blood pressure ≥ than 140/90 mm Hg at phase 9 clinic, with no previous history of hypertension or antihypertensive treatment, were categorised as non-treated hypertensives. Finally, participants receiving antihypertensive medication who had blood pressure at the phase 9 clinic ≥140/90 mm Hg currently receiving antihypertensive treatment were categorised as uncontrolled hypertensives.

Carotid-femoral PWV measurements were first included in the screening examinations at phase 9, (2008-2009). A total of 5,348 participants had at least one PWV measurement. After exclusion of participants with missing information in covariates or medication information, the final sample size was 4,998. According to tests of hypothesis (not shown), no significant differences in the exposure or outcome between the excluded group and the rest of the participants were found.

Figure 5 shows the collection dates, sample sizes and the correspondent phases at which relevant information for this objective was collected.

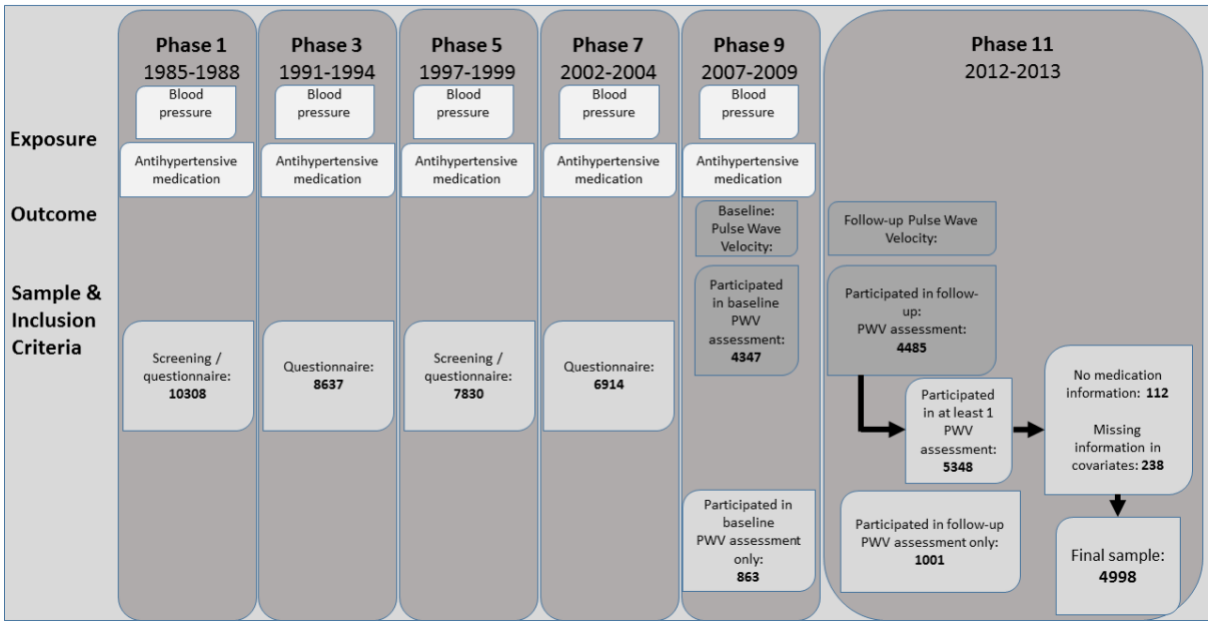


Figure 5. Sample selection for objective 5.2, progression of arterial stiffness according to baseline blood pressure control

5.10 Statistical analysis

5.10.1 Statistical Methods objective 5.1 Incident hypertension according to arterial stiffness category

A descriptive analysis was conducted for the eligible sample at phase 9. Subsequently, a logistic regression model was fitted using the STATA command *logistic* with incident hypertension at phase 11 as the outcome and cf-PWV as the exposure of interest. AS was used as a continuous and categorical variable in two different sets of models. The models were used to study the association between

arterial stiffness and the onset of hypertension 5 years later. A model adjusted with age, sex, and ethnicity was fitted initially, with incident hypertension at phase 11 as dependent variable. Stepwise variable selection was performed afterwards, including relevant sociodemographic variables, anthropometric measures, comorbidities and medication for comorbidities, as well as health behaviours which were added to subsequent models.

5.10.2 Statistical Methods for objective 5.2. Blood pressure control category and arterial stiffness progression.

A descriptive analysis showed the number of hypertensive participants from phase 1 to phase 11. Descriptive analyses were also conducted for the number of participants according to blood pressure control in the same phases and for the selected sample at phase 9. Multiple linear regression models were fitted to model the cross-sectional relationships between PWV and risk factors. Relevant covariates for fitting the model were chosen after the literature review. The basic model included age, sex, ethnicity, PWV, heart rate and mean arterial pressure. Afterwards, different comorbidities such as hypertension, hypercholesterolemia, or diabetes, among others, were included in the analysis. Health behaviours such as tobacco, alcohol consumption and physical activity were included in the final cross-sectional model. Tests for trends of linearity and departure from linearity were performed using likelihood ratio tests. The differences in PWV according to each category were estimated using the Stata command *margins*. The 5-year increase was calculated with a linear mixed model with an interaction term between the characteristic at the baseline and the time variable.

For assessing the association between 5-year increase in PWV and blood pressure control category, linear mixed effects models were used using the STATA command *mixed*. First, the rate of arterial stiffening was investigated building a basic model without covariates.

In its simplest form, ignoring possible risk factors, the linear mixed effects regression model for the measurement of outcome Y, on individual i, at occasion k, is given by:

$$Y_{ik} = \beta_0 + \beta_1 t_{ik} + v_{0i} + v_{1i} t_{ik} + \varepsilon_{ik}$$

Where β_0 = Overall population intercept

β_1 = Overall population slope with time

v_{0i} = Random effect of intercept for the i^{th} subject

v_{1i} = Random effect of slope for the i^{th} subject

t_{ik} = time of the k^{th} measurement for the i^{th} subject

ε_{ik} = error, assumed to be normally and independently distributed with mean 0 and common variance σ^2.

In the above model, the first two terms of the model are the fixed part of the model and the third and fourth terms are the random part of the model. The random part of the model allows each observation to have their own intercept and slope with time. In the present data, the outcome, Y, is pulse wave velocity which is measured just at two time points (k=2).

Now, focussing only on the fixed part of the model, we can extend the above model to incorporate the effect of a single risk factor on the outcome, as follows:

$$Y_{ik} = \beta_0 + \beta_1 t_{ik} + \beta_2 (\text{risk factor})_i + \beta_3 (\text{risk factor})_i * t_{ik}$$

In this model, the coefficient β_2, shows the cross-sectional association of the risk factor with the outcome, pulse wave velocity, at baseline. The term β_1 shows how the outcome, pulse wave velocity, changes with time and β_3 shows how this change with time is modified by levels of the risk factor, which was measured at baseline. In the current analysis, the risk factor of interest is blood pressure control category (BPCC) which is treated as a categorical variable. The basic model is then:

$$Y_{ik} = \beta_0 + \beta_1 t_{ik} + \beta_2 (BPCC)_i + \beta_3 (BPCC)_i * t_{ik}$$

Where HCC may be described by three variables comparing the blood pressure control categories with the normotensive untreated baseline category.

Given that PWV is closely related with mean arterial pressure and heart rate as part of the constitution of the measure, those two covariates were included in the models as time-varying covariates. The remaining covariates were included as time-invariant covariates for all the 5-year change models.

This model can be extended by adding in further risk factors into the model. Successive models were built including groups of risk factors, such as comorbidities or drugs for comorbidities, body constitution, and health factors.

$$Y_{ik} = \beta_0 + \beta_1 t_{ik} + \beta_2 (BPCC)_i + \beta_3 (BPCC)_i * t_{ik} + \beta_4 (covariate)_I + \beta_5 (covariate)_I * t_{ik}$$

Where the covariate terms may include terms for (i) body composition, (ii) comorbidity control and (iii) health behaviours.

For assessing the effect of age on the association between blood pressure control category and 5-year progression of PWV, an interaction term of age with time was added to the final model. Interactions with age were tested using likelihood ratio test. The analysis were carried out using Stata 16.0 (Stata Corp, College Station, Texas).

5.11 Results

5.11.1 Incident hypertension according to baseline cf-PWV

The selected sample for the assessing objective 5.1; consisted of 1728 participants with blood pressure ≤ 140/90 mm Hg and no history of hypertension from the beginning of the study. The 1,728 participants had their hypertension status checked at follow-up 5 years later (phase 11). As 136 participants were excluded due to missing observations on the exposure variable (cf-PWV), the final sample for the analysis was reduced to 1592. 1,145 out of the 1,592 were men and 447 women. The characteristics of the population sample included at phase 9, stratified by sex are shown in Table 11. At baseline, the mean age in men from the selected sample was not significantly different. The proportion of participants of non-white ethnicity was below 7% in both sexes. Mean SBP, DBP, MAP and waist circumference were higher in men. There were no significant differences in the BMI index between men and women, although men had a higher mean waist circumference. The prevalence of stroke, diabetes or the frequency of alcohol units consumed the last week between the two groups did not differ significantly between sexes. Mean pulse wave velocity at baseline and the proportion of new cases of hypertension at follow-up were higher in men

Table 11. Baseline characteristics of study sample at phase 9

Characteristic	Men (n=1145)	Women (n=447)
	% / Mean (SD)	% / Mean(SD)

Age (years)	64.2 (5.4)	63.7 (5.3)
Non-white ethnicity (%)	5.3	7.4
Systolic blood pressure (mm Hg)	121.9 (9.3)	116.7 (11.4)*
Diastolic blood pressure (mm Hg)	67.6 (7.2)	65.7 (8.3)*
Heart rate at baseline (bpm)	61.5 (10.8)	64.3 (11.0)*
Mean arterial pressure (mm Hg)	85.8 (7.2)	82.7 (8.5)*
Mean pulse wave velocity (m/s)	7.85 (1.6)	7.56 (1.5)*
Body Mass Index	25.3 (3.2)	25.3 (4.5)
Mean waist circumference (cm)	92.3 (9.3)	82.9 (10.9)*
Diabetes Mellitus type II (%)	4.8	5.6
Stroke (%)	0.4	0.5
Blood Cholesterol (mmol/l)	5.3 (0.9)	5.7 (1.0)*
Smoker (%)	6.4	4.3
Number of alcohol units last week	11.8 (11.1)	6.4 (7.2)*
Lipid lowering drugs (%)	18.3	16.3
Low physical activity level (%)	20.4	28.6*
Incident hypertension at follow-up (%)	35.0	27.3*

*Statistically significant differences compared to men

Mean pulse wave velocity in the selected sample, at baseline was 7.7 m/s (S.D.: 1.6) and ranged from 4.2 to 15.6 m/s. PWV was taken as being normally distributed, although there was some skew to the right (Figure 6).

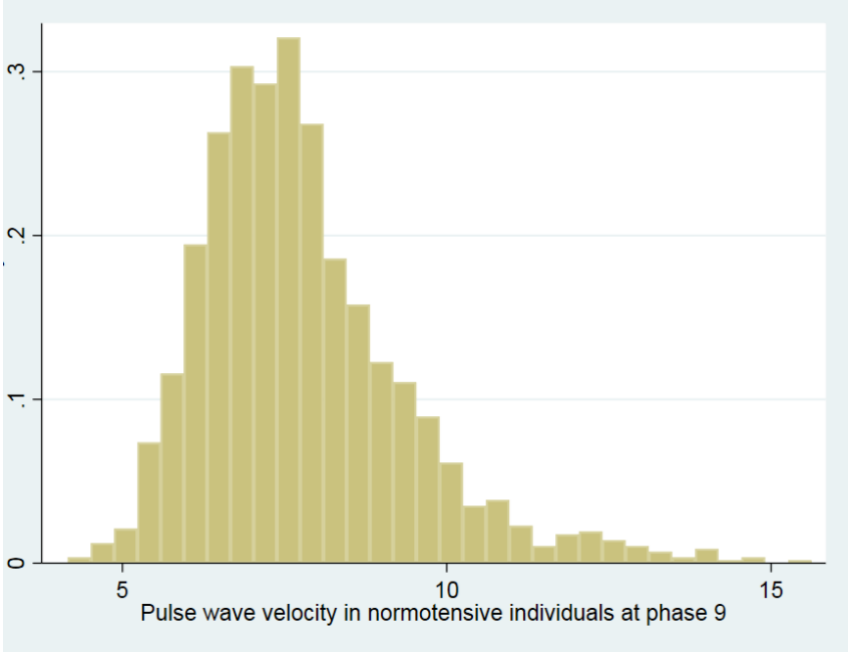


Figure 6. Distribution of pulse wave velocity among normotensive participants at phase 9

Mean SBP was 120.5 mm Hg (SD = 10.2). The spread in the selected sample for objective 2 was 80 to 139. The distribution was skewed to the left. As the range of systolic blood pressure is limited according to the criteria for the definition of hypertension of the European Guidelines for the management of arterial hypertension, the distribution of blood pressure for this subsample is truncated at 140 mm Hg (Figure 7).

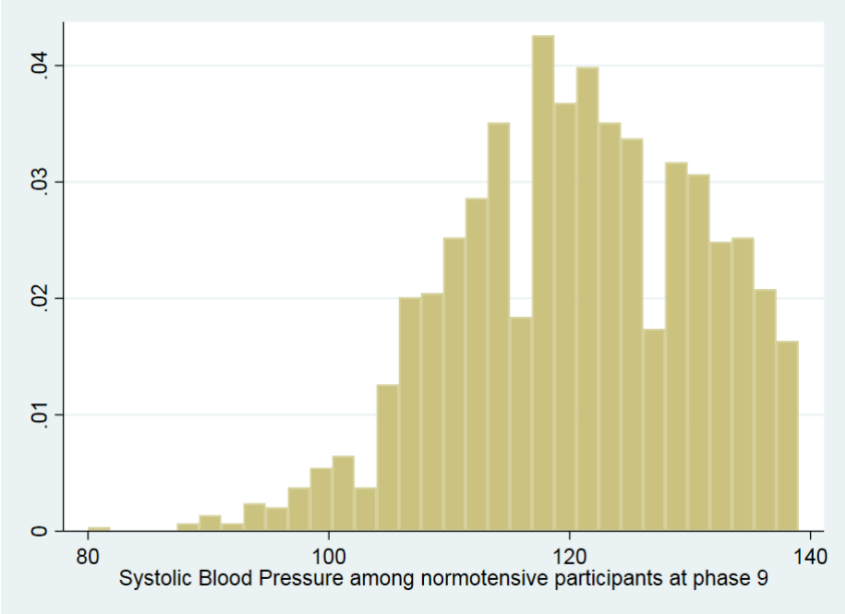


Figure 7. Distribution of systolic blood pressure among normotensive participants at phase 9

Figure 8 shows that the prevalence of hypertension (defined at each study phase as SBP/DBP≥140/90 or on antihypertensive medication) in the Whitehall II Study increased from 17.8% in phase 1 to 44% at phase 11.

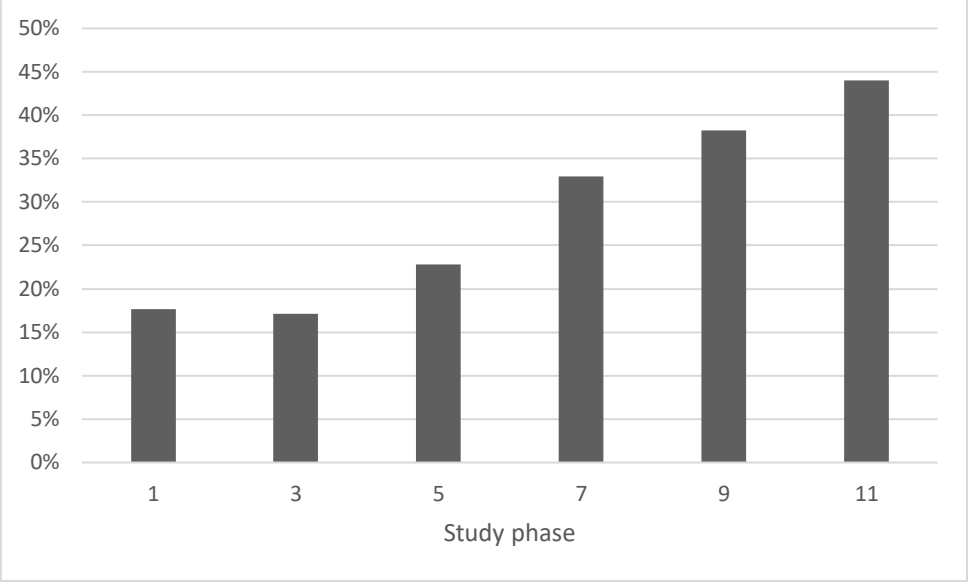


Figure 8. Prevalence of hypertension by phase in the Whitehall II study

The proportion of participants in each of the of blood pressure control categories within the sample is shown in *Figure 9*. It shows that the proportion of individuals with blood pressure below 140/90 or antecedents of blood pressure varied from 85% in phase 1 to 23.5% in phase 11. The proportion of treated hypertensives varied from

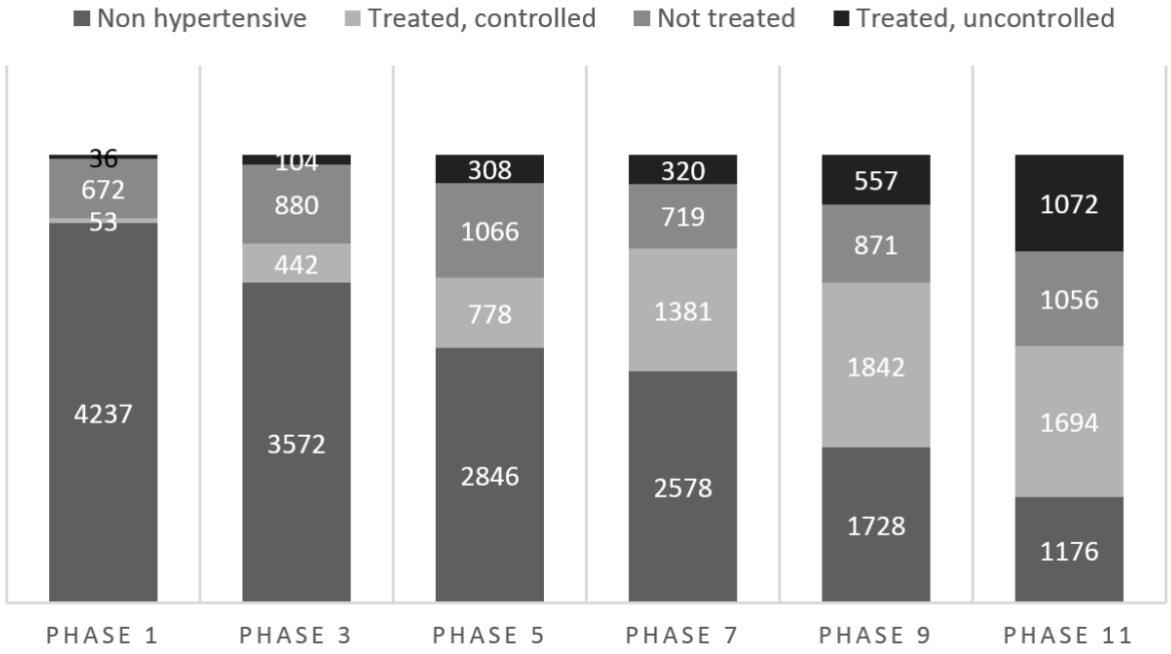


Figure 9.Evolution of blood pressure control categories from phase 1 to 11 in the selected sample

1.1% to 33.9% in the same period. The proportion of individuals with uncontrolled blood pressure despite receiving antihypertensive treatment rose from 0.7% at the cohort recruitment (phase 1), to 21.4% at follow up from this chapter (phase 11).

5.11.2 Association between arterial stiffness at baseline and incident hypertension at follow-up

Higher values of cf-PWV at baseline (phase 9) among normotensive participants, were associated with higher odds of incident hypertension at follow-up 5 years later (phase 11). Highest to lowest tertile of cf-PWV was significantly predictive of the progression to hypertension in normotensive untreated elder individuals (OR: 1.72; 95% CI 1.33-2.23) in models adjusted for age, sex, ethnicity, mean arterial pressure and heart rate. The association attenuated but remained after adjustment for BMI, waist circumference, metabolic and lifestyle confounders. After adjusting for systolic blood pressure, the odds of hypertension for the highest tertile remained different compared to the lowest tertile (OR: 1.60; 95% CI 1.15-2.22) (**Error! Reference source not found.**).

An additional series of models using cf-PWV as a continuous variable were fitted. The odds of incident hypertension are 1.19 per each metre per second of cf-PWV in the age, sex, ethnicity MAP and HR model (**Error! Reference source not found.**). The association between cf-PWV as a continuous variable and incident hypertension was not attenuated after adjusting for measurements of body composition and (models 2 and 3, **Error! Reference source not found.**) and weekly level of physical activity.

Table 12. Incident hypertension at phase 11 in non-hypertensives at phase 9

	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
PWV at phase 9 (n=1592)	Model 1		Model 2		Model 3	
Lowest tertile (PWV 4.15-7.4)	Ref.		Ref.		Ref.	
Middle tertile (PWV 7.41-8.91)	1.72 (1.33, 2.23)	<0.001	1.65 (1.27, 2.15)	<0.001	1.29 (0.98, 1.70)	0.069

Highest tertile (PWV 8.91-15.6)	2.49 (1.83, 3.37)	<0.001	2.38 (1.74, 3.26)	<0.001	1.60 (1.15, 2.22)	<0.01
PWV-Effect per 1 m/s	1.27 (1.18, 1.38)	<0.001	1.26 (1.17, 1.36)	<0.001	1.14 (1.05, 1.14)	<0.001

* Model 1 adjusted by age, sex, and ethnicity; Model 2 adjusted by model 1 + waist circumference and body mass index, blood cholesterol, diabetes, smoking status, alcohol units and lipid lowering drugs; Model 3 adjusted for model 2 + systolic blood pressure

5.11.3 Association between blood pressure control categories and arterial stiffness

5.11.3.1 Cross-sectional associations between sociodemographic factors and PWV

Before examining the relationships between blood pressure control and arterial stiffness, the associations between the baseline covariates measured at Phase 9 with the blood pressure control categories and with arterial stiffness were examined (**Table 13**). The proportion of men was higher in the group of non-controlled hypertensive participants than in the rest of the groups. Also, those participants of non-white ethnicity, with diabetes and individuals with a history of stroke were more likely to be in the uncontrolled hypertension category. Individuals with lower concentrations of blood cholesterol and higher body mass index tended to be in this group as well. Regarding body composition, waist circumference and body mass index tended to be higher in this group. In relation to lifestyle factors, the non-controlled hypertensive group had lower levels of physical activity, and more alcohol consumption. Regarding other factors of blood pressure control, the group of non-controlled hypertensives tended to have high blood pressure in a higher number of clinic visits, as well as a higher number of prescribed antihypertensive drugs, than the controlled hypertensive group.

The levels of arterial stiffness both at baseline and follow-up will be described as mean cf-PWV, rather than prevalence of arterial stiffness. Due to the lack of normal values for the population, defining a given threshold as prevalence would be arbitrary. The mean cf-PWV in the sample of 4998 participants at baseline was 8.4 m/s (SD= 2.0). In the group of normotensive participants, the baseline mean cf-PWV

was 7.8 metres per second (SD = 1.6). The baseline means of arterial stiffness were higher in all the other categories and can be seen in Table 13.

Table 13. Characteristics of the sample at phase 9 by level of Blood pressure control (n=4998)

Characteristic		Normotensive untreated (n=1728 except where given)	Controlled hypertensives (n=1842 except where given)	Untreated hypertensives (n=871 except where given)	Non-controlled hypertensives (n=557 except where given)
		% / Mean(SD)	% / Mean(SD)	% / Mean(SD)	% / Mean(SD)
Sex	Male	71.5	74.2	74.9	76.5
	Female	28.5	25.8	25.1	23.5*
Age		64.0(5.4)	66.0 (5.8)*	65.5 (5.6)*	67.7 (5.92)*
Ethnicity (n=4,998)	White	94.1	91.0	94.1	87.4
	Non-white	5.9	8.9	5.9	12.6
Systolic blood pressure (n=4,523)		120.5 (10.2)	123.6 (10.1)*	150.7 (10.5)*	149.3 (9.3)*
Diastolic blood pressure (n=4,523)		67.2 (7.5)	68.4 (7.9)*	80.9 (8.5)*	78.6 (9.5)*
Heart rate at baseline (n=4,194)		62.3(10.9)	63.4(10.9)*	67.1 (11.2)*	65.3 (11.9)*
Mean arterial pressure (n=4,523)		84.9 (7.7)	86.8 (7.9)*	104.2 (7.6)*	102.2 (7.8)*
Pulse Wave Velocity (n=4,194)		7.8(1.6)	8.5 (2.0)*	9.4 (2.1)*	9.9 (2.3)*
Body Mass Index		25.4 (3.7)	26.9 (4.0)*	26.5 (4.1)*	27.6 (4.4)*
Waist circumference (cm)		89.9 (10.9)	94.4 (11.3)*	93.9 (11.7)*	96.3 (11.5)*
Diabetes		5.0	14.5*	9.9*	21.7*
Stroke	Yes	0.6	2.5*	1.8*	4.9*
Blood Cholesterol (mmol/l)		5.4 (0.9)	5.0 (1.1)*	5.5 (1.0)	4.8 (1.1)*
Smoking status (n=4,995)	No	89.6	86.5	87.7	87.3
	Yes	10.4	13.5	12.3	12.8
Number of alcohol units last week		10.2 (10.2)	11.1 (15.6)*	11.7 (13.9)	10.7 (12.5)*
Lipid lowering drugs	Yes	17.7	42.5*	22.4*	56.0*
Physical activity level	High level	59.3	56.2	57.8	56.2
	Medium level	17.8	17.7	17.7	17.9
	Low level	22.9	26.1*	24.5	25.9
Number of phases with HBP		0	1.4 (1.0)+	1.9 (1.1)*+	2.8(1.2)+
Number of antihypertensive drugs		0	0.98 (0.98)+	0	1.7(0.8)+

*Significant difference with the normotensive untreated group. +Compared with the controlled hypertensive group

Regarding the cross-sectional association, results from the age, sex and ethnicity, heart rate and mean arterial pressure models showed a progressive increase in PWV per each additional year Specific results can be seen in the left-hand column of Table 14. Analysing age as a continuous variable, showed an increase in 0.14 m/s per year of age. A mean cross-sectional difference of 0.5 m/s in PWV was found in participants with prescription of antihypertensive drugs.

The group of participants with a type 2 diabetes mellitus diagnosis had a mean cross-sectional difference of 0.7 m/s than participants with no diabetes diagnosis. Body composition measured by body mass index or waist circumference showed a higher PWV. Current use of lipid lowering drugs meant a higher PWV in 0.29 m/s, as well as the use of antihypertensive drugs. Sex female and higher tertiles of blood cholesterol showed lower mean PWV by 0.18 m/s and 0.11 m/s than males and individuals in lower tertiles, respectively. The effect of age was 0.13 m/s per year.

5.11.4 Longitudinal associations between sociodemographic factors and PWV

In a sex, age, ethnicity, mean arterial pressure and heart rate model, continuous age showed a 5-year increase of 0.02 m/s per each year of age. The right-hand column of Table 14 shows that participants experience, on average, a 5-year increase in PWV of 0.05 m/s per year of age. Using 3 or more hypertensive drugs was associated with a 5-year increase of 0.29 m/s. The use of 1 or 2 classes of antihypertensive drugs was associated with higher PWV increase as well.

Table 14. Associations between sociodemographic, health factors and health behaviours and pulse wave velocity at baseline (2008-2009) and 5-year change in pulse wave velocity

Characteristic (individual association)	Pulse Wave Velocity at baseline (2008-2009)*			5-year increase in Pulse Wave Velocity (2012-2013)+		
	Mean	**Difference (95% CI)**	**P-value**	**Mean**	**Increase (95% CI)**	**P-value**
Age (per additional year of age)	0.14	--	<0.001	0.05	--.	<0.001
Sex						
Male	8.66	Ref.		0.50	Ref.	
Female	8.47	-0.18 (-0.27, -0.10)	<0.001	0.43	-0.07 (-0.23, 0.09)	0.37
Ethnicity						
White	8.60	Ref.		0.44	Ref.	
Non-white	9.02	0.42 (0.28, 0.56)	<0.001	0.59	0.27 (0.002, 0.53)	0.05
BMI						
Lowest third	8.31	Ref.		0.35	Ref.	
Second third	8.49	0.18 (0.07, 0.31)	0.001	0.39	0.04 (-0.12, 0.20)	0.6
Upper third	8.67	0.36 (0.23, 0.49)	<0.001	0.74	0.39 (0.22, 0.56)	<0.001
Average waist circumference						
Lowest third	8.20	Ref.		0.25	Ref.	
Second third	8.55	0.34 (0.22, 0.47)	<0.001	0.51	0.16 (-0.001, 0.32)	0.05
Upper third	8.71	0.51 (0.37, 0.64)	<0.001	0.82	0.57 (0.40, 0.73)	<0.001
Diabetes						
No	8.40	Ref.		0.42	Ref.	
Yes	9.15	0.74 (0.58, 0.91)	<0.001	0.88	0.46 (0.24, 0.69)	<0.001
Stroke						
No	8.48	Ref.		0.46	Ref.	
Yes	8.67	0.19 (-0.21, 0.59)	0.36	0.93	0.47 (-0.06, 0.99)	0.08
Blood cholesterol						
Lowest third	8.60	Ref.		0.67	Ref.	
Second third	8.36	-0.25 (-0.37, -0.12)	<0.001	0.40	-0.27 (-0.43, -0.11)	<0.001
Upper third	8.49	-0.11 (-0.2, -0.01)	0.09	0.33	-0.34 (-0.50, -0.18)	<0.001
Smoking						
No	8.48	Ref.		0.46	Ref.	
Yes	8.56	0.08 (-0.16, 0.31)	0.37	0.68	0.22 (-0.10, 0.53)	0.18
Occasional	8.49	0.01 (-0.44, 0.47)	0.94	0.65	0.19 (-0.42, 0.79)	0.61
Alcohol consumption						
No	8.46	Ref.		0.71	Ref.	
Yes	8.59	-0.13 (-0.27, -0.15)	0.08	0.38	-0.33 (-0.51, -0.16)	<0.05
Lipid drugs						
No	8.39	Ref.		0.36	Ref.	
Yes	8.69	0.29 (0.18, 0.40)	<0.001	0.72	0.36 (0.21, 0.51)	0.001
Number of antihypertensive drugs						
0	8.37	Ref.		0.36	Ref.	
<= 2	8.72	0.36 (0.28, 0.44)	<0.001	0.72	0.29 (0.14, 0.45)	<0.001
>= 3	8.87	0.50 (0.32, 0.69)	<0.001	1.24	0.88 (0.53, 1..238)	<0.05
Physical activity						
Low level	8.57	Ref.		0.49	Ref.	
Medium level	8.55	-0.02 (-0.17, 0.14)	0.84	0.43	-0.06 (-0.28, 0.15)	0.58
High level	8.44	-0.13 (-0.26, -0.006)	<0.05	0.47	-0.02 (-0.19, 0.15)	0.84

*Estimates are adjusted for age, sex, ethnicity, heart rate and mean arterial pressure + 5-year change models are adjusted for age, sex, ethnicity, mean arterial pressure and heart rate at follow-up

5.11.5 Cross-sectional association between blood pressure status category and PWV

The mean difference according to each blood pressure control category in an age, sex and ethnicity model and in successive changes to the model are shown in **Table 15**. The more relevant covariates in the model were the number of phases with hypertension, the number of classes of antihypertensive drugs, body mass index, alcohol consumption, lipid drug consumption. The left-hand column of Table 15 shows the cross-sectional relationship between baseline blood pressure control category and PWV. The age, sex and ethnicity model showed that compared to normotensive untreated individuals, mean PWV increase was 0.04 m/s (95% CI: -0.04,0.17 p:0.51) for controlled hypertensives, 0.20 m/s (95% CI: 0.06,0.35 p<0.001) untreated hypertensives and 0.25 (95% CI: 0.03,0.47 p<0.05) for uncontrolled hypertensives. After adjustments for adiposity measures, comorbidities and health behaviours in models 2 and 3, the association was not attenuated.

5.11.6 5-year change association between blood pressure control category and PWV

The effect of blood pressure control category on progression of arterial stiffness, as measured by PWV, is shown in the right-hand column of Table 15. A strong effect of blood pressure control category can be seen in the age, sex and ethnicity model. Compared to normotensive untreated participants, controlled hypertensives presented a 5-year increase of 0.25 m/s (95% CI 0.05-0.35). This effect was also showed in hypertensive untreated participants (0.49 m/s; 95% CI 0.27-0.36) and hypertensive uncontrolled participants (0.53 m/s; 95% CI 0.28-0.80), respectively.

After adjustment for baseline health behaviours, disease antecedents and body constitution, the association attenuated to insignificance for the untreated hypertensive category. The effect of the untreated hypertensive category in 5-year change in PWV was attenuated by one tenth in the fully adjusted model. The effect attenuated by one third in the uncontrolled hypertensive category, compared with the age, sex and ethnicity model.

The effect of age in the association between blood pressure control category and 5-year change in PWV was seen in additional models (not shown). After running a model with a timeXage interaction, a significant 5-year increase in PWV of 0.055 m/s per additional year of age was found (95% CI 0.04, 0.07). After comparing the rates of arterial stiffening between different tertiles of age at baseline, participants aged from 61 to 67 had an average 5-year increase in PWV of 0.23 m/s (95% CI 0.04, 0.07).

Table 15. Association of blood pressure control category with PWV at baseline and 5-year change in PWV

Blood pressure control category	Pulse wave velocity (m/s) at baseline (2008/2009)							5-year increase in pulse wave velocity (m/s) (2012-2013)					
	Model 1[1]		Model 2[2]		Model 3[3]		Person-Obs.	Model 1[1]		Model 2[2]		Model 3[3]	
Normotensive untreated	**Ref.**	**p.**	**Ref.**	**p.**	**Ref.**	**p.**		**Ref.**	**p.**	**Ref.**	**p.**	**Ref.**	**p.**
Hypertensive controlled	0.33 (0.25-0.42)	<0.001	0.25 (0.7-0.34)	<0.001	0.25 (0.16-0.33)	<0.001	8369	0.20 (0.05-0.36)	<0.05	0.16 (0.08-0.33)	<0.05	0.19 (0.03-0.35)	0.02
Hypertensive untreated	0.53 (0.38-0.68)	<0.001	0.49 (0.35-0.64)	<0.001	0.48 (0.34-0.63)	<0.001	8369	0.49 (0.27-0.69)	<0.001	0.49 (0.27-0.70)	<0.001	0.51 (0.29-0.73)	<0.001
Hypertensive uncontrolled	0.96 (0.79-1.12)	<0.001	0.84 (0.68-1.00)	<0.001	0.82 (0.66-0.99)	<0.001	8369	0.54 (0.28-0.80)	<0.001	0.44 (0.17-0.70)	<0.001	0.51 (0.24-0.77)	<0.001

[1]Adjusted for age, sex, ethnicity, mean arterial pressure and heart rate (Mean arterial pressure and heart rate are included as time-varying covariates) [2] Adjusted for model 1 plus smoking, physical activity, alcohol consumption and lipid lowering drugs [3]Model 5 plus diabetes, blood cholesterol, body mass index, waist circumference and stroke

5.11.6.1 Effect modification by social class

The role of social class was explored in the association between blood pressure control status categories and change in arterial stiffness. The results of these analyses can be seen in Table 16. The original association was not attenuated when employment grade, a proxy of social class, was used as an adjustment. After adjusting for age, sex, ethnicity and mean arterial pressure, the group of professional civil servants seemed to have a higher slope in arterial stiffening compared to civil servants in administrative and clerical positions. This association was not independent of health behaviours and body composition variables.

Table 16. Progression in arterial stiffness according to baseline blood pressure status and employment grade (n=4998)

Adjustments*	Increase in cf-PWV (m/s) per 5 years	p
Normotensive, untreated	Ref.	
Hypertensive, controlled	0.17 (0.13, 0.33)	0.033
Hypertensive, untreated	0.33 (0.07, 0.59)	<0.05
Hypertensive, non-controlled	0.43 (0.14, 0.73)	<0.01
Employment grade: administrative	Ref	
Employment grade: professional	0.19 (0.04, 0.34)	<0.05
Employment grade: clerical/support	0.13 (-0.13, 0.39)	0.34

*Model also adjusted for age, sex, ethnicity and mean arterial pressure

5.11.7 Discussion

This chapter aimed to study the association between blood pressure and progression in arterial stiffness in older individuals in the Whitehall II study. This includes the cross-sectional and the 5-year change in cf-PWV according to baseline blood pressure control category and the relationship between baseline arterial stiffness and incident hypertension. The findings presented here suggest that untreated hypertensives and uncontrolled hypertensives have a higher rate of arterial stiffening than normotensives. The preliminary results of the models investigating blood pressure progression according to arterial stiffness category suggested that

normotensive individuals with higher levels of arterial stiffness are more prone to develop hypertension. This is evidence of a bidirectional relationship between arterial stiffness and hypertension.

5.11.8 Baseline pulse wave velocity and incident hypertension

One of the objectives of this chapter was to investigate the bidirectional association between PWV and hypertension. The analysis performed for the first objective of the PhD thesis, which included analysis of an occupational cohort of elder individuals, showed that per each additional 1m/s higher PWV at baseline, there was a 1.19 higher odds of incident hypertension, independently of sociodemographic characteristics, comorbidities, and health behaviours. In addition, there was 1.7-fold increased odds for incident hypertension among individuals in the highest compared to lowest tertile of cf-PWV. The results support the hypothesis that normotensive individuals with higher arterial stiffness levels are more likely to progress to hypertension.

The result also provides evidence of a dose-response profile according to arterial stiffness and the potential for risk-stratification of normotensive untreated individuals. The finding is also coherent with physiological models explaining the potential role of arterial stiffness in the pathogenesis of hypertension.

The effect size that I found in this study was greater than the found in the study of Japanese workers (OR: 1.01), [246] although similar to the ones found in the Framingham study (OR: 1.30), and the ARIC Cohort (OR: 1.17) and smaller than the effect found in the Young Finns Study (OR: 1.96).

Regarding the statistical analysis, there are some differences between the analysis I used and those used by some researchers in the published papers. Ignoring the autocorrelation of repeated measures by using a conventional logistic regression

model instead of a mixed-effects logistic regression model, could result on overestimating the effect size and underestimating standard errors. [337]

The evidence reviewed and meta-analysed in this chapter shows an association between higher levels of arterial stiffness and higher odds of incident hypertension in individuals with normal blood pressure. All the meta-analysed studies addressing this question showed increased odds for becoming hypertensive, resulting in a pooled OR of 1.23 for incident hypertension among 10,677 normotensive untreated included individuals.

5.11.9 Baseline blood pressure category and 5-year change in arterial stiffness

The goal for the second objective of this chapter was to investigate the association between baseline blood pressure control category and the change in arterial stiffness at follow-up. This analysis showed that hypertension control status was associated with higher arterial stiffness at baseline and with a steeper increase in arterial stiffening at follow-up, independent of baseline sociodemographic factors, comorbidities, and health behaviours.

The age, sex, ethnicity model and the full model respectively showed a 5-year increase of 0.20 m/s and 0.19 m/s in cf-PWV of hypertensive controlled individuals in comparison to normotensive ones. A statistically significant continuous trend was also observed ($p<0.05$). This supports the hypothesis of poorly controlled hypertension leading to higher rates of arterial stiffening.

The BLSA could not find any impact of antihypertensive medications on the trajectories of PWV ($\beta= -0.04$; p: 0.37). This is a potential consequence of the sample size (n=775). [255] A similar issue occurred when Tedla et. al. suggest that type II diabetes attenuates the effect of antihypertensive treatment. This was

hypothesized after authors found that controlled hypertensive nondiabetics had arteries 3.9 times more distensible than uncontrolled hypertensive nondiabetics ($p<0.001$) but could not replicate this difference in uncontrolled and controlled hypertensives with diabetes (ADC: 0.2 p:0.93). This fact could be due to a true association or to the fact that the number of diabetic individuals was relatively small (n=230) [251]. The relatively large sample size of the Whitehall II study allowed me to test contrasts between blood pressure control subgroups. The comparison of absolute effect sizes is problematic due to the variety of adjustments to the models presented in published reports. Some studies report only the estimates resulting from age, and sex models, whereas others report the outcome of fully adjusted models. Even though, the difference in the trends of increase in arterial stiffness described in a similar way.

Although other studies included similar comparisons between controlled and uncontrolled hypertensives,[236] the added value of the approach is the addition of untreated individuals and the inclusion of the baseline arterial stiffness values in the models.

Most of the existent evidence failed to test effect modification of potentially relevant covariates such as dyslipidaemia or diabetes in the progression of arterial stiffness either for lack of sample size, representativity within the sample or model specification. Adiposity measures, like body mass index or waist circumference were the most relevant attenuators of the association showed by the models. Interestingly, although blood cholesterol did not show evidence of attenuation of the relationship, the use of lipid lowering drugs was associated with higher rate of arterial stiffness. These findings are similar to other reports showing that atherosclerosis may not play a major role in arterial stiffness.[241]

Among the included studies addressing the question of onset of hypertension according to baseline arterial stiffness there are three population-based cohorts and one occupational cohort study. The Whitehall II study is an occupational cohort of civil servants, likely having better standards of healthcare than the general population. For instance, there are more untreated hypertensives in the general population than in the study. [338] Under this assumption, the risk of progression to hypertension in individuals with lower quality of care could be higher than the risk showed in this chapter.

5.11.10 Strengths and limitations

Different characteristics can be named among the strengths of the Whitehall II study. The response rate is high. The data collection for arterial stiffness and hypertension made possible the use of longitudinal mixed models. In addition, the time span between measurements made it feasible to study a long-term evolution of arterial stiffness, which otherwise has been studied for shorter terms. Moreover, the Whitehall II study has a substantial sample size of observations of cf-PWV, compared with other studies trying to address the same questions regarding arterial stiffness.

There are some considerations to the findings presented and some limitations to be addressed. The strength of the effect of cf-PWV in hypertension varies from small to moderate. This could be also the result of the multi causality of hypertension. An additional consideration to the statistical analysis and defining hypertension as a categorical variable is that setting a cut-off value ignores the fact of the distribution of blood pressure as a continuous variable. This should be subject of analysis in further studies though is complicated by the large proportions currently using

antihypertensive. Regarding the meta-analysis and the pooled evidence, the high heterogeneity of the results should require the use of improved meta-analytic tools to better analyse the existing evidence.

Conclusions about the causality are limited, as the results derive from an observational study. Secondly, the self-reported medication in the study questionnaire could lead to underreporting due to recall bias.

5.11.10.1 Non-linear properties in the relationship blood pressure-PWV.

As mentioned in chapter 2 of this thesis, there is a variety of elastic and non-elastic components in the arterial wall. The elastic components mainly derived from the elastin fibres present in the tunica media and non-elastic components such as collagen and smooth muscle. The multiplicity of components in elastic arteries such as the aorta, provides a response to pressure that is not uniform and varies across different values of pressure. Young was the first to describe the stress/strain ratio, later known as the Young's modulus,[339] with a nonlinear relationship between distensibility and blood pressure. Physiological experiments have shown that, while at lower blood pressure values, the arteries are less stiff, or highly distensible, with a low variation in PWV from 0 mmHg until 100 mmHg; and an almost linear from 100 mmHg to 150 mmHg.[340] The change in volume per change in blood pressure tend to increase in an exponential way and stabilises after 150 mmHg. The explanation to this phenomenon is the progressive recruitment of collagen fibres as the accumulating pressure tends to deform the arteries and the collagen tends to preserve their structure. This means that the diameter will not increase beyond a certain area despite the increase in blood pressure. This also depends on the thickness and radius of the blood vessel.[341]

Despite a variety of models proposing a quadratic relationship, physicists and physiologists have studied the correlation between PWV and blood pressure, with more perfect linear relationships being shown in older age groups, and linear relationships with a higher slope in age groups below 30 years old.

5.11.10.2 Adjustments and confounding on blood pressure in the bidirectional relationship PWV-AS

As previously explained, PWV is closely related to blood pressure. Confounding is very likely when studying their bidirectional association unless appropriate adjustments are taken.

In relation to heart rate (HR) and PWV, with some research works in physiology suggesting that there is no relationship between them, most research has shown that higher HR induce a higher PWV, keeping the Mean Arterial Pressure constant.[342] In the same sense, the influences of HR on PWV tend to vary when the adjustments are done with MAP or Diastolic Blood Pressure. In physiological experiments in rats, MAP remained without significant changes when measured at both low and high HR.[343]

5.11.10.3 Advantages of the use of linear mixed models

As suggested in the statistical analysis section, linear mixed models, also called multilevel models with multivariate response types, are pertinent in the analysis of longitudinal change of a metric such as blood pressure within an individual or within a cluster of individuals, especially in comparison with conventional linear models. When assessing change between measurements over time, one of the commonly used approaches is to input the difference between a baseline measurement and the follow-up measurements, calculating a change score. If such a score would be analysed using a conventional linear regression, the change score variable would be necessarily associated to any of the variables that were used to build it, and to all the

other variables that are associated to them.[344] Linear mixed models for longitudinal change are useful in these situations because the models use centred observations between the datapoints. Using centred observations allows that the intercept and the slope are not biased, estimating a more accurate rate of longitudinal change.[345] Also, the models can accommodate the use of incomplete observations, i.e., individuals with different number of observations, maximising sample size, and finally, having a flexible covariance structure, which means they allow for its change over time, unlike conventional regression models.[346]

Chapter 6 Arterial stiffness, major adverse cardiovascular events and mortality in the Whitehall II study

6

6.1 Introduction

Aortic stiffness is a risk factor for major cardiovascular (CVD) events. The risk of such events including stroke or myocardial infarction, as well as cardiovascular and all-cause mortality, increases by 40% per each additional standard deviation of carotid-femoral pulse wave velocity (cf-PWV), the gold standard for measuring aortic stiffness.[173,285] The performance of standard risk models for prediction both of primary and secondary CVD events is improved when cf-PWV is added.[347]

Further research on the characteristics of cf-PWV as a predictor of CVD events is needed to explore its performance across subpopulations and determine which subgroups could benefit clinically after stratification based on this tool.

Although, the predictive value of a single cf-PWV measurement for major adverse cardiovascular events (MACE) is established, no studies have assessed risk using more than one measurement. Adding a second, follow-up cf-PWV measurement to model the risk profile of MACE of an individual may improve risk classification, compared to that provided by a single measurement since two measurements, separated by months or years, may provide a more accurate assessment of usual aortic stiffness.

Thus, the aim of this chapter is to study the association between arterial stiffness and the risk of Major Adverse Cardiovascular Events in the Whitehall II study. Using prospective time-to-MACE-event analysis, a comparison of survival models using a single and repeated measurements of cf-PWV will be carried out, together with an exploration of the role of cf-PWV as a predictor for non-CVD events and all-cause mortality. The role of antihypertensive medication as an effect modifier in the relationship between MACE and aortic stiffness will also be assessed. This is

relevant as it concerns part of the interrelationship between cf-PWV and hypertension that is being examined throughout this thesis.

6.2 Objectives:

The objectives of this chapter are to:

1. Determine the risk of Major Adverse Cardiovascular Events (MACE) according to carotid-femoral pulse wave velocity in the Whitehall II study.
2. Test whether adding a second measure of pulse wave velocity improves prediction models for MACE.
3. Study the predictive value of pulse wave velocity on non-cardiovascular mortality and all-cause mortality in participants of the Whitehall II study.
4. Investigate the role of antihypertensive medication as an effect modifier on the relationship between cf-PWV and MACE.

6.3 Rationale:

As it has been reported in other longitudinal studies, carotid-femoral cf-PWV is a risk predictor for MACE, non-cardiovascular and all-cause mortality events.[189,284] It is expected that the magnitude of the effect will be similar in the Whitehall II cohort. Repeated measurements of cf-PWV will add information and update risk status, thus more predictive accuracy may be provided by a model with two measurements of arterial stiffness than a model using a single measurement.

Given that in earlier chapters of this thesis an association was shown between antihypertensive medication status and the progression of arterial stiffness measured by cf-PWV, it is possible that antihypertensive medication may modify the effect of the relationship between cf-PWV and MACE. High blood pressure is a deleterious factor on the integrity of the arterial wall and a driver of arterial stiffness. Thus,

antihypertensive medication could modify the effect and potentially attenuate the effect of cf-PWV on the risk of MACE.

6.3.1 Specific hypotheses:

1. After taking account of conventional risk factors, higher carotid-femoral cf-PWV is associated prospectively with increased risk of MACE and some components of MACE such as stroke and CVD, non-cardiovascular mortality and all-cause mortality.
2. The inclusion of a second measurement of cf-PWV after 4 years improves risk prediction of MACE.
3. The larger the increase in cf-PWV between measurements over a 4-year period, the larger the event and mortality risks.
4. Antihypertensive medication is an attenuator of the association between aortic stiffness and MACE. The use of antihypertensive medication is associated with a reduced risk of MACE per each unit of cf-PWV.

6.4 Methods

6.4.1 Sample

The main sample included participants of the Whitehall II cohort who attended the research clinics at phase nine (2008/09) or phase eleven (2012/13) or both. Three subsamples were used: a subsample including participants with a measure of PWV at phase 9 as start of follow-up, another sample including participants with a measurement of phase 11 as start of follow-up and a third sample including participants with repeated measurements of cf-PWV, using the second measurement as start of follow-up.

6.4.2 Variables

6.4.2.1 Exposure: Arterial stiffness

The measurement of arterial stiffness was introduced in the Whitehall II study during the clinical examination at the phase 9 clinic in 2008/09 and was re-measured in the clinics at phase 11 in 2012/13. The method used was carotid-femoral cf-PWV, the gold standard technique. Cf-PWV is defined as the velocity of the pulse wave, generated by the heart during its contraction phase, per unit of time. To measure this velocity, tonometers able to detect the pulse wave are placed in the carotid artery and the femoral artery, covering all the length of the aorta artery.[348] The time taken for the pulse wave to travel between these points is measured. For details see the background chapter (0).

6.4.2.2 Outcome 1: Major Adverse Cardiovascular Events and components

MACE is a composite endpoint including fatal and non-fatal stroke, myocardial infarction, coronary heart disease and cardiovascular death. Transient ischaemic attacks are excluded from the definition. The definition of cardiovascular death includes deaths resulting from cardiovascular diseases coded from I00 to I99 in the International Classification of Disease, 10th revision[349] (see Appendices for chapter 6). The independent risk of stroke and coronary heart disease (CHD) according to cf-PWV, was assessed both individually and in joint models.

The date of the first MACE event in relation to the start of follow-up was used to classify participants into those with or without prevalent MACE. The group of prevalent MACE included participants with MACE before baseline who did not have further events. The group of incident MACE included participants without MACE history before the cf-PWV baseline but experienced an event during follow-up.

6.4.2.3 Outcome 2: Non-Cardiovascular mortality

All fatal events registered in the International Classification of Diseases, 10th version, which were not part of the cardiovascular definition (I00 to I99), were classified as non-CVD deaths.

6.4.2.4 Outcome 3: All-cause mortality

All fatal events that occurred during the follow-up, no matter the specific cause of death, were grouped as all-cause mortality.

6.4.2.5 Covariates

To adjust for confounding, covariates were selected after a literature search for relevant variables to the relationship of interest. All the estimates were adjusted for. Other well-known risk factors for MACE were also used as covariates. These included body composition variables: body mass index, mean waist circumference, plus blood cholesterol, use of lipid lowering drugs, relevant chronic diseases, type II diabetes mellitus, and the health behaviours. The risk models for non-CVD mortality and all-cause mortality events were also adjusted for mini-mental score, marital status, and prevalence of malignant cancers at baseline.[350] The definition of the variables is explained in the data structure section of this thesis (section 3.1).

6.5 Statistical analysis

6.5.1 Univariate analysis and model building strategy

Univariate analyses were performed as the first approach for model building. The log-rank test was used to test the differences between the survival curves of two or more groups. Categorical variables that were potentially relevant to the model were tested. If the log rank test was significant, these were included in the fully adjusted model.

For continuous covariates, unadjusted Cox-proportional hazards models were fitted to assess they should be included in the more complex models. Variables with crude models which p-value was up to 0.25 were included in further models. Some variables, such as heart rate and mean arterial pressure, were always included in the models, irrespective of their statistical significance, for their theoretical relevance.

6.5.2 Survival analysis

The time to MACE events, non-CVD mortality or all-cause mortality is specified as *t.* This time is 0 at the start of follow-up and is the time of the event or at censoring at the end of follow-up. The underlying hazard function at time t for the outcome over the follow-up period is given by $h_0(t)$. The general hazard, $h_j(t)$,for a given individual *j* with risk factors $X_j$is given by the Cox-proportional hazards model and has the general form:

$$h_j(t) = h_0(t)\exp(\beta_0 + X_j\beta)$$

The general form of the fully adjusted models are shown in the equation below. The model for MACE events is used below as an example. The differences in the adjustments for non-CVD mortality and all-cause mortality models are mentioned in the covariates section above.

$$\boldsymbol{MACE}\ h_j(t) = h_0(t)\exp(\beta_0 + sex_j\beta_1 + age_{baseline_j}\beta_2 + ethnicity_j\beta_3 + cfPWV_j\beta_4 + mean\ arterial\ pressure_j\beta_5 + heart\ rate_j\beta_6 + body\ mass\ index_j\beta_7 + waist\ circumference_j\beta_8 + type\ II\ diabetes_j\beta_9 + alcohol\ habits_j\beta_{10} + physical\ activity_j\beta_{11} + smoking_j\beta_{12})$$

6.5.3 Proportionality assumption

The proportional-hazards assumption was tested using the Schoenfeld analysis of residuals. This test checks that the log-hazard function is constant over time using the null-hypothesis of the zero slope with time. If the null-hypothesis of the zero

slope is rejected in the test, there is evidence of a violation of the proportional-hazards assumption.

6.5.4 Evaluation of model fit

Cox-Snell residuals vs. Nelson-Aalen cumulative hazard plot

Cox-Snell residuals are standardised residuals from the fitted model. These help to evaluate the overall fit of the model when plotted against the Nelson-Aalen cumulative hazard estimate. A model with a perfect fit should have a straight line with a slope of 1. A plot of the Cox-Snell residuals was plotted for each model to check the fit visually. These plots are shown in the Appendices for chapter 6.

6.5.5 Comparison of predictive power between survival models

The predictive power of survival models was compared using different approaches. The first approach was using Harrell's C-statistic. The C-statistic is an analogous measure to the area under the Receiver Operating Characteristic (ROC) curves. The additional benefit of including the former into the analysis is that is designed to test the ability of a prognostic tool to detect survival outcomes, whereas the latter better measures the capability of detecting the presence or absence of a disease.[351]

Where *i and j* are pairs of observations in the sample, η is the ranking of an observation among the sample, T is the survival time and *I* is the indicator function; Harrells' C statistic is defined as the proportion of pairs that are concordant divided by the number of pairs that are comparable. Pairs of observations with censored times (Δ_j) on them are not comparable.[352]

$$C = \frac{\sum_{i,j} I\,(\check{T}_i > \check{T}_j) \cdot I(\eta_j > \eta_i) \cdot \Delta_j}{\sum_{i,j} I\,(\check{T}_i > \check{T}_j) \cdot \Delta_j},$$

The values of the C-statistic vary between 0.5 and 1, defined as a non-predictive assessment and a predictive assessment, respectively.[353]

The statistics computed for the survival models in this chapter were calculated using post-estimation commands after fitting different models and the confidence intervals for these were calculated using bootstrapping 100 bootstrap samples after fitting the models. The differences between C-statistics were calculated using the “somersd” statistical package in Stata 16.[354]

6.6 Results

Figure 10 shows the numbers of participants with measurements of cf-PWV at phases 9 and 11 and the mean dates of the start of follow-up for MACE. The maximum number of fatal and non-fatal MACE is 544 from the clinic of phase 9 to the end of follow-up in March 2017. This number is reduced to 317 when participants with prevalent MACE at baseline are excluded from the sample. Additional details are shown in Table 17.

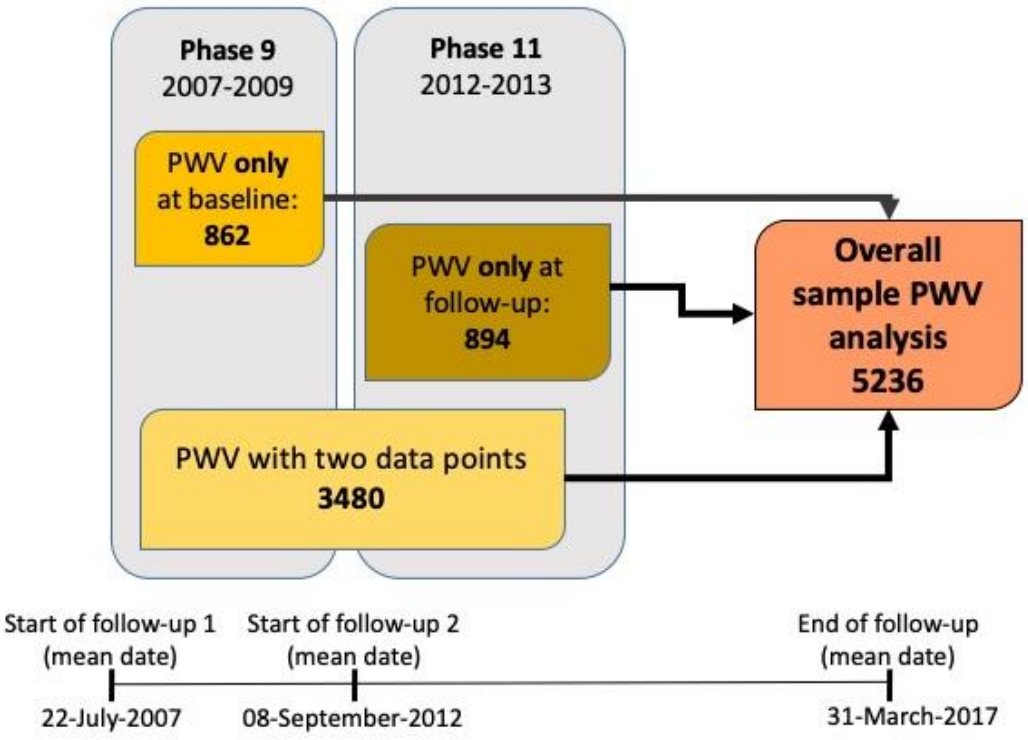


Figure 10. Follow-up time and samples used in the analysis

Table 17. Number of MACE according to cf-PWV measurements used in the analysis, in the whole sample and excluding participants with prevalent MACE*

			All participants		Excluding those with prevalent MACE	
Analysis	**cf-PWV measurements**	**Follow-up start**	**N**	**All MACE Events**	**N**	**Incident MACE Events**
1	Phase 9 only	22 - Jul - 07	4342	544	3548	317
2	Phase 11 only	04 - Feb -12	4374	445	3775	202
3	Phase 9 and 11	22 – Jul - 07	3480	389	3055	171
4	Phase 9 or 11	22 – Jul - 07	5236	700	4620	400

* Difference in the number of measurements is due to change of the number of participants at each phase of the study. The number of participants in analysis 3 is the lowest given that includes participants with two data points.

The number of events and the person-years accumulated were used to calculate incidence rates. As expected, all-cause mortality events had the highest rate, followed by non-fatal MACE. The specific rates can be seen in **Error! Reference source not found.**.

Table 18. Fatal and non-fatal incident rates for CVD and non-CVD events

Event	Events	Person-years	Rate (per 1.000)
All-cause mortality	721	46520	15.63
Cardiovascular mortality (fatal MACE)	54	38325	1.41
Non-cardiovascular mortality	219	38325	5.71
Non-fatal MACE	487	38325	12.7

6.6.1 Participants with one cf-PWV measurement at phase 9

The characteristics of the participants in Analysis 1 are summarised in Table 19. This sample of participants had a mean age of 65 years. There was a higher frequency of female participants of non-white ethnicity. Men tended to have higher mean systolic blood pressure and mean arterial pressure. Health behaviours such as drinking alcohol, and smoking were more prevalent in the male group whereas low physical activity was more frequent in the female group. Tables showing the characteristics for the samples used in Analyses 2 to 4 are given in the Appendices for chapter 6 (Appendix tables 7 to 9).

Table 19. Sociodemographic and biological characteristics and health behaviours of participants included in analysis number 1 (Analysis 1, N=4342)

Characteristic	Men (n=3234)	Women (n=1108)
	% / Mean (SD)	% / Mean (SD)
Age (years)	65.4 (5.7)	65.7 (5.7)
Ethnicity, non-white (%)	6.2	13.3
Systolic blood pressure (mm Hg)	129.3 (14.5)	124.4 (17.1)
Diastolic blood pressure (mm Hg) (n=4339)	70.3 (9.5)	68.5 (9.7)
Heart rate (bpm) (n=4339)	63.2 (11.2)	65.2 (10.7)
Mean arterial pressure (mm Hg) (n=4339)	90.5 (10.3)	87.1 (11.2)
Mean pulse wave velocity (m/s)	8.5 (2.0)	8.3 (2.0)
Body Mass Index (kg/m^2)	26.2 (3.5)	26.3 (4.9)
Mean waist circumference (cm)	94.9 (9.9)	85.4 (11.8)
Diabetes Mellitus type II (%)	11.1	10.9
Stroke (%)	1.9	1.1
Blood Cholesterol (mmol/l)	5.1 (1.0)	5.5 (1.0)
Smoker (%)	6.7	4.2
Alcohol drinker	12.7 (14.5)	6.0 (7.3)
Lipid lowering drugs (%)	32.7	24.7
Low physical activity level* (%)	20.4	28.6*

* Low physical activity level defined as less than 1 hour per week of moderately energetic activity and less than 1 hour per week of vigorous physical activity

6.6.2 Kaplan Meier curves

Survival curves by tertile of cf-PWV at phase 9 were plotted (Figure 11). The log rank test showed a statistically significant difference between the three curves ($p<0.001$). The probability of MACE was higher in the third tertile of cf-PWV compared to the first and second ones. After checking the Schoenberg residuals, the hazard was proportional.

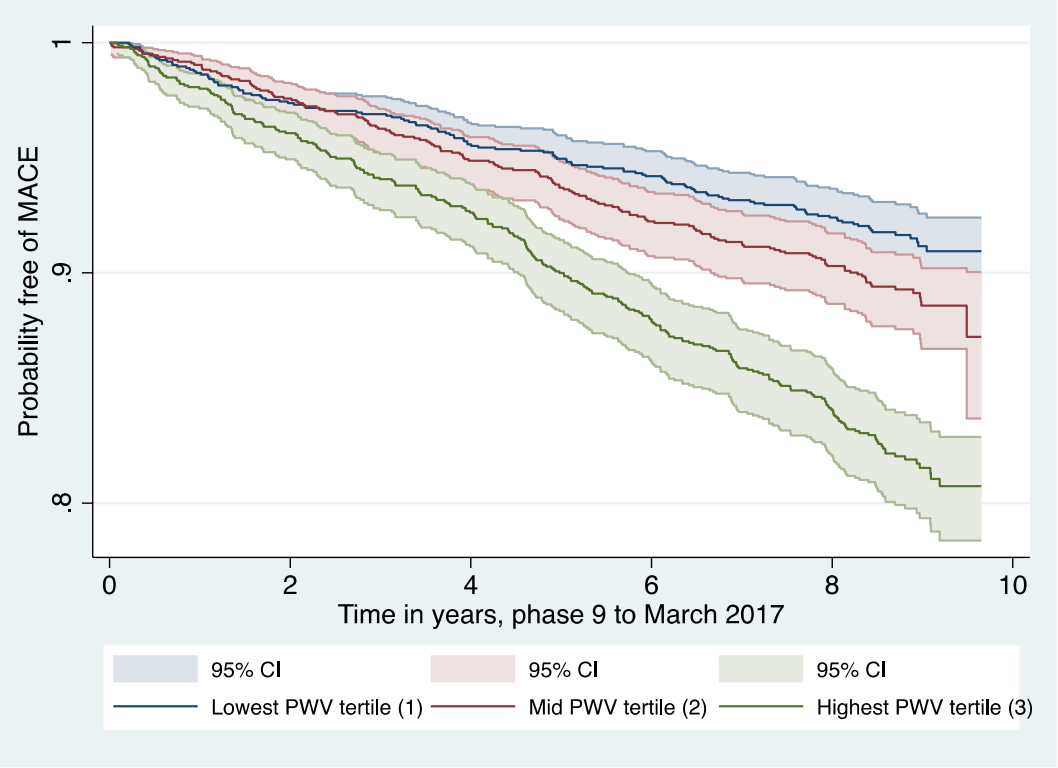


Figure 11. Kaplan-Meier survival curves for fatal and non-fatal MACE events by tertile of PWV at baseline (phase 9; 541 events, n=4,342).

6.6.3 Analysis 1: cf-PWV at phase 9 and risk of MACE (per 1 m/s increase cf-PWV)

Crude hazard ratios for fatal and non-fatal MACE were calculated using a Cox proportional hazards model. An unadjusted model was fitted using cf-PWV as a continuous variable, with a hazard ratio for MACE events of 1.18 per each metre per second of cf-PWV (not shown). Table 20 shows risk models between baseline cf-PWV and MACE. The base model adjusted for sociodemographic variables showed a 12% higher hazard per each additional metre per second of cf-PWV, this was attenuated to 11.8% when adjusting for all the selected covariates. When the sample was restricted to participants with incident MACE, the magnitude of the association changed to a 14.7% higher hazard. When assessed separately, the risk of CHD per unit of cf-PWV at baseline was 1.11 (95% CI 1.09, 1.20) when the model was

adjusted for sociodemographic factors, body composition and comorbidities. A model with the same adjustments assessing the risk of stroke showed a hazard ratio of 1.15 per cf-PWV unit (95% CI 1.09, 1.21).

Table 20. Analysis 1: cf-PWV at phase 9 and risk of MACE (per 1 m/s increase cf-PWV)

	All MACE (N= 4342, E=544)			Incident MACE only (N = 3548, E=317)		
Model*	**HR**	**95%CI**	**p**	**HR**	**95%CI**	**p**
Base model	1.120	1.076, 1.166	<0.001	1.150	1.072, 1.167	<0.001
Fully adjusted	1.118	1.072, 1.167	<0.001	1.147	1.088, 1.209	<0.001

***** The base model was adjusted for age, sex and ethnicity. The fully adjusted model is adjusted as for the base model plus body composition and comorbidities (BMI, waist circumference, total cholesterol, type II diabetes) and health behaviours (physical activity, smoking and alcohol consumption).

The above models were re-fitted after transforming cf-PWV to standard units. Table 21 shows that the risk of incident MACE is 37% higher per additional SD after adjusting for relevant covariates.

Table 21. Analysis 1: cf-PWV at phase 9 and risk of MACE (per 1 SD increase in cf-PWV)

	All MACE (N= 4342, E=544)			Incident MACE only (N = 3548, E=317)		
Model*	**HR**	**95%CI**	**p**	**HR**	**95%CI**	**p**
Base model	1.288	1.175, 1.412	<0.001	1.422	1.272, 1.590	<0.001
Fully adjusted	1.293	1.173, 1.425	<0.001	1.371	1.223, 1.536	<0.001

***** HR: Hazard Ratio. The base model was adjusted for age, sex and ethnicity. The fully adjusted model is adjusted as for the base model plus body composition and comorbidities (BMI, waist circumference, total cholesterol, type II diabetes) and health behaviours (physical activity, smoking and alcohol consumption).

6.6.4 Analysis 2: cf-PWV at phase 11 and risk of MACE

Table 22 shows the association between cf-PWV at the start of phase 11 and the risk of subsequent MACE in all participants and after excluding those with prevalent MACE at baseline. In the fully adjusted model, the risk of MACE for each unit increase in cf-PWV is 7% among all participants but is 13% higher among those participants free of prevalent MACE at baseline.

Table 22. Risk of MACE according to cf-PWV at phase 11 (m/s)

	All MACE (N= 4374, E=445)			Incident MACE only (N = 3775, E=202)		
Model*	**HR**	**95%CI**	**p**	**HR**	**95%CI**	**HR**
Base model	1.122	1.086, 1.160	<0.001	1.160	1.102, 1.221	<0.001
Fully adjusted	1.077	1.040, 1.117	<0.001	1.130	1.069, 1.194	<0.001

***** HR: Hazard Ratio. The base model was adjusted for age, sex and ethnicity. The fully adjusted model is adjusted as for the base model plus body composition and comorbidities (BMI, waist circumference, total cholesterol, type II diabetes) and health behaviours (physical activity, smoking and alcohol consumption).

Table 23 shows the same associations as above, but with the effects of cf-PWV expressed in standard units at phase 11. After adjustment for all covariates, the risk of MACE is 20% higher per each additional SD among all participants, but 36% higher for incident events only among those without prevalent MACE at baseline.

Table 23. Risk of MACE per SD of cf-PWV at phase 11

	All MACE (N= 4374, E=445)			Incident MACE only (N = 3775, E=202)		
Model*	**HR**	**95%CI**	**HR**	**HR**	**95%CI**	**HR**
Base model	1.340	1.233, 1.456	<0.001	1.455	1.279, 1.656	<0.001
Fully adjusted	1.207	1.102, 1.321	<0.001	1.360	1.183, 1.565	<0.001

***** HR: Hazard Ratio. The base model was adjusted for age, sex and ethnicity. The fully adjusted model is adjusted as for the base model plus body composition and comorbidities (BMI, waist circumference, total cholesterol, type II diabetes) and health behaviours (physical activity, smoking and alcohol consumption).

6.6.5 Analysis 3: Change between two cf-PWV measurements and risk of MACE events

The change in cf-PWV between phases 9 and 11 was normally distributed, with a mean change of 2.7 m/s. Because of biological variability, change in cf-PWV is negatively correlated with the first measurement of cf-PWV and the estimates of the effect of these changes in PWV on MACE outcomes will be confounded by the levels of the first cf-PWV measurement. To remove this correlation which is due to the effect of 'regression to the mean', change in PWV was defined as the residuals from the regression of cf-PWV at Phase 11 on cf-PWV at Phase 9. These residuals are uncorrelated with the cf-PWV measurements at Phase 9. Their distribution is shown in Figure 12

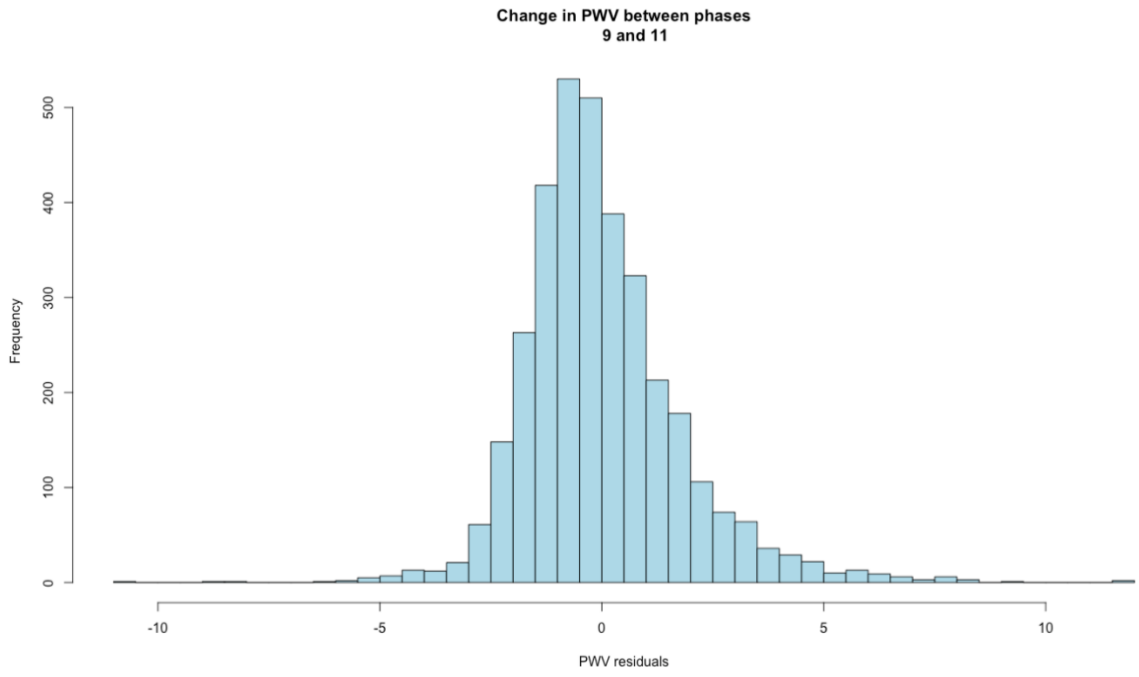


Figure 12. Distribution of the change in cf-PWV between phases 9 and 11

The sample on which change in cf-PWV was analysed consisted of 3,480 participants. This group was split into tertiles from lowest (tertile 1) to highest (tertile

3) change over time. The characteristics of the participants stratified by tertiles are shown in Table 24. The participants in the highest tertile had a mean age two years higher than the lowest tertile, tended to have higher systolic and diastolic blood pressure cf-PWV. There were significant differences in the proportion of participants with type II diabetes, and the use of lipid lowering drugs. The proportion of alcohol drinkers was higher in the lowest tertile of change in cf-PWV.

Table 24. Characteristics of individuals according to tertiles of change in cf-PWV^ between Phases 9 and 11 (Analysis 3, N=3,480)

Characteristic	Change in cf-PWV		
	Lowest Tertile (1) (N=1,160)	**Middle Tertile (2) (N=1,160)**	**Highest Tertile (3) (N=1,160)**
	% / Mean (SD)	% / Mean (SD)	% / Mean (SD)
Age (years)*	68.0 (5.3)	68.5 (5.4)	70.7 (5.8)
Ethnicity, non-white (%)	7.1	6.3	8.5
Systolic blood pressure (mm Hg)*	128.4 (15.1)	132.1 (15.6)	140.4 (17.0)
Diastolic blood pressure (mm Hg)	72.0 (9.3)	74.0 (8.8)	76.6 (10.0)
Mean arterial pressure (mm Hg)*	90.8 (10.2)	93.4 (9.9)	97.9 (11.2)
Heart rate (bpm)*	65.3 (11.1)	65.1 (10.4)	68.7 (11.4)
Body Mass Index (kg/m^2)	25.6 (3.8)	25.6 (3.6)	26.6 (3.9)
Waist circumference (cm)*	92.2 (11.5)	92.3 (10.9)	96.1 (11.6)
Diabetes Mellitus type II (%)*	7.7	7.8	13.5
Stroke (%)	1.6	1.9	1.9
Total Cholesterol (mmol/l)	5.1 (1.0)	5.2 (1.1)	5.1 (1.1)
Smoker (%)	2.1	2.7	3.1
Alcohol drinker (%)*	87.8	85.6	85.4
Antihypertensive medications	27.8	26.9	37.4
Lipid lowering drugs (%)*	36.9	37.8	42.5
Low physical activity level (%)	26.6	25.4	28.2

^ Change in cf-PWV is computed as the residuals from the regression of cf-PWV at phase 11 on cf-PWV at phase 9.
*Statistically significant difference between the 1st (lowest) and the 3rd (highest) tertile of change in cf-PWV.

The cumulative probability of time free of MACE events according to tertiles of change between measurements at baseline and follow-up can be seen in a Kaplan-Meier plot shown in Figure 13. There is an apparent higher risk of MACE according to the tertile of change between cf-PWV measurements. The results of further tests on this relationship using Cox models are shown afterwards.

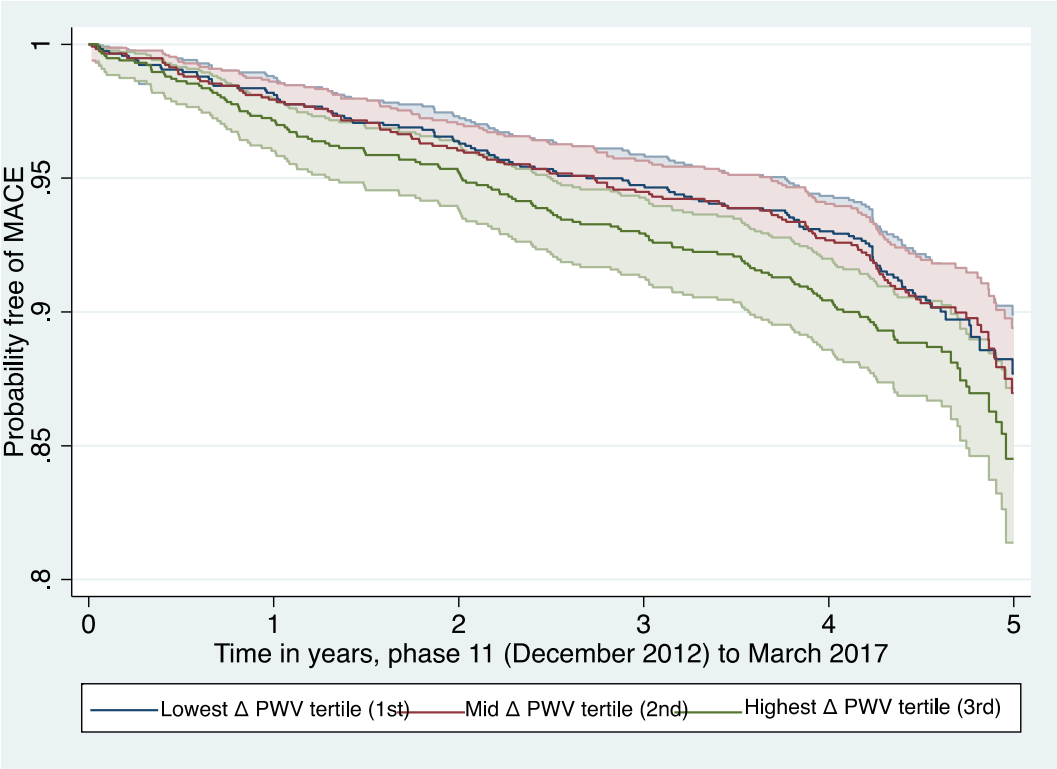


Figure 13. Survival probability according to change in cf-PWV between phase 9 and phase 11

After fitting Cox proportional-hazard models for the risk or MACE, there was a trend for an effect when assessing the risk of incident events in a model adjusted for sociodemographic variables only ($p<0.1$). This relationship was greatly attenuated after adjusting for comorbidities, body composition and health behaviours. The specific hazard ratios and confidence intervals can be seen in Table 25.

Table 25. Risk of MACE per change in cf-PWV between phase 9 and phase 11

	All MACE (N= 3,480; E=389)			Incident MACE only (N = 3,055; E=171)		
Model*	**HR**	**95%CI**	**p**	**HR**	**95% CI**	**p**
Base model	1.006	0.954, 1.061	0.813	1.069	0.991, 1.154	0.082
Fully adjusted	0.989	0.935, 1.045	0.706	1.015	0.934, 1.103	0.720

***** HR: Hazard Ratio. The base model was adjusted for age, sex and ethnicity. The fully adjusted model is adjusted as for the base model plus body composition and comorbidities (BMI, waist circumference, total cholesterol, type II diabetes) and health behaviours (physical activity, smoking and alcohol consumption).

6.6.6 Effect modification of antihypertensive medication on the relationship between cf-PWV and MACE.

In the chapter 5 of this thesis, an association between antihypertensive medications and a slower progression in the rate of arterial stiffness in normotensive participants was observed. This could also imply that the effect of one unit of PWV on the risk of MACE could vary according to the exposure to antihypertensive medication. A stratified analysis of the risk of MACE according to cf-PWV in participants with and without antihypertensive medication will be shown.

The hazard of MACE in participants using antihypertensive medication at the start of phase 9 is 2.3 times higher compared with participants not using antihypertensive medication. Among those free of prevalence MACE at phase 9, the hazard of incident MACE is 1.7 times higher in those using antihypertensive medication. Among all participants, we found the association between cf-PWV and MACE to be significantly stronger (p for interaction <0.001) among those not on antihypertensive medication compared to those taking antihypertensive medication However, after excluding participants with prevalent MACE, this interaction effect became non-significant (p=0.24) and the HR of MACE per m/s of PWV in participants with no antihypertensive medication was 1.18 (95% CI 1.10-1.26) whereas it was 1.07 (95% CI 1.00-1.12) in participants using antihypertensive medication. The models were

adjusted for sociodemographic factors, body composition, comorbidities and physical activity.

6.6.7 Predictive ability of the survival models using Harrell's C-statistic

The predictive ability of the fitted models using cf-PWV measured at phase 9 is shown in Table 26. After adding cf-PWV measured at phase 9 to all the different models, the predictive ability for fatal and non-fatal MACE was significantly improved compared to the models not including cf-PWV. This means that a single measurement of cf-PWV increases the predictive power of the risk models for cardiovascular disease in participants of the Whitehall II cohort.

Table 26. Change in concordance difference in models including and excluding Pulse Wave Velocity at baseline (phase 9)*

Model	C-statistic	p	95% Confidence interval	p for difference
Base model without cf-PWV at phase 9	0.6754	<0.001	0.6680, 0.7110	<0.01
Base model with cf-PWV at phase 9	0.6895	<0.001	0.6532, 0.6975	
Model 2 without cf-PWV at phase 9	0.7097	<0.001	0.6877, 0.7317	<0.01
Model 2 with cf-PWV at phase 9	0.7171	<0.001	0.6955, 0.7386	
Model 3 without cf-PWV at phase 9	0.7102	<0.001	0.6877, 0.7326	<0.01
Model 3 with cf-PWV at phase 9	0.7195	<0.001	0.6975, 0.7416	

* Base model adjusted for age, sex, ethnicity, mean arterial pressure and heart rate. Model 2 adjusted as for model 1 plus BMI, waist circumference, blood cholesterol and type II diabetes. Model 3 adjusted as for Model 2 with additional adjustment for alcohol consumption, physical activity and smoking status.

Some risk prediction models were fitted using the change between two measurements of cf-PWV at phase 9 and 11. Change between two measurements of cf-PWV did not improve the predictive power of survival models for MACE.
All the sequential adjustments, changes in predictive power assessment and the statistical significance are shown in Table 27.

Table 27. Differences in C-statistic values of models including and excluding change in cf-PWV between phases 9 and 11

Model	C-statistic	p	95% Confidence interval	p for difference
Base model without change in cf-PWV	0.6574	<0.001	0.6280, 0.6868	0.458
Base model with change in cf-PWV	0.6588	<0.001	0.6295, 0.6881	
Model 2 without change in cf-PWV	0.7091	<0.001	0.6814, 0.7368	0.573
Model 2 with change in cf-PWV	0.7093	<0.001	0.6817, 0.7370	
Model 3 without change in cf-PWV	0.7132	<0.001	0.6859, 0.7406	0.385
Model 3 with change in cf-PWV	0.7134	<0.001	0.6861, 0.7408	

[a] Base model adjusted for age, sex, ethnicity, mean arterial pressure and heart rate. Model 2 adjusted for model 1 plus BMI, waist circumference, blood cholesterol and type II diabetes Model 3 with additional adjustment for alcohol consumption, physical activity and smoking status.

6.6.8 Carotid-femoral Pulse Wave Velocity and risk prediction of non-cardiovascular mortality

Cox-models predicting the risk of non-cardiovascular mortality according to cf-PWV at phase 9 showed a 7% higher risk of per additional m/s of cf-PWV when fitting the base model, this prediction was not statistically significant after accounting for comorbidities, body composition and health-related behaviours. The results after adjustments can be seen in Table 28.

Table 28. Risk of non-cardiovascular mortality according to cf-PWV

	Non-CVD mortality (N = 4,222, E=214)*			
Model	HR	95% CI		p
Base model	1.078	1.063	1.104	0.03
Final model	1.058	0.988	1.132	0.10

Base model adjusted for age sex and ethnicity. Final model Base model plus mini-mental score, marital status, all malignant cancers, body mass index, waist circumference blood cholesterol, triglycerides and type II diabetes. *120 Participants were excluded from this model due to missing information on covariates.

6.6.9 Carotid-femoral Pulse Wave Velocity and risk prediction of all-cause mortality

cf-PWV predicted the risk of all-cause mortality. Table 29 shows that after adjusting for sociodemographic covariates, the risk of all-cause mortality was 11% higher per

each additional unit of cf-PWV. The risk of all-cause mortality events attenuated to 9% after adjusting for relevant comorbidities and health behaviours.

Table 29. Change in cf-PWV and risk of all-cause mortality (per m/s cf-PWV at phase 9)

Model	All-cause mortality (N= 4,342; E=688)		
	HR	**95%CI**	**p**
Base model	1.112	1.068, 1.165	<0.001
Fully adjusted model	1.097	1.056, 1.140	<0.001

Base model adjusted for age sex and ethnicity. Final model adjusted for base model plus mini-mental score, marital status, all malignant cancers, body mass index, waist circumference blood cholesterol, triglycerides, type II diabetes, fasting glucose, glycated haemoglobin, smoking status and alcohol consumption status.

6.6.10 Improvement of ASCVD scores by cf-PWV

A subsample composed of 3837 participants without history of MACE and with all the information to calculate the score of the Atherosclerotic Classification for CVD from the American Heart Association/American College of Cardiology was drawn from the main sample of participants at baseline and with a measurement of cf-PWV. There were 411 MACE events in this sample. The Net Reclassification Index was calculated, using the thresholds of the ASCVD risk: 7.5%, which is the lowest risk limit for recommending the use of lipid lowering drugs in the presence of a risk enhancer favouring that decision and 20% which is defined as high ASCVD risk indicating lipid lowering therapy. Calibration was measured using the Hosmer-Lemeshow test.

The risk of MACE increased across the quartiles of cf-PWV (**Figure 14**), with the highest quartile (cf-PWV >9.45 m/s) having an unadjusted hazard ratio (HR) of 2.99 (95% CI: 2.25 to 3.97) compared to the lowest. These associations remained after adjustment for the ASCVD score and endured stratification to intermediate risk group and exclusion of those on lipid-lowering and antihypertensive medications

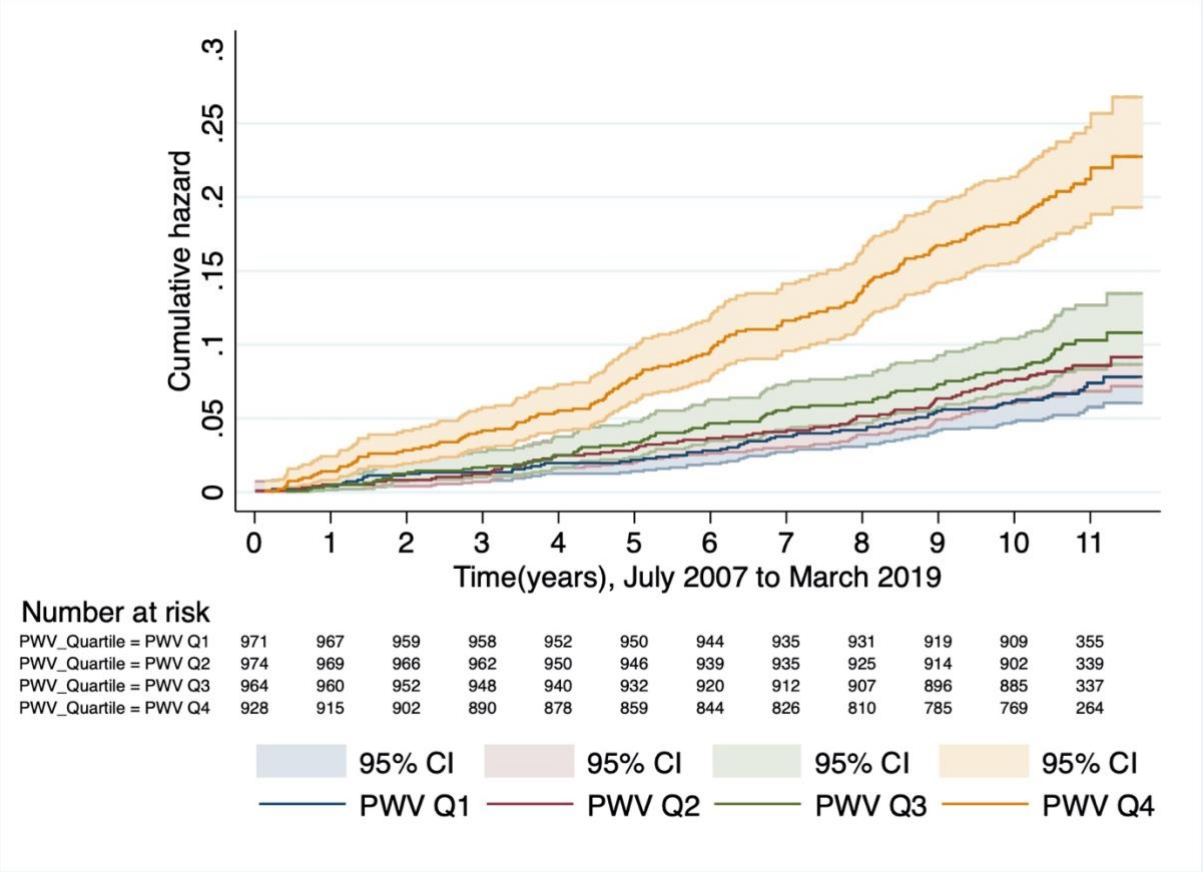


Figure 14. Cumulative hazard for MACE events according to quartiles of PWV

After adjustment for the ASCVD score the HR for the highest quartile was 1.93 (95% CI: 1.41 to 2.64). Using cf-PWV as a continuous measure, the unadjusted HR was 1.22 (95% CI: 1.17 to 1.26) for each 1 m/s higher cf-PWV. After adjustment for ASCVD risk factors, the association attenuated slightly to a HR of 1.12 (95% CI: 1.07 to 1.18) (Table 30). There was no evidence of non-linearity (p=0.33) or any interaction of cf-PWV with age (p=0.21).

After adding cf-PWV to the ASCVD score, there was a modest but significant improvement in the C-statistic of the model from 0.67 to 0.68 (Table 30).

Table 30.Association of MACE with ASCVD risk factors and aPWV and the predictive ability of two models excluding and including PWV (N=3837, Events= 411)

	HR	95%CI	p-value	C-statistic	95%CI	p-value
Model 1 (ASCVD components)						
Age	1.08	1.06, 1.09	<0.001	0.6680	(0.6423, 0.6938)	<0.001
Total cholesterol (per SD)	1.02	0.92, 1.13	0.71			
HDL-C (per SD)	0.89	0.79, 0.99	<0.05			
Antihypertensive use	1.61	1.30, 1.98	<0.001			
SBP (per SD)	1.15	1.04, 1.26	<0.01			
Smoker	1.67	1.18, 2.35	<0.001			
Incident diabetes	1.14	0.88, 1.48	0.319			
Female	0.74	0.57, 0.96	<0.05			
Model 2 (ASCVD components + cf-PWV)						
Cf-PWV (m/s) + Model 1	1.12	1.07, 1.18	<0.001	0.6784	(0.6525, 0.7044)	<0.001
Model 2-Model 1				0.0104	(0.0012, 0.0195)	<0.05

* Participants with history of MACE at baseline are excluded. ASCVD: Atherosclerotic Cardiovascular Disease; cf-PWV (m/s): carotid-femoral Pulse Wave Velocity at baseline (continuous measure in meters per second). SD: Standard Deviation. HDL-C: High-Density Lipoprotein cholesterol. SBP: Systolic blood pressure.

In an analysis including only the intermediate ASCVD group (7.5%-20%) or participants not on lipid-lowering or antihypertensive medications, the improvement in C-statistic was similar in magnitude although significant at the 0.1 level (Table 31).

Table 31. Participants with intermediate ASCVD risk score (7.5-20%) (208 events among 1966 participants)*						
Model 1 (ASCVD components)						
Adjustment	**HR**	**95%CI**	**p-value**	**C-statistic**	**95%CI**	**p-value**
Age	1.05	1.00, 1.09	<0.05	0.5808	(0.5402, 0.6211)	<0.001
Total cholesterol (per SD)	0.99	0.84, 1.17	0.92			
HDL (per SD)	0.93	0.78, 1.09	0.38			
Antihypertensive use	1.56	1.15, 2.11	<0.01			
SBP (per SD)	1.02	0.86, 1.20	0.83			
Smoker	1.18	0.69, 2.00	0.53			
Incident diabetes	1.09	0.63, 1.89	0.76			
Female	1.02	0.66, 1.56	0.94			
Model 2 (ASCVD components + cf-PWV)						
cf-PWV (m/s) + Model 1	1.13	1.06, 1.20	<0.001	0.6032	(0.5633, 0.6432)	<0.001
Model 2-Model 1				0.0225	(-0.004, 0.048)	0.09
Participants without lipid-lowering or antihypertensive medication (185 events among 2301 participants)^						
Model 3 (ASCVD components)				0.6463	(0.6077, 0.6849)	<0.001
Model 4 (Model 3 + cf-PWV)				0.6588	(0.6188, 0.6987)	<0.001
Model 4-Model 3				0.0125	(-0.0074, 0.0324)	0.22

* Participants with history of MACE at baseline and participants with ASCVD <7.5% or >20% are excluded. ^ Participant with current use of lipid lowering or antihypertensive medication are excluded. ASCVD: Atherosclerotic Cardiovascular Disease; cf-PWV (m/s): carotid-femoral Pulse Wave Velocity at baseline (continuous measure in meters per second). SD: Standard Deviation. HDL-C: High-Density Lipoprotein cholesterol. SBP: Systolic blood pressure.

Internal calibration of the risk prediction model was good. Table 32 shows that including cf-PWV as additional predictor led 11 (2.7%) extra MACE cases to be reclassified into a higher risk category (improving sensitivity) and 66 (1.9%) extra non-MACE cases to be classified into a lower risk category (improving specificity). The overall net reclassification improvement was 4.6% in the total sample (p= 0.04).

Table 32. Net reclassification improvement for MACE after the addition of aPWV to ASCVD risk factors*

ASCVD risk factors at baseline Subpopulation	ASCVD risk factors + cf-PWV				
Incident MACE cases	**<7.5%**	**7.5-20%**	**>20%**	**Total**	**NRI (95% CI)**
<7.5%	67	13	0	80	0.027 (-0.016, 0.070)
7.5-20%	21	183	33	237	
>20%	0	14	80	94	
Total	88	210	113	411	
No MACE during follow-up	**<7.5%**	**7.5-20%**	**>20%**	**Total**	
<7.5%	1322	109	0	1431	0.019 (0.007, 0.031)
7.5-20%	176	1456	77	1709	
>20%	0	76	210	286	
Total	1498	1641	287	3426	
Total NRI					0.046 (0.002,0.090) p=0.04

*Participants with history of MACE at baseline are excluded. ASCVD: Atherosclerotic Cardiovascular Disease.

The NRI increased to 11.3% when those on medication were excluded (Table 33**).**

Table 33. Net reclassification improvement for MACE after the addition of PWV to ASCVD risk factors* (185 events among 2301 participants).

ASCVD risk factors at baseline Subpopulation	ASCVD risk factors + cf-PWV				
Incident MACE cases	**<7.5%**	**7.5-20%**	**≥20%**	**Total**	**NRI (95% CI)**
<7.5%	54	15	0	69	0.081 (0.009, 0.152)
7.5-20%	14	80	16	110	
≥20%	0	2	4	6	
Total	68	97	20	185	
No MACE during follow-up	**<7.5%**	**7.5-20%**	**≥20%**	**Total**	
<7.5%	1076	89	0	1165	0.032 (0.015, 0.048)
7.5-20%	179	709	33	921	
≥20%	0	11	19	30	
Total	1255	809	52	2116	
Total NRI					0.113 (0.039, 0.187) P =0.02

*Participants with history of MACE at baseline and participants with lipid lowering and antihypertensive drugs are excluded. ASCVD: Atherosclerotic Cardiovascular Disease.

Cf-PWV was associated with major adverse cardiovascular events after adjustment for all ASCVD components and when stratified to intermediate risk group or to those not using lipid-lowering of antihypertensive medications. It also improved performance of the ASCVD score in predicting major adverse cardiovascular events. The net reclassification improvement after adding cf-PVW to ASCVD score, was 5% in the whole cohort and 11% in those not on lipid-lowering or antihypertensive medications. This shows that cf-PVW can identify elderly individuals with accelerated risk progression with a similar magnitude to that shown by coronary artery calcium, 11 and to show that adding cf-PWV to ASCVD score improves NRI. The improvement in NRI of 5 to 11% is comparable to other strong risk enhancers and suggests that health benefit could be achieved at population as well as clinical level. Earlier studies on improvement of the ASCVD score have shown that adding CAC and family history increased NRI by 12% and 5%, respectively.

6.6.11 Discussion

The extrapolation of findings from previous studies made me hypothesise that measurements of cf-PWV would predict Major Adverse Cardiovascular Events in the Whitehall II study and that models using two cf-PWV measurements would be more informative compared to those using a single cf-PWV measurement.
Carotid-femoral Pulse Wave Velocity did predict the risk of MACE in a dose-response relationship but using adding markers of change between two measurements of cf-PWV did not improve the predictive ability of the models.
The magnitude of the association with cf-PWV at baseline and MACE was stronger when using incident MACE as outcome compared with including participants with prevalent MACE in the analysis. This finding is contrary to what has been reported in the literature, where the risk of MACE events is usually higher in participants with

prevalent MACE compared to the risk of incident events.[356] In this sense, our findings indicate that cf-PWV predicts incident events better than it does recurrent events. It might be possible that all the events that integrate the composite endpoint of MACE, have a slightly different nature in their risk factors for the first and second event, as it has been reported for CVD events in some populations.[357]

When reporting cf-PWV in standard units, the magnitude of the risk for MACE events was of a similar magnitude of the risk of CVD events reported in previous meta-analysis.[286] This means that, despite Whitehall II participants being a selected population of civil servants, the findings on CVD risk could be extrapolated to other segments of the population. The risk of MACE events when using only the cf-PWV measurement at follow-up, shower a similar effect size. This shows that the risk prediction effect of cf-PWV is consistent.

The finding of a lack of risk prediction when using change in cf-PWV could be explained by the time proximity between measurements and thus the small magnitude in the change of cf-PWV. It could also be an indicator of cf-PWV being an indicator of cumulative risk rather than instantaneous risk. Given that the number of participants with two measurements of cf-PWV and the number of MACE events is smaller than in other models, the sample size could be a factor as well. The results suggest carotid-femoral cf-PWV, but not change in cf-PWV, predict MACE and all-cause mortality in the Whitehall II study. The predictive ability of the risk model is improved when adding a single measurement of cf-PWV but assessing the change between measurements did not improve the predictive ability of the models. In addition, the use of antihypertensive medication is associated with a reduction in the risk of MACE, although their association with cf-PWV was not different between the group taking and the group not taking this kind of drugs.

The HR per unit of cf-PWV is 1.15 in a crude model, and 1.08 (95%CI 1.04; 1.12) after adjustments for age, sex and ethnicity. The same effect size was found when assessing the effect of arterial stiffness on predicting the time to fatal CVD events. After adjusting for the same variables, the effect size was attenuated to a HR of 1.03 (0.91; 1.16). This apparent lack of effect was most likely explained by the lack of power due to the small number of fatal CVD events in the analysis time.

6.6.12 Limitations

The assessment of the change in discrimination between models using change in cf-PWV could be affected by the sample size of the population who had two measurements and the number of MACE events. Repeating this analysis with a higher number of events could be a way of disentangling whether the results are intrinsic to the change in PWV or are due to lack of power.

Chapter 7 Arterial stiffness and change in physical functioning markers in the Whitehall II study

7

7.1 Introduction

One of the functions of the arterial tree is to transport oxygen and nutrients to all the organs and tissues of the organism. This blood flow is regulated by many factors, being the mechanism that allows the body to sustain a given metabolic demand of one of these functions. In order to fulfil this function, vascular integrity must be conserved. Individuals with vascular abnormalities, such as aortic coarctation, usually have a reduced blood output through their aorta, being unable to fulfil the metabolic demands from the peripheral organs.[358]

This leads to the fact that individuals with low aortic output have a reduced aerobic or exercise capacity. This might not only result in a pathologic functional limitation, but also in a disability to perform activities from daily life in a way that is acceptable to their social environment. In other words, disability could be a consequence of vascular abnormalities.[359] The evidence about the functional limitation induced by vascular abnormalities varies from individual patient experience in the clinical settings to cohort studies. The Whitehall II study, an occupational cohort, has shown a cross-sectional association between increased carotid-femoral Pulse Wave Velocity (cf-PWV) and reduced scores of physical performances, such as walking speed and the physical component of the SF-36 questionnaire. [2] Cross-sectional evidence from the Framingham study, a population-based cohort, has indicated that loss of functionality is associated with a stiff aorta.[319] Possible associations between vascular stiffness and outcomes related to functionality, such as quality of life, strength, balance, or lean body mass, have been the subject of research, mainly in cross-sectional studies. Longitudinal associations have also been the focus of research, but no associations have been found. The exact effect size and strength of these associations are described in the review chapter. Therefore, the aim of this

chapter is to reproduce the cross-sectional association and to test the longitudinal associations between arterial stiffness, as measured by cf-PWV, and physical functioning related outcomes.

7.2 Objectives:

The objectives of this chapter are to:

1. Assess the cross-sectional relationship between cf-PWV and markers of physical functioning.
2. Assess the relationship between cf-PWV at baseline, change in cf-PWV and change in markers of physical functioning.
3. To estimate the relationship between cf-PWV and physical functioning in subgroups with target organ damage (chronic kidney disease, history of major adverse cardiovascular events, stroke).

7.3 Rationale:

Arterial stiffness, as measured by cf-PWV, is associated with loss of functionality and disability. This has been shown in cross-sectional studies, where reduced scores in functioning markers, such as walking speed or grip strength, are associated with higher cf-PWV values. Reduced ability to perform activities of daily living are also associated with stiffer arteries. In addition, markers of frailty, such loss of muscle mass, are associated with higher cf-PWV. The evidence from cohorts studying the relationship between arterial stiffness and change in physical functioning is less abundant and more diverging. Using information from participants of the Whitehall II study, this chapter will examine the cross-sectional association between low scores of physical functioning and high cf-PWV measurements. As stiffer arteries are associated with target-organ damage, the association between cf-PWV

measurements at baseline and repeated measurements of physical functioning will also be assessed.

7.4 Specific hypotheses

1. Arterial stiffness, as measured by cf-PWV, is cross-sectionally associated with objective and subjective markers of physical functioning or disability.
2. Arterial stiffness, as measured by cf-PWV, predicts change within repeated subjective and objective measurements of physical functioning.
3. The change in cf-PWV predicts a higher decline in physical functioning within a 9-year follow-up.
4. Participants with high blood pressure are more likely to have a decline in physical functioning over a long follow-up.
5. The association between cf-PWV and change in physical functioning is stronger in groups with target-organ damage, such as chronic kidney disease.

7.5 Methods

7.6 Analytic samples

The analytic sample for the analysis includes participants who attended the research clinics of phase 9 (2008-09), phase 11 (2012-13) or phase 12 (2015-16) and who have a measurement of cf-PWV at baseline and at least one measurement of each indicator of physical functioning at baseline and follow-up. A subsample including participants with target-organ damage was also analysed. A second subsample of participants with two measurements of cf-PWV was analysed when assessing the relationship between change in cf-PWV and change in markers of physical functioning. The timeline of the measurements in each research clinic can be seen in Figure 15. The characteristics of the participants integrating each sample can be seen in Table 34.

7.7 Variables

7.7.1 Exposure: Arterial stiffness

Arterial stiffness was first measured in the Whitehall study during the research clinic of phase 9 (2008-09) and was measured again in the clinic of phase 11 (2011-12). Carotid femoral-PWV is the gold standard of the measurement of arterial stiffness.[360] It is the speed of travel of the pulse wave along the arterial tree and defined as the time that the pulse wave takes to travel between the carotid and the femoral artery. In the Whitehall II study, cf-PWV was measured through arterial tonometry using the Sphygmacor® Atcor device.[361] Two different measurements were taken each time, if the difference between readings was greater than 0.5 m/s, a third reading was taken. More details about the definition and measurements of cf-PWV can be found in the methods section of the thesis.

7.7.2 Outcomes

7.7.2.1 Physical functioning

A variety of objective and subjective measures of physical functioning have been measured in the questionnaires and clinics of the collection phases 3 (1991-93), 5 (1997-99), 7 (2003-04), 9 (2008-09), 11 (2012-13) and 12 (2015-16). A variety of objective and subjective measures of physical function have been assessed within the cohort. I will use the last three measures to assess the association of arterial stiffness on the change in functioning. Some of the objective measures, such as chair rises and walking speed tests, are part of the Short Physical Performance Battery for assessing lower extremity function, proposed by Guralnik and colleagues.[362] Other objective measures of physical functioning that will be used in this chapter are the Medical Outcomes Study Short Form 36 [363], including both the physical and mental components. The final objective measurement that will be used in this chapter is the Fried's index of frailty. The objective measures of functioning

will be complemented using subjective measures of physical functioning also assessed in the study. Among these, the classification of Activities of Daily Living proposed by the staff of the Benjamin Rose Hospital [364] and the expansion of this list, the Instrumental Activities of Daily Living, proposed by Lawton and Brody [365]. All the objective measurements of physical functioning were assessed and performed as per the study protocols. The subjective measurements were applied according to the study questionnaires.

7.7.2.2 Repeated chair rises

Repeated chair rise exercises measure the ability and time taken for a person to repeatedly stand from a seating position and sit down again. These are done with a chair against a wall and the participant starting the test being sat down. After that, the participant was instructed to begin the test and stand from the chair without using their arms, then sitting down again as fast as possible until completing 5 chair rises. Participants who are not able to perform the test without using their arms, unable to hold balance or those for whom the nurse applying the test declared unsafe to perform it, were excluded from the test.

7.7.2.3 Walking speed

The gait test was performed along a marked area of 8 feet, free of obstacles, where the participants were instructed to perform timed walks both at a ‘normal’ self-selected pace and as fast as they can without running. The test was performed three times for each walking speed and every repetition of the test was timed. The protocols are an adapted version of the original test designed by Guralnik and colleagues.[366] The walking speed is reported in metres per second.

7.7.2.4 Grip strength

The grip strength test was performed in a seated position and measured in both dominant and non-dominant sides. With the elbow of the active arm in a flexion of 90 degrees and supported on a table. After explaining how the test would be performed, the nurse provided a dynamometer to the participants and was asked to apply as much force as they could during a single attempt. The participant applied full force for two seconds and had a rest of 1 minute after each attempt. To ensure repeatability, the test was performed three times for each participant and the highest of the average of the three measurements was used for the analysis.

7.7.2.5 Finger tapping

Finger tapping procedures in the Whitehall II study were done using the WPS Casio® MS-80 TV finger-tapping test, an electronic device that is a variation of the original test created by Halsted and Reitan.[367] The objective of the test was to make participants tap with their index fingers as fast as they could for a specific period. After identifying their dominant hand to the test administrator, the participant tapped the button of the device as many times as they were able for 10 seconds.

7.7.2.6 The 36-Item Short Form Survey (SF-36)

The Short-Form 36 survey is an instrument created within the development of the Medical Outcomes Study. This study intended to research how patient outcomes are affected by different types of healthcare, medical training specialties, social skills of healthcare personnel and developing tools for adequately following the health status of patients in medical practice.[368] The questionnaire was constructed to assess 8 different domains of health, such as the health problems that limit physical and social activities, body pain, limitation of the roles due to physical health problems, psychological distress and general mental health, energy cycles and self-perception of general health. Each section of the SF-36 questionnaire has its own scoring, with

each question being graded with Likert-type items. The number of items increased from 20 in the SF-20, to 36. This increase was designed as some of the domains were missing, or the questions included could not explore a given health domain with enough depth. This was the case for social differences in health, and changes in health status over time. Despite the increase in the number of items of the questionnaire, there are still some health aspects that have not been included in it, such as cognitive functioning, sleep disorders or health distress.

Once all the questions are answered, the scores from all the domains are calculated and added to either the physical component score (PCS) or the mental component score (MCS). The MCS is calculated by adding the weights of mental health, emotional, social function, and vitality scales.[369] The calculation of the PCS is performed prioritising the physical function, role-physical, bodily pain and general health domains.[370]

The Medical Outcomes 36 Questionnaire is a set of 36 questions that attempts to measure different aspects of functioning. Ten items aim to measure the state of physical functioning and 5 items attempt to measure the state of mental health. The score of the physical functioning component varies between 0 and 100, where 0 means that there is a limitation to perform every physical activity and 100 means that there are no limitations in any performing any activity.

The questionnaire is administered by the study personnel to the participants during the clinic or home visits.

7.7.2.7 Frailty

The measurements of frailty to be used in these analyses were assessed in the Whitehall II study during the research clinics of phases 9 (2007-09), 11 (2012-13) and 12 (2015-16). The criteria for frailty were adopted from the phenotype defined by

Fried and colleagues. It consists of 5 components including physical activity, walking speed, grip strength, weight loss and exhaustion. Low physical activity was defined as an energy expenditure of <383 calories a day. Slow walking speed was defined as 0.6 metres per second or less. The grip strength thresholds for frailty were defined according to the body mass index and sex of the participants. Low grip strength was defined as < 29 kg for male participants with a BMI < 24, <30 kg, for BMI between 24.1 and 28 and <32 kg for BMI >28. For female participants, low grip strength was defined as <17 for BMI <23, ≤ 17.3 for BMI between 23.1 and 26 and ≤18 for BMI between 26.1 and 29 and ≤21 kg for BMI ≥ 29.

Weight loss for frailty was defined as an unintentional reduction in weight of 10% since the last weight measurement (study clinic from previous phase). The definition of exhaustion is included in the Centre for Epidemiology Studies-Depression scale (CES-D). If the participants were feeling unable to carry on with their daily activities on 3 days or more during the week, the criteria of exhaustion were fulfilled.

7.7.2.8 Activities and Instrumental Activities of Daily Living

The questionnaires for assessment of physical limitation were introduced in the Whitehall II study in the questionnaire of phase 8 (2006) and continued at the research clinics of phases 9 (2008-09), 11 (2011-12) and 12 (2015-16). The 6 questions of Katz Index of Independence in Activities of Daily Living are included in the study questionnaire. These questions aim to monitor the ability of the participant to develop frequent self-care tasks. Either during the study questionnaires/clinics or home visits, the participants are asked whether they can dress, walk across a room, have a shower, eat independently, get in bed or seating in the toilet.[371] In the same session, the questions about Instrumental Activities of Daily Living are asked. These have to do with organisational skills, more specifically about preparing a hot meal,

grocery shopping, using the telephone, taking their medications, doing gardening or whether they have problems managing their money. The personnel administering the questionnaire asks whether these difficulties are due to a physical, or mental problem and will only take them into account if the problems are expected to last more than 3 months.

7.7.3 Target organ damage

Target organ damage was studied as an effect modifier of the potential relationship between cf-PWV and indicators of physical functioning. The markers of target organ damage were history of coronary heart disease before baseline or stroke before baseline and chronic kidney disease (CKD).

Serum creatinine in μmol/l was measured at the research clinic at baseline (Phase 9, 2011-12). It was converted to mg/dl using the conversion factor of the Chronic Kidney Disease Epidemiology Collaboration[372] (μmol/l*0.113) and then the glomerular filtration rate was calculated according to sex and age. The equation for men was: $141 \times (\text{serum creatinine}/09)^{-0.411} \times (0.993)^{\text{age at baseline}}$ if serum creatinine value was below 0.9 mg/dl. When serum creatinine was greater than or equal to 0.9 mg/dl the formula was $141 \times (\text{serum creatinine}/09)^{-1.209} \times (0.993)^{\text{age at baseline}}$. For women, the formula was $144 \times (\text{serum creatinine}/07)^{-0.329} \times (0.993)^{\text{age at baseline}}$ if creatinine was below 0.7 or $144 \times (\text{serum creatinine}/0.7)^{-1.209} \times (0.993)^{\text{age at baseline}}$ if serum creatine was greater than or equal to 0.7

CKD was defined as having a glomerular filtration rate at baseline lower than 60 mg/l.

7.8 Statistical analysis

Means and standard deviations were calculated for continuous variables and proportions were calculated for categorical variables. Tests of hypothesis were

calculated for differences in variables among groups. Paired t-tests and chi-squared tests were calculated accordingly.

Linear mixed models were used to estimate the association between standardised cf-PWV and indicators of physical functioning. The cross-sectional associations were assessed by adjusting the cf-PWV at baseline for covariates measured at baseline. The longitudinal association between cf-PWV at baseline and change in physical functioning over time was estimated by including an interaction term of cf-PWV with time and adjustment for covariates was made by adding interaction terms of each covariate with time. Time was modelled using the date of baseline measurement as time 0 and the date of the screening at the next clinic as the maximum time of follow-up ($t = date_{follow-up\ measurement} - date_{baseline\ measurement}$). The time was scaled in 5-year intervals to make it comparable with existing literature. For models that assessed change in physical functioning as a determinant of change in cf-PWV, the time 0 was taken as phase 11, the time where the second measurement of cf-PWV was taken. The linear mixed models were estimated using the command *mixed* on Stata 16, (Stata Corp, College Station, TX).

The linear mixed model for assessing the change in the physical functioning measurement (Y), on individual i, at occasion k is represented by the equation as follows:

$$Yik = \beta0 + \beta1tik + v0i + v1itik + \varepsilon ik$$

Where β_0 = Overall population intercept

β_1 = Overall population slope with time

v_{0i} = Random effect of intercept for the ith subject

v_{1i} = Random effect of slope for the ith subject

t_{ik} = time of the kth measurement for the ith subject

ε_{ik} = error, assumed to be normally and independently distributed with mean 0 and common variance σ^2.

The final model assessing the change in physical functioning according to cf-PWV at baseline is represented in the equation below:

$$Y_{(Physical\ functioning)} = \beta 0 + \beta 1_{\mathrm{tik}} + \beta 2_{(\mathrm{cf-PWV})\mathrm{i}} + \beta 3_{(\mathrm{cf-PWV})\mathrm{i}*\mathrm{tik}} + \beta 4_{(\mathrm{covariate})\mathrm{I}} + \beta 5_{(\mathrm{covariate})\mathrm{I}*\mathrm{t_{ik}}}$$

For these models, the terms for covariates included the sociodemographic variables: age, sex and ethnicity.

For the objective of modelling the change in cf-PWV and evaluating its association with the change in markers of physical functioning, we included participants who had a measurement of cf-PWV at phase 9 and 11. The change between measurements was modelled using the regression residuals of cf-PWV at phase 11 regressed on cf-PWV at baseline. This allowed us to have less biased estimates than those obtained by subtracting one the baseline measurement from the follow-up one. Then, equation from the mixed model to explain the change in physical functioning was modified to account for the change in PWV as follows:

$$Y\,\Delta(Physical\ functioning) = \beta 0 + \beta 1_{\mathrm{tik}} + \beta 2_{(\Delta\mathrm{cf-PWV})\mathrm{i}} + \beta 3_{(\Delta\mathrm{cf-PWV})\mathrm{i}*\mathrm{tik}} + \beta 4_{(\mathrm{covariate})\mathrm{I}} + \beta 5_{(\mathrm{covariate})\mathrm{I}*\mathrm{t_{ik}}}$$

7.9 Results

7.9.1 Sample characteristics

Around 7,000 participants of the Whitehall II cohort were present in at least one of the clinic phases of the study, either doing screening-only participation, questionnaire-only participation or both. The total sample of participants with measurements of cf-PWV at baseline and at least one measurement of each physical functioning indicator was 4,054 individuals. The screening periods for each phase and the mean dates of start and end of follow-up can be seen in Figure 15. For the analysis of the association between change in cf-PWV and change in physical functioning, a subsample of 3,372 individuals who had two measurements of cf-PWV taken at baseline and at follow-up 3-4 years later, at phase 11.

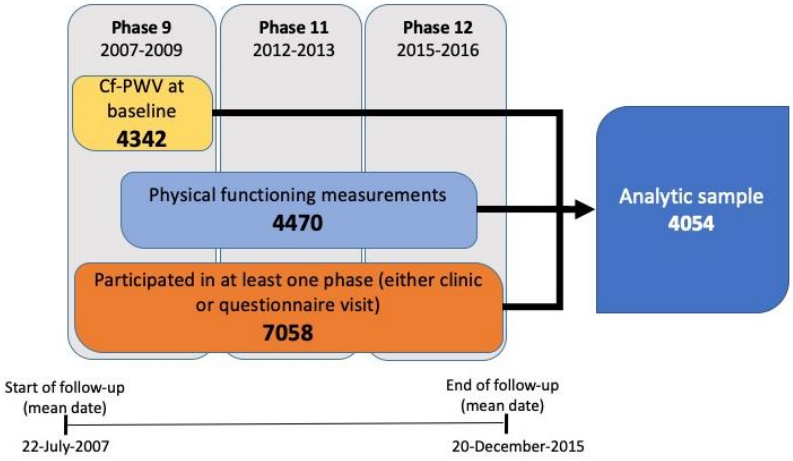


Figure 15. Analytic sample for assessing change in physical functioning according to cf-PWV

The sociodemographic characteristics, the distribution of some comorbidities, body composition and physical functioning measures by tertiles of cf-PWV at baseline are shown in Table 34. The highest tertile of cf-PWV has a greater proportion of male participants from non-white ethnicity and its participants tend to be older. Mean arterial pressure, heart rate and cf-PWV tended to be significantly different in the second and third tertiles compared to the first. Regarding comorbidities, participants

in the lowest tertile have a statistically significant lower proportion of stroke history, diabetes and use of antihypertensive treatment. The proportion of participants with coronary heart disease was highest in the highest tertile.

Regarding the measurements of physical functioning, participants on the highest tertile tended to have a lower score on the physical component of the SF-36 questionnaire, lung function and in the proportion of impaired instrumental activities of daily living. In addition, finger tapping speed and grip strength were also significantly lower. The time for chair rises was significantly higher in the highest two tertiles compared to the lowest tertile.

Table 34. Characteristics of the analytic sample according to tertiles of cf-PWV at baseline (N=4054)

Characteristic	Tertile 1 (N= 1,382) % / Mean (SD)	Tertile 2 (N=1,348) % / Mean (SD)	Tertile 3 (N=1,324) % / Mean (SD)
Age (years)	62.9 (4.8)	64.7 (5.4)*	67.8 (5.6)*
Sex (female)	28.2	24.5*	21.0*
Ethnicity (non-white) (%)	5.6	6.6*	10.4*
Mean arterial pressure (mmHg)	84.6 (9.2)	90.0 (9.2)*	94.1 (10.6)*
Heart rate (beats per minute)	59.7 (10.3)	63.6 (10.7)*	67.4 (11.1)*
PWV (m/s)	6.6 (0.60)	8.1 (0.4)*	10.7 (1.7)*
Antihypertensive treatment (%)	22.9	31.9*	40.3*
Stroke (%)	0.7	1.6*	2.1*
Type II Diabetes Mellitus (%)	4.9	9.6*	18.4*
Coronary heart disease (%)	9.2	9.5	12.7*
Walking speed (m/s)	1.16 (0.24)	1.14 (0.26)	1.09 (0.3)
SF-36 physical component score	51.1 (7.1)	49.8 (7.7)*	48.9 (8.1)*
SF-36 mental component score	53.4 (7.8)	54.0 (7.5)*	53.7 (8.3)
Maximum FEV (l)	3.11 (0.8)	3.0 (0.8)*	2.8 (0.8)*
Activities Daily Living (%)	5.6	5.9	7.4
Instrumental activities daily living (%)	7.1	7.3	11.2*
Frail (%)	1.0	1.4	1.7
Finger tapping (taps/min)	55.6 (10.1)	54.9 (11.1)	53.9 (10.9)*
Grip strength (kg)	37.6 (9.9)	37.6 (10.3)	36.4 (10.1)*
Chair rises (mean time, seconds)	10.4 (2.8)	10.9 (3.2)*	11.2 (3.3)*

*Statistically significant difference in means or proportions compared to the first tertile of cf-PWV

7.9.2 Cross-sectional association between cf-PWV and markers of physical functioning

Estimates for the cross-sectional association between arterial stiffness at baseline and physical function markers can be seen in Table 35.

All the physical function markers, apart from finger tapping speed and the mental component of the SF-36 questionnaire, were cross-sectionally associated with cf-PWV at baseline. Walking speed, lung function and physical component score of the SF-36 questionnaire were negatively associated with cf-PWV, whereas the time to perform chair rises and grip strength were positively associated with it. Most of the cross-sectional associations were replicated in each sex separately except for the

physical component of the SF-36, that was only observed in men. The association in women was marginally significant, although with a similar magnitude. Grip strength in both men and women showed a direct association with cf-PWV, with a negative, non-statistically significant association in both sexes.

The results from the models exploring the cross-sectional association between cf-PWV and frailty or activities of daily living at baseline can be seen in Table 36. The unadjusted estimates showed an association of cf-PWV with activities of daily living that was attenuated to non-significance after adjusting for the sociodemographic variables, age sex and ethnicity. Having one additional unit of cf-PWV was associated with 1.2 times higher odds of having problems performing at least one of the instrumental activities of daily living. The association was maintained even after adjusting for the same potential confounders.

Table 35. Cross-sectional association between cf-PWV and markers of physical functioning[1]

	Men and women		Men		Women	
Physical function score[2]	**Coefficient (95% CI)**	**P-value**	**Difference (95% CI)**	**P-value**	**Difference (95% CI)**	**P-value**
Walking speed	-0.017 (-0.025, -0.0089)	**<0.001**	-0.020 (-0.029, -0.011)	**<0.001**	-0.023 (-0.034, -0.008)	**<0.001**
Chair rises	0.018 (0.009, 0.027)	**<0.001**	0.018 (0.007, 0.028)	**<0.001**	0.028 (0.009, 0.046)	**<0.001**
Finger tapping	-0.11 (-0.45, 0.23)	0.53	-0.18 (-0.56, 0.20)	0.35	-0.76 (-1.42, -0.11)	**<0.05**
Grip strength	0.41 (0.07, 0.74)	**<0.05**	-0.11 (-0.42, 0.19)	0.46	-0.27 (-0.66, 0.12)	0.18
Lung function	-0.057 (-0.071, -0.022)	**<0.001**	-0.09 (-0.12, -0.07)	**<0.001**	-0.06 (-0.09, -0.03)	**<0.001**
Physical component SF-36	-0.47 (-0.72, -0.22)	**<0.001**	-0.52 (-0.08, -0.25)	**<0.001**	-0.56 (-1.14, 0.02)	0.06
Mental component SF-36	-0.21 (-0.46, 0.052)	0.12	-0.17 (-0.46, 0.11)	0.23	-0.45 (-1.03, 0.13)	0.13

1. Cf-PWV was transformed to standard units $\frac{(x-\bar{x})}{\sigma}$. 2. All the estimates are adjusted for age, sex, and ethnicity.

Table 36. Cross-sectional association between cf-PWV and frailty, activities, or instrumental activities of daily living[1]

	Men and women		Men		Women	
Physical function marker	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Frailty (frail vs. pre-frail/non-frail)[2]	1.17 (0.95, 1.46)	0.14	1.25 (0.94, 1.66)	0.12	1.17 (0,85, 1.60)	0.35
Frailty (frail vs. pre-frail/non-frail)[3]	1.04 (0.82, 1.32)	0.73	1.18 (0.89, 1.59)	0.25	1.09 (0.79, 1.50)	0.60
Activities of Daily Living [2]	1.15 (1.03, 1.28)	**<0.05**	1.11 (0.98, 1.27)	0.09	1.25 (1.02, 1.52)	**<0.05**
Activities of Daily Living [3]	1.11 (0.99, 1.25)	<0.1	1.07 (0.93, 1.24)	0.34	1.20 (0.97, 1.49)	0.09
Instrumental activities of Daily Living [2]	1.20 (1.09, 1.32)	**<0.001**	1.25 (1.11, 1.41)	**<0.001**	1.18 (1.00, 1.39)	**<0.05**
Instrumental activities of Daily Living [3]	1.19 (1.07, 1.32)	**<0.001**	1.20 (1.06, 1.37)	**<0.01**	1.16 (0.97, 1.38)	0.10

1. Cf-PWV was transformed to standard units $\frac{(x-\bar{x})}{\sigma}$. 2. Estimates are unadjusted 3. Estimates are adjusted for age, sex, ethnicity

7.9.3 Association between baseline cf-PWV and change in markers of physical functioning

The relationships between cf-PWV and change in physical functioning scores can be seen in Table 37. An association between cf-PWV at baseline and finger tapping speed was found in female participants, where there was an increase in 0.56 taps per minute per each additional standard deviation in cf-PWV. The association was not found when assessed only in men or in the whole sample.

The physical component of the SF-36 questionnaire decreased by 0.22 points per each standard deviation of cf-PWV after being adjusted for sociodemographic, body composition, comorbidities, and health behaviours per 5 years. When using cf-PWV as a continuous variable, there was a decrease in 0.10 units of the physical component of the SF-36 questionnaire per each m/s of cf-PWV per 5 years of follow-up (95%CI -0.21, -0.01). The association was independent of additional adjustments for comorbidities, body composition measures and health behaviours, attenuating to -0.21 points per SD of cf-PWV, (95% CI: -0.42, -0.02).

Results from the models for change in frailty, activities of daily living and instrumental activities of daily living can be seen in Table 38. The unadjusted estimates show that there are 1.25 increased odds of change from non-frailty to frailty phenotype in 5-year time per each SD of cf-PWV at baseline, although this association attenuated to non-significance after adjusting for sociodemographic variables. A relationship with a similar magnitude and effect size was observed with instrumental activities of daily living.

Table 37. Baseline cf-PWV and change in physical functioning

	Men and women		Men		Women	
Physical function score[1]	**Coefficient (95% CI)**	**P-value**	**Coefficient (95% CI)**	**P-value**	**Coefficient (95% CI)**	**P-value**
Walking speed	- 0.0026 (-0.0086, 0.0034)	0.39	-0.0029 (-0.01, 0.004)	0.41	-0.00041 (-0.011, 0.011)	0.94
Chair rises	0.0044 (-0.0031, 0.012)	0.26	0.0045 (-0.0046, 0.013)	0.36	0.0089 (-0.0059, 0.024)	0.24
Finger tapping	0.021 (-0.25, 0.29)	0.88	-0.21 (-0.52, 0.09)	0.18	0.56 (0.016, 1.10)	**0.04**
Grip strength	-0.033 (-0.19, 0.12)	0.68	0.016 (-0.17, 0.20)	0.87	0.034 (-0.21, 0.28)	0.79
Lung function	0.004 (-0.008, 0.016)	0.52	0.009 (-0.004, 0.024)	0.17	-0.00008 (-0.02, 0.021)	0.99
Physical component SF-36	-0.21 (-0.41, -0.013)	**<0.05**	-0.30 (-0.53, -0.075)	**<0.01**	0.09 (-0.034, 0.53)	0.68
Mental component SF-36	-0.018 (-0.18, 0.21)	0.86	0.033 (-0.18, 0.25)	0.76	-0.12 (-0.55, 0.32)	0.60

1. Cf-PWV was transformed to standard units $\frac{(x-\bar{x})}{\sigma}$. All the estimates are adjusted for age, sex and ethnicity.

Table 38. Change in frailty, activities or instrumental activities of daily living over 5 years, according to baseline cf-PWV

	Men and women		Men		Women	
Physical functioning marker	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Frailty (frail vs. pre-frail/non-frail)[1]	1.25 (1.00, 1.56)	**<0.001**	1.18 (0.76, 1.83)	0.75	1.19 (0.87, 1.64)	0.27
Frailty (frail vs. pre-frail/non-frail)[2]	1.08 (0.85, 1,38)	0.51	0.97 (.072, 1.31)	0.86	1.12 (0.77, 1.63)	0.58
Activities of Daily Living[1]	1.11 (0.98, 1.25)	0.12	1.17 (1.01, 1.36)	**<0.05**	0.95 (0.74, 1.24)	0.74
Activities of Daily Living[2]	0.95 (0.83, 1.09)	0.46	0.96 (0.82, 1.11)	0.56	0.85 (0.64, 1.12)	0.26
Instrumental activities of Daily Living [1]	1.24 (1.10, 1.39)	**<0.001**	1.17 (1.01, 1.34)	**<0.05**	1.18 (0.86, 1.64)	0.31
Instrumental activities of Daily Living [2]	0.95 (0.83, 1.09)	0.42	0.93 (0.79, 1.08)	0.35	0.99 (0.78, 1.25)	0.94

1. Estimates are unadjusted. 2. Estimates are adjusted for age, sex, ethnicity.

7.9.4 Relationship between change in cf-PWV and change in markers of physical functioning

Results of models assessing the relationship between change in cf-PWV and change in markers of physical functioning can be seen in Table 39 The only marker that showed an association with change was the physical component of the SF-36 questionnaire, where an increase in a unit of change in cf-PWV between baseline and follow up was associated with a decrease of 0.064 units in physical function score. The association was observed in the entire sample and only in men, after stratification by sex. There was an apparent trend in reduction of walking speed after an increase in cf-PWV, although it had borderline significance.

The results of the models assessing the relationship between change in cf-PWV and change in frailty or activities of daily living can be seen in Table 40. The change in cf-PWV was strongly associated with self-reported functional limitations in activities of daily living, even after adjusting for age, sex, and ethnicity. After stratification by sex, the relationship was observed only in men. Finally, change in cf-PWV was associated with functional limitation on Instrumental Activities of Daily Living, but it was attenuated to non-significance after adjusting for age, sex, and ethnicity.

Table 39. Change in cf-PWV and change in measurements of physical functioning[1]

	Men and women		Men		Women	
Physical function score	**Coefficient (95% CI)**	**P-value**	**Coefficient (95% CI)**	**P-value**	**Coefficient (95% CI)**	**P-value**
Walking speed	-0.001 (-0.006 - 0.003)	0.05	- 0.0012 (-0.0028, 0.0004)	0.14	- 0.002 (-0.004, 0.001)	0.23
Chair rises	-0.003 (-0.008 - 0.002)	0.08	0.0013 (-0.0006, 0.0033)	0.18	0.002 (-0.001, 0.005)	0.24
Finger tapping	-0.059 (-0.119 - 0.002)	0.05	- 0.061 (-0.129, 0.008)	0.08	- 0.039 (-0.166, 0.087)	0.54
Grip strength	-0.018 (-0.052 - 0.017)	0.31	- 0.009 (-0.051, 0.033)	0.68	- 0.045 (-0.102, 0.012)	0.11
Lung function	-0.0009 (-0.0035, - 0.0018)	0.51	- 0.002 (-0.005, 0.001)	0.28	0.002 (-0.003, 0.007)	0.37
Physical component SF-36	-0.064 (-0.108, -0.020)	**<0.01**	- 0.081 (-0.130, -0.032)	**<0.01**	- 0.002 (-0.100, 0.097)	0.98
Mental component SF-36	-0.003 (-0.046, 0.039)	0.89	0.0126 (-0.035, 0.060)	0.60	- 0.058 (-0.157, 0.041)	0.25

1. Estimates are adjusted for age, sex, ethnicity.

Table 40. Change in cf-PWV and change in frailty, activities or instrumental activities of daily living[1]

	Men and women		Men		Women	
Physical function score	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Frailty (frail vs. pre-frail/non-frail)[1]	1.00 (0.93, 1.07)	0.96	0.98 (0.90, 1.06)	0.58	1.05 (0.95, 1.15)	0.33
Frailty (frail vs. pre-frail/non-frail)[2]	0.98 (0.92, 1.04)	0.52	0.95 (0.86, 1.00)	0.09	1.04 (0.94, 1.14)	0.44
Activities of Daily Living[1]	1.08 (1.04, 1.14)	**<0.001**	1.07 (1.03, 1.11)	**<0.001**	1.08 (1.00, 1.16)	0.03
Activities of Daily Living[2]	1.24 (1.10, 1.39)	**<0.001**	1.06 (1.00, 1.10)	**<0.05**	1.09 (0.97, 1.24)	0.14
Instrumental activities of Daily Living[1]	1.04 (1.00, 1.07)	**<0.05**	1.04 (1.00, 1.08)	**<0.05**	1.03 (0.95, 1.12)	0.51
Instrumental activities of Daily Living[2]	1.00 (0.97, 1.03)	0.82	1.01 (0.98, 1.05)	0.63	0.98 (0.93, 1.04)	0.58

1. Estimates are unadjusted. 2. Estimates are adjusted for age, sex, ethnicity.

7.9.5 Analysis of the association within subgroups with target organ damage

Part of the subgroup analysis for change in physical functioning according to cf-PWV in different groups with target-organ damage can be seen in Table 41. All the models for participants with coronary heart disease, stroke and chronic kidney disease can be seen in the appendix. In participants with stroke, there was a 5-year increase of nearly 3 points in the mental component of the SF-36 questionnaire per each SD of cf-PWV. In participants with CKD, a 5-year decrease of 1 kg per each SD of cf-PWV was observed. Both associations with the mental component of SF-36 and grip strength were not observed when the change in the entire sample was assessed. Despite these findings, the tests for interaction were not significant for either the mental component of SF-36 in participants with history of stroke, nor in the change in grip strength in the subset with participants with CKD. An interaction effect of kidney function was found, but not when CKD (<60 ml/h) was parameterised as an indicator variable.

Table 41. Linear mixed models for change in physical functioning in subgroups with target-organ damage.

	Standardised physical function*	Coefficient (95% CI)	p-value
Stroke (N=62)	**Mental component SF-36**	**2.72 (1.05, 4.39)**	**<0.001**
CKD (N=198)	**Grip strength**	**-1.11 (-1.84, -0.39)**	**<0.001**

*Adjusted for age, sex, ethnicity and time scaled to 5-years.

7.10 Discussion

Arterial stiffness is cross-sectionally associated with lower scores in markers of physical functioning.[322] The existence of this cross-sectional association led me to hypothesise that individuals with high cf-PWV would have a steeper decline in

physical functioning compared to participants with low cf-PWV. In addition, I also hypothesised that participants with high blood pressure would experience on average a higher decline in physical functioning compared with participants with normal blood pressure. Finally, there was a specific hypothesis about how the relationship would unfold in groups with target-organ damage related to vascular dysfunction.

The descriptive statistics of the sample at baseline show the higher frequency of cardiovascular risk factors in the highest tertile of cf-PWV. As it has been explained in previous chapters, this cluster of vascular stiffening mechanisms such as glycated end products and some feedback mechanisms of increased blood pressure that in turn promotes an increased rate of arterial stiffening.[260]

The cross-sectional association between standardised cf-PWV and measures of physical function replicated some of the findings from previous reports of the Whitehall II study.[13,373] Increased aortic stiffness at baseline was associated with decreased lung function, walking speed and lower scores of the physical component of the SF-36 questionnaire. The associations seems to be consistent, as similar results were observed in a slightly different sample from the same cohort in a previous study.[2] Although we are unable to disentangle the temporality of the relationship in cross-sectional associations, lower walking speed after a stroke or vascular abnormalities resulting in motor impairment is a mechanism that has been described previously.[374] The relationship between lung function and arterial stiffness can be bidirectional, as the low pulmonary function can induce changes in vascular structure, and there could be a feedback mechanism of stiffer arteries into low cardiovascular function.[373]

As reported in table 34, the physical component of the SF-36 score was negatively associated with arterial stiffness after the follow-up period. This association was male-specific and supports the hypothesis of a higher decline in physical function. It has been hypothesised that subclinical vascular dysfunction precedes physiological impairments leading to functional limitation.[375] As a predecessor of functional limitation, cf-PWV could be used as an early marker to target and delay biological ageing in a risk population. Similar results were observed in another longitudinal study, where measures of arterial ageing as cf-PWV or intima media thickening at baseline were associated with objective measures of physical functioning (physical ability test),[362] but not with change in subjective measurements of physical functioning as ADL.[376] No associations were found between change in arterial stiffness and change in physical functioning assessments.

The change in arterial stiffness was also associated with a decrease in the physical component of the SF-36 questionnaire. The fact that the change is associated with a higher reduction in physical functioning could talk about the dose-response effect of arterial stiffness or could be attributable to higher change in participants with higher cardiovascular risk, such as participants with stroke. This pattern was also seen in the reported increase in the self-reported limitation of problems with activities of daily living.

After testing for interaction, I could conclude that the apparent differences in change in physical functioning according to arterial stiffness at baseline in subgroups with target-organ damage, reported in table 9, were due to chance. When talking specifically about kidney function, the interaction with chronic kidney

disease (GFR <60 ml/min) used as an indicator variable was not statistically significant, but a continuous interaction with GFR was ($p<0.05$). It is possible that individuals already within this group have reached a threshold of functional impairment, but others with subclinical vascular damage and kidney function can experience a decrease in functioning.

This chapter replicated the longitudinal association between cf-PWV at baseline and change in objective measures of physical functioning at follow-up and was the first study to show that change cf-PWV also predicts a decline in these objective measurements. Further research is needed to investigate why other tests of physical functioning such as walking speed or chair rises are associated with arterial stiffness only cross-sectionally but not longitudinally.

The associations between arterial stiffness and physical functioning tested in this chapter included scales and tests that are heterogeneous in the assessment and definition of physical functions. The instruments for self-reporting the ability to perform tasks necessary for the survival and social functioning of an individual, tend to measure not only the physical, but also the cognitive abilities and the mood or volition for performing such tasks. The physical performance battery, leaves aside the aspects related to mood and volition, focusing on the muscular and neurological abilities to perform movements. This difference between the ability to measure different activities was not necessarily reflected in the results of this chapter, as most subjective and objective markers were associated with stiffer arteries at baseline. Although the role of comorbidity was assessed when testing for interactions for chronic conditions and adjusting for the most relevant cardiovascular and musculoskeletal comorbidities, the result of this association

could be reflecting the poorer overall physical and psychological fitness of individuals with stiffer arteries, which could also be a result of the accumulation of allostatic load due to unmeasured comorbidities and cardiovascular ageing that was not measurable due to the cross-sectional nature of the study.

When testing the longitudinal associations between arterial stiffness and change in objective and subjective physical functioning, an association was expected with both types of measurements, but especially with objective measures, as arterial stiffness is directly related to physical and cardiovascular fitness. The lack of association with the change between tests included in the functioning battery could be related to the time between measurements and the lack of damage accumulation within the same period. The only prospective measure of physical function that was associated with arterial stiffness at baseline was the physical component of the SF-36 questionnaire. The explanation for this association together with the lack of change in other markers of physical functioning could be its ability to detect change in activities that are related to vascular damage and are not detected by other type of instruments. A more plausible explanation, however, could be that the association is confounded by comorbidities that were not included in the adjustments. As it appears to be independent from some of the main cardiovascular risk factors and comorbidities, traits in mental health such as catastrophising or depression, could be one of the explanatory factors for discordances between objective and subjective measures of physical functioning, leading to type I error.[377]

Chapter 8 Discussion

8 Discussion

This chapter outlines the findings of the thesis, integrating the findings from each chapter and stressing the main contributions of this research work to the current literature.

Potential threats to the internal validity of the findings such as bias and confounding are discussed first, whereas selection bias other issues potentially affecting external validity of the results are discussed afterwards. The final part of the discussion describes the clinical practice and health policy implications.

8.1 Research questions

Arterial stiffness is a marker of biological ageing and its progression, arterial stiffening, is a process that increases with age. Although there is a rate of stiffening that is considered normal, a number of factors could accelerate the process along the lifecourse.[260] Excluding the results of this thesis, the temporality of the association remained unclear, as some studies have reported that arterial stiffness precedes hypertension and other cohorts have shown evidence of a bidirectional association. The role of antihypertensive medication in the attenuation of the stiffening process was not clear in all the scenarios, as reports have been made about the lack of influence of antihypertensive treatment on the trajectories of stiffening. [255] Having observed these gaps in the evidence, I explored the differences in the rate of stiffening according to different blood pressure range categories and the role of antihypertensive medication into this association. Regarding cardiovascular risk, the most comprehensive meta-analysis of observational studies showed that arterial stiffness measured by its gold-standard,

cf-PWV, can be used as a surrogate marker of cardiovascular risk, [287] defined as incident major adverse cardiovascular events (MACE), such as myocardial infarction, stroke, or coronary heart disease, with a dose-dependent association. I wanted to replicate this association using data from the Whitehall II study while assessing the risk of major cardiovascular events associated with change in arterial stiffness between repeated measurements. As part of the measurement of cardiovascular risk, arterial stiffness seemed to improve the predictive ability of survival models after adjustment for the most common confounders such as the commonly used 10-year atherosclerotic cardiovascular disease score. This subsidiary question was investigated in another chapter.

Finally, arterial stiffness measured through cf-PWV is a marker of physical function. Its cross-sectional association with some objective and subjective measurements of physical functioning was assessed in an earlier analysis from this cohort.[13] This cross-sectional analysis generated a variety of hypotheses, but inferences about temporality of the association could not be drawn from it. Hence, a relevant question was to investigate whether follow-up measurements of cf-PWV predicted a decline in physical functioning.

Having identified the current gaps, the main aims of the present thesis were formulated as follows:

1. Determine the direction and temporality of the relationship between arterial stiffness and arterial hypertension and establish the degree to which this is modified by antihypertensive treatment.

2. Assess the risk of Major Adverse Cardiovascular Events according to baseline cf-PWV and according to change in cf-PWV between baseline and follow-up.
3. Assess the improvement in discrimination and calibration of predictive models of cardiovascular risk after the addition of cf-PWV.
4. Assess the relationship between PWV at baseline and change in PWV with change in objective and subjective measurements of physical functioning.

8.2 Summary of results:

8.2.1 Arterial stiffness, hypertension, and antihypertensive treatment.

Participants were grouped according to their blood pressure status and use of antihypertensive treatment at the time of study participation. Cross-sectionally, it was found that arterial stiffness increased linearly with blood pressure across the blood pressure status groups, being lowest in the group of participants with normal blood pressure and being highest in the group of participants with high blood pressure despite receiving treatment (i.e. treated uncontrolled participants). After a 4.3-year mean follow-up, an association between the blood pressure status group and the rate of increase in arterial stiffening was observed. In this case, the group of participants having normal blood pressure as a consequence of receiving antihypertensive medication (i.e. treated and controlled participants), were no different in their rate of stiffening than the normotensive ones. Compared to the normotensive group, participants not receiving antihypertensive medication but having a high blood pressure measurement (i.e. untreated hypertensive participants) presented the highest rate of increase in arterial stiffening, followed by

the treated uncontrolled participants who were receiving treatment but still had a high blood pressure measurement. The difference in the rate of stiffening between the uncontrolled and controlled hypertensive groups was not found after adjusting for body composition measurements and health behaviours. There was no evidence of any type of antihypertensive drugs, such as beta-blockers or ACE inhibitors being superior to each other on achieving higher modifications in the rate of arterial stiffening.

In summary, arterial stiffness at baseline was higher among uncontrolled hypertensive participants. After follow-up, the rate of increase in stiffening was higher in untreated participants. Evidence of bidirectionality was found, with evidence of high arterial stiffness at baseline preceding hypertension at follow-up.

8.2.2 Major adverse cardiovascular events and arterial stiffness.

The risk of major adverse cardiovascular events such as myocardial infarction, stroke, or coronary heart disease, either fatal or non-fatal, were predicted by cf-PWV in the Whitehall II study. The risk of incident MACE increased in a dose-dependent fashion and was present either between cf-PWV at baseline and risk of events until the first follow-up or cf-PWV at follow-up and risk of events at the end of the total follow-up period.

The change between measurements of arterial stiffness at baseline and follow-up was not associated with an increased risk of MACE. Adding measurements of change did not improve the C-statistic of the survival models.

Finally, the ability of cf-PWV to improve the predictive power and discrimination of the 10-year atherosclerotic cardiovascular disease score was tested. After adding

the elements of the ASCVD score, a discrete improvement in the prediction ability of the models was observed. Adding cf-PWV to the ASCVD score was associated with an increase in the Net Reclassification Improvement in the correct classification of participants with and without the MACE outcome.

The addition of cf-PWV to the 10-year ASCVD risk category could improve the risk classification of low and intermediate risk individuals improving risk stratification and the decision of potential statin treatment.

8.2.3 Arterial stiffness measured by cf-PWV, change in cf-PWV and change in markers of physical functioning.

Negative associations between physical functioning and change in cf-PWV were found cross-sectionally, more specifically with objective markers of functioning as walking speed, chair rises, grip strength, lung function and the physical domain of the SF-36 questionnaire. This cross-sectional between poor functioning and vascular damage could be the result of low cardiovascular status leading to functioning decline. Subjective markers of physical functioning as activities of daily living and instrumental activities of daily living were negatively associated with cf-PWV as well. Despite these cross-sectional associations, when the physical functioning effects were assessed longitudinally, there were fewer markers associated with cf-PWV.

Higher cf-PWV was associated with a prospective reduction in the score of the physical component of the SF-36 questionnaire, with the association being specific to men. Associations with subjective markers of physical functioning, such as instrumental activities of daily living, seemed to be confounded by age, sex or

ethnicity, since the direction of the associations changed after adjusting for these factors. A similar situation seemed to occur with frailty, where the association did not remain after performing the same adjustments.

When assessing the association between change in cf-PWV between baseline (phase 9) and follow-up (phase 11) and change in markers of physical functioning between phase 11 and phase 12, an increase in cf-PWV was associated with a decrease in the score of the physical component of the SF-36 questionnaire at phase 12. An increase in cf-PWV was associated with higher odds of presenting difficulties in performing at least one activity classified within the questionnaire of activities of daily living, a subjective marker of physical functioning OR= 1.04 (1.00, 1.07) per SD of cf-PWV. This association did not hold after adjusting for sex, age and ethnicity.

8.2.3.1 Cross-sectional and prospective associations between arterial stiffness measured by cf-PWV and markers of physical functioning.

Negative associations between cf-PWV and markers of physical functioning and change in cf-PWV were found cross-sectionally. Such markers of physical functioning were walking speed, chair rises, grip strength, lung function and the physical component of the SF-36 questionnaire. I will discuss the findings in the light of results from other studies, the potential roles of comorbidities, how the results between physical functioning measurements differed and the strength of the evidence presented in this thesis linking arterial stiffness and low scores of physical functioning.

8.2.3.2 Comparisons with previously reported findings How do these findings compare with previously reported studies

As it was shown in the literature review in section 2.4, most studies assessing this relationship were of cross-sectional nature. The specific outcome of physical functioning most frequently studied in the relationship with arterial stiffness was walking speed. Regarding the direction of the association, the results from this thesis were consistent with all the previously published studies that found a negative relationship, where a higher value of cf-PWV, was associated with lower walking speed. In other words, on average and cross-sectionally, individuals with stiffer arteries tended to walk slower.

Rather than the direction of the association, differences were found in the reported magnitude in the analyses of this thesis and that of studies published previously. Higher similarity of the association with the results shown by Brunner et. al was expected as that study analysed a very similar subgroup composed of individuals from the same cohort included in this thesis. Within the other 2 studies that analysed the association of that specific outcome, MUST study found an association smaller by two orders of magnitude using the 6-minute walking distance, while the dose effect could not be measured in the study published by Gonzales et. al,[323] as it performed a correlation analysis. The Mayo clinic found a smaller dose effect by one order of magnitude, quantifying not the speed but the total walked speed.

Finally, in a comparison with a related outcome, Gonzales et. al,[325] studied walking fatigability. Although the magnitude of the association is not directly comparable to

the results of this thesis, the direction of the association accounts for poorer cardiovascular fitness in the group with higher cf-PWV.

Another subgroup of studies of physical functioning and arterial stiffness is composed of three studies assessing the cross-sectional association between frailty and arterial stiffness. The Framingham study assessed the values of cf-PWV across categories of frailty. Their results were consistent with the results of this thesis, finding that individuals in higher categories of frailty were found to have higher cf-PWV, independently of their cardiovascular risk conditions.

Another study in a smaller group of individuals assessed the odds of frailty per standard unit of arterial stiffness. Although this thesis showed the same direction of the association it was not statistically significant. Finally, a related study assessed strength and lean body mass as outcomes; these were included in this thesis within the indexes of frailty. Grip strength was negatively associated to arterial stiffness, although this association was not independent of age and sex. Unlike these two studies, I did not find an association between frailty and cf-PWV. Sample size could be an explanation, given that only 1% of the sample was frail. A complementary explanation could be that the frailty processes are not related to vascular stiffness.

Comparing the prospective associations found in the literature with the results of this thesis, the Health ABC study found a strong relationship between sarcopenia and mean limb mass. Although sarcopenia is a building block of the concept of frailty, it was not assessed on its own in the analyses of this thesis. Grip strength is the closest concept to mean limb mass in this thesis. The direction of the

association was consistent with the findings of this study, although it was not independent of age, sex, and ethnicity.

Finally, den Ouden et al. studied the longitudinal changes in arterial stiffness, physical ability, and activities of daily living. Consistently with my results, they did not find a prospective association between change in activities of daily living and increase in cf-PWV. As in my study, the reason could be that the accumulation of vascular damage in a relatively short period, was not enough for having an impact in mobility.

Regarding evidence between grip strength and arterial stiffness, cross-sectional studies found an association between lower hand strength and high PWV. This association was counterintuitive when assessed cross-sectionally with higher PWV in higher grip strength but disappearing in longitudinal assessment.

8.2.3.3 Role of comorbidity

The role of cardiovascular comorbidity in the trajectories of physical functioning must be disentangled from the cross-sectional and prospective associations. The cross-sectional association between cardiovascular disease and physical functioning has been described with detail. Although the cross-sectional nature makes difficult to disentangle the temporality, it is likely that the association is the result of the cardiovascular damage, affecting the cardiovascular fitness and leading to more consequences of sedentary life.

Prospective studies can help to disentangle some of the effects. Heart failure impairs the ability to perform physical exercise, and physical tasks, which results in higher lifetime cardiovascular [378]

Other prospective studies have shown that the lower physical functioning at baseline predicts an increase in the risk of haemorrhagic and ischaemic strokes.[379] Prospective studies of venous thromboembolism also show that the trajectories of physical functioning tend to decrease, and the odds of frailty are higher after the presence of a thrombotic event.

Looking at the longitudinal trajectories of other comorbidities such as hypertension, and their relationship with physical functioning might help to disentangle the prospective roles of arterial stiffness, blood pressure and functional limitation. Higher systolic blood pressure is more hazardous in individuals without physical limitations measured by the ADL score, whereas the risk of higher SBP is lower in individuals with the presence of physical limitations.[380] This suggests that the associations between functioning and arterial stiffness could be modified by other comorbidities and should be analysed in subgroups in future analyses.

8.2.3.4 Arterial stiffness, cardiovascular related disability, and frailty

The concept of cardiovascular related disability refers to the functional impairments secondary to diseases of the heart, arteries, and veins. Myocardial infarction and stroke are some of the most common cardiovascular conditions and are one of the main causes of mortality and disability in the world. Other conditions such as cerebrovascular disease, congenital heart disease, heart failure, deep venous thrombosis, or atherosclerosis, tend to be more chronic and produce higher disability. With the improvement in the survival rate after acute cardiovascular events, and the chronic nature of other cardiovascular diseases, the prevalence of

disability tends to increase, especially in middle-income and low-income countries.[381]

This is important in relationship to frailty, with many of the risk factors among the cardiovascular conditions listed above. Chronic cumulative damage, cardiovascular ageing, and reduced functional capacity of the heart and elastic arteries are features of frailty. Lower resiliency in other body systems, such as sarcopenia or osteoporosis, are also present in musculoskeletal ageing. This lack of resiliency of the cardiovascular system is associated with lower physical activity, higher dependency, and mood problems, thus promoting a vicious cycle that further decrease cardiovascular fitness, muscle loss, and decrease in functional capacity. Using biomarkers and other diagnostic tools that enable early detection and tracking of cardiovascular conditions has been established as one strategy to prevent the onset of frailty.[382]

8.3 Main contributions to the field

8.3.1 Evidence

This thesis brought novel evidence about findings that were not consistent within the literature. A bidirectional relationship between blood pressure and arterial stiffness was found, and the role of the antihypertensive modified the trajectories of arterial stiffening in hypertensive individuals with controlled and uncontrolled blood pressure.

Regarding CVD risk, this thesis showed that cf-PWV improved the predictive power and risk prediction of traditional CVD risk factors.

Carotid femoral PWV is cross-sectionally associated with most measurements of physical functioning but cf-PWV and change in cf-PWV were only prospectively associated with the mental component of the SF-36 questionnaire.

The associations found between cf-PWV, higher cardiovascular risk and lower physical functioning, as well as its attenuation by the proper use of antihypertensive medication show evidence that cardiovascular ageing can be modified.

8.3.2 Contributions to understanding the topic and potential explanations

It was found in this thesis that antihypertensive treatment attenuates of the rate of vascular ageing, a finding that was not consistent in the reviewed studies. These inconsistent findings in other cohorts could be explained by lack of comparisons between different subgroups of treatments (i.e. only comparing treated vs. untreated but not comparing controlled hypertensives vs. uncontrolled ones). This comparison tended to be studied in detail in studies of groups of patients with resistant hypertension. Other studies did observe the attenuation in arterial stiffening by antihypertensive medication in the whole sample but not in subgroup analysis due to small sample size. I was able to show this analysis in subgroups with higher risk of arterial stiffening as participants with type II diabetes or kidney disease.

The change between measurements of cf-PWV was not associated with the risk of MACE. The lack of statistical power given the small number of events could be an explanation for this. An alternative explanation could be that the change between baseline and follow-up cf-PWV measurements reflects a 4-year risk accumulation

compared to a lifetime risk accumulation captured in a single cf-PWV measurement.

Arterial stiffness and arterial stiffening were associated with change in physical functioning score but not with change in mental functioning or individual functioning scores. The first explanation would be that the physical health measure is a proxy of general health and bodily pain, which might be affected before the mental health score. This causality pattern is supported by findings from the same cohort and mendelian randomisation studies.[383,384] Comparison of rates of change between different risk markers specific to motor pathways rather than those specific to cognitive pathways the ones affected earlier, could explain why the physical quality of life measurements are affected earlier than mental ones.

As the SF-36 is a global measure of physical health, this would explain why more specific indicators such finger tapping or standing tests would not be asssociated.

8.3.3 Research directions

An immediate step would be modelling the influence of time spent without hypertension control or with high blood pressure on the rate of arterial stiffening and modelling the potential reversibility of the process. This can be done using Markov modelling that is beyond of the scopes of this thesis.

A systematic review and meta-analyses of studies with repeated measurements of arterial stiffness, cardiovascular events and physical functioning, would be useful to disentangle whether the lack of predictive ability of repeated measures of arterial stiffness is due to small sample size or due to a lack of risk accumulation between measurements.

Replication of longitudinal findings in the relationship of arterial stiffness with physical functioning in other cohorts is needed to understand which are the shared mechanisms and which specific characteristics of physical functioning can be best predicted by measurements of arterial stiffness.

The findings about the improvement of discrimination and calibration provided by cf-PWV in the 10-year ASCVD risk score provided could be subject to validation studies in clinical populations.

8.4 Validity

The internal and external validity of the findings of this thesis will be described in this section.

8.4.1 Internal validity and bias

8.4.1.1 Misclassification

In line with the findings of the analytic chapter 4, it is likely that a small proportion of normotensive and prehypertensive participants progressed towards hypertension soon after the clinical screening, increasing the probability of misclassification of the exposure.[239,385] To address this potential bias, a sensitivity analysis was performed using the stricter hypertension criteria from the American Heart Association and by classifying participants with history of hypertension as controlled hypertensive participants. The differences in baseline arterial stiffness and in the rate of arterial stiffening between normotensive and hypertensive individuals were maintained after the sensitivity analysis changing the hypertension definitions.

Among the covariates used to adjust for confounding, some of the self-reported covariates could be subject to the influence of misclassification, especially the ones related to health-related behaviours. For example, as alcohol consumption in the study was measured through the study questionnaire and participants reported the number of units of alcohol they consumed during the last week, alcohol intake could be underestimated. [386] A way to counteract this was to adjust for alcohol drinking status rather than the number of alcohol units in sensitivity analysis. The magnitude and direction of the associations were not affected, with similar alcohol consumption patterns to other British cohorts.[387]

History of smoking showed weak or no associations with arterial stiffness in most of the analysis of this thesis either when analysing it as history of smoking or using cumulative measures as “pack-years”. Current smokers at the first phase of the study were more likely to have died or not participate in some clinical tests. [388] Longer survival of non-smokers or ex-smokers in the cohort can be an explanation of this lack of association. Smoking has been found to increase AS in the short-term, but not in the long-term. [389] Further analyses of smoking were not within the scope of this thesis.

Although self-reported physical activity is subject to overreporting in physically inactive individuals and underreporting in physically active ones, different increasing levels of physical activity were associated with a lower rate of increase in arterial stiffening. This was consistent with previous findings in this cohort [390] as well as other studies.[391]

In general, participants of the Whitehall II study seem to be interested about their health status and the medical checks provided by the study personnel, which

contributes to them being engaged and careful when providing the information on study questionnaires.[392]

The influence of selective attrition on the relationship between hypertension and arterial stiffness would likely attenuate the magnitude of the associations with both cardiovascular and non-cardiovascular outcomes, as participants who left the study in early phases have 1.5 higher odds of cardiovascular and all-cause mortality.[393]

8.4.1.2 Confounding

Most of the confounding factors in the relationship between hypertension and arterial stiffening were accounted for in the models built to assess the different associations. Age was the strongest confounder, given that it is associated with high blood pressure, cardiovascular mortality and low scores of physical functioning. Other non-modifiable factors as family history of hypertension were not associated with arterial stiffness or arterial stiffening. Among the modifiable factors, the strongest confounders in this association included in the models were selected after the literature review, including cardiovascular comorbidities or their medication, body composition measurements and health behaviours. Despite this, other modifiable factors as stress or its markers, social deprivation, diet, sleep pattern or prenatal conditions remain to be tested and could be part of residual confounding. These factors were not included in the models in order to keep them as parsimonious as possible and not diluting the associations due to overadjustment. In addition, these factors are not involved in the main causal pathways of this association, as it is shown in Directed Acyclic Graphs reported in

the literature.[394] Another reason for not including them in the main analysis was to follow the aims and objectives of the thesis. Genetic and epigenetic conditions such as telomere shortening have been described to play a role in vascular ageing as well.[395]

8.4.1.3 Random error

Currently, the Whitehall II study has one of the biggest longitudinal samples of arterial stiffness measurements in the world. This reduces the likelihood of random error being a threat to the validity of the analyses performed to answer the main questions and hypotheses of this thesis. Power calculations for the linear mixed regression models assessing the rate of progression in arterial stiffening according to blood pressure status, and the survival analyses studying the risk of MACE according to a single measurement of cf-PWV showed that the number of available observations provided enough power to detect differences between groups. There was low power to test other hypotheses, such as the risk of MACE according to change between cf-PWV measurements assessed in chapter 5. After adjusting the survival models for sex, age and ethnicity, the estimated HR for cf-PWV was 1.10 and not statistically significant. This was concordant with the power analyses for the survival models, which estimated enough power to detect a minimum HR of 1.40 with the available sample size. It is unknown whether the lack of association is real or is the effect of type II error.

In the measurement of the exposure, cf-PWV, random error will be present in the measurement of path-length. Path-length is defined as the length of the aortic

segment travelled by the pulse wave. It can be measured clinically, by using a tape measure, or using imaging methods such as magnetic resonance.[396,397] When it is measured clinically, the distance from the carotid artery to the sternal notch is measured, as well as the distance from the femoral artery to the sternal notch. The final path length is calculated by subtracting these two distances. Studies assessing the validity of the determination of the travel distance using a tape measure found that BMI could be a variable associated to influence random error within the cf-PWV values, although its magnitude is minimal. [398] In addition to clinical training, the staff has been trained in the use of standard, reproducible methods for research. This further diminishes the likelihood of this measurement error.

Influence of change in body composition measurements between baseline and follow-up on measurement error is unlikely in this analysis as adjustments were only made for baseline and not follow-up BMI and follow-up waist circumference measurements.

The influence of random error in the measurement of cf-PWV itself was minimised by including a third measurement in those cases where the first and second measurements presented a difference larger than 0.5 m/s.

There were some threats to internal validity in this thesis. Misclassification of exposure were analysed, and their role was ruled out after performing sensitivity analyses. The literature reviews and refining of hypotheses before the start of the thesis ensured that most of the confounding factors were taken into account in the analyses. Measurement error was minimised by the quality of the data used for this thesis.

8.4.2 External validity and selection bias

The response rate of 73% at recruitment of the Whitehall II study, [392] is beyond the expectations for most researchers in observational studies. [399] The response rate for the study phase 9, which was the baseline of follow-up for most analysis of this thesis, was 72.3% in alive participants and 84.5% in eligible ones (alive and who have not withdrawn from the study), selection bias due to loss of follow-up seems unlikely. This goes in line with other research outputs of the study showing very small or no difference due to of loss of follow-up in CVD related variables. [400]

In addition to verifying the response rate by itself, influence of selection bias should be further examined by checking the differences between responders and non-responders. [331] Regarding this, all-cause mortality within the study tends to be higher in the non-responders to the initial recruitment phase, compared to half-responders and responders. [330,393]

After comparing the population with home visits to the rest of the sample, some determinants of cf-PWV, such as age and systolic blood pressure, were on average, higher than those of the participants who visited the research clinic. Although other important determinants such as type II diabetes or self-reported stroke were not different, it is likely that not assessing cf-PWV in participants with home screenings attenuated the effect size of the association between hypertension and progression in arterial stiffness. The hypothesis was supported by an early analysis,[13] showing a discrete increase in the effect size of the association after adding multiply imputed cf-PWV in the participants with home visits. There was no change in its direction or the strength of the association.

Also, a proportion of participants of the study at phase 9 did participate in the study clinic but did not get a cf-PWV measurement. Their age at clinical screening was on average 10 months greater than that of those who had participated, but their anthropometric measurements, or comorbidities did not differ.

Despite the likely presence of the 'healthy worker effect', where the participants of a given occupational cohort have a reduced frequency of health conditions compared to the rest of the population, comparative studies have shown that the strength of the association between a variety of chronic conditions and the risk of cardiovascular disease are similar in this occupational cohort and in a wide population-based study as the British Regional Heart Study. [332]

Despite the generalisability of the findings, other factors should be observed when extrapolating these results. Explaining factors would be the milder life course trajectories of SBP and DBP, higher prevalence of antihypertensive treatment and an interaction showing increased arterial stiffening with age in the Whitehall II study. [401] Hence, the effects of blood pressure on promoting arterial stiffness are likely to be attenuated in this study compared to similar cohorts. The proportion of participants with incident hypertension before the baseline of the analyses of this thesis was 24% for participants aged between 60 and 70, whereas in similar groups of studies like ELSA it was around 37%.[402] The participants of this cohort were born between 1933 and 1953. As the peak of the coronary heart disease pandemic of the 20th century occurred during the 1970s in the UK, most of the members of this cohort were entering midlife after the peak of the CHD epidemic.[403]

Although a number of associations in this thesis were sex-specific, being only observed in men. The fact that the Whitehall II study has a relatively low proportion of female participants (26.8%), does not seem to affect the external validity of the findings, as the physiological mechanisms and causal pathways related to arterial stiffness were not different between sexes. [404] In this case of shared mechanisms of disease, lower representativity of a particular subgroup would not pose a threat for external validity. [405]

The low proportion of participants of non-white ethnicity might lead to generalisability problems to non-Caucasian populations.

Most of the analyses in this thesis showed that participants of non-white ethnicity have higher CVD risk, CVD-related events and higher cf-PWV. Despite this higher risk, this category is composed of different ethnic groups (e.g., black, Southeast Asian, etc.), which makes the risk heterogeneous. A higher MACE incidence but lower mortality in Londoners from Southeast Asian descent is an example of this heterogeneity.[406]

8.5 Causality

In epidemiology, cause is defined as a condition that precedes and is necessary or sufficient to produce another condition, disease, or outcome.[405] Ultimately, these analyses intended to provide evidence of arterial stiffness being a condition that in part explained the onset or increased development of MACE events or decreased physical functioning, as well as hypertension being a necessary condition for an increased rate in arterial stiffening. Although a formal analysis of causal inference was not performed in this thesis, the adjustment of the associations for potential

confounders was indeed an analysis meant to establish a degree of causality [407]

Some of the Bradford Hill criteria and how were them fulfilled will be discussed in this section. This is not intended to work as a checklist to demonstrate causality, as no checklist could entirely fulfil such objective (Rothman, 1998). Rather, this subsection discusses the presence of causality elements in the three main hypotheses of the thesis.

Elements of a cause-effect relationship of blood pressure status and the degree of arterial stiffening were observed in the results from chapter 4.

The temporality in the association between hypertension and arterial stiffening is bidirectional. Participants with high blood pressure status had higher rate of arterial stiffening compared to participants with controlled or without blood pressure. In the other direction, participants in the highest (3rd) tertile of arterial stiffness showed higher odds of progression into hypertension. The effect size varied from moderate to strong, as participants with uncontrolled blood pressure or untreated blood pressure had twice the rate of increase after follow-up as the comparison group. Similar associations have been found in other cohorts, speaking about its consistency. The associations between arterial stiffness, hypertension, and mortality, tended to be more specific to circulatory physiological measures (mean arterial pressure, heart rate) and body composition measurements (BMI, waist circumference). Health behaviours and inflammatory markers that influence arterial stiffness and hypertension separately, were not factors that affected this association.

There is a high dose-response relationship in this relationship, with arterial stiffness having almost a linear relationship with blood pressure and we can see that

influence on individuals reaching control of blood pressure with antihypertensive medications or in participants without hypertension. There seems to be an additional element beyond the level of blood pressure, as uncontrolled participants seem to have an additional rate of increase in arterial stiffening. The association is biologically plausible, as there are multiple mechanisms linking hypertension to a higher rate of stiffening, mainly the fracture of elastic fibres in arteries being exposed to continuous high blood pressure, leading to a lower overall stiffness. Although the unidirectional associations between hypertension and arterial stiffness have been consistently found in separate studies, reproducing similar associations with different sizes of effect, although the finding of bidirectional associations and a role of antihypertensive medication on it are not consistent, which is a novel finding of this thesis described previously.

The bidirectionality in the relationship between arterial stiffness and hypertension is biologically plausible, as there are many pathological mechanisms described in both ways. Studies of mendelian randomisation have shown that genetic, heritable components predict some shared traits between PWV and blood pressure.[408] Increased arterial pulsatility or decoupling of the RAS system are physiological mechanisms of hypertension that induce stiffness, whereas stiffening mechanisms such as increased vascular resistance, arterial inflammation, atherosclerotic plaques, calcium deposition, fractured elastin fibres are inducers of hypertension.

Regarding temporality in the association between cf-PWV and MACE, high AS preceded MACE. I ensured this by excluding recurrent events in the survival analyses. These were associated in a dose-response fashion. Participants with higher average arterial stiffness having a relative risk 5 times higher than the ones

with low arterial stiffness. The biological mechanisms involved in MACE, specially in haemorrhagic events, are shared with these related to the onset of hypertension, fracturing elastic fibres and diminishing the arterial compliance, hence making the arterial wall more likely to rupture. Arterial stenosis and atherosclerotic conditions can explain the occurrence of ischaemic types of MACE. The effect size is similar to what has been seen in other studies, with the association of arterial stiffness with the outcome of MACE being strong and behaving in a dose-response fashion, with higher scores of arterial stiffness associated with a higher incidence of MACE. The fact that arterial stiffness predicts both all-cause and non-CVD mortality talks about moderate specificity of the association or that arterial health is related to processes beyond the cardiovascular system. Many elements of causality in this relationship, especially a clear temporality, allow to conclude that it is likely that arterial stiffness has a causal role in the development of fatal and non-fatal events. I will finally discuss the application of causality criteria to the association between arterial stiffness and physical functioning. Temporality was observed in the decrease of the SF-36 physical component summary score, as high arterial stiffness as well as arterial stiffening preceded a decrease in physical functioning. There are two main pathways explaining biological plausibility of this association: first, arterial stiffness because of the relationship between cardiovascular disease and clinical damage in the nervous system. The first pathway most likely operated in individuals with high cardiovascular leading to MACE with motor impairment as a consequence. The second would be explained by subclinical neurological damage, as is the case with pulsatile pressure affecting cerebral vessels and leading to white matter injury.[409,410] The association was found with one measure of physical

functioning, but not with others, this might have to do with the mechanisms enabling the decrease in physical functioning and talk about the specificity of the association with the most vigorous physical activities listed in the SF-36 questionnaire, rather than the less vigorous activities from the clinical screening seen in a dose-response fashion. Considering all the elements of causality in this association, further research needs to be done to elucidate the role of arterial stiffness as a marker of ongoing cardiovascular disease or an independent risk factor contributing to decreased physical functioning and why affects one form of physical functioning and not others.

8.5.1 Causal inference and genetic studies

Genetics also play a role in the associations between arterial stiffness, hypertension, and major cardiovascular events. Causal inference studies are helping to disentangle some of these relationships.

Seminal genetic studies on this topic estimated that 36% and 12% of the variability in arterial features and cf-PWV, respectively, were directly attributable to genes and heritage [411]

Although the description of this amount of variability in PWV attributable to genes suggests that there are multiple potential targets for the prevention and treatment of arterial stiffness, the identification of genes that are related to PWV does not necessarily mean that these are immediate objectives for gene therapy. This is mainly explained by the polygenic nature of conditions involved and the small effect sizes of individual genes usually reported. [412]

Genetic studies with relatively small samples have reported the heritability of specific genes and transcription factors relative to PWV such as arterial calcification or vascular smooth cell tone. [413] A GWAS sub study in the Framingham cohort studied the association between gene expressions in arterial stiffness and blood pressure. This study generated hypotheses for genetic regions that could be explored, although without conclusive associations with genomic-wide significance [414]

Other GWAS and multi-OMICS studies have suggested the presence of specific nucleotide variants in the gene CIB2 that were responsible for higher arterial calcification and higher cf-PWV, finding a causal relationship between the presence of the genotype, DNA methylation and the expression of the CIB2 genes.[415]

One of the most extensive meta-analyses for genetic studies in arterial stiffness including more than 23.000 observations from 11 cohorts, showing GWAS for cf-PWV that resulted in the isolation of the BCLB11 (B-cell leukemia 11b) transcription factor, which was associated with higher arterial stiffness and higher risk for CVD.[416] Other studies have replicated its presence in vascular smooth cells, regulating the function in their cytoskeletal actin and vascular stiffness.[417]

Other cohort studies have shown cross-sectional and longitudinal associations between candidate genes and cross-sectional arterial stiffness measured as carotid distensibility and arterial diameter, as well as progression in both quantities over time, finding that the expression of genes coding for arterial matrix proteins were associated with arterial stiffness, while on a longitudinal analysis, the

increase in the expression of the ACE gene and the NOS3 gene were associated with a decrease in carotid distensibility.[418]

Finally, another study from the same cohort has tried to assess the independent heritability of phenotypes of arterial stiffness, as well as their relationship with the onset and progression of hypertension. The analysis of mendelian randomisation supported the findings from other observational cohorts showing the existence of a bidirectional relationship, with over 60% of heritability in PWV and around 50% for systolic blood pressure components.[419]

8.6 Strengths

The Whitehall II study is an occupational cohort with a high response rate, not only in the initial recruitment phase, but also across the 12 different research phases of the study. The study has substantial numbers of observations of cf-PWV. This sample size allows for a variety of subgroup comparisons and stratification without losing statistical power. In addition, the measure of cf-PWV being used is the gold standard for the assessment of arterial stiffness, as it is quick, non-invasive and reflects in-vivo studies with invasive techniques. [420]

Regarding the accuracy of blood pressure measurements, the protocols for the assessment of blood pressure in the research clinics include some strategies such as allowing for resting time after arrival and promoting a relaxed environment with increased communication between research staff and study participants, all of which have been shown to reduce the impact of white coat hypertension. [421]

In addition to the high-quality data from the Whitehall II study, the NHS Hospital Episode Statistics is a robust data source of clinical records. The use of HES-

linked data has enabled me to improve the accuracy in the assessment of the risk of hard cardiovascular outcomes using cf-PWV as an exposure in some participants who have been lost to follow-up.

Measures of physical function are not only self-reported questionnaires such as the SF-36, but also standardised tests of physical performance. The consistent and standardised application of the study protocols enable the repeatability and consistency of these measurements.

8.7 Limitations

One of the limitations of the timing of the measurements of arterial stiffness in this cohort is that they only capture the process of arterial stiffness after the start of midlife, whereas some transitions regarding blood pressure tend to happen before that age. Earlier measurements would have added evidence about the bidirectionality of the relationship between arterial stiffness and hypertension.

Another limitation is the lack of statistical power to detect whether changes in the magnitude of arterial stiffness are related to changes in CVD risk. Solving the questions about change would be an important way of monitoring cardiovascular risk.

Whitehall II is a relatively healthy cohort in terms of cardiovascular disease risk, which might be reflected in lower predictive power of risk models.

The association between AS progression and blood pressure status might be affected by some degree of misclassification, as it is challenging to model the exact time spent with hypertension between baseline and follow-up.

This thesis does not intend to suggest that hypertension is the only relevant risk factor for arterial stiffness. Although most of the common risk factors for arterial stiffness and arterial stiffening were considered, inflammation is a relevant topic that was not analysed. A reason for this was the lack of strong associations between inflammation markers such as C-reactive protein or Interleukin-6, and cf-PWV in this cohort.[373]

Despite that the access to HES data is a valuable tool for the assessment of non-fatal and fatal clinical events in participants with loss to follow-up, its utility still has limitations. More than 24% of deaths occur in care homes and another 22% occur in private homes. [422]

Regarding physical functioning, a potential drawback of self-reported measurements of limitations in physical functioning is that they are subjective, thus potentially overestimating, or underestimating functioning. This variation could be higher according to the type of cardiovascular or metabolic condition of the individual and to age group.[423]

8.7.1 How weak or strong is the evidence that PWV relates to physical functioning

Although the associations currently assessing arterial stiffness and physical functioning have been independent of the main cardiovascular risk factors, the independence is not evidenced when the associations have been assessed longitudinally. Another weakness of the evidence is the heterogeneity of the populations in which it has been assessed. Most of the studies include samples smaller than 200 individuals and are cross-sectional, which could contribute to the

already existing burden of cardiovascular disease accumulated in the studied groups.

Most of the participants come from inpatient populations, cohorts from patients with kidney disease, or individuals with already onset cardiovascular disease, being prone to selection bias.

In addition, there is a high heterogeneity of the outcomes that were being evaluated. Muscle fatigability was an outcome measured in one study. This cannot be directly compared to the standard test of walking speed or walking on a treadmill machine. In the subjective markers of functioning, health related quality of life might not be equivalent to the quality of life measured by the SF-36 questionnaire.

8.8 Clinical implications

Pulse wave velocity can be a marker of progression in the treatment of hypertension. Thus, monitoring the success of antihypertensive therapy and the subclinical progression of its consequences, as target-organ damage, could be a task that could rely on primary care settings using cheaper and more widely available tools compared with cardiovascular imaging, mostly available at secondary care, as it could be the case with cf-PWV. As cf-PWV adds information to blood pressure measurements, encouraging joint monitoring could be an improvement in clinical practice, especially in prehypertensive patients.

The follow-up could be focused on general cardiovascular risk as well. The improvement of cf-PWV on the 10-year cardiovascular risk scores could be

reflected in the intermediate or long-term, the use of cf-PWV in the medical consultation of global cardiovascular risk and supporting decisions as initiation of statin treatments in populations with low and intermediate cardiovascular risk, where treatment decisions tend to be difficult and can fall into grey areas. As this marker is a correlate of physical functioning and a predictor of decline in some physical functioning scores, its inclusion in the physical examination of elderly patients could be a measurement of vascular ageing as a global assessment of health and predictor of cardiovascular risk.

8.8.1 Practical utility of cf-PWV in clinical practice in light of the ASCVD analyses

Finally, the results of the risk reclassification of individuals using the ASCVD stratification in chapter 6 show that using cf-PWV improves the performance of the AHA/JACC pooled chain equations, especially in risk groups where the decision of initiation of primary prevention therapies might not be clear. The improvement in NRI of 5% to 11% is comparable to other strong risk enhancers and suggests that health benefit could be achieved at population as well as clinical level. Earlier studies on improvement of the ASCVD score have shown that adding CAC and family history increased NRI by 12% and 5%, respectively,[424] whereas ankle-brachial index and C-reactive protein did not show improvements. Currently, determination of CAC score is the gold standard recommendation when the treatment decision of lipid-lowering medication is uncertain.[425] However, CAC measurement is dependent on ionizing radiation and computed tomography (CT) scanners are often not available in primary care. The cost per gained quality

adjusted life-year (QALY) using CAC measurements (USD $35,000-$48,000) exceeds the cost-effectiveness recommendations in the UK, 40 making its use in primary care less feasible. In comparison, cf-PWV is less expensive, does not rely on ionizing radiation, and can be performed by trained technicians. This stresses the need of further studies on applicability of cf-PWV in risk stratification in primary care settings.

8.9 Research implications

Further studies assessing the ability of cf-PWV for risk stratification in cardiovascular disease events should be done before recommending its implementation in clinical practice. An additional suggestion for future studies would be carrying out some of these validations in multi-ethnic populations.

The possibility of including cf-PWV as another risk enhancer for low and intermediate risk individuals in the atherosclerotic classifications of the American Heart Association and a potential cost-effectiveness evaluation against coronary calcium would help to find alternatives for risk stratification and medical decision making in primary CVD prevention.

8.10 Policy implications

There are different recommendations for health policy that can be formulated from the results of this thesis.

The relatively high proportion of untreated hypertensive and uncontrolled hypertensive participants in this relatively healthy cohort lead us to infer that the burden of this types of hypertension can be lower than in the general population.

Health policy should be aimed at improving and strengthening programmes for increasing awareness for the treatment of hypertension is a way of reducing the progression of arterial stiffening on a population level. Wide availability of measurement facilities and programmes for lending blood pressure measurement equipment at home to the general population can be the subject of policy measures.

The findings related to the risk of Major Adverse Cardiovascular Events inferred from a single measurement of cf-PWV, showed its relevance as a risk predictor in participants with low and intermediate cardiovascular risk measured by the 10-year ASCVD score. Even though, there are still some clinical validations that remain to be done until arterial stiffness measurement can be adequately used in daily clinical practice. The health policy recommendations on that sense would be to fund more studies that can provide the required validations. Cost-effectiveness studies reported the cost of a CAC score scan and associated costs near $160 US Dollars in 2014, [425] although prices in commercial clinics in the UK round £476. [427] Prices for one measure cf-PWV are not yet available commercially, but we could infer that these would be lower as the commercial price of a cf-PWV measuring device is 6 times lower the price of a CT-scanner (AtCor Medical, Australia), being a non-invasive measurement requiring only one healthcare professional to take it and does not require exposure to radiation or follow-up tests that might not be conclusive.

Lower physical functioning and a steeper decline in physical function were predicted by high arterial stiffness, meaning that research or inclusion of arterial stiffness monitoring could be a tool in programs for monitoring healthy ageing. In

line with the WHO health policies on that subject, the ability of health services abilities to prevent or control chronic conditions should be improved. Programmes that explore the use of markers of low-physical function such as arterial stiffness can be helpful in promoting the compression of morbidity in old age.

8.11 Conclusion

The overall aim of this thesis was to test the bidirectional relationship between arterial stiffness and hypertension, as well as establishing how arterial stiffness measures predict major cardiovascular events and decline in physical functioning. After adjustment for the most relevant cardiovascular risk factors, arterial stiffness measured by cf-PWV predicted the development of incident hypertension. In an opposing way, not being treated for hypertension or not achieving a therapeutic level of blood pressure after receiving treatment, predicted a higher increase in arterial stiffness over time.

Arterial stiffness measured by cf-PWV also improved the performance of traditional cardiovascular factors in models predicting the risk of cardiovascular events in intermediate and low-risk individuals from the cohort. The comparison between repeated measurements of cf-PWV showed that a single measurement of cf-PWV has sufficient information to establish the cardiovascular risk of an individual, not needing repeated measurements to perform this prediction.

Lastly, cf-PWV and change in cf-PWV predicted the decline in physical functioning over time., although many of the functioning indicators were not independently associated and the effect was not observed after adjusting for them.

This thesis underscores the importance of cf-PWV as a marker of high cardiovascular risks and its potential implementation in clinical practice.

Chapter 9 Appendix

9 Appendix

9.1 Co-authored outputs

9.2 Other outputs

9.3 Appendixes for chapter 5

Appendix. Incident hypertension at phase 11 in non-hypertensives at phase 9

Incident hypertension	Odds ratio	P>|z|	[95% conf. interval]	
PWV lower tertile				
PWV middle tertile	**1.71931**	**0.000**	**1.325392**	**2.230305**
PWV highest tertile	**2.485633**	**0.000**	**1.830862**	**3.374571**
Age at baseline	**1.029455**	**0.008**	**1.007472**	**1.051919**
Sex	.7744163	0.052	.5986388	1.001807
Ethnicity	1.163182	0.516	.7370401	1.835709

Incident hypertension	Odds ratio	P>|z|	[95% conf. interval]	
PWV lowest tertile				
PWV middle tertile	**1.658240**	**0.000**	**1.272487**	**2.160932**
PWV highest tertile	**2.352499**	**0.000**	**1.720017**	**3.217557**
Age	**1.033936**	**0.003**	**1.011565**	**1.056802**
Sex	.6663647	0.024	.4678535	.9491047
Ethncity	1.148778	0.553	.7264886	1.816533
Waist circumference	.9844851	0.237	.9592792	1.010353
Body mass index	1.080824	0.031	1.006948	1.160119

Incident hypertension	Odds ratio	P>|z|	[95% conf. interval]	
PWV lowest tertile				
PWV middle tertile	**1.653993**	**0.000**	**1.26851**	**2.156617**
PWV highest tertile	**2.381827**	**0.000**	**1.738839**	**3.262577**
Age	**1.033335**	**0.004**	**1.010658**	**1.05652**
Sex	**.6509915**	**0.020**	**.4533723**	**.9347503**
Ethnicity	1.162832	0.527	.7290165	1.854798
Waist circumference	.9843542	0.236	.9590232	1.010354
Body Mass Index	**1.080051**	**0.034**	**1.005901**	**1.159667**
Cholesterol	1.054615	0.398	.9321675	1.193147
Type II Diabetes	.7487609	0.282	.4421184	1.268083
Current smoker	1.197695	0.445	.7540042	1.902474
Alcohol units	.997974	0.722	.9868721	1.009201
Statins	1.249597	0.164	.9127735	1.710713

Incident hypertension	Odds ratio	P>|z|	[95% conf. interval]	
PWV lowest tertile				
PWV middle tertile	**1.292287**	**0.069**	**.9799114**	**1.704243**
PWV highest tertile	**1.600165**	**0.005**	**1.149859**	**2.226818**
Age	**1.033437**	**0.005**	**1.00997**	**1.057448**
Sex	.8515266	0.402	.5845027	1.240538
Ethnicity	1.114264	0.664	.6836944	1.815993
Waist circumference	.9831026	0.212	.9571622	1.009746
Body mass index	1.049595	0.197	.975223	1.129639
Blood cholesterol	1.026227	0.693	.9024943	1.166923
Type II diabetes	.8007491	0.425	.4636442	1.382955
Smoker	1.422437	0.158	.8724213	2.319208
Alcohol units	.9980685	0.745	.9864871	1.009786
Statins	1.186226	0.304	.8563492	1.643176
Systolic blood pressure	**1.071825**	**0.000**	**1.057158**	**1.086696**

9.4 Appendices for chapter 6

Appendix 1. Codes from the International Classification of Diseases (ICD) for cardiovascular diseases

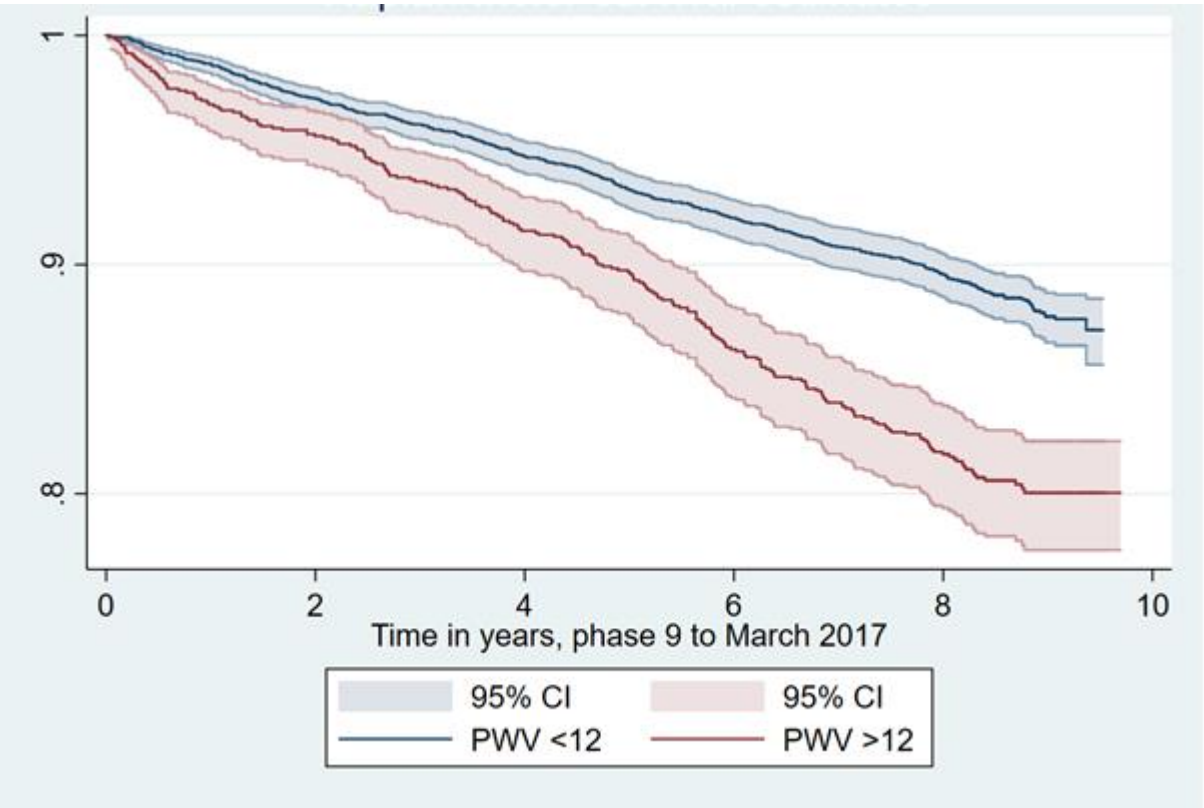
ICD code	Description
I00-I02	Acute rheumatic fever
I05–I09	Chronic rheumatic heart diseases
I10–I15	Hypertensive diseases
I20–I25	Ischemic heart diseases
I26–I28	Pulmonary heart disease and diseases of pulmonary circulation
I30–I52	Other forms of heart disease
I60–I69	Cerebrovascular diseases
I70–I79	Diseases of arteries, arterioles and capillaries
I80–I89	Diseases of veins, lymphatic vessels and lymph nodes, not elsewhere classified
I95–I99	Other and unspecified disorders of the circulatory system

Appendix 2. Codes from the International Classification of Diseases (ICD) for non-cardiovascular diseases

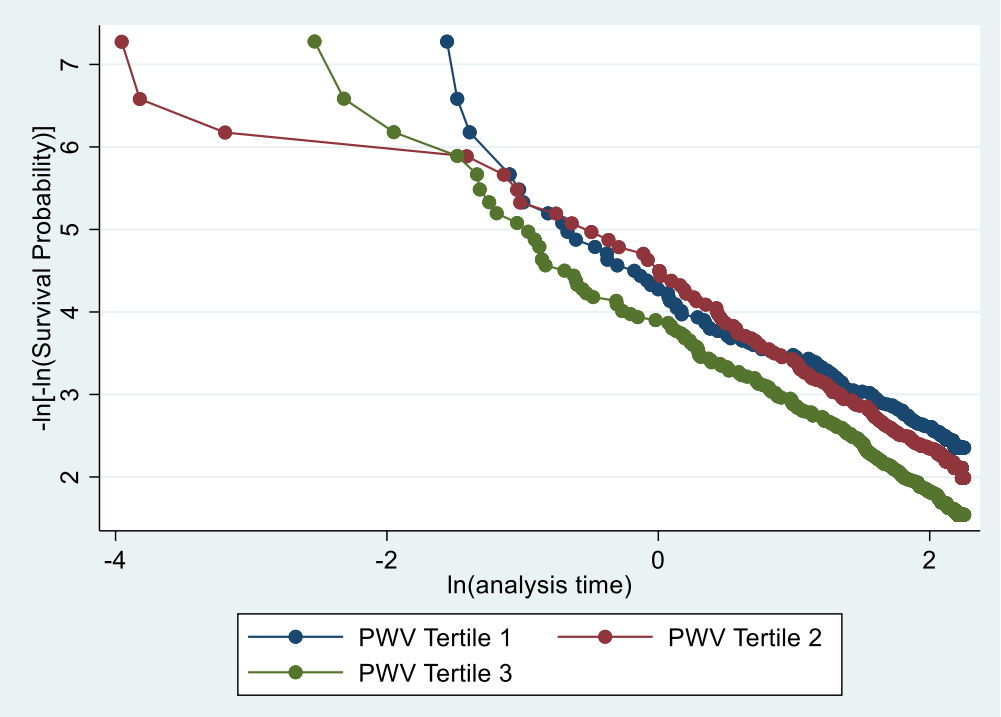
ICD code	Description
A00-B99	Infectious and parasitic diseases
C00–D48	Chronic rheumatic heart diseases
D50–D89	Hypertensive diseases
E00–E90	Ischemic heart diseases
F00–F99	Pulmonary heart disease and diseases of pulmonary circulation
G00-G99	Diseases of the nervous system

H00-H59	Diseases of the eye and adnexa
H60-H95	Diseases of the ear and mastoid process
	Diseases of the respiratory system
K00-K93	Diseases of the digestive system
L00-L99	Diseases of the skin and subcutaneous tissue
M00-M99	Diseases of the musculoskeletal system and connective tissue
N00-N99	Diseases of the genitourinary system
V01-Y98	External causes of morbidity and mortality

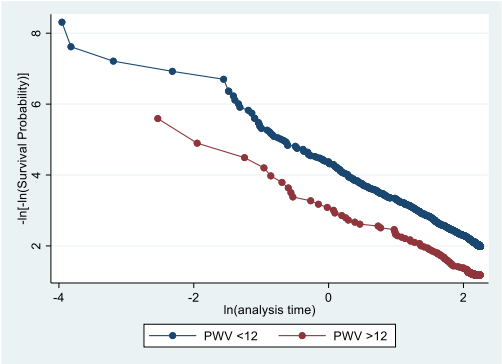
Appendix 3. Cumulative Hazard estimate for PWV as categorical variable



Appendix 4: Log-log plot of survival according to PWV tertiles



Appendix 5: Log-log plot of PWV as dichotomical variable



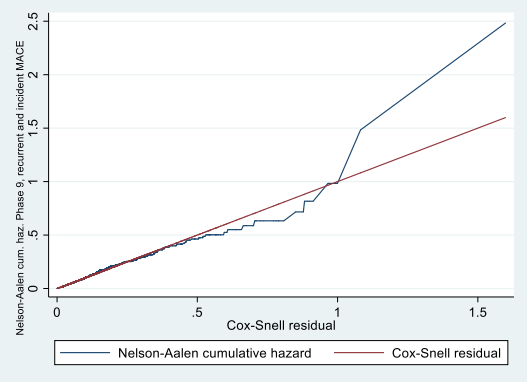
Appendix 6. Harrel's C-statistic for model comparison

Phase	C-Statistic	Somers' D
Phase 9 recurrent	0.7214	0.4429
Phase 9 incident	0.6937	0.3874
Phase 11 recurrent	0.7427	0.4854
Phase 11 incident	0.7031	0.4061
Residual method recurrent	0.7194	0.4389
Residual method incident	0.6896	0.3792

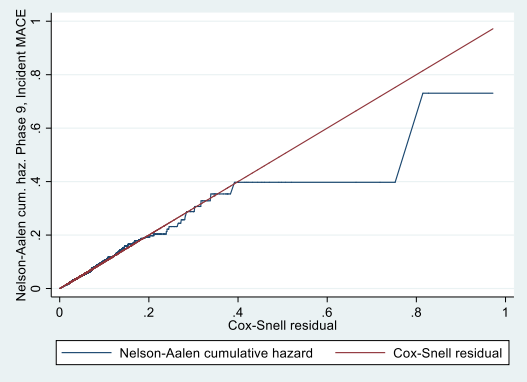
Appendix 7. Test of proportional-hazards assumption for each model

Model			
	Chi2	**df**	**P**
Phase 9 incident and recurrent MACE	27.79	14	0.01
Phase 9 incident MACE	11.29	14	0.66
Phase 11 incident and recurrent MACE	14.44	14	0.42
Phase 11 incident MACE	10.63	14	0.71
Residual method recurrent	28.84	14	<0.01
Residual method incident	16.32	14	0.19

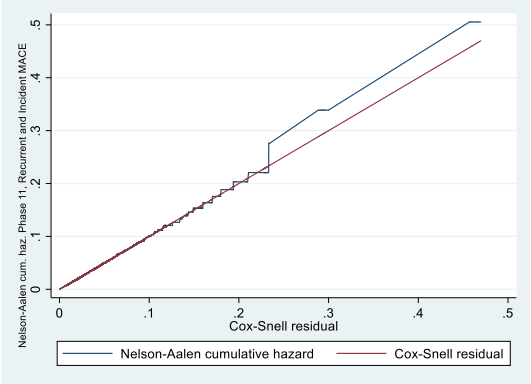
Appendix 8. Nelson-Aalen cumulative hazard estimators for Cox-Snell residuals for model for incident MACE according to PWV in phase 9



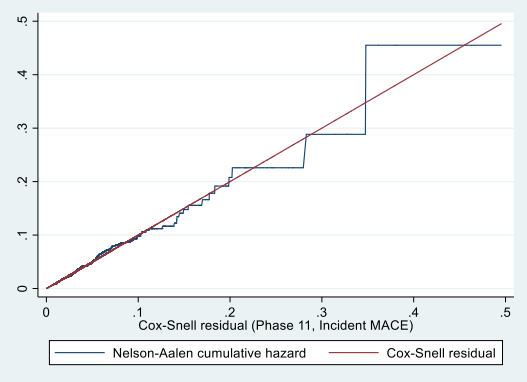
Appendix 9. Nelson-Aalen cumulative hazard estimators for Cox-Snell residuals for model for incident MACE according to PWV in phase 9



Appendix 10. Nelson-Aalen cumulative hazard estimators for Cox-Snell residuals for model for incident MACE according to PWV in phase 11


Nelson-Aalen cum. haz. Phase 11, Recurrent and Incident MACE
0
.1
.2
.3
.4
.5
0
.1
.2
.3
.4
.5
Cox-Snell residual
Nelson-Aalen cumulative hazard
Cox-Snell residual

Appendix 11. Nelson-Aalen cumulative hazard estimators for Cox-Snell residuals for model for incident MACE according to PWV in phase 11



Appendix 12 . Model using only participants with recurrent PWV at phase 9

Model (adjustments)	PWV (m/s)			PWV (standard units)		
	Haz. Ratio	[95% Conf.	Interval]	Haz. Ratio	[95% Conf.	Interval]
M1	1.02	0.96	1.09	1.05	0.90	1.23
M1 + BMI	1.01	0.95	1.08	1.03	0.88	1.20
M2 + Stroke	1.01	0.95	1.08	1.03	0.88	1.20
M3 + Waist circumference	1.02	0.95	1.09	1.04	0.89	1.22
M4 + Blood Cholesterol	1.00	0.93	1.07	0.99	0.85	1.17
M5 + Type II diabetes mellitus	1.01	0.94	1.08	1.02	0.87	1.20
M6 + alcohol consumption	1.02	0.95	1.10	1.05	0.89	1.24
M7 + physical activity	1.02	0.95	1.10	1.05	0.89	1.24
M8 + smoking status	1.02	0.95	1.10	1.05	0.89	1.24

***p<0.001**p<0.01*p<0.05

Appendix 13. Risk of MACE events according to Pulse Wave Velocity at phases 9, 11 or combined

											95% CI			PA (df)	C-Statistic
Phase (N-events)	**Hazard Ratio**	95% CI		PA (df)	C-Statistic	**Hazard Ratio**	95% CI		PA (df)	C-Statistic	Hazard Ratio	95% CI		PA (df)	C-Statistic
Phase 9* **(N= 4339 E = 541)**	1.0894	1.0485	1.1318	0.77 (3)	0.6779	1.0927	1.0490	1.1381	0.17 (4)	0.6788	1.0646	1.0205	1.1106	0.16 (9)	0.7305
Phase 11+ (N= 4371 E = 442)	1.0786	1.0426	1.1157	0.34 (3)	0.6847	1.0921	1.0537	1.1319	0.28 (4)	0.6875	1.0459	1.0066	1.0868	0.25 (9)	0.7526
Phase 11 and 9, residuals^‡ **(N = 3479 E= 314)**	1.0182	0.9610	1.0788	0.55 (3)	0.673	1.0154	0.9564	1.0782	0.26 (4)	0.6730	0.9953	0.9377	1.0567	0.17 (9)	0.7480

* Adjusted for age and sex +Adjusted for age, sex and systolic blood pressure ^Adjusted for sex, age, systolic blood pressure blood cholesterol, HDL cholesterol, smoking status, type II diabetes and antihypertensive medication ‡Models combining PWV at phases 9 and 11 used the regression residual method.

Appendix 14. Risk of MACE events according to Pulse Wave Velocity at phases 9, 11 or combined

Phase (N-events)	Hazard Ratio	95% CI		PA (df)	C-Statistic	Hazard Ratio	95% CI		PA (df)	C-Statistic	Hazard Ratio	95% CI		PA (df)	C-Statistic
Phase 9† (N= 4339 E = 541)	1.1205 (***)	1.0761	1.1667	0.007 (6)	0.6895	1.1030 (***)	1.0577	1.1504	0.005 (11)	0.7190	1.1102 (***)	1.0639	1.1585	0.02 (14)	0.7233
Phase 11+ (N= 4369 E = 442)	1.1121 (***)	1.0730	1.1524	0.08 (6)	0.7032	1.0708 (***)	1.0312	1.1119	0.1607 (11)	0.7388	1.0711 (***)	1.031	1.112	0.42 (14)	0.7427
Phase 11 and 9, residuals^‡ (N = 3479 E= 314)	1.0295	0.9691	1.0937	0.2572 (6)	0.6824	1.0470	0.9974	1.0990	0.4584 (11)	0.7329	0.9927	0.9339	1.0552	0.6596 (14)	0.7361

***p<0.001**p<0.01*p<0.05 † Adjusted for age, sex, ethnicity, mean arterial pressure and heart rate +Adjusted for age, sex and systolic blood pressure ^ Additional adjustment for Body Mass Index, Stroke, Waist circumference, blood cholesterol, Type II Diabetes Mellitus ‡, Additional adjustment for alcohol consumption, physical activity, and smoking status

Appendix 15. Risk of MACE events according to PWV measurements at phase 9 and 11

Age, sex, ethnicity model (N=3478 E=314)	Haz. Ratio	Std. Err.	P-value	[95% Conf. Interval]
Sex	0.511891	0.082198	0.001	0.373675 0.70123
Ethnicity	1.679623	0.293317	0.003	1.192791 2.365155
Age at phase 11	1.096259	0.010718	0.001	1.075452 1.117468
Mean arterial pressure, phase 11	0.997283	0.005364	0.613	0.986825 1.007852
Heart rate, phase 11	0.991018	0.005232	0.087	0.980816 1.001326
C-statistic	0.6808			
Model 1 (N=3478 E=314)	Haz. Ratio	Std. Err.	P-value	[95% Conf. Interval]
Residuals, PWV phase 9 and 11	1.029591	0.031763	0.345	0.969182 1.093766
Sex	0.513645	0.082501	0.001	0.374924 0.703691
Ethnicity	1.67217	0.292168	0.003	1.187286 2.355081
Age at phase 11	1.093916	0.010998	0.001	1.072572 1.115685
Mean arterial pressure, phase 11	0.996121	0.005497	0.481	0.985405 1.006953
Heart rate, phase 11	0.990488	0.005267	0.072	0.980219 1.000864
C-statistic	0.6824			

Model with residuals + PWV at phase 9 (N=3478 E=314)	Haz. Ratio	Std. Err.	P-value	[95% Conf. Interval]
Residuals, PWV phase 9 and 11	1.050464	0.030659	0.092	0.99206 1.112306
Sex	0.529456	0.085236	0.001	0.386187 0.725876
Ethnicity	1.554782	0.274029	0.012	1.100642 2.196307
Age at phase 11	1.073959	0.011928	0.001	1.050833 1.097595
Mean arterial pressure, phase 11	0.991665	0.00559	0.138	0.98077 1.002682
Heart rate, phase 11	0.986606	0.005367	0.013	0.976144 0.99718
Pulse wave velocity, phase 9	1.118607	0.030902	0.001	1.059651 1.180843

C-statistic	0.6910

Model with residuals + PWV at phase 11 (N=3478 E=314)	Haz. Ratio	Std. Err.	P-value	[95% Conf. Interval]
Residuals, PWV phase 9 and 11	0.920663	0.036121	0.035	0.852521 0.994252
Sex	0.529456	0.085236	0.001	0.386187 0.725876
Ethnicity	1.554782	0.274029	0.012	1.100642 2.196307
Age at phase 11	1.073959	0.011928	0.001	1.050833 1.097595
Mean arterial pressure, phase 11	0.991665	0.00559	0.138	0.98077 1.002682
Heart rate, phase 11	0.986606	0.005367	0.013	0.976144 0.99718
Pulse wave velocity, phase 11	1.140986	0.037091	0.001	1.070557 1.216048
C-statistic	0.6910			

Appendix 16. Risk of MACE according to PWV at phases 9 and 11.

Model with residuals + PWV at phase 9 and 11 (N=3478 E=314)	Haz. Ratio	Std. Err.	P-value	[95% Conf. Interval]
Residuals, PWV phase 9 and 11	1.05046	0.03065	0.092	0.99206, 1.112306
Sex	0.52945	0.08523	0.001	0.386187, 0.725876
Ethnicity	1.55478	0.27402	0.012	1.100642, 2.196307
Age at phase 11	1.07395	0.01192	0.001	1.050833, 1.097595
Mean arterial pressure, phase 11	0.99166	0.00559	0.138	0.98077, 1.002682
Heart rate, phase 11	0.98660	0.00536	0.013	0.976144, 0.99718
Pulse wave velocity, phase 9	1.11860	0.03090	0.001	1.059651, 1.180843
C-statistic	0.6910			
Model with PWV at phase 9 and 11 (N=3478 E=314)	Haz. Ratio	Std. Err.	P-value	[95% Conf. Interval]
Sex	0.52945	0.08523	0.001	0.386187, 0.725876
Ethnicity	1.55478	0.27402	0.012	1.100642, 2.196307
Age at phase 11	1.07395	0.01192	0.001	1.050833, 1.097595
Mean arterial pressure, phase 11	0.99166	0.00559	0.138	0.98077, 1.002682
Heart rate, phase 11	0.98660	0.00536	0.013	0.976144, 0.99718
Pulse wave velocity, phase 9	1.07277	0.03576	0.035	1.004911, 1.145217
Pulse wave velocity, phase 11	1.05046	0.03065	0.092	0.99206, 1.112306
C-statistic	0.6910			

Appendix 17. Risk of MACE according to PWV at phase 9

Age, sex, ethnicity model (N=4336, E=539)	Haz. Ratio	Std. Err.	P>|z|	[95% Conf.	Interval]
Sex	.5462338	.0642843	0.000	.4337142	.6879447
Ethnicity	1.657627	.2183218	0.000	1.280493	2.145835
Age at phase 9	1.095984	.0081604	0.000	1.080106	1.112096
Mean arterial pressure at phase 9	1.002519	.0043044	0.558	.9941182	1.010991
Heart rate at phase 9	.9871731	.0042523	0.003	.9788738	.9955428
C-statistic	0.6775				

Age, sex, ethnicity model + PWV (N=4336, E=539)	Haz. Ratio	Std. Err.	P>|z|	[95% Conf.	Interval]
PWV at phase 9	1.120503	.0231037	0.000	1.076124	1.166713
Sex	.5583804	.0658337	0.000	.4431723	.7035384
Ethnicity	1.579934	.2090003	0.001	1.219097	2.047573
Age at phase 9	1.077327	.0087779	0.000	1.06026	1.09467
Mean arterial pressure at phase 9	.9967052	.0044136	0.456	.9880922	1.005393
Heart rate at phase 9	.9812882	.0044598	0.000	.9725859	.9900684
C-statistic	0.6895				

Model 2 (N=4336, E=539)	Haz. Ratio	Std. Err.	P>|z|	[95% Conf.	Interval]
PWV at phase 9	1.148069	.0291939	0.000	1.092253	1.206738
Sex	.5279587	.0777058	0.000	.3956569	.7045003
Ethnicity	1.617566	.2583034	0.003	1.182869	2.212012
Age at phase 9	1.065399	.0107361	0.000	1.044563	1.08665
Mean arterial pressure at phase 9	.9900158	.0052839	0.060	.9797135	1.000426
Heart rate at phase 9	.9803465	.0054551	0.000	.9697128	.9910967
Residuals, PWV phase 9 and 11	1.027807	.0256599	0.272	.9787248	1.07935
C-statistic	0.6872				

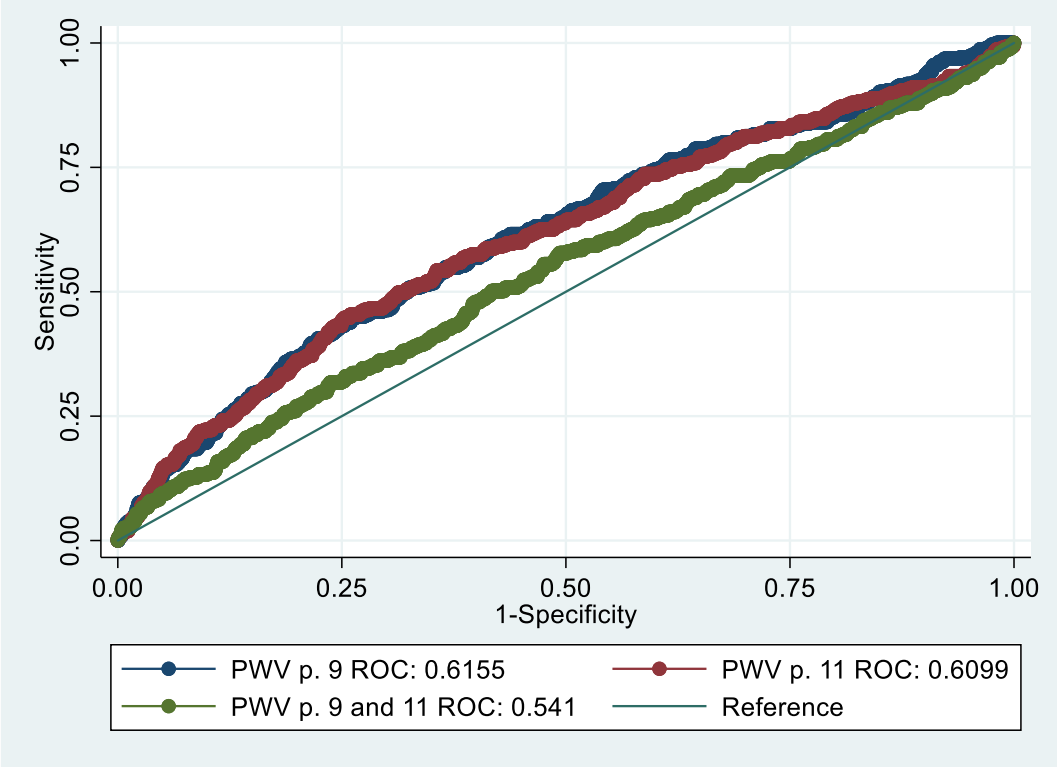
Age, sex, ethnicity model (N=4396, E=442)	Haz. Ratio	Std. Err.	P>|z|	[95% Conf.	Interval]
Sex	.4871463	.0642326	0.000	.3762049	.630804
Ethnicity	1.660813	.2472313	0.001	1.240536	2.223474
Age at phase 11	1.099563	.0090336	0.000	1.081999	1.117412
Mean arterial pressure at phase 11	.9904981	.0045182	0.036	.981682	.9993934
Heart rate at phase 11	.9899458	.0043147	0.020	.9815253	.9984386
C-statistic	0.6882				

Appendix 18. Risk of MACE according to PWV at phase 11.

Model 1 (N=4396, E=442)	Haz. Ratio	Std. Err.	P>|z|	[95% Conf.	Interval]
PWV at phase 11	1.112196	.0199597	0.000	1.073755	1.152012
Sex	.5005481	.0661267	0.000	.3863624	.6484803
Ethnicity	1.558102	.2329505	0.003	1.162341	2.088615
Age at phase 11	1.075067	.0098469	0.000	1.055939	1.094541
Mean arterial pressure at phase 11	.9836124	.0046488	0.000	.9745429	.9927663
Heart rate at phase 11	.9851192	.0043753	0.001	.9765811	.993732
C-statistic	0.7032				

Model 2 (N=4396, E=442)	Haz. Ratio	Std. Err.	P>|z|	[95% Conf.	Interval]
PWV at phase 11	1.140986	.0370905	0.000	1.070557	1.216048
Sex	.529456	.0852357	0.000	.3861866	.7258762
Ethnicity	1.554782	.2740288	0.012	1.100642	2.196307
Age at phase 11	1.073959	.0119282	0.000	1.050833	1.097595
Mean arterial pressure at phase 11	.9916654	.0055899	0.138	.9807698	1.002682
Heart rate at phase 11	.986606	.0053665	0.013	.9761438	.9971804
Residuals, PWV phase 9 and 11	.9206634	.0361209	0.035	.8525213	.9942521
C-statistic	0.6910				

Appendix 19. ROC curves for MACE and PWV at phases 9 and 11.



Appendix 20. ROC analysis for MACE events according to PWV at phases 9, 11 and combined

	Obs	ROC	Std. Err.	[95% Conf. Interval]
Pulse Wave Velocity, phase 9	3480	0.6155	0.0173	0.5816, 0.6493
Pulse Wave Velocity, phase 11	3480	0.6099	0.0177	0.5753, 0.6445
Pulse Wave Velocity at phase 9 and 11, residuals	3480	0.5410	0.0178	0.5060, 0.5759
Ho: area(PWV at phase 9) = area(PWV at phase 11) = area(PWV at phases 9 and 11)				
chi2(2) = 26.67	Prob>chi2 = 0.0000			

9.5 Appendixes for chapter 7

Appendix 1. SF-36 Questions

GENERAL HEALTH DOMAIN

a) In general, would you say your health is:

Excellent, Very Good, Good, Fair, Poor

b) Compared to one year ago, how would you rate your health in gen now?

Much better now than one year ago

Somewhat better now than one year ago

About the same as one year ago

Somewhat worse now than one year ago

Much worse than one year ago

LIMITATIONS OF ACTIVITIES

c) The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

a) Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

b) Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

c) Lifting or carrying groceries

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

d) Climbing several flights of stairs

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

e) Climbing one flight of stairs

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

f) Bending, kneeling, or stooping

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

g) Walking more than a mile

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

h) Walking several blocks

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

i) Walking one block

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

j) Bathing or dressing yourself

Yes, Limited a lot; Yes, Limited a little; No, Not limited at all

PHYSICAL HEALTH PROBLEMS

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

a) Cut down the amount of time you spent on work or other activities

Yes; No

b) Accomplished less than you would like

Yes; No

c) Were limited in the kind of work or other activities

Yes; No

d) Had difficulty performing the work or other activities (for example, it took extra effort)

Yes; No

EMOTIONAL HEALTH PROBLEMS

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

a. Cut down the amount of time you spent on work or other activities

Yes; No

b. Accomplished less than you would like

Yes; No

c. Didn't do work or other activities as carefully as usual

Yes; No

SOCIAL ACTIVITIES

a) Emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all, Slightly, Moderately, Quite a bit, Extremely

PAIN

a) How much bodily pain have you had during the past 4 weeks?

None, Very mild, Mild, Moderate, Severe, Very severe

b) During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

None, Very mild, Mild, Moderate, Severe, Very severe

ENERGY AND EMOTIONS

These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks

a. Did you feel full of pep?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

b. Have you been a very nervous person?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

c. Have you felt so down in the dumps that nothing could cheer you up?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

d. Have you felt calm and peaceful?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

e. Did you have a lot or energy?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

f. Have you felt downhearted and blue?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

g. Did you feel worn out?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

h. Have you been a happy person?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

i. Did you feel tired?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

SOCIAL ACTIVITIES

a. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time, Most of the time, A good bit of the time, Some of the time, A little of the time, None of the time

GENERAL HEALTH

11. How TRUE or FALSE is each of the following statements for you?

a. I seem to get sick a little easier than other people

Definitely true, Mostly true, Don't know, Mostly false, Definitely false

b. I am as healthy as anybody I know

Definitely true, Mostly true, Don't know, Mostly false, Definitely false

c. I am as healthy as anybody I know

Definitely true, Mostly true, Don't know, Mostly false, Definitely false

d. I expect my health to get worse

Definitely true, Mostly true, Don't know, Mostly false, Definitely false

e. My health is excellent

Definitely true, Mostly true, Don't know, Mostly false, Definitely false

9.6 Additional outputs of the thesis

9.6.1 Co-supervision MSc Thesis

UCL
INSTITUTE FOR EPIDEMIOLOGY AND HEALTH CARE

MSc POPULATION HEALTH
DISSERTATION COVER SHEET
2017/8

CANDIDATE NUMBER: **WVQW8**
TYPE OF DISSERTATION: **Secondary Data Analysis**
DISSERTATION TITLE: Telomere length, frailty and physical functioning in Whitehall II

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