Physiological systemic and cerebrovascular determinants of the risk of stroke, chronic cerebral ischaemia and vascular cognitive impairment

Thesis submitted for degree of Doctor of Medicine

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ABSTRACT

Stroke and dementia are the commonest cause of neurological disability in the UK, but despite optimal secondary prevention there remains a >10% risk of recurrent, major cardiovascular events over 5 years. Furthermore, chronic damage to the small vessels in the brain, 'Small Vessel Disease,' is associated with up to 30% of strokes and 40% of dementia, yet the underlying mechanisms are unclear and there is no specific proven treatment.

Our previous work demonstrated that haemodynamic indices beyond mean blood pressure are associated with an increased risk of either stroke, small vessel disease and dementia, including blood pressure variability, arterial stiffness and cerebral arterial pulsatility. These indices are all associated with age and a history of hypertension, and represent intermediate cardiovascular phenotypes of vascular aging. However, their distribution in populations at risk, associated risk factors and relationship to cardiovascular events and small vessel disease remains poorly defined.

Within the OXVASC population-based cohort of all patients with cardiovascular events in a population of >92,000 in Oxfordshire, we have carried out physiological tests in consecutive, consenting patients presenting to the TIA and minor stroke clinic at the 1 month follow-up visit, since September 2010. This cohort undergo detailed clinical review and face-to-face follow-up to 10 years, and undergo measurement of arterial stiffness and aortic pressure by applanation tonometry (Sphygmocor), continuous non-invasive beat-to-beat blood pressure (Finometer) and cardiac monitoring for 5-10 minutes and transcranial ultrasound for assessment of cerebral blood flow velocity and cerebral arterial pulsatility (DWL DopplerBox).

In this population, I demonstrated that beat-to-beat blood pressure variability (BPV) is normally distributed at younger ages, but becomes progressively more positively skewed in older men and women, with an initial fall from younger patients to over 50, before a marked average rise, implying a transition from 'healthy' endogenous forms of increase BP variability in young patients to unhealthy, excessive BPV. Beat-to-beat BPV is particularly associated with increased arterial stiffness, a marker of vascular aging, but also shows a complex relationship with increase body mass index, which is linearly associated in men but increased both in underweight and overweight women. Beat-to-beat BP variability also progressed significantly over the age of 55 in 179 patients over five years. Finally it was associated with an increased risk of recurrent vascular events, independent of age and other risk factors.

In the second half of the thesis, I assessed the effect of arterial stiffness and its interaction with pulsatility of blood flow to the brain. In the UK Biobank study (chapter 7), increased arterial stiffness resulted in an earlier transition from mid-life hypertensive phenotypes with high diastolic and systolic blood pressures to a falling diastolic BP and a linearly rising systolic blood pressure (SBP) with age, at about age 55.

In OXVASC, aortic stiffness, aortic pulsatility and cerebral pulsatility increased with age, with a particularly strong association between aortic and cerebral pulsatility, partially mediated by aortic stiffness (chapter 8). All three progressed at older ages despite treatment, predominantly after 55 years for cerebral pulsatility (chapter 9).

Finally, cerebral arterial pulsatility was associated with cerebral small vessel disease and predicted the risk of recurrent cardiovascular events more strongly than other physiological indices, and independent of age and cardiovascular risk factors, although this relationship was not mediated by small vessel disease.

Overall, arterial stiffness, beat-to-beat BP variability and cerebral arterial pulsatility were all associated with vascular aging and a history of hypertension, they progressed after the age of 55 and were predictive of the risk of recurrent stroke. These reflect vascular aging and are key potential targets that may mediate the persistent cardiovascular risk in the elderly, despite optimal medical management.

Declarations

I certify that this thesis entitled ‘was performed whilst I was a postgraduate student affiliated with the University of Oxford.

I declare that this thesis is my own work. Having designed the protocol and established the physiological assessments within the OXVASC cohort during my DPhil, I have continued to supervise them since 2013 and carried out or directly oversaw the studies since 2017. I developed and performed the majority of the cleaning and analysis of physiological data. I helped acquire some of the clinical data for the OXVASC study, but as with any large epidemiological study, the majority of this has been acquired by the wider OXVASC research team. However, all statistical analysis of the relationships between physiological, demographic and clinical outcomes has been performed by myself. The analysis of the Biobank data utilizes open access data produced by the very large UK Biobank study, but all further analysis was performed by myself. Finally, this thesis was drafted in its entirety by myself, and reviewed by my adviser Prof Rothwell. Sections of this thesis have been published in peer-reviewed scientific journals, but have not been used in the application for or submission of any other degree.

Acknowledgements

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Papers Produced from this Work (in reverse chronological order)

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- Webb AJS, Lawson A, Wartolowska K, Mazzucco S, Rothwell PM. BMI and arterial stiffness are associated with greater beat-to-beat blood pressure variability after TIA or minor stroke. Stroke. Apr 9th 2021
- Webb AJS, Lawson A, Wartolowska K, Mazzucco S, Rothwell PM. Progression of beat-to-beat blood pressure variability despite best medical management. Hypertension 2020. 193-201
- Webb AJS. Progression of arterial stiffness is associated with mid-life diastolic blood pressure and transition to late life hypertensive phenotypes. JAHA 2020. 9(1)
- Webb AJS, Lawson A, Li L, Mazzucco S, Rothwell PM. Physiological determinants of residual cerebral arterial pulsatility on best medical treatment after TIA or minor stroke. JCBFM. Nov 5th 2020.
- Webb AJS, Lawson A, Mazzucco S, Rothwell PM. Age and sex distribution of beat-to-beat blood pressure variability after transient ischemic attack and minor stroke: A population-based study. IJS. Nov 9th, 2020
- Webb AJS, Mazzucco S, Li L, Rothwell PM. Prognostic Significance of Blood Pressure Variability on Beat-to-Beat Monitoring After Transient Ischemic Attack and Stroke. Stroke. 2018. 49:62-67.

Papers in Preparation

- Webb AJS, Lawson A, Wartolowska K, Mazzucco S, Rothwell PM. Progression of aortic stiffness and pulsatility determines cerebral arterial pulsatility despite best medical management. Stroke, 2021 (Under Review)

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CHAPTER ONE

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1.1 Stroke and Hypertension

1.1.1 The burden of stroke and hypertension

Stroke is the leading cause of morbidity in the United Kingdom and the third leading cause of death. Approximately 1,200,000 people are living with the consequences of stroke, with 150,000 new strokes per year,[1] including 56,000[2] fatal events. It costs the National Health Service approximately 6% of its total budget[3] in direct costs, and is a major cause of dementia.[4] As result, its prevention and treatment as part of the National Stroke Programme and the NHS Long Term Plan[5] is an integral component of the new Integrated Stroke Delivery Networks.

Hypertension is one of the most prevalent diseases worldwide and the strongest modifiable risk factor for stroke. It affected over a quarter of the world's population in 2000 and is set to rise to approximately 29% of the population by 2025, affecting 1.56 billion people.[6, 7] A mean systolic blood pressure (SBP) >115mmHg explains 50-60% of the worldwide population attributable risk of stroke[8-10] and an increment of 20mmHg SBP doubles the risk of stroke between 40-69 years of age.[8] Randomisation to a treatment which reduces mean SBP results in a significant reduction in cardiovascular events with a 5-6mmHg decrease in diastolic pressure (DBP) resulting in a 33-50% reduction in stroke and a 4-22% reduction in acute coronary events.[11] Nonetheless, only approximately 50% of patients with hypertension receive treatment in both the UK[12] and the rest of the world,[13] resulting in a significant excess burden of cerebrovascular disease.[14]

1.1.2 Hypertension and stroke subtypes

The pathophysiological relationship between hypertension and stroke is complex. Firstly, hypertension causes accelerated atherosclerosis, contributing to the pathogenesis of large artery disease;[15] secondly, it causes hypertensive and ischaemic heart disease resulting in atrial fibrillation[16] and post-infarction mural thrombosis, with subsequent cardioembolic stroke; thirdly it is associated with arteriosclerosis, lipohyalinosis and the development of leukoaraiosis, lacunar strokes and vascular

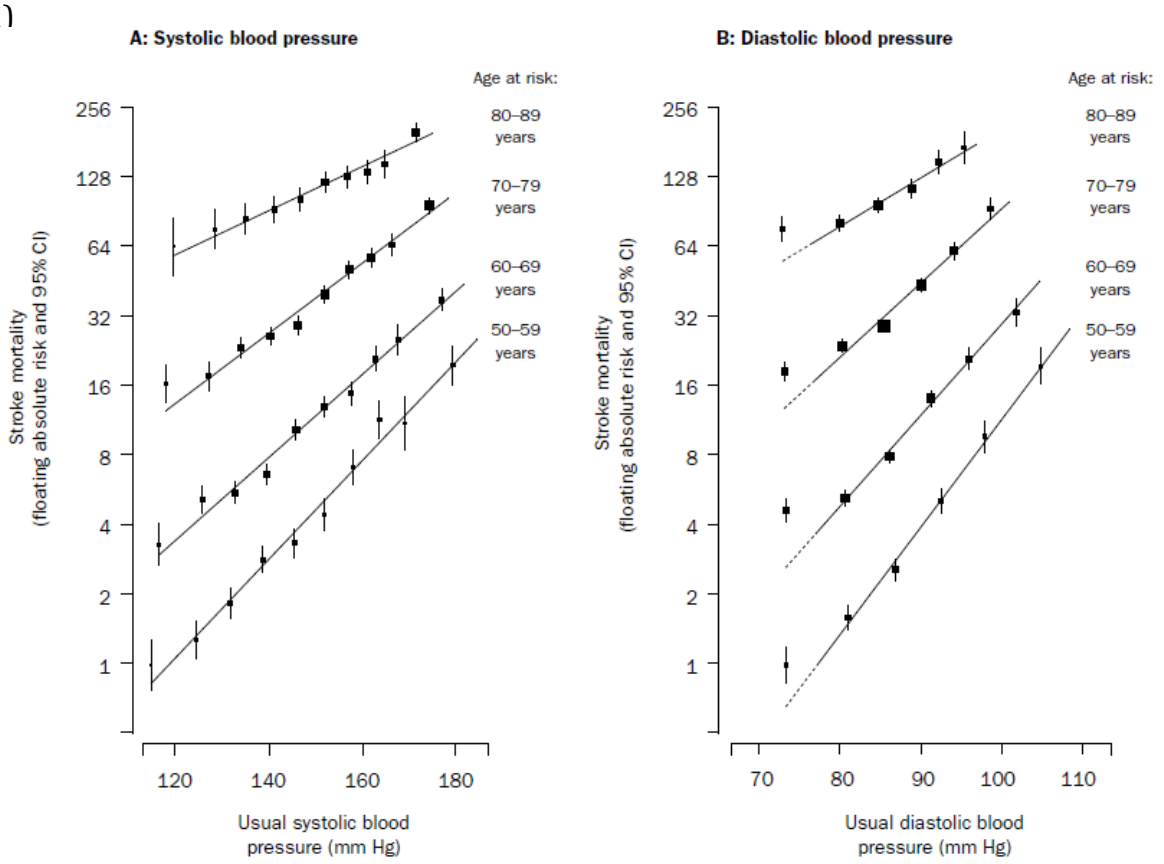
dementia.[17] A strong relationship between small vessel disease and hypertension has been suggested by hospital-based cohort studies, although these studies probably overestimate this relationship when population based cohorts are also included.[18] Nonetheless, there are clear relationships between hypertension and all aetiological subtypes of stroke.

1.2 Hypertension as a risk factor

1.2.1 Usual blood pressure

Although there had been extensive research into the relationship between blood pressure and cardiovascular disease, a precise quantification of the relationship between DBP and cardiovascular events was only reached in 1990 with the concept of 'Usual' blood pressure. [11, 19] This population-based statistical measurement uses the 'regression dilution ratio' to adjust the risk relationship between measured blood pressure and cardiovascular events for the effect of regression to the mean. This systematic bias occurs when patients at the extremes of a distribution tend to have blood pressures closer to the population mean at follow up, independent of intervention, resulting in an under-estimation of the risk of subsequent cardiovascular events for a given blood pressure. Using this technique, it has been shown that there is a continuous relationship between average DBP or SBP and vascular mortality,[8] coronary artery disease,[8, 19] stroke,[8, 19] heart failure,[20] peripheral artery disease[21] and end-stage renal failure.[22] In more than 1 million adults,[8] for each 20mmHg decrease in SBP or 10mmHg in the DBP, the rate of vascular mortality at 40-49 years of age is halved, with one-third less at 80-89 years old, with a greater decrease in stroke mortality (hazard ratio (HR)=0.36, 95% CI 0.32-0.40) than in ischaemic heart disease (HR=0.49, 0.45-0.53). This relationship is continuous down to at least 115mmHg / 75mmHg, is similar for all vascular death (HR=0.43, 0.38-0.48), and is even seen for non-vascular causes of death (HR=0.88, 0.87-0.89). The relative risk for all outcomes decreases with age, but as the risk increases with age, the absolute increase in risk with increasing blood pressure is greater in older individuals.

1)



2)

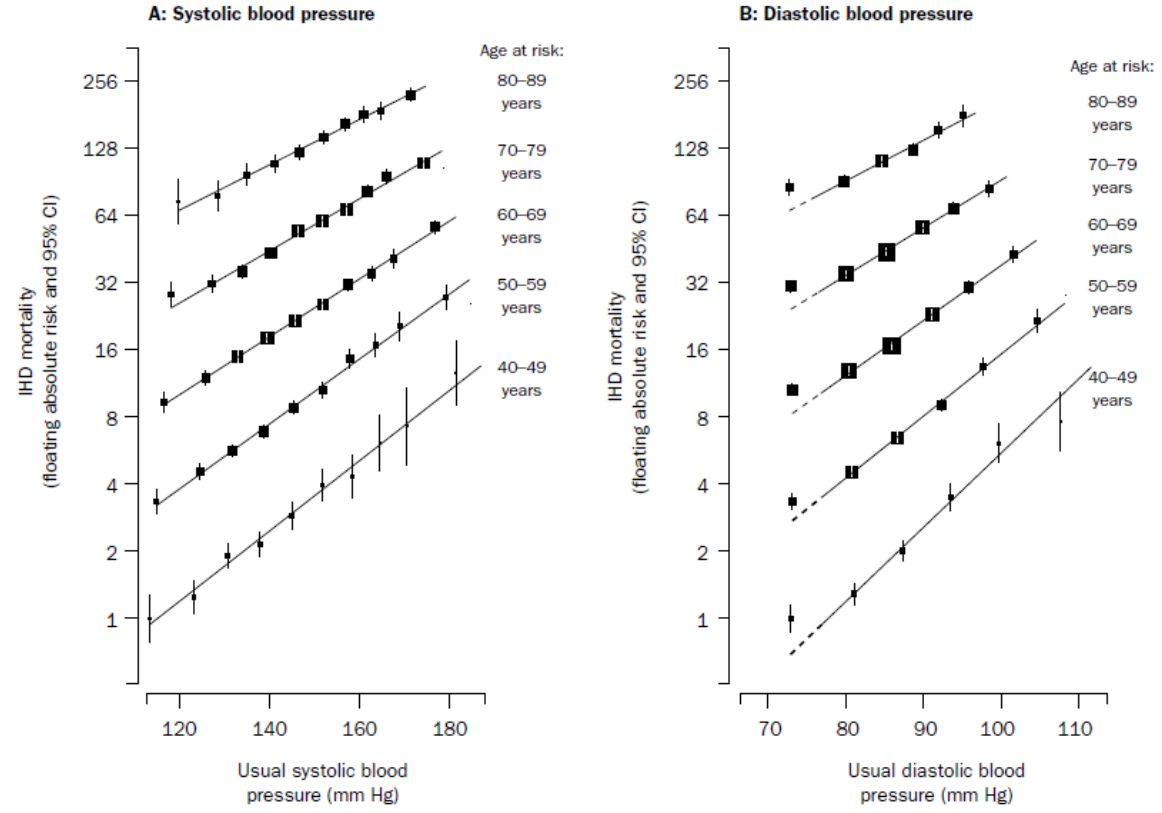


Figure 1.1 Mortality rate in each decade of age versus usual blood pressure at the start of the decade for 1) Stroke and 2) Ischaemic heart disease. Derived from Lewington et al.[9]

1.2.2 The importance of systolic blood pressure

DBP was viewed as the key determinant of risk until the mid-1990s, with the earliest derivations of 'Usual' blood pressure being based solely upon DBP.[11, 19] This was probably due to the influence of the Epidemiology Pooling Project,[23] clinical teaching and the impact of the early randomised clinical trials of antihypertensive drugs, recruitment to which was almost entirely based upon DBP. This trial criterion resulted from the FDA only requiring drug companies to show a reduction in DBP, not SBP. However, even as early as 1971 the Framingham study had demonstrated that SBP was more informative for predicting coronary artery disease risk than DBP in a specified population.[24] Stamler et al. then demonstrated the importance of SBP across the entire US population,[25] and a number of studies in elderly populations have since demonstrated an inverse relationship between DBP and cardiovascular outcomes for any given SBP.[26-28] The most precise estimate available, from the Prospective Collaborative Study Group, showed that diastolic and systolic BP were equally informative in the prediction of stroke and coronary artery disease, although this analysis was dominated by studies in younger individuals.[8] Overall, these studies reflect the fact that DBP falls with age whilst SBP rises, causing an increase in the prevalence of isolated systolic hypertension.[26, 29] Systolic hypertension is therefore the most prevalent form in this highest risk group, resulting in a greater proportional burden of disease due to systolic hypertension than diastolic hypertension. This has led to a number of trials in patients with isolated systolic hypertension,[30, 31] that have demonstrated that the treatment of isolated systolic hypertension >160mmHg still has significant benefits with a 13% reduction in total mortality, a 23% reduction in coronary events and a 30% reduction in stroke,[28] with similar effects even in the very elderly.[32]

1.2.3 Hypertension and the risk of dementia

Dementia and cognitive impairment are similarly associated with more than 40 million people worldwide suffering from dementia,[33] at a rate rising significantly as the average age of the population increases. Dementia occurs frequently as a consequence

of a clinically evident stroke, with a 10% risk after a first stroke rising to a >30% risk after a second stroke[4] but is also independently associated with a history of hypertension, with a 18-25% increased risk of dementia with a diagnosis of hypertension in in midlife.[34] Furthermore, treatment of hypertension with some antihypertensive agents, including calcium channel blockers or angiotensin receptor blockers, may reduce the incidence of dementia,[35] although overall treatment of hypertension has a less clear effect on dementia incidence[36] despite more intensive treatment reducing the incidence of mild cognitive impairment in the recent SPRINT-mind trial.[37] Furthermore, in addition to an association with acute stroke, dementia and cognitive impairment are particularly strongly associated with intermediate markers of cerebrovascular risk that are also strongly associated with hypertension, including cerebral small vessel disease and arterial stiffness.[38]

The discordance between the association between hypertension and dementia and the relative lack of efficacy of antihypertensive treatment may be explained by intervention occurring too late or by cognitive impairment resulting from established and persistent intermediate markers of cardiovascular injury independent of ongoing blood pressure level, such as arterial stiffness, increased arterial pulse pressure and small vessel disease. However, the direct relationship between these intermediate markers and resulting cognitive impairment remains poorly defined, and no specific intervention exists to specifically target these intermediate phenotypes.

1.3. Treatment of hypertension

1.3.1 The efficacy of blood pressure reduction

The earliest trials of blood-pressure lowering drugs demonstrated that the acute control of blood pressure in the setting of malignant hypertension provided significant reductions in mortality, renal failure and heart failure.[39-42] However doubts persisted about whether chronic control of less severe hypertension would be of benefit, especially in patients with established cerebrovascular disease due to concerns that a reduction in blood pressure would impair cerebral perfusion. The first small, randomised controlled

trials demonstrated that new blood pressure lowering therapies such as chlorothiazide[43] or pronethanol[44] were safe. Then a series of trials from the Veterans Administration demonstrated a relative risk reduction in all cardiovascular events of >90% for active treatment of patients with a DBP >115mmHg,[45, 46] and 73% for patients with a DBP 90-114mmHg, with similar effects in stroke survivors.[47] This started 30 years of intensive investigation of antihypertensive agents versus placebo in a wide range of clinical circumstances, from the first Australian National Blood Pressure study[48] through to the MRC trials in mild hypertension, [49] and the elderly,[50] amongst others,[51, 52] all demonstrating a significant reduction in cardiovascular risk from the reduction of average blood pressure in a wide range of patients, with a greater reduction with more aggressive treatment.[53, 54] Pooled analyses of these trials confirmed the relationship demonstrated by the observational studies described above, with randomisation to a treatment which lowers DBP by 5-6mmHg reducing incidence of stroke by 35-40% and coronary artery disease by 20-25%.[11, 55]

The early trials focused on diuretics and beta-blockers in comparison to placebo, demonstrating the efficacy of blood pressure lowering at different levels of baseline blood pressure,[56] in men and women,[57] in isolated systolic hypertension,[30] in patients with

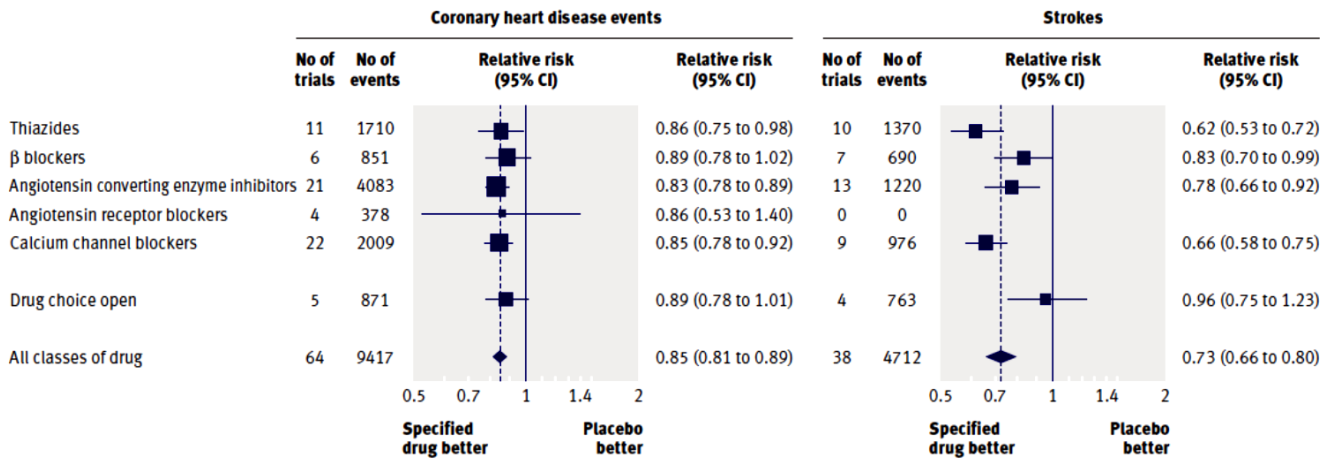


Figure 1.2 Relative risk reduction following randomisation to each class of antihypertensive drug compared to placebo for coronary heart disease and stroke. Derived from Law et al.[68]

diabetes,[58] and for many other demographic characteristics. Across all patient groups, blood pressure lowering was shown to be efficacious in the reduction of subsequent cardiovascular events, including in the secondary prevention of stroke, with a 24% reduction in the risk of recurrent stroke across all trials[59] and a 43% reduction in the risk of recurrent stroke following randomisation to a combination of perindopril and indapamide in particular.[60] Whether to reduce blood pressure below 130/80 was still debated, with proponents for and against the existence of a J-shaped curve, although virtually all trials have demonstrated a continuous reduction in the subsequent risk of stroke with lowering blood pressure, without an apparent threshold below which there is no benefit. This is in contrast to coronary artery disease in which some trials suggest an increased mortality at lower BP levels,[61-63] but subsequent trials such as Cardio-SIS[64] demonstrated that treating to a target below 130/80 in hypertensive people is still associated with further reductions in mortality (HR=0.50, 0.31-0.79). A series of trials then specifically sought to address whether more intensive control to a target of 130/80 or less was beneficial, including the ACCORD trial in diabetes, SPS3 after lacunar stroke and SPRINT in the wider population at moderate cardiovascular risk. SPRINT demonstrated that a more intensive BP target of <130mmHg was associated with a significant reduction in both the composite outcome of all cardiovascular events (HR=0.75; 95%CI, 0.64-0.89; p<0.001) and all-cause mortality (HR= 0.73; 95% CI, 0.60-0.90; p=0.003). After stroke, SPS3 failed to conclusively demonstrate benefit from more intensive blood pressure lowering, with non-significant reductions in all stroke (hazard ratio 0·81, 95% CI 0·64–1·03, p=0·08), disabling or fatal stroke (0·81, 0·53–1·23, p=0·32), and the composite outcome of myocardial infarction or vascular death (0·84, 0·68–1·04, p=0·32), but a significant reduction in haemorrhagic stroke risk (0·37, 0·15–0·95, p=0·03),[65] which is consistent with the benefit of antihypertensive treatment for patients with normal blood pressure in the PROGRESS trial.[66] As a result of these trials, there is an increased adoption of lower targets in the AHA guidance,[67] and after stroke in the UK guidance,[68] although guidelines differ.

1.3.2 Residual risk of cardiovascular events despite secondary prevention

In addition to improved control of hypertension after stroke, overall secondary prevention of stroke has advanced significantly in the past three decades. Randomised trials have demonstrated the benefits of interventions to prevent recurrent stroke, including carotid endarterectomy,[69] carotid stenting[70] and PFO closure.[71] Furthermore, medical treatments have been proven to significantly reduce the rate of recurrent stroke and all cardiovascular events, including anticoagulation for atrial fibrillation,[72] the development of novel oral anticoagulants to replace warfarin,[73] effective lipid lowering with statins[74] and early dual antiplatelet therapy.[75] However, the rate of recurrent stroke and all cardiovascular events remains high. In the TIA-Registry, the rate of recurrent stroke was 5.1% at 1 year[76] and 9.5% at 5 years,[77] with a rate of all cardiovascular events of 6.2% and 12.9% respectively.

In the EXPRESS study, acute treatment within 24-48 hours of a TIA compared to treatment after approximately 7 days was associated with up to an 80% reduction in the risk of early stroke.[78] However, despite initiating intensive investigation and secondary prevention measures, there remains a significant long term risk of recurrent stroke and all cardiovascular events. Even following the introduction of intensive blood pressure control to a target of 130/80 or less via Bluetooth-dependent home blood pressure monitoring, the risk all cardiovascular events remains above 10%. This inability to prevent recurrent events despite near optimal initiation of secondary prevention is likely to reflect a number of factors including poor compliance with treatment, incomplete efficacy of control of target risk factors by current treatments and complications of treatments, such as haemorrhagic complications of antithrombotic treatment.[79] Alternatively, recurrent stroke and cardiovascular events may reflect mechanistic pathways independent of current secondary prevention targets. Such pathways may include established intermediate cardiovascular phenotypes, where cardiovascular injury

has occurred secondary to primary risk factors and continues to increase the risk of further cerebrovascular events despite control of the original risk factor, such as arterial stiffness,[80] blood pressure variability[81] or cerebral small vessel disease.[82] These pathways may therefore represent further treatment targets in high risk patients with established cardiovascular disease. However, to understand the role of intermediate cardiovascular phenotypes and their potential as treatment targets, it is critical to define the temporal pattern of their development, their distribution within the population, risk factors leading to their development and their association with subsequent events, and whether these associations are truly causative intermediate steps or confounding associations.

1.4 Intermediate cardiovascular phenotypes

1.4.1 Hypertension and intermediate phenotypes

Although acute or recent elevations in blood pressure may directly result in cerebrovascular events, either through vessel rupture resulting in acute intracerebral haemorrhage[83] or induced cerebral vasospasm, the 50-60% population attributable risk of stroke[8] is likely to result predominantly from the long-term effects of hypertension on the vasculature. As such, hypertension is associated with a number of intermediate cardiovascular phenotypes that may also subsequently result in acute vessel occlusion or rupture, leading to stroke. If sustained exposure to elevated blood pressure is indeed causative of these phenotypes, and in turn these are causative of cerebrovascular events or dementia, then not only blood pressure level but duration of exposure (ie age) is critical. As such, these intermediate phenotypes can be seen as a form of vascular aging, accelerated by hypertension.

If the hypothesized relationship between long-term hypertension, intermediate cardiovascular phenotypes and clinical outcomes is correct, then prevention of this long-term hypertension-associated clinical outcomes could result either from early, sustained control of blood pressure or from modification of intermediate cardiovascular phenotypes. However, to test this hypothesis, and therefore to develop effective

interventions beyond reduction of blood pressure level, requires an understanding of their distribution amongst the population, their association with hypertension compared to other cardiovasucular risk factors, the relative age-dependent time-course of hypertension and each intermediate phenotype, their individual prognostic value and vitally their relationship with each other and interactions in prediction of clinical outcomes. With this understanding, it is possible to develop interventions to test in interventional studies to both formally test the hypothesis and determine their utility as treatment targets.

A wide range of potential intermediate phenotypes have been identified including vessel calcification, cerebral autoregulation, cerebral reactivity, atrial fibrillation, heart failure, carotid intimal media thickness etc. However, 3 specific phenotypes have been identified that are particularly closely related to hypertension and cerebrovascular injury, and are closely associated with each other: cerebral small vessel disease, arterial stiffness and blood pressure variability.

1.4.2 Cerebral small vessel disease

Chronic vascular injury to the small blood vessels in the brain ('small vessel disease,' SVD)[84] is seen as white matter hyperintensities on MRI imaging and affects the majority of patients over the age of 65 and nearly everyone by 80 years of age.[85] It causes 30% of ischaemic strokes,[86] 40% of dementia[87] and is associated with late-onset refractory depression[88], intracerebral haemorrhage, and a significant functional decline in physical abilities.[89] With modern brain imaging techniques, multiple imaging changes are seen as injury to the small vessels in the brain, including enlarged perivascular spaces, cerebral atrophy and cerebral microbleeds[90] and even apparently normal appearing white matter shows evidence of microstructural damage (diffusion tensor imaging, DTI)[91]. However, despite the high prevalence and significant morbidity the population-specific prevalence and pathophysiology is poorly understood.

Cerebral SVD is strongly associated with age and a history of hypertension,[85] with weaker evidence for an association with diabetes and a limited number of genetic

associations identified in genome-wide association studies.[92] Some cohorts have demonstrated an association between markers of SVD and particulate air pollution,[93] suggesting a potentially increased susceptibility in urban environments. Physiologically, cerebral SVD has been associated with chronic hypoperfusion of the white matter,[94] increased stiffness of the aorta and increased pulsatility of blood flow to the brain,[95] with evidence that the latter reflects increased transmission of the aortic waveform through stiffened vessels to the brain. Similarly, dysfunction of intracerebral vessels has been demonstrated through impaired reactivity of blood vessels and breakdown of the blood brain barrier.[96]

White matter hyperintensities, the commonest manifestation of cerebral small vessel disease, are particularly strongly associated with a history of hypertension,[97] arterial stiffness[95] and blood pressure variability,[98] their progression is reduced by effective antihypertensive treatment in post-hoc analyses of randomized controlled trials[99] and subgroups of prospective trials[37] and they are strongly associated with an increased risk of cerebrovascular events[100] and dementia.[38] As such, they may represent a key intermediate phenotype between hypertension and cerebrovascular and cognitive outcomes, but equally may represent an intermediate phenotype between the other intermediate phenotypes such as arterial stiffness or blood pressure variability and clinical outcomes.

1.4.3 Arterial stiffness and cerebral pulsatility

Arterial stiffness is associated with recurrent cardiovascular events and all-cause mortality, independent of age, blood pressure and cardiovascular risk factors.[80, 101] It is strongly associated with age and a history of hypertension,[102] is often described as a measure of 'vascular age' and features in clinical guidelines as a marker of hypertensive end-organ damage.[103, 104] Studies that have assessed the longitudinal relationships between age, arterial stiffness and blood pressure have largely reported an association between increased SBP and progression of arterial stiffness,[102, 105, 106] although some have reported no evidence of a direct relationship,[107] or sex-specific

differences in longitudinal progression of arterial stiffness.[108] However, studies have not reported the bidirectional interactions between increased SBP or DBP and progression of arterial stiffness and the modification of longitudinal changes in BP by increasing arterial stiffness in mid-life.

In addition to being a marker of hypertensive end-organ damage,[107] arterial stiffness may directly induce end-organ injury through reduced damping of the systolic pressure wave, increased pulse wave reflection from the periphery, and impaired Windkessel function of the aorta,[109] resulting in increased systolic pressures, increased pulsatility of aortic blood pressure and greater transmission of pulsatile flow to the distal circulation.[110] The low-resistance arterial beds in the kidney and brain are particularly susceptible to the increased pulsatility of aortic blood flow, resulting in a strong association between aortic stiffness and cerebral arterial pulsatility with acute lacunar stroke,[111] white matter injury[95] and the associated sequelae of decline in mobility, late-onset refractory depression and dementia.[89]

1.4.4 Blood pressure variability

The importance of visit-to-visit blood pressure variability in predicting the subsequent risk of stroke was demonstrated in a series of papers in the Lancet and Lancet Neurology in March 2010.[112-114] Prof Peter Rothwell presented analyses of five prospective cohorts of patients with hypertension or previous TIA or stroke (from the UK-TIA,[115] ESPS 2,[116] Dutch-TIA[117] and ASCOT-BPLA[118] trials) and two randomized controlled trials in hypertensive patients (ASCOT-BPLA[118] and MRC-elderly[50]). In the five prospective cohorts, there was a marked increase in the risk of stroke with increasing deciles of visit-to-visit variability in blood pressure, measured as either standard deviation (SD), the coefficient of variation (SD/mean) or other derived measures of variability, including variation independent of the mean (VIM). In the UK-TIA cohort, there was a >6 fold increase in the subsequent risk of stroke comparing the highest decile of SD with the lowest (HR=6.22, 95% CI 4.16-9.29), which was even greater when excluding

patients with previous stroke (HR=8.23, 5.51-12.30) or evidence of previous infarction on CT (HR=10.44, 6.64-16.38), figure 1.6. The risk of stroke was most strongly predicted

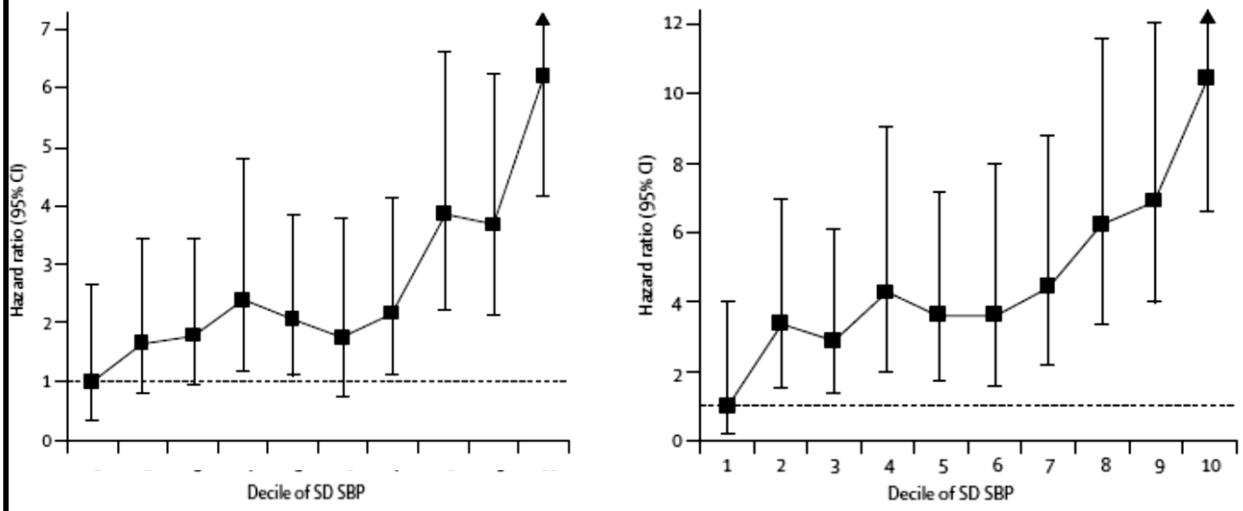


Figure 1.3 Hazard ratios for risk of any subsequent stroke by deciles of SD SBP based in the UK-TIA trial, with the first decile as the reference category. The left panel includes all patients. The panel on the right excludes patients with previous stroke. Derived from Rothwell et al.[20]

by the maximum blood pressure, adjusted for mean SBP (HR=15.01, 6.56-34.38). This demonstrates the importance of episodic hypertension, with severe episodic hypertension (lowest SBP <140 ; highest >180) being at greater risk than patients with stable hypertension (all readings >140mmHg) even though they had a lower mean SBP.

Visit-to-visit variability in SBP was a stronger predictor of the risk of stroke with increasing precision of measurement (top decile of SD-SBP versus bottom decile in the UK-TIA trial: 1-7 visits HR=6.22, 4.16-9.29; 1-10 visits HR=12.08, 7.40-19.72). The relationship was seen across all five cohorts, and it persisted with adjustment for mean SBP, age, sex and other baseline risk factors. Furthermore it was a stronger predictor of outcome than short-term, SD of daytime SBP measured in the ASCOT-BPLA 24 hour ABPM substudy, with only very weak predictive values for within-visit variability in SBP or the 'white-coat' effect. Finally, visit-to-visit variability was also a strong predictor of coronary events, heart failure and angina, although not to the same extent as stroke.

1.4.5 Randomised treatment reduces visit-to-visit variability and stroke risk

ASCOT-BPLA randomised 19257 hypertensive patients aged 40-79 with 3 or more additional cardiovascular risk factors to amlodipine with or without perindopril

compared to atenolol with or without bendroflumethiazide, resulting in a significantly lower visit-to-visit variability in the amlodipine-based arm. Adjustment for differences in mean SBP alone explained less than one third of the difference in the risk of stroke between the groups, whilst adjustment for both mean SBP and SD-SBP explained all of the difference in subsequent risk of stroke (unadjusted HR=0.78, 0.67-0.90; adjusted for mean SBP HR=0.84, 0.72-0.98, adjusted for mean SBP and SD-SBP HR=0.99, 0.85-1.16). In the MRC-elderly trial, randomisation to a beta-blocker resulted in a significantly greater SD-SBP than randomization to a thiazide diuretic, with a slightly greater CV even compared to placebo. This correlated with a significantly greater risk of stroke in the beta-blocker group during the first two years of follow-up, and a greater risk even than the placebo group, in spite of a significant reduction in blood pressure. Only following the addition of other antihypertensives to a beta-blocker was there a reduction in visit-to-visit variability in SBP, and a reduction in the risk of stroke compared to the placebo group.

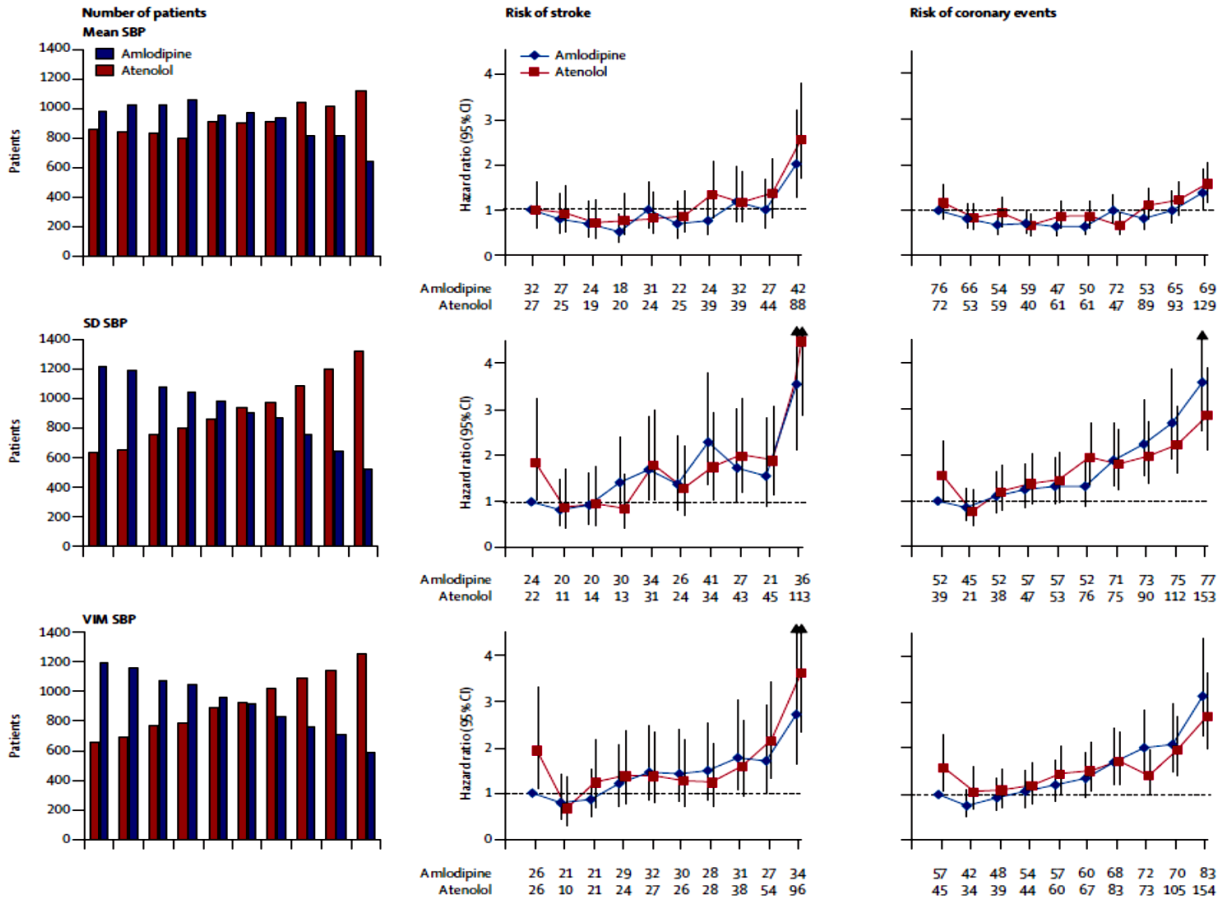
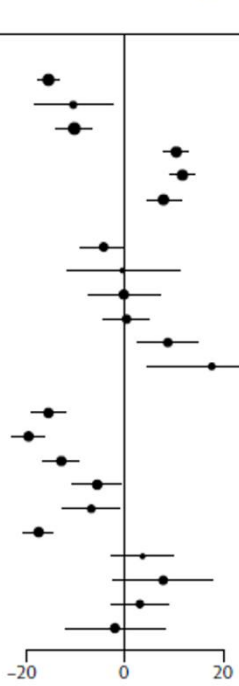


Figure 1.4 Distribution of patients in the two treatment groups in ASCOT-BPLA according to deciles of mean, SD and VIM of SBP (left) and the association of each of these variables with risk of stroke (middle) and risk of coronary events (right). The middle and right columns show the hazard ratios (95% CI) for risk of stroke and acute coronary events, by deciles of the same parameters. SBP=systolic blood pressure. VIM=variation independent of mean. Derived from Rothwell et al.[20]

The accompanying systematic review and meta-analysis[81] assessed the effects of all different classes of antihypertensive medication on intra-individual variability in SBP in published trial reports. As no study at this time reported intra-individual variability in systolic blood pressure by randomized drug class, this study used the variation in SBP across each drug group (the standard deviation of the mean SBP at a specific time point) to derive a within-trial treatment effect on variability in SBP as the group variance ratio (G-VR= the ratio of the variance of mean SBP in each drug group). Further analyses of the 5 observational cohorts reported by Prof Rothwell demonstrated that in a linear regression model, intra-individual SBP variability explained more than 50% of the variance in group SD at a specific timepoint, with G-VR being a reliable surrogate measure of antihypertensive effects on within-individual variability in SBP in the ASCOT-BPLA and the MRC trials.[113]

This meta-analysis demonstrated that randomization to a calcium channel blocker was associated with the greatest reduction in VR compared to either placebo or all other drug classes, with smaller reductions in VR with thiazide and thiazide-like diuretics but increases in VR with ACE inhibitors, angiotensin receptor blockers and most significantly with beta-blockers (figure 1.6). These effects of antihypertensive drugs on variability in SBP were very similar to their effects on the subsequent risk of stroke seen in all trials. Furthermore, in trials reporting both VR and stroke risk, there was a strong relationship between randomized treatment effect on variability in SBP and risk of stroke, independent of mean SBP (r^2=0.473 $p<0.0001$, VR $p=0.014$, difference in mean SBP $p=0.038$). Further systematic reviews have since demonstrated that the correlation between drug class effects on VR and stroke risk is not due to confounding by atrial fibrillation,[119] persists when the agents are used in combination with other agents[120] and is dose dependent.[120] Finally, non-selective beta-blockers increase variability in SBP more than selective beta-blockers and are probably associated with a greater stroke risk as a result.[121]

Figure 1.5 Pooled estimates of within-trial comparisons between drug classes at follow-up as difference in percentage change in coefficient of variation compared to baseline.



	Patients	Trials	Change in CV (95% CI)	p value				
				Pooled	Heterogeneity	Drug A	Drug B	Sign test
Drug class vs all other drug classes								
CCB	106697	94	−15·50% (−17·60 to −13·40)	<0·0001	<0·0001	71	23	<0·0001
CCBND	2753	39	−10·59% (−18·52 to −2·65)	0·0074	0·0001	20	13	0·296
DD	81772	69	−10·32% (−14·06 to −6·58)	<0·0001	<0·0001	29	20	0·253
ARB	39447	47	10·28% (7·73 to 12·83)	<0·0001	<0·0001	18	29	0·145
ACEI	76064	125	11·52% (8·99 to 14·04)	<0·0001	<0·0001	52	73	0·074
BB	39392	78	7·69% (4·23 to 11·15)	<0·0001	<0·0001	25	53	0·002
Drug class vs placebo								
CCB	11294	34	−4·46% (−8·88 to −0·05)	0·0427	0·16	24	9	0·0148
CCBND	736	6	−0·57% (−12·03 to 10·90)	0·9077	0·26	3	3	1·0000
DD	12187	20	−0·30% (−7·66 to 7·06)	0·9335	0·0074	10	10	1·0000
ARB	6756	13	0·20% (−4·60 to 4·99)	0·9315	0·48	5	8	0·5811
ACEI	7074	43	8·58% (2·33 to 14·82)	0·0135	<0·0001	15	27	0·0896
BB	1210	19	17·41% (4·30 to 30·52)	0·0066	0·78	4	15	0·0192
Drug class vs drug class								
CCB vs BB	20306	13	−15·54% (−19·01 to −12·07)	<0·0001	0·51	11	2	0·0225
CCB vs ACE	24520	41	−19·63% (−23·04 to −16·22)	<0·0001	<0·0001	28	13	0·0288
CCB vs ARB	19635	17	−13·06% (−16·78 to −9·34)	<0·0001	0·18	14	3	0·0127
CCB vs DD	25323	14	−5·75% (−10·77 to −0·73)	0·0171	0·5	11	3	0·0574
DD vs BB	4076	9	−6·96% (−12·59 to −1·33)	0·0072	0·54	8	1	0·0391
DD vs ACE	26616	15	−17·49% (−20·41 to −14·58)	<0·0001	0·19	12	3	0·0352
DD vs ARB	498	3	3·44% (−2·85 to 9·72)	0·0546	0·87	0	3	0·2500
ARB vs BB	9847	5	7·60% (−2·51 to 17·71)	0·0619	0·14	3	2	1·0000
ARB vs ACE	3167	22	2·92% (−2·81 to 8·65)	0·3011	0·3	9	13	0·5235
ACE vs BB	4071	33	−2·06% (−12·14 to 8·03)	0·6822	0·45	15	18	0·7277

However, a major limitation of visit-to-visit, or even day-to-day, BP variability is the long period of assessment required to measure it, limiting its use in the acute assessment and treatment of patients. More recently, variability in blood pressure from one beat to the next over 5 minutes has been identified as a possible alternative method of assessment of BP variability. It shares very similar demographic and physiological associations with day-to-day BP variability[122] and responds similarly to antihypertensive medication[123] and has prognostic value in predicting outcome after acute stroke.[124] However, its distribution across the population, association with other intermediate phenotypes and prognostic value for recurrent events is unknown.

1.5.1 Summary and Aims

Despite major advances in prevention of cerebrovascular events, there remains a high rate of recurrent cerebrovascular events despite effective management. Furthermore, the rate of vascular cognitive impairment and dementia remains high, with no proven preventative strategies beyond prevention of stroke itself. Hypertension underlies over half of all strokes, yet good control of hypertension is not sufficient to

prevent future detrimental clinical outcomes. This may be explained by a failure to consistently control hypertension from an early enough stage or by the development of established cardiovascular intermediate phenotypes that result from hypertension and cause these clinical outcomes but are not reversed by control of blood pressure. These include cerebral small vessel disease, arterial stiffness and pulsatility and blood pressure variability. Treatments to target these intermediate cardiovascular manifestations of 'vascular aging' therefore have the potential to tackle a significant burden of this residual risk. However, our understanding of the timecourse of the distribution and timecourse of these phenotypes and their association with subsequent clinical events remains limited. Therefore, in this thesis, I aim to:

1. Determine the age-sex distribution of beat-to-beat blood pressure variability, arterial stiffness and arterial pulsatility
2. Identify demographic associations and potential determinants of beat-to-beat blood pressure variability, arterial stiffness and arterial pulsatility.
3. Establish the rate of progression of these indices with time in high risk patients.
4. Estimate the risk of recurrent events associated with elevation in each of these measures.

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CHAPTER TWO

The physiological substudy of the OXVASC Phenotyped cohort

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2.1 Introduction

This thesis aims to characterize novel physiological markers of vascular aging that act as intermediate risk factors between age or hypertension with cardiovascular events and dementia. These represent potential novel treatment targets in addition to effective control of classical risk factors, and include beat-to-beat blood pressure variability,[1] arterial stiffness and arterial pulsatility,[2] both systemically and in the cerebral circulation. However, to determine their potential as both intermediate risk factors and targets for treatment, it is critical to understand their distribution in the population; the risk factors they are determined by; their rate of progression, potential for amelioration; whether they are associated with future cardiovascular events and ultimately whether there are interventions to prevent their progression and future cardiovascular events.

To answer these questions requires a large, prospective cohort study in which these physiological measures are recorded at baseline, extensive recording of clinical baseline data, repeated studies after a prolonged period of time, and prospective follow-up for recurrent cardiovascular events with a high rate of ascertainment. To have sufficient power to determine the clinical significance of these risk factors, independent of other classical risk factors, this population needs to be sufficiently high risk with a high enough incidence of recurrent cardiovascular events. To this end, prospective physiological assessments were introduced within the world-leading, population-based Oxford Vascular Study (OXVASC).

2.2 The OXVASC Study

OXVASC is a population-based study of all vascular events (TIA, stroke, coronary and peripheral vascular) in nearly 100,000 residents in Oxfordshire.[3] Established in 2002 it is the only such study that does not exclude older age groups, with >50% of recruits aged over 75 and 30% over 80. The study population is larger than similar cohorts (e.g. 8,500 in Framingham)[4] and includes the practice populations

of about 100 primary care physicians (>99% of the actual population), rather than selected volunteers. All patients registered with these 9 GP practices who experience a cardiovascular event are identified via multiple modes of ascertainment, including hot pursuit (review of acute hospital admissions), cold pursuit (review of GP records, coroner records and hospital bereavement records) and via an acute TIA / minor stroke referral clinic run by the OXVASC study for all patients within this population.

Phase-1 of OXVASC (2002-2012) ascertained over 6,000 patients with acute vascular events and 1,000 controls, providing a core of clinical, imaging, and biobank data. Patients were ascertained daily in the acute phase and have face-to-face follow-up to 10 years. In Phase-2 (2012-2022), patients continued to be ascertained as in phase 1, but for patients referred to the OXVASC study acute TIA clinic they underwent maximal testing and interventions and so constitute the OXVASC Phenotyped cohort. Participants were recruited at the daily emergency clinic, following a referral after attendance at the Emergency Department or from primary care, usually within 24 hours. All consenting patients underwent a standardised medical history and examination, ECG, blood tests and a stroke protocol MRI brain and contrast-enhanced MRA (or CT-brain and carotid Doppler ultrasound or CT-angiogram). All patients were assessed by a study physician, and reviewed by the senior study neurologist (PMR) and are followed-up face-to-face at 1, 3, 6 and 12 months, and 2, 5 and 10 years.



Figure 2.1 The OXVASC Population

Since September 2010 , as part of the OXVASC Phenotyped Cohort, a routine prospective cardiovascular physiological assessment is performed at the 1 month follow-up visit, constituting the physiological subgroup of the OXVASC Phenotyped Cohort.[1] Participants were excluded if they were under 18 years, had severe cognitive impairment, were pregnant, had autonomic failure, or had a recent myocardial

infarction, unstable angina, heart failure (NYHA 3-4 or ejection fraction <40%) or untreated bilateral carotid stenosis (>70%). OXVASC is approved by the Oxfordshire Research Ethics Committee A.

2.3 OXVASC Physiological Assessments

2.3.1 Development of the study

The OXVASC study demonstrated the remarkable effectiveness of rapid assessment and early intervention to prevent early recurrent stroke. In the EXPRESS substudy of OXVASC, Prof Rothwell demonstrated that rapid assessment of patients with TIA reduced the early rate of recurrent stroke by up to 80% (see figure 2.2).[5]

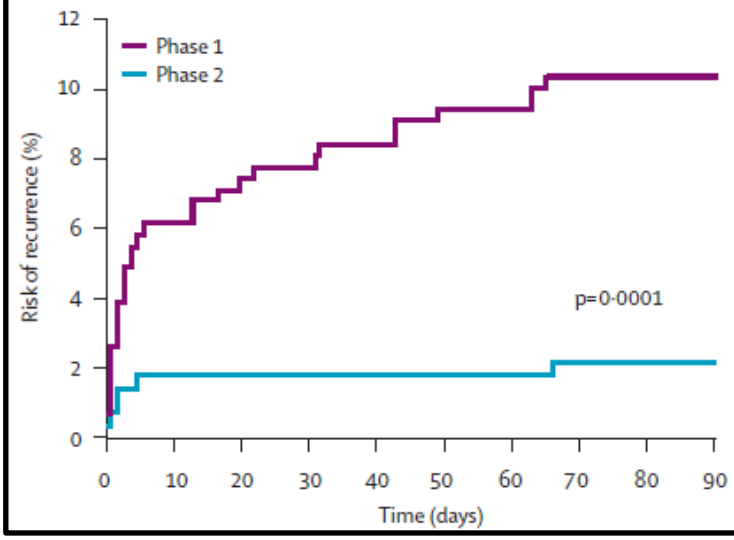


Figure 2.2 Risk of recurrent stroke in the EXPRESS study. In Phase 1 between 2002-2005, patients were assessed via the normal referral NHS pathway, often after up to a 7 day interval. In Phase 2 between 2005-2007, patients were assessed as soon as possible, usually within 24-48 hours.

However, despite early intensive risk factor control, a significant risk of recurrent stroke and cardiovascular events remains (see Chapter 1). To explain this residual long-term risk, and identify treatment targets to prevent it, additional interventions and investigations were introduced including systematic MRI imaging of patients where there were no contraindications, Bluetooth-enabled telemetric blood pressure monitoring (the COMMIT study), routine 5-day ambulatory R-tests for atrial fibrillation and echocardiography, to form the OXVASC Phenotyped cohort. In September 2010, a pilot study was initiated, during the author's DPhil, to assess the cardiovascular physiology of patients in more detail to identify additional risk factors after optimal

control. The initial 50 patient pilot study included continuous, non-invasive beat-to-beat blood pressure and ECG monitoring, applanation tonometry for pulse wave velocity and pulse wave analysis measurement, orthostatic testing, the cold pressor test, a mental arithmetic stress test and a GTN challenge. After 50 patients, the GTN challenge was stopped, but transcranial Doppler ultrasound (TCD) was introduced to measure cerebral pulsatility, followed by TCD monitoring to assess cerebral autoregulation and reactivity to CO2 challenges (hyperventilation and breath-holding). 260 patients were assessed by the author during his DPhil by August 2013,[1] after which the protocol was streamlined and incorporated in the routine follow-up appointments of the OXVASC study, supervised by the author but performed by a vascular scientist or other trained clinicians. This retained the core protocol, but removed the orthostatic testing and cold pressor test, and continues to be performed at the 1 month follow-up visit in all OXVASC participants.

Since April 2017, all participants in the physiological substudy have been invited to undergo a repeat physiological assessment when they attend for their 5 year follow-up appointment, under the current OXVASC physiological protocol.

2.3.2 The assessment procedure

OXVASC patients attend the 1 month follow up clinic if they have been reviewed at the OXVASC acute TIA/minor stroke clinic or if they are assessed as a late ascertainment due to not presenting to medical services acutely, or presenting with a cerebrovascular event out of the region. At the 1 month follow-up appointment they have clinical assessment, echocardiography, ambulatory ECG and BP monitoring and a physiological assessment. The physiological assessment is performed in a temperature controlled (21-23^{0}C) room. Consenting, consecutive patients lie supine on a comfortable couch for approximately 45 minutes to undergo a series of physiological assessments, usually performed by 2 operators.

All monitoring data are acquired via export of analogue waveforms from each device to a Powerlab 8/35, with acquisition of blood pressure, ECG and CO2 waveforms at 200Hz and transcranial ultrasound waveforms at 100Hz.

2.3.3 Applanation tonometry

Radial artery pulse waveforms are recorded using a pressure tonometer and designated software (SphygmoCor, At-Cor Medical, Sydney, Australia), usually from the right radial artery.[2] Briefly, mean values of approximately 10 radial pulse waves are used to generate a corresponding average, central aortic pressure waveform with a validated mathematical transfer function. A minimum of 10 radial artery pulse waveforms are required by the software to calculate the aortic systolic, diastolic and pulse pressure. The system also calculates the augmentation index, reflecting the increase in the systolic pressure due to reflection of the outgoing pressure waveform from the periphery returning to collide with the forward travelling wave.[6, 7] Two recordings of adequate quality are taken, up to a maximum of 5 readings if good quality records are not possible, with quality assessed by an 'operator index' greater than 85, measuring the reproducibility of the arterial signal

Aortic arterial stiffness is determined as the carotid-femoral pulse wave velocity (cfPWV).[8] This requires recording of the pulse wave by applanation tonometry with the SphygmoCor device from both carotid and femoral arteries, with concurrent recording of the ECG. The aortic length is estimated as the distance from the sternal notch to the femoral recording site, minus the distance from the sternal notch to the carotid recording site. The time of arrival of each arterial waveform is determined by the interval between the QRS complex of the ECG and the intersection between the lowest pressure immediately before each beat and a tangent to the steepest rise in the arterial pressure wave (the 'intersecting tangent method'). The cfPWV is calculated as the time of transmission divided by the estimated aortic length. For quality control, two readings are taken. If they are within 1m/s of each other, and the variability (SD) of each reading is below 1 m/s then an average of both readings is taken. Otherwise a third reading is

taken and the median of the three readings is accepted as the average, excluding any measurements that are clearly artefactual.[8, 9]

2.3.4 Transcranial ultrasound

TCD is performed with DiaMon 2MHz probes, firstly with a handheld probe to best identify the middle cerebral and basilar arteries, with application of ultrasound gel over the temporal (in front of the ear) and suboccipital windows (at the nape of the neck). The M1 segment is identified between 45-55mm depth as the segment of the artery with blood flow towards the probe, slightly superficial to a site of bifurcation into another vessel with flow away from the probe (the anterior cerebral artery), with blood flow towards the probe ideally extending for at least 1cm within the skull. The basilar artery is identified via the trans-foramen magnum bone window, identifying blood flowing away from the probe at >75mm. The maximal velocity in these segments will be identified and peak, trough and mean blood flow velocities will be derived from the wave form envelope, taking the average of at least 5 cardiac cycles. From these measures Gosling's pulsatility index will be derived as the primary measure of cerebral pulsatility ((peak velocity - trough velocity) / mean velocity). Where bone windows are adequate (ie MCA blood flow can be insonated via the temporal bone), bilateral monitoring insonation of the middle cerebral artery will be established with 2 probes held to the head a with a comfortable headset, with spectra acquired by a DWL Doppler Box to a dedicated laptop. This waveform is acquired over 10 minutes with concurrent heart rate and blood pressure monitoring (see below). End-tidal carbon dioxide monitoring is performed with AD Instruments Gas Analyser ML206 via nasal CO2 sampling cannulae

2.3.5 Beat-to-beat blood pressure

Continuous ECG and BP are recorded using a Finometer Midi, with a pressure cuff from the middle phalanx of the middle finger of the left hand. If a good quality trace can not be obtained, the hand is warmed, or the finger cuff is moved to a proximal

phalanx or to the index finger.[10] After a period of at least 5 minutes of rest and after achievement of a satisfactory BP signal from the monitor and the stabilization of BP at the same level, recordings will be performed for up to 10 minutes (minimum 5 minutes). The device incorporates a pressure cuff and photoplethysmograph, and provides a fluctuating input pressure to ensure a stable volume of blood in the finger as determined by the photoplethysmograph, with the required input pressure therefore matching the intrarterial pressure in the finger (the 'volume-clamp' method).[11]

A standard brachial blood pressure is obtained twice with a calibrated device (A&D UA-767 BT) from the contralateral arm prior to recording, and the Finometer trace calibrated to this reading by 2 point calibration. During recording, the machine's inbuilt calibration mechanism is switched off to avoid interruption of data.

2.4 Processing of physiological data

2.4.1 Derivation of physiological indices

All data are exported from the initial recording equipment (Finometer, transcranial ultrasound, ECG and CO2 monitoring) via a Powerlab 8/35, acquired at 1kHz. Data are then exported from the Powerlab software to Matlab and processed via bespoke in-house software. The continuous BP waveform is calibrated via two-point calibration to at least 2 brachial readings. QRS complexes of the ECG waveforms are identified, and R-R intervals which change by more than 2 standard deviations of the change in R-R intervals are flagged as potential ectopic beats. These are then manually reviewed, and the ectopic R-R intervals are linearly interpolated between adjacent normal beats.

Peaks and troughs of blood pressure and transcranial ultrasound waveforms are extracted by peak detection, median filtered, filtered with a low pass, zero-phase shift Butterworth filter, linearly interpolated across ectopic beats and manually reviewed, followed by linear interpolation of artefactual beats. After first-order

cleaning of physiological data, beat-to-beat blood pressure is derived over 5 minutes of supine rest and any linear trend across 5 minutes is removed. BPV is then derived as the coefficient of variation of the detrended waveform.

Up to 15 beats of the transcranial ultrasound waveform envelope are manual reviewed, artefactual beats are excluded and of the remaining beats, the peak and trough values are detected to calculate the peak (PSV) and trough (EDV) velocities and the Gosling's pulsatility index (PSV-EDV)/(mean velocity).

2.4.2 Statistical analysis

The derived physiological indices are finalized and included in a defined dataset for each analysis, including demographic and clinical data from the OXVASC study and recurrent event data where these are available.

Frequency of clinical characteristics are compared by t-tests or ANOVA for discrete data. Relationships between cross-sectional continuous variables are analysed by general linear models, for univariate analysis and adjusted for age, sex and clinical characteristics. For discrete indices as outcomes, logistic regression is used for binary data, ordinal regression for ordinal outcomes and Cox-Proportional Hazards for analysis of time to event survival data, specifically for recurrent events. Finally, for longitudinal, repeated assessments, analyses are performed by mixed-effect linear models, with an autoregressive covariance structure to account for repeated measures.

2.5 Summary

Incorporation of a prospective physiological assessment within the highly phenotyped OXVASC study's population based cohort, with repeated measures at 5 years, provides a unique opportunity to determine the characteristics of novel physiological indices after optimal risk factor control, and to assess their potential as predictors of recurrent cerebrovascular events and cognitive decline. As such, it has the potential to identify novel treatment targets to be tested in future clinical trials.

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CHAPTER THREE

Age and sex distribution of beat-to-beat blood pressure variability after TIA and minor stroke

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3.1 Summary

Beat-to-beat blood pressure variability (BPV) is associated with increased stroke risk but its importance at different ages is unclear. This chapter describes the age-sex distribution of BPV in patients with TIA or minor stroke.

In consecutive patients within 6 weeks of TIA or non-disabling stroke (Oxford Vascular Study), non-invasive BP was measured beat-to-beat over 5 minutes (Finometer). The age-sex distribution of BP variability (rCV, residual coefficient of variation) was determined for systolic (SBP) and diastolic (DBP) BP. The risk of top-decile BPV was estimated (logistic regression), unadjusted and adjusted for age, sex and cardiovascular risk factors.

In 908 of 1013 patients, excluding 54 in atrial fibrillation and 51 with low quality recordings, rCV was positively skewed with a median SBP-rCV of 4.2% (IQR 3.2 – 5.5) and DBP-rCV of 3.9% (3.0 – 5.5), with 90th centile thresholds of 7.2% and 7.3%. Median SBP-rCV was higher in patients under 50 years (4.5, 3.0 - 5.3) compared to 60-70 years (4.1, 3.2 - 5.2), but rose to 4.5% (3.5 – 6.9) above 80 years, with an increasingly positive skew. The proportion of patients with markedly elevated BPV in the top-decile increased signifcantly per decade (OR 1.72, $p<0.001$), after adjustment for sex and risk factors.

Median beat-to-beat BPV fell in midlife, reflecting loss of physiological, organised BPV. However, rates of markedly elevated BPV significantly increased with greater age, suggesting BPV may be particularly important in older patients.

3.2 Introduction

As described in the introduction, patients with episodic hypertension have a high risk of stroke,[1, 2] residual visit-to-visit variability in blood pressure (BPV) on treatment has a poor prognosis despite good control of mean BP,[3] and benefits of some antihypertensive drugs in the prevention of stroke may partly result from reduced variability in SBP.[4, 5] Strong associations for visit-to-visit BP variability have been found with recurrent cardiovascular events,[6] diabetes,[7] renal impairment [8] and cognitive decline,[9] with similar predictive value of BP variability on day-to-day home readings,[10, 11] and a significant reduction with specific antihypertensives.[4, 5] However, both visit-to-visit and home BP variability require a prolonged period of assessment, good patient compliance and follow-up visits. As such, they are of limited use in acute assessment.

Variability in blood pressure from one beat to the next (beat-to-beat BPV) enables BPV assessment at a single visit and has a similar physiological profile to home day-to-day BPV[12]. However, normal values for beat-to-beat BPV in at-risk individuals and their distribution across age and sex groups are unknown. Furthermore, beat-to-beat BPV is itself composed of multiple components from physiological rhythms reflecting breathing and intact autonomic function to increased BPV associated with stiff arteries and impaired baroreceptor function in older patients with impaired compensatory mechanisms.[12] To assess the potential clinical utility of beat-to-beat BPV, it is necessary to understand the distribution of beat-to-beat BPV in at risk populations and whether potentially-pathological, markedly-increased BPV is increased in specific groups.

Therefore, we determined the normative age-sex distribution of beat-to-beat BPV variability in patients with a TIA or minor stroke.

3.3 Methods

3.3.1 Study population

Consecutive, consenting patients with TIA or minor stroke were recruited between September 2010 and October 2019, to the Oxford Vascular Study (OXVASC) Phenotyped Cohort.[11, 12] Participants were recruited at the daily emergency clinic, following a referral after attendance at the Emergency Department or from primary care, usually within 24 hours. The OXVASC population consists of >92,000 individuals registered with 100 primary-care physicians in Oxfordshire. All consenting patients underwent a standardised medical history and examination, ECG, blood tests and a stroke protocol MRI brain and contrast-enhanced MRA (or CT-brain and carotid Doppler ultrasound or CT-angiogram). All patients were assessed by a study physician, and reviewed by the senior study neurologist (PMR) and are followed-up face-to-face at 1, 3, 6 and 12 months, and 2, 5 and 10 years.

As part of the OXVASC Phenotyped Cohort, after 15-20 minutes supine rest, beat-to-beat BPV was measured over 5 minutes in a quiet, dimly-lit, temperature-controlled room (21-23^{0}C). Continuous ECG and non-invasive blood pressure were acquired at 200Hz (Finometer, Finapres Medical Systems, The Netherlands), via a Powerlab 8/30 (LabChart Pro, ADInstruments, USA). Waveforms were preferentially recorded from the middle phalanx of the middle finger. Automated calibration ('Physiocal') was performed until the recording was stable, but turned off during each test. Estimated brachial waveforms (Finometer) were calibrated offline by linear regression to 2-3 supine, oscillometric brachial readings, performed immediately prior to the monitoring period on the contralateral arm, with manual exclusion of artefacts. If necessary the finger cuff was moved to an adjacent finger or the proximal phalanx of the same finger, or the hand was warmed. Two sitting clinic BPs, 5 minutes apart, were measured at ascertainment and one month in the non-dominant arm, by trained personnel.

3.3.2 Analysis

BPV on beat-to-beat monitoring was calculated over 5 minutes. Ectopic beats and artefacts were automatically detected, visually reviewed and removed by linear interpolation of R-R interval. Blood pressure artefacts were automatically detected and manually reviewed, and removed by linear interpolation to adjacent normal beats, with in-house software. Patients in atrial fibrillation during the recording were excluded. Systolic and diastolic BPV were calculated as the standard deviation (SD) and the coefficient of variation (CV=SD/mean), before and after de-trending of the recording about a linear regression (rCV). All recordings were reviewed blinded to clinical data to assess for the quality of recording (3- excellent quality; 2- adequate quality for analysis; 1-unuseable, poor quality recording).

Distributions of BP indices were described by histograms for all patients, stratified by sex and age. Normality of distributions was assessed by the Shapiro-Wilk test. Summary values for non-normal distributions ($p<0.05$) were summarized as the median, skewness, inter-quartile range and 90^{th} centile.

Models were performed for univariate associations; adjusted for age and sex and for age, sex and cardiovascular risk factors (current smoking, history of hypertension, diabetes).

3.4 Results

3.4.1 Study Population

1031 assessments were performed in 1013 eligible, consecutive patients between September 2010 and October 2019, with 18 patients assessed twice. 54 / 1013 (5.3%) patients were in atrial fibrillation during the recording, whilst 51 (5%) patients had inadequate recordings. Patients with atrial fibrillation or poor recording quality were older, had higher blood pressure and were more likely to have hypertension (table 2.1).

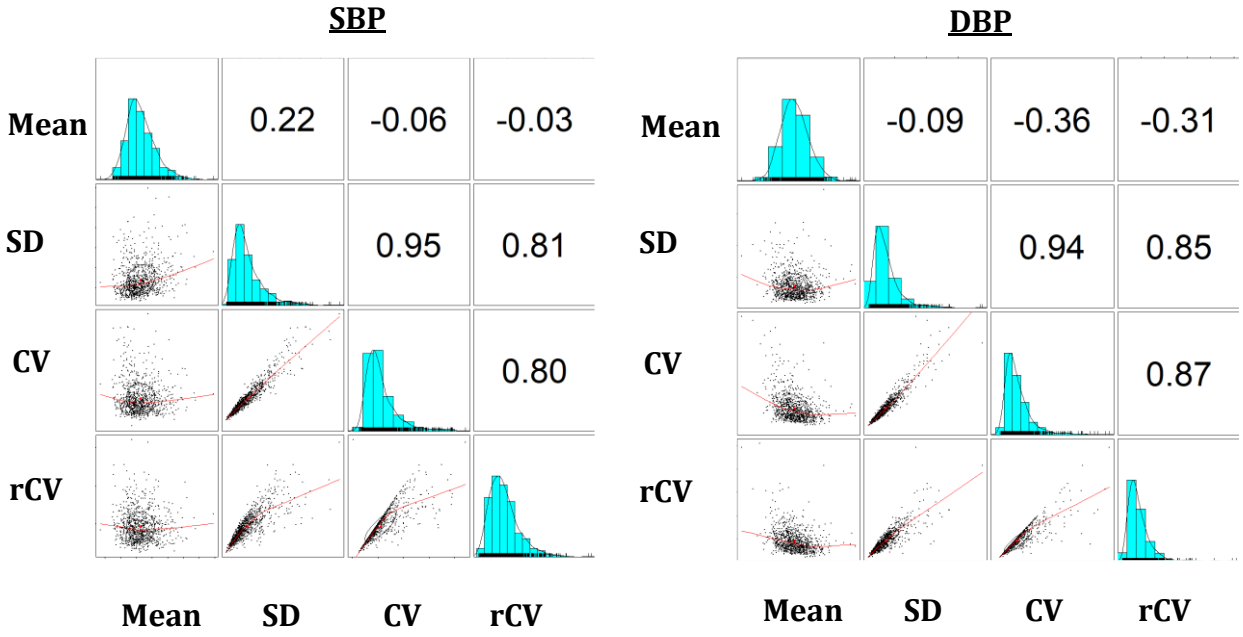
Table 3.1 Demographics of included participants. Participants in AF or with poor quality recordings were excluded. p-values from ANOVA for continuous variables, chi-squared tests for categorical variables. AF = atrial fibrillation; BP = blood pressure.

	All	Included	In AF	Poor data	p-val
	1013	908 (90)	54 (5)	51 (5)	
Age	67 (13.3)	66.1 (13.3)	77.8 (8.9)	72.2 (11.1)	<0.001
Sex	427 (42)	387 (43)	18 (33)	22 (44.9)	0.35
Hypertension	453 (44)	390 (43)	36 (67)	27 (55.1)	<0.001
Diabetes	116 (11)	104 (11.5)	7 (13)	5 (10.2)	0.88
Smoking					
- Current	160 (15)	148 (16)	6 (11)	6 (12.2)	0.39
- Ever	535 (53)	478 (53)	32 (59)	24 (49)	0.62
Family History AF	303 (30)	274 (30)	12 (22)	17 (34.7)	0.12
- During test	54 (5.3)	-	54 (100)	-	-
- Ever	74 (7)	37 (4.1)	35 (65)	2 (4.1)	<0.001
Dyslipidaemia	314 (31)	277 (31)	20 (37)	16 (32.7)	0.67
Heart Failure	16 (1.6)	10 (1.1)	6 (11)	-	<0.001
Systolic BP	133 (19)	132 (18)	137 (24)	145 (27)	<0.001
Diastolic BP	78 (12)	78 (11)	83 (15)	77 (8.9)	0.01

3.4.2 Distribution of Blood Pressure Variability

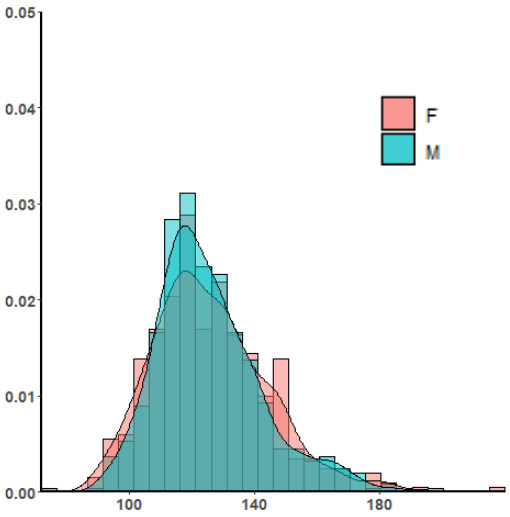
Mean SBP was strongly correlated with SD of SBP (figure 3.1), but with no correlation with CV-SBP or CV-DBP, before or after detrending. However, there was correlation between mean-DBP and SD-DBP, with an inverse correlation between CV-DBP and mean DBP before and after detrending.

Figure 3.1. Associations between indices of mean BP and BP variability. Scatter plots with loess-smoothed regression lines, histograms with density curves and r values for the linear associations are presented for mean, standard deviation (SD), coefficient of variation (CV) and residual CV after de-trending of data (rCV), for both systolic (SBP) and diastolic (DBP) blood pressure.

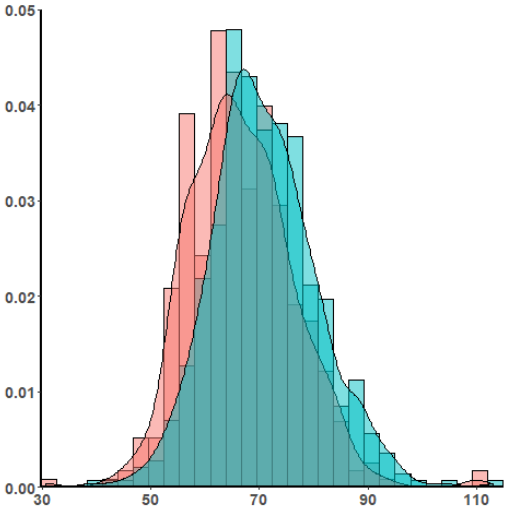


Mean SBP and DBP were normally distributed across the population but all indices of BPV were positively skewed (figure 3.2), with a similar skew for maximum BP values and a greater skew for DBP than SBP. There was a persistent but reduced skew after detrending of recordings (table 3.2). Median rCV-SBP was greater than median rCV-DBP, but the distribution of rCV-DBP was more skewed, with a higher threshold for the top decile of the population. Although mean DBP was greater in men than women (figure 3.2), there was minimal difference in the distribution of CV (figure 3.2).

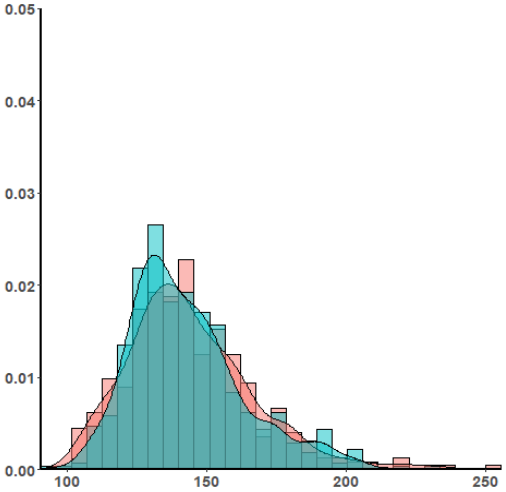
Figure 3.2. Distribution of blood pressure variables, stratified by sex. Histograms and density curves are given for men and women, for the distribution of mean, maximum and coefficient of variation (CV) of systolic (SBP) and diastolic (DBP) blood pressure. M= male; F = female.



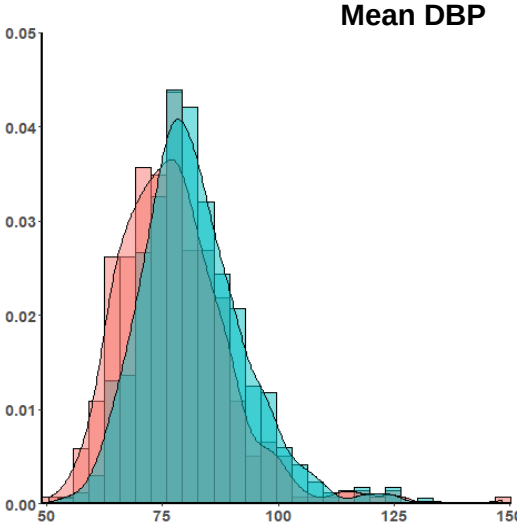
Mean SBP



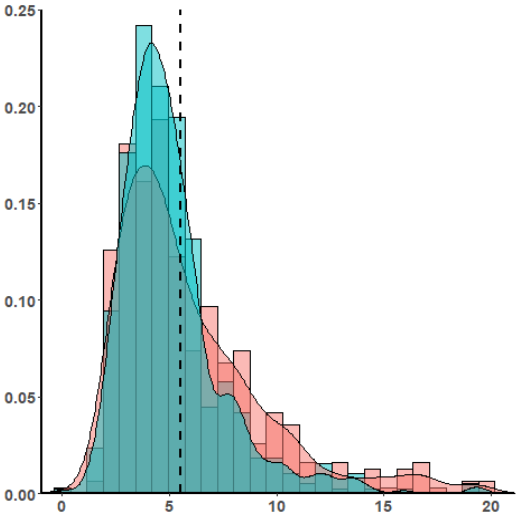
Mean DBP



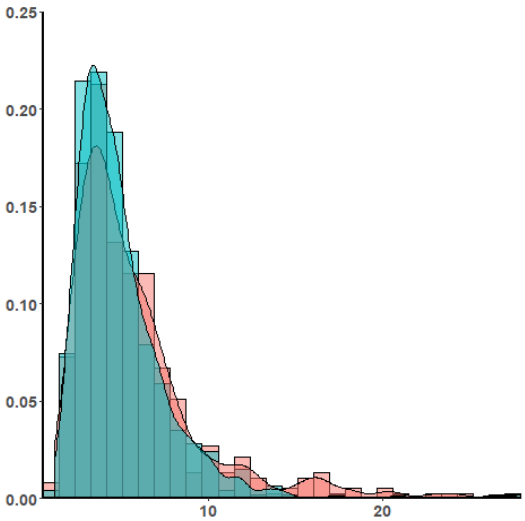
Max SBP



Max DBP



CV SBP



CV DBP

Table 3.2 Age-dependent distribution of residual coefficient of variation (rCV) for systolic and diastolic blood pressure. Results the median value, inter-quartile range (IQR), 90th centile and skewness. P-values for normality (Shapiro-Wilk).

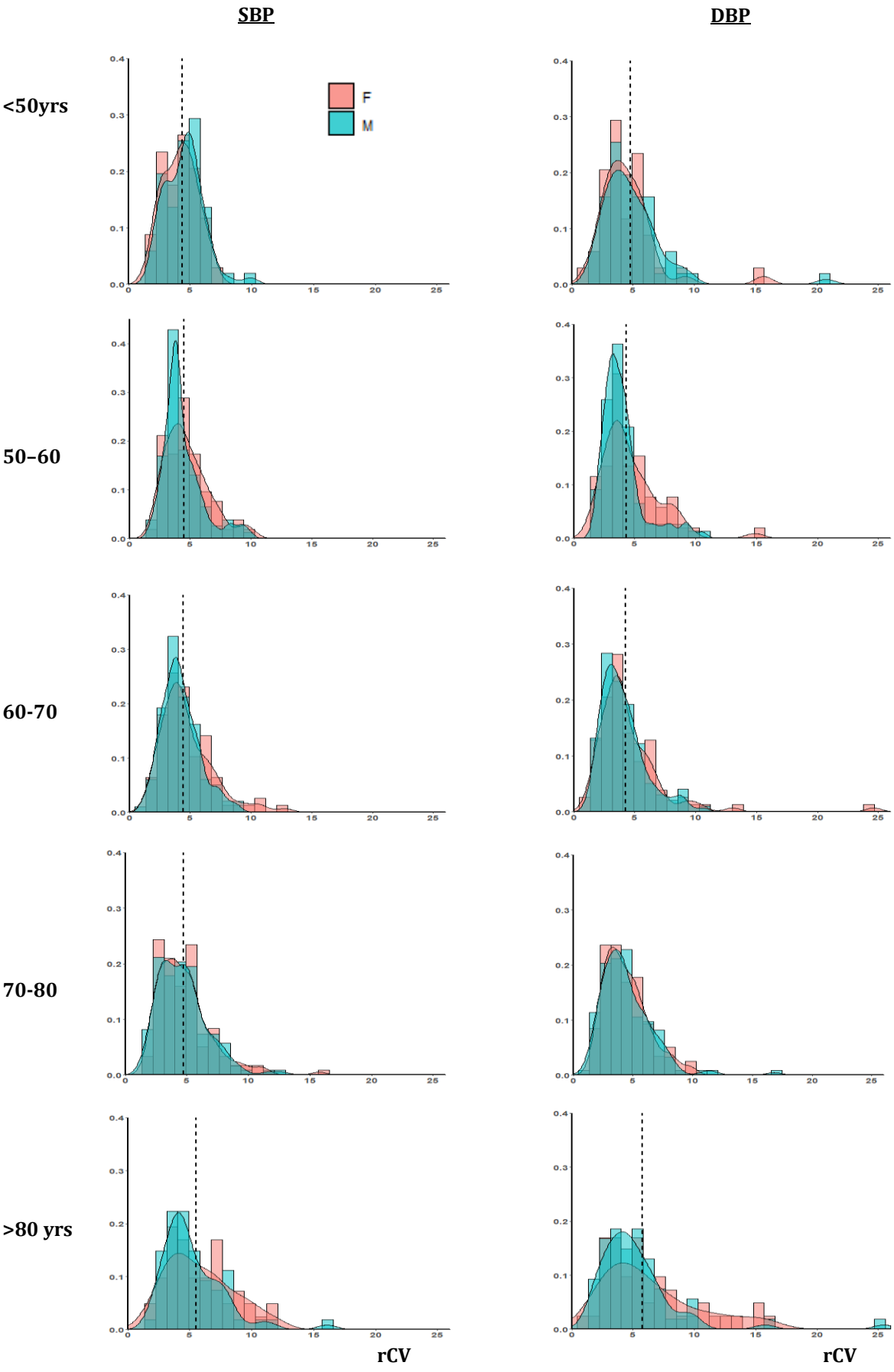
		rCV-SBP				rCV-DBP			
	n	Median (IQR)	90%	p-val	Skew	Median (IQR)	90%	p-val	Skew
All	908	4.2 (3.2 - 5.5)	7.18	<0.001	1.4	3.9 (3.0 - 5.5)	7.34	<0.001	2.8
Age:									
<50	110	4.5 (3.0 - 5.3)	6.17	0.006	0.5	4.3 (3.2 - 5.7)	6.82	<0.001	3.0
50-60	162	4.0 (3.4 - 5.3)	6.97	<0.001	1.2	3.7 (2.9 - 4.9)	7.46	<0.001	1.8
60-70	222	4.1 (3.2 - 5.2)	6.72	<0.001	1.3	3.7 (2.8 - 5.1)	6.69	<0.001	3.5
70-80	293	4.4 (3.1 - 5.6)	7.36	<0.001	1.4	4.0 (3.0 - 5.5)	6.85	<0.001	1.6
>80	121	4.5 (3.5 - 6.9)	8.47	<0.001	1.2	4.7 (3.3 - 6.6)	9.72	<0.001	2.2

3.4.3 Relationship of blood pressure variability to age and sex

The median value of rCV-SBP and rCV-DBP increased across age groups. However, there was a greater median rCV in patients under 50 compared to patients aged between 50 and 60, although the distribution of CV was more 'normal.' However, rCV was more positively skewed with increasing age, with a progressive increase in the top decile of rCV across groups, particularly in patients over the age of 80, suggestive of a non-physiological increase in BPV (figure 3.3).

In contrast to the greater linear increase in mean SBP with age in women than men, the non-linear change in rCV was similar for both men and women but was more marked for DBP, with a nadir of rCV-DBP occurring at a greater age in women than men (figure 3.3). However, there was minimal difference in the median rCV-DBP in hypertensive patients, diabetics and smokers, with only a slightly greater CV-SBP in smokers and diabetics (table 3.1).

Figure 3.3. Distribution of BP variability, stratified by age. BPV is presented as histograms and density curves for the residual coefficient of variation after de-trending of data (rCV) over 5 minutes, stratified by sex, systolic (SBP) and diastolic (DBP) blood pressure.



The risk of being in the top decile was associated with increased age and sex in univariate analyses for rCV-SBP but not for rCV-DBP, with a similar pattern for mean SBP and an inverse association for mean DBP (table 3.3). After adjustment for age, the association with sex was not significant. The same pattern of association was seen for general linear models for the association between demographics measures and rCV.

Table 3.3. Demographic associations with being in the top decile of blood pressure or blood pressure variability. Odds ratios (OR) are presented per decade with p-values (p-val) from logistic regression, unadjusted and adjusted for age / sex, or age, sex and cardiovascular risk factors. rCV= residual coefficient of variation after linear de-trending.

	Systolic BP				**Diastolic BP**			
	Mean		**rCV**		**Mean**		**rCV**	
	OR	**p-val**	**OR**	**p-val**	**OR**	**p-val**	**OR**	**p-val**
Unadjusted								
Age	**1.68**	**<0.001**	**1.57**	**<0.001**	**0.83**	**0.019**	**1.08**	**0.37**
Female	**1.08**	**0.744**	**1.58**	**0.044**	**0.53**	**0.008**	**1.20**	**0.429**
Adjusted (A/S)								
Age	**1.64**	**<0.001**	**1.55**	**<0.001**	**0.86**	**0.061**	**1.06**	**0.454**
Female	**1.01**	**0.978**	**1.50**	**0.079**	**0.55**	**0.011**	**1.18**	**0.461**
Adjusted (CVrf)								
Age	**1.64**	**<0.001**	**1.72**	**<0.001**	**0.84**	**0.066**	**1.09**	**0.36**
Female	**1.03**	**0.902**	**1.42**	**0.142**	**0.53**	**0.011**	**1.09**	**0.724**

3.5 Discussion

In this high risk population, beat-to-beat BP variability over 5 minutes was positively skewed with a median rCV of 4.2% for systolic BP and 3.9% for diastolic blood pressure. However, there was a non-linear relationship between rCV and age with a fall in BPV in younger patients, likely reflecting loss of intact physiological mechanisms, followed by a high rate of markedly-increased BPV in older age-groups, implying these patients may be at particular risk from excess BPV.

Despite studies demonstrating that visit-to-visit and day-to-day BP variability is associated with an increased risk of cardiovascular events[1-4, 7, 9, 13-15], few studies have determined the prognostic significance of beat-to-beat blood variability[16] despite its wide-spread use in the assessment of autonomic function.[17] One study demonstrated that beat-to-beat BPV was increased in acute stroke and associated with poor outcome,[16] albeit with SD as the principle index of BPV. Beat-to-beat BPV was also associated with markers of end-organ injury in this population,[12] in limited studies in other populations[18],[10] and in limited studies using intra-arterial blood pressure.[19]

There is still no agreement as to the optimal measure of BPV or a description of its distribution in at-risk populations. In this study, we confirmed that SD was strongly correlated with mean BP, whilst CV-SBP was not significantly associated with mean-SBP, despite a weak negative correlation between mean DBP and CV-DBP. However, due to the baseline drift in BP during recordings, due either to measurement artefact or a physiological reduction in baseline BP, de-trending about a linear regression significantly reduced the artefactual component of the positive skew of the population. Although previous studies have also derived 'variation independent of the mean',[1, 3] this is a population-specific measurement that can not be applied in individuals or be used to derive normative values.

BPV was very positively skewed and this increased with age. As such, dichotomized thresholds for markedly-elevated BPV are likely to have prognostic

significance. Given an increased risk of recurrent events in the top quartile of BPV in this population,[11] with a particularly high risk in the top decile of BPV in analyses of visit-to-visit BPV,[2] 75% and 90% population thresholds for rCV-SBP are likely to be clinically applicable. Furthermore, the association between age and beat-to-beat BPV was markedly non-linear, consistent with reports from the ASCOT-BPLA and UK-TIA trials,[1, 2] with similar associations between age and other outcomes.[20,21] As such, simple linear adjustment for age in epidemiological models of BPV and similar variables, as is the norm, will consistently fail to adequately adjust for age in prognostic models, resulting in systematic bias. Either stratified models or non-linear approaches[20, 22] are therefore essential to develop age-specific prognostic estimates for key predictors of future cardiovascular risk.

Despite the positive skew of the distribution of BPV, BPV was greater in the youngest patients compared to patients over 50 years old, with a more normal distribution. However, as age increased there was a progressively greater skew of the population with an increasing threshold for the top decile of rCV-SBP across age strata. This likely reflects different components of BPV, with a fall in physiological, ordered fluctuations in younger patients reflecting an intact autonomic nervous system, followed by later-life development of increased, more randomly-distributed BPV in patients with failure of compensatory mechanisms.

There are limitations to this study. Firstly, all patients were assessed for a cerebrovascular event, limiting generalizability to other groups. However, this population is at an increased risk of recurrent stroke[23] associated with increased beat-to-beat BPV.[11] Secondly, 5% of patients did not have adequate recordings, despite methods to improve quality, particularly in elderly patients who may be at a particularly increased risk of stroke. As such, the prevalence of elevated BPV and associated cardiovascular risks may be underestimated. Thirdly, beat-to-beat BPV was measured in a highly controlled environment, using expensive equipment (Finometer). Development of more cost-effective methods would be essential to apply beat-to-beat

BPV to routine clinical practice. Fourthly, patients with atrial fibrillation were excluded from the analysis due to confounding by the randomly-distributed variation in the R-R interval and the increased stroke risk due to embolism. More patients will be required to investigate the determinants and significance of beat-to-beat BPV in patients with AF. Finally, we extensively cleaned and de-trended the data, improving precision of measurement but also limiting its applicability to clinical practice. As such, further development is required to standardize methods of acquisition, data cleaning and analysis of beat-to-beat BPV in a cost-effective and practical method.

Overall, we determined the distribution of beat-to-beat BPV in a relevant, high-risk population with TIA or minor stroke, providing normative values and characterization of the distribution by age and demographic group. This lays the foundation for further research to characterize the physiological determinants of BPV and to differentiate BPV reflecting intact, protective physiological processes from abnormal BPV associated with an increased risk of stroke. This will allow further analysis of the prognostic significance of beat-to-beat BPV in large populations, and trials to determine its validity as a target for treatment. Finally, it will be critical to develop practical methods to measure BPV in a cost-effective manner in clinical environments.

Beat-to-beat BP variability is a promising method to characterize BP variability at the time of a single patient assessment. We have characterized the distribution of BPV in a high-risk population, identifying a high rate of markedly-elevated BP in older patients. As well as providing evidence of different forms of BPV, this provides a robust foundation for future studies to better characterize the prognostic significance of beat-to-beat BPV and its utility as a treatment target.

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CHAPTER FOUR

BMI and arterial stiffness are synergistically associated with beat-to-beat blood pressure variability after TIA or minor stroke

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4.1 Summary

Blood pressure variability (BPV) is associated with an increased risk of cardiovascular events whilst beat-to-beat assessment enables rapid assessment of BPV. It increases, with a positive skew, in older patients but the underlying causes of elevated BPV are unclear.

In consecutive patients within 4-6 weeks of TIA or non-disabling stroke (Oxford Vascular Study), continuous non-invasive BP was measured beat-to-beat over 5 minutes (Finometer). Arterial stiffness was measured by carotid-femoral pulse wave velocity (PWV, Sphygmocor). After automated and manual data cleaning, associations between BP variability (rCV, residual coefficient of variation), demographic factors and arterial stiffness were determined for both systolic (SBP) and diastolic (DBP) BP, by ANOVA and linear models. Relationships between demographic factors and arterial stiffness were determined by interaction terms and mediation.

Among 1013 patients, 54 (5.3%) were in AF and 51 (5%) had low quality recordings. In a general linear model including the remaining 908 participants, SBPV was most strongly associated with age ($p=0.00003$), BMI ($p=0.003$), and arterial stiffness ($p=0.008$), with weaker independent associations with current smoking ($p=0.01$) and a low diastolic BP ($p=0.046$). However, whilst there was a linear increase in SBPV with BMI in men, in women SBPV was lowest for a BMI in the normal range, but was greater below 20 or above 30 (ANOVA $p=0.012$, BMI-sex interaction $p=0.03$). Although BMI and PWV were partially independent, increased PWV mediated approximately 32% of the relationship between increased BMI and SBPV ($p<0.001$).

Vascular aging, manifest as arterial stiffness, was a strong determinant of increased SBPV and partially mediated the effect of increased BMI. However, although high BMI was independently associated with SBPV in both sexes, a low BMI was associated with increased SBPV only in women.

4.2 Introduction

As demonstrated in chapter 3, beat-to-beat blood pressure variability is highest in older patients, in both men and women, with a marked positive skew that increases at older ages, identifying a subgroup of patients with particularly marked BPV. However, there was also a small decline in BPV between the youngest patients and those over 50. However, other determinants of increased BPV, and patients at risk of high BPV, isn't clear. Furthermore, beat-to-beat BPV is itself composed of multiple components contributing to total BPV, from physiological rhythms reflecting breathing and intact autonomic function to increased BPV associated with stiff arteries and impaired baroreceptor function in older patients with impaired compensatory mechanisms.[1] To assess the potential clinical utility of beat-to-beat BPV, and to identify populations at an increased risk of elevated BPV, it is necessary to understand the determinants of beat-to-beat BPV in at risk populations, and how this varies by demographic characteristics.

Therefore, in the physiological cohort of the OXVASC Phenotyped study, we determined association between beat-to-beat BPV variability with arterial stiffness and major cardiovascular risk factors and which demographic features determine a likely pathological excess of beat-to-beat BPV.

4.3 Methods

4.3.1 Study population

As in previous chapters, consecutive, consenting patients with TIA or minor stroke (NIHSS <5) were recruited between September 2010 and October 2019, as part of the Phenotyped Cohort of the Oxford Vascular Study (OXVASC).[1, 2] Participants were recruited at the OXVASC daily emergency assessment clinic,[3] following a referral after attendance at the Emergency Department or from primary care, usually within 24 hours. Patients were referred after transient neurological symptoms or symptoms consistent with a minor stroke, not requiring direct admission to hospital.

As part of the OXVASC Phenotyped Cohort, a routine prospective cardiovascular physiological assessment is performed at the 1 month follow-up visit between September 2010 and September 2019. Participants were excluded if they were under 18 years, had severe cognitive impairment, were pregnant or had autonomic failure, a recent myocardial infarction, unstable angina, heart failure (NYHA 3-4 or ejection fraction <40%) or untreated bilateral carotid stenosis (>70%). OXVASC is approved by the Oxfordshire Research Ethics Committee.

As in chapter 3, after 15-20 minutes supine rest, beat-to-beat BPV was measured over 5 minutes in a quiet, dimly-lit, temperature-controlled room (21-23^{0}C). Continuous ECG and non-invasive blood pressure were acquired at 200Hz (Finometer, Finapres Medical Systems, The Netherlands). Waveforms were preferentially recorded from the middle phalanx of the middle finger. Automated calibration ('Physiocal') was performed until the recording was stable, but turned off during each test. Estimated brachial waveforms (Finometer) were calibrated offline by linear regression to 2-3 supine, oscillometric brachial readings, performed immediately prior to the monitoring period on the contralateral arm, with manual exclusion of artefacts. In patients with a significant deterioration in recording quality during the first five minutes, the test was stopped, and the calibration procedure repeated. If necessary the finger cuff was moved to an adjacent finger or the proximal phalanx of the same finger, or the hand was warmed with a hand warmer. Prior to physiological assessment, two sitting clinic BPs, 5 minutes apart, were measured at ascertainment and one month in the non-dominant arm.

4.3.2 Analysis

BPV on beat-to-beat monitoring was calculated over 5 minutes. Ectopic beats and artefacts were automatically detected from the R-R interval of the ECG, visually reviewed and removed by linear interpolation of R-R interval. Blood pressure artefacts were automatically detected, manually reviewed, and removed by linear interpolation to

adjacent normal beats with in-house software. Patients in atrial fibrillation during the recording were excluded. Systolic and diastolic BPV were calculated as the standard deviation (SD) and the coefficient of variation (CV=SD/mean), before and after de-trending of the recording about a linear regression across 5 minutes. All recordings were reviewed blinded to clinical data, after automated and manual cleaning, to assess recording quality (3- excellent; 2- adequate for analysis; 1-unuseable, poor quality recording), based upon the presence of artefacts or drift in the baseline measurement.

Associations with demographic indices were determined by general linear models and by logistic regression for the top decile of BPV. Models were performed for univariate associations; adjusted for age and sex and for age, sex and cardiovascular risk factors at study entry (current smoking, history of hypertension, diabetes).

4.4 Results

4.4.1 Study Population

This analysis includes the same patients as described in chapter 3, with 1031 assessments performed in 1013 eligible, consecutive, consenting patients between September 2010 and October 2019, with 18 patients assessed twice after separate clinical presentations. Again, the 54 (5.3%) patients in atrial fibrillation were excluded and 51 (5%) patients had inadequate recordings. Patients with atrial fibrillation or poor quality were older, had higher BP and were more likely to be hypertensive (table 3.1).

As described in chapter 3, beat-to-beat SBP variability increased with age with a slightly greater SBPV in women, that was not significant after adjustment for age. However, the other key univariate and adjusted associations of increased beat-to-beat SBPV were increased BMI and increased arterial stiffness (table 4.1 and 4.2). After adjustment for age, sex and other cardiovascular risk factors, current smoking was also associated with increased SBPV. A history of hypertension, diabetes or dyslipidaemia were not associated. Only age was associated with DBPV in univariate analysis, although BMI was associated with DBPV after adjustment (table 4.2).

Table 4.1. Linear models for baseline demographic measures and systolic blood pressure (SBP) level and variability. SBP variability is analysed as the residual coefficient of variation (rCV), unadjusted or adjusted (adj) for age and sex, or for age, sex and cardiovascular risk factors (CV-RFs). Independent variables include hypertension (HTN) reported by patients at baseline, or defined by patient report and current treatment at baseline. Current treatment and pulse wave velocity measures are given at the time of the physiological assessment. p-val – p value; LDL – low density lipoprotein; HDL – high density lipoprotein; TSH – thyroid stimulating hormone; BMI – body mass index.

	Mean SBP						SBP rCV					
	Unadjusted		Adj. Age+Sex		Adj CV-RFs		Unadjusted		Adj. Age+Sex		Adj CV-RFs	
	β	p-val	β	p-val	β	p-val	β	p-val	β	p-val	β	p-val
Age	0.34	<0.001*	0.34	<0.001*	0.35	<0.001*	0.02	0.001*	0.02	0.001*	0.02	<0.001*
Female	0.47	0.692	-0.13	0.909	-0.15	0.898	0.27	0.039*	0.24	0.064	0.26	0.057
HTN Reported	7.83	<0.001*	5.51	<0.001*	6.33	<0.001*	0.03	0.804	-0.11	0.415	-0.06	0.728
HTN Treated	5.04	<0.001*	1.93	0.171	2.26	0.124	-0.07	0.636	-0.26	0.111	-0.25	0.139
Diabetes	1.62	0.377	1.21	0.499	1.09	0.547	0.31	0.123	0.33	0.109	0.39	0.056
Dyslipidaemia												
- Reported	1.4	0.274	-0.53	0.675	-0.87	0.526	-0.15	0.278	-0.23	0.119	-0.24	0.115
- Treated	1.52	0.313	-0.69	0.645	-1.85	0.239	-0.15	0.383	-0.24	0.162	-0.27	0.123
Smoking												
- Ever	-0.13	0.91	0.39	0.738	-0.75	0.561	0.04	0.793	0.11	0.418	0.01	0.958
- Current	-0.6	0.708	3.09	0.055	3.61	0.041*	0.18	0.311	0.41	0.026*	0.42	0.036*
Height	-3.89	0.877	2.83	0.908	3.52	0.885	-1.12	0.689	-0.24	0.932	-0.06	0.982
Weight	-0.01	0.732	0.02	0.443	0.01	0.571	0	0.21	0.01	0.024*	0.01	0.032*
BMI	-0.1	0.411	0.02	0.842	0.01	0.938	0.03	0.046*	0.03	0.01*	0.04	0.005*
Creatinine	0	0.958	-0.06	0.085	-0.06	0.077	0	0.503	0	0.294	0	0.243
Cholesterol	-0.16	0.68	0.1	0.789	0.17	0.654	0.01	0.759	0.01	0.805	0.03	0.506
LDL	-0.01	0.555	-0.01	0.564	-0.01	0.593	0	0.282	0	0.229	0	0.285
HDL	1.16	0.323	0.32	0.793	0.71	0.563	-0.05	0.704	-0.2	0.127	-0.2	0.15
TSH	0.23	0.487	0.13	0.684	0.19	0.558	-0.05	0.139	-0.06	0.105	-0.05	0.13
On antihypertensive	4.18	0.002*	1.11	0.42	-5.96	0.168	-0.04	0.805	-0.21	0.175	0.16	0.736
On statin	1.11	0.439	-0.79	0.578	-1.19	0.736	-0.2	0.215	-0.28	0.078	-0.58	0.143
Pulse Wave Velocity	2.43	<0.001	2.29	<0.001	2.35	<0.001	0.14	<0.001	0.11	0.002	0.11	0.002

Table 4.2. Linear models for baseline demographic measures and diastolic blood pressure (DBP) level and variability. DBP variability is analysed as the residual coefficient of variation (rCV), unadjusted or adjusted (adj) for age and sex, or for age, sex and cardiovascular risk factors (CV-RFs). Current treatment and pulse wave velocity measures are given at the time of the physiological assessment. p-val – p value; LDL – low density lipoprotein; HDL – high density lipoprotein; TSH – thyroid stimulating hormone; BMI – body mass index.

	Mean DBP						DBP rCV					
	Unadjusted		Adj. Age+Sex		Adj CV-RFs		Unadjusted		Adj. Age+Sex		Adj CV-RFs	
	β	p-val	β	p-val	β	p-val	β	p-val	β	p-val	β	p-val
Age	-0.11	<0.001	-0.1	<0.001	-0.11	<0.001	0.01	0.156	0.01	0.186	0.02	0.03
Female	-4.38	<0.001	-4.2	<0.001	-4.29	<0.001	0.26	0.121	0.24	0.144	0.21	0.224
HTN Reported	0.96	0.144	1.93	0.004	2.42	0.002	0.06	0.697	0	0.997	0.17	0.412
HTN Treated	-0.4	0.599	0.67	0.394	1.19	0.145	-0.16	0.409	-0.26	0.19	-0.19	0.367
Diabetes	-1.2	0.242	-1.57	0.114	-1.59	0.116	-0.05	0.839	-0.03	0.894	0.05	0.86
Dyslipidaemia												
- Reported	-0.75	0.292	-0.67	0.34	-0.39	0.605	-0.19	0.288	-0.22	0.228	-0.13	0.496
- Treated	-1.32	0.116	-0.93	0.263	-1.05	0.229	-0.34	0.101	-0.4	0.062	-0.4	0.077
Smoking												
- Ever	0.91	0.17	0.01	0.99	-0.14	0.843	-0.08	0.637	-0.03	0.882	-0.08	0.66
- Current	1.83	0.038	0.27	0.759	0.43	0.662	0.1	0.666	0.23	0.323	0.3	0.234
Height	15.68	0.258	8.84	0.516	8.74	0.522	-10.81	0.002	-10.17	0.004	-10.07	0.004
Weight	0.02	0.112	0	0.681	0	0.826	0	0.591	0	0.263	0	0.192
BMI	0.09	0.179	0.05	0.396	0.08	0.246	0.03	0.116	0.03	0.066	0.04	0.018
SBP at 1 month	0.16	<0.001	0.17	<0.001	0.17	<0.001	0	0.707	0	0.706	0	0.552
DBP at 1 month	0.39	<0.001	0.38	<0.001	0.38	<0.001	-0.02	0.013	-0.02	0.012	-0.02	0.028
Creatinine	0	0.894	-0.03	0.056	-0.03	0.059	0	0.721	0	0.37	0.01	0.294
Cholesterol	0.2	0.345	0.35	0.088	0.31	0.141	-0.01	0.92	-0.01	0.799	-0.01	0.843
LDL	-0.01	0.501	0	0.679	0	0.658	0	0.545	0	0.484	0	0.562
HDL	-0.58	0.375	1.17	0.076	1.22	0.069	-0.04	0.825	-0.18	0.298	-0.21	0.233
TSH	0.22	0.236	0.24	0.17	0.26	0.148	-0.04	0.362	-0.04	0.341	-0.04	0.37
On antihypertensive	-1.08	0.15	0.05	0.953	-4.97	0.039	-0.09	0.629	-0.2	0.32	0.5	0.424
On statin	-1.47	0.067	-1.04	0.186	-1.4	0.475	-0.4	0.045	-0.46	0.025	-0.78	0.122
Pulse Wave Velocity	0.15	0.32	0.57	0.001	0.66	<0.001	0.13	<0.001	0.14	0.002	0.16	<0.002

4.4.2 The distribution of BPV by BMI groups

BMI was the strongest clinical risk factor associated with increased BPV. There was a significant difference in BPV between standardly defined BMI groups in women (ANOVA p=0.012), with a U-shaped relationship, such that SBPV in women with a BMI between 25-30 was significantly lower than women with a BMI below 20 (post-hoc Tukey HSD p=0.04) or greater than 30 (p=0.0499), see figure 4.1. It was not associated with mean or maximum SBP but only with SBPV (figure 4.2), and was negatively correlated with age (p<0.0001). The elevated SBPV in women with a low BMI did not reflect a specific excess of high BPV in this group, with a similarly shaped distribution of SBPV across different levels of BMI. In contrast, there was no difference between absolute BMI categories in men (figure 4.1), with a linear increase in BPV when BMI was defined by quintiles (figure 4.2). This pattern predominantly reflected differences in weight, with a more linear increase in BPV across quintiles of weight in men, with no change across quintiles of height.

Figure 4.1. Mean values of rCV for systolic (SBP) and diastolic (DBP), stratified by sex and standard thresholds for BMI. Results are presented as the mean and confidence interval for each group. rCV is calculated as the coefficient of variation of de-trended recordings.

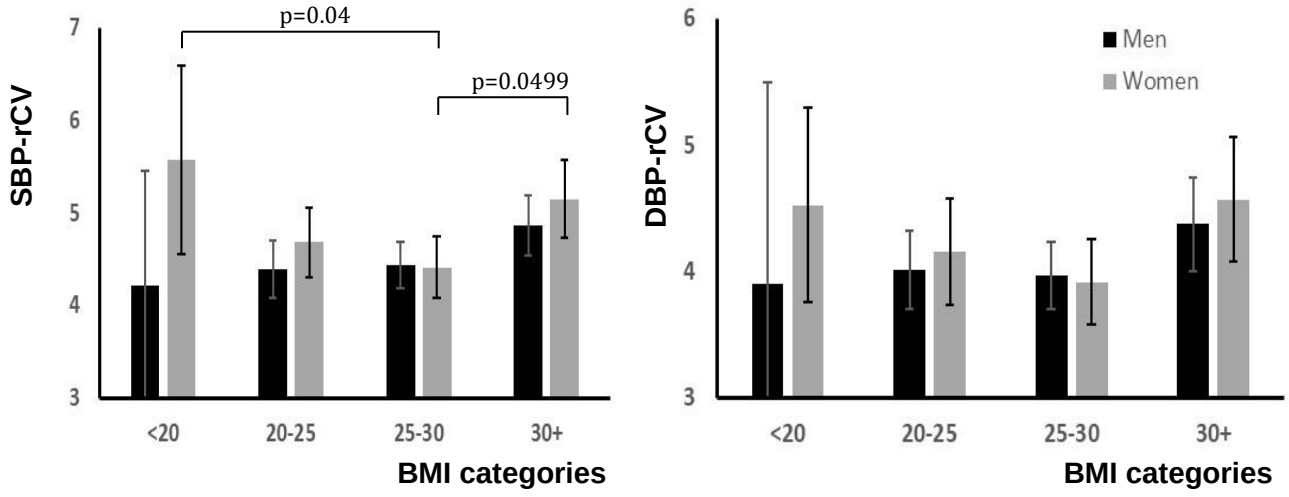
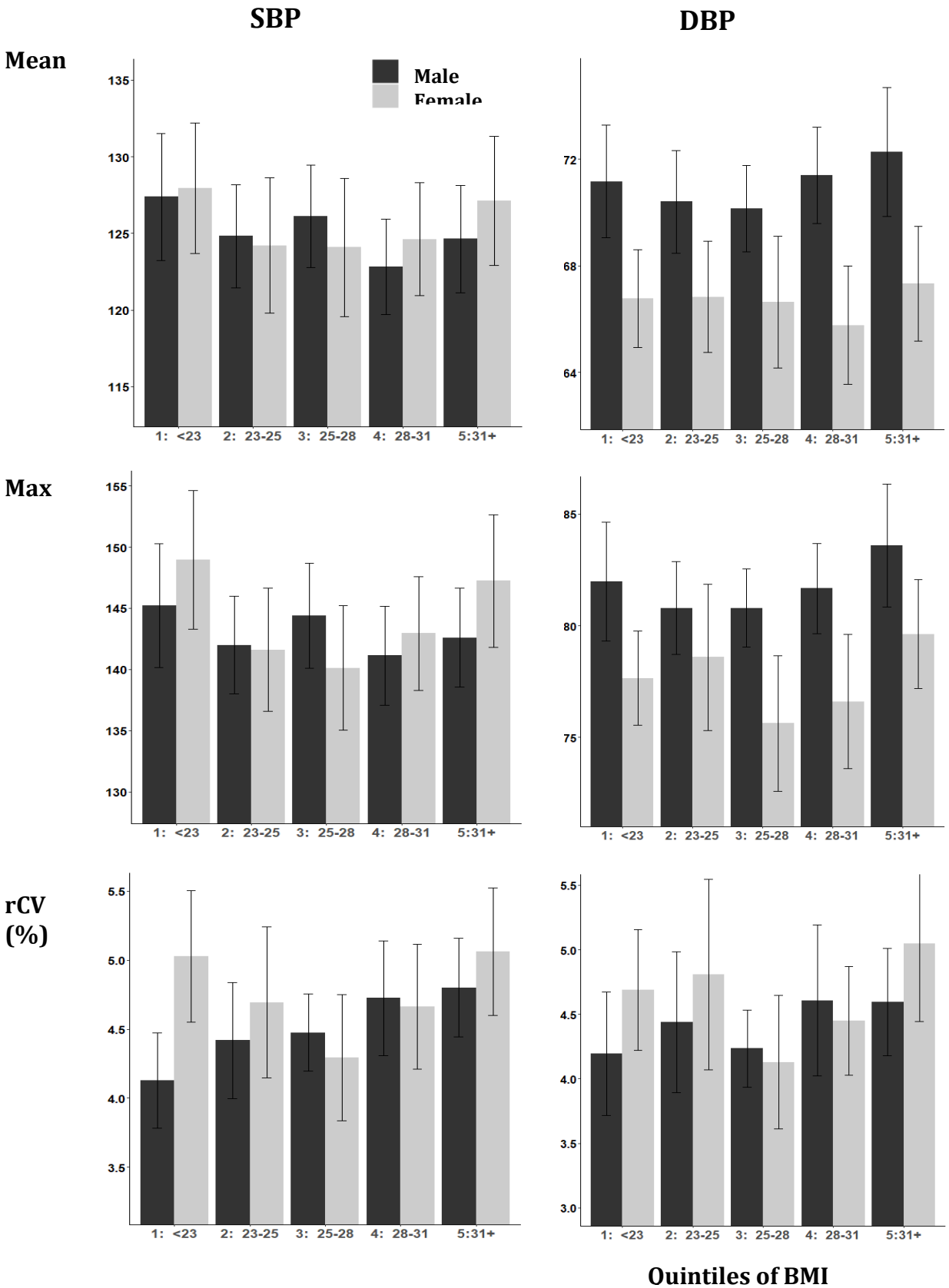


Figure 4.2. Mean values of mean, maximum and rCV for systolic (SBP) and diastolic (DBP) blood pressure, stratified by sex and quintiles of BMI. Results are presented as the mean and confidence interval for each group. rCV is calculated as the coefficient of variation of de-trended recordings. Y-axes are scaled to the inter-quartile range of the whole population for each index.



4.4.3 The relationship between BPV and BMI

The difference in the pattern of change with BMI between sexes was evident in a multivariate model, with a significant association between BMI and SBPV or DBPV when including all indices significantly associated with BPV after adjustment for age, sex and CV risk factors (table 4.2). Furthermore, there was a significant interaction between BMI with sex for SBPV, but not for DBPV, reflecting the linear increase in SBPV in men but not women. This was confirmed on stratifying the population by sex, with a significant association between BMI and BPV in men, exceeding even the association between age and BPV, whilst in women there was no overall linear association with the only strong determinant being the relationships between age and low DBP with SBPV (table 4.2), reflecting the non-linear pattern in women.

4.4.4 The relationship between BPV and arterial stiffness

Arterial stiffness was the factor most strongly associated with BPV, even compared to age. A linear increase in SBPV with arterial stiffness was seen in both men and women (figure 4.3), with a steeper relationship in women than in men but with no significant interaction with sex (p=0.38). This association was sufficiently strong that there was no association between age and SBPV after adjustment for PWV (adjusted for age and sex: age p=0.19; sex p=0.007; PWV p=0.002).

Figure 4.3: Relationship between increased carotid-femoral pulse wave velocity beat-to-beat BP variability, stratified by sex. Results are presented as the mean and confidence interval for each group. rCV is calculated as the coefficient of variation of de-trended recordings. Y-axes are set to the 95% range of each measure across the whole population.

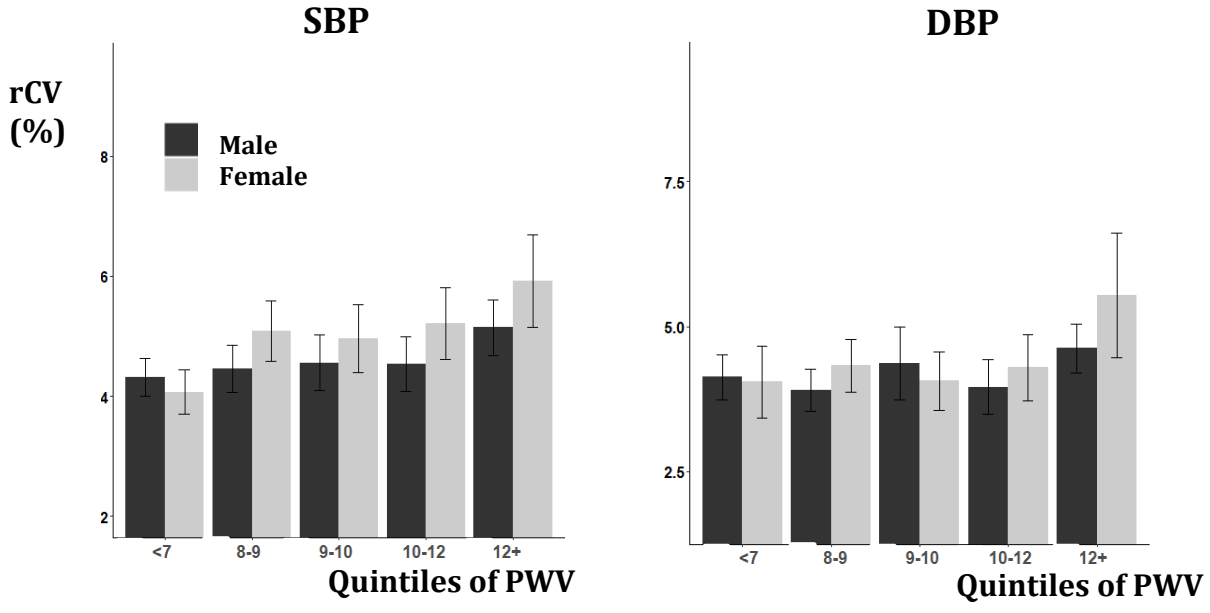


Table 4.3. Multivariate associations and interactions between BMI and sex for variability in systolic (SBP) and diastolic (DBP) blood pressure. Odds ratios (OR) and p-values (p-val) are given from binomial logistic regression, unadjusted and adjusted for age and sex, or for age, sex and cardiovascular risk factors (smoking, diabetes, history of hypertension). BP variability is measured as the residual coefficient of variation after de-trending of data (rCV).

	Entire Population				**Stratified by Sex**			
	SBP		**DBP**		**SBP**		**DBP**	
	β	**p-val**	**B**	**p-val**	**B**	**p-val**	**β**	**p-val**
Model 1					***Men***			
Age	0.02	0.0003**	0.05	0.33	0.02	0.01*	0.05	0.49
Female	0.22	0.11	0.69	0.65	-	-	-	-
DBP	-0.01	0.046*	-0.07	0.21	-0.01	0.29	-0.05	0.48
Smoking	0.47	0.01*	3.6	0.09	0.39	0.07	3.0	0.23
BMI	0.04	0.003**	0.35	0.02*	0.07	0.0002**	0.49	0.02*
Model 2					***Women***			
Age	0.02	0.0002**	0.06	0.31	0.03	0.004**	0.07	0.43
Female	1.78	0.015*	9.1	0.26	-	-	-	-
DBP	-0.01	0.03*	-0.07	0.18	-0.02	0.04*	-0.12	0.22
Smoking	0.50	0.01*	3.7	0.08	0.66	0.06	4.9	0.20
BMI	0.07	0.0003**	0.51	0.02*	0.02	0.45	0.2	0.35
BMI * Female	-0.06	0.03*	-0.31	0.29	-	-	-	-
Model 3								
Age	0.015	0.06	-0.01	0.88				
Female	2.31	0.01*	1.29	0.21				
DBP	-0.01	0.07	-0.07	0.29				
Smoking	0.60	0.008*	0.47	0.07				
PWV	0.10	0.008*	0.11	0.01*				
BMI	0.06	0.01*	0.029	0.28				
BMI * Female	-0.07	0.03*	-0.042	0.27				

4.4.5 Interactions between BPV, BMI and arterial stiffness

There was no univariate association between BMI and PWV (β=0.03, p=0.17), but after adjustment for age and sex there was a strong association (β=0.06, p<0.001), which persisted after adjustment for other cardiovascular risk factors (β=0.05, p=0.008). In a combined model allowing for the sex*BMI interaction, BMI and PWV independently predicted SBPV, with no residual significant association between either age or DBP with SBPV, although associations remained for sex and smoking. There was no interaction between smoking and BMI for BPV, and no association between thyroid stimulating hormone level and either BMI or BPV. In a causal mediation analysis allowing for adjustment for age and sex, there was also a significant indirect mediation effect of the relationship between BMI and SBPV by PWV (ACME 0.008 p<0.001; ADE 0.016 p=0.3; total effect 0.024), explaining 32% of the relationship between BMI and SBPV. There was no significant indirect mediation by BMI of the relationship between PWV and SBPV (ACME p=0.12, 10% variance explained).

4.5 Discussion

In an at-risk population with recent TIA or minor stroke, beat-to-beat BP variability over 5 minutes was associated with increased BMI and arterial stiffness. There was a linear increase in SBPV with BMI in men, but both low and high BMI were associated with increased SBPV in women. PWV and BMI were independently associated with SBPV, but PWV also mediated a proportion of the effect of BMI on SBPV. Associations between current smoking and increased SBPV persisted despite adjustment, but associations between both age and falling DBP were not significant after adjustment for PWV, indicating a likely primary role for arterial stiffness in mediating the relationship between age, DBP or BMI with beat-to-beat BPV.

Despite the large number of studies demonstrating that visit-to-visit and day-to-day BP variability are associated with an increased risk of cardiovascular events[4-13], few studies have determined the prognostic significance of beat-to-beat blood variability,[14] despite its wide-spread use in the assessment of autonomic function in

both research and clinical practice,[15, 16] whilst beat-to-beat BPV was associated with markers of end-organ injury and vascular aging, both in this population, [1] and in limited studies in other populations.[17] This was also consistent with limited studies using intra-arterial continuous blood pressure measurements.[18, 19]

However, there is as yet little evidence identifying the determinants and clinical characteristics of patients with increased beat-to-beat BPV. We previously demonstrated in a much smaller sample that beat-to-beat BPV was associated with aortic stiffness and pulsatility, as well as with markers of cardiovascular reactivity,[1] and that SBPV increases with age with an increased skew of the distribution of SBPV in a subset of the population. In this analysis, increased BMI was the strongest clinical factor associated with increased SBPV. However, the relationship between BMI and SBPV was complex, with a linear association in men. However, in women there was a U-shaped relationship, with increased SBPV in women with both a reduced and increased BMI compared to normal BMI, consistent with an increased risk of mortality for patients when both over and underweight. Although this could reflect 'physiological' variability in women with a reduced BMI that could be beneficial, the marked positive skew of the distribution of SBPV in women with a BMI below 20 suggests that this is not the case, and that the elevation in SBPV is driven by patients with an excess of pathological SBPV, even in women with a reduced BMI. This may reflect increased autonomic instability and sympathetic overactivity in obese patients, but could reflect reverse causation, with frailer patients being predisposed both to being underweight and to having increased BPV. Alternatively, elevated SBPV may mediate some of the relationship between obesity or being underweight and cardiovascular risk, and represent a new treatment target.

This study confirmed the previously demonstrated association between arterial stiffness and beat-to-beat BPV, with no significant association between age and SBPV after adjustment for PWV. Furthermore, the mediation analysis suggests that it is partly increased arterial stiffness in patients with increased BMI that results in increased

SBPV, although this does not explain the increased SBPV in patients with reduced BMI. This also implies that weight loss in the obese has the potential to reduce cardiovascular morbidity, and stroke in particular, through reductions in arterial stiffness and therefore reductions in blood pressure variability. Furthermore, given the negative correlation between age and BMI, this supports the hypothesis that the rising incidence of stroke in younger patients, and women in particular, may partly be driven by increasing weight that may partly be mediated by increased SBPV.

There are limitations to our study. Firstly, all patients were assessed after a cerebrovascular event, limiting generalizability to other disease groups. However, this population are at an increased risk of recurrent stroke,[3] and this risk has been shown to be associated with beat-to-beat BPV in this group.[2] Secondly, 5% of patients did not have adequate recordings, despite repeated measures to improve quality, particularly in elderly patients who may be at a particularly increased risk of stroke. As such, the prevalence of elevated BPV may be underestimated, with consequent underestimation of the risk associated with elevated BPV. Thirdly, we measured beat-to-beat BPV in a highly controlled environment, using expensive equipment. Development of more cost-effective methods would be essential to apply beat-to-beat BPV to routine clinical practice. Fourthly, we extensively cleaned and de-trended the data, improving precision of measurement but also limiting its direct applicability to clinical practice. As such, further development is required to standardize methods of acquisition, data cleaning and analysis of beat-to-beat BPV in a practical method for use in clinical practice. Finally, obesity may cause systematic bias in the assessment of PWV through artificially increasing the measured distance between the carotid and femoral applanation sites, whilst also affecting accuracy of blood pressure measurement.

Overall, beat-to-beat SBPV reflected both age-associated arterial stiffness and changes in BMI. This suggests a potential role for weight loss to reduce SBPV in the obese, with a resulting reduction in cardiovascular risk, but further research is required in under-weight women to determine why SBPV may be increased, and whether this is

a marker of frailty or an independent treatable factor. Furthermore, it implies that measures that reduce SBPV, such as amlodipine,[6] may be particularly effective at reducing cardiovascular risk in patients who are overweight, or women who are underweight.

Beat-to-beat BP variability reflects increased arterial stiffness and an increased BMI in both sexes, and a low BMI in women alone. This may be a potentially modifiable mechanism resulting in the increased risk of cardiovascular events due to increased arterial stiffness and alterations in BMI.

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CHAPTER FIVE

Progression of beat-to-beat blood pressure variability despite best medical management after TIA and stroke

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5.1 Summary

Variability in blood pressure is associated with recurrent stroke, despite control of hypertension, is assessable in a single visit from beat-to-beat, and beat-to-beat blood pressure is associated with demographic indices that increase the risk of recurrent stroke. However, no study has identified rates and determinants of progression of beat-to-beat BP variability and which patient groups are particularly at risk.

In consecutive patients within 6 weeks of TIA or non-disabling stroke (Oxford Vascular Study), continuous non-invasive BP was measured beat-to-beat over 5 minutes (Finometer). Arterial stiffness was measured by carotid-femoral pulse wave velocity (PWV, Sphygmocor). Repeat assessments were performed at the 5 year follow-up visit and agreement determined by intraclass correlation coefficient (ICC). Rates of progression of systolic and diastolic BPV and their determinants, stratified by age group and sex, were estimated by mixed-effect linear models, adjusted for age, sex and risk factors.

188 of 310 surviving, eligible patients had repeat assessments after a median of 5.8 years. PWV was highly reproducible but SBPV and DBPV were not (ICC 0.71,0.1 and 0.16 respectively). However, all three progressed significantly (PWV 2.39%, $p<0.0001$; SBPV 8.36%, $p<0.0001$; DBPV 9.7, $p<0.0001$). The rate of increase of PWV, SBPV and DBPV all increased significantly with age ($p<0.0001$), with a greater positive skew. Progression of SBPV and DBPV were also particularly associated with female sex (PWV $p=0.00035$; SBPV $p<0.0001$; DBPV $p<0.0001$) and aortic mean SBP (SBPV $p=0.037$, DBPV $p<0.0001$).

Beat-to-beat BP variability progresses significantly in high-risk patients, particularly in older patients with elevated aortic systolic pressure. Targetting beat-to-beat BPV and its progression in these patients represents a potential new therapeutic target to reduce cardiovascular risk.

5.2 Introduction

Patients with increased blood pressure variability have a high risk of recurrent stroke[1, 2] whilst residual visit-to-visit variability in blood pressure (BPV) on treatment has a poor prognosis despite good control of mean BP.[1, 2] Beat-to-beat BP variability enables BPV to be assessed in a single visit and has similar physiological associations to longer-term forms of BPV.[3] Furthermore, beat-to-beat BPV increased significantly with age (chapter 3) and was associated with other physiological and demographic indices that increase the risk of stroke (chapter 4). If proven to be associated with the risk of recurrent stroke, then it has the potential to be an important novel treatment target to reduce the residual risk of recurrent stroke after optimal secondary prevention (chapter 1). However, an optimal treatment target progresses over time, and that progression should parallel the risk of harmful outcomes. As such, treatments would have the potential to reduce both the absolute value of BPV and its progression. However, no study has specifically determined the rate of progression of BPV with time.

Therefore, in the OXVASC Physiological cohort, I determined the rate of progression of beat-to-beat BPV, its consistency over time and the clinical and physiological associations underlying progression of BPV.

5.3 Methods

5.3.1 Study population

The initial cohort included consecutive, consenting patients with TIA or minor stroke were recruited between September 2010 and September 2019, as part of the Phenotyped Cohort of the Oxford Vascular Study (OXVASC). Participants were recruited at the OXVASC daily emergency assessment clinic, following a referral after attendance at the Emergency Department or from primary care, usually within 24 hours. Patients were referred after transient neurological symptoms or symptoms consistent with a minor stroke, not requiring direct admission to hospital. As part of the OXVASC Phenotyped cohort, a routine prospective cardiovascular physiological assessment is performed at the 1 month follow-up visit. Since August 2017, all surviving participants still registered with OXVASC primary-care physicians are eligible to undergo a repeat physiological assessment when attending for their 5 year follow-up visit. Participants undergoing a repeat study as part of an assessment for a recurrent cerebrovascular event more than 2.5 years after their initial physiological assessment could also be included. Participants were excluded if they were under 18 years, cognitively impaired (MMSE<23), pregnant, had autonomic failure, a recent myocardial infarction, unstable angina, heart failure (NYHA 3-4 or ejection fraction <40%) or untreated bilateral carotid stenosis (>70%). OXVASC is approved by the Oxfordshire Research Ethics Committee.

The repeat assessments were performed under the same conditions as the baseline studies, at rest in a quiet, dimly-lit, temperature-controlled room (21-23^{09}C). After 15-20 minutes supine rest, beat-to-beat BPV was measured over 5 minutes in a quiet, dimly-lit, temperature-controlled room (21-230C). Continuous ECG and non-invasive blood pressure were acquired at 200Hz (Finometer, Finapres Medical Systems, The Netherlands), via a Powerlab 8/30 with LabChart Pro software (ADInstruments, USA). Waveforms were preferentially recorded from the middle

phalanx of the middle finger. Automated calibration ('Physiocal') was performed until the recording was stable, but turned off during each test. Estimated brachial waveforms (Finometer) were calibrated offline by linear regression to 2-3 supine, oscillometric brachial readings, performed immediately prior to the monitoring period on the contralateral arm, with manual exclusion of artefacts. Applanation tonometry (Sphygmocor, AtCor Medical, Sydney, Australia) was used to measure carotid-femoral pulse wave velocity (aortic-PWV), aortic augmentation index and aortic systolic and diastolic blood pressure and pulse pressure (ao-SBP, ao-DBP, ao-PP).

5.3.2 Analysis

Consistency in measures between baseline and follow-up were determined by intraclass correlation coefficients and linear regression, and visually by Bland-Altman plots. Significant changes in indices between baseline and follow-up were assessed by paired t-test. Rates of progression of measures of arterial stiffness, aortic blood pressure and cerebral blood flow velocity were determined by linear mixed effect modelling, with autoregressive covariance to account for repeated measures. Models were assessed for age and gender, with rates of progression determined by the interaction with the time interval between visits, as continuous indices and stratified by age group (<55, 55-65, 65-75, >75). Potential determinants of absolute values and rates of progression were assessed, unadjusted and adjusted for age, gender and cardiovascular risk factors (smoking, dyslipidaemia, diabetes, hypertension).

Analyses were performed in R and Matlab r2018, using in-house software.

5.4 Results

5.4.1 Study Population

188 of the 310 eligible patients with more than 5 years of follow up, out of 908 eligible patients with baseline BPV, were reviewed at a median of 5.8 years after the initial assessment. Of the included patients, 150 had arterial stiffness assessments at baseline and follow-up, whilst 170 had beat-to-beat BPV. Demographic characteristics were similar between patients undergoing arterial stiffness and beat-to-beat BPV (table 5.1).

Table 5.1. Population of patients with repeat studies at approximately 5 years follow-up, undergoing measures of arterial stiffness

.	PWV	SBPV	p
N	150	170	
Age	66 (10.9)	65 (11.1)	0.44
Female	57 (38)	66 (38.8)	0.97
Hypertension	112 (74.7)	128 (75.3)	1
Diabetes	19 (12.7)	23 (13.5)	0.95
Atrial fibrillation	13 (8.7)	12 (7.1)	0.74
Dyslipidaemia	116 (77.3)	133 (78.2)	0.95
Current Smoker	13 (8.7)	21 (12.4)	0.38
Past Smoker	67 (44.7)	80 (47.1)	0.75
Medications	-	-	-
Antiplatelet	119 (79.3)	135 (79.4)	1
Antihypertensives	109 (72.7)	123 (72.4)	1
Statins	111 (74)	127 (74.7)	0.99
Weight (Kg)	77.6 (14.8)	79.3 (17.2)	0.51
BMI (Kg/m2)	26.6 (4.1)	27.1 (4.8)	0.31
Creatinine	78.4 (19.8)	77.6 (21.8)	0.42
SBP (mmHg)	149.3 (24.8)	148.7 (24)	0.78
DBP (mmHg)	85.3 (13.7)	85.5 (14.2)	0.44

5.4.2 Reproducibility and progression of BPV

Arterial stiffness (PWV) was highly reproducible within individuals over the five years of follow-up (table 5.2), but SBPV and DBPV were only weakly reproducible. However, there was a significant mean increase in PWV, SBPV and DBPV, with a greater positive skew of the distribution for SBPV and DBPV at follow-up (Figure 5.1). In contrast, HRV and BRS were more reproducible than beat-to-beat BP variability, but there was no significant progression of HRV during follow-up, with a marginal fall in BRS (table 5.2).

Figure 5.1. Change in distribution of arterial stiffness (pulse wave velocity), SBPV and DBPV

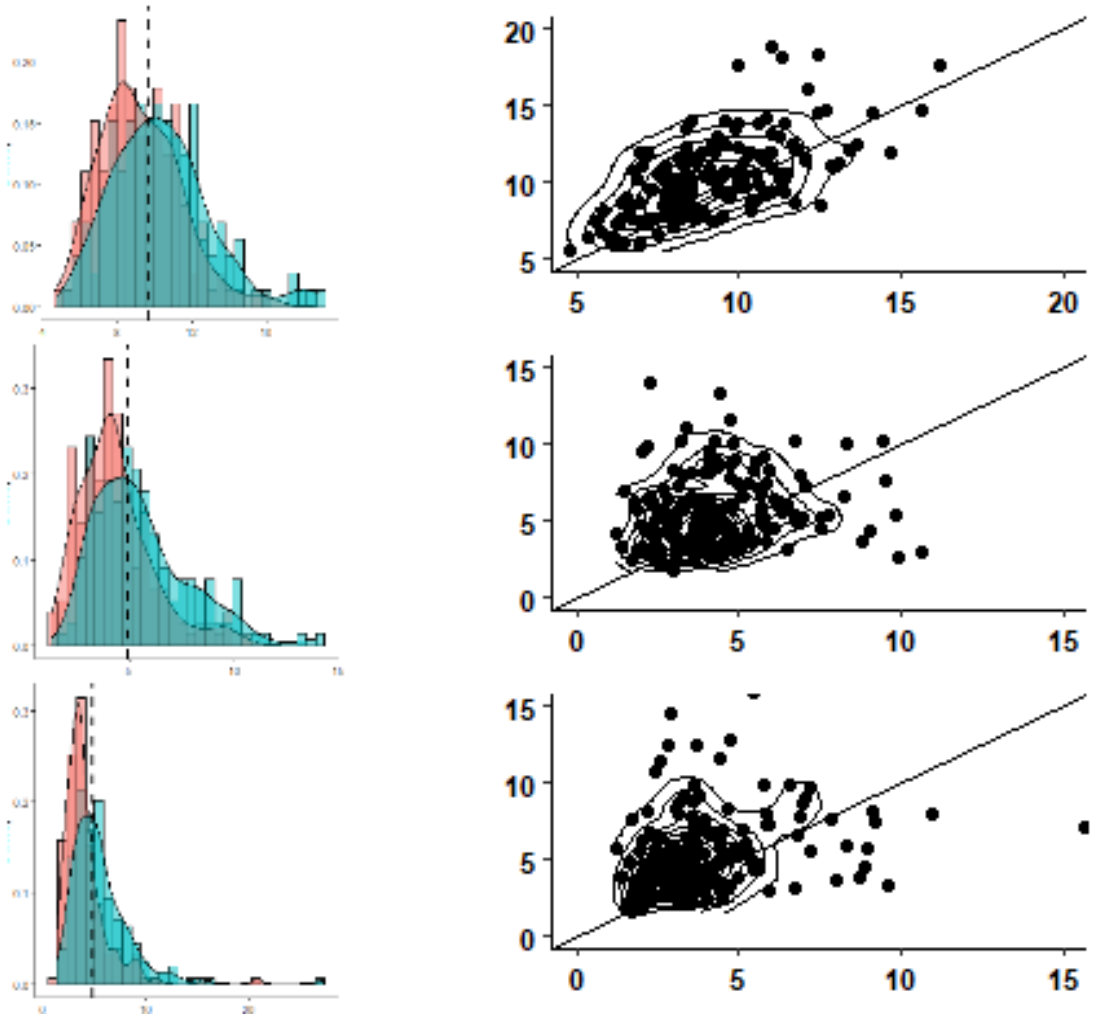


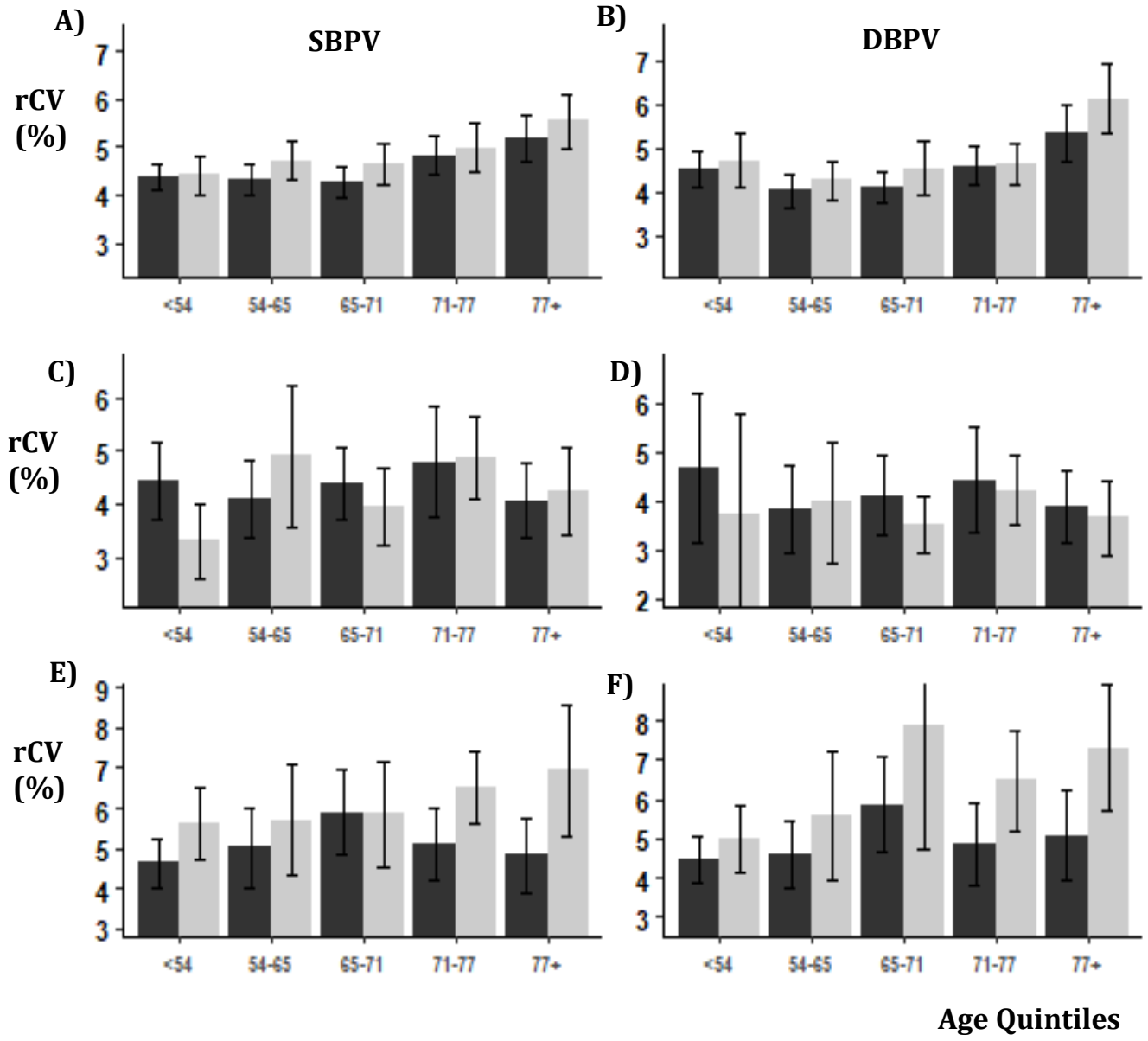
Table 5.2. Reproducibility and mean progression of arterial stiffness, aortic blood pressure and cerebral blood flow velocity.

Index	ICC	ICC confidence	r2	p	Baseline Mean	FU Mean	t-test p	Annual change	Annual change (%)
PWV	0.71	(0.62 - 0.78)	0.42	<0.001	9.2	10.3	<0.001	0.19	2.39
SBP mean	0.50	(0.35 - 0.61)	0.11	<0.001	120	122	0.48	-0.15	0.06
DBP Mean	0.19	(-0.04 - 0.37)	0.04	0.006	71	65.2	<0.001	-0.98	-1.17
SBPV	0.10	(-0.16 - 0.30)	0.02	0.056	4.3	5.52	<0.001	0.21	8.36
DBPV	0.16	(-0.08 - 0.34)	0.03	0.026	4.1	5.5	<0.001	0.24	9.7
HRV	0.49	(0.35 - 0.60)	0.11	<0.001	4.1	4.46	0.25	0.05	6.87
BRS	0.42	(0.23 - 0.57)	0.09	<0.001	10	8.03	0.049	-0.42	3.7

5.4.3 Cross-sectional relationship between age and BPV

In the 908 patients with baseline measurements, SBPV and DBPV were greater at older ages in both sexes, although the relationship was non-linear with no clear increase with age between the youngest quintiles (figure 5.2). This cross-sectional relationship was still evident at follow-up visits in women, although not in men. However, in those patients returning for follow-up, there was no cross-sectional association between age and SBPV at baseline (p=0.56), reflecting greater attrition of patients with higher baseline SBPV or arterial stiffness, with SBPV and PWV predicting loss to follow-up, even after adjustment for age and gender (SBPV p=0.048; PWV p=0.021).

Figure 5.2. Cross sectional distribution of blood pressure variability by age and gender. Panels A-B show SBP and DBP variability for the whole population at baseline; C-D show SBPV and DBPV at baseline for participants who returned for follow-up; Panels E-F show SBPV and DBPV at follow-up. Age groups (<55, 55-65,65-75, >75), for men (black) and women (grey).



5.4.4 Determinants of progression of BPV

In mixed effect linear models, PWV was greater in older patients and increased at a greater rate at older ages (figure 5.3). Similarly, the rate of increase for SBPV and DBPV increased significantly with increasing age and female gender (table 5.3), although due to the lower reproducibility of BPV and the greater attrition of older patients with greater SBPV at baseline, there was no cross-sectional association between age and SBPV (figure 5.3). In contrast, there was no significant progression in mean aortic SBP and a consistent small fall in aortic DBP across ages (figure 5.4), although the rate of change of mean DBP did not increase with increasing age. A history of diabetes was associated with both a greater cross-sectional PWV and a greater rate of progression of PWV, but did not predict increased SBPV, DBPV or their progression. However, other demographic indices including AF, smoking, weight were associated with greater BPV cross-sectionally but a decrease in BPV with time, likely reflecting regression to the mean or attrition bias.

Progression of both SBPV and DBPV were associated with an elevated aortic systolic and diastolic blood pressure, and particularly with an increased aortic pulsatility, both before and after adjustment for age and gender (table 5.4), but were not associated with aortic stiffness. Following adjustment for age, gender, cardiovascular risk factors, PWV and the other aortic measure, progression of SBPV and DBPV were still associated with an elevated baseline aortic SBP (SBPV $p=0.037$, DBPV $p<0.001$) but not with baseline DBP (SBPV $p=0.53$, DBPV $p=0.15$).

Figure 5.3. Progression of arterial stiffness (pulse wave velocity), SBPV and DBPV by age and gender. Panels A-C show individual changes during follow-up, and summary estimates within age groups (<55, 55-65,65-75, >75), for all patients (black), for men (blue) and women (red). Panels D-F show the average rate of progression within each age group, divided by age and gender

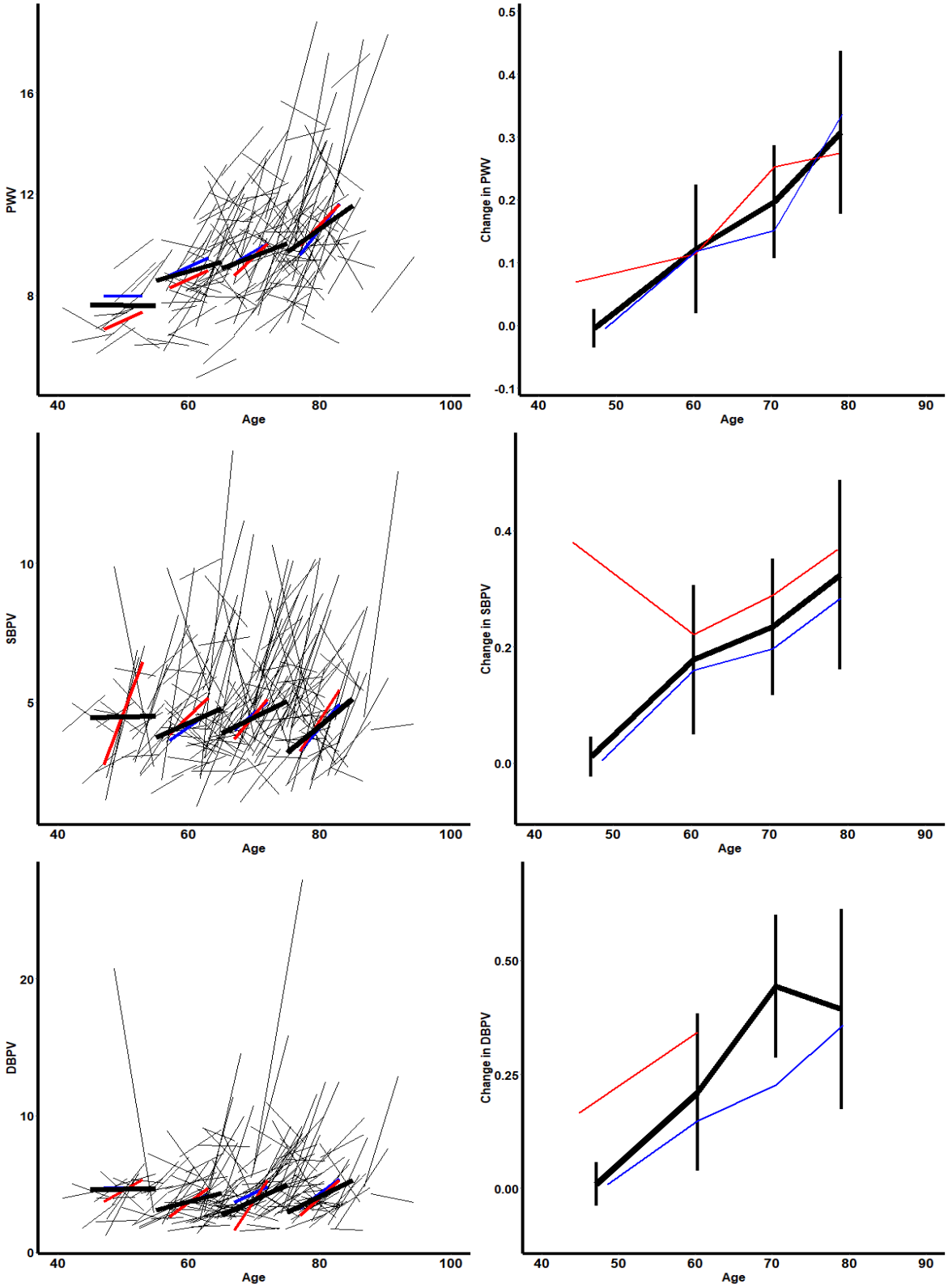


Figure 5.4 Progression of mean SBP and DBP, heart rate variability and baroreceptor sensitivity by age and gender. Panels A-D show individual changes during follow-up, and summary estimates within age groups (<55, 55-65,65-75, >75), for all patients (black), for men (blue) and women (red). Panels E-H show the average rate of progression within each age group, divided by age and gender

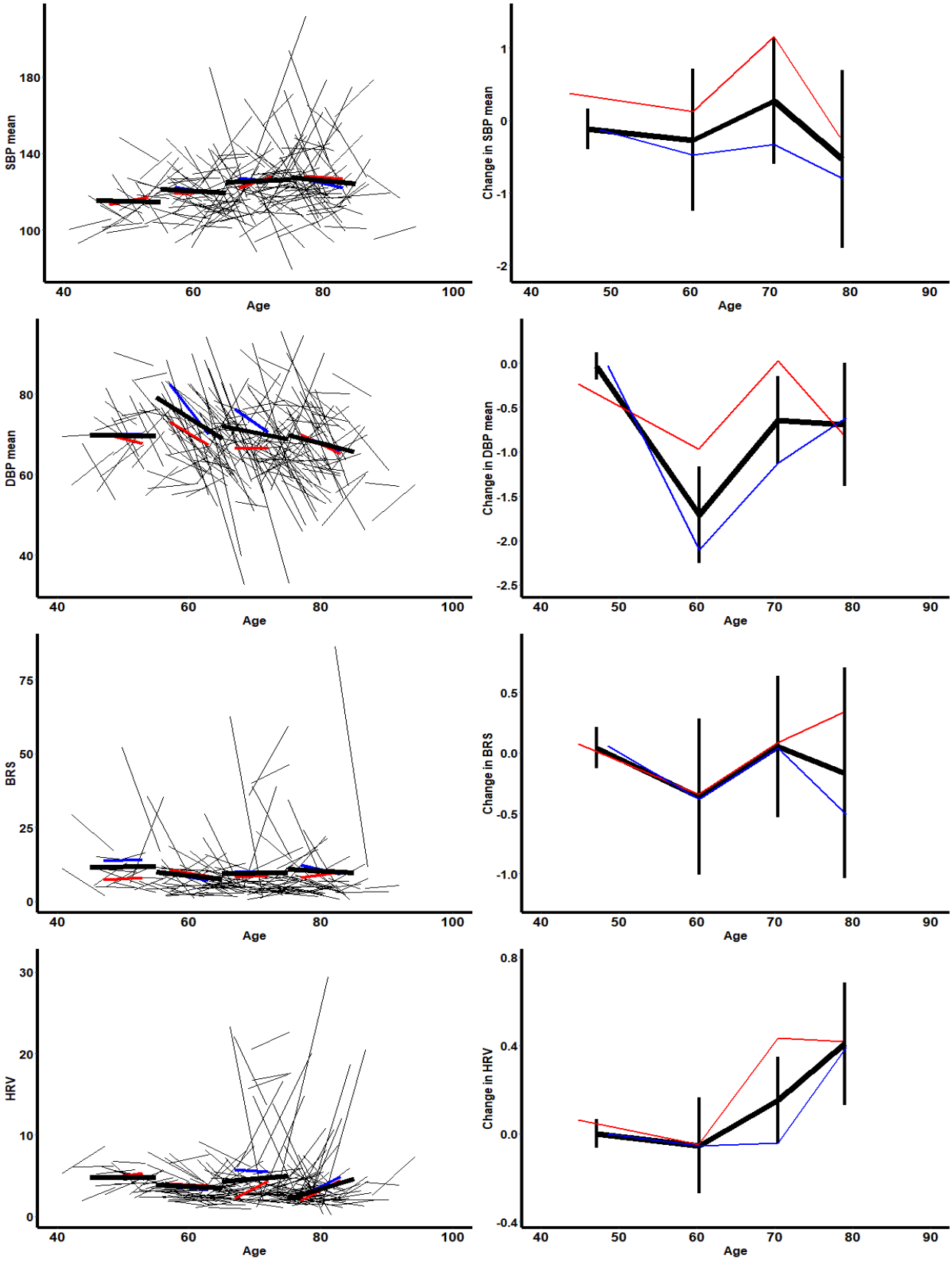


Table 5.3. Association between demographic characteristics and progression of key indices of arterial stiffness and pulsatility.

Index	PWV		PWV change		SBPV		SBPV change		DBPV		DBPV change	
	b	p	delta b	delta p	b	p	delta b	delta p	b	p	delta b	delta p
Female	-0.59	0.068	0.18	0.0004	-0.53	0.068	0.28	<0.0001	-0.95	0.022	0.46	<0.0001
Age	0.075	<0.0001	0.009	<0.0001	-0.012	0.32	0.011	<0.0001	-0.024	0.17	0.017	<0.0001
Hypertension	0.78	0.045	-0.054	0.41	0.026	0.94	-0.13	0.1	0.17	0.74	0.0077	0.94
Diabetes	0.93	0.071	0.2	0.013	0.048	0.92	0.13	0.21	-0.0047	0.99	0.17	0.22
AF history	-1.1	0.081	0.22	0.033	-1.1	0.058	0.29	0.031	-0.81	0.33	0.49	0.009
Dyslipidaemia	0.3	0.47	-0.13	0.05	0.17	0.68	-0.18	0.029	0.2	0.72	-0.19	0.11
Smoking	0.37	0.26	-0.16	0.0002	0.71	0.019	-0.14	0.01	1.3	0.0023	-0.24	0.00084
Current Smoking	0.045	0.94	0.051	0.62	-0.16	0.74	0.22	0.044	0.42	0.55	0.034	0.82
Weight	0.027	0.016	-0.0055	0.0007	0.026	0.0089	-0.0082	<0.0001	0.034	0.018	-0.011	<0.0001
BMI	0.078	0.05	-0.0098	0.16	0.025	0.47	-0.012	0.13	0.023	0.65	-0.013	0.24
Creatinine	0.014	0.099	-0.0051	<0.0001	0.028	0.0001	-0.0056	<0.0001	0.037	0.0005	-0.0065	<0.0001
Cholesterol	0.29	0.03	-0.062	0.0008	0.29	0.014	-0.1	<0.0001	0.31	0.067	-0.11	0.00039
Antihypertensive	0.61	0.11	-0.03	0.64	0.23	0.51	-0.16	0.041	0.4	0.42	-0.029	0.78
Statin	0.33	0.39	-0.087	0.17	0.47	0.2	-0.18	0.019	0.49	0.35	-0.18	0.093

Table 5.4. Association between physiological characteristics and progression of key indices of arterial stiffness and pulsatility.

Index	PWV		PWV change		SBPV		SBPV change		DBPV		DBPV change	
	b	p	delta b	delta p	b	p	delta b	delta p	b	p	delta b	delta p
Heart Rate	-0.0043	<0.0001	0.0004	0.059	-0.0009	0.38	0.00086	0.0005	0	0.84	0.00087	0.009
HRV	-0.0011	0.98	0.0028	0.73	0.13	0.01	-0.015	0.12	0.24	0.0005	-0.011	0.37
PWV	-	-	-	-	0.068	0.4	0.022	0.16	0.032	0.78	0.07	0.0017
Ao SBP	0.013	0.13	0.0047	<0.0001	-0.0032	0.73	0.0066	0.0001	-0.0029	0.82	0.012	<0.0001
Ao DBP	-0.01	0.48	0.011	<0.0001	-0.05	0.0016	0.0067	0.04	-0.054	0.016	0.014	0.003
Ao PP	0.031	0.007	0.004	0.05	0.019	0.12	0.0079	0.0009	0.019	0.27	0.015	<0.0001
BRS-LF	-0.034	0.024	0.005	0.058	-0.042	0.01	0.0086	0.006	-0.027	0.24	0.016	0.0004
BRS-HF	-0.028	0.0041	0.004	0.011	-0.027	0.008	0.0072	<0.0001	-0.02	0.15	0.012	<0.0001
BRS seq	-0.0077	0.53	0.004	0.18	0.0029	0.82	-0.0008	0.8	0.0085	0.69	-0.0011	0.83

5.5 Discussion

Variability in systolic and diastolic blood pressure increased at an increasing rate with increasing age, female gender and a greater aortic blood pressure, in this first study of progression of beat-to-beat variability in SBP and DBP. Specifically, there was a greater positive skew of SBPV and DBPV at follow-up compared to baseline. This paralleled the greater rate of increase in arterial stiffness with age but PWV did not predict the increase in blood pressure variability, despite both PWV and SBPV predicting a greater loss to follow-up. Overall, aortic systolic blood pressure was particularly associated with an increase in SBPV or DBPV, after adjustment for demographic factors, arterial stiffness and DBP.

Visit-to-visit,[2] day-to-day[4, 5] and diurnal blood pressure variability[6, 7] all predict the risk of stroke and future cardiovascular events, independent of mean blood pressure. Calcium channel blockers and thiazide-like diuretics reduce BPV,[8-10] with an associated difference in the rate of recurrent cardiovascular events. However, these methods require repeated assessments and the magnitude of the prognostic value of BPV compared to mean BP alone is uncertain, limiting their current use in clinical practice.[11] Beat-to-beat blood-pressure variability can be assessed in a single clinic visit, is cross-sectionally associated with clinical markers of cardiovascular risk (chapters 3 and 4) is associated with similar underlying physiological processes as day-to-day blood pressure variability[3] and predicts outcome after acute stroke.[12] This study demonstrates that beat-to-beat BPV progresses significantly over time, despite excellent risk factor control and predicting loss to follow-up, and this progression parallels progression of arterial stiffness. This supports the potential of beat-to-beat BPV as a treatment target and its utility for acute assessment of patients, but also suggests that even more intensive blood pressure control to levels suggested in the SPRINT trial [13] may have greater benefit. Furthermore, BPV increased quicker with age

with a greater skew, potentially identifying that more elderly patients risk of recurrent events is particularly related to BPV.

Despite similar physiological and demographic associations,[3] beat-to-beat BPV measures BPV over a much shorter time period. Therefore, it is not affected by diurnal or seasonal influences,[14] variability in patient adherence to medication[15] or day-to-day environmental factors such as weekday vs weekend readings.[16] Instead, it predominantly reflects current autonomic function,[17] and is the primary input in determining resting state baroreceptor sensitivity and dynamic cerebral autoregulation.[18] It may therefore more directly reflect loss of compensatory mechanisms in older patients, as suggested by the greater loss to follow-up of patients with either increased BPV or increased arterial stiffness and as a key determinant of the short-term gain of baroreceptor function and cerebral autoregulation. As such, if its prognostic utility is confirmed, it could provide a more efficient method of determining the same prognostic information as longer forms of BPV. Alternatively, it may provide different or additional information and may be affected by different treatment options, whether short-term mechanical[19] or pharmacological interventions,[20].

This study does have some limitations. It is still relatively small due to the high rate of attrition in a high-risk population, but it is the first study to assess beat-to-beat BP variability at an interval of 5 years and, despite the moderate size, associations were strong and consistent with previous evidence. Secondly, due to the age and frailty of the population, only 50% of the baseline population could be reassessed at 5 years, as many were deceased or had moved out of the area, with BPV at baseline predicting greater attrition at follow-up, thus resulting in a likely conservative underestimation of the cross-sectional and longitudinal associations between age and BPV. However, this rate of attrition also implies the potential prognostic significance of BPV. Thirdly, due to the low reproducibility of BPV there was evidence of regression to the mean, with the opposite direction of the cross-sectional and longitudinal associations with specific

predictors. However, despite this, regression to the mean was not evident for age, gender and aortic BP, the most important predictors of progression of BPV. Finally, we haven't shown that progression of BPV itself predicts the risk of recurrent cardiovascular events, or that it is amenable to treatment.

This study has now demonstrated that beat-to-beat BPV is determined by demographic and physiological processes and progresses significantly over time. As such, it is a promising prognostic factor and potential treatment target, both for interventions to directly reduce BPV and by preventing its progression. However, it is important to replicate this in other populations, demonstrate that progression of BPV independently predicts future risk and that beat-to-beat BPV is amenable to intervention with a resulting reduction in future events.

Beat-to-beat BP variability progresses over 5 years in high risk patients with recent TIA or minor stroke, with higher rates of progression in older patients and patients with higher blood pressures, despite excellent risk factor control. This therefore identifies patient groups most likely to be at an increased risk related to progression of BPV, and therefore those in whom interventions to reduce beat-to-beat BPV may best be targeted.

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CHAPTER SIX

Prognostic significance of blood pressure variability on beat-to-beat monitoring after TIA and stroke

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6.1 Summary

Visit-to-visit and day-to-day BP-variability (BPV) predict an increased risk of cardiovascular events, but only reflect one form of BPV. Beat-to-beat BPV can be rapidly assessed and might also be predictive.

In consecutive patients recruited within 6 weeks of TIA or non-disabling stroke (Oxford Vascular Study), BPV (CV, coefficient of variation) was measured beat-to-beat over 5 minutes (Finometer), day-to-day over 1 week on home monitoring (HBPM, 3 readings, 3 times daily) and on awake ambulatory BP monitoring (ABPM). BPV after 1 month of standard treatment was related (Cox proportional hazards) to recurrent stroke and cardiovascular events over 1-5 years, adjusted for mean SBP.

Among 520 patients, 26 had inadequate beat-to-beat recordings and 22 patients were in AF. 405 patients had all forms of monitoring. Beat-to-beat BPV predicted recurrent stroke and cardiovascular events independently of mean SBP (HR per group SD, stroke: 1.47, 1.12-1.91, p=0.005; cardiovascular events: 1.41, 1.08-1.83, p=0.01*)*, including after adjustment for age and gender (stroke 1.47,1.12-1.92, p=0.005) and all risk factors (1.40,1.00-1.94, p=0.047). Day-to-day BPV was less strongly associated with stroke (adjusted HR=1.29, 0.97-1.71, p=0.08) but similarly with cardiovascular events (1.41, 1.09-1.83, p=0.009). BPV on awake ABPM was non-predictive (stroke 0.89, 0.59-1.35, p=0.59; cardiovascular events 1.08, 0.77-1.52, p=0.65). Despite a weak correlation (r=0.119, p=0.02), beat-to-beat BPV was associated with risk of recurrent stroke independently of day-to-day BPV (1.41, 1.05-1.90, p=0.02).

Beat-to-beat BPV predicted recurrent stroke and cardiovascular events, independently of mean SBP and risk factors, but short-term BPV on ABPM did not. Beat-to-beat BPV may be a useful additional marker of cardiovascular risk.

6.2 Introduction

Patients with episodic hypertension in clinic after a previous TIA or stroke have a high risk of recurrent stroke,[1, 2] residual visit-to-visit variability in blood pressure (BP) on antihypertensive treatment has a poor prognosis despite good control of mean BP,[3] and benefits of some antihypertensive drugs in the prevention of stroke may partly result from reduced variability in SBP.[3, 4] Home day-to-day BP variability (HBPM-BPV) is similarly associated with an increased stroke risk,[5, 6] particularly for variability in morning BP,[6] and is reduced by similar medications. In contrast, short-term BP variability on awake ambulatory BP monitoring (ABPM) is only weakly predictive of cardiovascular events,[2] as is within-visit variability in office BP, with short-term BPV also correlating poorly with visit-to-visit BPV.[1,2] However, the predictive value of beat-to-beat BP variability over 5 minutes has not been determined.

Beat-to-beat BPV over 5 minutes is only weakly correlated with day-to-day BPV on HBPM and premorbid visit-to-visit BPV, but shares the same physiological associations, suggestive of a similar pathophysiology.[7] Increased beat-to-beat BPV,[8] and diminished baroreceptor sensitivity (derived from beat-to-beat BP monitoring),[9] are potentially associated with a worse outcome after a major acute stroke, and may be associated with an increased risk of recurrent events.[8] Beat-to-beat BPV is increased at older ages (chapter 3), associated with markers of increased cardiovascular risk including arterial stiffness and BMI (chapter 4) and progresses over time (chapter 5). However, previous studies were small with significant methodological problems. Therefore, we determined the predictive value of beat-to-beat BPV in a prospective cohort of patients with recent TIA or minor stroke.

6.3 Materials and Methods

6.3.1 Study population

This analysis was performed in a subset of the OXVASC Physiological cohort with complete clinical follow-up. For this population, patients were recruited between September 2010 and September 2015 from the Oxford Vascular Study (OXVASC)[10] TIA and minor stroke clinic. The OXVASC population consists of 92,728 individuals registered with 100 primary-care physicians in Oxfordshire, UK.[10] All consenting patients underwent a stroke protocol MRI brain and contrast-enhanced MRA (or CT-brain and carotid Doppler ultrasound or CT-angiogram), an echocardiogram and 5 day ambulatory cardiac monitoring. All patients were reviewed by a study physician, the diagnosis verified by the senior study neurologist (PMR), aetiology determined by a panel of stroke neurologists and were followed-up face-to-face at 1, 3, 6 and 12 months, and up to 2, 5 or 10 years. Recurrent events were determined at face-to-face follow-up and by multiple overlapping methods of ascertainment, including daily review of hospital admissions, review of death certificates and coroner's records, manual review of GP records and linkage to hospital event statistics and death registries.

Participants were excluded if they were under 18 years, cognitively impaired (MMSE<23), pregnant, had cancer, autonomic failure, a recent myocardial infarction, unstable angina, heart failure (NYHA 3-4 or ejection fraction <40%) or untreated bilateral carotid stenosis (>70%) and were excluded from this analysis if they had atrial fibrillation during testing. The study was approved by the Oxfordshire Research Ethics Committee.

6.3.2 Blood Pressure Measurement

Two sitting clinic BPs, 5 minutes apart, were measured at ascertainment and one month in the non-dominant arm, by trained personnel after five minutes of rest. From ascertainment, patients agreeing to perform home BP monitoring (HBPM) performed three home readings over 10 minutes, three times daily (after waking, mid-

morning and evening) with a Bluetooth-enabled, regularly-calibrated, telemetric IEM Stabil-o-Graph or A&D UA-767 BT. Patients were instructed to relax in a chair for 5 minutes before measuring BP in the non-dominant arm, or the higher-reading arm when the mean SBP differed by >20mmHg. Anonymised measures were securely transmitted via Bluetooth radio and a mobile phone to a password-protected website (t+ Medical, Abingdon, UK) and medication prescribed according to guidelines,[11] most frequently with perindopril, indapamide or amlodipine, to a target of <130/80. The day before the one month follow-up, ABPM was performed with an A&D TM-2430 monitor in the non-dominant arm. BP was measured every 30 minutes during the day and 60 minutes at night.

Beat-to-beat BPV was measured over 5 minutes at the ascertainment visit or 1 month clinic in a quiet, dimly-lit, temperature-controlled room (21-23^{0}C). Continuous 3-lead ECG and finger arterial BP were acquired at 200Hz (Finometer MIDI) via a Powerlab 8/35 (ADInstruments), from the non-dominant arm when possible. Automated calibration was performed until the recording was stable, but turned off during each test, and readings calibrated offline to the mean of 2 supine, oscillometric brachial readings.

6.3.3 Analysis

BPV on beat-to-beat monitoring was calculated over 5 minutes. Ectopic beats and artefacts were automatically detected, visually reviewed and removed by linear interpolation. Patients in atrial fibrillation during the recording were excluded. Variability was calculated as the coefficient of variation about a linear regression across 5 minutes to remove drift in the waveform (rCV). HBPM variability was derived from the last 7 days of recording prior to the 1 month follow-up visit, from the average SBP or DBP calculated from the last two readings of each cluster of three. Awake BP variability on ABPM was derived after automated and manual exclusion of artefacts according to standard criteria.[12] BP variability was derived as the coefficient of variation (CV=SD/mean) and the residual CV (rCV) about a moving average on HBPM.

Reproducibility of BPV on HBPM was determined in 100 patients between the 2nd and 3rd week of monitoring as Pearson's r and intraclass correlation coefficient (ICC). In 50 patients, beat-to-beat BPV was measured at baseline and the one month visit according to the same protocol to determine reproducibility of measurement by Pearson's r and the intraclass correlation coefficient (ICC).

Risk of recurrent cardiovascular events were determined per unit increase in mean and variability in SBP or DBP and per standard deviation for the population for each method of measurement by Cox Proportional Hazards Regression, with and without adjustment for age, gender and major cardiovascular risk factors (hypertension, diabetes, family history, smoking, atrial fibrillation), and in combined models adjusting for other measures of BPV. The effect of adjustment of beat-to-beat and day-to-day BPV for regression to the mean was estimated by scaling the difference between mean BPV for each quartile of BPV and the population mean by the ICCcoefficient.[13]

6.3.4 Literature Review

Pubmed and EMBASE were searched from inception until 1st March 2017 with the terms ("blood pressure" OR "BP" OR "hypertension" OR "BPV") AND ("baroreflex" OR "BRS" OR "baroreflex sensitivity") AND ("stroke" OR "cerebr*" OR "prognosis" OR "death" OR "mortality" OR "cerebrovascular accident" OR "cerebrovascular event" OR "cerebrovascular" OR "leukoaraiosis" OR "white matter hyperintensities" OR "white matter disease" OR "small vessel disease"). All papers reporting recurrent cardiovascular events per unit of beat-to-beat BP variability were identified.

6.4 Results

6.4.1 Study Population

Of 520 patients, 26 had poor quality recordings due to excessive ectopics or poor quality finometer recordings due to poor peripheral circulation, whilst 22 were excluded from beat-to-beat analyses due to AF during the recording, leaving 472 patients with valid beat-to-beat recordings. 466/520 patients had adequate HBPM (2.9 readings per cluster for median 29 days) and 461/520 had adequate ABPM (table 1), with 405

patients with adequate monitoring undergoing all forms of recording. There were weak positive associations between BPV measured with different methods (beat-to-beat CV vs HBPM rCV r=0.119, p=0.017; beat-to-beat CV vs awake SBP CV r=0.04, p=0.37;HBPM rCV vs awake SBP CV r=0.20, p<0.001) but limited associations with demographics (table 6.1).

Table 6.1 Demographics of patients with adequate beat-to-beat recording.

	Quartiles of beat-to-beat systolic BPV					
	1 (n=118)	2 (n=118)	3 (n=118)	4 (n=118)	All (n=472)	p-value
Age	64.9 (13.2)	66.2 (13.2)	65.7 (11.7)	67.9 (14.6)	66.2 (13.2)	0.36
Male (%)	60 (51)	69 (59)	74 (63)	70 (60)	273 (58)	0.28
Diabetes	8 (6.8)	9 (7.6)	16 (13.6)	16 (13.6)	49 (10.4)	0.17
Family History*	30 (32)	32 (31)	26 (25)	22 (21)	110 (27)	0.24
Hyperlipidaemia	36 (31)	36 (31)	31 (26)	25 (21)	128 (27)	0.31
Current smoker	18 (15)	17 (15)	17 (14)	21 (18)	73 (16)	0.87
Beat-to-beat:						
SBP Mean	127 (18)	125 (19)	125 (19)	125 (20)	126 (19)	0.84
SBP rCV	2.2 (0.5)	3.3 (0.3)	4.5 (0.3)	7.0 (2.0)	4.3 (2.1)	<0.001*
DBP Mean	74 (9)	72 (11)	70 (10)	69 (13)	71 (11)	0.002*
DBP rCV	2.4 (1.3)	7.5 (4.3)	4.1 (1.1)	7.5 (4.3)	4.3 (3.0)	<0.001*
HBPM SBP rCV						
SBP Mean	122 (15)	122 (21)	123 (17)	123 (17)	123 (19)	0.78
SBP rCV	4.7 (2.1)	4.4 (1.8)	4.6 (1.6)	4.8 (2.0)	4.6 (1.9)	0.33
Awake ABPM:						
SBP Mean	126 (11)	127 (15)	128 (12)	128 (12)	128 (12)	0.47
SBP CV	12 (3.2)	12 (3.9)	12 (3.8)	12 (3.6)	12 (3.6)	0.90
Asleep ABPM:						
SBP Mean	116 (15)	113 (20)	113 (18)	116 (15)	114 (17)	0.40
SBP CV	11 (4.8)	11 (5.7)	11 (4.0)	11 (5.0)	11 (4.9)	0.99
Creatinine	74 (21)	79 (22)	78 (21)	83 (23)	79 (22)	0.021*
BMI	27 (5.1)	26 (3.8)	28 (5.6)	27 (4.9)	27 (4.9)	0.09
Cholesterol	5.1 (1.3)	5.3 (3.0)	5.2 (1.2)	5.0 (1.2)	5.2 (1.9)	0.48

6.4.2 Associations between BPV, mean BP and cardiovascular events

Increased BPV on beat-to-beat monitoring in the 405 patients undergoing all forms of monitoring was associated with an increased risk of ischaemic stroke, any stroke and all cardiovascular events, independently of mean SBP (table 6.2), before and after adjustment for age and sex, with a significant association with the risk of recurrent ischaemic stroke remaining after adjustment for other cardiovascular risk factors (HR per SD=1.40, 1.00-1.94, p=0.047). Relationships were similar for all patients undergoing each form of monitoring, and largely unchanged by adjustment for mean SBP. The hazard ratio per 1% increase in rCV for stroke was 1.24 (1.07 - 1.43, p=0.004). BPV on home monitoring was not as strongly associated with stroke, but was associated with all-cause mortality and a composite of death and cardiovascular events (table 6.2). Beat-to-beat DBPV was not predictive of recurrent events, although home DBPV weakly predicted all cause mortality (table 6.3). In contrast to beat-to-beat and home monitoring, BPV on ABPM did not predict recurrent events (table 6.2) but mean SBP on all three methods of measurement strongly predicted the risk of future events (table 6.4).

Table 6.2 Hazard Ratio for cardiovascular events per SD of systolic rCV.

			Adjusted for mean SBP		Adjusted for Mean + RFs	
	Measure	**Ev**	**HR (95%CI)**	**p-val**	**HR (95%CI)**	**p-val**
Ischaemic Stroke	Bt-to-bt	26	1.47 (1.10 - 1.97)	0.009	1.49 (1.11 - 2.00)	0.007
	Day-to-day		1.22 (0.88 - 1.69)	0.24	1.24 (0.88 - 1.73)	0.22
	Awake		0.89 (0.59 - 1.35)	0.59	0.93 (0.61 - 1.43)	0.75
Any Stroke	Bt-to-bt	31	1.47 (1.12 - 1.91)	0.005	1.47 (1.12 - 1.92)	0.005
	Day-to-day		1.29 (0.97 - 1.71)	0.08	1.27 (0.95 - 1.71)	0.11
	Awake		0.93 (0.64 - 1.36)	0.72	0.96 (0.65 - 1.41)	0.82
All cause mortality	Bt-to-bt	22	1.18 (0.82 - 1.70)	0.36	1.09 (0.75 - 1.58)	0.65
	Day-to-day		1.40 (1.02 - 1.93)	0.04	1.21 (0.83 - 1.76)	0.31
	Awake		1.09 (0.72 - 1.66)	0.69	1.02 (0.64 - 1.62)	0.94
CV Death or MACE	Bt-to-bt	33	1.41 (1.08 - 1.83)	0.01	1.39 (1.07 - 1.81)	0.01
	Day-to-day		1.41 (1.09 - 1.83)	0.009	1.33 (1.01 - 1.74)	0.04
	Awake		1.08 (0.77 - 1.52)	0.65	1.16 (0.80 - 1.69)	0.42
Death or MACE	Bt-to-bt	46	1.27 (1.00 - 1.61)	0.05	1.23 (0.96 - 1.56)	0.1
	Day-to-day		1.42 (1.14 - 1.76)	0.002	1.31 (1.03 - 1.66)	0.02
	Awake		1.01 (0.76 - 1.35)	0.94	1.03 (0.75 - 1.42)	0.84

	Measure	Ev	Unadjusted		Adjusted for Age/Gender	
			HR (95%CI)	p-val	HR (95%CI)	p-val
Ischaemic Stroke	Bt-to-bt	31	1.27 (0.98 - 1.66)	0.07	1.24 (0.95 - 1.63)	0.11
	Day-to-day	31	0.97 (0.64 - 1.48)	0.90	1.00 (0.64 - 1.54)	0.98
	Awake	31	1.39 (0.99 - 1.95)	0.06	1.49 (1.03 - 2.16)	0.03
Any Stroke	Bt-to-bt	34	1.22 (0.96 - 1.55)	0.10	1.20 (0.95 - 1.52)	0.13
	Day-to-day	33	1.06 (0.77 - 1.46)	0.74	1.06 (0.76 - 1.48)	0.74
	Awake	33	1.14 (0.83 - 1.57)	0.42	1.16 (0.83 - 1.63)	0.39
All cause mortality	Bt-to-bt	24	1.20 (0.90 - 1.60)	0.21	1.09 (0.81 - 1.46)	0.58
	Day-to-day	28	1.42 (1.07 - 1.88)	0.02	1.28 (0.91 - 1.81)	0.16
	Awake	26	1.32 (0.94 - 1.85)	0.11	1.24 (0.88 - 1.76)	0.22
CV Death or MACE	Bt-to-bt	37	1.21 (0.96 - 1.52)	0.10	1.16 (0.92 - 1.46)	0.22
	Day-to-day	38	1.21 (0.92 - 1.58)	0.17	1.18 (0.89 - 1.57)	0.25
	Awake	38	1.27 (0.96 - 1.68)	0.10	1.36 (1.01 - 1.84)	0.04
Death or MACE	Bt-to-bt	53	1.16 (0.94 - 1.43)	0.17	1.09 (0.88 - 1.34)	0.44
	Day-to-day	55	1.23 (0.99 - 1.54)	0.07	1.16 (0.91 - 1.48)	0.22
	Awake	54	1.26 (0.99 - 1.59)	0.06	1.31 (1.02 - 1.67)	0.04

Table 6.3 Hazard Ratio for cardiovascular events per SD of diastolic rCV.

	Measure	Ev	Unadjusted		Adjusted for Age/Gender	
			HR (95%CI)	p-val	HR (95%CI)	p-val
Ischaemic Stroke	Bt-to-bt	23	1.55 (1.15 - 2.09)	0.004	1.56 (1.15 - 2.12)	0.005
	Day-to-day	22	1.61 (1.20 - 2.16)	0.002	1.58 (1.17 - 2.13)	0.003
	Awake	23	1.65 (1.19 - 2.30)	0.003	1.68 (1.21 - 2.35)	0.002
Any Stroke	Bt-to-bt	34	1.44 (1.08 - 1.92)	0.01	1.44 (1.07 - 1.93)	0.02
	Day-to-day	33	1.56 (1.19 - 2.06)	0.002	1.56 (1.17 - 2.07)	0.002
	Awake	33	1.60 (1.17 - 2.17)	0.003	1.59 (1.16 - 2.19)	0.004
All cause mortality	Bt-to-bt	24	1.47 (1.10 - 1.98)	0.01	1.43 (1.04 - 1.96)	0.03
	Day-to-day	28	1.40 (1.03 - 1.92)	0.03	1.64 (1.16 - 2.32)	0.005
	Awake	26	1.52 (1.08 - 2.15)	0.02	1.42 (0.95 - 2.12)	0.09
CV Death or MACE	Bt-to-bt	37	1.61 (1.24 - 2.08)	<0.001	1.59 (1.21 - 2.08)	<0.001
	Day-to-day	38	1.71 (1.33 - 2.19)	<0.001	1.73 (1.33 - 2.25)	<0.001
	Awake	38	1.67 (1.25 - 2.22)	<0.001	1.60 (1.19 - 2.16)	0.002
Death or MACE	Bt-to-bt	53	1.62 (1.31 – 2.00) 1.59 (1.28 - 1.97) 1.69 (1.33 - 2.15)	<0.001	1.56 (1.24 - 1.95)	<0.001
	Day-to-day	55		<0.001	1.66 (1.32 - 2.08)	<0.001
	Awake	54		<0.001	1.58 (1.23 - 2.04)	<0.001

Table 6.4. Hazard Ratio for cardiovascular events per SD of mean SBP

6.4.3 Risk of recurrent events per quartile of BPV

There was a significant increase in the absolute risk of recurrent stroke or all cardiovascular events across quartiles of BPV (figure 6.1). Furthermore, beat-to-beat and day-to-day BPV were both moderately reproducible in 50 and 100 patients respectively (ICC: HBPM 0.614 p<0.001, beat-to-beat 0.503 p<0.001) resulting in a similar increase in the association between 'usual' BPV on beat-to-beat and home monitoring following correction for regression dilution (figure 6.2).

Figure 6.1. Absolute risks of recurrent stroke or major cardiovascular events by quartile of BPV on each form of monitoring.

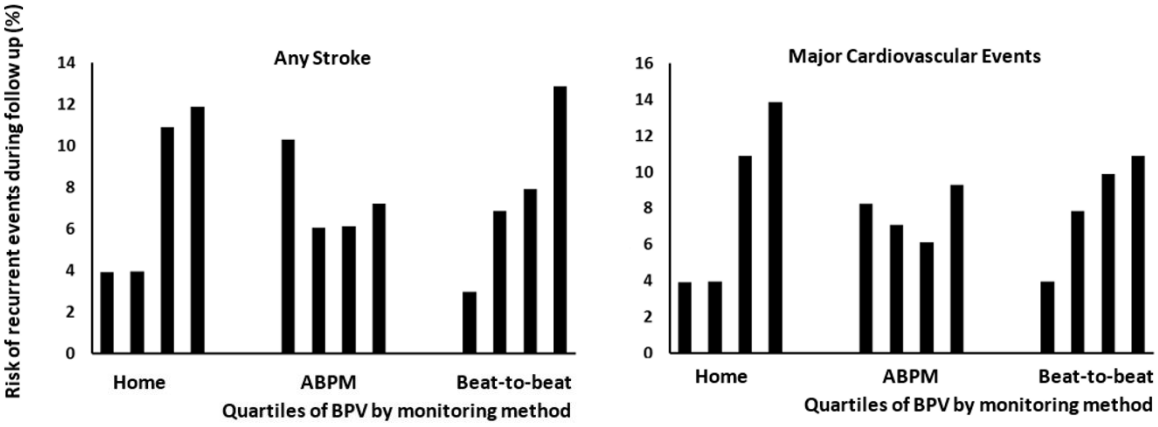
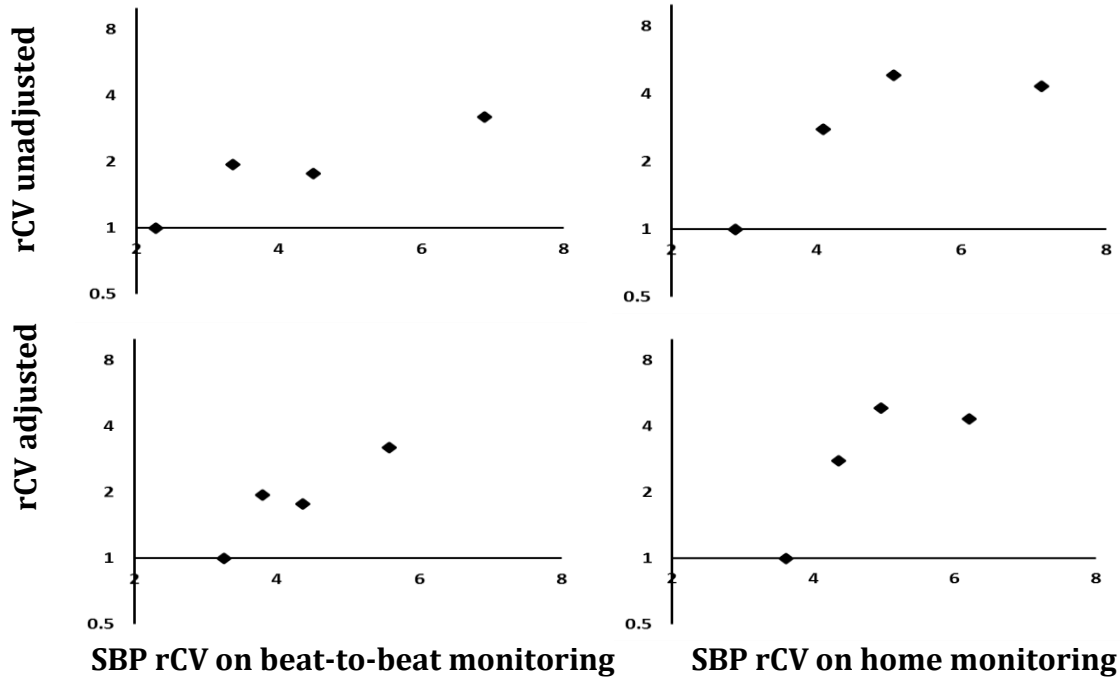


Figure 6.2. Absolute risks of recurrent stroke or major cardiovascular events by quartile of BPV on each form of monitoring, with and without adjustment for regression dilution bias



6.4.4 Independence of predictive value of beat to beat and home BPV

In models including both beat-to-beat and HBPM BPV, beat-to-beat BPV was more predictive of the risk of recurrent stroke, whereas BPV on HBPM was more predictive of the risk of all cardiovascular events (table 6.5).

Table 6.5. Differences in mean SBPV on beat-to-beat and home day-to-day monitoring for patients experiencing recurrent events. Results are presented as mean (standard deviation) for patients affected or unaffected by each outcome event. P-values (p-val) are presented for t-tests. MACE = Major adverse cardiovascular events.

	Beat-to-beat BPV			Home BPV		
Event	**Unaffected**	**Affected**	**p-val**	**Unaffected**	**Affected**	**p-val**
Ischaemic Stroke	**4.68 (2.4)**	**6.24 (3.6)**	**0.001**	**4.75 (1.8)**	**5.19 (1.7)**	**0.21**
Any Stroke	**4.66 (2.4)**	**6.16 (3.6)**	**0.001**	**4.74 (1.8)**	**5.34 (1.6)**	**0.06**
All cause mortality	**4.70 (2.5)**	**6.14 (3.3)**	**0.006**	**4.73 (1.8)**	**5.45 (1.5)**	**0.03**
CV Death or MACE	**4.66 (2.4)**	**5.97 (3.5)**	**0.002**	**4.71 (1.7)**	**5.46 (1.9)**	**0.01**
Death or MACE	**4.60 (2.4)**	**5.98 (3.5)**	**0.03**	**4.68 (1.8)**	**5.44 (1.7)**	**0.02**

6.4.5 Systematic review of predictive value of BPV

219 abstracts of 960 search responses were potentially relevant, with 34 papers reviewed in full. No study reported the risk of recurrent cardiovascular events per change in beat-to-beat BPV. As in a previous meta-analysis,[14] the risk of a poor outcome after acute stroke was associated with both SBPV (HR 1.07, 0.9-1.2) and DBPV (HR 1.33, 1.1-1.7),[8],[15] whilst a reduced baroreceptor sensitivity was associated with poor outcome after stroke[9] or myocardial infarction.[16, 17]

6.5 Discussion

BPV predicted the risk of recurrent stroke and all cardiovascular events on 5 minutes of beat-to-beat BP monitoring, with an approximately 4-fold increase in risk between the lowest and highest quartile of the population, with broadly similar predictive power to BPV on day-to-day monitoring.

Residual visit-to-visit variability in blood pressure (BP) on antihypertensive treatment has a poor prognosis despite good control of mean BP, with an increased risk of stroke and all cardiovascular events,[1, 2] and benefits of some antihypertensive drugs in the prevention of stroke appear to be due partly to reduced variability in SBP.[3, 4] However, more rapid assessment and control of BPV would be clinically useful, especially in the acute phase after TIA or stroke. BPV on home BP monitoring is also predictive of recurrent strokes and all cardiovascular events[5, 6] and can be assessed over days, but still poses practical challenges in retrieving and analysing equipment and readings. Our study shows that a rapid, 5 minute assessment of beat-to-beat BPV may have similar prognostic significance compared to HBPM. If affected by antihypertensive medication in the same way as visit-to-visit and HBPM BPV, beat-to-beat BPV could be a useful index to guide antihypertensive treatment decisions. However, we found only a weak correlation between BPV on different methods of measurement, yet they were independently related to outcomes. This is consistent with the weak relationship between within-visit and between-visit BPV in previous analyses of the ASCOT trial.[2] Therefore, BPV on beat-to-beat and home monitoring may well be complementary measures, potentially reflecting different pathophysiologiocal mechanisms leading to stroke.

Previous studies demonstrated that home and beat-to-beat BPV are associated with a similar underlying physiological phenotype,[7] including increased arterial stiffness, aortic pulsatility, reduced baroreceptor gain and increased cardiovascular reactivity to stress. Beat-to-beat BPV is also associated with increased age, BMI and arterial stiffness (chapters 3 and 4), consistent with increased vascular aging. Furthermore, patients

with an acute stroke have increased beat-to-beat BP variability and reduced baroreceptor gain,[18] which is associated with increased mortality,[9] and is partly dependent upon stroke location.[19] However, the precise mechanism by which BPV is associated with an increased risk of recurrent stroke is unclear. This may reflect either the effects of associated physiological indices (arterial stiffness, pulsatility, cerebrovascular reactivity) or direct effects of beat-to-beat BPV itself. However, beat-to-beat BPV is a composite measure of multiple physiological processes including irregular, episodic components and rhythmic components related to breathing and to underlying autonomic rhythms (ie low frequency oscillations at 0.04-0.15Hz),[20] and its prognostic significance may also reflect multiple pathophysiological processes.

Our study has some limitations. Firstly, some patients were excluded due to poor quality recordings, either due to poor peripheral circulation, excess ectopy or AF during the recording. However, this reflects the strength of study which included an consecutively recruited, unselected elderly population with acute events. Second, although statistical power to compare different measures of BPV was limited by the relatively small number of recurrent vascular events, the study is nevertheless the largest study of the prognostic significance of beat-to-beat SBP in stroke patients. Thirdly, BPV was estimated after initiation of antihypertensive treatment, which may affect BPV and its association with recurrent events. However, this also largely removes the confounding effects of inadequate mean blood pressure control. Finally, repeated assessments for calculation of reproducibility of measures were performed after initiation of treatment. However, this would be expected to cause an underestimate of reproducibility.

Beat-to-beat BPV is therefore an appealing measure to increase our understanding of both physiology and cerebrovascular risk prediction but its potential use in clinical practice is limited by the need for continuous BP monitoring, specialist analysis, a need for validation in other cohorts and normative values and thresholds for pathologically-relevant BPV, requiring further research. Furthermore, its utility will

ultimately depend upon its capacity to alter management through improved risk predictive or the identification and monitoring of a novel treatment target.

Beat-to-beat BPV was a novel predictor of the risk of recurrent stroke and may be complementary to BPV on day-to-day home BP monitoring, may aid in risk stratification and may help identify independently treatable mechanisms to reduce the risk of stroke.

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CHAPTER SEVEN

Progression of arterial stiffness is associated with mid-life diastolic blood pressure and transition to late life hypertensive phenotypes

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7.1 Summary

Hypertension-associated cardiovascular events are particularly associated with elevated systolic blood pressure (SBP) in late-life reflecting increasing arterial stiffness, which is associated with beat-to-beat blood pressure variability, cerebral pulsatility and small vessel disease. However, long-term interactions between SBP, diastolic BP (DBP) and arterial stiffness in development of late-life hypertensive phenotypes remain unclear.

In UK Biobank, I determined associations between arterial stiffness index (ASI), SBP, DBP and their progression; and transition from normotension (<140/90mmHg) to hypertension or elevated ASI (>10m/s). Associations were determined by general linear and logistic regression, adjusted for cardiovascular risk factors and variability of measurements across follow-ups. Mean values of baseline SBP, DBP and ASI were determined stratified by deciles of age, BP and ASI, with confidence intervals determined by bootstrapping. In 169,742 participants at baseline, ASI was more strongly associated with DBP than SBP, before and after adjustment for risk factors (β: SBP=-0.01, p<0.001; DBP=0.06, p<0.001), whilst DBP was more strongly associated with progression of ASI (n=13,761, SBP β=0.013 p=0.01; DBP β=0.038 p<0.001). Baseline ASI was associated with increasing SBP during follow-up (β=0.02, p<0.001) but not DBP (β=0.0004, p=0.39), but was associated with a younger age of transition from rising to falling DBP (highest vs lowest quartile: 51.2, 49.9–52.3 vs 60.4, 59.6–61.3, p<0.001). ASI predicted the development of isolated systolic hypertension (ISH OR: 1.30, 1.22 - 1.39), particularly after adjustment for measurement variability (OR 2.29).

Mid-life DBP was the strongest predictor of progression of arterial stiffness, whilst arterial stiffness was associated with earlier transition to a falling DBP. Prevention of long-term harms associated with arterial stiffness may require more intensive control of mid-life DBP.

7.2 Introduction

Systolic blood pressure is the principal focus of modern control of hypertension due to the strong short-term and mid-term associations with acute vascular events,[1] the proven efficacy of hypertensive treatment for isolated systolic hypertension [2-4] and due to the greater population attributable burden of SBP-associated cardiovascular events, reflecting the high incidence of both cardiovascular events and isolated systolic hypertension in the elderly.[5] Specifically, SBP tends to increase and DBP decrease in late-life. [6] Therefore short term associations with SBP in late-life may not reflect the long-term attributable risk of cardiovascular events due to mid-life diastolic hypertension, particularly if the risk associated with mid-life DBP is mediated by late-life SBP.

Arterial stiffness is associated with recurrent cardiovascular events and all-cause mortality, independent of age, blood pressure and cardiovascular risk factors.[7, 8] It is strongly associated with age, a history of hypertension,[9] beat-to-beat blood pressure variability (chapter 4), cerebral arterial pulsatility and small vessel disease.[10] is often described as a measure of 'vascular age' and features in clinical guidelines as a marker of hypertensive end-organ damage.[11, 12] Studies that have assessed the longitudinal relationships between age, arterial stiffness and blood pressure have largely reported an association between increased systolic blood pressure and progression of arterial stiffness,[9, 13, 14] although some have reported no evidence of a direct relationship,[6] or sex-specific differences in longitudinal progression of arterial stiffness.[15] However, studies have not reported the bidirectional interactions between increased SBP or DBP and progression of arterial stiffness and the modification of longitudinal changes in BP by increasing arterial stiffness in mid-life.

In addition to being a marker of hypertensive end-organ damage,[6] arterial stiffness may directly induce end-organ injury through reduced damping of the systolic pressure wave, increased pulse wave reflection from the periphery, and impaired

Windkessel function of the aorta,[16] resulting in increased systolic pressures, increased pulsatility of aortic blood pressure and greater transmission of pulsatile flow to the distal circulation.[17] The low-resistance arterial beds in the kidney and brain are particularly susceptible to the increased pulsatility of aortic blood flow, resulting in a strong association between aortic stiffness and cerebral arterial pulsatility with acute lacunar stroke,[18] white matter injury[10] and the associated sequelae of decline in mobility, late-onset refractory depression and dementia.[19] To develop strategies to prevent end-organ injury, it is critical to understand the magnitude and direction of the relationship between BP and arterial stiffness, and the optimal period for intervention to prevent increased arterial stiffness.

The UK Biobank cohort is the largest, mid-life community-based cohort with measures of blood pressure and arterial stiffness in nearly 170,000 participants.[20] Furthermore, Biobank includes repeated measures in a large subset of the population, allowing estimation of the consistency of arterial stiffness and blood pressure measures and the effect of variability in these measures on epidemiological associations over prolonged periods, providing a unique opportunity to understand the longitudinal interactions between arterial stiffness, SBP and DBP. This allows estimation and adjustment of analyses for variability in measurements, determination of cross-sectional associations at baseline using age as a surrogate for longitudinal changes in a very large population, and validation of these associations by determining direct associations between baseline measures and within-individual progression over time in a subgroup of the population. This also allows estimation of clinically relevant blood pressure measures, including diagnosis of hypertension and median age for transition to late-life phenotypes.

This study therefore aims to determine the relationship between mid-life systolic or diastolic blood pressure and progression of arterial stiffness, and the converse relationship between arterial stiffness and longitudinal changes in blood pressure.

7.3 Methods

7.3.1 Study Population

UK Biobank recruited >500,000 community-based participants aged 40 to 69 years old in 22 centres between 2006 and 2010.[20] It includes baseline assessments of health status, lifestyle and environmental factors, cognitive status and physical measures, including blood pressure and arterial stiffness, with repeated assessments in 20,000 – 35,000 participants. Medical history of any diagnoses made by a doctor (diabetes, previous cardiovascular events or other medical conditions) were self-reported by participants at baseline and each follow-up. All data used in these analyses are available from UK Biobank upon direct application by valid researchers and further materials can be obtained upon application to the corresponding author. UK Biobank was approved by the UK North West Multi-centre Research *Ethics* Committee and all participants provided informed written consent.

Arterial stiffness index (ASI) in the UK Biobank study was measured using the PulseTrace PCA2 (CareFusion,San Diego, CA) (Field-ID 21021)[21] at baseline between 2009 and 2010, with repeated measures at a single centre between 2012 and 2013 and at 3 centres between 2014-2019. The PulseTrace PCA2 uses finger photoplethysmography to obtain a pulse waveform during a 10 to 15 second measurement. The measurement was repeated on a larger finger or on the thumb if less than two thirds of the waveform was visible or if the waveform did not stabilise within 1 minute. ASI is derived from the interval between the forward and presumed aortic-reflected reverse travelling pulse wave, standardised to the standing height. Systolic blood pressure and diastolic blood pressure were measured twice at baseline and each follow-up by a trained nurse after the participant had been at rest for at least 5 minutes in the seated position with a digital sphygmomanometer (Omron 705 IT; OMRON Healthcare Europe B.V., Hoofddorp, Netherlands) with a suitably sized cuff; the average of the two systolic or diastolic blood pressure measures were used in all

analyses. Pulse pressure was calculated as the systolic minus diastolic pressure and mean blood pressure (MBP) as DBP plus 1/3 of the pulse pressure.

Socioeconomic, health, lifestyle and environmental factors were recorded at baseline through a self-administered touchscreen questionnaire, supported by face-to-face interviews. All data were downloaded from UK Biobank, imported into R for analysis. Values exceeding physiologically plausible levels, or 4 standard deviations from the mean, were excluded as outliers.

7.3.2 Analysis

I firstly determined cross-sectional associations between BP and arterial stiffness in the larger population available at baseline, and in the larger follow-up population at the second follow-up. Secondly, I described the evolution of these cross-sectional associations with increasing age through stratification of the population. To directly assess longitudinal relationships in the smaller group of participants with ASI measured at baseline and at least one follow-up visit, I determined associations between baseline measures and progression of each index during follow-up, as percentage change of the dependent variable per annum. Finally, I determined the predictive value of baseline indices for the risk of progression to clinically-defined hypertension or excessive arterial stiffness.

To assess factors affecting reliability of analyses, the consistency of measurements across follow-visits was first estimated by intraclass correlation coefficients , by linear correlation and by Cronbach's alpha. Mean differences were estimated and compared by paired t-tests to assess for progression in indices over time. Participants were further stratified by the presence of a 'notch' in the ASI waveform between the systolic and diastolic phase of the cardiac cycle. Mean differences between assessment centres at baseline and follow-up were calculated, and compared by one-way ANOVA. To minimise between-centre variation, analyses were repeated for each index following standardisation of values at each centre to the

distribution of values across all centres at that visit: standardising each value by the number of standard deviations from the mean of its centre to the mean and number of standard deviations of the whole population.

To determine the rate of progression of each index, participants were pooled across the two follow-up visits as different participants returned at each follow-up, with limited numbers assessed at both follow-ups. Progression of each index of interest (ASI, SBP, DBP and PP) was standardised as the percentage change per annum. For the limited participants attending both follow-ups, the longer follow-up was used to calculate the rate of progression.

Relationships between baseline and follow-up indices at each visit, and between baseline and change between visits, were determined by general linear models, unadjusted and adjusted for age and sex; age, sex and the baseline index and for age, sex, baseline index, and cardiovascular risk factors (cholesterol, current or ever smoking status, diabetes, blood pressure). Models were repeated stratified by age above or below 60. Non-linear interactions were assessed by addition of a squared-term for age to univariate associations (between-model comparison by ANOVA) and through stratification of results by quartiles of a second index. To determine the maximum point of non-linear associations between BP and age, the maximum inflection point was identified by solving the third-order fitted polynomial across deciles, with confidence intervals determined by bootstrapping. The impact of regression dilution was assessed by non-parametric estimation of the regression dilution ratio, through division of the population into 5 evenly-spaced bins of the index of interest ((mean in highest group at follow-up – mean of lowest group at follow-up)/((mean in highest group at baseline – mean in lowest group at baseline)).[1] Secondly, parametric adjustment was performed by adjustment of the regression coefficient (or its logarithm in logistic regression) by the reliability ratio. The effect upon the proportion of variance explained by an association was estimated by dis-attenuation of the regression

coefficients (r_{xy} / (rr_x*$rr_{y)}$ where rr is the reliability ratio). Finally, multivariate adjustment for measurement variation was estimated via regression calibration.[22]

To assess the risk of progression to clinically-defined forms of hypertension or excessive arterial stiffness, normotension was defined by baseline BP (<140/90), a reported diagnosis of hypertension or use of antihypertensives, with subdivision of the population into mixed hypertension or isolated systolic hypertension. Elevated ASI was defined as >10m/s, consistent with European guidelines for carotid femoral pulse wave velocity.[11] The effect of baseline measures on the risk of transition between hypertensive states or to elevated arterial stiffness at either follow up was determined by logistic regression, with and without adjustment for cardiovascular risk factors or the effect of regression dilution.

Analyses were performed in R and SAS.

7.4 Results

7.4.1 Study Population

The 169,742 / 502,536 UK Biobank participants with arterial stiffness (ASI) measured at baseline at 10/22 Biobank centres were largely similar to the remaining population (table 7.1), with clinically insignificant differences for most measures, albeit statistically significant differences given the large population size. 20199 participants who returned at the first follow-up visit were similar to the baseline population, whilst 31,418 participants who returned for the second follow-up visit after a median of 8.5 years were younger when recruited with lower SBP and BMI, fewer smokers and fewer having hypertension or diabetes. Only 1,731 participants with ASI at baseline attended both follow-ups and had ASI measured, whilst 13,210 participants had ASI and BP measured at baseline and at least one follow-up.

Table 7.1. Baseline characteristics of patients assessed at baseline and returning for the second follow-up visit. Results are shown only for values at the baseline visit for all participants, participants with ASI measured at baseline and for participants with ASI measured at follow-up. Values are reported as mean (SD) or number (percentage). Differences between groups are compared by t-tests for continuous measures and chi-squared tests for discrete values. ASI = arterial stiffness index; MI = myocardial infarction; p-val = p value; SBP = systolic blood pressure; DBP = diastolic blood pressure; HTN = hypertension; LpA = Lipoprotein a; BMI = body mass index; HDL = high density lipoproteins; LDL = low-density lipoproteins.

Characteristic	All Participants (n=502536)	ASI at baseline (n= 169742)	p-val	Had ASI at 2nd FU (n=31418)	P-val vs Base
Baseline Age (years)	56.5 (8.1)	56.8 (8.2)	<0.0001	55 (7.5)	<0.0001
Male (n/%)	229134 (45.6)	77730 (45.8)	0.045	15495 (49.3)	<0.0001
Diabetes (n/%)	26402 (5.3)	9741 (5.7)	<0.0001	870 (2.8)	<0.0001
Smoking (n/%)					
- Current	52979 (10.6)	17081 (10.1)	<0.0001	2029 (6.5)	<0.0001
- Ever	226049 (45.1)	75428 (44.4)	<0.0001	12380 (39.4)	<0.0001
Hypertension (n/%)	135762 (27.1)	46113 (27.2)	0.0564	6433 (20.5)	<0.0001
Previous Events					
- Stroke (n/%)	7668 (1.5)	2491 (1.5)	0.0183	247 (0.8)	<0.0001
- MI (n/%)	11608 (2.3)	3783 (2.2)	0.0074	375 (1.2)	<0.0001
Creatinine (µmol/L)	72.3 (18.5)	72.6 (18.4)	<0.0001	72.5 (14.1)	0.6492
Cholesterol (mmol/L)	5.7 (1.1)	5.7 (1.1)	0.0052	5.7 (1.1)	<0.0001
HDL (mmol/L)	1.4 (0.4)	1.5 (0.4)	<0.0001	1.5 (0.4)	0.9559
LDL (mmol/L)	3.6 (0.9)	3.5 (0.9)	<0.0001	3.6 (0.8)	<0.0001
LpA (nmol/L)	44.6 (49.2)	44.9 (49.1)	0.0287	44.1 (49)	0.0158
Weight (Kg)	78.1 (15.9)	78.2 (16)	<0.0001	77.4 (15.1)	<0.0001
BMI (Kg/m^2)	27.4 (4.8)	27.5 (4.8)	0.0066	26.7 (4.3)	<0.0001
SBP (mmHg)	137.8 (18.7)	137.8 (18.6)	0.8892	135.4 (17.6)	<0.0001
DBP (mmHg)	82.2 (10.2)	82.1 (10.1)	<0.0001	81.5 (9.9)	<0.0001
Pulse Pressure (mmHg)	55.6 (13.6)	55.7 (13.6)	0.0007	53.8 (12.4)	<0.0001
ASI (m/s)	9.3 (3.1)	9.3 (3.1)	-	9.2 (3)	0.0005
HbA1C (mmol/mol)	36.1 (6.7)	36.3 (6.9)	<0.0001	35.1 (5.2)	<0.0001
HTN treatment (n/%)	104006 (26)	36235 (27)	<0.0001	4415 (16.4)	<0.0001

7.4.2 Variability of blood pressure and arterial stiffness measures over time

To assess the effect of visit-to-visit variability on subsequent models, variability of measurement of ASI was significant across follow-ups (alpha <0.4), with greater consistency (>0.6) for SBP or DBP between follow-ups (table 7.2). Variability was lower for measures between baseline and the first follow-up than the second follow-up. Variability of ASI improved slightly when only including participants with a 'notch' in the arterial waveform at both baseline and follow-up, despite a relatively low agreement between visits for the presence of a notch (Cohen's kappa: 0.25).

Table 7.2. Variability of blood pressure and arterial stiffness for baseline results vs the first or second follow-up visit. The intra-class correlation coefficient (ICC), its confidence interval (CI) and Cronbach's alpha are reported , with the mean increase from baseline to follow-up and the R^2 for a univariate linear regression. All analyses are significant at the p<0.001 level except for analyses with a *.

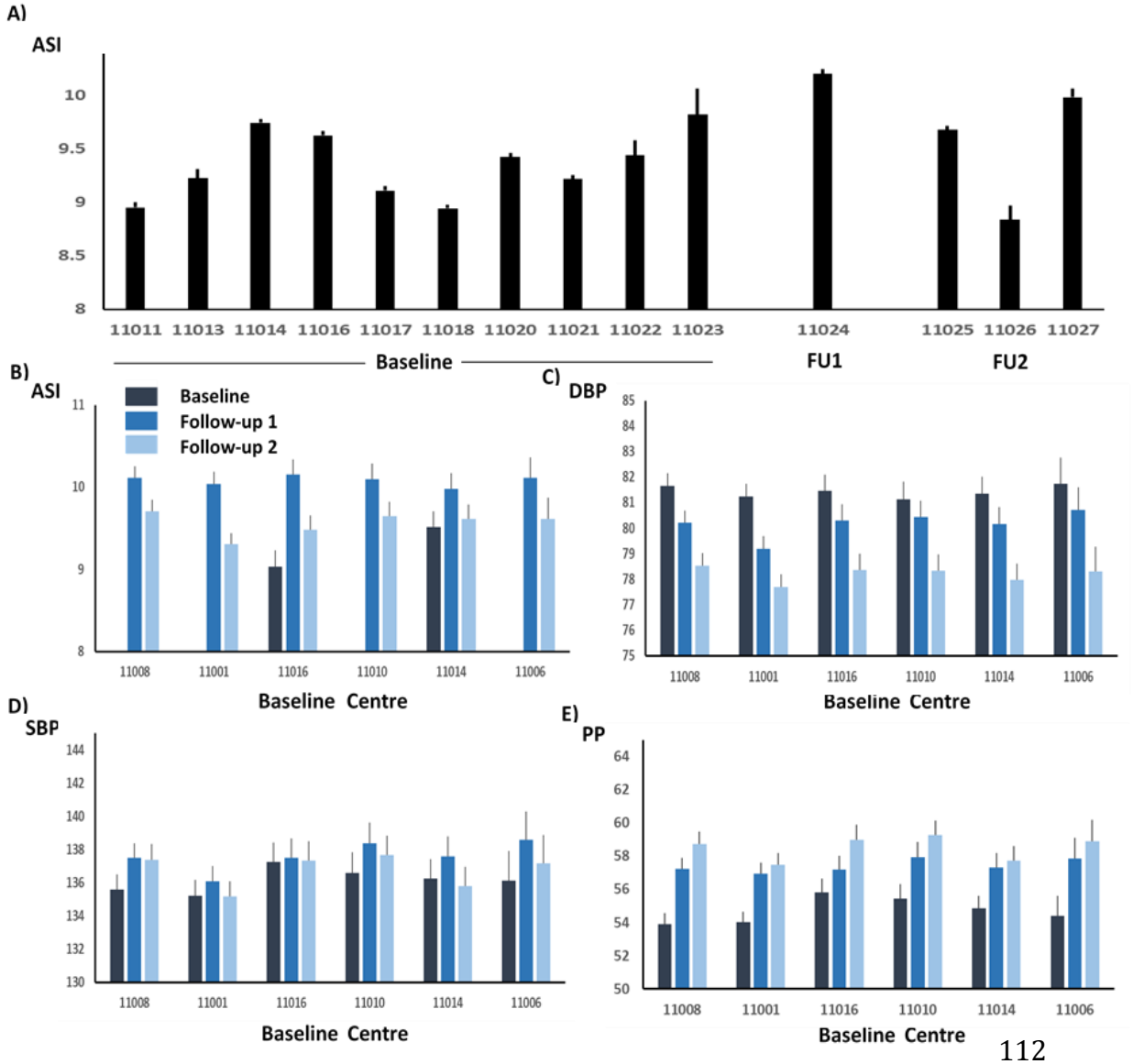
Characteristic	Baseline to Second Follow-up (10823)				
	ICC	CI	Alpha	Mean Increase	R^2
Absolute Values					
SBP	**0.58**	**0.57 - 0.58**	**0.74**	**2.55**	**0.34**
DBP	**0.51**	**0.50 - 0.52**	**0.70**	**-2.89**	**0.30**
Pulse Pressure	**0.52**	**0.51 - 0.53**	**0.74**	**5.41**	**0.35**
Arterial Stiffness	**0.18**	**0.16 - 0.20**	**0.33**	**0.50**	**0.04**
- No notch	**0.12**	**0.03 - 0.20**	**0.23**	**-0.92**	**0.02**
- Notched	**0.26**	**0.24 - 0.28**	**0.42**	**0.53**	**0.07**
- By Centre	**0.18**	**0.17 - 0.20**	**0.33**	**0.31**	**0.04**

7.4.3 Variability in BP and ASI measurement between centres

In addition to the random variability in measures, there were systematic differences between visits, with a mean increase in SBP, PP and ASI and a fall in DBP between visits (table 7.1). Furthermore, there were systematic differences between centres for concurrently measured ASI and SBP, which was not evident for

DBP and PP. Specifically, upon stratifying participants attending the first follow-up visit (carried out at one centre) by the centre they attended at baseline, there was greater variation between baseline centres for baseline SBP and ASI measures than for measures taken at the first follow-up (figure 7.1). There was also a systematically greater mean SBP and ASI at follow-up 1 than either baseline or follow-up 2 (figure 7.1), including for the subset of participants attending all three visits. However, standardising values to a global distribution only marginally increased the consistency of measures (table 7.2).

Figure 7.1. Recorded BP and arterial stiffness measures at baseline and follow up, stratified by baseline Biobank assessment centre. Variation in mean indices at each centre is shown for mean ASI (A+B), DBP (C), SBP (D) and pulse pressure (E) at baseline, first follow-up and second follow-up, stratified by the baseline centre attended. Significant variation between centres is seen for ASI and SBP, with greater between-centre variation for measures taken at baseline than measures taken at follow-up for both SBP and ASI.



7.4.4 Cross-sectional associations between arterial stiffness and blood pressure

At baseline, ASI and BP related measures were strongly associated with age, with the weakest linear association between age and DBP, and were greater in men, ever-smokers, and with increased creatinine, weight or BMI (table 7.3). However, despite an approximately linear relationship with age across all patients for baseline SBP, PP and ASI, age was non-linearly associated with DBP, with increasing DBP in lower deciles of age and falling DBP in later deciles (figure 7.2), with addition of a squared term for age to the univariate association between age and DBP significantly increasing the amount of explained variance (univariate r^2=0.0015 to r^2=0.007, p<0.001, ANOVA). The maximum mean DBP occurred at 55 years old in men and 58.3 years in women.

Figure 7.2. Interactions between age, blood pressure and arterial stiffness (ASI) at baseline. Values of mean systolic (SBP) and diastolic (DBP) blood pressure, pulse pressure (PP) and ASI are shown by deciles of age (panels A-D) or ASI (panels E-F), stratified by quartiles of ASI (A+B), SBP (C), DBP (D) or age (E+F)

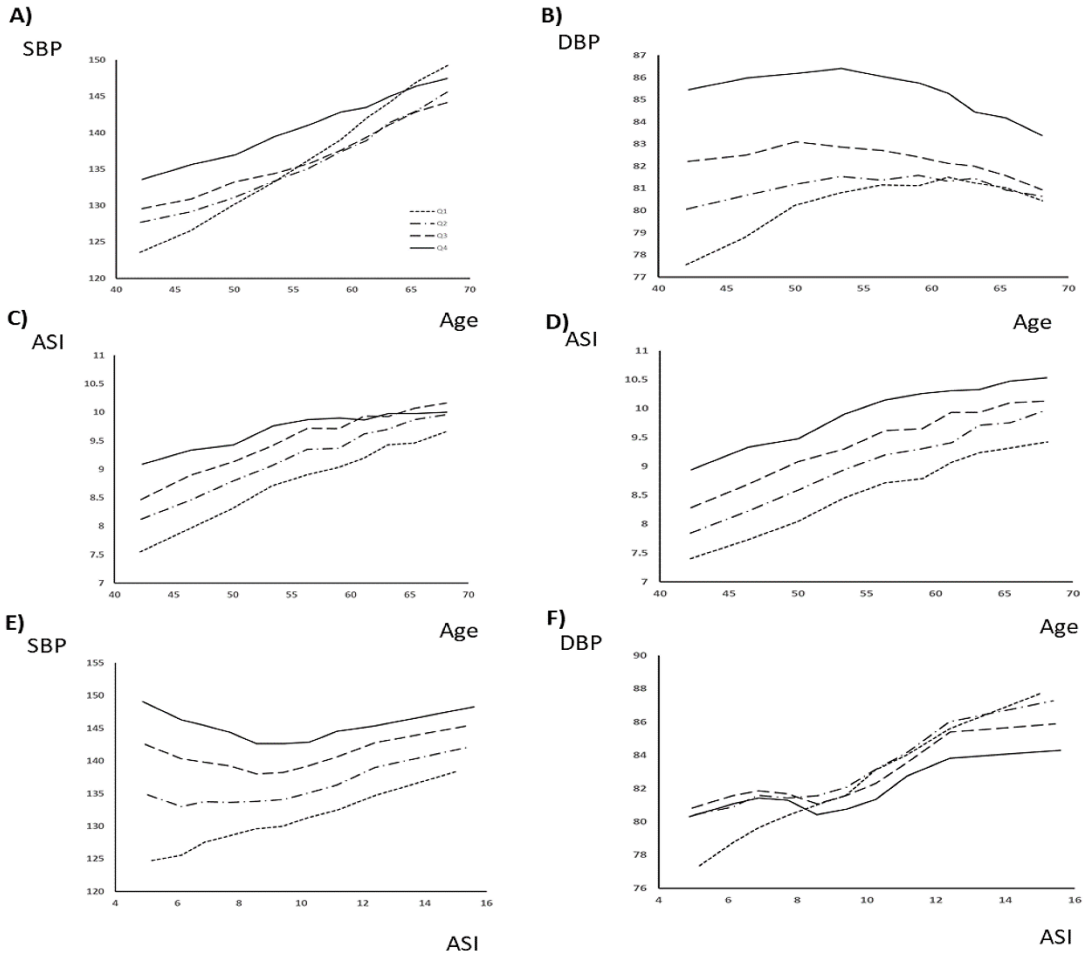


Table 7.3. Associations between blood pressure and arterial stiffness at baseline with demographic features. Beta-coefficients and r^2 are shown for univariate general linear models. In models adjusted for age / sex or for age / sex / cardiovascular risk factors (diabetes, current smoking and cholesterol). The beta-coefficient for each independent variable (by row) with the dependent variable (by column) is given, with the R^2 squared change compared to the models including only age and sex; or age, sex and cardiovascular risk factors. Associations are reported for the population in whom arterial stiffness index (ASI) was recorded at baseline. SBP=systolic blood pressure; DBP = diastolic blood pressure, PP = pulse pressure. All associations are significant, except where indicated *p>0.05. n=169,742. ASI = arterial stiffness index; SBP = systolic blood pressure; DBP = diastolic blood pressure; PP = pulse pressure; MBP = mean blood pressure; HTN = hypertension; BMI = body mass index; Dependent variables are shown in each column, with independent variables listed by row.

	SBP		**DBP**		**PP**		**ASI**	
	β	**ΔR^2**	**β**	**ΔR^2**	**β**	**ΔR^2**	**β**	**ΔR^2**
Univariate								
Age	0.75	0.106	0.05	0.002	0.7	0.174	0.08	0.043
Female Sex	3.96	0.022	2.38	0.027	1.59	0.007	0.85	0.038
Diabetes	0.94	0.002*	-2.34	0.001	3.22	0.005	-0.05	0.002*
HTN	12.91	0.094	5.52	0.058	7.33	0.058	0.7	0.01
Smoking								
Base	-2.75	0.002	-0.65	0	-2.09	0.002	0.7	0.005
Ever Smoked	1	0.001	0.07	0*	0.93	0.001	0.61	0.01
Creatinine	0.06	0.004	0.03	0.004	0.03	0.002	0.02	0.009
Cholesterol	1.72	0.011	1.16	0.017	0.55	0.002	0	0*
BMI	0.73	0.035	0.59	0.077	0.14	0.003	0.07	0.013
Weight	0.2	0.03	0.18	0.081	0.02	0.001	0.03	0.03
SBP			0.38	0.488	0.62	0.715	0.03	0.024
DBP	1.28	0.488			0.28	0.045	0.06	0.034
MBP	1.42	0.837	0.79	0.863	0.46	0.149	0.05	0.034
PP	1.16	0.715	0.16	0.045			0.02	0.006
ASI	0.94	0.024	0.6	0.034	0.34	0.006		
+ Age / Sex								
SBP			0.41	0.502	0.59	0.56	0.01	0.004
DBP	1.25	0.452			0.25	0.035	0.05	0.022
MBP	1.38	0.734	0.81	0.855	0.41	0.108	0.03	0.014
PP	1.17	0.596	0.17	0.041			-0.01	0.001
ASI	0.4	0.007	0.52	0.02	-0.11	0.005		
+ CV RFs								
SBP			0.4	0.454	0.60	0.544	0.01	0.004
DBP	1.21	0.402			0.21	0.026	0.05	0.021
MBP	1.38	0.678	0.81	0.795	0.40	0.093	0.03	0.013
PP	1.13	0.561	0.13	0.056			-0.01	0.003
ASI	0.32	0.026	0.48	0.049	-0.15	0.01		

At baseline, ASI was positively correlated with all BP indices, with a stronger association with DBP than SBP, before and after adjustment for cardiovascular risk factors (figure 7.2, table 7.3). There was a stronger association with DBP in a model including age, sex, cardiovascular risk factors and both BP indices (β: SBP=-0.01, p<0.001; DBP=0.06, p<0.001). MBP and DBP were highly correlated (table 7.3), and associations between ASI and DBP or MBP were similar, but slightly weaker for MBP. There were modest but clinically significant increases in the magnitude of associations following adjustment for regression dilution by either non-parametric or parametric methods (table 7.4), with the greatest increases in the magnitude of relationships between ASI and measures of blood pressure (*β* unadjusted 0.94; adjusted 3.09) reflecting the greater variability of ASI.

7.4.5 Adjusted associations between arterial stiffness and blood pressure

The positive association between ASI with baseline SBP or PP significantly diminished after adjustment by age, sex and cardiovascular risk factors, but remained significant for DBP, particularly in the highest deciles of arterial stiffness (figure 7.2), with no effect of adjusting for heart rate (SBP β=0.023, p=0.2; DBP β=0.06, p<0.001). Associations were stronger for participants with notched ASI waveforms and when standardising by centre attended, although the overall magnitude of the associations were limited by the greater variability of ASI. Adjustment for variability of ASI increased these associations and the proportion of explained variance (table 7.4), with correction for variability of ASI increasing the relationship between ASI and baseline SBP from 0.94 mmHg per m/s ASI to 3mmHg per m/s ASI, and the proportion of explained variance from 2% to 10%.

Table 7.4. Effect of adjustment of baseline associations between blood pressure, arterial stiffness and demographic characteristics, adjusted for measurement variation between baseline and the second follow up visit.
Adjustment for measurement variation is derived from the internal variability estimated in patients with ASI measured at baseline and the second follow-up visit. The beta-coefficient for the general linear model is reported for univariate associations, adjusted non-parametrically (see methods) or parametrically by Cronbach's alpha. Differences in R^2 values are determined by dis-attenuation. Multivariate adjustment for measurement variation is performed by multi-variate regression calibration. SBP=systolic blood pressure; DBP = diastolic blood pressure; MBP = mean blood pressure; HTN = hypertension; PP = pulse pressure; BMI = body mass index; n=169,742

	SBP			**DBP**			**PP**			**ASI**		
	β_{raw}	β_{α}	β_{np}	β_{raw}	β_{α}	β_{np}	β_{raw}	β_{α}	β_{np}	β_{raw}	β_{α}	β_{np}
Effect on β												
Age	0.75	0.75	0.75	0.05	0.05	0.05	0.70	0.70	0.70	0.08	0.08	0.08
Creatinine	0.06	0.08	0.07	0.03	0.04	0.03	0.03	0.04	0.03	0.02	0.03	0.02
Cholesterol	1.72	2.19	2.29	1.16	1.48	1.54	0.55	0.70	0.73			
BMI	0.73	0.82	0.76	0.59	0.66	0.62	0.14	0.16	0.15	0.07	0.08	0.07
SBP				0.38	0.53	0.48	0.62	0.87	0.79	0.03	0.04	0.04
DBP	1.28	1.85	1.59				0.28	0.40	0.35	0.06	0.09	0.07
PP	1.16	1.67	1.74	0.16	0.23	0.24				0.02	0.03	0.03
ASI	0.94	3.09	2.92	0.6	1.97	1.86	0.34	1.12	1.06			
Effect on r^2	r^2	r^2_{dis}		r^2	r^2_{dis}		r^2	r^2_{dis}		r^2	r^2_{dis}	
Age	0.106	0.144		0.002	0.003		0.174	0.236		0.043	0.130	
Creatinine	0.004	0.006		0.004	0.007		0.002	0.003		0.009	0.031	
Cholesterol	0.011	0.019		0.017	0.030		0.002	0.003		0.000	0.000	
BMI	0.035	0.050		0.077	0.115		0.003	0.004		0.013	0.042	
SBP				0.488	0.94		0.715	1.316		0.024	0.099	
DBP	0.488	0.94					0.045	0.087		0.034	0.146	
PP	0.715	1.308		0.045	0.086					0.006	0.024	
ASI	0.024	0.099		0.034	0.146		0.006	0.025				
+ Age / Sex	*β*	*p-val*		*β*	*p-val*		*β*	*p-val*		*β*	*p-val*	
SBP				0.72	<0.001		1.03	<0.001		0.02	<0.001	
DBP	2.42	<0.001					0.50	<0.001		0.09	<0.001	
PP	2.42	<0.001		0.34	<0.001					-0.01	<0.001	
ASI	2.10	<0.001		2.67	<0.001		-0.57	<0.001				
+ CV RFs												
SBP				0.72	<0.001		1.05	<0.001		0.02	<0.001	
DBP	2.51	<0.001					0.50	<0.001		0.09	<0.001	
PP	2.42	<0.001		0.34	<0.001					-0.01	<0.001	
ASI	2.15	<0.001		2.75	<0.001		-0.60	<0.001				

There was a complex interaction between age, ASI and SBP or DBP (figure 7.2). When split by quartiles of baseline ASI, at all ages baseline DBP was greater, but an increased ASI was associated with an earlier mean age of transition from a rising to falling DBP (age at peak DBP by ASI quartile: highest quartile 51.2, 95% CI

49.9 – 52.3; second 51.5, 50.0 – 53.3; third 57.6, 55.5 – 59.1; lowest 60.4, 59.6 – 61.3 years). In contrast, although baseline SBP increased with age in all quartiles of ASI, this increase was steepest for patients at lower levels of initial ASI (figure 7.2). As DBP remained low, this group had the greatest increase in PP across ages. Upon stratifying by age, there was a linear relationship between ASI and baseline SBP in the lowest quartile of age, but a U-shaped relationship in older quartiles. This did not reflect a different pulse-waveform phenotype with age (figure 7.3) but may be explained by the similar relationships with SBP variability across all visits with a U-shaped relationship in older age groups between ASI and with SD of SBP or DBP (figure 7.4).

Figure 7.3 Average pulse waveforms by tertile of Arterial stiffness index (ASI) at baseline across the whole population, stratified by quartile of age.

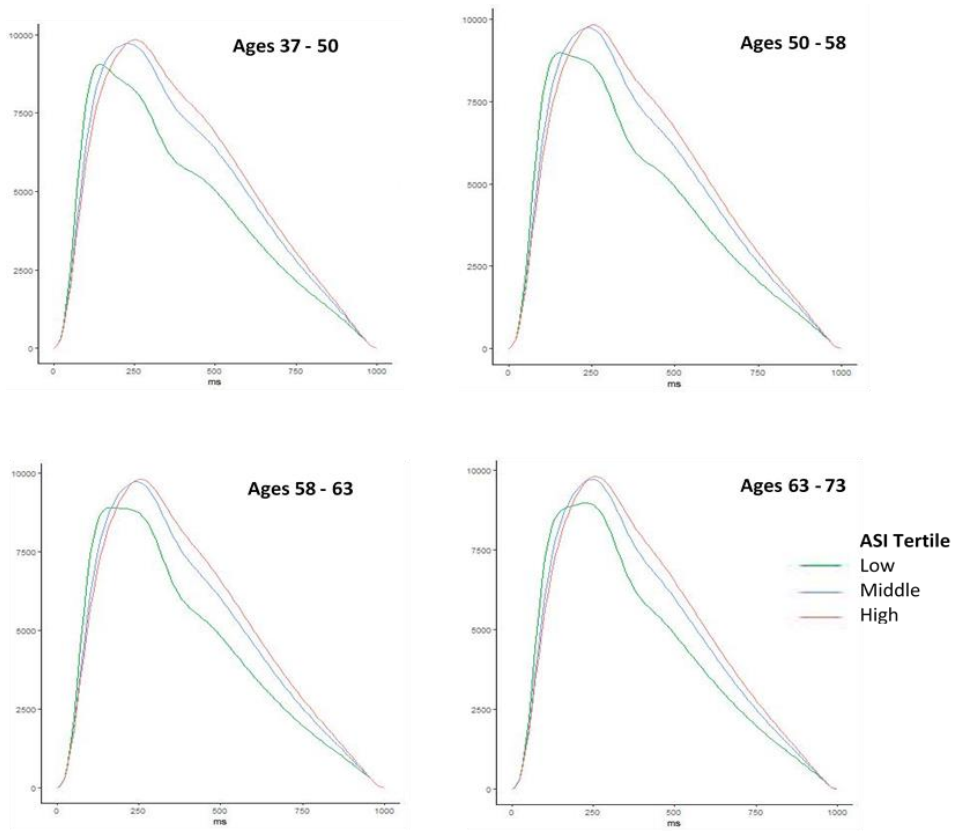
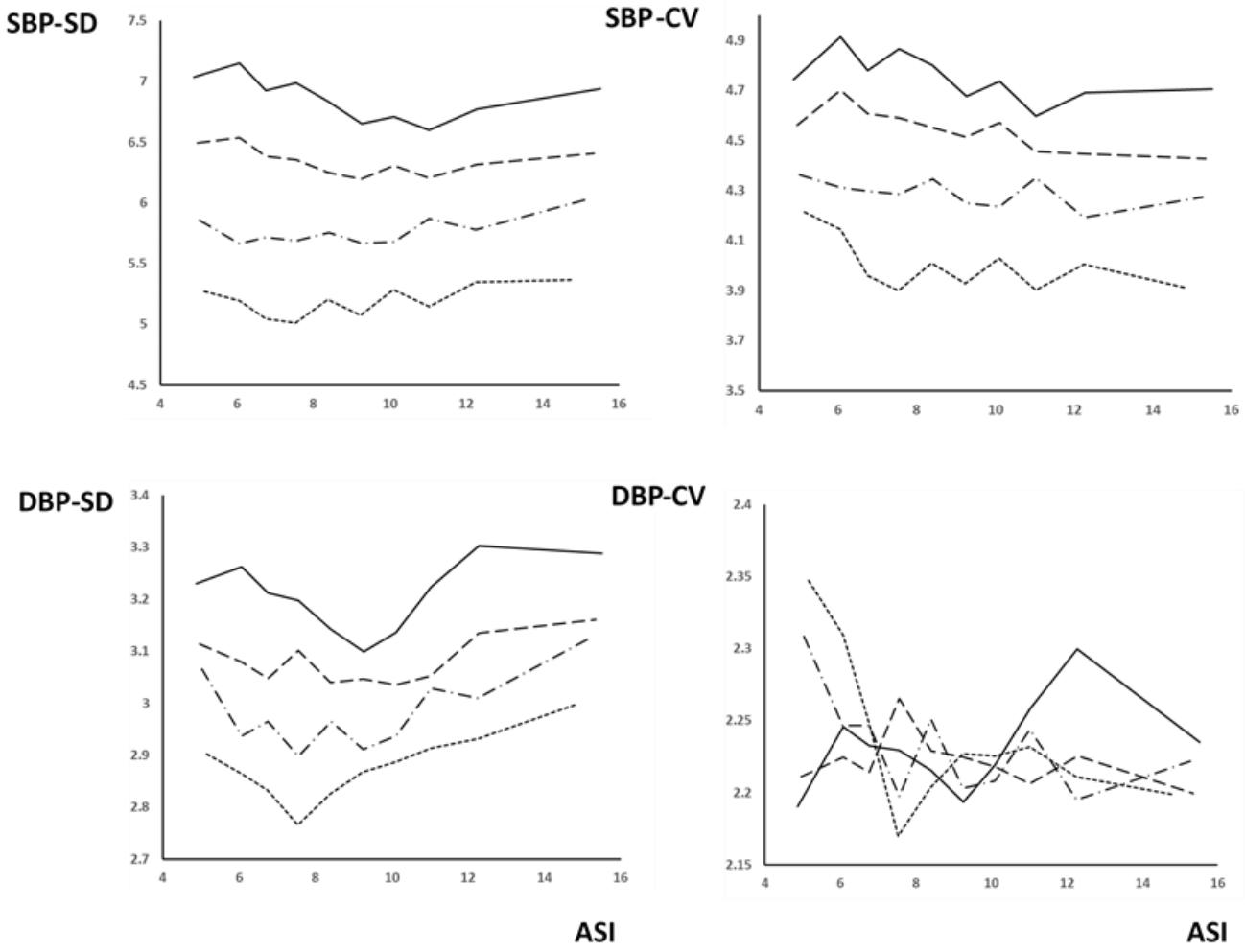


Figure 7.4. Relationship between baseline arterial stiffness index (ASI), age and blood pressure variability at baseline. ASI is divided into deciles, and stratified by quartiles of age, plotted against standard deviation of systolic BP (SBP-SD) or diastolic BP (DBP-SD) or their coefficients of variation (CV)



7.4.6 Longitudinal relationships between ASI and blood pressure

In 13,761 participants with ASI measured at baseline and at least one follow-up (13,210 with BP measures), percentage change in ASI per annum at either follow up was negatively associated with increasing age, due to the strong baseline association between age and ASI and regression to the mean of participants with high ASI. Following adjustment for baseline ASI, increasing age was associated with an increase in ASI at follow-up (table 7.4). Similarly, following adjustment for baseline ASI, baseline SBP and DBP were associated with an increase in ASI across visits, before and after adjustment for age and risk factors (table 7.5). There was no significant effect on the relationship between baseline SBP or DBP with increase in ASI across visits after

Table 7.5. Associations between measures of blood pressure and arterial stiffness at baseline, with percentage change in these indices per annum at follow-up, adjusted for baseline measures, standardised by centre and including only patients with notched ASI recordings at baseline and follow-up. Beta-coefficients and r^2 are shown for univariate general linear models. In models adjusted for age / sex or for age / sex / cardiovascular risk factors (CV RFs = diabetes, current smoking and cholesterol) the beta-coefficient for each key index (row) with the dependent variable (by column) is given, with R^2 squared change compared to the models including only age and sex; or age, sex and CV RFs.

	Percentage Change in Indices per annum at either follow-up							
	SBP		**DBP**		**PP**		**ASI**	
Baseline Indices	**β**	**ΔR^2**	**β**	**ΔR^2**	**β**	**ΔR^2**	**β**	**ΔR^2**
Univariate								
Age	0.02	0.011	-0.02	0.01	0.10	0.044	0.03	0.001
Female	0.2	0.007	0.15	0.004	0.27	0.003	1.19	0.015
Diabetes	-0.2	0*	-0.15	0*	-0.64	0.001*	1.08	0*
HTN	0.3	0.004	0.12	0*	0.59	0.004	0.53	0.001
Smoking								
Base	0.07	0*	0.17	0*	-0.03	0*	0.16	0*
Ever Smoked	0.11	0.001	-0.01	0*	0.3	0.002	0.3	0*
Creatinine	0.01	0.004	0.01	0.001	0.01	0.004	0.03	0.003
Cholesterol	-0.02	0*	-0.04	-0.001*	0.01	0.002*	0.03	-0.002*
BMI	0.03	0.006	0.02	0.002	0.06	0.006	0.04	0.001
Weight	0.01	0.007	0.01	0.004	0.02	0.006	0.03	0.006
SBP	-0.04	0	-0.01	0.005	0.01	0.002	0.03	0.006
DBP	0	0*	-0.07	0	0.01	0.002	0.07	0.01
MBP	0.01	0.004	-0.03	0.005	-0.05	0	0.06	0.009
PP	0	0*	-0.01	0.005	-0.11	0	0.02	0.001
ASI	0.04	0.004	0.01	0*	0.1	0.007	-1.42	0
+ Age / Sex								
SBP	-0.04	0	-0.01	0.002	0.01	0.001	0.02	0.002
DBP	0.01	0*	-0.07	0	0.01	0.001	0.06	0.006
MBP	0.01	0*	-0.02	0.002	-0.06	0	0.05	0.006
PP	-0.01	0*	-0.01	0.002	-0.13	0	0	0*
ASI	0.02	0.001	0.01	0*	0.03	0*	-1.53	0
+ CV RFs								
SBP	-0.05	-0.001	-0.01	0	0.01	0.002*	0.02	-0.001
DBP	0.01	-0.001*	-0.07	-0.002	0.01	0.002*	0.06	0.003
MBP	0.01	0.001	-0.02	0.001	-0.058	0.001	0.05	0.005
PP	-0.01	-0.001*	-0.01	0	-0.13	0.002	0	-0.002*
ASI	0.01	-0.001*	0.01	-0.002*	0.02	0.002*	-1.54	-0.002
+ SBP + DBP								
PP	0	-0.001*	-0.01	-0.002	-0.14	0.001	-0.07	-0.003
ASI	0.01	-0.001*	0	-0.003*	0.02	0.001*	-1.57	-0.003

adjustment for baseline heart rate (SBP β=0.02, p<0.001; DBP β=0.06, p<0.001). Furthermore, although use of hypertensive medications at baseline was associated with an increased risk of progression of ASI during follow-up (β=0.44, p=0.008), use of antihypertensive medications was associated with reduced progression of ASI after adjustment for age, sex and cardiovascular risk factors but this was not significant (β=-0.38, p=0.16). In participants younger than 60 years old, baseline DBP was more strongly associated with the increase in ASI at follow-up than in participants older than 60 years (Table 7.6) but there was minimal difference in the association between baseline SBP and change in ASI in participants younger than or older than 60 years. Associations between progression of ASI were still slightly stronger for DBP than MBP such that DBP was the stronger predictor of increases in ASI during follow-up. In contrast, ASI at baseline did not predict an increase in DBP at follow-up, but did predict an increase in SBP and PP, before and after adjustment for cardiovascular risk factors.

ASI and baseline blood pressure were associated with a greater risk of transition to any hypertension, isolated systolic hypertension or raised arterial stiffness (table 7.6), with progression to hypertension being more strongly associated with a raised systolic or diastolic BP at baseline, as expected. This association was increased after adjusting for regression dilution, with a 2.5 fold increase in the risk of hypertension at follow up per 1.7m/s ASI (the standard deviation of ASI in normotensive participants, table 7.6). Finally, both baseline ASI and baseline SBP or DBP were associated with transition to a raised ASI at follow-up, with ASI the strongest predictor after adjustment for regression dilution.

Table 7.6. Risk of progression to hypertension or increased arterial stiffness index (ASI) at either follow-up. Results are presented for progression from baseline normotension ('Norm') to any hypertension (HTN) or isolated systolic hypertension(ISH), from mixed hypertension (SDH) to ISH; and from low ASI (<10m/s) to high ASI (>10m/s). Values are odds ratios (OR) per standard deviation of the dependent variable (by row) with 95% confidence intervals (CI). Univariate models are presented, with subsequent models presenting the OR for each dependent variable adjusted for age, sex and cardiovascular risk factors ('partially adjusted') or adjusted for age, sex, cardiovascular risk factors and SBP and DBP ('adjusted for BP'). Finally, univariate hazard ratios are reported adjusted for variability at follow-up by parametric (Cronbach's alpha) or non-parametric methods (regression dilution ratio). Numbers are reported for patients with BP data at baseline and follow up, and ASI at baseline. SBP = systolic blood pressure; DBP = diastolic blood pressure

	Norm → HTN (n=13635)		**SDH → ISH** (n=13169)		**Norm → ISH** (n=13210)		**ASI Transition** (n=13761)	
	OR	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**	**OR**	**95%CI**
Univariate								
ASI	1.36	(1.28 - 1.44)	0.98	(0.90 - 1.07)	1.31	(1.23 - 1.39)	1.22	(1.17 - 1.27)
SBP	2.59	(2.49 - 2.69)	1.40	(1.33 - 1.48)	2.59	(2.47 - 2.71)	1.25	(1.19 - 1.3)
DBP	1.82	(1.76 - 1.88)	0.74	(0.70 - 0.78)	1.39	(1.35 - 1.45)	1.21	(1.16 - 1.27)
Age	1.51	(1.47 - 1.56)	1.46	(1.38 - 1.54)	1.73	(1.67 - 1.80)	1.32	(1.26 - 1.39)
Female Sex	1.17	(1.15 - 1.19)	1.04	(1.01 - 1.08)	1.10	(1.07 - 1.12)	1.12	(1.09 - 1.16)
Diabetes	1.05	(1.02 - 1.09)	1.00	(0.94 - 1.07)	1.04	(1.00 - 1.08)	1.04	(0.98 - 1.10)
Smoking current	1.02	(0.99 - 1.06)	0.98	(0.92 - 1.04)	0.97	(0.93 - 1.01)	1.04	(0.98 - 1.09)
Cholesterol	1.12	(1.09 - 1.15)	0.98	(0.93 - 1.03)	1.16	(1.12 - 1.20)	1.04	(0.99 - 1.08)
Partially adjusted								
ASI	1.18	(1.11 - 1.26)	0.92	(0.84 - 1.01)	1.11	(1.04 - 1.20)	1.16	(1.11 - 1.22)
SBP	2.49	(2.39 - 2.60)	1.31	(1.24 - 1.39)	2.45	(2.34 - 2.58)	1.13	(1.08 - 1.19)
DBP	1.88	(1.82 - 1.95)	0.76	(0.71 - 0.80)	1.44	(1.39 - 1.50)	1.17	(1.12 - 1.23)
Also adjusted for BP								
ASI	1.11	(1.04 - 1.18)	0.96	(0.87 - 1.06)	1.07	(1.00 - 1.15)	1.16	(1.11 - 1.22)
SBP	2.17	(2.06 - 2.27)	1.21	(1.14 - 1.29)	2.64	(2.49 - 2.79)	1.00	(0.93 - 1.08)
DBP	1.27	(1.22 - 1.33)	1.06	(1.02 - 1.10)	0.89	(0.84 - 0.93)	1.17	(1.09 - 1.26)
Adjusted for visit-to- visit variability	Para	Non-Para	Para	Non-Para	Para	Non-Para	Para	Non-Para
ASI	2.61	2.54	0.94	0.95	2.31	2.25	2.78	1.84
SBP	4.79	3.34	2.32	1.53	4.78	3.34	1.35	1.32
DBP	2.52	2.10	0.33	0.69	1.67	1.51	1.30	1.26

7.5 Discussion

In nearly 170,000 people, including more than 13,000 participants with repeated measures over 4-9 years, there were strong associations between baseline SBP and DBP with arterial stiffness, with stronger associations for DBP than SBP. Mid-life DBP was the strongest predictor of increases in arterial stiffness over time, whilst greater arterial stiffness was associated with increases in SBP. However, arterial stiffness was associated with an earlier age of transition to a falling DBP and was associated with the risk of transition to hypertension or elevated arterial stiffness during follow-up.

The strong associations between arterial stiffness, hypertension,[6] a consistently rising systolic blood with age and a parabolic relationship with diastolic blood pressure are well-established.[23, 24] The demonstrated stronger relationship with future arterial stiffness for mid-life steady components of blood pressure contrasts with the prognostic importance of pulsatile blood pressure components (isolated systolic blood pressure, pulse pressure) in the elderly.[24-26] However, if both the mid-life risk associated with increased DBP and the late life increase in pulsatile pressures are both ultimately due to mid-life hypertension then principally targeting late-life pulsatile blood pressure is sub-optimal. However, there have been relatively few studies directly measuring the associations between mid-life blood pressure and progression of arterial stiffness over prolonged periods,[9, 13-15] none that we are aware of that directly compare mid-life DBP with SBP and none that adequately assessed the effect of arterial stiffness on the age of transition to late-life hypertensive phenotypes.[27, 28] Although a number of interventions to reduce arterial stiffness have been examined,[29-31] optimal prevention of late-life harms may therefore depend on treating mid-life factors that lead to arterial stiffening, prior to the transition to late-life phenotypes.

In UK Biobank, arterial stiffness is associated with increased blood pressure at all ages, with any hypertension predicting progression of arterial stiffness in the

future, consistent with limited results from smaller studies.[6, 9] However, baseline DBP was the strongest predictor of progression of arterial stiffness, with increased stiffness associated with an early transition to a fall in DBP, supporting the control of mid-life DBP to prevent later arterial stiffening. Although there were similar associations for mean BP, the associations remained stronger for DBP, and DBP was therefore preferable as a covariate in adjusted models. The stronger relationship between mid-life diastolic BP and progression of arterial stiffness reflects the steady component of blood pressure, that may drive maladaptive large vessel changes with increases in collagen and calcification and a reduction in elastin. Alternatively, elevated mid-life DBP may reflect distal vasoconstriction, increasing systemic resistance due to either metabolic factors (smoking, obesity) or a primary small vessel arteriopathy, increasing wave-reflection and secondary haemodynamic stress on large vessels.

The apparent linear relationship between baseline arterial stiffness and the future increase in SBP was relatively weak, particularly after adjustment for age or baseline SBP, but this partly reflected the non-linear relationship between SBP and ASI in older age groups. In this group, there was a U-shaped relationship between ASI and SBP associated with increased BP variability, implicating factors independent of ASI that result in an increase in SBP, for example, due to 'white-coat' hypertension[32] or age-related baroreceptor dysfunction. Alternatively, it is possible that calculation of ASI, which utilises the morphology of the waveform rather than direct measurement of the pulse wave transit time, may have underestimated arterial stiffness in some elderly patients with increased SBP, reflecting previous reports of sub-optimal correlation between ASI and PWV.[21] However, the mean waveform across ASI tertiles was consistent across ages, suggesting that increase in SBP at the second follow up reflected direct effects on SBP, rather than misclassified arterial stiffness (figure 7.3).

A significant limitation of arterial stiffness, as measured in UK Biobank, is the greater variability of ASI compared to previous reports of the variability of arterial stiffness,[21, 33] and compared to the variability of BP measurements in UK Biobank. This partly reflects the use of the PulseTrace device. Although this device was simple to apply in a large population and is correlated with arterial stiffness, it is less reproducible and only indirectly reflects large artery stiffness rather than being a direct measurement. This both reduces the power of the analyses to detect a significant effect of ASI and increases the possibility that the associations between demographic indices or blood pressure with ASI and change in ASI may be due to physiological factors other than arterial stiffness. However, the findings are consistent with previous reports of relationships between arterial stiffness, age and blood pressure,[9] although these studies did not assess progression in such a large population, or the effect of stiffness on age of transition of DBP. Secondly, it reduces the estimated effect size for associations between ASI and clinical outcomes, other haemodynamic indices or progression to hypertension in this cohort. Finally, due to the reliability ratio of ASI being less than 0.5 and its strong covariance with BP, there is an inherent limit to the reliability of conclusions that aim to adjust for measurement variation, which can be viewed as indicative only.[22] Nonetheless, these analyses were consistent with the overall findings, biologically plausible and demonstrate that conclusions reached without assessing the likely impact of measurement error are likely to be even more misleading, even if the absolute magnitude of adjusted associations remains uncertain. This also demonstrates the need to adjust for differential measurement error in epidemiological studies, and in UK Biobank in particular.

There are limitations to this analysis. The UK Biobank population is a community-selected population of patients, resulting in ascertainment bias and a healthier population than the general population.[20, 34] This is particularly pronounced for individuals re-attending for MRI imaging at the second follow-up. This limits external

applicability to the whole population but given the very large size of the study, and internal consistency of the demonstrated associations, the effect of the relationships between BP and ASI are likely to be similar for the majority of individuals. Secondly, there were systematic differences in UK biobank between centres. This resulted in a centre-specific elevation in ASI and SBP at the first follow-up. This may reflect a centre specific factor resulting in temporary rather than sustained elevations in SBP and ASI, such as environmental causes of sympathetic activation at that centre compared to other centres. However, standardising results according to centre attended, or including centre as a random effects variable, had no significant impact on the conclusions. Finally, the apparent strength of linear associations were often small, albeit with high significance levels due to the large size of the population. However, I have reported r^2 change for most indices compared to a baseline model, producing a lower apparent strength of association than the r^2 for the whole model. Furthermore, the low proportion of variance explained was partly due to the greater variability of measures, with significant improvements in the magnitude of association and the proportion of explained variance after adjustment for measurement variation. Finally, the true magnitude of differences is clinically significant despite relatively weak statistical associations. As such, any treatments affecting these indices at the population level are likely to be associated with significant population-wide clinical benefits, beyond that implied by the statistical strength of association.

The principal implication of this work is that targeting hypertension to prevent arterial stiffening is likely to be effective at reducing detrimental clinical outcomes, but the optimal long-term target is likely to be mid-life blood pressure, and diastolic blood pressure in particular. Studies in older populations may be better targeted at de-stiffening strategies or haemodynamically-focussed treatments that limit the distal effects of increased arterial stiffness.[35] Thirdly, the apparent elevation in SBP in a subset of older individuals with low ASI needs further investigation to validate

this finding in other populations with other methods of measuring arterial stiffness, and to identify the underlying determinants and prognostic significance of elevated SBP in the absence of elevated arterial stiffness. Finally, characterisation of the relationship between arterial stiffness and blood pressure in UK Biobank is necessary to understand the effects of blood pressure and arterial stiffness on end-organ damage, including effects on cardiac and brain imaging measures and clinical outcomes. In particular, future analyses need to allow for differential measurement error between variables, and non-linear relationships between age, blood pressure and arterial stiffness.

Mid-life diastolic blood pressure is the strongest predictor of progression of arterial stiffness, with elevated arterial stiffness associated with elevated systolic and diastolic blood pressure at all ages, an earlier transition from a rising to falling diastolic blood pressure and development of late-life hypertensive phenotypes. Hypertension predicts progression of arterial stiffness, but elevated systolic blood pressures persists in a subgroup of elderly patients despite normal arterial stiffness index. Finally, future epidemiological analyses, particularly in UK Biobank, must allow for the effects of the variability of measurements in predictive analyses of end-organ damage and future cardiovascular events.

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CHAPTER EIGHT

Physiological determinants of residual cerebral arterial pulsatility on best medical treatment after TIA or minor stroke

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8.1 Summary

Cerebral arterial pulsatility is strongly associated with cerebral small vessel disease (SVD) and lacunar stroke, yet its dependence on central versus local haemodynamic processes is unclear.

In consecutive patients on best medical management, 4-6 weeks after TIA or non-disabling stroke (Oxford Vascular Study), arterial stiffness (PWV), aortic systolic (aoSBP), diastolic (aoDBP) and pulse pressures (aoPP) were measured by applanation tonometry (Sphygmocor). Middle cerebral artery peak (MCA-PSV) and trough (MCA-EDV) flow velocity and Gosling's pulsatility index (MCA-PI) were measured by transcranial ultrasound (TCD, DWL DopplerBox). Associations between demographic factors, arterial stiffness and haemodynamic indices were determined by general linear models, adjusted for age, sex and risk factors, with potential pathways assessed by mediation analysis.

In 981 participants with either aortic or MCA measures, there was no association between aoSBP and MCA-PSV ($p=0.09$) or between aoDBP and MCA-EDV ($p=0.28$), after adjustment for confounders. Although aoDBP and MCA-EDV both fell with age, there was a greater fall with age in aoDBP in men (interaction $p=0.0004$) versus a greater fall in MCA-EDV in women ($p=0.04$), whilst AoSBP increased with age ($p<0.0001$) in both sexes but MCA-PSV did not. In contrast, aoPP explained 37% of the variance in MCA-PI before adjustment for confounders ($p<0.0001$) with 50% of the variance explained by the fully adjusted model ($p<0.0001$). Arterial stiffness partially mediated the relationship between aoPP and MCA-PI (24%, $p<0.0001$).

Absolute aortic pressure and middle cerebral artery blood flow were poorly correlated, with differing patterns of association with demographic characteristics, but pulsatility in the aorta and MCA were strongly correlated, suggesting that pulsatility of cerebral blood flow is principally dependent upon transmission of aortic pulsatility to the brain.

8.2 Introduction

Small vessel disease accounts for approximately 30% of strokes and 40% of dementia[1] and is associated with acute lacunar stroke,[2] progressive cognitive decline,[3] late-onset refractory depression,[4] functional impairment in daily living[5] and increased mortality.[6] White matter hyperintensities are highly prevalent in the population, affecting over half of people over the age of 65 and the majority of people over 85. However, even patients with advanced imaging changes can remain functionally independent,[7] indicating a pre-clinical stage of the disease where intervention may prevent progression of SVD and resulting clinical morbidity. White matter hyperintensities are strongly associated with a history of hypertension and are particularly associated with markers of vascular aging, including aortic stiffness and pulsatility of blood flow in the aorta and the cerebral circulation.[8] As such, cerebral pulsatility reflects the late-life, hypertensive phenotype that may ultimately reflect mid-life diastolic hypertension (chapter 7).

Identifying methods to reduce cerebral arterial pulsatility and associated clinical harms would be informed by characterising the physiological processes responsible for elevated pulsatility, understanding the role of pulsatility in leading to clinical harm and identifying potential treatment targets. Pulsatility of cerebral blood flow is associated with distal arterial resistance and increased aortic stiffness and pulsatility of aortic blood flow. However, it is unclear what the magnitude of the association between cerebral arterial pulsatility and aortic blood pressure is, whether the relationship between aortic pulsatility and cerebral blood flow velocity is consistent across demographic groups and whether targeting aortic pulsatility and arterial stiffness could reduce cerebral arterial pulsatility and small vessel disease.

In a large population with recent TIA or minor stroke with optimised blood pressure control and best medical treatment, we compared the population distribution and association between cerebral pulsatility and aortic haemodynamic measures.

8.3 Methods

8.3.1 Study population

Consecutive, consenting patients with TIA or minor stroke were recruited between September 2010 and September 2019, as part of the Oxford Vascular Study (OXVASC).[9, 10] Participants were recruited at the OXVASC daily emergency assessment clinic, following a referral after attendance at the Emergency Department or from primary care, usually within 24 hours. Patients were referred after transient neurological symptoms or symptoms consistent with a minor stroke, not requiring direct admission to hospital.

As part of the OXVASC Phenotyped Cohort, a routine prospective cardiovascular physiological assessment is performed at the 1 month follow-up visit.[9, 10] Participants were excluded if they were under 18 years, cognitively impaired (MMSE<23), pregnant, had autonomic failure, a recent myocardial infarction, unstable angina, heart failure (NYHA 3-4 or ejection fraction <40%) or untreated bilateral carotid stenosis (>70%).

Physiological tests were performed at rest in a quiet, dimly-lit, temperature-controlled room (21-23^{0}C). Applanation tonometry (Sphygmocor, AtCor Medical, Sydney, Australia) was used to measure carotid-femoral pulse wave velocity (aortic-PWV), aortic augmentation index and aortic systolic and diastolic blood pressure and pulse pressure (ao-SBP, ao-DBP, ao-PP).[17] Where applanation tonometry was not possible due to bilateral carotid stenosis or technical failure, pulse wave analysis and arterial stiffness was determined with a vicorder. The distribution of PWV and aortic measures determined by vicorder were transformed to match the population distribution of measures derived from a Sphygmocor, estimated in a subset of the population in whom both tests were performed, with sensitivity analyses performed restricted to patients undergoing assessment of PWV by Sphygmocor alone. TCD (Doppler Box, Compumedics DWL, Singen, Germany) was performed with a 2MHz probe at the temporal bone window on the same side as carotid applanation, where

possible. The waveform envelope was acquired at 100Hz simultaneously with ECG and blood pressure at 200Hz (Finometer, Finapres Medical Systems, The Netherlands), via a Powerlab 8/30 with LabChart Pro software (ADInstruments, USA). The MCA was insonated at the site of peak velocity closest to 50mm, or if this was not adequate, at the depth giving the optimal waveform, excluding vessels with velocity transitions and magnitude indicative of a focal MCA stenosis. All waveforms were visually inspected and beats corrupted by artefact were excluded. Absolute peak, trough and mean velocities were calculated as the average of the remaining beats during a 15 second window, from the envelope of the spectrum. MCA pulsatility was calculated as Gosling's pulsatility index (MCA-PI= (systolic CBFV-diastolic CBFV) / mean CBFV).

8.3.1 Analysis

Associations between demographic or physiological indices were determined by general linear models for continuous variables, logistic regression for binary outcomes and ordinal regression for ordinal outcomes. Models were performed for univariate associations; adjusted for age and sex; age, sex and cardiovascular risk factors and for cardiovascular risk factors and interactions between age and sex and cardiovascular risk factors. Relationships between systemic haemodynamic measures and cerebral pulsatility were determined by general linear models, with mediation analysis to identify the potential pathway of effect. Mean values and associations were determined stratified by age (in quintiles) and sex, with formal assessment of interactions through general linear models.

Analyses were performed in R (packages *tidyverse*, *mediation, emmeans*), Matlab r2015 or Windows Excel.

8.4 Results

8.4.1 Study Population

Of 1013 eligible participants, 981 had either arterial stiffness or TCD performed. 849 patients had TCD recordings (excluding 104 with no bone windows

and 60 participants due to machine failure, lack of availability or participant refusal), of whom 801 had higher quality recordings. 936 patients had arterial stiffness measures whilst 767 patients underwent both tests. Patients with either TCD or arterial stiffness measures were younger than those with neither, were more likely to be in heart failure and were more likely to be women (table 8.1).

Table 8.1. Distribution of demographic indices is shown for patients undergoing pulse wave analysis or transcranial ultrasound

	All n (%)	Had either PWV Or TCD n (%)	p-val
	1013	981	
Age	67 (13.3)	66.9 (13.4)	<0.001
Sex	457 (45.1)	433 (44.1)	<0.001
Hypertension	782 (77.2)	761 (77.5)	0.11
Diabetes	119 (11.7)	112 (11.4)	0.07
Smoking			
- Current	164 (16.2)	158 (16.1)	0.69
- Ever	542 (53.5)	524 (53.4)	0.75
FHx of stroke	303 (29.9)	298 (30.3)	0.07
AF			
- During test	54 (5.3)	50 (5.1)	0.07
- Ever	74 (7.3)	69 (7)	0.07
Dyslipidaemia	819 (80.8)	791 (80.5)	0.33
Heart Failure	16 (1.6)	14 (1.4)	0.031

Table 8.2. Associations with demographics variables. Results are presented for general linear models as univariate analyses, and following adjustment for age, sex and cardiovascular risk factors.

	Aortic Measures								Cerebral Measures			
	SBP		DBP		PP		PSV		EDV		PI	
	B	p	B	p	B	p	B	p	B	p	β	p
Univariate												
Age	0.41	<0.001	-0.11	<0.001	0.48	<0.001	-0.17	0.001	-0.35	<0.001	0.008	<0.001
Female Gender	3.74	0.003	-2.95	<0.001	7.68	<0.001	7.08	<0.001	2.26	0.003	0.039	0.008
Diabetes	-0.004	1	-0.545	0.6	1.827	0.28	3.037	0.17	-0.177	0.88	0.061	0.009
Smoking												
- **Ever**	-0.95	0.45	0.11	0.87	-0.66	0.54	-1.22	0.38	-0.46	0.55	0.002	0.9
- **Current**	-0.74	0.67	1.95	0.03	-2.6	0.07	1.87	0.32	2.71	0.007	-0.07	<0.001
HTN	5.99	<0.001	0.07	0.93	5.49	<0.001	-5.08	0.002	-6.37	<0.001	0.11	<0.001
MI	-0.32	0.91	-3.64	0.012	1.87	0.44	1.34	0.68	-2.5	0.15	0.099	0.003
Dyslipidaemia	-0.62	0.64	-0.96	0.17	1.43	0.21	-1.93	0.2	-2.91	<0.001	0.06	<0.001
Creatinine	0.009	0.74	-0.012	0.43	0.012	0.63	-0.064	0.06	-0.086	<0.001	0.001	<0.001
Weight	-0.03	0.2	0.02	0.16	-0.04	0.014	-0.02	0.33	0.01	0.66	-0.001	0.028
BMI	-0.18	0.14	0.09	0.19	-0.25	0.022	-0.18	0.21	0.03	0.71	-0.003	0.02
Adjusted												
Age	0.41	<0.001	-0.11	<0.001	0.48	<0.001	-0.17	0.012	-0.35	<0.001	0.008	<0.001
Female Gender	3.74	0.007	-2.95	<0.001	7.68	<0.001	7.08	<0.001	2.26	<0.001	0.039	0.009
Diabetes	-0.004	0.95	-0.545	0.29	1.827	0.14	3.037	0.026	-0.177	0.56	0.061	0.001
Smoking												
- **Ever**	-0.95	0.33	0.11	0.34	-0.66	0.91	-1.22	0.7	-0.46	0.73	0.002	0.39
- **Current**	-0.74	0.015	1.95	0.3	-2.6	0.035	1.87	0.63	2.71	0.66	-0.07	0.72
HTN	5.99	0.09	0.07	0.09	5.49	0.95	-5.08	0.021	-6.37	<0.001	0.114	0.08
MI	-0.32	0.44	-3.64	0.003	1.87	0.85	1.34	0.16	-2.5	0.68	0.099	0.1
Dyslipidaemia	-0.62	0.08	-0.96	0.45	1.43	0.33	-1.93	0.86	-2.91	0.55	0.06	0.48
Creatinine	0.009	0.47	-0.012	0.15	0.012	0.73	-0.064	0.3	-0.086	0.6	0.001	0.26
Weight	-0.03	0.74	0.02	0.97	-0.04	0.79	-0.02	0.8	0.01	1	-0.001	0.53
BMI	-0.18	0.74	0.09	0.25	-0.25	0.23	-0.18	0.15	0.03	0.46	-0.003	0.18

8.4.2 Demographic associations of aortic and cerebral blood flow

A history of hypertension was associated with increased SBP, DBP and aortic PP, but was also associated with a lower PSV and EDV but a significantly raised PI, before and after adjustment for age and other cardiovascular risk factors (table 8.2). A history of diabetes was associated with increased PSV and MCA-PI, but there was no difference in EDV. Although current smoking was weakly associated with cerebral pulsatility before adjustment, and a lower SBP and aortic pulse pressure after adjustment for confounders, there were no independent associations between smoking with cerebrovascular indices after adjustment for risk factors.

8.4.3 Age and sex distribution of aortic stiffness and pulsatility

The age and sex distributions of aortic stiffness and aortic pulsatility were very similar to the distributions for MCA-PI, with age being particularly associated with increased PWV, aortic pulse pressure and MCA-pulsatility index (figures 8.1 and 8.2). MCA-PI and aortic PP were both greater in women than men but this difference attenuated after adjustment for age, reflecting the difference in age distributions between the sexes. However, although MCA-PI increased with age similarly in both sexes, aortic-PP increased slightly more rapidly with age in women than men, whilst there was no significant difference in the increase in PWV between sexes (figure 8.1, table 8.3).

Figure 8.1. Age and sex distributions of aortic stiffness and cerebral arterial pulsatility.

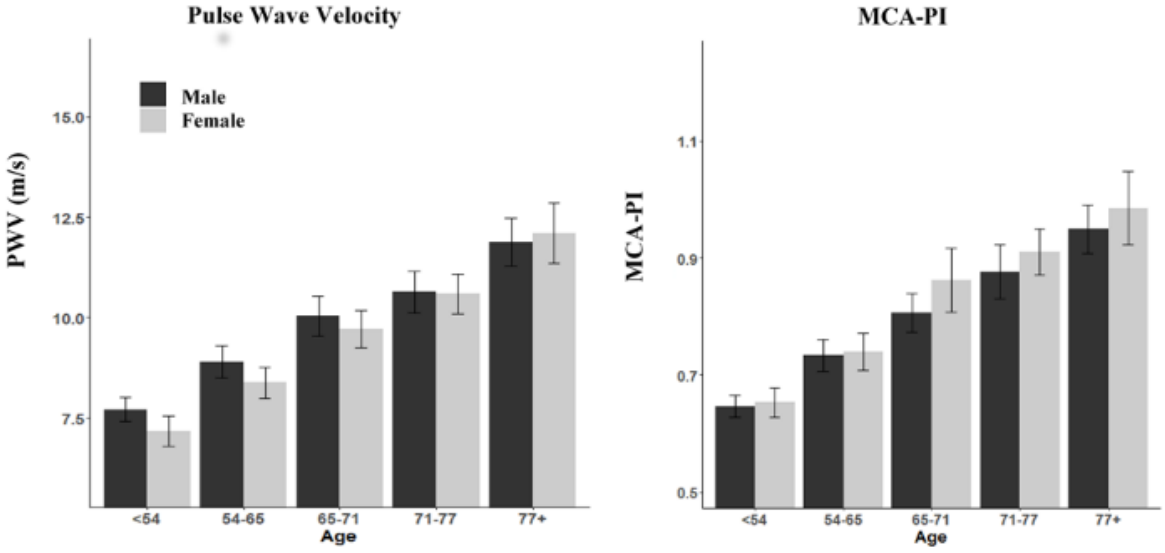


Figure 8.2. Distributions of cerebral physiological indices and aortic indices, and the relationship between them. Results are presented as histogram and kernel density plot, stratified by gender, and for the scatter plot between the cerebral and systemic measures. Results are presented for aortic systolic blood pressure (SBP), aortic diastolic blood pressure (DBP), aortic pulse pressure (PP), MCA peak systolic velocity (PSV), MCA end diastolic velocity (EDV) and MCA pulsatility index (PI).

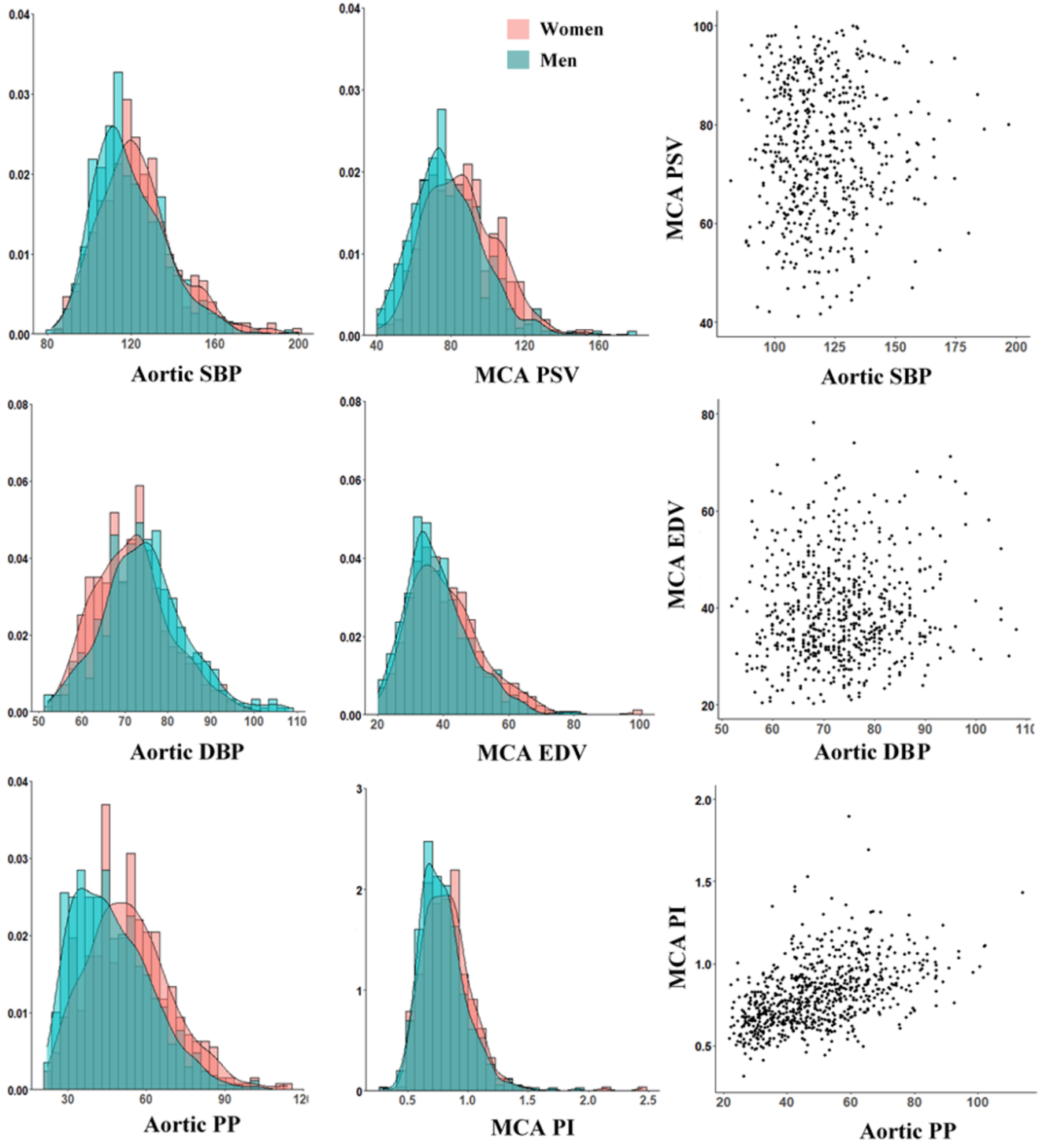


Table 8.3. Relationship between age and sex with key physiological indices. Results of general linear models are presented for each index including age and sex (model 1), age and sex and the interaction between them (model 2) and for model 2 also adjusted for cardiovascular risk factors.

	Model 1			Model 2		Adjusted	
	R^2	Age (p)	Sex (p)	R^2	Interact.	R^2	Interact.
PWV (m/s)	0.31	<0.0001	0.21	0.31	0.041	0.35	0.08
HR (bpm)	0.01	0.83	0.002	0.01	0.24	0.03	0.36
SBP	0.08	<0.0001	0.58	0.10	<0.0001	0.10	<0.0001
DBP	0.05	0.018	<0.0001	0.07	<0.0001	0.07	0.001
Ao. SBP	0.10	<0.0001	0.005	0.11	<0.0001	0.12	0.001
Ao. DBP	0.05	<0.0001	<0.0001	0.06	0.002	0.05	0.004
Ao. PP	0.20	<0.0001	<0.0001	0.20	0.027	0.21	0.036
Ao AIX	0.28	<0.0001	<0.0001	0.28	0.026	0.30	0.03
MCA PSV	0.05	<0.0001	<0.0001	0.05	0.21	0.05	0.29
MCA EDV	0.23	<0.0001	<0.0001	0.23	0.022	0.24	0.04
MCA PI	0.29	<0.0001	0.02	0.29	0.25	0.31	0.22

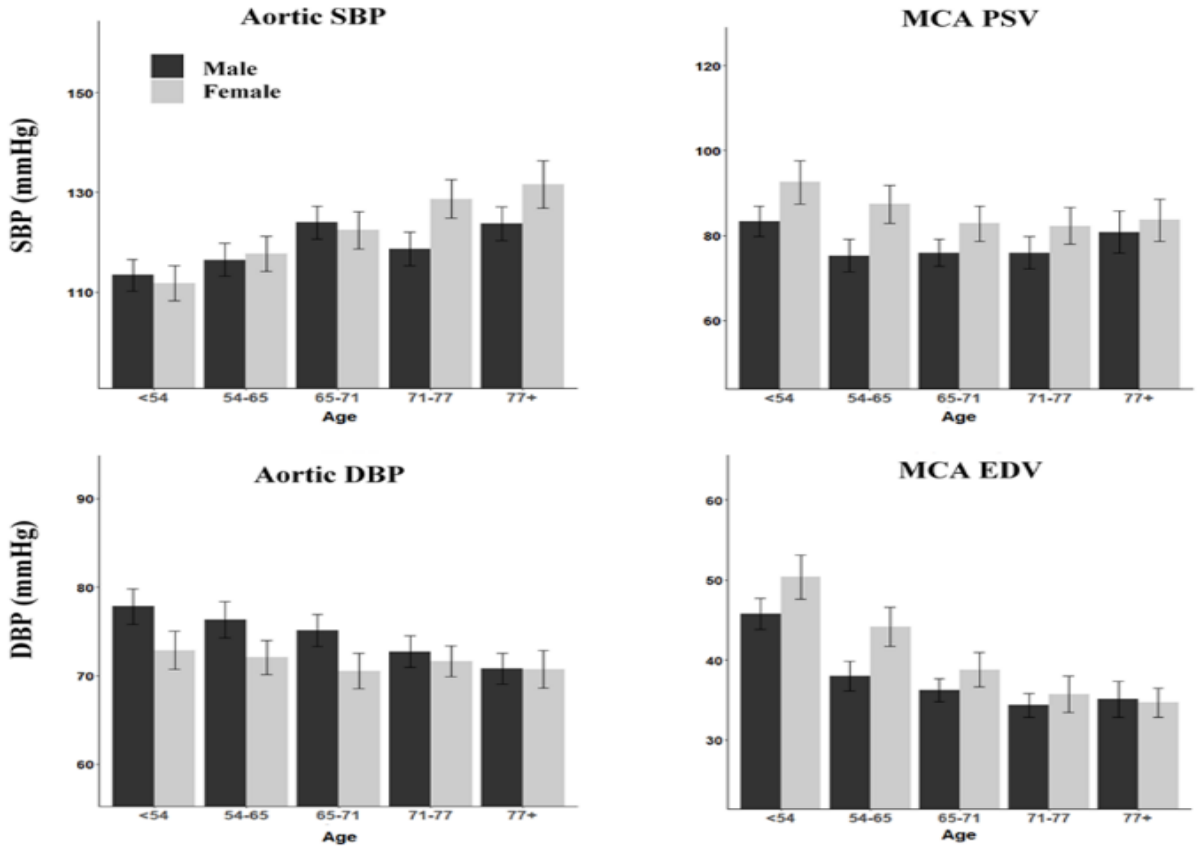
8.4.4 Age and sex distribution of aortic and cerebral blood flow indices

In contrast to the relatively consistent age-sex distributions for systemic and central measures of pulsatility, absolute values of blood pressure and cerebral blood flow velocities differed. Aortic SBP increased with age in both sexes, but with a stronger relationship in women than men and a significant interaction between age and sex (table 8.3). However, MCA-PSV fell with age in women but in men there was little change across age-groups. In contrast, EDV and DBP both fell with age but the fall in EDV was more pronounced in women (p=0.0004), compared to a limited fall in aortic DBP in women versus men (table 8.4, figure 8.3).

Table 8.4. Relationships between cerebral blood flow indices and systemic physiological indices. Results are presented for general linear models as univariate analyses, and following adjustment for age, sex and cardiovascular risk factors.

	MCA PSV				MCA EDV				MCA PI			
	Univariate		Adjusted		Univariate		Adjusted		Univariate		Adjusted	
	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p
PWV	0.003	0.10	0.006	0.54	0.11	<0.001	0.007	0.10	0.22	<0.001	0.015	<0.001
Ao. AiX	0.005	0.03	0.005	0.068	0.003	0.069	-0.002	0.018	0.051	0	0.01	0.68
Ao. SBP	0	0.41	0.006	0.09	0.03	<0.001	0.001	0.42	0.097	<0.001	0.022	<0.001
Ao. DBP	0.006	0.027	0.006	0.076	0.006	0.03	0.001	0.28	0.07	<0.001	0.014	<0.001
Ao. PP	0.005	0.024	0.014	0.003	0.05	<0.001	0	0.05	0.23	<0.001	0.08	<0.001
Ao. MBP	0.001	0.20	0	0.87	0	0.28	-0.004	0.99	-0.002	0.74	0.024	0.34
MCA PSV	-	-	-	-	0.62	<0.001	0.53	<0.001	0.01	0.037	0.03	<0.001
MCA EDV	0.62	<0.001	0.66	<0.001	-	-	-	-	0.18	<0.001	0.08	<0.001
MCA PI	0.01	0.003	0.034	<0.001	0.18	<0.001	0.04	<0.001	-	-	-	-

Figure 8.3. Age and sex distributions of cerebral and aortic physiological indices. Results are presented as the mean and confidence interval of each index, for each quintile of age split by sex. Results are presented for aortic systolic blood pressure (SBP), aortic diastolic blood pressure



There were at best weak associations between SBP with PSV and DBP with EDV, before and after adjustment for risk factors (table 8.5). In contrast, there was a strong association between aortic PP and MCA PI, which persisted after adjustment for age, sex and cardiovascular risk factors. There were similarly strong associations with aortic pulse wave velocity, with weaker associations with aortic augmentation index.

8.4.5 Multivariate associations of pulsatility and stiffness

In multivariate analyses of all haemodynamic aortic indices, there were only weak associations between absolute aortic pressure values and MCA PSV or EDV, whether aortic BP was defined by aortic SBP and aortic DBP or aortic mean pressure. However, aortic pulse pressure was a significant predictor of absolute cerebral blood flow velocity after adjustment for age and sex and cardiovascular risk factors, albeit with loss of this association following adjustment for heart rate and augmentation index (table 8.5).

In contrast, aortic pulse pressure was particularly strongly associated with MCA-PI, predicting 37% of the variance in cerebral pulsatility index, with an inverse association with mean aortic BP. Addition of age, sex and cardiovascular risk factors only explained a further 6% of the variance in MCA-PI, with a small additional effect of adjusting for heart rate and augmentation index. The fully adjusted model explained nearly 50% of the variance in MCA-PI (table 8.5). Similar effects were found for models defining aortic BP as aortic SBP and DBP rather than pulse pressure and MBP. Within the models, aortic PP remained the strongest predictor of MCA-PI, with a standardised beta coefficient of 0.37 exceeding the standardised beta-coefficient for age (0.30).

In addition to the strong relationship between ao-PP and MCA-PI, PWV predicted an earlier arrival of the pulse wave at the MCA (MCA arrival time $p<0.0001$), including after adjustment for age and sex ($p=0.003$). Furthermore, aortic PWV partially mediated the relationship between aortic PP and MCA-PP, explaining 24% of the relationship between aoPP and MCA-PI ($p<0.0001$), although this fell to 9% after adjustment for age and sex ($p<0.0001$).

Table 8.5. Physiological determinants of cerebrovascular indices. Results of general linear models are presented for each cerebral blood flow index (PSV, EDV and PI) for model 1: including aortic pulse wave velocity (PWV), aortic pulse pressure (Aor-PP) and Aortic mean blood pressure (Aor-MBP); or for model 2: including all factors in model 1 plus Aortic augmentation index (Aor-Aix) and R-R interval, the inverse of heart rate. Both models are presented unadjusted for clinical characteristics, adjusted for age and sex and adjusted for age, sex and cardiovascular risk factors (diabetes, history of hypertension, current smoking, ever smoking).

	MCA Peak Velocity						**MCA Trough Velocity**						**MCA Pulsatility Index**					
	Physiology		**Adj Age/Sex**		**Adj CV RFs**		**Physiology**		**Adj Age/Sex**		**Adj CV RFs**		**Physiology**		**Adj Age/Sex**		**Adj CV RFs**	
	β	**p**	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**	**β**	**p**
Model 1	**R^2 =**	**0.01**	**R^2 =**	**0.06**	**R^2 =**	**0.07**	**R^2 =**	**0.12**	**R^2 =**	**0.24**	**R^2 =**	**0.25**	**R^2 =**	**0.37**	**R^2 =**	**0.42**	**R^2 =**	**0.43**
PWV	-0.08	0.054	0.06	0.216	0.05	0.383	-0.27	<0.001	-0.05	0.301	-0.03	0.564	0.31	<0.001	0.18	<0.001	0.12	0.003
Aor-PP	0.13	0.007	0.14	0.007	0.15	0.006	-0.2	<0.001	-0.11	0.016	-0.08	0.111	0.51	<0.001	0.39	<0.001	0.37	<0.001
Aor-MBP	-0.07	0.129	-0.08	0.09	-0.07	0.149	0.12	0.005	0.06	0.151	0.05	0.22	-0.3	<0.001	-0.23	<0.001	-0.22	<0.001
Model 2	**R^2 =**	**0**	**R^2 =**	**0.06**	**R^2 =**	**0.07**	**R^2 =**	**0.12**	**R^2 =**	**0.22**	**R^2 =**	**0.23**	**R^2 =**	**0.41**	**R^2 =**	**0.46**	**R^2 =**	**0.49**
PWV	-0.03	0.47	0.13	0.021	0.12	0.043	-0.26	<0.001	-0.04	0.449	-0.01	0.825	0.38	<0.001	0.25	<0.001	0.19	<0.001
Aor-PP	0.06	0.253	0.08	0.208	0.07	0.241	-0.17	0.002	-0.1	0.069	-0.06	0.28	0.39	<0.001	0.31	<0.001	0.26	<0.001
Aor-MBP	-0.08	0.096	-0.09	0.105	-0.08	0.167	0.1	0.035	0.04	0.398	0.02	0.63	-0.32	<0.001	-0.25	<0.001	-0.23	<0.001
Aor-Aix	0.07	0.112	0.07	0.175	0.09	0.103	-0.02	0.589	0.02	0.601	0.04	0.393	0.13	<0.001	0.06	0.123	0.08	0.058
R-R int.	0	0.926	0.05	0.307	0.06	0.205	-0.09	0.039	-0.04	0.304	-0.05	0.28	0.13	<0.001	0.11	0.001	0.15	<0.001

8.5 Discussion

In a large population of patients with TIA or minor stroke, cerebral arterial pulsatility was strongly associated with aortic pulsatility and stiffness, with similar distributions by age, sex and cardiovascular risk factors. In contrast, there were minimal to weak relationships between absolute measures of aortic pressure (SBP or DBP) and absolute measures of cerebral blood flow. There were additional independent associations between cerebral pulsatility with measures of arterial stiffness and heart rate, with small additional effects of age, sex or cardiovascular risk factors. Overall, nearly half of the variance in cerebral pulsatility across the population was explained, suggesting a strong dependence of cerebral pulsatility on aortic pulsatility and arterial stiffness.

Middle cerebral arterial pulsatility is strongly associated with cerebral small vessel disease,[8, 11] lacunar stroke[12, 13] and the risk of recurrent stroke, independently of age and sex, and independent of hypertension and diabetes. Cerebral pulsatility is a potential therapeutic target but its reduction requires a more detailed understanding of its determinants. Although previously assumed to principally reflect changes in distal resistance in the brain,[14] our study demonstrates a strong dependence of MCA pulsatilty on aortic pulse pressure and further demonstrates that this relationship is dependent upon arterial stiffening, particularly due to central stiffening (aortic PWV) with a likely contribution of increased wave reflection from peripheral vessels (augmentation index). Partial mediation of the aortic cerebral pulsatility relationship by arterial stiffness, and the association of aortic stiffness with early arrival time of the pulse, also implies that increased aortic stiffness increases transmission of the aortic waveform to the brain, increasing pulsatility in the middle cerebral artery.

Our study is the first and largest of its type to assess systemic cardiovascular physiology with cerebral haemodynamic function in the same population of high-risk individuals with TIA or minor stroke. As such, it provides the best description

available of the comparative distribution of changes in arterial stiffness and cerebral physiological indices in this patient group, with similar distributions of aortic and cerebral indices with increasing age and by sex. Furthermore, it confirms associations with a history of hypertension and diabetes, but limited association with dyslipidaemia or a history of current or previous smoking, reaffirming the importance of effective blood pressure control in early to mid-life[15] and consistent with recent reports of a strong dependence of arterial stiffness and associated vascular changes on mid-life hypertension.[15]

In contrast to the strong associations between aortic pulsatility with cerebral arterial pulsatilty, we demonstrated a comparatively weak association between absolute blood pressure indices and absolute measures of cerebral blood flow velocity. However, these patients had undergone significant blood pressure control that may have resulted in a short-term blood pressure reduction but persistent effects of previous hypertension on the cerebral vasculature, resulting in discordance between current aortic and cerebral values. This shifting of the 'set-point' of the relationship between absolute aortic pressures and cerebral blood flow velocities implies an important role of local determinants of absolute blood pressure and absolute blood flow, such as cerebrovascular tone and stiffness. In contrast, the strong association between aortic and cerebral pulsatility, despite blood pressure lowering, implies that effects on systolic and diastolic pressures in an individual occur in parallel with a preserved relative difference, such that cerebral pulsatility still reflects enhanced transmission of the persistently pulsatile aortic waveform to the brain. Interventions to reduce cerebral pulsatility are therefore likely to require a reduction in aortic pulsatility,[16] a reduction in the speed and magnitude of wave reflections or a reduction in arterial stiffness itself.[17] The Eclipse study suggested that a vasodilating phosphodiesterase inhibitor, cilostazol, reduced cerebral arterial pulsatility,[18] but did not also assess effects on aortic blood pressure and therefore could not determine the site of action. Similarly, the CAFÉ study[16] suggested a

greater effect of vasodilating antihypertesive medications on aortic blood pressure than vasoconstricting treatments, but did not assess cerebral blood flow. A number of trials are currently underway to assess the effects of vasodilators on cerebrovascular function, including TREAT-SVDs, LACI-1/2[19] and the OxHARP trial, the latter of which will specifically determine the effect of vasodilators on cerebral arterial pulsatility in patients with mild to moderate white matter hyperintensities.

Our study does have some limitations. Firstly, it was performed over a prolonged period in unselected patients in a follow-up clinic. It therefore included a large number of frail and elderly participants in whom physiological assessments could not always be performed and who are more prone to artefacts due to poor temporal bone windows. However, this population therefore also reflects the broad spectrum of patients most at risk of future events, and includes the more elderly and frail patients at the greatest risk of progression of small vessel disease. Secondly, in this study we have not reported the relationship with small vessel disease and clinical outcomes. However, this study was focused on the possible cause of increased cerebral pulsatility, which has previously been shown to strongly correlate with small vessel disease. With longer follow-up in this population, we will be able to determine the direct and indirect associations of each component of this pathway with clinical outcomes. However, ultimately an interventional study will be needed to prove the effect of changes in blood pressure or aortic pulsatility on cerebral haemodynamics and resulting clinical outcomes.

The relationships between arterial stiffness, aortic pulsatility, cerebrovascular pulsatility, small vessel disease and stroke risk, as well as potential associated effects of cerebrovascular reactivity and autoregulation, represents a constellation of physiological mechanisms that are potential targets to prevent progression of cerebral small vessel disease, recurrent stroke and cognitive decline. Our study clarifies the underlying mechanisms that can be targeted to reduce cerebral pulsatility, their relative importance and their comparative distribution within the

population, with a key focus on aortic stiffness and pulsatility. As such, it provides a framework to understand the premorbid factors leading to the changes in this population, a baseline for ongoing assessment of risk and disease progression, and a series of therapeutic targets to test in clinical trials, both to determine their physiological effects and ultimately their effects on clinical outcomes.

Cerebral arterial pulsatility is strongly associated with aortic pulsatility, which in turn depends upon aortic stiffness and augmentation of aortic pressure. This study has defined the relative distribution of these indices within the population, demonstrating potential for significant impact at the population level through treatments to reduce cerebral arterial pulsatility through targeting aortic and systemic arterial pulsatility.

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CHAPTER NINE

Progression of aortic stiffness and cerebral pulsatility despite best medical management

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9.1 Summary

Increased cerebral arterial pulsatility is associated with cerebral small vessel disease, recurrent stroke and dementia despite best medical treatment. However, no study has identified rates and determinants of progression of stiffness and pulsatility.

In consecutive patients within 6 weeks of TIA or non-disabling stroke (Oxford Vascular Study), arterial stiffness (PWV) and aortic systolic (aoSBP), diastolic (aoDBP) and pulse pressures (aoPP) were measured by applanation tonometry (Sphygmocor), whilst middle cerebral artery peak (MCA-PSV) and trough (MCA-EDV) flow velocity and Gosling's pulsatility index (MCA-PI) were measured by transcranial ultrasound. Repeat assessments were performed at the 5 year follow-up visit and agreement determined by intraclass correlation coefficients. Rates of progression and their determinants were determined by mixed-effect linear models, adjusted for cardiovascular risk factors.

188 of 310 surviving, eligible patients had repeat assessments after a median of 5.8 years. PWV, aoPP and MCA-PI were highly reproducible (ICC 0.71,0.59 and 0.65 respectively), with progression of PWV (2.4%, $p<0.0001$) and aoPP (3.5%, $p<0.0001$) but not MCA-PI overall (0.93, $p=0.22$). However, PWV increased at a faster rate with increasing age (0.009m/s/yr/yr, $p<0.0001$), whilst aoPP and MCA-PI increased significantly above 55 years (aoPP $p<0.0001$, MCA-PI $p=0.009$). Higher aortic SBP and DBP predicted a greater rate of progression of PWV and aoPP, but not MCA-PI, although current MCA-PI was strongly associated with concurrent aoPP ($p<0.001$).

Arterial pulsatility and aortic stiffness progressed significantly after 55 years of age despite best medical treatment. Progression of stiffness and aortic pulsatility was determined by high blood pressure, but MCA-PI predominantly reflected current aortic pulsatility. Treatments targetting cerebral pulsatility may need to principally target aortic stiffness and pulsatility to have the potential to prevent cerebral small vessel disease.

9.2 Introduction

Small vessel disease is associated with acute lacunar stroke,[1] progressive cognitive decline,[2] late-onset refractory depression,[3] functional impairment in daily living[4] and increased mortality,[5]. White matter hyperintensities are highly prevalent in the population, affecting over half of people over the age of 65 and the majority of people over 85. However, even patients with advanced imaging changes can remain functionally independent,[6], providing an opportunity for intervention to prevent progression of SVD and subsequent clinical events. White matter hyperintensities are particularly associated with a history of hypertension and markers of vascular aging,[1] such as aortic stiffness and pulsatility of blood flow in the aorta and the cerebral circulation.[7] However, despite suggestive post-hoc analyses of clinical trials,[8, 9], no dedicated prospective study has identified treatments to reduce progression of cerebral small vessel disease.[10, 11]

In addition to understanding the cross-sectional relationships between aortic stiffness, aortic pulsatility and cerebral pulsatility (chapter 8), reducing their effects and the potential to reduce resulting clinical harms depends upon understanding the rate of progression and identification of the physiological processes responsible for elevated pulsatility. In cross-sectional analyses, cerebral arterial pulsatility was strongly associated with aortic pulsatility, a relationship that is partially mediated by arterial stiffness (chapter 8), implying increased transmission of aortic pulsatility to the brain through stiff vessels may underlie cerebral small vessel disease.[7] However, in chapter 8, I provided no evidence of the temporal relationship between arterial stiffness, aortic pulsatility and cerebral pulsatility and their progression over time. Furthermore, an accurate estimate of the rate of progression and its determinants is critical for the planning and interpretation of clinical trials to prevent progression of arterial stiffness and pulsatility,[12] particularly after control of classical cardiovascular risk factors such as hypertension.

I therefore determined the rates of progression of residual arterial stiffness, aortic pulsatility and cerebral arterial pulsatility and their determinants over 5 years in a population of patients with recent TIA or minor stroke, after optimal control of blood pressure and cardiovascular risk factors according to current guidelines.

9.3 Methods

9.3.1 Study Population

Consecutive, consenting patients with TIA or minor stroke were recruited between September 2010 and September 2019, as part of the Phenotyped Cohort of the Oxford Vascular Study (OXVASC).[13, 14] As before, participants were recruited at the OXVASC daily emergency assessment clinic, following a referral from primary care or after attendance at the Emergency Department, usually within 24 hours. Patients were referred after transient neurological symptoms or symptoms consistent with a minor stroke, not requiring direct admission to hospital.

Since August 2017, all surviving participants still registered with OXVASC primary-care physicians are eligible to undergo a repeat physiological assessment when attending for their 5 year follow-up visit (see chapter 5). Participants undergoing a repeat study as part of an assessment for a recurrent cerebrovascular event more than 2.5 years after their initial physiological assessment could also be included. Participants were excluded if they were under 18 years, cognitively impaired (MMSE<23), pregnant, had autonomic failure, a recent myocardial infarction, unstable angina, heart failure (NYHA 3-4 or ejection fraction <40%) or untreated bilateral carotid stenosis (>70%). OXVASC is approved by the Oxfordshire Research Ethics Committee. Physiological tests were performed at rest in a quiet, dimly-lit, temperature-controlled room (21-23^{0}C). Applanation tonometry (Sphygmocor, AtCor Medical, Sydney, Australia) was used to measure carotid-femoral pulse wave velocity (aortic-PWV), aortic augmentation index and aortic systolic and diastolic blood pressure and pulse pressure (ao-SBP, ao-DBP, ao-PP).[13] TCD (Doppler Box, Compumedics DWL, Singen, Germany) was performed

with a 2MHz probe at the temporal bone window on the same side as carotid applanation, where possible.

9.3.2 Analysis

Consistency in measures between baseline and follow-up were determined by intraclass correlation coefficients and linear regression. Significant changes in indices between baseline and follow-up were assessed by paired t-test. Rates of progression of measures of arterial stiffness, aortic blood pressure and cerebral blood flow velocity were determined by linear mixed effect modelling, with autoregressive covariance structure to account for repeated measures. Analyses were stratified by age and gender, with rates of progression determined by the interaction with the time interval between visits, as continuous indices and stratified by age group (<55, 55-65, 65-75, >75). Potential determinants of absolute values and rates of progression were assessed, unadjusted and adjusted for age, gender and cardiovascular risk factors (smoking, dyslipidaemia, diabetes, hypertension).

9.4 Results

9.4.1 Study Population

188 of 310 patients were seen for follow up at a median of 5.8 years after the initial assessment. Of the included patients, 150 had arterial stiffness assessments at baseline and follow-up, whilst 139 had TCD performed on both occasions. Demographic characteristics were similar between patients undergoing arterial stiffness and TCD measures (Table 9.1).

Table 9.1. Demographics of patients with repeat studies after 5 years follow-up. The table shows distribution of patients who underwent pulse wave velocity (PWV) measures or cerebral pulsatility measures (PI) at baseline and follow-up. Results are reported as frequency (percentage) or as mean (standard deviation). The p-value (p) is shown for t-tests or a test of proportions. BMI = body mass index; SBP =systolic blood pressure; DBP = diastolic blood pressure.

.	PWV	PI	p
n	150	139	
Age	66 (10.9)	65 (10.9)	0.4
Female	57 (38)	50 (36)	0.81
Hypertension	112 (74.7)	102 (73.4)	0.91
Diabetes	19 (12.7)	13 (9.4)	0.48
Atrial Fibrillation	13 (8.7)	12 (8.6)	1
Dyslipidaemia	116 (77.3)	107 (77)	1
Current Smoker	13 (8.7)	16 (11.5)	0.54
Past Smoker	67 (44.7)	64 (46)	0.91
Medications	-	-	-
Antiplatelet	119 (79.3)	108 (77.7)	0.85
Antihypertensives	109 (72.7)	97 (69.8)	0.68
Statins	111 (74)	103 (74.1)	1
Weight (Kg)	77.6 (14.8)	78.9 (16.2)	0.52
BMI (Kg/m2)	26.6 (4.1)	26.8 (4.6)	0.57
Creatinine	78.4 (19.8)	76.4 (19.2)	0.36
SBP	149.3 (24.8)	150.9 (24.8)	0.45
DBP	85.3 (13.7)	86.4 (13.7)	0.4

9.4.2 Reproducibility and progression of arterial stiffness and pulsatility

Arterial stiffness (PWV), aortic pulsatility and cerebral arterial pulsatility were all highly reproducible within individuals over the five years of follow-up (table 2), with PWV the most reproducible index. Absolute indices of aortic blood pressure or cerebral blood flow velocity were also signficantly reproducible, but less so than pulsatility or arterial stiffness measures, with weaker correlations between baseline and follow-up values (table 9.2, figure 9.1).

There were significant increases in arterial stiffness and aortic pulsatility between baseline and follow-up, with the greatest annual increase in aortic pulse pressure (3.5% per year) and only a non-significant, increase in cerebral arterial pulsatility across all patients (0.93%, table 9.2). The increase in aortic pulsatility reflected a significant fall in aortic DBP (table 9.2), with a rise in aortic SBP (p=0.01), but both cerebral peak and trough flow velocities fell with time (table 9.2).

Table 9.2. Reproducibility and progression of arterial stiffness, aortic blood pressure and cerebral blood flow velocity.

Index	ICC	ICC 95%CI	r^2	p	Baseline Mean	FU Mean	t-test p	Annual change	Annual change (%)
PWV	0.71	(0.62 - 0.78)	0.42	<0.001	9.18	10.3	<0.001	0.19	2.4
Aortic SBP	0.45	(0.29 - 0.57)	0.10	<0.001	117	122	0.0068	0.80	0.87
Aortic DBP	0.47	(0.32 - 0.59)	0.10	<0.001	74.1	72.2	0.016	-0.31	-0.24
Aortic PP	0.59	(0.47 - 0.68)	0.23	<0.001	42.8	49.4	<0.001	1.1	3.5
MCA PSV	0.59	(0.47 - 0.69)	0.18	<0.001	81.8	80	0.29	-0.36	0.23
MCA EDV	0.70	(0.60 - 0.77)	0.3	<0.001	39.5	37.7	0.017	-0.36	-0.39
MCA PI	0.65	(0.54 - 0.74)	0.24	<0.001	0.808	0.827	0.22	0.0037	0.93

9.4.3 Change in arterial stiffness with age

Carotid-femoral PWV was higher at older ages, with no significant yearly increase in patients below the age of 55, but PWV increased at a greater annual rate with increasing age (figure 9.2). Although pulse wave velocity was greater in men at each age, the rate of increase in PWV with age was similar in men and women (figure 9.1). Similarly, both aortic pulse pressure and cerebral arterial pulsatility increased at a greater rate above the age of 55 with no significant increase before 55 years (>55 vs < 55: aoPP p=0, MCA-PI p=0.009), although the rate of change of aoPP or MCA-PP above 55 years was not consistent across age groups (figure 9.2).

Figure 9.1. Distribution and reproducibility of middle cerebral artery pulsatility index (PI), aortic pulse wave velocity (PWV) and aortic pulse pressure (AoPP).

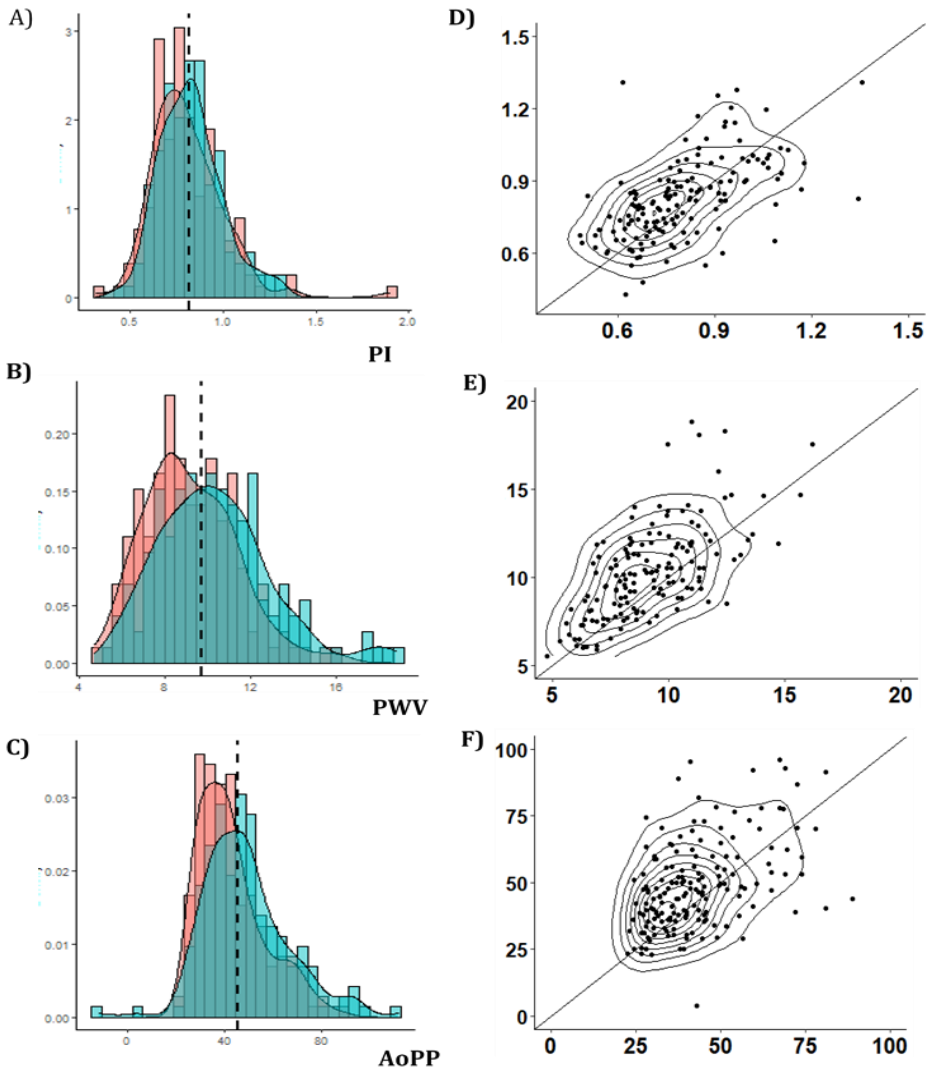
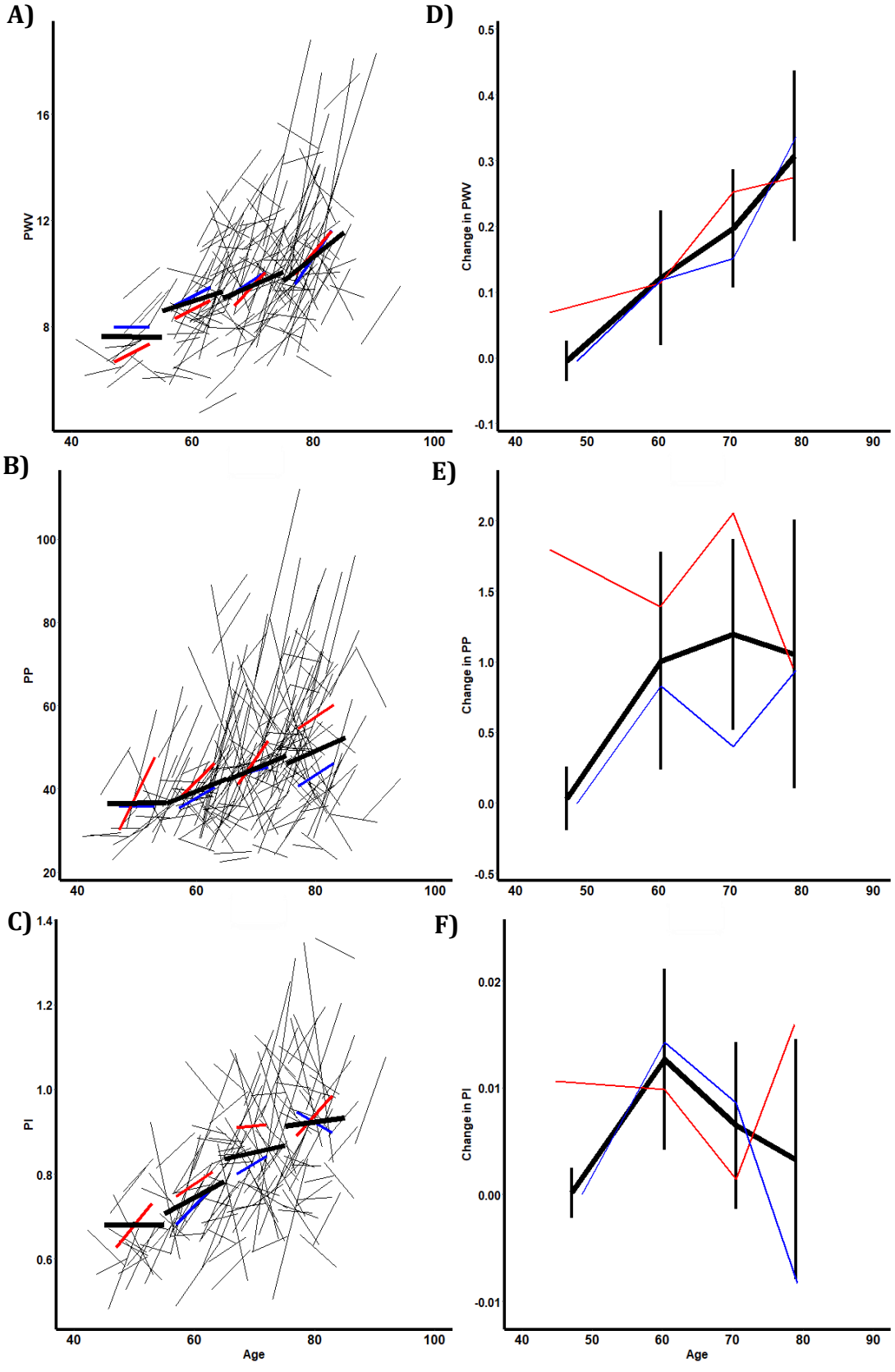


Figure 9.2. Progression of aortic blood pressure and cerebral blood flow velocity by age and gender. Panels A-C show individual changes during follow-up, and summary estimates within age groups (<55, 55-65,65-75, >75), for all patients (black), for men (blue) and women (red). Panels D-F show the average rate of progression within each age group, stratified by age



Aortic pulsatility was significantly greater in women than men and increased at a faster rate (Table 9.3), whilst MCA-PI was non-significantly greater in women than men with no difference in the rate of increase (Figure 9.2, Table 9.3). The rise in aortic and cerebral pulsatility at older ages was paralleled by a higher aortic SBP with increasing age, with a higher rate of increase at older ages. In contrast, PSV and EDV were lower at older ages, and were lower in men compared to women, whilst the rate of change in PSV and EDV was similar across ages (table 9.4, figure 9.3).

Figure 9.3. **Progression of aortic blood pressure and cerebral blood flow velocity by age and gender.** Summary estimates within age groups (<55, 55-65,65-75, >75) are shown for all patients (black), for men (blue) and women (red).

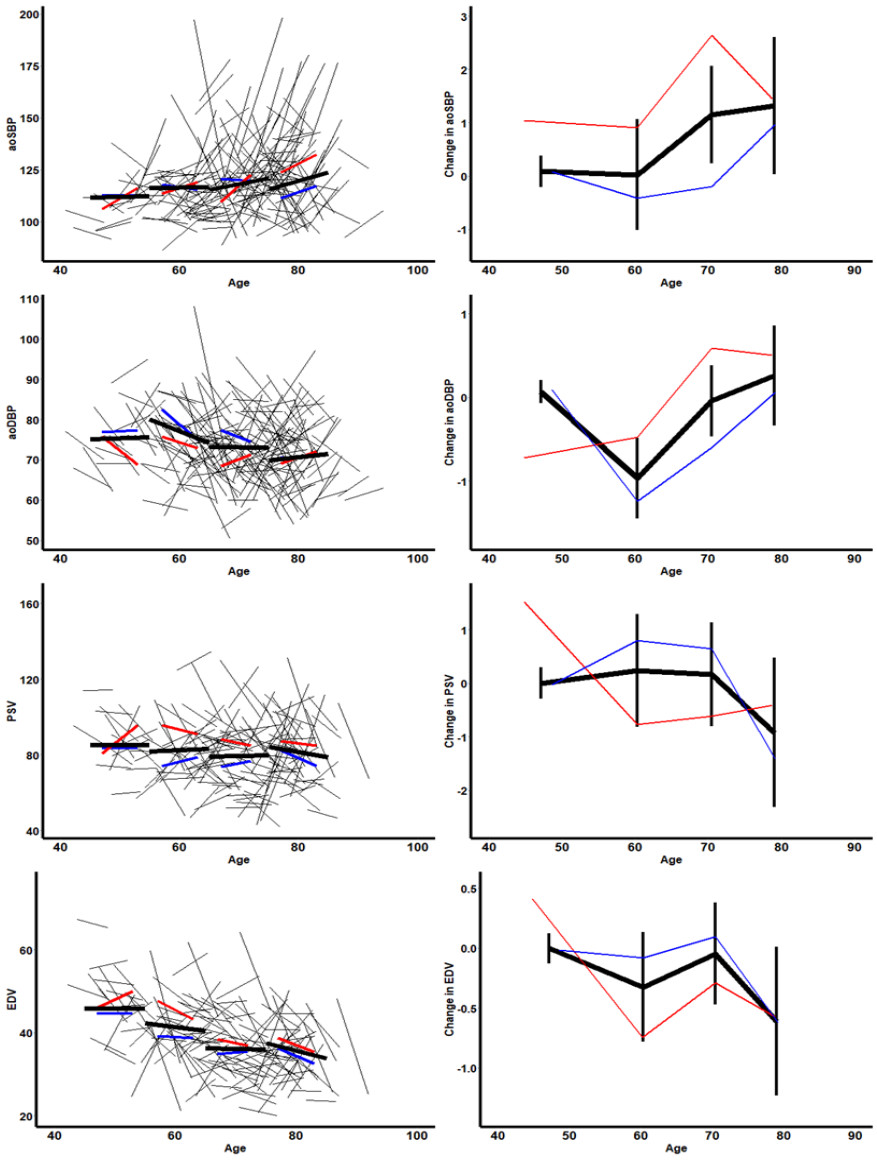


Table 9.3. Associations between demographic characteristics and progression of key indices of arterial stiffness and pulsatility. Results from mixed effect linear models, adjusted for age, gender and cardiovascular risk factors are shown, for absolute level and progression (change) of aortic pulse wave velocity (PWV), aortic pulse pressure (PP) and middle cerebral artery pulsatility index (PI). B = unstandardized beta-coefficient; p= p-value. Beta-coefficients and p-values for change in each index are derived from the interaction term between the index of interest and the time interval between assessments.

Index	PWV		PWV change		PP		PP change		PI		PI change	
	b	p	b	p	b	p	b	p	b	p	b	p
Female	-0.59	0.068	0.18	0.00035	3.4	0.092	1.6	<0.0001	0.024	0.34	0.0078	0.049
Age	0.075	<0.0001	0.009	<0.0001	0.36	<0.0001	0.045	0.00023	0.0067	<0.0001	0.00029	0.038
Hypertension	0.78	0.045	-0.054	0.41	5	0.043	0.022	0.96	0.048	0.092	-0.0081	0.082
Diabetes	0.93	0.071	0.2	0.013	-3	0.35	0.62	0.28	0.049	0.27	0.00036	0.96
Atrial Fibrillation	-1.1	0.081	0.22	0.033	-5.6	0.14	1.2	0.1	-0.096	0.033	0.00098	0.9
Dyslipidaemia	0.3	0.47	-0.13	0.05	-2.9	0.27	0.1	0.83	0.0053	0.86	-0.0023	0.66
Smoking	0.37	0.26	-0.16	0.00021	7.1	0.00084	-0.94	0.002	0.068	0.0068	-0.0082	0.013
Current Smoking	0.045	0.94	0.051	0.62	1.6	0.63	0.29	0.66	-0.0043	0.91	0.0027	0.71
Weight	0.027	0.016	-0.0055	0.00072	0.03	0.67	-0.024	0.028	-0.0011	0.2	0	0.44
BMI	0.078	0.05	-0.0098	0.16	-0.15	0.54	0.036	0.43	-0.0031	0.27	0	0.95
Antihypertensive	0.61	0.11	-0.03	0.64	3.4	0.15	0.35	0.43	0.044	0.11	-0.0078	0.088
Statin	0.33	0.39	-0.087	0.17	-1.2	0.62	0.11	0.81	0.016	0.58	-0.0029	0.55

Table 9.4. Asssociation between demographic measures and progression of absolute measures of aortic blood pressure and cerebral blood flow velocity. Results from mixed effect linear models, unadjusted for covariates, for absolute level and progression (change) of aortic systolic (SBP) and diastolic (DBP) blood pressure and middle cerebral artery peak (PSV) and end-diastolic (EDV) flow velocity. B = unstandardized beta-coefficient; p= p-value. Beta-coefficients and p-values for change in each index are derived from the interaction term between the index of interest and the time interval between assessments.

Index	SBP		SBP Change		DBP		DBP Change		PSV		PSV change		EDV		EDV change	
	b	p	b	p	b	p	b	p	b	p	b	p	b	p	b	p
Female	1.1	0.67	1.8	<0.001	-2.3	0.072	0.18	0.4	10	<0.001	-0.43	0.38	4.1	0.002	-0.39	0.069
Age	0.29	0.01	0.041	0.011	-0.08	0.18	-0.004	0.58	-0.27	0.035	-0.007	0.67	-0.37	<0.001	-0.012	0.12
Hypertension	6	0.052	0.51	0.4	0.94	0.54	0.5	0.079	-0.85	0.8	0.22	0.7	-1.6	0.29	0.36	0.15
Diabetes	-3.7	0.36	0.11	0.88	-0.5	0.81	-0.54	0.14	15	0.006	-0.72	0.43	4.5	0.062	-0.097	0.81
AF	-2.5	0.6	1	0.27	3.1	0.2	-0.11	0.81	1.1	0.84	-0.92	0.35	3.2	0.19	-0.36	0.41
Dyslipidaemia	-7.8	0.018	0.8	0.21	-4.9	0.003	0.7	0.021	3	0.41	0.25	0.7	1.6	0.33	0.2	0.47
Smoking ever	3.8	0.15	-0.39	0.33	-3.3	0.013	0.55	0.004	0.009	1	-0.008	0.98	-2.1	0.14	0.28	0.11
Current Smoking	5.3	0.21	-0.79	0.36	3.7	0.081	-1.1	0.009	0.021	1	0.31	0.73	-0.14	0.95	0.14	0.72
Weight	0.082	0.34	-0.03	0.037	0.05	0.24	-0.006	0.41	-0.13	0.18	0.033	0.03	-0.03	0.5	0.018	0.009
BMI	0.006	0.98	-0.004	0.94	0.15	0.33	-0.039	0.17	-0.27	0.42	0.11	0.083	-0.02	0.89	0.043	0.12
BP lowering drugs	3.9	0.19	1	0.075	0.37	0.8	0.7	0.013	-1.9	0.56	0.18	0.75	-2	0.19	0.35	0.15
Statin	-5.5	0.071	0.96	0.1	-4.2	0.006	0.85	0.002	2.6	0.46	-0.053	0.93	1.1	0.49	0.079	0.76

9.4.5 Change in arterial stiffness and pulsatility with physiological measures

Elevated aortic SBP, DBP and pulse pressure were all associated with a greater rate of progression of PWV (table 9.5), but cross-sectionally, higher PWV was associated with a lower concurrent DBP reflecting the decrease in DBP with time and age. After adjustment for heart rate and other aortic blood pressure values, only a higher aortic mean pressure was associated with a greater rate of increase in PWV with time (p=0.048). Similarly, higher aortic SBP and DBP, elevated heart rate and a greater cerebral pulsatility were associated with a greater rate of increase in aortic pulse pressure, with persistent associations with progression of aortic pulse pressure and aortic systolic and diastolic blood pressure, even after adjustment for arterial stiffness and aortic blood pressure (p=0.0001 for both).

In contrast to aortic measures, a greater rate of progression of MCA-PI was only associated with increased age and female gender (table 9.4) and a lower cerebral end-diastolic velocity (table 9.6), with no association between blood pressure level or arterial stiffness with progression of MCA-PI before or after adjustment for age, gender and cardiovascular risk factors. However, there were strong cross-sectional associations between MCA-PI and a concurrent high aortic SBP, low aortic DBP and high aortic PP.

Table 9.5. Associations between physiological characteristics and progression of key indices of arterial stiffness and pulsatility. Results from mixed effect linear models, adjusted for age, gender and cardiovascular risk factors are shown, for absolute level and progression (change) of aortic pulse wave velocity (PWV), aortic pulse pressure (PP) and middle cerebral artery pulsatility index (PI). Beta-coefficients and p-values for change in each index are derived from the interaction term between the index and the time interval between assessments. b = unstandardized beta-coefficient; p= p-value; SBP = systolic blood pressure; DBP =diastolic blood pressure; MCA = middle cerebral artery; PSV = peak systolic velocity; EDV = end-diastolic velocity; PI = pulsatility index.

Index	PWV		PWV change		PP		PP change		PI		PI change	
	B	p	b	p	b	p	b	p	b	p	b	p
Heart Rate	-0.004	<0.001	0.0003	0.059	0.014	0.044	0.003	0.034	<0.001	0.0098	0	0.86
HRV	-0.001	0.98	0.0028	0.73	0.084	0.79	-0.062	0.28	-0.008	0.02	0.0002	0.69
PWV					1.9	0.0003	0.059	0.5	0.011	0.079	0	0.96
Ao SBP	0.013	0.13	0.0047	8e-04	0.62	<0.001	0.016	<0.001	0.0014	0.034	0.0001	0.18
Ao DBP	-0.01	0.48	0.011	1e-04	0.1	0.26	0.082	<0.001	-0.003	0.0057	0.0002	0.46
Ao PP	0.031	0.007	0.0038	0.05					0.0044	<0.001	0	0.60
MCA PSV	-0.008	0.37	0	0.77	0.13	0.037	-0.008	0.5	0.0031	<0.001	0	0.18
MCA EDV	-0.021	0.27	-0.005	0.057	-0.003	0.98	-0.051	0.0086	-0.003	0.0068	0	0.014
MCA PI	0.44	0.66	0.44	0.0054	30	<0.001	3.2	0.0023				

Table 9.6. Asssociation between physiological measures and progression of absolute measures of aortic blood pressure and cerebral blood flow velocity. Results from mixed effect linear models, unadjusted for covariates, for absolute level and progression (change) of aortic systolic (SBP) and diastolic (DBP) blood pressure and middle cerebral artery peak (PSV) and end-diastolic (EDV) flow velocity. B = unstandardized beta-coefficient; p= p-value. Beta-coefficients and p-values for change in each index are derived from the interaction term between the index of interest and the time interval between assessments.

Index	SBP		SBP Change		DBP		DBP Change		PSV		PSV change		EDV		EDV change	
	b	p	b	p	b	p	b	p	b	p	b	p	b	p	b	p
Heart Rate	0.0074	0.4	0.0017	0.38	-0.007	0.14	-0.001	0.13	0.012	0.2	-0.001	0.46	-0.001	0.79	0	0.35
HRV	0.24	0.56	-0.052	0.49	0.15	0.46	0.017	0.65	-0.61	0.13	-0.029	0.69	-0.085	0.63	0.0028	0.94
PWV	2.5	0.0002	-0.021	0.85	0.64	0.051	-0.084	0.14	-0.76	0.31	0.037	0.78	-0.63	0.052	0.016	0.77
Ao SBP					0.38	<0.001	-0.016	<0.001	0.034	0.68	<0.001	0.97	-0.032	0.38	-0.004	0.49
Ao DBP	1.1	<0.001	0.082	<0.001					-0.28	0.042	-0.009	0.72	-0.027	0.67	-0.006	0.62
Ao PP	1.1	<0.001	0.011	0.21	0.071	0.14	0.011	0.21	0.21	0.058	-0.009	0.65	-0.036	0.47	-0.007	0.44
MCA PSV	0.034	0.67	-0.001	0.95	-0.1	0.011	0.0068	0.38					0.35	<0.001	0.0046	0.26
MCA EDV	-0.14	0.36	-0.029	0.25	-0.15	0.059	0.022	0.094	1.9	<0.001	-0.027	0.065				
MCA PI	20	0.027	3.1	0.029	-9.7	0.032	-0.1	0.89	43	<0.001	-1.6	0.22	-12	0.0022	-0.75	0.22

9.5 Discussion

This is the first study of progression of aortic stiffness, aortic pulsatility and cerebral arterial pulsatility in patients with recent TIA or minor stroke, despite good blood pressure control. Over five years, aortic stiffness and aortic systolic pressure rose whilst aortic diastolic pressure fell, with a resulting increase in aortic pulsatility. Cerebral pulsatility rose above the age of 55, reflecting a fall in end-diastolic velocity with no rise in peak systolic velocity. The rate of rise in PWV and aortic pulsatility was associated with age and elevated aortic blood pressure, but only age, female gender and a low end-diastolic flow velocity predicted an increased rate of rise in MCA-PI, although current MCA-PI level was strongly associated with concurrent aortic pulsatility.

In previous cross-sectional analyses in this population,[7] cerebral small vessel disease was associated with cerebral arterial pulsatility, which was principally associated with aortic stiffness and transmission of increased aortic pulsatility to the brain. Furthermore, there were similar distributions of aortic stiffness, aortic pulsatility and cerebral arterial pulsatility by age and sex, and the cross-sectional relationship between aortic pulsatility and cerebral arterial pulsatility was partially mediated by arterial stiffness (chapter 8).[17] This longitudinal follow-up study confirms the strong concurrent association between aortic pulsatility, aortic stiffness and cerebral arterial pulsatility, and further demonstrates that aortic blood pressure level predicts a greater increase in the rate of progression of aortic stiffness and pulsatility. As such, although an acute reduction in aortic pulsatility may reduce cererbal arterial pulsatility directly, control of mid-life elevated aortic blood pressure, and diastolic blood pressure in particular, may reduce progression of arterial stiffness and aortic pulsatility and therefore future cerebral small vessel disease and its sequelae (chapter 7). Furthermore, this population had undergone intensive blood pressure monitoring and treatment, with a minimal difference between treatment at the time of initial assessment

and at follow-up five years later.[18] Therefore this study demonstrates that residual aortic blood pressure and pulsatility was still associated with increased cerebral pulsatility despite excellent control of blood pressure and other risk factors, presenting a potentially modifiable risk factor in addition to current standard treatment.

The majority of the progression in arterial stiffness and pulsatility occurred after the age of 55, with steadily increasing rates of progression of arterial stiffness consistent with previous studies in hypertensive populations. Both aortic and cerebral pulsatility were greater above 55 years, but with a limited ongoing increase in the rate of progression thereafter. This apparent transition at approximately 55 years old is consistent with the average age of transition to late-life hypertensive phenotypes (chapter 7),[19] and the stronger relationship between mid-life elevated diastolic blood pressure with cerebral white matter hyperintensities,[20] compared to greater SBP and pulsatility above the age of 55. Furthermore, although DBP fell as arterial stiffness increased, a higher aortic DBP was still the strongest driver of progression of PWV, and therefore a subsequent fall in DBP. This indicates the likely critical role for control of mid-life DBP to prevent later progression of aortic stiffness and pulsatility, and resulting cerebral pulsatility.

Although no systemic haemodynamic measures were specifically associated with progression of cerebral pulsatility, current cerebral pulsatility was most strongly associated with concurrent aortic pulsatility. As such, targetting both excess aortic systolic pressure and diastolic pressure in mid-life whilst not excessively reducing diastolic pressure in late life, may reduce progression of aortic pulsatility and therefore later-life cerebral arterial pulsatility. This may be possible with peripheral vasodilators that reduce wave reflection from the peripheral circulation. For example, in the CAFE study,[21] amlodipine reduced aortic systolic pressure to a greater extent than a beta-blocker, whilst cilostazol reduced cerebral pulsatility in the ECLIPSE study.[22] Currently, isosorbide mononitrate and cilostazol are being assessed in the LACI-1/LACI-2 studies (ISRCTN 14911850),[23] antihypertensives in the TREAT-SVDs trial (NCT03082014) and sildenafil in the OxHARP trial (NCT03855332).

This study does have some limitations. Firstly, it is relatively small, with only 139 participants having TCD performed at baseline and follow-up. However, it is still significantly larger than any other available study recording both aortic and cerebral indices, and other studies have not assessed progression of these measures in this patient population.[24, 25] Furthermore, despite the moderate size, associations were strong and consistent with previous evidence whilst building upon it. Secondly, not all participants underwent both TCD and arterial stiffness measures. This is inevitable in a pragmatic study including all patients with TIA or minor stroke, due to the relatively high incidence of participants with poor bone windows for TCD or, for PWV, significant large artery disease or increased BMI. Thirdly, due to the age and frailty of the population only 50% of the baseline population could be reassessed at 5 years, as many were deceased or had moved out of the area. This may result in underestimation of the degree of progression of age-related variables due to attrition of higher risk patients with greater progression, but any resulting bias is likely to be conservative. Furthermore, the median age of the population was relatively old for a follow-up study (mean age 66 at baseline, 71 at follow-up), supporting the relevance of these results to the population most at risk. Finally, without repeat brain imaging, I was unable to assess whether progression of cerebral arterial pulsatility mediates the relationship between arterial stiffness, aortic pulsatility and progression of cerebral small vessel disease.

Overall, the strong relationship between residual aortic blood pressure after intensive blood pressure treatment and longitudinal progression of arterial stiffness and pulsatility supports the need for further research to determine the temporal relationship with other key physiological mechanisms affected in small vessel disease, such as blood pressure variability, cerebral autoregulation and cerebrovascular reactivity, that represent alternative potential treatment targets. Further trials will then be necessary to determine if modifying cerebral pulsatility or other mechanisms translates to a reduction

in progression of cerebral small vessel disease, proceeding to large clinical trials to determine if such interventions translate to meaningful clinical effects.

Arterial pulsatility and aortic stiffness progressed significantly over the age of 55 in a population of patients with TIA or minor stroke. Progression of aortic measures was most strongly associated with age and elevated aortic blood pressure, whilst only age, sex and low diastolic cerebral blood flow velocity predicted progression of cerebral arterial pulsatility. However, aortic pulsatility was the strongest association of concurrent cerebral pulsatility, identifying a potentially treatable mechanism to limit progression of cerebral small vessel disease.

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CHAPTER TEN

Increased cerebral arterial pulsatility and the risk relationship between leukoaraiosis and cerebrovascular events

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10.1 Summary

Aortic pulsatility is associated with cardiovascular events and middle cerebral arterial pulsatility is associated with cerebral white matter hyperintensities. However, it is unclear if cerebral pulsatility predicts recurrent cerebrovascular events and if it mediates the association between white matter hyperintensities and cerebrovascular events.

Patients were recruited from the Oxford Vascular Study within 6 weeks of a TIA or minor stroke with ascertainment of recurrent events face-to-face up to 5 years. Leukoaraiosis was categorised on MRI with the Fazekas scale. MCA pulsatility (Gosling's index: MCA-PI) was measured by transcranial ultrasound, whilst aortic pulse wave velocity (ao-PWV) and aortic systolic, diastolic and pulse pressure (SBP, DBP, PP) were determined by applanation tonometry (Sphygmocor).

In 411 patients, MCA-PI was strongly correlated with aortic pulsatility ($p<0.001$), with increasing aortic stiffness accentuating the association ($p=0.001$). Severity of leukoaraiosis was associated with MCA-PI and aortic PWV (Fazekas: MCA-PI 2.10, 1.65 - 2.66, $p<0.001$; PWV 2.02, 1.63-2.50, $p<0.001$), particularly in periventricular regions, with weaker associations with aortic SBP, DBP and pulse pressure. After adjusting for aortic and cerebral BP and pulsatility, as well as age and gender, MCA-PI was the only haemodynamic index associated with recurrent cerebrovascular events (HR=1.55, $p=0.02$) and all cardiovascular events (HR1.53, $p=0.049$). Severe leukoaraiosis was associated with an increased risk of cerebrovascular events, but this did not mediate the relationship between MCA-PI and outcome.

Cerebral arterial pulsatility reflected transmission of aortic pulsatility to the brain through stiff vessels and predicted recurrent cerebrovascular events independent of other haemodynamic indices and severity of white matter hyperintensities. Cerebral arterial pulsatility may represent a modifiable treatment target to reduce the effects of hypertension induced end-organ damage on the brain.

10.2 Introduction

Aortic stiffness reflects hypertension-induced end-organ damage and is associated with white matter hyperintensities and recurrent cerebrovascular events,[1] whilst the presence of white matter hyperintensities is associated with an increased risk of stroke,[2] dementia,[3] and functional decline.[4] However, the aetiology of white matter hyperintensities and their relationship with cerebrovascular events is not yet fully understood. The relative importance of haemodynamic factors as opposed to a primary microangiopathy[5] in the development of leukoaraiosis is unclear and associations with age, hypertension and diabetes are consistent with both processes (chapter 8). We previously demonstrated that leukoaraiosis is associated with increased middle cerebral artery pulsatility (MCA-PI), potentially reflecting increased transmission of aortic pulsatility to the brain through stiffened conduit vessels,[6] whilst other studies have similarly found a relationship between increased middle cerebral artery (MCA) pulsatility, white matter hyperintensities and the presence of previous lacunar infarction in patients with hypertension[7]. However, these relationships strongly covary with age and are susceptible to residual confounding, whilst no study has addressed the risk relationship between cerebral arterial pulsatility and recurrent cerebrovascular events and how this interacts with severity of white matter hyperintensities.

Therefore we performed the first study assessing the dependence of leukoaraiosis on arterial stiffness and pulsatility in both the aorta and middle cerebral artery in patients with recent TIA or minor stroke, and determined the extent to which these factors differentially predict the severity of leukoaraiosis and the risk of recurrent cerebrovascular events.

10.3 Methods

10.3.1 Study Population

Consecutive consenting and eligible participants within 6 weeks of a TIA or minor stroke (NIHSS<5) were recruited to the physiological substudy of the Oxford Vascular Study (OXVASC)[8] between January 2011 and September 2015,[9] from the acute TIA and stroke clinic associated with the study and followed up face-to-face for a minimum of 6 months. This includes the population with complete ascertainment of recurrent events upon closing of the database for this analysis. Participants were excluded if they were under 18 years, unable to have an MRI scan, cognitively impaired (MMSE<23), pregnant or had had a recent myocardial infarction (<1 month), unstable angina, heart failure (NYHA 3-4 or ejection fraction <40%) or untreated severe bilateral carotid stenosis (>70%) or occlusion. The study was approved by the Oxfordshire Research Ethics Committee.

MRI scans were performed during the acute clinical assessment on a 3T MRI system (Siemens Magnetom Verio) according to a standardised protocol using vendor-designed sequences. The protocol comprised T2-weighted TSE and FLAIR sequences, diffusion and susceptibility-weighted images, a post contrast T1-weighted spin-echo 2D sequence as well as a time-of-flight MRA of the intracranial vessels and a contrast enhanced MRA of the large neck arteries.

All axial T2 scans were scored according to a modified version of the Fazekas[10] scale by an experienced observer blinded to clinical and physiological data, as this score is the simplest, most commonly used and well-validated semi-quantitative score for leukoaraiosis. Scans were also graded by the ARWMC[11] score to demonstrate the consistency of the results. For comparison, the Fazekas and ARWMC scores were also categorised into four approximately equally sized groups (Fazekas: none 0, mild 1, moderate 2, severe ≥3; ARWMC: none 0, mild 1-3, moderate 4-9, severe ≥10).

Physiological tests were performed at rest in a quiet, dimly-lit, temperature-controlled room (21-23^{0}C). Applanation tonometry (Sphygmocor, AtCor Medical,

Sydney, Australia) was used to measure carotid-femoral pulse wave velocity (aortic-PWV), aortic augmentation index and central aortic systolic, diastolic and pulse pressure (ao-SBP, ao-DBP, ao-PP). TCD (Doppler Box, Compumedics DWL, Singen, Germany) was performed with a handheld 2MHz probe at the temporal bone window on the same side as carotid applanation. The waveform envelope was acquired at 100Hz simultaneously with ECG and blood pressure at 200Hz (Finometer, Finapres Medical Systems, The Netherlands), via a Powerlab 8/30 with LabChart Pro software (ADInstruments, USA). The MCA was insonated at 50mm, or if this was not adequate, at the depth giving the optimal waveform. All waveforms were visually inspected and beats corrupted by artefact were excluded. MCA pulsatility was calculated as Gosling's pulsatility index (MCA-PI= (systolic CBFV-diastolic CBFV) / mean CBFV).

10.3.2 Analysis

Differences between patient groups in continuous variables were assessed by t-tests or ANOVA, with tests for linear trend for severity of leukoaraiosis, whilst differences in frequencies were compared by chi-squared tests. Univariate relationships between continuous variables were assessed by linear regression. Multivariate predictors of continuous physiological outcomes were determined by general linear models, whilst predictors of relationships between leukoaraiosis severity and either clinical or physiological measures were assessed with ordinal regression. Recurrent cardiovascular events were ascertained by multiple overlapping methods including hot and cold pursuit and face-to-face follow up at 1,3,6,12 and 60 months, and reviewed by a panel of stroke phyicians or neurologists. Risk of recurrent events were determined by Cox Proportional Hazards regression and standardised to the standard deviation across the population for each physiological index. Outcomes included recurrent cerebrovascular events (definite or probable stroke or TIA), all cause mortality and major adverse cardiovascular events (MACE=CV death, stroke, myocardial infarction, acute limb ischaemia, acute aortic aneurysm event). All models were performed for univariate associations and for models combining principal

physiological indices (aortic BP, cerberal blood flow, aortic and cerebral pulsaitlity and aortic stiffness), with and without adjustment for age and gender.

All analyses were performed in Matlab R2010a, SPSS 17.0 and Microsoft Excel 2010.

10.4 Results

10.4.1 Study Population

411 / 448 eligible patients had adequate temporal bone windows for TCD, of whom 367 / 411 (89%) had an MRI, with the remainder having a CT scan. Of patients undergoing MRI, 30% had no leukoaraiosis, 45% had mild – moderate leukoaraiosis and 25% had severe leukoaraiosis (table 10.1).

Table 10.1. Demographic characteristics of patients with adequate TCD measurements, according to severity of leukoaraiosis.

	Fazekas Scale Score				
	0 (n=111)	1 (n=79)	2 (n=86)	≥3 (n=91)	p-value
Age	58.3 (15)	64.9 (17)	70.8 (9.7)	74.6 (8.5)	<0.001
Male	76 (67)	47 (54)	43 (51)	70 (58)	0.11
Hypertensive	26 (37)	26 (51)	29 (60)	57 (75)	<0.001
Diabetes	10 (8.8)	10 (11.5)	9 (10.5)	14 (11.5)	0.90
Family History*	21 (21.6)	28 (37.8)	20 (27.4)	29 (26.4)	0.13
Hyperlipidaemia	25 (21.9)	23 (26.4)	28 (32.6)	38 (31.1)	0.30
Atrial Fibrillation	2 (1.8)	6 (6.9)	11 (12.9)	16 (13)	0.007
Current smoker	27 (24)	10 (12)	8 (9.4)	15 (12)	0.016
Blood Pressure:					
Systolic	148 (21)	147 (22)	150.5 (20)	158 (24)	0.001
Diastolic	87 (14)	86.6 (14)	84.4 (12)	86.3 (12)	0.49
Creatinine	77 (18)	76.5 (23)	78.8 (24)	88 (26)	<0.001
BMI	26.6 (4.5)	27.3 (5)	27.5 (5.5)	27.1 (4.5)	0.63

10.4.2 Associations between demographics, leukoaraiosis and haemodynamics

Severity of leukoaraiosis was associated with increasing age, history of hypertension, atrial fibrillation, creatinine and baseline systolic blood pressure (Table 10.1). The same demographic variables were strongly associated with aortic pulse wave velocity, aortic pulse pressure and middle cerebral artery pulsatility index, except for no significant association with atrial fibrillation and no relationship between aortic pulse pressure and creatinine (table 10.2). In addition, pulsatility in both the aorta and MCA were greater in women whilst MCA-PI and PWV were greater in diabetics.

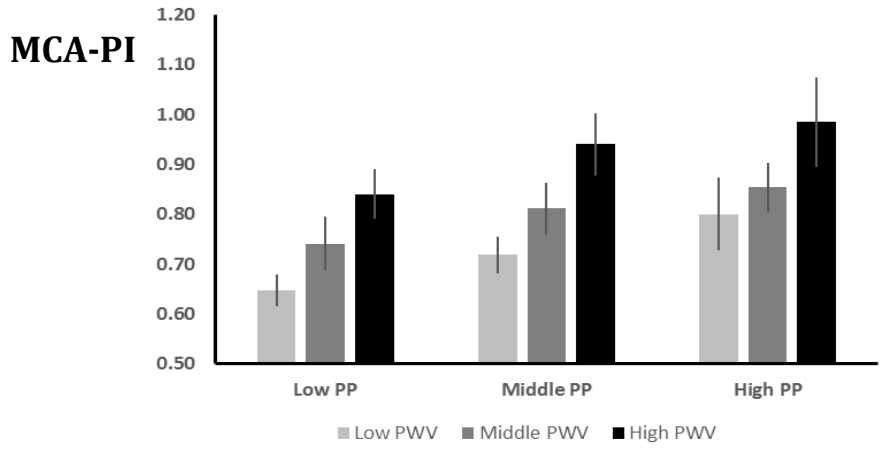
		MCA-PI	p-val	PWV	p-val	Aortic PP	p-val
Age (per 10 yrs		0.08	<0.001*	1.08	<0.001*	4.8	<0.001*
Gender	M	0.79	0.03*	9.6	0.82	42.4	<0.001*
	F	0.84		9.5		48.8	
Hypertensive	N	0.76	<0.001*	8.9	<0.001*	40.8	0.001*
	Y	0.87		10.8		47.4	
Diabetes	N	0.80	0.004*	9.4	<0.001*	44.8	0.87
	Y	0.90		11.1		45.2	
Family History	N	0.81	0.80	9.7	0.80	44.6	0.63
	Y	0.81		9.6		45.4	
Hyperlipidaemia	N	0.79	0.001*	9.5	0.14	45.2	0.57
	Y	0.86		10.0		44.2	
Atrial Fibrillation	N	0.81	0.18	9.6	0.84	44.8	0.71
	Y	0.86		9.7		45.8	
Current smoker	N	0.82	0.11	9.7	0.11	45.7	0.03*
	Y	0.77		9.1		40.7	
Systolic BP (per 10mmHg)		0.02	<0.001*	0.20	<0.001*	1.9	<0.001*
Diastolic BP (per 5mmHg)		-0.016	<0.001*	-0.035	0.52	-0.13	0.66
Creatinine (per 10mmol)		0.016	<0.001*	0.30	<0.001*	0.13	0.72

Table 10.2. Relationship between demographic and haemodynamic variables.

10.4.3 Relationship between systemic and cerebral haemodynamic measures

There were no significant relationships between aortic systolic or diastolic BP with peak systolic (PSV) or end diastolic (EDV) MCA flow velocities respectively, either as a linear regression or as a linear trend across tertiles (figure 10.2), except for a weak relationship between aortic DBP and MCA-EDV. However, there was a strong relationship between aortic pulse pressure or pulsatility index with MCA pulsatility index ($p<0.001$). This relationship persisted within each tertile of population age, whether chronological age or 'vascular age' as estimated by aortic stiffness (figure 10.1). However, whilst there was no significant interaction between age and aortic pulsatility in predicting MCA-PI, there was a significant interaction between aortic PWV and aortic pulse pressure in predicting MCA-PI (aortic PP $p<0.001$; aortic PWV<0.001; PP x PWV $p=0.001$), with a greater than expected MCA-PI in the highest tertile of PWV compared to lower tertiles of PWV as aortic PP increased (figure 10.1).

Figure 10.1. Effect of stratification by aortic stiffness (PWV) or age on the relationship between aortic and cerebral pulsatilty.



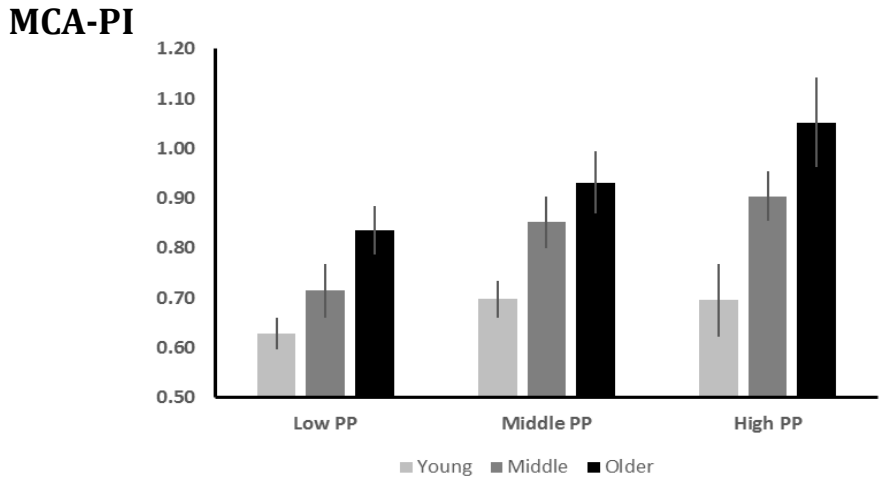
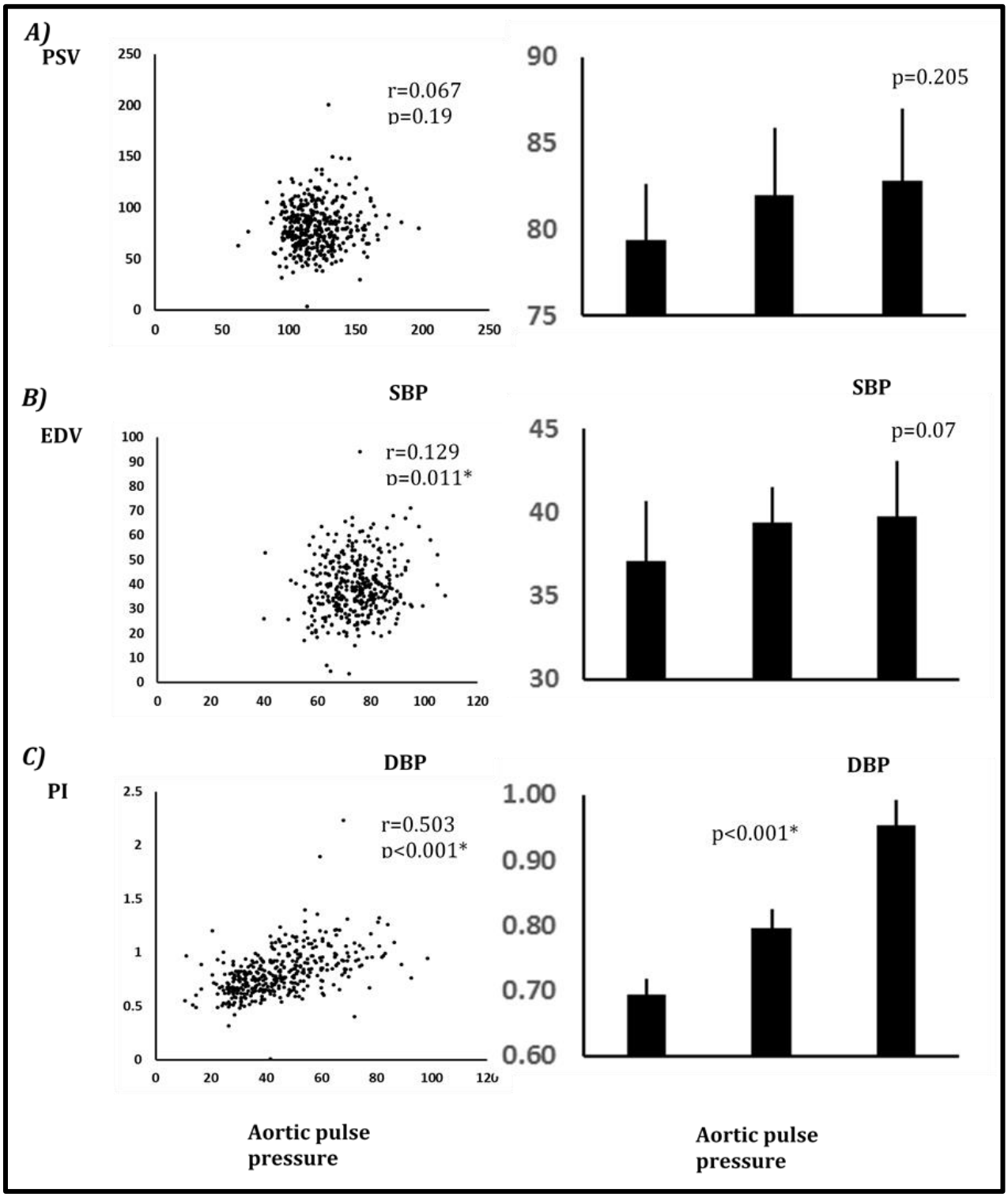


Figure 10.2. Relationship between aortic blood pressure parameters and measured cerebral blood flow velocity on TCD. Relationships are shown for aortic SBP vs MCA peak flow velocity (PSV, panel A), aortic DBP vs end-diastolic flow velocity (EDV, panel B) and aortic pulsatility vs MCA pulsatiliity index (panel C), as a simple scatter plot and by tertile of aortic measures



10.4.4 Physiological associations with severity of leukoaraiosis

Patients with leukoaraiosis had significantly greater MCA-pulsatility (0.86 vs 0.74, p<0.0001), aortic PWV (p<0.0001) and aortic pulse pressure (p<0.0001). Increasing severity of leukoaraiosis was particularly associated with increased pulsatility in the MCA and the aorta. However, in the aorta this reflected both an increased SBP and low DBP, but in the MCA this was primarily driven by a reduced MCA diastolic flow velocity. Aortic pulse wave velocity was similarly strongly associated with severity of leukoaraiosis. These associations were stronger for periventricular lesions than for deep white matter lesions (table 10.3).

Table 10.3. Physiological associations with severity of leukoaraiosis.

	Periventricular		Deep White Matter		Total score	
	OR (95% CI)	p-val	OR (95% CI)	p-val	OR (95% CI)	p-val
Age	3.24 (2.52 - 4.16)	<0.001	2.51 (1.98 - 3.17)	<0.001	2.79 (2.23 - 3.50)	<0.001
Creatinine	1.31 (1.10 - 1.57)	0.0024	1.34 (1.12 - 1.60)	0.001	1.38 (1.16 - 1.65)	<0.001
SBP clinic	1.24 (1.04 - 1.49)	0.02	1.23 (1.03 - 1.48)	0.02	1.24 (1.04 - 1.48)	0.02
DBP clinic	0.89 (0.74 - 1.06)	0.20	0.92 (0.77 - 1.10)	0.38	0.89 (0.75 - 1.06)	0.19
Aortic SBP	1.37 (1.14 - 1.66)	0.001	1.13 (0.94 - 1.37)	0.18	1.26 (1.05 - 1.51)	0.01
Aortic DBP	0.85 (0.71 - 1.02)	0.08	0.76 (0.63 - 0.91)	0.004	0.79 (0.66 - 0.94)	0.009
Aortic PP	1.61 (1.33 - 1.96)	<0.001	1.43 (1.18 - 1.73)	<0.001	1.53 (1.26 - 1.85)	<0.001
Aortic Aix	1.19 (1.00 - 1.42)	0.049	1.09 (0.92 - 1.30)	0.33	1.12 (0.94 - 1.34)	0.20
MCA PSV	0.87 (0.71 - 1.07)	0.20	0.95 (0.82 - 1.10)	0.50	0.79 (0.64 - 0.96)	0.02
MCA EDV	0.54 (0.43 - 0.68)	<0.001	0.51 (0.40 - 0.64)	<0.001	0.51 (0.41 - 0.64)	<0.001
MCA PI	2.37 (1.85 - 3.03)	<0.001	1.80 (1.42 - 2.27)	<0.001	2.10 (1.65 - 2.66)	<0.001
PWV	2.17 (1.76 - 2.69)	<0.001	1.54 (1.26 - 1.87)	<0.001	2.02 (1.63 - 2.50)	<0.001

10.4.5 Association between leukoaraiosis and recurrent events

In univariate analyses, severe leukoaraiosis was associated with an increased risk of recurrent stroke or all cardiovascular events (MACE), before and after adjustment for age and gender (figure 10.3), although there was no significant increase in the risk of stroke or MACE in the mild leukoaraiosis group compared to patients with no leukoaraiosis. Both aortic stiffness (PWV) and MCA-PI were significantly associated with the risk of recurrent cerebrovascular events, death or all major cardiovascular events (table 10.4), whilst aortic pulsatility was associated with death, and SBP with death or major cardiovascular events.

Table 10.4. Physiological associations with recurrent cardiovascular events. Hazard ratios per standard deviation increment in each physiological index (cox proportional hazards regression) are shown for recurrent stroke or TIA, all cause death or major cardiovascular events (cardiovascular death, stroke, myocardial infarction, acute peripheral vascualr disease).

	Cerebrovascular (60)		All cause death (25)		MACE (35)	
	HR (95% CI)	p-val	HR (95% CI)	p-val	HR (95% CI)	p-val
Age	1.38 (1.03 - 1.85)	0.031*	3.39 (1.76 - 6.51)	<0.001*	1.77 (1.16 - 2.71)	0.008*
Creatinine	1.25 (0.99 - 1.57)	0.06	1.56 (1.15 - 2.11)	0.004*	1.46 (1.09 - 1.95)	0.01*
SBP clinic	1.21 (0.94 - 1.54)	0.13	1.94 (1.39 - 2.71)	<0.001*	1.37 (1.01 - 1.85)	0.044*
DBP clinic	0.96 (0.74 - 1.24)	0.74	1.04 (0.69 - 1.58)	0.86	0.83 (0.58 - 1.18)	0.3
Aortic SBP	1.13 (0.89 - 1.45)	0.32	1.44 (0.99 - 2.1)	0.05	1.1 (0.79 - 1.52)	0.58
Aortic DBP	0.94 (0.74 - 1.2)	0.63	0.93 (0.6 - 1.42)	0.73	0.83 (0.6 - 1.16)	0.27
Aortic PP	1.17 (0.91 - 1.51)	0.22	1.57 (1.06 - 2.33)	0.025*	1.31 (0.95 - 1.81)	0.1
Aortic Aix	1.01 (0.8 - 1.27)	0.94	1.12 (0.85 - 1.49)	0.41	1.06 (0.8 - 1.39)	0.7
MCA PSV	1.18 (0.93 - 1.49)	0.17	0.96 (0.63 - 1.45)	0.84	1.02 (0.73 - 1.43)	0.92
MCA EDV	0.97 (0.74 - 1.26)	0.8	0.69 (0.44 - 1.07)	0.09	0.69 (0.48 - 1)	0.05
MCA PI	1.33 (1.09 - 1.63)	0.006*	1.42 (1.05 - 1.92)	0.024*	1.65 (1.33 - 2.04)	<0.001*
PWV	1.36 (1.08 - 1.72)	0.009*	1.78 (1.26 - 2.51)	0.001*	1.43 (1.05 - 1.93)	0.023*

10.4.6 Independence of associations between haemodynamic indices, leukoaraiosis and recurrent events

In combined models, cerebral haemodynamic indices and aortic stiffness were independently associated with leukoaraiosis, but were no longer associated with leukoaraiosis after adjusting for age. In contrast, for recurrent cerebrovascular events and all cardiovascular events, only MCA-PI predicted the risk of recurrent events, with no significant attenuation of this relationship after adjusting for age and gender. In contrast, all cause death was associated with aortic stiffness and age (table 10.5).

Finally, although severe leukoaraiosis and MCA-PI were both predictive of recurrent cerebrovascular events or MACE, these associations were largely independent, with only a small, non-significant reduction in the association between severe leukoaraiosis and recurrent events after adjustment for MCA-PI (figure 10.3), and a comparable small increase in the association between MCA-PI and outcomes after adjustment for leukoaraiosis. Additional adjustment for age and gender had no significant impact, and reduced any mediation between MCA-PI and leukoaraiosis.

Figure 10.3. Interaction between leukoaraiosis and MCA pulsatility with the risk of stroke or cardiovascular events.

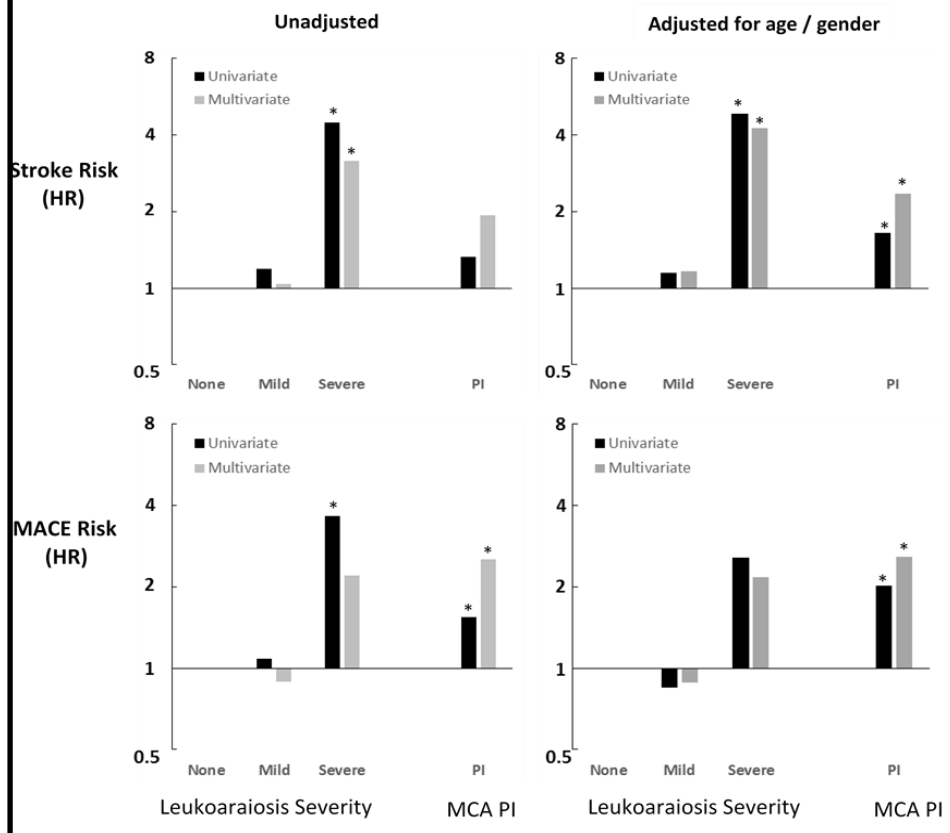


Table 10.5. Relative strength of physiological associations with leukoaraiosis and cardiovascular events. Hazard ratios per standard deviation increment in each physiological index (cox proportional hazards regression) are shown for recurrent stroke or TIA, all cause death or major cardiovascular events (cardiovascular death, stroke, myocardial infarction, acute peripheral vascualr disease), with odds ratios for increased Fazekas score per standard deviation. Models include all physiological indices presented, with or without age and gender.

	Fazekas score		Cerebrovascular Events (60)		All cause death (25)		MACE (35)	
	HR	p-val	HR	p-val	HR	p-val	HR	p-val
MCA EDV	0.70 (0.55 - 0.90)	0.005*	1.18 (0.87 - 1.60)	0.28	0.87 (0.51 - 1.50)	0.63	1.11 (0.74 - 1.68)	0.61
MCA PI	1.43 (1.05 - 1.93)	0.022*	1.40 (1.03 - 1.90)	0.033*	0.99 (0.59 - 1.64)	0.96	1.51 (1.03 - 2.21)	0.034*
Aortic DBP	0.86 (0.68 - 1.08)	0.20	1.00 (0.75 - 1.32)	0.97	0.90 (0.56 - 1.45)	0.67	1.00 (0.68 - 1.47)	0.99
Aortic PP	1.12 (0.87 - 1.45)	0.38	1.00 (0.72 - 1.40)	0.99	1.36 (0.82 - 2.26)	0.24	1.01 (0.65 - 1.55)	0.97
PWV	1.44 (1.15 - 1.82)	0.002*	1.28 (0.97 - 1.70)	0.08	1.55 (1.04 - 2.32)	0.032*	1.28 (0.88 - 1.85)	0.19
MCA EDV	0.86 (0.66 - 1.12)	0.25	1.28 (0.94 - 1.74)	0.11	1.10 (0.62 - 1.93)	0.75	1.25 (0.84 - 1.87)	0.28
MCA PI	1.13 (0.82 - 1.57)	0.46	1.38 (1 - 1.89)	0.047*	0.84 (0.45 - 1.58)	0.59	1.49 (1.01 - 2.18)	0.043*
Aortic DBP	0.98 (0.76 - 1.25)	0.85	1.04 (0.77 - 1.4)	0.82	0.95 (0.54 - 1.69)	0.87	1.06 (0.70 - 1.61)	0.78
Aortic PP	1.02 (0.78 - 1.34)	0.87	1.01 (0.71 - 1.43)	0.95	1.34 (0.74 - 2.40)	0.33	1.06 (0.67 - 1.68)	0.80
PWV	1.18 (0.91 - 1.53)	0.22	1.19 (0.87 - 1.63)	0.27	1.22 (0.77 - 1.95)	0.40	1.14 (0.75 - 1.74)	0.53
Female	1.00 (0.62 - 1.60)	0.99	0.74 (0.41 - 1.35)	0.33	0.61 (0.20 - 1.85)	0.38	0.46 (0.19 - 1.10)	0.08
Age	2.42 (1.76 - 3.33)	<0.001*	1.3 (0.86 - 1.95)	0.21	3.59 (1.56 - 8.26)	0.003*	1.46 (0.84 - 2.53)	0.18

10.5 Discussion

Middle cerebral artery pulsatility was associated with cerebral white matter hyperintensities, recurrent cerebrovascular events and all cardiovascular events, independent of other haemodynamic indices, age and gender, but did not explain the relationship between severe leukoaraiosis and recurrent events. As in the larger population in chapter 8, MCA pulsatility was strongly associated with aortic pulsatility, with a stronger relationship between aortic and cerebral pulsatility as aortic stiffness increased, implying greater transmission of the aortic waveform to the cerebral circulation.

White matter hyperintensities on MRI are associated with an increased risk of lacunar stroke,[1] cognitive impairment[3] and long-term functional decline,[4] and have an extremely high prevalence in the elderly, such that the majority of patients over the age of 70 will have some degree of white matter injury.[10] They have primarily been associated with hypertension and diabetes, yet despite their epidemiological significance, the underlying pathophysiology is poorly understood, there is no proven treatment to prevent progression and the pathological relationship between leukoaraiosis and the risk of cerebrovascular events, cognitive impairment and physical decline is poorly delineated. A number of studies have demonstrated a strong relationship between pulsatility of cerebral blood flow and the presence of lacunar stroke[1] or white matter hyperintensities,[7] and in chapter 8 we demonstrated that increased cerebral pulsatility was best explained by transmission of pulsatile aortic blood to the brain through stiff conduit vessels. Alternatively, a number of studies have suggested that increased pulsatility is an effect of changes in distal resistance due to the presence of white matter injury, subsequent to underlying endothelial dysfunction.[1]

Despite evidence of the relationship between cerebral pulsatility and chronic white matter hyperintensities, and a strong relationship between systemic pulse pressure and the risk of cardiovascular events,[12] this is the first study to demonstrate a strong prognostic relationship between cerebral arterial pulsatility and recurrent

cerebrovascular and total cardiovascular events, so identifying cerebral pulsatility as a novel risk factor that may be a better index of recurrent risk than currently available haemodynamic measures. This is potentially modifiable (chapter 9), and could provide a treatment target. However, there appeared to be largely independent relationships between white matter hyperintensities or cerebral pulsatility with recurrent events, suggesting, at least in part, that there are different pathophysiological relationships underlying chronic ischaemic cerebrovascular injury and recurrent cerebrovascular events.

The physiological mechanism of cerebral arterial pulsatility is dependent upon the degree of pulsatility generated at the aortic root, its dampening by compliance/elasticity of the intervening vasculature and the impact of downstream resistance to flow. This is further complicated by the complex interaction between augmentation of the diastolic phase through the Windkessel effect of elastic vessels and the impact of reflection of the forward travelling pressure wave from the vascular bed distal to the site of measurement. Previous studies suggested that this primarily reflected distal vascular resistance,[5] but the strong relationship between aortic pulsatility and cerebral pulsatility, and the non-linear augmentation of this relationship with increasing aortic stiffness, suggests that aortic pulsatility and its transmission through stiff conduit vessels is the principle determinant of measured MCA pulsatility. This contrasts with the weak relationship between absolute measures of aortic blood pressure and cerebral blood flow velocity. However, the absolute flow velocity at any phase of the cardiac cycle reflects the high intra- and inter-individual variability in measured TCD flow velocities due to which precise vessel is imaged, variability of vessel calibre, presence of focal stenoses and investigator factors such as angle of insonation (chapter 9). However, pulsatility is effectively a ratio normalised to mean flow velocity, and therefore, in a preserved waveform, is largely independent of these factors, generating an index that is both more reliably measured, and even when optimally measured, is a more consistent reflection of underlying physiology.

The pathophysiological relationship between pulsatility and either recurrent events or white matter hyperintensities is less clear. Pulsatility could be intrinsic to the pathophysiology of these outcomes through barotrauma to small vessels at peak systolic velocities, hypoperfusion at low end-diastolic flow or secondary effects such as changes in glymphatic removal of cerebral waste products.[13] Indeed, although the association between pulsatility and outcomes may reflect unmeasured confounders, the strength of association and its independence of major demographic factors and other haemodynamic indices suggests a likely causative component of the process. However, the prognostic component blood flow pulsatility could either be high systolic peaks, low diastolic troughs, or a combination of these factors in different conditions.

The study has limitations. Firstly, there is a relatively high rate of patients without bone windows in whom valid TCD measures were not available, due to the high proportion of elderly patients in the population, which may particularly exclude patients at greater cardiovascular risk. However this reflects the principal strength of the study in consecutively recruiting patients from an unbiased population with TIA or minor stroke across all age groups, and exclusion of the highest risk patients is most likely to result in reduction in power and a conservative estimate of risk relationships. Secondly, despite this being the largest population reported with aortic and cerebral haemodynamics as well as recurrent cardiovascular events, the total number of events is still relatively modest. Nonetheless, robust risk relationships were demonstrable, independent of likely confounders. Thirdly, aortic blood pressure was not directly measured simultaneously with TCD, however the transformation of the radial arterial waveform assessed by applanation tonometry is well validated as a non-invasive method.

This study implies that there are a number of potential targets to prevent the development and progression of cerebral white matter hyperintensities and recurrent cerebrovascular events. As cerebral pulsatility reflects aortic stiffness and pulsatility, measures to prevent the development of aortic stiffness by more intensive treatment of

early life hypertension, and to reduce aortic stiffness through 'de-stiffening' strategies are needed. Secondly, identifying which known drugs with haemodynamic effects have beneficial effects on cerebral pulsatility without excessively reducing minimum BP and trugh EDV could reduce future risk. Finally, further research is required to characterise the mechanism by which aspects of pulsatility of blood flow results in acute events and chronic cerebrovascular injury.

Cerebral pulsatility is strongly associated with leukoaraiosis and recurrent cerebrovascular events, but does not fully mediate the association between chronic white matter changes and outcomes. It reflects increased aortic pulsatility and its transmission through stiff conduit vessels, and therefore is a potential treatment target to reduce the impact of established hypertension-induced end-organ damage on the brain.

10.6 References

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CHAPTER ELEVEN

Conclusions and Future Work

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11.1 Introduction

Secondary prevention of stroke and cardiovascular disease has progressed significantly in the past twenty years, but despite early assessment[1] and intensive risk factor control, the risk of recurrent cardiovascular events remains greater than 10% over 5 years[2], with a 10% risk of dementia after a first stroke rising to 33% risk after a second stroke.[3] Despite improvements in stroke mortality, stroke remains the second largest cause of mortality internationally,[4] and in combination with vascular dementia, represents the leading cause of morbidity in the UK. Therefore, identification of novel markers of future risk, development of new treatments and refinement of current treatments has the potential to significantly reduce overall morbidity and mortality in the population. In particular, hypertension explains up to 60% of the population attributable risk of stroke[5, 6] but control of hypertension only reduces stroke risk by a relative risk reduction of 30-40% in the short-term.[7] Furthermore, the high residual risk in the population after intensive risk factor control[2] suggests that blood pressure reduction alone does not fully ameliorate the risk resulting from prolonged hypertension and the resulting vascular injury. As such, there are likely to be hypertension-induced intermediate risk factors that can be identified and targeted to reduce future cardiovascular either through better prevention by improved control of hypertension or by treatments targeting these intermediate phenotypes.

This thesis therefore aimed to characterise potential intermediate cardiovascular phenotypes that are associated with increasing age and a history of hypertension, often described as components of ‘vascular aging, to determine their potential as independent risk factors for recurrent stroke, mediators of the persistent hypertension-associated vascular risk and treatment targets after control of classical risk factors. Confirmation of this role therefore requires demonstration of their association with age and premorbid hypertension, characterisation of clinical and physiological associations, the rate of progression with time and ultimately whether they are associated with future cerebrovascular risk. Furthermore, it is important to

determine to what extent these strongly covarying markers of vascular aging may be independent of each other and so to what extent they may play a causative role. Those intermediate cardiovascular phenotypes that progress over time, and are most strongly and independently associated with a history of hypertension and cardiovascular risk can then be targeted in future interventional trials to reduce the future risk of stroke and dementia.

The thesis therefore used the OXVASC study to characterise three leading and related physiological consequences of hypertension that may mediate the relationship between historic hypertension and the risk of recurrent stroke: blood pressure variability, arterial stiffness and arterial pulsatility.

11.2 Summary of Key Findings

11.2.1 Beat-to-beat blood pressure variability

Professor Rothwell previously demonstrated that variability in blood pressure from one clinic visit to the next was associated with a significantly increased risk of stroke, myocardial infarction and death.[8-10] To operationalise the use of blood pressure variability, we subsequently demonstrated in the OXVASC population that variability in blood pressure can validly be measured from day-to-day at home and from beat-to-beat over 5 minutes, and that the physiological determinants of both forms of BPV are similar, suggesting a common underlying mechanism.[11] Furthermore, medication effects on day-to-day BPV were similar to the effects of antihypertensives on visit-to-visit BPV.[12-14] However, the distribution, determinants and predictive value of beat-to-beat BPV were not defined.

This thesis demonstrates that beat-to-beat BPV was particularly strongly associated with age in both genders (chapter 3), but that this relationship was non-linear with a marginally greater BPV in the youngest patients compared to patients over 50, before a steady rise in BPV with age. Furthermore, the distribution becomes progressively more positively skewed with increasing age, implying that multiple determinants of BPV exist that contribute to the overall measurement. In particular,

there are likely to be ‘healthy’ forms of BPV in the young, which give way to erratic and excessive BPV with loss of compensatory mechanisms in older patients, who are therefore most likely to be at risk due to increased BPV. Consistent with this hypothesis, the age related increase in BPV was particularly associated with increased arterial stiffness (chapter 4) and increased BMI, although BPV was also increased in women with a low BMI, potentially reflecting loss of muscle mass in older women or detrimental effects of excessive weight loss on the vasculature. Finally, its progression over time was associated with increased aortic blood pressure and autonomic dysfunction (chapter 5). Together, these associations support BPV as a reflection of prior hypertension as well as an aging vasculature with loss of compensatory mechanisms including vessel compliance and autonomic function.

In addition to pathological BPV as a consequence of hypertension, age and age-related loss of compensatory mechanisms, beat-to-beat BPV predicted the risk of recurrent stroke (chapter 6) to a greater extent even than day-to-day BPV, although with a similar pattern of association. Finally, beat-to-beat BPV progressed significantly over 5 years and predicted loss to follow-up, implying its utility as a measure of frailty (chapter 5). Therefore, measures to reduce BPV, either through control of aortic systolic blood pressure to prevent its progression or through interventions to directly reduce BPV have the potential to reduce future risk, even in the context of good control of mean BP, and represents a potentially important novel target to reduce the risk of stroke and dementia.

11.2.2 Arterial Pulsatility

Cerebral arterial pulsatility, measured as Gosling’s pulsatility index, was also very strongly associated with age, with a linear increase in pulsatility with age in both sexes (chapter 8). This was very similar to the distribution of arterial stiffness with age, and cerebral pulsatility was strongly associated with aortic stiffness. However, it was even more strongly associated with pulsatility of aortic blood pressure, with

arterial stiffness partially mediating the relationship between aortic and cerebral pulsatility, consistent with previous evidence suggesting aortic stiffness increases transmission of aortic pulsatility to the brain.[15] In particular, absolute cerebral blood flow velocities were only weakly related to absolute aortic pressures, but pulsatility was well preserved. Furthermore, in longitudinal analyses (chapter 9), although cerebral artery pulsatility progressed a limited amount in all patients, in those over 55 years there was significant progression, whilst baseline aortic pressure predicted progression of aortic stiffness and pulsatility but not cerebal pulsatility. Rather cerebral pulsatility was strongly associated with concurrent aortic pulsatility.

Finally, the clinical relevance of cerebral pulsatility was indicated by its association with cerebral small vessel disease and the risk of recurrent cardiovascular events, independent of age, gender, classical risk factors and aortic stiffness. Furthermore, the association with clinical events was not mediated by the small vessel disease implying separate pathophysiological pathways.

Overall, cerebral arterial stiffness is a very strong candidate target as a novel treatment target. It is strongly associated with other markers of risk, but also predicts future events independent of these markers, it is associated with a history of elevated blood pressure, remains significant after good control of BP and although there was evidence of only limited progression across the population, preventing progression in those who do so has the potential to reduce the burden of subsequent clinical events.

11.2.3 Arterial stiffness

In the OXVASC study, carotid femoral pulse wave velocity was also very strongly associated with a history of hypertension and age, with a strong linear association between age and PWV in both genders. It progressed significantly with time, and the rate of progression increased at increasing ages. However, although it predicted severity of white matter hyperintensities and the risk of death, independently of cerebral and aortic pulsatility, this diminished following adjustment

for age, consistent with the strong collinearity between age and PWV and indicative of the use of PWV as a marker of 'vascular aging.'

In UK Biobank, I also demonstrated that increasing arterial stiffness, as arterial stiffness index, modified the timecourse of progression of blood pressure with higher ASI resulting in an earlier transition of diastolic blood pressure from its rise in mid-life to its fall in late life, reflecting the stiffness of the aorta itself. Furthermore, increases in ASI were particularly associated with a history of hypertension.

11.2.4 Covariance and independence between markers of vascular aging

Although the analyses in this thesis largely approach the three different measures independently, both in terms of their distributions, clinical associations, progression and prognostic value, it is also evident that there is a close relationship between them. All three measures are strongly associated with age and a history of hypertension, and their progression is driven by increased aortic blood pressure. The three factors are therefore all indicators of both chronological age and the accumulated injury due to hypertension, representing a form of vascular aging. Furthermore, all have predictive value, independent of each other and of classical risk factors, indicative of their potential as novel therapeutic targets.

However despite some independence, both blood pressure variability and arterial pulsatility are very strongly associated with arterial stiffness, whilst PWV partially mediates the translation of aortic pulsatility into cerebral arterial pulsatility. Physiologically, this is best explained by a predominant effect of age and hypertension on aortic stiffness, resulting in increasing systolic pressures, falling diastolic pressures, greater transmission of aortic pulsatility to the periphery and associated loss of vascular compliance impairing autonomic control of blood pressure. Other factors may contribute, but aortic stiffness is therefore central to the concept of intermediate cardiovascular phenotypes, with persistent vascular injury despite control of blood pressure that continues to increase the risk of stroke and

other cardiovascular events. Therefore, although long-term control of blood pressure from an earlier age is paramount to prevent this, its inevitable occurrence in a large number of patients implies the need for novel treatments to ameliorate its effects.

This thesis would therefore strongly support both interventions to reduce aortic stiffness itself, in addition to long-term blood pressure control, and treatments to target likely physiological sequelae of aortic stiffness, including arterial pulsatility and blood pressure variability. Both of these measures may be directly improved by haemodynamic interventions, whether vasodilators for arterial pulsatility[16] or calcium channel blockers for blood pressure variability,[12] which is vital given relatively limited success in interventions to directly reduce aortic stiffness itself.[17, 18] However, arterial stiffness and pulsatility are very strongly tied together, both in distribution and associations, although the non-linear association between age and BPV supports additional factors that drive increases in BPV that are not related to arterial stiffness, such as cardiovascular reactivity,[11] that warrant additional research to assess their potential as treatment targets.

11.3 Research Implications: Ongoing Work

11.3.1 Progressing understanding of cardiovascular intermediate phenotypes

The results presented in this thesis aim to characterise the distribution and clinical associations of beat-to-beat blood pressure variability, arterial stiffness and cerebral arterial pulsatility, their progression and associations with recurrent cardiovascular events. However, a number of key questions remain to understand these indices. The current rate of recurrent events in fully-ascertained follow-ups is still relatively low, with only a proportion of the population currently included in the analyses of recurrent events, limiting our ability to determine the true magnitude of independence between the different phenotypes. We continue therefore to recruit patients to the OXVASC study, and to assess more patients with physiological tests,

to provide additional power to understand the independence of these measures, and whether they differ in the types of recurrent events predicted. Furthermore, we are also assessing whether there are significant differences between different stroke aetiologies and these indices.

In addition, we continue to reassess patients after 5 years to increase the size of the population with repeat visits to improve the power of these analyses, and to allow us to determine the predictive value of physiological indicators for other indices such as new onset atrial fibrillation.

However, these are not the only cardiovascular indices measured in the physiological substudy of the OXVASC Phenotyped cohort, and there are a number of other intermediate phenotypes that many play a crucial role in the residual risk after good risk factor control. In addition to the indices discussed, we perform assessments of cerebral autoregulation and cerebral reactivity[19], cardiovascular reactivity to stress and most patients undergo detailed brain imaging. Therefore, similar analyses to this thesis will also be performed to determine the potential importance of these measures. Furthermore, these indices need refinement to provide more precise measures, such as measures which discriminate pathological blood pressure variability from healthy blood pressure variability.[20] Finally, these indices need validation in other datasets, and we are currently using the UK Biobank study to determine whether cerebral pulsatility, arterial stiffness and elevated blood pressure is associated with increased cerebral small vessel disease.

11.3.2 Identifying potential interventions

The epidemiological associations identified in this thesis demonstrate potential novel risk factors for future clinical harm, independent of classical risk factors. However, as all the results are associations, it is impossible to definitively determine true independence between these indices, and whether they play a causative role in recurrent cardiovascular events and cognitive impairment. This requires interventional

studies that specifically improve these cardiovascular intermediate phenotypes, and in which improvement of these phenotypes results in a reduction in future cardiovascular events or dementia. However, there is currently very weak evidence for interventions to improve outcomes in small vessel disease. Therefore, we are currently carrying out two clinical trials to identify interventions with the potential to fulfil this role.

In the TREAT-SVDs study, we are testing whether three different antihypertensive agents (amlodipine, losartan, atenolol) improve variability in blood pressure and cerebral reactivity in patients with lacunar stroke. Building upon earlier work demonstrating that amlodipine reduces visit-to-visit blood pressure variability,[12] this trial will assess effects of the three drugs in 105 patients in a crossover design on home BP variability from day-to-day. This is a multicentre trial in 5 centres in Europe, but in Oxford we are carrying out a substudy to assess drug class effects on beat-to-beat BP variability and arterial stiffness.

In the OxHARP clinical trial, we are testing the effects of three weeks of treatment with sildenafil (a vasodilating phosphodiesterase 5 inhibitor) versus cilostazol (a PDE3 inhibitor) versus placebo in a crossover, double blind randomised trial in 75 patients with a lacunar or cryptogenic stroke. The primary outcome is cerebral arterial pulsatility, but we are also determining effects on cerebrovascular reactivity, cerebral autoregulation, blood pressure variability (home and beat-to-beat) and arterial stiffness. This will therefore determine whether these treatments improve these cardiovascular intermediate phenotypes and have the potential to reduce future risk of stroke and dementia.

11.4 Research Implications: Future Research

The work in this thesis provides a strong foundation for further studies. In addition to further refinement of the indices reported in this thesis, and the ongoing investigations into other intermediate phenotypes, there is a need to identify other markers of cardiovascular risk in addition to classical risk factors and these

physiological indices. It is also vital to assess the role of these indices in populations other than patients with TIA or minor stroke. As such, a critical avenue of research will be to operationalise these assessments to provide a simpler mode of measurement, to enable them to be deployed in a primary prevention setting to determine their importance in moderate risk individuals.

However, the most important avenue of further research planned is the translation of the results of this work to clinical practice. This requires the development of methods of measurement of these indices within a clinical framework, and a program of work to this end is currently being planned. Secondly, it is vital to identify treatments that can improve these indices and have the potential to be applied in clinical practice. The OxHARP and TREAT clinical trials are archetypal studies for this, but a range of further agents exist that warrant further assessment, including endothelin-1 antagonists (ie bosentan), drugs to reduce the cardiac cycle length and so reduce pulsatility, agents to directly reduce arterial stiffness (alegebrium) and drugs that may directly improve autonomic function.

However, ultimately the proof of this approach will require large, randomised clinical trials to demonstrate a reduction in cerebrovascular events and cerebral small vessel disease with such agents. If the OxHARP trial confirms beneficial effects of either sildenafil or cilostazol, this would strongly support development of such a trial, initially to determine effects on cerebral small vessel disease in a reasonable population but ultimately to assess the effects in a large clinical trial with clinical endpoints.

10.5 Conclusions

The relationship between a long-standing history of hypertension and cardiovascular events is not completely abolished despite control of blood pressure to current targets, which may be explained by established vascular injury that independently increases the risk of future events. Intermediate cardiovascular phenotypes including arterial stiffness, beat-to-beat blood pressure variability and

arterial pulsatility are all determined by a history of hypertension and increasing age, progress over time and predict the risk of future cerebrovascular events, despite excellent control of classical risk factors. They are strongly associated with each other but are also independently associated with markers of cardiovascular disease and recurrent events. As such, they represent potential novel treatment targets to reduce the risk of recurrent cerebrovascular events, particularly in addition to currently recommended secondary prevention measures. Current trials will determine whether commonly used medications ameliorate these intermediate cardiovascular phenotypes, and have the potential to reduce the risk of future stroke and dementia.

11.6 References

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