The Characterisation and Treatment of Resistant Hypertension

Submitted by
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I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

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Abstract

Hypertension is a highly prevalent condition and, as a risk factor for vascular disease in particular, a leading contributory cause of death worldwide. Recent consensus guidelines suggest that moderate and severe (grade II and III) hypertension should be treated rapidly to achieve targets though, prior to the inception of this thesis, the evidence for the safety and efficacy of this approach, together with the physiological consequences of rapid hypertension treatment in moderate and severe disease, was limited.

This thesis explores the clinical consequences of an 18-week treatment programme for individuals with grade II and III hypertension, using guideline-recommended pharmacological treatment, delivered over an accelerated timeframe. The blood pressure response to treatment is reported, together with the tolerance and safety of the protocol, as defined by the protocol completion rate, frequency of medication side effects and clinically significant adverse events. The programme also provided an opportunity to study health-rated quality of life in patients with moderate and severe hypertension and the effect of rapid treatment on health-related quality of life. This allowed for the first validation (according to modern standards) of an English language disease-specific instrument for measuring health-related quality of life in hypertension, following translation of the original MINICHAL disease-specific instrument from the original Spanish.

In addition, the clinical treatment programme provided an opportunity to study the microvascular response to rapid treatment of moderate and severe hypertension, particularly with relevance to the rarefaction of hypertension and its reversal with treatment. Moreover, the morphological and functional myocardial consequences of treatment were determined, using cardiac MR imaging.

Accordingly, this thesis presents evidence supporting the rapid treatment of moderate and severe hypertension, providing an opportunity for this to be studied in future investigations, with the aim of exploring whether this approach is prognostically advantageous for patients.

Contents

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Abbreviations

ABP: Ambulatory Blood Pressure
ABPM: Ambulatory Blood Pressure Monitoring
ACEi: Angiotensin Converting Enzyme inhibitor
ACE-R: Addenbrooke's Cognitive Examination-Revised
ACR: Albumin/Creatinine Ratio
AER: Albumin Excretion Rate
ARB: Angiotensin Receptor Blocker
AVA: Automated Vascular Analysis
BMI: Body Mass Index
BP: Blood Pressure
BSA: Body Surface Area
CCB: Calcium Channel Blocker
CMR: Cardiac Magnetic Resonance
CoV: Coefficient of Variation
DASBP: Daytime Average Systolic Blood Pressure
dBP: diastolic Blood Pressure
DENSE: Displacement Encoding with Stimulated Echoes
DOT: Directly-Observed Therapy
ECs: Endothelial Cells
ECFs: Endothelium-derived Constricting Factors
ECG: Electrocardiogram
EDRFs: Endothelium-Derived Relaxing Factors
EMPRO: Evaluating the Measurement of Patient-Reported Outcomes
EPCs: Endothelial Progenitor Cells
eNOS: Endothelial Nitric Oxide Synthase
ESC: European Society of Cardiology
GFR: Glomerular Filtration Rate
HRQoL: Health-Related Quality of Life
ICC: Intraclass Correlation Coefficient
ISOQOL: International Society of Quality of Life Research
ISPOR: International Society for Pharmacoeconomics and Outcomes Research
LA: Left Atrial

LED: Light-Emitting Diodes

LV: Left Ventricular

LVEDV: Left Ventricular End-Diastolic Volume

LVEDVi: Left Ventricular End-Diastolic Volume index

LVEF: Left Ventricular Ejection Fraction

LVESV: Left Ventricular End-Systolic Volume

LVESVi: Left Ventricular End-Systolic Volume index

LVH: Left Ventricular Hypertrophy

MDRD: Modification of Diet in Renal Disease study

MFI: Microvascular Flow Index

MR: Magnetic Resonance

MRI: Magnetic Resonance Imaging

NICE: The National Institute for Health and Care Excellence

NIHR: National Institute for Health Research

NO: Nitric Oxide

NSAIDs: Non-Steroidal Anti-Inflammatory Drugs

OCT: Optical Coherence Tomography

OPS: Orthogonal Polarised Spectral

PAT: Pulse Amplitude Tomography

PORH: Post-Occlusive Reactive Hyperaemia

PPV: Proportion of Perfused Vessels

PROQOLID: Patient-Reported Outcome and Quality of Life Instruments Database

PVD: Perfused Vessels Density

PWV: Pulse Wave Velocity

RHI: Reactive Hyperaemic Index

ROS: Reactive Oxygen Species

sBP: systolic Blood Pressure

SCMR: Society of Cardiovascular Magnetic Resonance

SDF: Sidestream Dark Field

SENC: Strain-Encoded imaging

SHR: Spontaneously Hypertensive Rat

SM: Somatic Manifestations

SOP: Standard Operating Procedure

SSFP: Steady State Free Precession

StM: State of Mind

TVD: Total Vessels Density

UK: United Kingdom

US: United States

VA: United States Department of Veterans Affairs

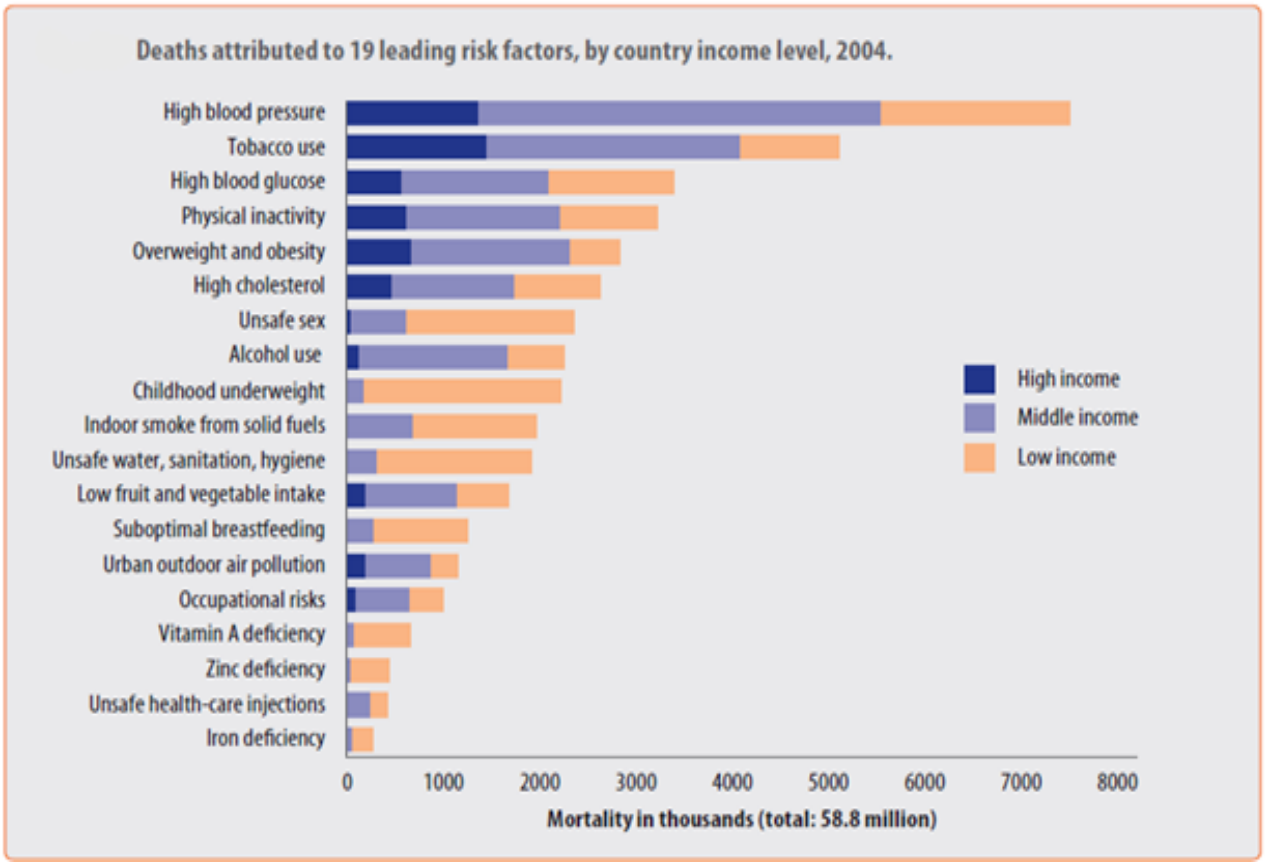
VEGF: Vascular Endothelial Growth Factor

WHO: World Health Organisation

Chapter 1 Introduction

Hypertension affected approximately 1 billion people worldwide in the year 2000, with over 1.5 billion individuals expected to be affected by 2025 (Kearney et al., 2005). As a major stimulus for atherosclerosis, hypertension is a major contributor to diseases responsible for considerable morbidity and mortality worldwide. This is highlighted by a World Health Organisation (WHO) analysis of risk factor exposure and causal associations, finding that hypertension is the leading contributory cause of death worldwide, greater than the combined individual contributions from tobacco and alcohol (WHO, 2009) (Figure 1.1).

Figure 1.1: WHO analysis of disease risk factors and their causal associations. Reproduced from "Global Health Risks: Mortality and burden of disease attributable to selected major risks"(WHO, 2009).



The term "essential hypertension" was first coined in the early 20th century as it was felt that resistance arteries constricted, producing an increase in hydrostatic pressure within the circulatory system as a necessary compensatory mechanism

of the body, rather than a disease in itself. This followed the historical ontological concept of medicine, which advocated that changes in body functions were simply compensatory mechansms not to be treated. It was on this basis that febrile response to infections was encouraged to postpone death in the 18th century, by eminent physicians such as Herman Boerhaave (Burton, 1743). Likewise, the belief that hypertension was a compensatory mechanism of the body in order to provide sufficient blood flow to organs afflicted by disease continued into the 20th Century (White, 1937) (cited in (Fuchs and Whelton, 2020)). Even eminent physicians of the time, such as Sir William Osler, discouraged early efforts to treat hypertension:

> "It is well to recognise that the extra pressure is a necessity … Get it out of your heads, if possible, that the high pressure is the primary feature, and particularly the feature to treat." – (Osler, 1912).

However, by the second half of last century, evidence to support hypertension as an important primary disease was growing. With the advent of the first antihypertensive medication in the 1950s, it was demonstrable that treatment of a cohort of individuals with malignant hypertension significantly improved an otherwise dire prognosis for this condition (Leishman, 1959). A subsequent early randomised controlled study for the use of antihypertensive agents in severe hypertension was terminated prematurely, having demonstrated a number needed to treat of just 4 in order to prevent stroke, myocardial infarction or death over the course of one year's treatment (Freis, 1967).
The benefit of antihypertensive therapy, chiefly thiazide diuretics and beta-blockers, was confirmed through 14 unconfounded randomised controlled trials over the following 20 years, concluding in a comprehensive meta-analysis showing a reduction in stroke and cardiovascular risk afforded by pharmacological BP reduction (Collins et al., 1990).

Subsequent trials of newer agents, such as calcium channel blockers and angiotensin converting enzyme inhibitors, could therefore not employ a treatment-naïve control group. The most common study designs for newer agents were therefore either the addition of the new agent (versus placebo) to existing therapy or direct comparison with the older agent as the control group (Zanchetti

et al., 2015). The reduction in cardiovascular risk associated with BP reduction was found to be independent of regimen used in a meta-analysis of 46 randomised controlled trials, including a total of over 230,000 patients (Law et al., 2009), with a slight advantage for regimens using calcium-channel blockers for the prevention of stroke.

The morbidity and mortality benefit of antihypertensive therapy therefore appears to be predominantly related to BP reduction itself, rather than specific drug properties (Zanchetti et al., 2015). The magnitude of this effect has been shown to be approximately a halving of cardiovascular disease risk for each incremental reduction in blood pressure by 20/10mmHg down to 115/80mmHg (Lewington et al., 2002). Accordingly, consensus guidelines recommend BP goals of <140/90mmHg in treated patients (Mancia et al., 2013, NICE, 2011) (Table 1.1), though targets may be reduced further in future iterations of guidelines with growing evidence that more intensive BP reduction may be beneficial, particularly in higher risk individuals (Wright et al., 2015).

Table 1.1: Classification and treatment targets for hypertension Adapted from NICE 2011 and ESC 2013 guidelines (NICE, 2011, Mancia et al., 2013).

Category	Office systolic BP (mmHg)	Office diastolic BP (mmHg)	Ambulatory BP (mmHg) daytime average
Optimal	<120	<80	
Normal§	120-129	80-84	
High normal§	130-139	85-89	
Grade I hypertension§	140-159	90-99	≥135/85
Grade II hypertension§	160-179	100-109	≥150/95†
Grade III (severe†) hypertension§	≥180	≥110	
Target if age <80 years	<140	<90‡	<135/85†
Target if age ≥80 years	<150	<90	<145/85†

§highest category from either systolic or diastolic measurement defines classification.
†NICE guidance only. ‡ESC recommends <85mmHg in patients with diabetes mellitus.

1.1 BP control on a population level

In a clinical trial setting, significant reductions in office BP can be achieved with simple pharmacological interventions, averaging approximately 10/5mmHg in blinded interventional trials versus placebo (Zanchetti et al., 2015). Interestingly, BP also consistently falls in the placebo groups of controlled trials (Beckett et al., 2008, Lithell et al., 2003, Liu et al., 2005).
However, there remain concerns that the marked BP reductions and associated improvements in morbidity and mortality seen in clinical trials may not be translatable to routine clinical care (Singer et al., 2002, Sigmund et al., 2020). One potential challenge in translating clinical trial outcomes to usual clinical care may be a greater adherence to treatment in clinical trials compared with usual care. For example, it is notable that only 50% patients in specialist hypertension clinics are adherent to medication regimens in systematic testing (Hameed et al., 2016). There is therefore scope for a potential Hawthorne effect during trial participation (Adair, 1984), increasing medication adherence and lifestyle modification during the duration of the study and thereby improving BP control beyond that seen in routine care.
Additionally, enrolment based on single office BP measurements may lead to a regression to the mean (Bland and Altman, 1994) during follow-up and an appearance of greater BP reduction in some studies. Previous large-scale studies have predominantly used single office BP measurements as outcome data, though there is clear evidence to show that 24-hour mean BP is more closely associated with target-organ damage and prognosis (Parati et al., 1987, Conen and Bamberg, 2008, Fagard et al., 2008). Such reliance on office, rather than ambulatory BP to determine the treatment efficacy is thus an important limitation of historical antihypertensive studies and therefore their applicability to clinical practice.

The pursuit of mortality data as a “hard clinical endpoint” in contemporary trials may also affect observed BP reductions. As trials pursue adequate statistical power for these outcomes, there has been a tendency to focus on higher-risk individuals during study design, as opposed to the lower-risk majority, to ensure a higher event rate (Williams, 2005). Such a skewed patient group selection may affect the profile of response to BP treatment compared to the population as a whole.

The limits of generalising clinical trial data to routine care is underlined by the fact that only 37% of all patients (treated and untreated) with hypertension achieve BP targets, as shown in a cross-sectional study of Health Survey for England surveys (Falaschetti et al., 2014). This deficiency cannot be ascribed to idiosyncrasies of the English healthcare system as population surveys throughout the world have shown similarly inadequate BP control in hypertensive individuals (Joffres et al., 2013, Wolf-Maier et al., 2004, Volpe et al., 2007).

1.2 Barriers to successful treatment on hypertension

The reasons for poor control on a population basis are likely to be due to a combination of lifestyle factors, non-adherence to medication, secondary causes, clinical inertia (reluctance of the attending clinician to intensify treatment) and true treatment-resistant hypertension (Doumas et al., 2014). Each of these aspects will be considered in more detail below.

1.2.1 Lifestyle factors

Lifestyle factors with deleterious effects on blood pressure include: excessive alcohol (Teresa Aguilera et al., 1999, Ohira et al., 2009), high dietary sodium intake (He et al., 2013), obesity, lack of exercise, medication (including NSAIDs (Johnson et al., 1994) and ciclosporin (Robert et al., 2010)) and liquorice ingestion (Omar et al., 2012).

It is well-established that lifestyle adaptations can lower BP. Among the most rigorous lifestyle interventions is the “DASH diet”, which, at its inception, was deployed stringently on 151 participants for whom all food consumption was controlled over an 8-week period (Appel et al., 1997). Adherence to the

intervention was close to 100% and BP was reduced by an average of 5.5/3mmHg over the control group. However, it is clear that this form of intensive intervention and control over a sustained period of time is not feasible in routine clinical care. In the subsequent randomised controlled PREMIER trial, for which participants did not relinquish control over food intake but still received intensive lifestyle modification coaching including 18 face-to-face contact episodes over 6 months covering all potential lifestyle interventions for hypertension, systolic BP was reduced by only 4mmHg (Appel et al., 2003).

As such, it can be concluded that the cumulative effect of systematically addressing all lifestyle factors is modest. Furthermore, such intensive support as seen in trials of lifestyle intervention for hypertension is not practical from a healthcare delivery standpoint for the vast majority of individuals. It is therefore expected that lifestyle modifications alone are not sufficient to achieve target BP without pharmacological therapy in the vast majority of hypertensive subjects. This is reflected in consensus guidelines (NICE, 2011, Mancia et al., 2013), which suggest drug-treatment and lifestyle adaptations should begin in parallel for those with significant hypertension.

1.2.2 Non-adherence

Poor adherence to medication regimens is particularly understandable for a condition in which the majority of patients are asymptomatic. Incorporation of urinary drug metabolite testing and directly-observed therapy (DOT) into specialist hypertension clinics has revealed complete or partial non-adherence to prescribed therapy of 25-50% (Tomaszewski et al., 2014, Hameed et al., 2016). However, as participants were drawn from referrals to specialist clinic, rates of non-adherence may be higher than in the general population of treated hypertensives. Furthermore, the degree of prior knowledge of participants undergoing adherence testing is not clear from either study. Such prior knowledge of testing, if present, would have the potential to affect behavioural patterns just prior to testing and thus confound results. Adherence to antihypertensive therapy in all patients treated for hypertension and unaware of testing is unknown.

It can be postulated that, through the assessment of patients' sense of wellbeing at the time of receiving their diagnosis of hypertension and by monitoring their

wellbeing during treatment, individuals likely to be non-adherent to medication could be identified. By doing so, psychological intervention could be targeted to these individuals, with the aim of determining their rationale for non-adherence, mitigating non-adherence in this group and improving BP control on a population level. Whether such a psychological intervention would be effective is the subject of ongoing research (OUTREACH study, cited in (Poulter et al., 2020)).

Instruments for the measurement of health-related quality of life (HRQoL) require validation in accordance with internationally agreed standards (Valderas et al., 2008, Reeve et al., 2013). Specifically, instruments must have undergone an assessment of overall concept, reliability, validity, responsiveness to change, interpretability, burden of administration, alternative modes of administration and cross-cultural and linguistic adaptations. Accordingly, the EMPRO tool, which uses these concepts (Valderas et al., 2008), can be used to choose between alternative instruments when selecting an appropriate questionnaire for the assessment of HRQoL, either as part of a research study or in a clinical setting. Additionally, instruments for the assessment of HRQoL must be employed within the linguistic and cultural setting for which they have been validated. The steps required for appropriate translation and cross-cultural adaptation of an instrument have been outlined by the International Society for Pharmacoeconomics and Outcomes Research (ISPOR) task force and include the production of two forward translations, reconciliation of these two versions, back-translation to the native language, reconciliation with the original instrument, cognitive debriefing with a sample of individuals reflecting the demographics of the target population and harmonisation with existing validated versions of the instrument (Wild et al., 2005). These practical steps require that translations are always undertaken by individuals whose native language is the target language for translation. Subsequent psychometric analysis should include an assessment of internal consistency, test-retest reliability, construct validity (through co-administration with a generic questionnaire and association with variables known to affect HRQoL) and responsiveness to change (application within a study during which HRQoL is expected to change).

Disease-specific instruments for the assessment of HRQoL are often preferred to generic instruments, given their greater responsiveness to change (Patrick and Deyo, 1989). However, the only published English language disease-specific instrument for the assessment of HRQoL in hypertension is the Bulpitt-Fletcher

questionnaire (Bulpitt and Fletcher, 1990), which has an excessive administration time (20-40 minutes), contains redundant items and is outdated with regards the terminology used, cultural values and expected medication side effects explored, given that its inception was more than 30 years ago. Although not included in the original article (Bulpitt and Fletcher, 1990), the Bulpitt-Fletcher instrument has been validated in terms of responsiveness to change, through its inclusion in studies of hypertension treatment (Tedesco et al., 1999, Sharman et al., 2013). However, no analysis of its construct validity or internal consistency has been conducted.

Several disease-specific instruments for exploring HRQoL in hypertension have been validated in other languages and translating such an instrument would prove an efficient alternative to developing a new instrument de novo in English. Reviewing the Patient-Reported Outcome and Quality of Life Instruments Database (PROQOLID), the options for adaptation of a validated instrument are the HYPER-31 instrument in Italian (Bamfi et al., 1998), the CHAL instrument (Spanish) (Roca-Cusachs et al., 1992), or its abridged version, the MINICHAL instrument (Badia et al., 2002). Of these, given its brevity and complete validation, the MINICHAL appears the most attractive target for adaptation, noting also that it has already been adapted and validated in Brazilian Portuguese (Schulz et al., 2008).

The translation, adaptation and validation steps described above have not been undertaken for a disease-specific instrument for use in English for patients with hypertension. Therefore, validating such an instrument would be of value within the hypertension research community and for clinical use.

1.2.3 Secondary hypertension

The prevalence of an underlying disease leading directly to hypertension ('secondary hypertension') is again poorly understood. The most common causes include renovascular disease and primary hyperaldosteronism (Sinclair et al., 1987), though a thorough investigation should include exclusion of Cushing's syndrome, phaeochromocytoma, thyroid and parathyroid abnormalities and coarctation of the aorta (Omura et al., 2004).

Cross-sectional studies have estimated a prevalence of secondary hypertension in treated hypertensive patients of <1-12% (Persu et al., 2014, Daugherty et al.,

2012). This variability reflects the lack of a standard regimen for exclusion of secondary hypertension, variability of the environments in which the assessment is made and divergence of clinical practice.

1.2.4 Clinical inertia

Clinical inertia can be defined as the failure to initiate, intensify or change therapy despite clinical evidence and consensus guidelines suggesting that this is the appropriate course of action.

The risk of clinical inertia in hypertension is relatively high as few patients present with symptoms. This means that unsubstantiated reasons for not intensifying treatment are easier to employ by the treating clinician with assent from the patient. The precise impact of clinical inertia in hypertension is difficult to quantify due to the variability in the design of studies investigating this phenomenon (Faria et al., 2009). However, in one such study, antihypertensive medication was not intensified in 86.9% of clinical consultations where a BP above target was documented, as determined by a retrospective cohort study of over 7000 patients in the United States (Okonofua et al., 2006).

Reluctance of clinicians to intensify anti-hypertensive treatment may reflect an over-estimation of the effectiveness of present care, a fear of over-burdening patients with additional medication and associated potential for drug side effects and inconvenience to the patient, unfamiliarity with using multiple drug combinations, or an underestimation of the benefits of optimal BP control (Phillips et al., 2001).

It can be proposed that clinical inertia may be largely overcome through enforcement of medication intensification via a pre-defined protocol, largely removing humanistic factors in the attending clinician from determining whether medication commencement or intensification is warranted. As such, studies investigating protocol-directed therapy in hypertension have sought to demonstrate that these programmes improve the proportion of patients achieving BP targets.

1.3 Evidence for protocol-directed treatment

Protocoled treatment programmes aim to ameliorate the effect of clinical inertia by mandating a rigorous stepped care approach according to pre-defined goals. The effect of allied healthcare professional-led care in hypertension, which usually employs such protocols, has been well-studied and is the subject of a recent Cochrane review (Clark et al., 2021), which found evidence of improved outcomes with nurse prescribers from non-UK healthcare settings.

Though this review includes an exploration of the effect of introducing protocols to limit clinical inertia, the analysis primarily determines the benefit of all allied healthcare professionals delivering clinical care for chronic conditions, using doctor-led care as the comparator. The observed effect therefore includes a component from training these allied professionals in up-to-date guidance before such programmes are introduced, the use of disease-focussed clinics promoting greater familiarity with hypertension management, and the longer consultation times often afforded to such clinics which encourage a stronger clinician-patient relationship and greater potential for patient education during clinic time.

Similarly, a meta-analysis by the United States Department of Veterans Affairs (VA) (Shaw et al., 2013) also noted an improvement in achieving target office BP for patients attending nurse-led hypertension clinics, with an odds ratio of 1.41 for achieving target BP in favour of nurse-led protocols versus usual care. Although more specific to studies of nurse-led protocols rather than all allied professionals, the VA meta-analysis does not clarify the role of protocol-directed therapy itself in improving target BP attainment.

Kasier Pemanente, a healthcare consortium in the United States with 8 million patient members, have demonstrated that large-scale implementation of a treatment algorithm for hypertension intervention improved the proportion of patients achieving target office BP from 54% to 85% over a 6-year period (Sim et al., 2014). Though this retrospective study lacked a control group and is thus subject to confounders such as the general trend for BP control to have improved over this period across the developed world, it points more closely to the advantages afforded by using protocols in hypertension treatment as, unlike the studies analysed by Clark *et al.* and Shaw *et al.*, the range of professionals delivering the care was not significantly changed.

Only two randomised controlled trials have been undertaken to specifically explore the effect of protocol-directed therapy for hypertension management. Firstly, a cluster-randomised controlled trial of 2104 patients conducted in primary care in Canada studied the effect of implementing a simplified stepped-care algorithm for BP treatment, with control practices continuing with usual care. This showed a significant difference in the proportion of patients achieving office target BP in the intervention practices, with 64.7% participants at BP target in practices using the protocol versus 52.7% at target in control practices (Feldman et al., 2009).

Within this study, delivery of the protocol was doctor-led and therefore the positive effect could be ascribed to the implementation of the protocol rather than by whom it was delivered. However, the observed difference may have been affected by intra-cluster correlation of confounding factors, including from education of doctors regarding the new protocol together with refreshing their knowledge of antihypertensive treatment more rigorously in those assigned to use the new protocol to guide clinical decision-making. This is likely to have occurred despite the investigators' intention to supply consistent education and expert guidance to both groups. Reporting of the uptake of educational materials and advice between the two groups, which may have acted to refute this assertion, was not published.

In another such pragmatic multicentre study in Australia, 1562 patients were randomised (1:2 ratio) to usual care or intensive stepwise drug intervention (Stewart et al., 2012). Clinical care continued to be doctor-led and a total of 2337 participants were recruited. In the intervention arm, 36.2% patients achieved office target BP after 26 weeks' treatment compared with 27.4% participants in the control arm. This favours a significant benefit of the antihypertensive treatment protocol. The low numbers of participants achieving target BP compared with observational studies is likely due to the fact that stringent BP targets were employed (including $\leq$125/75mmHg in those with proteinuria and $\leq$130/80mmHg in those with any other form of end-organ damage) and that only 4 visits consisting of 10-15 minute consultations over 26 weeks were undertaken by each participant.

Therefore, investigation of the effect of rigorous stepped care protocols in the management of hypertension has shown an improvement in the proportion of treated patients achieving BP targets, an effect which is likely, at least in part, to

be through the mitigation of clinical inertia. Although the two randomised trials were undertaken in Canada and Australia respectively, if a similar study were undertaken in the United Kingdom (UK) it is unlikely that the healthcare framework and treated population would differ sufficiently to produce conflicting results. It is therefore reasonable to consider these findings generalisable to the UK. However, the difficulty in robustly blinding the participants and the inherently impossible task of blinding treating clinicians to the intervention has undermined the potential for a double-blinded randomised trial to further investigate this effect.

1.4 Putative benefits of rapid hypertension treatment

As raised BP is associated with adverse cardiovascular outcomes, it is evident that those individuals with higher BP measurements due to a delay in achieving target values are at greater risk of cardiovascular events cumulatively during this delay. Additionally, there is now growing evidence from retrospective studies to suggest that timely control of BP prevents later adverse events, providing a legacy benefit after BP control has been achieved.

This concept is supported by analysis of the VALUE study (Julius et al., 2004), which was designed to test whether regimens based on valsartan or amlodipine had differing effects on cardiovascular outcomes despite equivalent BP reduction. Although the VALUE study was unable to assess this objective due to significantly greater BP reduction in the amlodipine arm of the trial, serial mean matching for BP has allowed retrospective analysis of cardiovascular outcomes for a mixed group of amlodipine and valsartan-treated patients (Weber et al., 2004). This analysis has shown that reaching BP target by 6 months significantly favoured improved cardiovascular outcomes for the duration of follow-up (up to 6 years). Moreover, initial positive response to treatment only 1 month after commencing an antihypertensive regimen also conferred a significant advantage in terms of combined cardiac events, stokes and death for the remainder of the study, regardless of antihypertensive agent used.

A similar effect was noted on retrospective analysis of the Syst-Eur trial (Staessen et al., 1997), which included a control group of untreated patients during the 6-month intervention period and demonstrated, after a median of 6 years open-

label follow-up, that earlier treatment reduced subsequent cardiovascular events long-term (Staessen et al., 2004).

Both of these findings are tempered by their nature as post-hoc analyses which were not pre-defined: they are subject to the bias inherent in selecting the analysis after completion of the study. This criticism can be countered by two subsequent studies which have explored the *a priori* hypothesis that timely treatment of BP leads to improved cardiovascular outcomes, though both of these were also retrospective analyses.

Firstly, a retrospective analysis of primary care electronic notes from over 3000 patients demonstrated that those who suffered a cardiac event were less likely to be at BP target at every point of time during follow-up (Gradman et al., 2013). Subsequently, a further retrospective analysis, on this occasion of over 88,000 primary care case notes, showed that a delay in intensifying treatment in response to high BP measurements confers a significantly increased risk of cardiovascular events or all-cause mortality, even when this delay is only 6-18 weeks (Xu et al., 2015).

Unfortunately, as retrospective analyses, there is a potential for introduction of bias. For example, it is not clear whether or not those that responded more quickly to treatment had other favourable disease characteristics, such as an absence of end-organ damage or vascular dysfunction.

Thus retrospective analysis and *post-hoc* interpretation of major clinical trial findings have suggested morbidity and mortality benefits from timely BP reduction; this has not yet been examined by a prospective randomised controlled trial. Though such a trial would be theoretically possible, the number of participants required to meet clinical endpoints together with the time necessary to complete the investigation has meant that this has yet to be undertaken. Furthermore, it would not be practically possible to blind either the clinician or patient to antihypertensive treatment which is more rapid than usual care, introducing a further confounder to interpretation of the results of such a study.

1.5 Microvascular associations of hypertension

1.5.1 Overview of hypertension haemodynamics

Hydrostatic pressure within the arteries is generated through contraction of the left ventricle to eject blood, forcing it through the vessels against resistance to flow (total peripheral resistance). It is these two factors which therefore underpin blood pressure. This can be illustrated as:

$$\text{Mean arterial pressure} \propto \text{cardiac output} \times \text{total peripheral resistance}$$

As cardiac output is determined by the sum of heart rate and stroke volume (the amount of blood ejected by the left ventricle in one contraction), the above formula can be expanded to:

$$BP \propto \text{heart rate} \times \text{stroke volume} \times \text{total peripheral resistance}$$

In established chronic hypertension, cardiac output has been found to be normal or slightly reduced, whereas total peripheral resistance is invariably raised. This contrasts with early hypertension, which appears to be characterised by an increase in cardiac output as a result of raised heart rate, as shown in several studies employing invasive measurement of haemodynamic parameters (Bello et al., 1967, Julius et al., 1971, Andersson et al., 1983, Lund-Johansen, 1991). The finding of raised heart rate in early hypertension may be a consequence of sympathetic overactivity, with subsequent reduction in cardiac output and development of the established high total peripheral resistance state occurring at least partly as a consequence of down-regulation of beta-adrenoceptors (Julius, 1991).

Studies investigating the relative contribution of different vessel types to the overall peripheral vascular resistance are affected by methodological limitations which have yet to be completely overcome (Christensen and Mulvany, 2001). Chief amongst these is that the vast majority of studies have used animal models, particularly rats, with the exclusion of larger animals including humans. In

addition, the process of measuring fractional pressure changes within vessels using micropuncture with glass pipettes (Wiederhielm et al., 1964) or indwelling catheters (Christensen and Mulvany, 1993) is likely also to affect the measured pressure, as may the process of tissue exposure and the administration of anaesthetic agents. These limitations have resulted in heterogeneous findings in the precise relative contributions of different vessel types to peripheral vascular resistance in health and disease. Despite this, it is accepted as consensus that most resistance to flow at rest occurs in arteries of 100-400μm diameter and these vessels are therefore commonly referred to as “resistance arteries” (Duling, 1991).

The relative contributions of different vessel types to peripheral vascular resistance in the hypertensive state has been investigated, predominantly in the rat model. In the most part, the profile of resistance across different vessel types has been found to be maintained in hypertensive rats when compared with normotensive controls in the cerebral circulation of 4-5-week-old rats (Bohlen, 1987) and the skeletal muscle of mature animals (DeLano et al., 1991, Christensen and Mulvany, 1994, Roy and Mayrovitz, 1984). In the mesentery and mature cerebral circulation, 100-400μm diameter arteries appear to provide a greater degree of resistance in hypertensive versus normotensive rats (Meininger et al., 1986, Bohlen and Lash, 1994, Harper and Bohlen, 1984), indicating a degree of protection of the capillary bed from raised pressure. The anomaly of these studies is the study of Kanatsuka and colleagues (Kanatsuka et al., 1991), who investigated the pressure profile in the coronary circulation of anaesthetised hypertensive cats, finding a greater pressure drop in the distal microvasculature (<150μm diameter vessels) compared with the 150-400μm diameter arteries.
These studies are likely to be affected by a heterogeneity in pressure-measuring techniques, anaesthesia, species and organ studied, contributing to the variable results described. Despite this, it is clear that the predominant site of resistance is arteries of 100-400μm diameter in both healthy and hypertensive animals. Although these arteries continue to dissipate the same or a slightly greater fraction of pressure in the hypertensive state, this is not sufficient to prevent transmission of the higher pressure to distal vessels (arterioles and capillaries) in hypertension.

Experimentally, high capillary pressure in hypertension has been demonstrated using glass micropipettes to puncture nailfold capillaries and directly measure intraluminal capillary pressure in human subjects (Williams et al., 1990). This technique has also been used to demonstrate that raised capillary pressure is particular to established hypertension rather than short-term BP fluctations, as transient elevation of BP triggered by exercise is not transmitted to nailfold capillaries due to effective autoregulation by resistance arteries (Shore et al., 1993).

As the site of molecular exchange between the intravascular space and tissue fluid through their single-cell layer endothelial barrier, capillaries are vulnerable to this increase in hydrostatic pressure, resulting in hyperfiltration and consequent preclinical tissue oedema.

1.5.2 Resistance artery structure in hypertension

Distinct structural features of the peripheral circulation in hypertensive subjects were first proposed by Richard Bright, a 19th century English physician, following his examination of necropsy specimens (Bright, 1836) (cited in (Feihl et al., 2008)).

Further study in the modern era led to the conclusion that resistance arteries underwent hypertrophic remodelling. This followed investigation in spontaneously hypertensive rats (SHR) and transgenic (mRen-2)27 (renin TGR) rats with in situ pressure-diameter studies of mesenteric arterial branches (Struijker-Boudier et al., 1996), demonstrating increased arterial media cross-sectional area, reduced distensibility and increased wall tension in both hypertensive rat strains compared with controls. This appeared to confirm previous in vitro studies of mesenteric bed resistance arteries obtained from SHR rats, showing hypertrophy of the arterial media when compared with samples from control animals (Mulvany et al., 1978).

However, studies in humans with essential hypertension have not demonstrated hypertrophic remodelling of resistance vessels as found in animal models of hypertension. In contrast, eutrophic remodelling, characterised by a reduction in lumen diameter but no change in media thickness, has been demonstrated in arterial samples obtained from gluteal subcutaneous vessels in a sample of 15

patients (Aalkjaer et al., 1987b) and is in fact in-keeping with Bright's original studies of post-mortem human specimens.

Similarly, eutrophic remodelling has been demonstrated in other vascular beds, including omental arteries (Rosei et al., 1995). In the retina, eutrophic remodelling has also been observed in hypertensive subjects when compared with normotensive controls (Ritt et al., 2008). However, Ritt *et al.* used scanning laser Doppler flowmetry for this observation. This technique allows visualisation of vessels in vivo and therefore does not allow for determination of maximal luminal diameter in fully relaxed conditions. The luminal narrowing reported in this study may therefore be in part due to a functional, rather than purely structural abnormality, and is not directly comparable to ex vivo studies.

Despite the argument that there is significant heterogeneity of techniques used to measure vessel structure in different tissues, subsequent studies have shown reasonable correlation between these methods, in particular between in vivo retinal scanning laser Doppler flowmetry and subcutaneous vessel myography of ex vivo subcutaneous vessel samples (Rizzoni et al., 2012). It can therefore be concluded that these structural arterial changes are likely to occur with a degree of homogeneity in the majority of vascular beds in humans.

The case for similar vascular remodelling across different tissues is further supported by use of the rat model, SHR, in which the disruption of resistance artery structure is similar in mesenteric, femoral, cerebral and coronary tissue (Thybo et al., 1994). Although, as discussed previously, the structural alterations in the hypertensive rat consist of hypertrophic rather than eutrophic remodelling, it can be postulated that this model may represent a specific subset of hypertensive disease. This is supported by the fact that subjects with some secondary causes of hypertension, particularly renovascular hypertension, tend to have hypertrophic remodelling of resistance arteries, as determined by micromyograph analysis of biopsies from 70 participants (Rizzoni et al., 1996).

It is less likely that hypertrophic remodelling represents an end-stage of the hypertensive disease process, developing from eutrophic remodelling in severe disease only, as human studies demonstrating eutrophic remodelling have included participants with severe hypertension (such as the average inclusion criteria blood pressure of 175/104mmHg by Aalkkjaer et al. 1987) and hypertension severity was not significantly different between secondary and essential hypertension groups in Rizzoni et al. 1996. However, the difficulty in

determining duration of hypertension as a distinct variable for severity of hypertension has meant that this has not been controlled for in previous studies examining resistance artery structure. Thus, a process of longstanding eutrophic remodelling developing into hypertrophic remodelling as a consequence of hypertension duration, rather than severity, cannot be excluded.

The importance of resistance artery remodelling is underscored by the finding that increased media:lumen ratio is an independent predictor of cardiovascular adverse events, as demonstrated in a sample of 128 high risk individuals using a Cox proportional hazard model to adjust for known cardiovascular risk factors including blood pressure (Rizzoni et al., 2003). It has previously been argued that these structural changes may contribute to the genesis of high intra-vascular pressure, possibly through amplification of high pressure through the physical properties of a narrowed lumen, as determined by Laplace's Law (Schiffrin, 1992). This is supported by examination of the SHR model, showing that resistance artery hypertrophy in the model occurs at 4 weeks, prior to the development of high blood pressure (Korner et al., 1991). However, as discussed above, the deficiencies in the SHR model in providing an accurate reflection of vascular processes in human hypertension are now well-known. Furthermore, regional normotension induced by an arterial ligature in the SHR model results in the normotensive limb developing arterial morphology which is indistinguishable from controls at 12 and 24 weeks (Bund et al., 1991). In humans, isolated resistance subcutaneous resistance vessels from normotensive offspring of hypertensive parents do not show morphological changes associated with hypertension (Aalkjaer et al., 1987a), thus further supporting the conclusion that structural disruption of resistance artery morphology occurs as a consequence of hypertension rather than as a pre-cursor to the disease process.

1.5.3 Resistance artery function: myogenic tone

Mechanosensors in vascular smooth muscle cells of resistance arteries are able to exert a vasoconstrictor response directly in response to raised intraluminal pressure (Davis and Hill, 1999). This response, termed myogenic tone, is essential for regulating blood flow and pressure in the capillary bed.

Exaggerated myogenic tone has been demonstrated in vitro using aortic samples obtained from the SHR model (Fitzpatrick and Szentivanyi, 1980). Such studies

have used either an isometric myograph or an isobaric pressure-perfusion system (Mulvany and Aalkjaer, 1990), both of which determine maximal myogenic responsiveness rather than responses at normal physiological pressures. When studied at normal physiological distending pressures, mesenteric arteries isolated from adult SHRs showed no difference in contractility under isobaric conditions when compared with arteries from normotensive rats (Izzard et al., 1996).

Thus, it could be reasonably concluded that the remodelling of resistance arteries should be viewed as a structural adaptation which does not appear to affect the myogenic response of these vessels at physiological pressures.

1.5.4 Endothelial function: a key determinant of vascular health

The pioneering work by Nobel laureate Robert F. Furchgott demonstrated an integral role for the vascular endothelium in affecting the vasodilatory response to acetylcholine of isolated rabbit aorta (Furchgott and Zawadzki, 1980) and laid the foundation for considering the endothelium as a highly active organ involved in a plethora of vascular processes.

Endothelial dysfunction is characterised by an imbalance of endothelium-derived constricting factors (EDCFs) such as angiotensin II and endothelins, and endothelium-derived relaxing factors (EDRFs), the most significant and well-characterised of which is nitric oxide (NO) (Feletou and Vanhoutte, 2006). Such an imbalance is maintained and amplified by the production of reactive oxygen species (ROS) and the superoxide anion, the latter of which scavenges NO to form peroxynitrite, which further reduces NO production by inhibiting endothelial nitric oxide synthase (eNOS) (Endemann and Schiffrin, 2004). ROS triggers uncoupling of the essential cofactor BH4 from eNOS, causing it to switch to superoxide production rather than NO (Landmesser et al., 2003), and thereby instigating a positive feedback loop which results in markedly reduced NO bioavailability.

Aside from being a potent vasodilator, NO is integral to other endothelial functions, being antithrombotic, antiproliferative and inhibitory to leukocyte adhesion (Cooke and Dzau, 1997). As such, an imbalance between NO (and other EDRFs) and ECRFs, has been implicated in a wide range of disease processes, including heart failure (Maguire et al., 1998), hypercholesterolaemia

(Drexler et al., 1991, Chowienczyk et al., 1992), type 2 diabetes mellitus (Hink et al., 2001) and hyperhomocysteinaemia (Chambers et al., 1999). With additional associations between NO pathway perturbation and other atherosclerotic risk factors, including aging (Celermajer et al., 1994), cigarette smoking (Heitzer et al., 1996) and family history of premature coronary artery disease (Clarkson et al., 1997), it is possible that endothelial dysfunction provides a common link between these factors and atherogenesis (Vallance and Chan, 2001), leading to acute events such as myocardial infarct and stroke.

As with other risk factors for ischaemic heart disease, extensive evidence links endothelial dysfunction and hypertension. In rat models of hypertension, endothelial dysfunction of conduit (upstream) and resistance arteries has been demonstrated in SHR (Michel et al., 2008, Sekiguchi et al., 2002, Yang et al., 2004), the two-kidney one-clip model (Lee et al., 1995, Stankevicius et al., 2002), Dahl salt-sensitive rat (Zhou et al., 1999, Zhou et al., 2001) and steroid-treated hypertensive rats (Cordellini, 1999).

In humans with essential hypertension, a blunted response to endothelium-dependent vasodilatory agents has been shown in the forearm vasculature (Linder et al., 1990, Panza et al., 1990), together with an enhanced constriction response with inhibition of eNOS (Panza et al., 1993, Calver et al., 1992). A similarly blunted endothelial response has been demonstrated in the coronary circulation of patients with essential hypertension (Egashira et al., 1995, Treasure et al., 1993).

On examination of the Framingham cohort, the degree of blunting of the endothelium-dependent vasodilatory response was proportional to the degree of hypertension (Benjamin et al., 2004). The importance of endothelial dysfunction in hypertension is underscored by the finding that endothelium-dependent forearm vasodilatation is an independent predictor of cardiovascular events in never-treated subjects with essential hypertension (Perticone et al., 2001).

Whether hypertension is the cause or consequence of endothelial dysfunction remains the subject of debate (Bleakley et al., 2015). Endothelial dysfunction, as determined by brachial flow-mediated dilatation, has been detected in children as young as 8 years (Celermajer et al., 1992) though endothelial dysfunction early in life was not found to predict future development of hypertension in a large

cohort of 3500 ethnically-diverse participants after adjustment for covariables, the largest such study to date (Shimbo et al., 2010).

McAllister and colleagues found that the vasoconstrictor response to the NOS-inhibitor L-NMMA was diminished in normotensive offspring of hypertensive parents when compared with those of normotensive parents (McAllister et al., 1999), suggesting that basal NO production is reduced in these individuals. However, it must be noted that the study included only 12 pairs of data and that urinary albumin, serum creatinine and serum total cholesterol were noticeably higher in the experimental group versus controls (though each difference in individual variables was below the level of statistical significance). Furthermore, although the study was designed to detect a difference between the two groups for endothelium-dependent vasodilatation elicited through intra-arterial infusion of acetylcholine, no significant difference was found.

It is also clear that high BP can trigger endothelial dysfunction, as shown by measurement of endothelium-dependent vasodilatation triggered by intra-arterial vasodilators following 1-hour of sustained hypertension with intra-arterial noradrenaline infusion in 8 healthy volunteers (Millgård and Lind, 1998). Similarly, blunting of the endothelial vasodilatory response, as measured using flow-mediated dilatation, also occurs following hypertension induced by 30 minutes' exercise (Jurva et al., 2006).

Thus, the case for endothelial dysfunction occurring as a consequence, rather than a cause of hypertension, is certainly stronger at the present time.

1.5.5 Reversibility of endothelial dysfunction in hypertension: a therapeutic target?

If endothelial dysfunction post-dates the development of hypertension, it is logical to propose that treatment of hypertension may alleviate the endothelial dysfunction. This hypothesis has been tested by a plethora of studies demonstrating that endothelial function can be improved by a variety of antihypertensive drug classes (Table 1.2). In addition, lifestyle interventions used in hypertensive subjects, such as increased fruit and vegetable intake and low salt intake, are associated with improved endothelial function (Jablonski et al., 2009, McCall et al., 2009).

Table 1.2: Antihypertensive drug classes shown to improve endothelial function (excluding studies enrolling patients with heart failure or known left ventricular dysfunction)

Drug Class	Medication (Study)	Population (Sample size)	Measure of endothelial dysfunction
Calcium channel blockers (CCB)	Felodipine (Mervaala et al., 1997)	SHR (n=40)	In vitro mesenteric artery response to acetylcholine
	Nifedipine (Luscher et al., 2009)	Stable coronary artery disease (n=454)	Coronary artery diameter response to acetylcholine
	Amlodipine (He et al., 2014)	Essential hypertensior (n=24)	Brachial artery flow-mediated dilatation
Angiotensin converting enzyme inhibitors (ACEi)	Quinapril (Mancini et al., 1996)	Stable coronary artery disease	Coronary artery diameter response to acetylcholine
	Enalapril (Motz and Strauer, 1996)	Hypertensive patients (n=15)	Coronary flow reserve
	Ramipril (Mervaala et al., 1997)	SHR (n=40)	In vitro mesenteric artery response to acetylcholine
	Various (Higashi et al., 2000)	Hypertensive patients (n=26)	Brachial artery flow-mediated dilatation

	Perindopril (Sekuri et al., 2003)	Newly-diagnosed grad I-II hypertension (n=29	Brachial artery flow-mediated dilatation
	Perindopril (Bots et al., 200	Stable coronary artery disease (n=333)	Brachial artery flow-mediated dilatation
	Captopril (Kim et al., 2009	Stable angina pectoris (n=87)	Brachial artery flow-mediated dilatation
Angiotensin receptor blocker (ARB)	Losartan (Prasad et al., 2000)	Stable coronary artery disease (n=31)	Brachial artery flow-mediated dilatation
	Telmisartan (Hinoi et al., 2008)	Essential hypertensior (n=40)	Coronary flow reserve
Direct renin antagonists	Aliskiren (Fukutomi et al., 2014)	Elderly patients with hypertension (n=105)	Brachial artery flow-mediated dilatation
Vasodilating beta-blockers	Nebivolol (Tzemos et al., 2001)	Essential hypertension (n=12)	Brachial artery flow-mediated dilatation
	Nebivolol or carvedilol (Zepeda et al., 2012)	Mild-moderate essential hypertension (n=57)	Brachial artery flow-mediated dilatation
	Carvedilol (Kelly et al., 2012)	Obesity and (pre)hypertension (n=25)	Digital reactive hyperaemic index (RHI)
Aldosterone antagonists	Spironolactone (Nishizaka et al. 2004)	Hypertension and hyperaldosteronism (n=80)	Brachial artery flow-mediated dilatation

	Eplerenone (Sanz-Rosa et a 2005)	Spontaneously hypertensive rats (n=8)	In vitro aortic respons to acetylcholine

Thus, in a variety of settings and with different measures, CCBs, vasodilatory beta-blockers and antihypertensive agents that act on the renin-angiotensin system have all been shown to improve endothelial function. Published evidence for an improvement in endothelial function with thiazide diuretics or older generation beta-blockers (such as atenolol) is lacking and, for those studies which incidentally include these classes of agents as comparator groups, no improvement in endothelial function was demonstrated (Higashi et al., 2000, Tzemos et al., 2001).

Given the previously described association of endothelial dysfunction and adverse cardiovascular outcomes, it can be postulated that agents that improve endothelial function may be associated with better outcomes for a given BP reduction than those with a neutral effect on endothelial function. This hypothesis is supported by the findings of the LIFE study (Dahlof et al., 2002), which prospectively compared an ARB (losartan)-based regimen with atenolol-based therapy in over 9000 participants, finding a significant benefit in cardiovascular morbidity and mortality for the ARB-based regimen. In a subsequent open-label study directly comparing antihypertensive treatment with either an ACEi or a thiazide diuretic, the ACEi regimen resulted in a reduction in cardiovascular events, particularly in men (Wing et al., 2003).

Comparison of a CCB-based regimen with a diuretic-based regimen in high risk individuals in the ACCOMPLISH trial (Jamerson et al., 2008) identified that the former was associated with fewer cardiovascular events. However, the CCB-based group in this trial achieved a significantly lower mean final BP than the thiazide-based group, meaning that BP-independent benefits for amlodipine-based regimens cannot be conclusively inferred.

A theoretical advantage of renin-angiotensin system modulators or CCB-containing antihypertensive regimens is, however, controversial, and not supported by a large meta-analysis of antihypertensive trials (Law et al., 2009). However, this meta-analysis contains a significant degree of heterogeneity in the

studies examined (for example, testing the heterogeneity of direct comparison studies using CCB agents in hypertension to prevent coronary artery disease reveals I^2=43%). Furthermore, the analysis includes studies examining antihypertensive agents in conditions other than essential hypertension, such as heart failure and pre-existing coronary artery disease, adding to the perceived limitations of this, the largest meta-analysis of hypertension treatment to date.

Thus, the larger randomised controlled trials, such as the LIFE study (Dahlof et al., 2002), may provide a more accurate reflection of a direct comparison of different antihypetensive agents due to the homogeneity in overall design, inclusion critera, intervention and outcome definitions inherent within a single study. Whether some antihypertenisve agents confer a significant prognostic advantage over others through mitigtion of the endothelial dysfunction of essential hypertension therefore remains unclear, though the prognostic benefits of BP reduction itself are irrefutable.

1.5.6 Rarefaction of hypertension

A consistent finding in studies exploring the vascular associations of essential hypertension has been that of microvascular rarefaction: a reduction in density of vessels in the microvasculature (Feihl et al., 2006). Rarefaction can be described as functional (present but not perfused at rest) or structural (anatomically absent). The rarefaction of hypertension was first described in humans by Ruedemann (Ruedemann, 1933), who noted an abnormally low density of small conjunctival vessels in hypertensive subjects. Since then, animal models have confirmed these findings, firstly in ex vivo preparations of the mesentery of 20-week-old SHR (Henrich et al., 1978).

Subsequent in vivo studies have confirmed this finding initially in the cremaster muscles of 6-week-old SHR animal models (Chen et al., 1981, le Noble et al., 1990) and the gracilis muscles of 16-week-old SHRs (Prewitt et al., 1982). The latter study was able to demonstrate that, in mature rats, capillary rarefaction was both structural and functional in nature by determining structural capillary density following intra-arterial administration of sodium nitroprusside.

Using the same animal model, capillary rarefaction has been observed in different tissue beds, including the myocardium (Rakusan et al., 1994, Pu et al., 2003) and in cutaneous vessels (Haack et al., 1980). The only organ reported to not

demonstrate consistent capillary rarefaction in the SHR model is the brain (Lin et al., 1990), perhaps as a reflection of the essential physiological need to prevent raised intra-cerebral capillary pressure and consequent parenchymal damage.

Rarefaction has also been demonstrated in other rat models of hypertension: Goldblatt renovascular models (Levy et al., 2001, Kobayashi et al., 1999) and in hypertension induced by angiotensin II (Sabri et al., 1998). Interestingly, in normotensive Sprague-Dawley rats who undergo surgery to experimentally induce aortic coarctation, rarefaction of distal arterioles occurs in cremaster muscle of rats with coarctation as compared with cremaster muscle obtained from sham surgery control animals (Boegehold et al., 1991). In this study, aortic coarctation was achieved using constricting silver clips placed proximally to the renal artery ostia. As the cremaster muscle is a hindlimb structure, it is downstream to the constricting ring and thus has a normal perfusion pressure despite hypertension in other vascular beds. This finding suggests that, in the rat model, capillary rarefaction is not simply a reactive phenomenon to raised perfusion pressure.

In humans, the development of direct intravital capillary video microscopy has allowed visualisation of human cutaneous capillaries in health and disease (Shore, 2000). This technique has been used to demonstrate that essential hypertension is associated with capillary rarefaction in the cutaneous circulation of the fingertip (Gasser and Buhler, 1992). In severe hypertension (mean BP 180/109mmHg), cutaneous capillary density in the forearm is reduced by approximately 20% compared with normotensive controls (Prasad et al., 1995). Furthermore, nailfold capillary rarefaction has been demonstrated in the study of 20 subjects with mild untreated hypertension (Cheng et al., 2008b). In a smaller study of elderly participants with predominantly isolated systolic hypertension, a trend towards capillary rarefaction in the cutaneous microcirculation of the finger was detected, though this was not statistically significant (James et al., 2006).

These studies were performed at rest and therefore reflect functional capillary rarefaction. Further studies have used post-occlusive hyperaemia and venous occlusion to demonstrate structural capillary rarefaction in the cutaneous microcirculation of subjects with essential hypertension (Antonios et al., 1999c, Serne et al., 2001). Venous occlusion yielded significantly higher capillary density results than post-occlusive hyperaemia in these studies and should therefore be considered as the more accurate measure of total structural capillary density.

Capillary rarefaction also occurs in other organs in essential hypertension, having been demonstrated ex vivo in skeletal muscle from subjects with essential hypertension (Henrich et al., 1988). Furthermore, recent advances in retinal imaging has facilitated the measurement of a marked reduction in the number of terminal arteriolar branches as an indicator of rarefaction, in essential hypertension (Hughes et al., 2006).

A new method of visualising the human sublingual microcirculation in vivo using side stream dark field imaging (Microscan®) has been shown to be reproducible in healthy volunteers and subjects with clinical conditions known to disrupt the microcirculation (Hubble et al., 2009, Reynolds et al., 2013). This technique has yet to be applied to investigate the human sublingual microcirculation in essential hypertension. Therefore, the question as to whether sublingual microvascular rarefaction occurs in essential hypertension (and whether this improves with treatment) remains unanswered.

Capillary rarefaction may antedate the onset of sustained hypertension given its presence in subjects with normal BP and hypertensive parents versus those with normotensive parents (Noon et al., 1997). In addition, rarefaction has been demonstrated in individuals with “high normal” blood pressure (Antonios et al., 1999b), and normotensive adult offspring of hypertensive parents (Antonios et al., 2003). A role for capillary rarefaction *in utero* in the pathogenesis of hypertension is supported by the finding of reduced capillary density in newborn infants of hypertensive mothers versus infants of normotensive mothers (Antonios et al., 2012).

However, the studies provide conflicting results as to whether the early rarefaction of hypertension is a structural or functional in nature. Whereas Antonios *et al*. (2012) and Noon et al. (1997) described a normal density of perfused capillaries at rest with rarefaction only at maximal capillary density with venous occlusion in the study group, the Antonios *et al.* studies of 1999 and 2003 describe physiological rarefaction at rest in addition to rarefaction with venous occlusion. Therefore, it remains unclear as to whether the observed capillary rarefaction at rest in those at risk of hypertension but without clinical manifestations of the disease is due to a structural absence of capillaries or a physiological consequence of reduced microvascular perfusion. As the studies report relatively small numbers of participants in their experimental groups (n=18-25), it can be

speculated that larger future studies may help to clarify this discrepancy in their findings.

1.5.7 Mechanism of rarefaction

The finding of rarefaction in hypertensive patients has led to speculation that the pathophysiology of essential hypertension involves a primary defect in angiogenesis (Feihl et al., 2006). This is supported by the study of circulating bone-marrow-derived endothelial progenitor cells (EPCs), first described in 1997 (Asahara et al., 1997) and now thought to play a key role in vascular repair and angiogenesis (Hristov et al., 2003a). In the SHR and deoxycorticosterone acetate (DOCA)-salt hypertensive rat models, EPC senescence is increased compared with control animals, as determined using β-galactosidase staining (Imanishi et al., 2005). On the contrary, enhanced EPC function has been detected in a mouse model of renovascular hypertension (Salguero et al., 2008), though subsequent study of renovascular hypertensive pigs has revealed that this is likely to be a transitory compensatory mechanism, as EPC function during persistent hypertension tends to reduce after initial activation (Zhu et al., 2011).
EPCs isolated from the blood of human subjects with hypertension show accelerated senescence and reduced telomerase activity (Imanishi et al., 2005). Furthermore, EPCs derived from subjects with pre-hypertension (defined as office systolic BP =130-139mmHg or office diastolic BP = 85-89mmHg) also show impaired function in vitro when compared with control sample EPCs (MacEneaney et al., 2011). Although this study originally defined pre-hypertension as office systolic BP = 120-139mmHg or diastolic BP = 80-89mmHg, a group which showed no difference in EPC function versus controls, the post-hoc definition of pre-hypertension which did demonstrate a significant difference in EPC function is more in keeping with the definition found in current consensus guidelines in the UK and Europe (NICE, 2011, Mancia et al., 2013).
A recent study has determined that reduced angiogenesis, as evidenced by reduced EPC function, occurs in those at risk of hypertension in later life by virtue of being born of hypertensive pregnancies (Yu et al., 2016). However, the study group included a significant number of subjects born of pregnancies complicated by pre-eclampsia and other disorders specific to pregnancy, for which the primary pathological processes are markedly different to essential hypertension. In these

circumstances, it is possible that the determinants of hypertension in offspring later in life are different from those for children of uncomplicated pregnancies. Whether reduced EPC activity occurs in normotensive individuals who are at risk of essential hypertension due to being offspring of parents with essential hypertension is an intriguing research question which has not yet been addressed.

Further evidence for a primary role for attenuated angiogenesis in the development of essential hypertension is provided by the finding that anti-angiogenic chemotherapy agents, which are used increasingly in the treatment of solid tumours, trigger hypertension in treated patients (Mourad and Levy, 2011).

Opposing the argument that defective angiogenesis is responsible for the rarefaction of hypertension is that capillary rarefaction can be found in animal models where the hypertension is clearly a secondary phenomenon, such as the renovascular models and surgically-induced aortic coarctation (Boegehold et al., 1991, Levy et al., 2001, Kobayashi et al., 1999). It may therefore be concluded that either impaired angiogenesis occurs as a consequence of hypertension (though not via a direct pressure-dependent mechanism), or that rarefaction and impaired angiogenesis represent a common feature of both essential and secondary hypertension, being a primary defect in the former condition.

Capillary and arteriolar rarefaction results in raised peripheral resistance (Greene et al., 1989) and is associated with increased capillary pressure (Tooke et al., 1991). The direct consequences of this include the disruption of oxygen, nutrient and metabolite exchange with tissue, which contributes to the target organ damage occurring in the disease. As such, rarefaction, and by extension impaired angiogenesis, provides an important potential therapeutic target for antihypertensive therapy.

1.5.8 Does rarefaction reverse with antihypertensive treatment?

Studies exploring the reversal of capillary rarefaction with antihypertensive treatment have produce conflicting findings depending on the class of antihypertensive, animal model and methodology used.

In the SHR, nifedipine (a CCB) has been shown to attenuate (Rakusan et al., 1994) or even completely reverse (Turek et al., 1987) cardiac capillary

rarefaction. In a renovascular rat model of hypertension, another CCB agent, benidipine, has also be demonstrated to significantly reduce the degree of cardiac capillary rarefaction (Kobayashi et al., 1999).

Studies investigating the effects of ACEi and ARB agents on microvascular rarefaction have produced more inconsistent findings. In the SHR, one study has shown no effect on cardiac capillary density with either ACEi or ARB (Pu et al., 2003) whereas an earlier study had shown a significant increase in cardiac capillary density in the same model treated with an ACEi (Unger et al., 1992). The discrepancy in this case may be due to the timing of treatment, with Pu *et al.* commencing treatment when the animals were aged 10 weeks and Unger and colleagues treating from birth. Certainly in rats in whom hypertension has been induced through administration of angiotensin II, co-administration of the ARB, losartan, can prevent development of hypertension and cardiac capillary rarefaction (Sabri et al., 1998).

The thiazide diuretic, indapamide, has also been shown to ameliorate capillary rarefaction in cardiac tissue of hypertensive rats. In the 1 kidney-1 clip renovascular rat model, indapamide (with or without the addition of an ACEi) increased cardiac capillary density, whereas monotherapy ACEi had no effect. In the SHR, both ACEi and indapamide trended towards affecting an increase in cardiac capillary density, with the combination of the two medications acting synergistically to induce a significant increase in cardiac capillary density (Rakusan et al., 2000).

A more recent study aimed to address this heterogeneity of findings by directly comparing the effects of an ACEi (enalapril), ARB (losartan), CCB (nifedipine) and beta-blocker (atenolol) on functional and structural capillary density of SHR skin and skeletal muscle (Sabino et al., 2008). All medications, except atenolol, were found to increase capillary density, though nifedipine had no effect on structural rarefaction in the heart and enalapril did not reverse structural rarefaction in the cutaneous microvasculature.

Centrally-acting antihypertensive agents have also been investigated in this regard in the SHR model (Nascimento et al., 2010): monotherapy with clonidine, rilmenidine or moxonidine for 28 days reversed capillary rarefaction in skeletal muscle and cutaneous tissue. This raises the possibility that reversal of capillary rarefaction by antihypertensive agents is mediated directly via a reduction in BP. However, the finding that superoxide scavenger antioxidants are also able to

attenuate structural capillary rarefaction in skeletal muscle of the SHR (Kobayashi et al., 2005), possibly through the inhibition of apoptosis in these vascular beds, raises the possibility that some antihypertensive agents may be acting additionally to increase capillary density in hypertensive subjects through an antioxidant effect. This may explain the variable findings between different antihypertensive classes described above.

In humans, few studies have specifically investigated the effect of antihypertensive agents on the capillary rarefaction of hypertension. In two cross-sectional studies, nailfold capillary density was found to be reduced, even in subjects treated for hypertension with an assortment of antihypertensive agents (Cheng et al., 2008a, Penna et al., 2008). However, in both studies a significantly higher mean BP in the treated hypertensive group compared with the normotensive controls was reported, which would explain the difference between the two groups in terms of capillary density. A further cross-sectional study found that subjects with treated hypertension had higher cutaneous capillary density than normotensive controls (Debbabi et al., 2006). These findings are surprising and are certainly limited by methodology used by the authors. In particular, single photographs were used to determine capillary density, rather than the conventional 2-5 minutes of continuous video, potentially introducing a significant confounder to results. A subsequent further open label cross-sectional study by the same group reaffirmed these findings in almost 200 participants, whilst adding that only subjects receiving a combination of ACEi and thiazide diuretic demonstrated a significant increase in structural cutaneous capillary density versus normotensive controls (Debbabi et al., 2010).

Given the conflicting findings of these studies as a whole, and the inherent confounding factors inherent in cross-sectional studies, it is difficult to draw definitive conclusions from these results.

Only 2 studies to date have addressed these limitations by completing linear interventional studies to investigate the effects of antihypertensive treatment on capillary rarefaction in humans. The first of these assessed capillary density in the retinas of 25 never-treated hypertensive subjects allocated to receive either amlodipine or lisinopril in a double-blind randomised trial (Hughes et al., 2008). After 52 weeks' treatment, microvascular density was found to have increased in both treatment groups, with no significant difference between the two groups.

A further interventional study aimed to determine the effects of treatment with either an ARB (olmesartan) or vasodilatory beta-blocker (metoprolol) on cutaneous capillary density (Kaiser et al., 2013). After 26 weeks' treatment, capillary density was found to have increased in both treatment groups, though not back to that found in control subjects. The results indicate a similar increase in capillary density after post-occlusive reactive hyperaemia (PORH) in all groups when compared with resting capillary density, and therefore indicate that structural rarefaction, rather than functional rarefaction, improved with treatment. Of note, not all participants were treatment-naïve prior to enrolment, which may have affected the response of these subjects to treatment within the study. Furthermore, PORH was employed to determine maximal (structural) capillary density, a technique which is known to recruit fewer capillaries than venous occlusion and thus under-estimates structural capillary density. The surprising finding by the authors that minimal additional recruitment occurred with PORH compared to rest images underscores this limitation to the results generated. As such, the conclusions drawn from this study should be done so with caution and further prospective studies investigating the effect of antihypertensive treatment on the microvasculature of the skin, together with other vascular beds, are needed to corroborate or refute these findings.

In conclusion, studies investigating whether capillary rarefaction is reversible with antihypertensive treatment have produced conflicting results, though the weight of evidence suggests that CCBs and agents which block the renin-angiotensin-aldosterone axis are most effective at doing so, particularly in the rat model. Other interventions which lower BP, such as reducing salt intake, have also been shown to ameliorate the rarefaction of hypertension (He et al., 2010), lending support to the conjecture that at least part of this effect on the microvasculature appears to be directly mediated by the reduction in BP. However, the heterogeneous effects of different classes of antihypertensive agents, and the positive effect of antioxidants in reducing capillary rarefaction in the rat model, suggests that part of the reduction in rarefaction may be mediated by BP-independent mechanisms. However, all of the studies in humans have focussed on grade I-II hypertension. Whether capillary rarefaction reverses with treatment in grade II-III hypertension is not known. If capillary rarefaction proves to be irreversible in more advanced disease, this may provide, at least in part, a physiological basis for true treatment resistance in hypertension. If this were shown to be the case, consideration would

need to be given to the development of treatments that specifically target the microvasculature in subjects with reduced baseline capillary density.

1.7 Aims of the MD project

It is not known whether rapid treatment of newly-diagnosed grade II-III hypertension is safe and effective, though retrospective studies have suggested it may confer a prognostic advantage over delayed treatment. The over-arching aim of the project is to investigate this through the development and application of a new 18-week treatment programme, aimed to reduce BP effectively using a stepwise protocol.

The primary research question is therefore whether the conduct of such a programme is feasible, as determined by whether sufficient participants can be recruited to the study and complete the 18-week protocol.

The secondary aims of the project are to explore the physiological and clinical effects of such a programme. Accordingly, the BP reductions achieved during the 18-week treatment protocol will be determined and the proportion of indiviudals at target after 18 weeks compared with the proportion at target with usual care in the community. Furthermore, adverse events during treatment will be closely monitored and documented.

A nested experimental medicine study will utilise the treatment programme to assess whether capillary rarefaction is reversible with antihypertensive treatment in grade II-III essential hypertension and whether left ventricular mass (and mechanics) change rapidly within the short timeframe described. The programme also provides an opportunity to develop and validate a disease-specfic instrument for the measurement of HRQoL in hypertension.

1.8 Objectives

1. Assess whether a treatment protocol, delivered over 18 weeks, is feasible for the management of treatment-naïve moderate-severe hypertension.
2. Determine if cutaneous capillary density changes after 18 weeks' antihypertensive treatment in moderate-severe hypertension.
3. Compare different microvascular beds, particularly cutaneous and sublingual vessels, in hypertensive subjects before and after treatment.

4. Determine whether myocardial structure and function, as assessed by cardiac Magnetic Resonance Imaging (MRI), changes following 18 weeks' hypertension treatment.
5. Validate the English language adaptation of a hypertension disease-specific instrument for measurement of HRQoL.

Chapter 2 Methods

2.1 Study design

I planned to enrol participants into an 18-week treatment programme, to examine the feasibility and safety of a rapid treatment programme for newly-diagnosed grade II and III hypertension from a clinical standpoint. Using the design of a nested experimental study, I aimed to explore the cardiac and microvascular changes occurring between enrolment and after 18 weeks of treatment.

2.2 Clinical treatment protocol

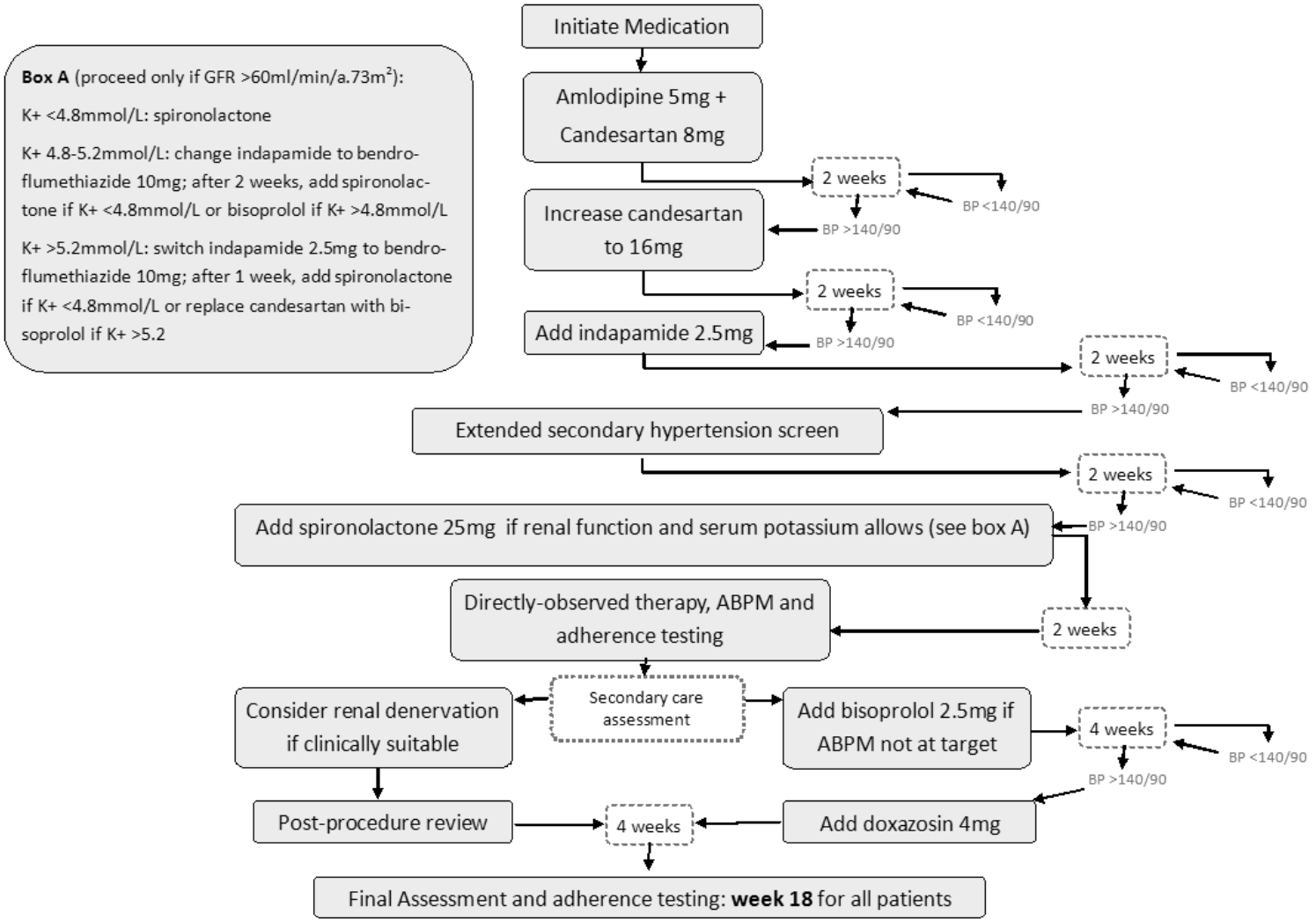
Subjects never treated for hypertension were encouraged to be referred for possible study enrolment after a single usual care office BP measuring ≥170mmHg. To facilitate referral to the study, General Practitioners and secondary care doctors in the referring area were contacted and invited to meet the study team. In order to enable timely referrals before antihypertensive treatment was started, a software program for SystmOne was developed, which alerted clinicians using this system to refer to the study should they enter clinical details compatible with study enrolment. In addition, through engagement with local industry and University research events, the study was widely publicised, thereby promoting referrals from all available sources.
Following referral from their usual care practitioner with an office systolic BP measurement ≥170mmHg, subjects aged 18-79 years were considered for enrolment provided their ambulatory daytime average systolic BP measured ≥150mmHg when performed by the study team, thus defining grade II-III hypertension (Table 1.1). Office BP was also measured by the study team during the screening visit, though this was not used to decide study inclusion.

The exclusion criteria for enrolment in the study were in-keeping with those for renal denervation as it was envisaged at the planning stage that renal denervation would be a key component of the study:

- Previous or current prescription of any medication used in the study protocol or previous pharmacological treatment of hypertension with any agent, regardless of duration of treatment.
- Glomerular filtration rate (GFR) <60 ml/min/1.73m^2 by the Modification of Diet in Renal Disease Study (MDRD) formula.
- Previous renal artery intervention.
- Bleeding diathesis
- Haemoglobin <10 g/dl
- Platelet count <100 x10^9/l
- Inability to provide informed consent
- Pregnancy or breast-feeding
- Hypertension-related event (including stroke or acute kidney injury) within the preceding 3 months
- Any condition, including hypertensive urgency, which requires more immediate BP lowering or tailored anti-hypertensive strategy at enrolment.

Once enrolled, participants began pharmacological antihypertensive treatment, initially with the combination of an angiotensin receptor blocker (candesartan 8mg once daily) and dihydropyridine calcium channel blocker (amlodipine 5mg once daily). Thereafter, treatment followed a defined programme (Figure 2.1), with medication changes in-keeping with consensus guidelines (NICE, 2011, Mancia et al., 2013), though over an accelerated timeframe of 18 weeks.

Figure 2.1: 18-week treatment protocol for enrolled subjects with grade II or III hypertension



For each visit after initiation of antihypertensive treatment, office BP was measured and treatment intensified in a stepwise manner, following the protocol, should the participant's BP be above target. Visits were undertaken by a doctor or study nurse at the Diabetes and Vascular Research Centre, Exeter, using the mobile clinic or in the participant's own home, depending on the participant's preference and availability.

2.2.1 Standardisation of Office BP and other measurements

As the primary outcome for the clinical aspect of the study, it was critical to ensure that office BP was measured in a uniform manner.

Patients were asked to take their usual medications on the day of each study visit. They were asked not to consume caffeine or over the counter medicine within 12 hours of the visit and removed tight or restrictive clothing from their arm. BP measurement took place following 5 minutes in a seated position (NICE, 2011, O'Brien et al., 2003); the participant and clinician did not speak whilst the cuff was inflating and deflating and it was ensured that the participant's legs were uncrossed (Pickering et al., 2005). Throughout the study, the same automated BP device was used to measure BP from the same arm (the arm with the highest reading, identified at their enrolment visit), using a cuff of an appropriate size. The first BP measured in this manner at each visit was removed from analysis, as per current clinical guidelines (NICE, 2011).

Throughout, office BP was measured in accordance with Standard Operating Procedures (SOPs) drafted specifically for the study (Appendix A-B), using an OMRON M6 device (Omron Healthcare Europe B.V. Hoofddorp, Netherlands). Ambulatory BP was measured using an A&D TM-2430 system (A&D Instruments Limited, Abingdon, UK), together with its associated analysis software (Doctor Pro TM-2430-13, A&D Instruments Limited, Abingdon UK)(Appendix C). Prior to analysis, participants was asked for their sleep/wake times and these were used to determine average daytime and average nocturnal BP metrics.

Height was measured in accordance with a pre-defined SOP (Appendix D). Weight and body composition was determined using a Tanita Body Composition Analyzer BC-418 MA (Tanita Europe BV, Amsterdam, Netherlands)(Appendix E).

Albumin excretion rate (AER) was calculated from the average of two overnight timed urine samples, using the COBAS method (Roche Diagnostics Limited, Burgess Hill, UK). Standard formulae were employed to determine AER and albumin:creatinine ratio (ACR) from samples collected.

Given the propensity for the aldosterone:renin ratio measurement to be affected by posture (Tiu et al., 2005) and degradation of renin at room temperature (Locsei et al., 2009), a SOP was developed to ensure standardisation of this measurement in all participants (Appendix F).

Epworth scores were calculated prior to enrolment for all participants, to exclude the presence of obstructive sleep apnoea. This was undertaken using a standard questionnaire (Appendix G), with a score >10 regarded as abnormal (Johns, 1993).

For the avoidance of doubt, the SOPs given in Appendix C-E and Appendix G were not drafted by the candidate, being in place before the period of study commenced.

2.2.2 Lifestyle interventions

Participants received lifestyle advice at every visit, given the proven benefit of lifestyle adaptations in terms of BP control (Appel et al., 2003). This included encouragement to address alcohol, salt, caffeine and liquorice intake, should these be significant factors in each individual case. Furthermore, participants were encouraged to consume a healthy diet and stop smoking (if applicable). If there was a need to supply lifestyle educational material, British Heart Foundation-approved booklets were provided.

2.2.3 Secondary hypertension

At enrolment, participants underwent a physical examination to determine signs of endocrine disorders, such as hyperthyroidism, acromegaly and Cushing's syndrome. In addition blood samples for thyroid and renal function were taken.

In subjects aged <45 years at enrolment, an extended secondary hypertension screen was performed. This included serum aldosterone/renin ratio, 24-hour urinary metanephrines and 24-hour urinary free cortisol. These investigations

were also undertaken in subjects aged ≥45 years who were found to be resistant to treatment within the protocol (Figure 2.1).

2.2.4 Management of medication intolerance

Participants were encouraged to report medication side effects and were offered the opportunity to benefit from a low threshold for changing medications should intolerance be determined.

Specifically, should the addition of any antihypertensive agent precipitate a >30% rise in serum creatinine, the new medication was discontinued without delay. Hypokalaemia and hyperkalaemia were managed according to a pre-defined protocol in-keeping with contemporary clinical standards (Rosano et al., 2018).

If lightheadedness was reported, this prompted measurement of 24-hour ambulatory BP, with de-escalation of antihypertensive medication if this determined relative hypotension. Should a medication provoke mild side effects, it was proposed that these often resolve with continued treatment, though the study team member was permitted to reduce the frequency of medication administration if needed.

It was noted that the dihydropyridine calcium channel antagonist, amlodipine, was used as an initial agent in the treatment protocol, though causes ankle oedema in 10-20% patients (Osterloh, 1989). Therefore, when ankle swelling whilst taking amlodipine was reported, this was changed to the alternative dihydropyridine calcium channel antagonist, lercanidipine.

In addition, if participants developed gynaecomastia as a consequence of spironolactone, it was planned to change this medication to eplerenone. As bisoprolol can be associated with peripheral coldness, this was changed to an alternative cardiac-selective beta-blocker, nebivolol, if this particular symptom was reported.

Overall, deviation from the standardised protocol was permitted for any participant following discussion with the study investigators, with consultant clinician support.

2.2.5 Withdrawal of participants from study treatment

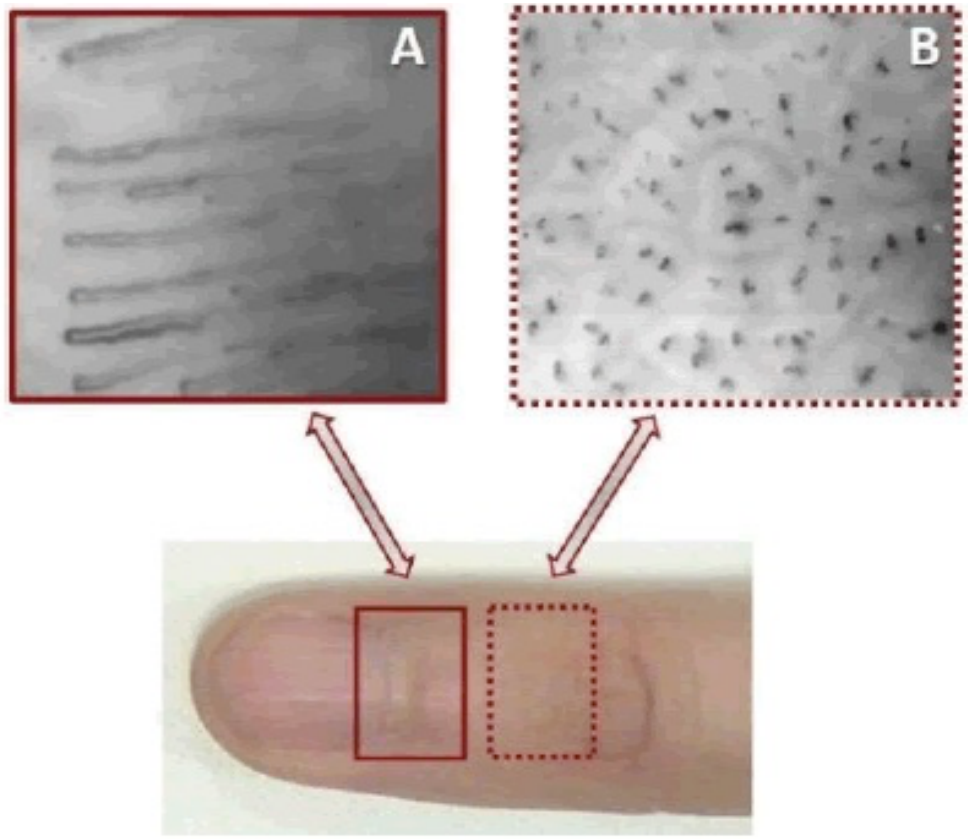
At the time of informed consent and enrolment, participants were told that they had the right to withdraw from the study and/or treatment at any time, without giving reason. In addition, it was planned to withdraw participants from the study should they become ineligible (as dictated by *a priori* inclusion and exclusion criteria), deviated significantly from the treatment protocol, were non-adherent to treatment, withdrew consent or were lost to follow-up. Nevertheless, intention-to-treat analysis of study outcomes was employed, including those who withdrew from study participation, when possible.

2.3 Capillaroscopy

Cutaneous capillary number was determined using capillary video microscopy (capillaroscopy) at the time of study enrolment and after 18 weeks of antihypertensive treatment.

Historically, rudimentary microscopy was first used to visualise capillaries *in vivo* by Johan Christophorous Kolhaus in 1663, who described “small vessels in the nailfold of man” (Gilje et al., 1953). Thereafter, Giovanni Rasori, an Italian academic and physician, used a magnifying glass to describe an “inexorable knot of capillary loops” in conjunctivae (Rossi and Rasori, 1955), including in this description the finding of “abnormally reddish” areas of inflammation. Modern capillaroscopy allows *in vivo* assessment of perfused capillaries through illumination of skin by a light source under which haemoglobin appears black. The nailfold is often chosen as the site of analysis as this is easily accessible and, in this region, the terminal capillary loops lie parallel to the surface of the skin, allowing straightforward visualisation (Cutolo et al., 2005). When dorsal digital cutaneous capillaries are visualised, the terminal capillary loops lie perpendicular or oblique to the surface of the skin and can therefore be counted for a known area (Figure 2.2), facilitating determination of cutaneous capillary density.

Figure 2.2: Cutaneous capillaroscopy of the nailfold (A) and dorsal finger (B), adapted from (Fedorovich et al., 2018)



For capillaroscopy in the Diabetes and Vascular Research Centre, University of Exeter, a black and white charge-coupled camera (LDH 0703/30, Philips, Eindhoven, Netherlands) is mounted onto a microscope fitted with a 10x objective lens and 0.5x c-mount reduction lens. A 100W mercury vapour light (Leica Microsystems, Wetzier, Germany) is used, which has an emission spectrum similar to the absorption spectrum of haemoglobin (370-450nm). As such, perfused capillaries appear black, though capillaries which are not perfused are not visualised. Images are displayed in real-time on a black and white monitor (VM-920K, Hitachi, Tokyo, Japan) and recorded on sVHS video (AG7350, Panasonic, Tokyo, Japan) for post-hoc analysis.

2.3.1 Standardised capillaroscopy protocol

Before capillaroscopy can be performed on an individual they must be acclimatised in a temperature-controlled environment, given the thermoregulatory properties of skin and therefore the capacity for ambient and

skin temperature to affect perfused capillary density (Shore, 2000). Acclimatisation was standardised for at least 30 minutes within a laboratory where the temperature was maintained at 23 ±1°C. In addition, subjects were asked to refrain from smoking and from consuming caffeinated beverages for 12 hours beforehand and were asked to remain nil by mouth overnight, prior to consuming breakfast of two slices of wholemeal toast with butter, provided by the study team immediately before the start of acclimatisation.

To facilitate the examination, subject were supine with their left upper limb at heart level and supported by a padded table. The middle finger was fixed with a reusable mould and clear nail varnish applied to the dorsal surface of the finger, proximal to the nail fold, to mitigate the scattering of light at the interface between the skin and atmosphere. A digital cuff was applied to the middle finger proximally.

For each sequence of data acquisition, 6 microscopy fields were analysed for 1 minute each, following an anticlockwise cycle and ensuring that the field of view returned to the original position at the end of the acquisition process. For each field, the microscope fine focus was adjusted to varying depths to ensure that all capillaries were seen.

The above routine was undertaken firstly at rest, in order to determine functional (resting) capillary density. Thereafter, structural (total) capillary density was examined following 10 minutes of venous occlusion, inflating the digital cuff to 50mmHg. This method was selected, rather than post-occlusive reactive hyperaemia, as previous investigations have determined that capillary measurement during venous occlusion has a greater yield for the determination of structural capillary density (Antonios et al., 1999a, Serne et al., 2001).

2.3.2 Capillaroscopy data analysis

Recordings were analysed post-hoc, using a sheet of acetate on which was drawn a 119mm diameter circle. The acetate was held on the monitor screen and, for each field analysed, the total number of visible capillaries within the circle, including those capillaries only visible transiently, were marked. Firstly these were counted for the data acquired at rest and averaged for the 6 fields acquired. Using the known area of the circle and the final magnification value (169x), capillary density was derived from the average capillary number.

Thereafter, data acquired during venous occlusion was analysed, as above. This provided values for structural (total) capillary density, as opposed to functional (resting) capillary density. Capillary reserve could then be calculated by determining the percentage of additional capillaries perfused following venous occlusion.

Prior to acquiring data for the study, the accuracy and reproducibility of the above techniques and measurements were validated against departmental standards using previously acquired and validated recordings. Unfortunately, given the time between undertaking this analysis and the completion of the research project, this data was lost and is therefore not available for presentation. Nevertheless, contemporaneously, the results were reviewed by the supervising team and felt to be acceptable.

2.4 Cardiac MRI

MRI studies were undertaken at the Exeter Magnetic Resonance Research Centre, St Luke's Campus, University of Exeter, using the Philips 1.5T MRI scanner contained therein (Philips Intera, Philips Healthcare, Best, Netherlands). A 5-channel surface phased array coil was used for data acquisition.

Prior to the examination, participants were specifically consented for administration of gadolinium as the contrast agent, including the rare association with nephrogenic systemic fibrosis (Kuo et al., 2007) though the risk of this was minimised through exclusion of subjects with chronic kidney disease stage IIIa or greater prior to enrolment. Studies were aborted in cases of claustrophobia, though a further attempt with the participant entering the scanner prone was considered in each case, where appropriate.

2.4.1 Sequence protocol

The study MRI protocol was finalised following discussion with the UK Biobank cardiac imaging lead, Prof Steffen Petersen, to benefit from their expertise in streamlining imaging processes and providing cost- and time-effective MRI data for analysis.

As such, the protocol consisted of localisers, followed by 4-chamber, 2-chamber and 3-chamber long axis steady-state free precession (SSFP) imaging. This facilitated acquisition of a short axis stack, which was used for quantification of left ventricular volumes and mass, with absolute values indexed to body surface area. T1-weighted gradient echo sequences were employed to determine renal and adrenal anatomy, aiming to exclude adrenal adenomas as a secondary cause of hypertension in particular. SSFP imaging of the entire thoracic aorta was supplemented by a dedicated aortogram after intravenous administration of gadolinium (0.15mmol/kg gadopentate dimeglumine, Gadovist®, Bayer Healthcare Pharmaceuticals, Wayne NJ, USA), delineating the aorta and renal arteries and thereby excluding coarctation and renal artery stenosis as causes of secondary hypertension.

8-10 minutes after the administration of gadolinium, gradient echo phase-sensitive inversion recovery sequences in basal, mid and apical short axis slices, together with 1 long axis plane, were used to evaluate for the presence of late gadolinium enhancement, indicating focal myocardial fibrosis, infarction or infiltration (Vohringer et al., 2007).

2.4.2 Volume calculations

MRI data were analysed initially using Extended MR Workspace (Philips Healthcare, Best, Netherlands) to planimeter end-diastolic epicardial area, end-diastolic endocardial area and end-diastolic epicardial area in each short axis slice covering the entire left ventricle from mitral valve to apex, in-keeping with standard protocols (Kramer et al., 2013). Left atrial volumes were calculated using the biplane area length method, which has been shown to be accurate and reproducible, when compared with the biplane modified Simpson and prolate ellipse methods (Jiamsripong et al., 2008).

The distance from the middle of the atrioventricular ring to the left ventricular apex in end-diastole, as measured in the 4-chamber long axis steady state free precession (SSFP) sequence, was defined as left ventricular long-axis length. Similarly, the 4-chamber view was used to define left ventricular short-axis length, measured as the distance between endocardial surfaces at the level of the papillary muscle insertion point in end-diastole. The ratio of left ventricular short

axis length to left ventricular long axis length was then calculated to define left ventricular sphericity index, as previously described (Maceira et al., 2006).

2.4.2 Feature tracking

The MRI protocol also allowed for post-hoc analysis of myocardial strain using feature tracking, undertaken using specialist software (TomTec Imaging Systems, 2-dimensional CPA MR, Cardiac Performance Analysis, Unterschleissheim, Germany).

Strain is a measurement of myocardial contraction and relaxation and is defined as the change in myocardial segment length as a ratio to its original length. This allows deformation to be defined and quantified in a specific direction of myocardial contraction in relation to the cardiac axis: longitudinal, circumferential and radial (Scatteia et al., 2017). As such, longitudinal strain represents the shortening of the myocardium from base to apex, radial strain is the deformation of the myocardium inwards towards the blood pool and circumferential strain describes the circular deformation around the axis of the ventricle. Moreover, the rate of deformation in each direction can be defined and expressed as strain rate. Through this precise quantification of myocardial function, cardiac diseases can be detected before becoming apparent with volume quantification techniques (Negishi et al., 2014, Negishi et al., 2013).

Feature tracking is a post-processing technique which traces tissue voxels throughout the cardiac cycle, thereby isolating and calculating strain in each dimension described above, using inhomogeneities in tissue brightness or anatomical features within the image (Hor et al., 2011, Hor et al., 2010, Schuster et al., 2016). The methodology necessitates manual tracing of left ventricular endocardial and epicardial borders, with the initial contour set in end-diastole. This process is performed for the 4-chamber view long axis SSFP cine and then repeated in three short axis planes: apical, mid-ventricle and basal. The most apical plane still showing the blood pool and the most basal plane showing myocardium circumferentially were identified and the planes adjacent to these in the direction of the mid-cavity chosen for analysis, as previously described (Kowallick et al., 2016) and given that the most apical and basal slices have shown poorer intra-observer and inter-observer reproducibility for calculation of strain parameters (Kowallick et al., 2014). The slice equidistant between the

selected apical and basal slices was identified as the mid-ventricular cine for analysis.

Following manual tracing in the end-diastolic frame, the software automatically traces the endocardial and epicardial borders throughout the cardiac cycle, though the tracking performance was manually reviewed and the process above repeated if the tracking of endocardial and epicardial borders appeared imprecise. This procedure was repeated 3 times with the results calculated as the mean of these 3 measurements.

In addition to longitudinal, radial and circumferential strain as described above, the left ventricle also produces forward blood flow through twist: clockwise rotation of basal segments relative to anticlockwise rotation of apical segments (when viewed from the apex) (Esch and Warburton, 2009). Basal and apical rotation, reported in degrees, can be assessed using feature tracking with good intra-observer and inter-observer reproducibility (Kowallick et al., 2016, Kowallick et al., 2014). By convention, apical rotation is positive and net rotation between basal clockwise rotation (ϕ_{base}) and apical anticlockwise rotation (ϕ_{apex}) defines "twist":

$$Twist = \phi_{apex} - \phi_{base}$$

This measurement can be normalised for left ventricular length as the distance between the basal and apical imaging planes (D) to define "torsion" in degrees/cm:

$$Torsion = \frac{\phi apex - \phi_{base}}{D}$$

Feature tracking was selected as the preferred technique for use in the study, over other methods for the quantification of myocardial strain using MRI, given the fact that competing techniques require the acquisition of additional sequences or employ time-consuming non-automated post-processing (Scatteia et al., 2017), limiting their use clinically and therefore the applicability of the results in the present study to real-world medicine.

For example, MRI tagging, which involves the production of visible orthogonal lines on the myocardium throughout cine imaging, is subject to fading of these

lines and low spatial resolution. In addition, the acquisition of tagging sequences is time-consuming and can involve long breath-holds for patients. Phase velocity mapping, used clinically for the assessment of valvular heart disease, has also been repurposed to assess myocardial deformation, though is constrained by poor temporal resolution (Simpson et al., 2013). Displacement encoding with stimulated echoes (DENSE), which employs encoding of displacement into the phase of the image, and strain-encoded imaging (SENC), which uses tagging parallel to the imaging plane, are both limited by fading of the deformation data during the cardiac cycle (Gao et al., 2014, Osman et al., 2001). Therefore, given its real-world applicability, excellent temporal resolution and lack of impact on overall scanning time, MRI feature tracking provided the best solution for exploration of myocardial mechanics within the study.

A SOP was drafted to enable standardisation of the procedure for feature tracking analysis, including for future projects (Appendix H).

2.4.3 Strain reproducibility

To assess intra-observer reproducibility of strain parameters within the study, the feature tracking analysis procedure was repeated to produce a mean of 3 separate analyses for each set of images from 10 normotensive healthy control subjects and 10 participants prior to and following antihypertensive treatment. Image analysis was performed on 3 occasions with the coefficient of variation determined for each participant and averaged as shown (Table 2.1). Once intra-observer variability had been established as in-keeping with previous published data (Kowallick et al., 2014), the feature tracking analysis protocol was applied to all studies retrospectively.

Table 2.1: Intra-subject reproducibility of feature tracking parameters in 10 normotensive controls, in 10 hypertensive subjects before treatment and in 10 hypertensive subjects after treatment

Strain parameter		CoV (%)		
		Control subjects	Pre-treatment	Post-treatment
Radial (short axis)	Endocardial	13.7	15.0	13.6
	Mean	13.7	15.0	13.6
Radial (long axis)	Endocardial	21.6	17.3	24.2
	Mean	21.6	17.3	24.2
Longitudinal	Endocardial	9.6	8.1	7.8
	Mean	10.4	7.0	8.1
Basal circumferential	Endocardial	3.0	4.2	4.5
	Mean	3.6	4.8	3.1
Mid circumferential	Endocardial	2.7	5.4	3.9
	Mean	3.1	6.4	3.3
Apical	Endocardial	5.0	4.9	4.9
circumferential	Mean	5.0	5.4	4.8
Rotation (apical)	Endocardial	32.2	29.2	31.5
	Mean	24.1	23.7	28.2
Rotation (basal)	Endocardial	48.5	39.5	49.0
	Mean	39.1	41.2	41.6
Twist	Endocardial	18.9	22.5	25.7
	Mean	15.3	19.3	24.6
Torsion	Endocardial	23.2	22.5	25.7
	Mean	15.3	19.3	24.6

Mean: average of endocardial and epicardial measurements; CoV: Coefficient of variation

2.4.4 MRI training

Prior to commencing the MRI aspect of the study, training in performing and interpreting cardiac MRI was undertaken at the Royal Bournemouth Hospital and, once in post, the Royal Devon and Exeter Hospital, achieving Society of Cardiovascular Magnetic Resonance (SCMR) Level 2 standard (Kim et al., 2018). Though this was not formally awarded, ongoing assessment of competency was undertaken by two SCMR Level 3 accredited cardiologists (Dr A. Ludman and Prof N. Bellenger). For feature tracking, training was provided by the vendor (Tomtec Imaging Systems, Unterschleissheim, Germany), with the majority of the analysis being completed by the software's artificial intelligence. Through continued training within the hospital MRI unit and at the St Luke's campus, University of Exeter, together with MRI-related coursework, Level 3 accreditation was awarded by SCMR at the end of the project.

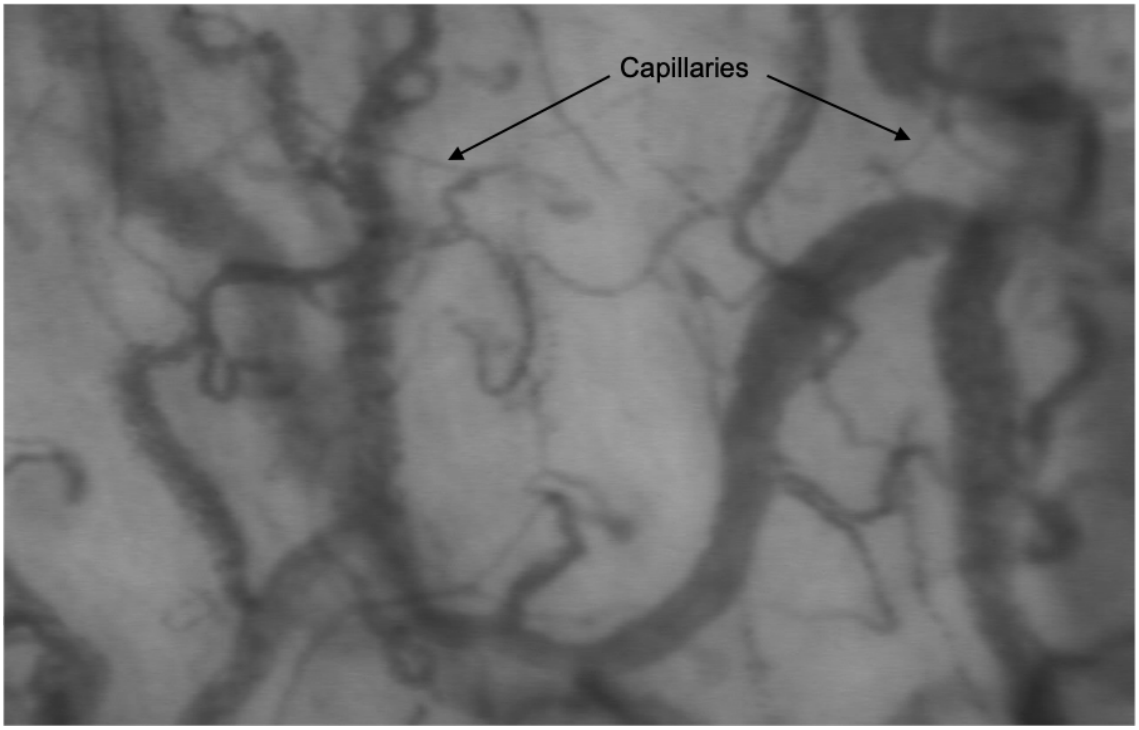
2.5 Sidestream dark field imaging

Visualising the human microcirculation *in vivo* had previously been limited to cutaneous and retinal vessels, until the development of orthogonal polarised spectral (OPS) imaging (Slaaf et al., 1987). This technique used polarised light focussed by a lens onto a target of approximately 1mm diameter. Received light was then collected by the same lens and a charge-coupled camera used to form an image (Groner et al., 1999). Unfortunately, use of this technology was limited by the fact that it required a bulky light source and by the distortion of acquired images by internal light scattering (Ince, 2005).

Building on OPS technology, sidestream dark field (SDF) imaging uses dark-field illumination from the side, obviating the problem of surface reflections by ensuring that there is minimal direct optical contact between the sensing central core of the probe and the light-emitting diodes (LEDs) (Ince, 2005). The handheld probe contains 6 concentrically-placed LEDs with a central sensing lens and charge-coupled camera (Goedhart et al., 2007), providing an overall magnification of 380x (Treu et al., 2011). The LEDs emit light with a wavelength of 530nm, which is absorbed by haemoglobin within erythrocytes (Ince, 2005). Accordingly, perfused capillaries, arterioles and venules within the field of view appear black,

compared with a grey/white background (Figure 2.3). Commonly, the sublingual microcirculation is chosen for examination, as this is accessible in subjects without causing discomfort. This technology is portable, allowing it to be used in a variety of clinical settings, including the emergency department (Trzeciak et al., 2007, Filbin et al., 2014), operating theatre (Bansch et al., 2014) and intensive care unit (Trzeciak et al., 2007, Donati et al., 2013).

Figure 2.3: Sidestream dark field video snapshot of the sublingual microcirculation in a healthy individual using Microscan, showing red blood cells within capillaries, venules and arterioles



2.5.1 Imaging protocol

SDF imaging was acquired during the study using a handheld SDF device (Microscan MicroVision, Medical, Wallingford, USA) with a 5x objective lens and 0.16 aperture number. Data was collected on the day of enrolment to the clinical study and after 18 weeks of antihypertensive treatment.

Procedural techniques followed established practice within the department, which has demonstrated good inter-observer and intra-observer reproducibility in healthy volunteers (Hubble et al., 2009). Specifically, using a disposable cap, the

probe was placed lateral to the frenulum and approximately 3-4cm from the base of the tongue. Once contact with the mucosa was made, the probe was withdrawn slightly whilst maintaining the image, to ensure that extrinsic compression did not affect microvascular blood flow. In order to optimise image quality, brightness and focus were adjusted throughout and care was taken to avoid movement artefact. For each participant, 5 videos were acquired of 15 seconds each, from which the 3 clearest and most stable videos were chosen for offline analysis, in-keeping with expert consensus (De Backer et al., 2007).

2.5.2 Data analysis

Offline analysis was conducted using dedicated Automated Vascular Analysis (AVA) software (AVA 3.0, Academic Medical Center, University of Amsterdam, Netherlands). Firstly, movement artefact correction was applied, prior to manual tracing of all vessels in a defined region of interest. The diameter of these vessels was estimated using a tool within the software package, dividing these into small (1-10μm), medium (11-20μm) and large (21-50μm).

Tracing of vessels facilitated determination of vessel density, with this further classified into total vessel density (which includes all vessels seen and is analogous to structural capillary density), perfused capillary density (which includes vessels only with visible flow and is analogous to functional capillary density) and proportion of perfused vessels (which reflects capillary reserve).

In addition, microvascular flow index (MFI) was calculated. This is a semi-quantitative measure which requires the operator to grade each of 4 quadrants in the field of interest in terms of the predominant type of blood flow seen within vessels (0 = no flow; 1 = intermittent flow; 3 = continuous flow; 4 = hyperdynamic flow). MFI was then determined as the mean of the flow values for the 4 quadrants (Spronk et al., 2002, Boerma et al., 2005, De Backer et al., 2007).

Although MFI has good reproducibility (Boerma et al., 2005, Trzeciak et al., 2007), it does not differentiate between a difference in the proportion of capillaries perfused versus a change in the nature of flow through a static number of perfused capillaries. Further measurements were therefore made using the concept of constructing three equidistant horizontal and vertical lines onto the dataset. By dividing the number of vessels crossing the lines by the total length of the lines, an estimate of vessel density was produced (de Backer score), which

was categorised to produce metrics for the total number of capillaries and the proportion of perfused capillaries at rest (De Backer et al., 2002, De Backer et al., 2007).

2.5.3 Reproducibility

Intra-observer reproducibility was assessed through analysis of three volunteer subjects on three occasions. On each visit, 5 videos were acquired of 15 seconds, with the clearest 3 videos selected for offline analysis. All consensus parameters were calculated for each visit: total vessel density, perfused vessel density, proportion of perfused vessels, MFI and de Backer score. The mean and standard deviation between visits were then determined for each subject, facilitating the calculation of the coefficient of variation for each individual. The overall coefficient of variation for each measurement was calculated as the mean of all 3 control subjects examined (Table 2.2) and was found to be in-keeping with previous data examining these parameters in a cohort of 16 health volunteers in the same institution as the present study (Hubble et al., 2009).

Table 2.2: Intra-subject reproducibility of SDF imaging parameters in normotensive control subjects

SDF imaging parameter		CoV (%) Subject 1	Subject 2	Subject 3	Overall
Small vessels	TVD	5.61	15.57	6.52	9.23
(0-10 µm)	PVD	4.24	17.49	8.37	10.04
	PPV	2.02	1.71	2.29	2.01
	MFI	1.62	0	1.62	1.08
Other vessels	TVD	12.43	3.77	26.72	14.30
(11-50 µm)	PVD	13.36	6.22	26.19	15.26
	PPV	1.77	4.86	4.78	3.80
	MFI	4.95	3.27	4.41	4.21
All vessels	TVD	2.64	13.44	4.75	6.95
	PVD	2.34	15.21	5.49	7.68

	PPV	1.93	1.97	2.70	2.20
	MFI	3.27	1.62	2.95	2.61
Grid crossings		8.75	4.69	0.37	4.60
Grid length		6.53	4.69	10.12	7.11
De Backer score		4.72	8.60	10.69	8.00

SDF: sidestream dark field; CoV: coefficient of variation; TVD: total vessels density; PVD: perfused vessels density; PPV: proportion of perfused vessels; MFI: microvascular flow index.

2.6 Patient-reported outcome measures

Acceptability of the treatment protocol was monitored through administration of health-related quality of life (HRQoL) instruments at enrolment and after 8, 10 and 18 weeks of treatment. This included the generic EQ-5D-5L linear scale, the EQ-5D-5L summary index (UK values), together with the disease-specific Bulpitt-Fletcher instrument. All instruments were self-administered with the questionnaires provided to participants at the appropriate appointments and returned by post.

In tandem, the Spanish language disease-specific MINICHAL instrument (Figure 2.4) was adapted into English, following internationally-accepted standards (Valderas and Alonso, 2008, Wild et al., 2005, Reeve et al., 2013). Psychometric evaluation was enabled through the co-administration of the adapted questionnaire with other instruments as detailed above. Administration at week 8 and at week 10 after study enrolment enabled test-retest evaluation of the English-language MINICHAL instrument. Furthermore, instrument administration before and after 18 weeks of the intensive treatment protocol determined whether this strategy affected HRQoL, contributing to the assessment of acceptability of this protocol for patients.

Figure 2.4: Original Spanish language MINICHAL instrument for evaluation of health-related quality of life in subjects with hypertension

Cuestionario de Calidad de Vida de la Hipertensión Arterial (MINICHAL)

	Marque una cruz en la casilla que elija, sólo una por línea			
¿En los últimos 7 días...	**No, en absoluto**	**Sí, algo**	**Sí, bastante**	**Sí, mucho**
1. ha tenido dificultades para conciliar el sueño?				
2. ha tenido dificultades para continuar con sus relaciones sociales habituales?				
3. le ha resultado difícil entenderse con la gente?				
4. siente que *no* está jugando un papel útil en la vida?				
5. se siente incapaz de tomar decisiones y empezar nuevas cosas?				
6. se ha notado constantemente agobiado y en tensión?				
7. tiene la sensación de que la vida es una lucha continua?				
8. se siente incapaz de disfrutar sus actividades habituales de cada día?				
9. se ha sentido agotado y sin fuerzas?				
10. ha tenido la sensación de que estaba enfermo?				
11. ha notado dificultades al respirar o sensación de falta de aire sin causa aparente?				
12. se le han hinchado los tobillos?				
13. ha notado que orina más a menudo?				
14. ha notado sequedad de boca?				
15. ha notado dolor en el pecho sin hacer ningún esfuerzo?				
16. ha notado una sensación de entumecimiento u hormigueo en alguna parte del cuerpo?				
¿Diría usted que su hipertensión y el tratamiento de la misma afecta a su calidad de vida?				

(Roca-Cusachs et al., 2003)

Psychometric analysis of the English-language MINICHAL instrument included determination of internal consistency through Cronbach's alpha and correlation between items within the instrument. Test-retest reliability was evaluated though calculation of the intraclass correlation coefficient for questionnaires completed after 8 and 10 weeks of treatment, between which there was no change in medication. Construct validity of the instrument was assessed by determining the association of questionnaire results with variable known to affect HRQoL in hypertension, such as heart rate, BMI and gender.

2.7 Cognitive function

Cognitive function was assessed before and after treatment within the protocol, with administration of the Addenbrooke's Cognitive Examination-Revised (ACE-R) questionnaire (Mioshi et al., 2006) and the Revised Trail Making Tests A and B (Reitan, 1958).

The ACE-R, as an examination of cognitive function, has been adopted into clinical practice and is widely-used in research settings (Crawford et al., 2012). The instrument is scored out of 100, with 5 cognitive domains: attention and orientation, memory, verbal fluency, language and visuospatial skills. Higher scores within the test indicate greater cognitive function. Within the study the ACE-R was administered by an appropriately-trained research nurse, within a timeframe of 10-15 minutes.

Trail making tests consist of asking participants to draw lines in order to connect circles on a single sheet of paper in the appropriate order. The test consists of 2 parts: Part A consists of the numbers 1-25 within the circles, which should be connected in ascending order and without lifting the pen from the paper. For Part B, both numbers (1-13) and letters (A-L) are contained within 25 circles, with participants asked to connect the circles in ascending order but alternating between numbers and letters (i.e. 1-A-2-B-3-C-4-D etc.). Both trails are scored as the amount of time needed to complete the exercise; there are no deductions for errors, though the task of completing the trails is supervised and participants are asked to correct errors as they occur. In this manner, trail making tests have been shown to be sensitive indicators of cognitive function (Reitan and Wolfson, 2004), specifically the domains of visual search and motor speed skills for Part

A. Part B also measures these domains, though is additionally an assessment of higher level cognitive skills, including executive control and cognitive flexibility (Crowe, 1998, Arbuthnott and Frank, 2000, Kortte et al., 2002).
Through the administration of these assessments before and after intensive treatment of moderate-severe hypertension, we aimed to determine whether this management strategy affected the cognitive abilities of participating subjects in the short-term.

2.8 Statistical analysis

Statistical analysis was performed throughout the project using STATA v14.1-16.0 (StataCorp, College Station, Texas, USA).
Normality of results was assessed through visual inspection of the distribution. This also allowed identification of outliers, with a sensitivity analysis performed once outliers were excluded, to ensure that these had no significant effects on the conclusions presented.
Continuous baseline and outcome variables were presented as means (± standard deviation) for parametric data and medians (with interquartile range) for non-parametric data. Categorical and binary data were presented as counts and percentages. Data were not log-transformed.
Given the design of the project as a before-and-after interventional study, parametric data were analysed using a paired t-test. Non-parametric data were analysed using Wilcoxon's signed ranks test, proportions using a one-sample test of proportions and categorical data with McNemar's test. Univariate linear regression models were employed to determine the relationship between independent and outcome (dependent) variables, with the β coefficient reported as the degree of change in the outcome variable for every unit of change in the independent variable.
For the assessment of HRQoL instrument validity, internal consistency was determined using Cronbach's alpha, test-retest reliability with Intraclass Correlation Coefficient (ICC), construct validity through correlation with alternative questionnaires (Spearman correlation) and independent variables known to affect HRQoL in the study group (Wilcoxon's signed rank test or

Pearson's correlation coefficient depending on normality) and responsiveness to change using a paired t test and Cohen's d.

The threshold value for alpha (α), the probability of a type I error (incorrectly rejecting the null hypothesis) was set as 0.05, using two-sided P values. For multiple comparisons, a Bonferroni correction was applied and reported in the relevant tables. Power (1-β), where β is the probability of a type II error (incorrectly failing to reject the null hypothesis), was established as 0.80, when this was able to be set a priori.

Noting that most results were derived from the before-and-after nature of the study, it was intended that these data be graphically represented as line graphs where possible. However, where this did not illustrate the findings sufficiently, box and whisker plots were utilised. In this case, the horizontal line within the box represents the median, the upper and lower boundaries of the box represent the interquartile range and the whiskers represent the range of values (outliers are plotted individually as dots). In terms of associations between variables, these were illustrated using scatter graphs in the standard manner.

For assessment of reproducibility of a measurement, the Coefficient of Variation (CoV) was calculated using the formula below:

$$\text{CoV (\%)} = (\text{standard deviation} / \text{mean}) \times 100$$

2.8.1 Sample size considerations

The overall project was designed as a feasibility study of an 18-week intensive treatment programme for individuals with treatment-naïve grade II and grade III hypertension. Given the absence of a control group, the efficacy of the treatment could not be assessed, beyond a comparison with data reported by the Health Survey for England on the control of BP in hypertensive subjects with usual care (Falaschetti et al., 2014). Therefore, the principle objective of the project was to explore whether the conduct of such a programme was feasible, as determined by whether sufficient participants could be recruited to the study and complete the 18-week protocol.

In this sense, the tolerability of the protocol was a key outcome measure. A priori, this was defined as the drop-out rate from the treatment protocol overall, though also includes the incidence of adverse events and medication discontinuation, in-keeping with its accepted definition in medical practice (Stanulovic et al., 2022). In defining tolerability in this manner, it is accepted that the self-selected group of well-motivated participants recruited to the study may compromise the applicability of the results to the wider population, though unfortunately this is a confounding factor which cannot be mitigated.

As a feasibility study with these aims, it was felt that 50 participants would provide sufficient signal to inform the key outcomes, with this initial recruitment revised down from the initial target of 100 participants at the outset of the project (as described below). In light of this recruitment target, the statistical power of the separate components of the project could be determined.

Clinical study:

The principle aim of the clinical study was to determine the feasibility of the project. Thus the study aimed to determine whether 50 participants could be recruited, whether BP could be reduced with comparable control rates to usual care and whether the protocol was well-tolerated, as defined above. Accordingly, it was not appropriate to conduct a statistical power calculation, as this was a feasibility study, rather than an assessment of the efficacy of treatment.

Microvascular study:

This nested experimental medicine component of the project aimed to determine whether cutaneous structural capillary density changed with treatment over 18 weeks. To calculate the power of recruiting 50 participants to the study as a whole, it was assumed that 10% patients would either not consent to paired capillaroscopy at the initial and final visits or yield non-diagnostic data in this regard. For comparison, a study of treatment with olmesartan, an angiotensin receptor blocker, was identified, with this finding a 1.7 standard deviation increase in capillary density after 24 weeks' treatment (Kaiser et al., 2013). Accordingly, with a plan sample size of 45 patients, a two-tailed paired t test was predicted to provide a power of 0.83 for this endpoint. This calculation assumed that the study was able to elicit the same reduction in capillary density using combination antihypertensive therapy and over a shorter timeframe than the reference study.

Cardiac MRI study:

The component of the project exploring the myocardial response to the programme was designed to determine whether left ventricular mass index changed with treatment over 18 weeks. A previous study using cardiac MRI was identified, defining a 0.65 standard deviation change in left ventricular mass index after 52 weeks' antihypertensive treatment (Reichek et al., 2009). Assuming that 75% participants would be able to undergo paired MRI studies, 38 participants would facilitate the detection of a 0.55 standard deviation difference with a power of 0.90 using a two-tailed paired t test. This calculation assumed that a shorter period of higher intensity treatment would produce a similar reduction in left ventricular mass index in comparison with the reference study.

HRQoL instrument study

For the validation of the MINICHAL instrument, no pre-specified power calculation was indicated, with the anticipated recruitment of 30 participants felt to provide sufficient signal to validate the instrument, in accordance with current dogma.

2.8 Evolution of the project over time

At the time of its inception, it was envisaged that the study would result in a significant number of participants undergoing renal denervation. Accordingly, the exclusion criteria for enrolment reflected this prospect. However, it became clear shortly after starting the study that few participants would prove consistently resistant to treatment within the protocol and thereby become eligible for the procedure. Incidentally, this occurred after the publication of the SYPLICITY HTN-3 trial (Bhatt et al., 2014) which found no significant difference in 6-month BP decline between intervention and control groups. Nevertheless, it was felt important to continue with the a priori treatment protocol and exclusion criteria for the duration of the study and therefore these were not adjusted for the entirety of the project.

In addition, the original recruitment target was 100 participants, as documented in the ISRCTN registry entry (ISRCTN number: 57475376; assigned 25/0/2015).

However, particularly given the burden of the initial and final visits, which included detailed phenotyping of each participant's vascular function, it was evident that this would not be feasible within the necessary timeframe. Therefore, this was reduced to 50 participants following a meeting of the study team, which was still felt to be sufficient to inform the key endpoints as described above.

Furthermore, during the initial period of recruitment, it was envisaged that participants might be reluctant to visit the research centre on a 2-4 weekly basis for 18 weeks. The first patients to be recruited were therefore offered the option of visits in their own home or the mobile clinic van, with these visits primarily conducted by myself, rather than the study nurse (prior to a change in nursing personnel midway through the study). In total, 4 patients received their follow-up visits outside of the research centre. Given concerns that the change in BP measurement location would affect the results, a decision was taken to standardise follow-up visits in the research centre, particularly as it became clear that the various engagement strategies with primary and secondary care were enabling sufficient participant recruitment. After completion of the study, a sensitivity analysis was undertaken to ensure that the practice of initial participants receiving their follow-up visits in locations other than the research centre had no effect on the overall results.

Chapter 3 Rapid Treatment of moderate to severe hypertension using a novel protocol in a single centre, before and after interventional study

3.1 Abstract

Rapid treatment to target in hypertension may have beneficial effects on long-term outcomes. This has led to a new recommendation in the 2018 European hypertension guidelines for patients with stage II/III hypertension to be treated to target within three months. However, whether it is feasible and safe to quickly manage treatment-naïve Stage II/III hypertension to target was unclear.

We examined this using a single centre before and after interventional study, treating newly-diagnosed, never-treated, stage II/III hypertensive patients with a daytime average systolic ABP ≥150mmHg to target within 18 weeks. The proportion at office target BP at 18 weeks was determined, together with office and ambulatory BP change from baseline to after the intervention. The protocol was designed to maximise medication adherence, including a low threshold for treatment adaptation. Safety was evaluated through close monitoring of adverse events and protocol discontinuation.

55 participants were enrolled with 54 completing the protocol. 69 ±12.3% were at office target BP at their final visit, despite a high average starting BP of 175/103mmHg, as a consequence of significant reductions in both office and ambulatory BP. Of those at office target BP, 51% were above target on ambulatory measurement. Adherence testing demonstrated that 92% of participants were adherent to treatment at their final visit.

The accelerated management of treatment-naïve Stage II/III hypertension is feasible and safe to implement in routine practice and there is no evidence to suggest it causes harm. Further large-scale randomised studies of rapid, adaptive treatment, including a cost-effectiveness analysis, are required.

3.2 Summary Table

What is known about the topic?	• Retrospective data has indicated that rapidly achieving BP targets may benefit long-term cardiovascular outcomes. • Recent international consensus guidelines have recommended treatment to target within 3 months for moderate and severe hypertension.
What this study adds	• Achieving target BP in moderate and severe hypertension following only 18 weeks' treatment is feasible and safe. • Medication adherence within this study was higher than adherence reported in observational studies of hypertension treatment. • In those at target on office BP measurement, a high proportion of treated individuals were above target on ambulatory BP measurement.

3.3 Background

The nature and intensity of blood pressure (BP) lowering in high-risk hypertensive individuals (Wright et al., 2015), is a source of interest and debate. Achieving historical guideline BP targets has proven challenging, with only 63% of patients with treated hypertension in England achieving the national target of office BP <140/90mmHg (Falaschetti et al., 2014). Following the recent American Heart Association/American College of Cardiology and European Society of Hypertension guidelines and recommendations for more stringent blood pressure control (Whelton et al., 2017, Williams et al., 2018), the challenge is now greater. Protocol-directed therapy may improve the effectiveness of hypertension treatment by mitigating clinical inertia: the failure to initiate, intensify or change therapy despite clinical evidence and guidelines to support this. The magnitude of clinical inertia in hypertension treatment is unknown (Faria et al., 2009), however antihypertensive medications were not intensified as needed in 86.9% of clinical consultations in over 7000 patients in one US study (Okonofua et al., 2006).

The STICH-care (Feldman et al., 2009) and the VIPER-BP studies (Stewart et al., 2012), investigated the impact of protocol-directed therapy on hypertension treatment response. Both randomised controlled trials showed a higher proportion of grade I hypertension patients achieved target BP in the protocol-directed therapy arm. However, whether protocol-directed therapy is feasible and effective in treatment-naïve participants with moderate or severe hypertension is unknown.

Early BP control may confer better outcomes (Gradman et al., 2013). Furthermore, control of grade II/III hypertension within 3 months of diagnosis has been recommended in the recent European guidelines for the treatment of hypertension (Williams et al., 2018). Rapid treatment to target may therefore offer an advantage, though the data remain surprisingly minimal on whether such an approach is safe, effective or well-tolerated.

We therefore devised a treatment protocol for patients with grade II/III hypertension constructed with the aim of participants reaching target BP within 18 weeks of their diagnosis. The protocol was designed to maximise drug tolerance, thereby optimising treatment adherence and BP control. As a supplement to the protocol, we aimed to monitor the cognitive function of

participants through testing at the time of enrolment and after 18 weeks' treatment, given the previous demonstration of an independent inverse correlation between BP and cognitive function in longitudinal studies (Elias et al., 1993, Kilander et al., 1998, Skoog et al., 1996)

3.4 Methods

This was an open-label, single-centre, before and after interventional study design.

Participants were recruited from 22 primary care practices or from secondary care in the county of Devon, United Kingdom. Referred subjects were eligible for screening if they were aged 18-79 years, had an office systolic BP of ≥170mmHg and had never previously received antihypertensive treatment.

Exclusion criteria were: Glomerular Filtration Rate (GFR) <60ml/min/1.73m^2, previous renal artery intervention, haemoglobin <10g/dl, platelet count <100 x10^9/l, bleeding diathesis, pregnancy or breastfeeding, inability to provide informed consent, hypertension-related event (including stroke or acute kidney injury) within the preceding 3 months, or any condition, including hypertensive urgency, requiring more immediate BP lowering or tailored antihypertensive strategy at enrolment. These were developed to allow for renal denervation, should this become indicated for individual participants with hypertension resistant to pharmacological treatment, as outlined within the treatment protocol.

At screening, subjects underwent 24-hour ambulatory BP monitoring (ABPM) and were eligible for trial participation if this confirmed at least grade 2 hypertension with a daytime average systolic BP (DASBP) measurement ≥150mmHg as per current guidelines (NICE, 2011). For ABPM measurement, an A&D TM-2430 system was employed (A&D Instruments Limited, Abingdon, UK), with appropriate analysis software (Doctor Pro TM-2430-13, A&D Instruments Limited, Abingdon, UK).

All participants gave informed consent and followed a treatment protocol using an antihypertensive medication pathway designed to maximise tolerance within an accelerated time frame, with appointments every 2-4 weeks over an 18-week period (Figure 2.1). At every visit they also received lifestyle advice in accordance with British Heart Foundation guidance. Screening visits were undertaken by research nurses, enrolment and week-18 visits by the research team (including a doctor and research nurses, with the aid of technicians) and follow-up visits by either a doctor or research nurse. Initially, participants were offered the opportunity to undertake follow-up visits in their own home or in a mobile clinic van. However, this was adjusted mid-study to allow for follow-up visits solely in the research centre, to remove the potential confounding variable of a change in

environment from affecting the results. For clarity, all enrolment and week-18 visits were undertaken in the research centre without exception and it was during these visits that the key outcome measurements were made.

Prior to treatment, all participants underwent 12-lead ECG, Epworth scoring and venous blood sampling for full blood count, electrolytes, liver function tests, HbA1c, fasting glucose and fasting lipid profile. Serum was stored for subsequent more complete secondary hypertension screening in case patients proved resistant to treatment. Participants also consented to two overnight urine collections for microalbuminuria using the COBAS method (Roche Diagnostics Limited, Burgess Hill, UK).

Before and after completion of the protocol, body fat percentage and weight (for BMI) was determined using a Tanita Body Composition Analyzer BC-418 MA (Tanita Europe BV, Amsterdam, Netherlands).

Furthermore, cognitive function testing was undertaken at the time of enrolment and after 18 weeks using the Addenbrooke's Cognitive Examination-Revised (ACE-R) questionnaire (Mioshi et al., 2006) and the Revised Trail Making Tests A and B (Reitan, 1958).

Patients under the age of 45 years underwent rigorous screening for secondary hypertension at the outset, whilst those over the age of 45 underwent screening if they proved to be drug resistant (as defined by a failure to respond to the first three antihypertensive agents in the protocol). This included cardiac magnetic resonance (CMR) imaging to exclude aortic coarctation, renal artery stenosis and adrenal adenomata, blood testing for thyroid function, calcium, renin, aldosterone and renal function and 24-hour urine testing for metanephrines and free cortisol levels.

If proven to be resistant to treatment, medication adherence was assessed by directly observing the participant ingesting each medication sequentially, with one hour between each medication and urine drug metabolite levels measured immediately prior to drug ingestion and at the same time on the following day. Urinary drug metabolite levels were also determined at the final visit.

Visit BP measurements were performed using an Omron M6 device (Omron Healthcare Europe B.V. Hoofddorp, Netherlands), after 5 minutes seated with feet on the floor, in a quiet environment using an appropriately sized cuff. Following an initially discarded reading, the average of three subsequent

readings was taken. The arm with the highest BP reading was determined at the initial screening visit and used for all subsequent BP measurements.
At all visits prior to or following medication changes, serum creatinine and electrolytes were determined to check for electrolyte disturbances or reduction in GFR. The protocol was followed as in Figure 2.1.

3.4.1 Medication intolerances

With a view to increasing adherence we aimed to minimise medication intolerance and prevent the association of the use of antihypertensive drugs with adverse effects. We employed a low threshold for changing or stopping medication. In the case of lightheadedness, moderate-severe symptoms triggered withdrawal of the most recently added drug, whereas mild symptoms were investigated with ABPM. If this demonstrated excessive control or dipping, drugs were also de-escalated.
Ankle swelling led to withdrawal or exchange of the calcium channel blocker; spironolactone was converted to eplerenone if gynaecomastia occurred and we maintained a low threshold for stopping medication for any other symptoms, including non-specific complaints such as lethargy or sleep disturbance. A creatinine increase of >30% from baseline led to discontinuation of the most recently introduced drug.

3.4.2 Study endpoints

The primary endpoint for the study was the proportion of participants achieving target office BP at 18 weeks.
The tolerability of the protocol was determined by assessing the proportion of participants who did not complete the protocol as outlined.
Key other secondary endpoints included the median number of antihypertensive medications prescribed at the end of the 18-week period, the proportion of participants diagnosed with secondary hypertension and the proportion with non-adherence to antihypertensive treatment

3.4.3 Sample size and statistical analysis

Using data from the Health Survey for England, it is reported that 63% of patients with known hypertension presently achieve consensus guidelines targets (Falaschetti et al., 2014). In this study, we anticipated that a similar proportion could be achieved using our treatment programme in just 18 weeks.

Baseline and outcome data are presented as means (standard deviation) or medians (interquartile range) for continuous data depending on the normality of the data and counts (percentages with 95% confidence intervals) for categorical and binary variables. To facilitate meaningful statistical analysis of endpoints, including safety, we planned to recruit at least 50 participants, allowing for predicted participant dropout prior to the 18 weeks.

Parametric data were analysed using a paired t-test; non-parametric data were analysed using Wilcoxon's signed ranks test, proportions using a one-sample test of proportions and categorical data with McNemar's test. A two-sided *P* value threshold <0.05 was considered statistically significant. For multiple comparisons, a Bonferroni correction was applied.

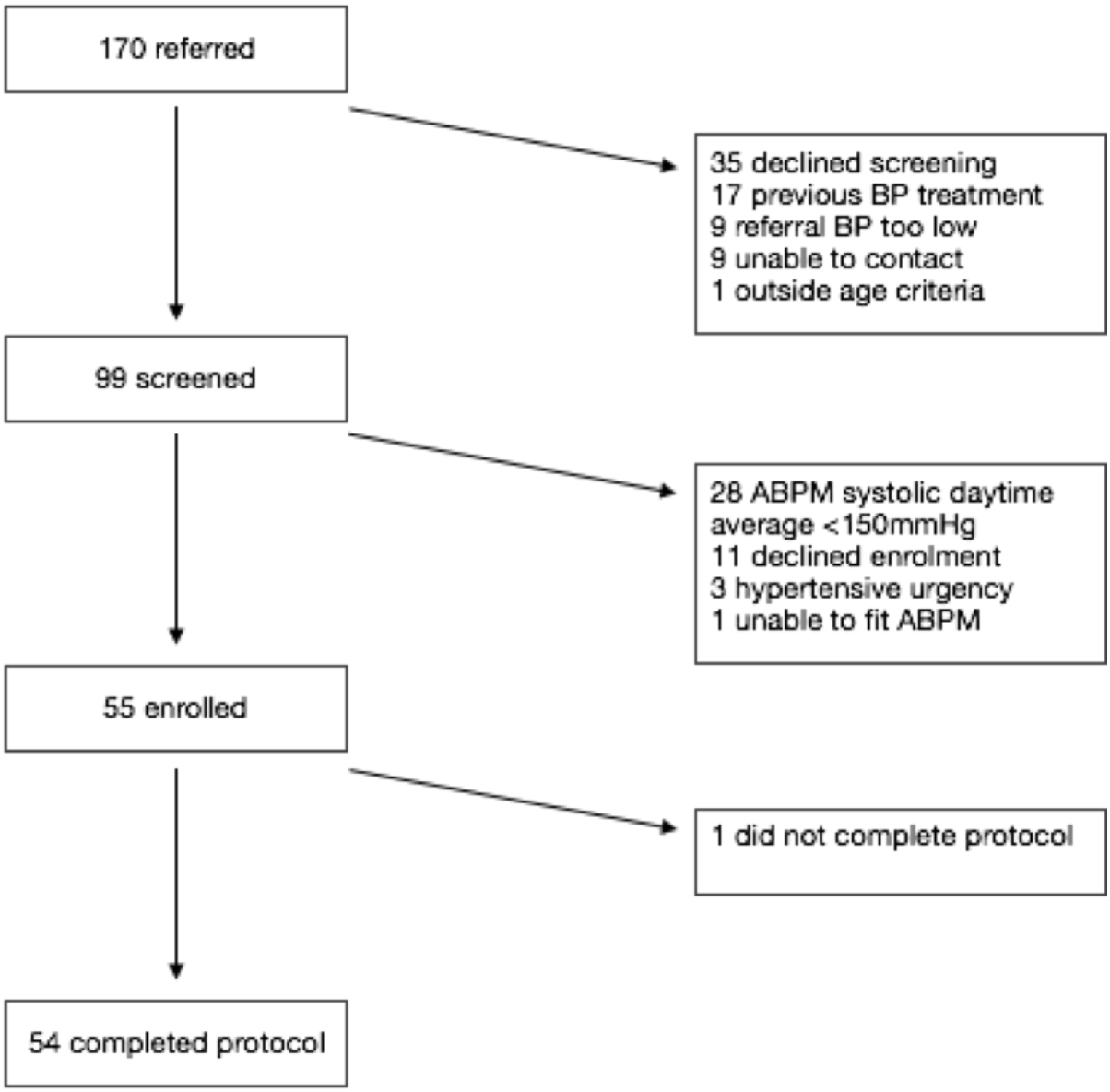
Statistical analysis was performed using STATA v14.1 (StataCorp, College Station, Texas, USA).

3.5 Results

Recruitment took place from July 2015 to February 2017, during which time 170 potential participants were referred to the study. The basic referral criteria were not met in 27 subjects (referral office BP too low (9 patients); previously treated with antihypertensive medication (17); outside of age criteria (1)). Despite best efforts, 9 participants did not respond to contact from the study team, whilst 35 subjects declined a screening appointment after telephone consultation which described the study and what participation involved.

At screening, 11 declined study enrolment after face-to-face discussion of the protocol. A further 28 patients had DASBP measurement <150mmHg and therefore did not satisfy the BP inclusion criteria for the study. One patient was unable to undergo ABPM, 3 required immediate treatment for hypertensive urgency and 1 described an inability to tolerate tablet ingestion. The remaining 55 participants gave informed consent and were recruited to begin the treatment programme (Figure 3.1).

Figure 3.1: Flowchart showing study recruitment of treatment-naïve subjects with moderate-severe hypertension, aged 18-80 years and subsequently fulfilling enrolment criteria at screening



Of the 55 enrolled participants, 54 completed the treatment programme as outlined in Figure 2.1, with 1 patient withdrawing consent after 14 weeks of treatment. The following results therefore pertain to the remaining group of 54 participants. The mean age of this group was 59 ± 11 years and 22 (40%) were female. Obstructive sleep apnoea was excluded in all participants: median Epworth Score = 5 (interquartile range: 3-8). 3 participants underwent follow-up visits in their own home and 1 patient was seen for follow-up visits in the mobile clinic van (outside his place of work). All other follow-up visits were with the

Diabetes and Vascular Research Centre, Exeter. As such, a sensitivity analysis was undertaken to determine whether the outcome of these 4 patients affected the results, with no significant impact found.

The characteristics of enrolled patients before and after treatment are given in Table 3.1.

Table 3.1: Participant characteristics

Variable	Before treatment	After treatment	P value
Office systolic BP (mmHg)	175 ± 16	132 ± 12	<0.0001
Office systolic BP <140mmHg (n;%)	0	41 (76)	
Office diastolic BP (mmHg)	103 ± 11	80 ± 9	<0.0001
Office diastolic BP <90mmHg (n;%)	6 (11)	46 (85)	
Office BP <140/90mmHg (n;%)	0	37 (69)	
Daytime average systolic BP (mmHg)	163 ± 11	135 ± 10	<0.0001
Daytime average systolic BP <135mmHg (n;%)	0	26 (48)	
Daytime average diastolic BP (mmHg)	93 ± 9	78 ± 7	<0.0001
Daytime average diastolic BP <85mmHg (n;%)	14 (26)	43 (80)	
Daytime average BP <135/85mmHg (n;%)	0	24 (44)	
Heart rate (bpm)	70 ± 11	66 ± 9	<0.006*
Inter-arm systolic BP difference ≥10mmHg (n;%)	6 (11)	4 (7)	
BMI (kg/m^2)	29.9 ± 5.6	29.9 ± 5.4	0.93
Waist circumference (cm)	103 ± 13	103 ± 13	0.99
% body fat	34.5 ± 8.5	34.4 ± 8.2	0.80

Current smoker (n;%)	6 (11)	6 (11)	1.00[#]
Alcohol (units/week)	7 (1-15)	4 (1-10)	0.59*
Weekly exercise (hours)	4 (1-7)	5 (2-8)	0.96
Fasting total cholesterol (mmol/L)	5.5 ± 1.1	5.5 ± 1.2	0.54
Fasting glucose (mmol/L)	5.5 ± 0.6	5.5 ± 0.6	0.93
HbA1c (mmol/mol)	38 ± 3.4	38 ± 3.7	0.60
Serum sodium (mmol/L)	140 ± 2.0	138 ± 2.2	<0.0001
Serum potassium (mmol/L)	4.6 ± 0.4	4.4 ± 0.5	0.004
Creatinine (μmol/L)	75 ± 13	77 ± 14	0.09
Urine albumin/creatinine ratio	0.825 (0.5-2.7)	0.7 (0.5-1.4)	0.0094
Urine albumin excretion rate (mg/day)	8.5 (5-18.5)	7 (5-16)	0.017
Angiotensin receptor blocker(n;%)	0	46 (85)	n/a
Calcium channel blocker(n;%)	0	53 (98)	n/a
Thiazide diuretic(n;%)	0	31 (57)	n/a
Aldosterone antagonist(n;%)	0	11 (20)	n/a
α-blocker(n;%)	0	3 (6)	n/a
β-blocker(n;%)	0	3 (6)	n/a

Characteristics of 54 participants with moderate-severe hypertension before and after 18 weeks' antihypertensive treatment with P values determined between groups using a paired t test unless otherwise indicated (Expressed as mean ± standard deviation, proportion ± 95% confidence interval or median and interquartile range; *Wilcoxon's signed ranks test; [#]one-sample test of proportions). With Bonferroni correction, p<0.0017 considered significant.

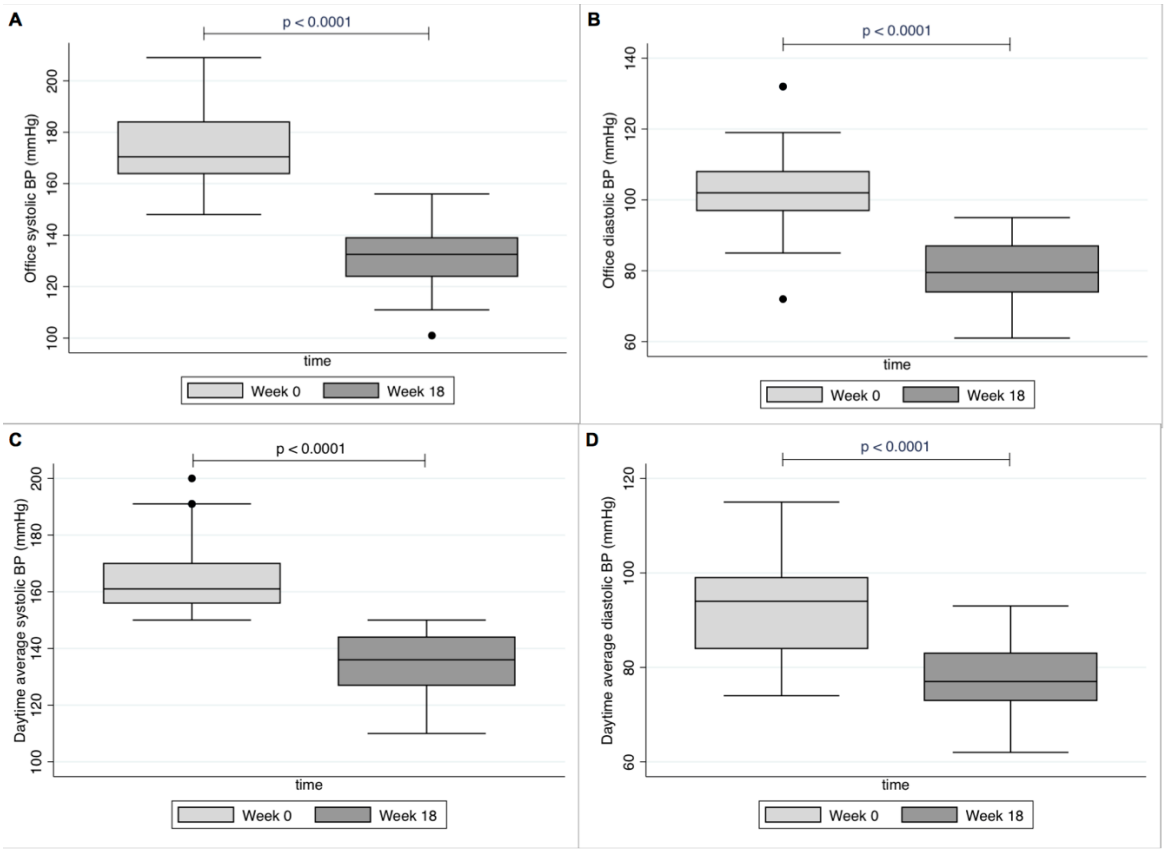
3.5.1 Primary endpoint and BP reduction

Despite the high average screening office BP for the cohort of 175/103mmHg, 69% (n=37) achieved target at 18 weeks. Marked reductions in office BP were seen in both systolic and diastolic readings (Figure 3.2A-B), with mean office BP

being 175 ± 16 / 103 ± 11mmHg when taken by the study team at enrolment, reducing to 132 ± 12 / 80 ± 9mmHg at week 18 (p<0.0001). This was well-tolerated by participants.

The protocol targeted office BP; however, ambulatory BP also significantly improved during the study period **(**Figure 3.2C-D) with 44% participants at the target of <135/85mmHg on ABPM at week 18. Mean daytime average ambulatory BP reduced from 163 ± 11 / 93 ± 9mmHg at study enrolment to 135 ± 10 / 78 ± 7mmHg at week 18 (p<0.0001).

Figure 3.2: BP reduction for 54 participants with never treated moderate-severe hypertension before and after completing an 18-week treatment protocol. (A Office systolic BP; B Office diastolic BP; C Daytime average systolic BP; D Daytime average diastolic BP)



Overall, of the 37 patients who achieved target on office blood pressure at 18 weeks, 19 (51%) participants were not at target BP on ABPM, defined as ≥135/85mmHg (indicating masked hypertension). Furthermore, 6 (11%) of the total number of 54 participants were not at office target at week 18 but were at target BP on ABPM (indicating white coat hypertension).

3.5.2 Safety and tolerability

The protocol was well-tolerated, with only one participant withdrawing during the study. Medication intolerances requiring drug discontinuation are summarised in Table 3.2.

Table 3.2: Intolerance of medication during treatment

	ARB (n=54)	Amlodipine (n=54)	Indapamide (n=35)	BFZ (n=2)	Spironolactone (n=14)	Doxazosin (n=4)
Light-headedness	1 (2)	0	5 (14)	1 (50)	2 (14)	1 (25)
Lethargy	2 (4)	1 (2)	0	0	2 (14)	0
Ankle swelling	0	3 (6)	0	0	0	0
Cr increase ≥30%	5 (9)	0	1 (3)	0	0	0
Total intolerant	8 (15)	4 (7)	6 (17)	1 (50)	4 (29)	1 (25)

Intolerance of medication used in a rapid treatment protocol in 54 newly-diagnosed treatment-naive patients with moderate-severe hypertension (Number(%); ARB: angiotensin receptor blocker (Candesartan); BFZ: bendroflumethiazide; Cr: creatinine; no intolerance to either bisoprolol or lercanidipine)

Creatinine increased by >30% after the introduction of candesartan in 5 participants and in 1 participant after the addition of indapamide. In each case, the newly-introduced medication was withdrawn and renal function returned to normal within 2 weeks. None of these patients had evidence of renovascular disease on CMR.

There was no significant increase in either HbA1c or fasting glucose at week 18 (Table 3.1). There were no episodes of syncope in participants throughout the study period.

3.5.3 Non-adherence

Once patients were taking 3 antihypertensive medications, if their office BP remained ≥140/90mmHg, adherence testing was performed with directly observed therapy (DOT) (median point of assessment: 16 weeks). In the 11 participants proven to be resistant to treatment, urinary drug levels before and after DOT showed that all were adherent to treatment immediately prior to this appointment.

In addition, urine drug testing was performed at the final (18 week) visit for 51 of the 54 patients (94%), as urine samples were not stored for three patients. Due to the drug not being measured by the urinary assay, we were unable to confirm adherence for lercanidipine in the three patients receiving this drug. However, it is noteworthy that these patients were adherent to all their other antihypertensive medications. In the remaining 48 patients we demonstrated non-adherence for 4 patients to one of their medications (whilst they were adherent to their other prescribed antihypertensive drugs); one to candesartan, one to bendroflumethiazide and two to doxazosin. Thus, in those 48 patients in whom we were able to fully assess adherence, 44 (92%) were adherent to all of their antihypertensive therapy.

3.5.4 Cognitive function

Cognitive function testing was complete in 51 participants, being incomplete in 3 participants due to timing restrictions on testing days. This found a trend towards cognitive improvement in participants following 18 weeks of intensive antihypertensive treatment, particularly in terms of fluency and visuospatial ability, though no metric reached statistical significance (Table 3.3).

Table 3.3: Cognitive function in 51 participants with moderate-severe hypertension before and after 18 weeks' treatment. Expressed as mean standard deviation or median (interquartile range); P value from paired t-test.

Cognition parameter	Pre-treatment	Week 18	P value
Addenbrooke's attention score	18 (18-18)	18 (18-18)	0.62
Addenbrooke's memory score	25 (24-26)	25 (24-26)	0.56
Addenbrooke's fluency score	12 (10-13)	12 (11-13)	0.07
Addenbrooke's language score	25 (24-26)	26 (25-26)	0.28
Addenbrooke's visuospatial score	16 (15-16)	16 (15-16)	0.07
Addenbrooke's total score	95 (92-97)	96 (93-97)	0.07
Mini Mental State Examination	30 (29-30)	30 (29-30)	0.38
Trail time A (s)	35 ± 11.8	32 ± 11.0	0.09
Trail time B (s)	68 ± 27.7	66 ± 23.1	0.60

3.5.5 Response to lifestyle advice

Intensive lifestyle advice and behavioural modification support was offered at every appointment, with British Heart Foundation literature also provided at enrolment for all participants. Despite this, there was no significant change in BMI, body fat percentage, arm circumference, smoking status, patient-reported alcohol intake or weekly exercise at week 18 compared with enrolment (Table 3.1).

3.5.6 Secondary hypertension

Seven participants were aged under 45 years at enrolment and therefore underwent immediate extended testing to exclude secondary hypertension - all testing was negative. A further 16 participants underwent extended secondary hypertension screening after the third drug introduction step in the protocol (as previously defined) - two of these were found to have biochemical profiles consistent with Conn's syndrome. Aside from this, following blood, urine and

CMR assessments, no other diagnoses of secondary hypertension were made, suggesting a 3.7% prevalence of secondary hypertension in the entire cohort.

3.5.7 Number of antihypertensive medications prescribed

A mean of 2.7 medications were prescribed per participant at their final visit. Of the 23 (43%) patients prescribed three medications at their final visit, 6 (26%) were above office target BP. A further 8 (15 ±7.2%) were prescribed more than three medications at their final visit indicating that, of all participants completing the study, 14 (26%) could be described as being resistant to antihypertensive treatment according to the standard office BP definition (above target on ≥3 antihypertensive medications at optimal doses and of different classes (including a diuretic) or at target on 4 or more antihypertensive medications at optimal doses (Calhoun et al., 2008)). None of these reached the a priori protocol threshold for considering renal denervation.

3.6 Discussion

Our study demonstrates for the first time that rapid management of treatment-naïve moderate-severe hypertension via an 18-week, dedicated protocol is feasible and there is no evidence of harm using this approach. Overall, 69% of participants achieved an office BP target of <140/90mmHg at week 18, a comparable figure to data from the Health Survey for England, which reported a 63% control rate to <140/90mmHg for patients with treated hypertension in 2011 (Falaschetti et al., 2014). This control rate also compares to contemporaneous data published concerning hypertension treatment in London, which suggests the "rule of halves" for BP control is still relevant today, albeit in an urban, mixed ethnicity population (Wu et al., 2019). However, of those at target on office BP in the present study, 51% were found not to be at target on ABPM as defined as daytime average BP <135/85 mmHg (Mancia et al., 2013).

By nature of the selection criteria for the study, the cohort exhibited a higher starting office BP than that of a normal hypertensive population. Screening office BP for this cohort averaged 175/103mmHg, compared to that of 164/95mmHg in the ASCOT BPLA study (Dahlof et al., 2005).

Using an average of 2.7 medications per patient, mean ± standard deviation office systolic BP reduction was 43 ± 15mmHg and mean office diastolic BP reduction was 23 ± 9mmHg over 18 weeks. This compares favourably with a mean 18mmHg office systolic BP reduction after one year's treatment with an average of 2.8 medications in the SPRINT trial intensive treatment group (Wright et al., 2015). These substantial and rapid office BP reductions in our study were well-tolerated.

We demonstrated adherence in 44 out of 48 patients in whom it was possible to assess this at the final visit. This excellent adherence may have been due to, at least in part, the design and delivery of the protocol, though the design of the study without a control arm means that this conclusion cannot be definitively drawn. Alternatively, this cohort with treatment-naïve grade II/III hypertension may have been more receptive to pharmacological therapy than comparable cohorts, which is plausible given the early onset of aggressive treatment following diagnosis.

3.6.1 Ambulatory BP response

The prevalence of masked hypertension was ~35% in our sample following antihypertensive treatment, as defined by office BP <140/90mmHg and daytime ABPM ≥135/85mmHg. This finding is similar to previous cross-sectional study of over 12,000 patients, determining a 30.5% prevalence of masked hypertension in non-diabetic subjects on antihypertensive treatment (Franklin et al., 2013).
In this study we targeted office BP, as per international guidelines. However, despite achieving this target in 69% of patients, more than half of those at office target were above target on ABPM. This raises the question of whether successful completion of a protocol-directed antihypertensive treatment programme should be decided by office BP alone. Residual elevation of ambulatory BP is associated with raised cardiovascular risk and could partly explained the phenomenon of "residual risk" described in treated high-risk individuals (Zanchetti, 2009).

3.6.2 Safety and tolerability

The impact of protocol-directed therapy in hypertension treatment has been investigated by two randomised controlled trials. Firstly, a cluster-randomised controlled trial of 2104 patients conducted in primary care in Canada studied the effect of implementing a simplified stepped-care algorithm for BP treatment (the STITCH-care protocol), with control practices continuing with usual care. A majority of patients had only mild hypertension and treatment success was determined after 6 months. The intervention practices were found to achieve target office BP in 64.7% participants versus 52.7% in control practices (Feldman et al., 2009).
In the VIPER-BP study, 1562 patients in Australia with uncontrolled hypertension were randomised to protocol-directed treatment over 26 weeks versus usual care (Stewart et al., 2012). Over 60% of enrolled patients were already treated for hypertension and the mean entry BP indicated that most had mild hypertension. Subsequently, 36.2% patients achieved office target BP after 26 weeks' treatment in the intervention group, versus 27.4% in the control group, a difference which reached statistical significance. However this was at the

expense of high rates of treatment side effects and participant withdrawals (Clark and McManus, 2012).

The mean office BP of participants at randomisation in the VIPER-BP study was 149/87mmHg and in those receiving the intervention in the STITCH-Care study the mean entry office BP was 155/88mmHg. In our study, the mean office BP taken at the time of enrolment by the study team was 175/103mmHg. As such, our study demonstrates that an accelerated protocol-directed approach is feasible in those with moderate-severe hypertension, building on the previous data from STITCH-Care and VIPER-BP demonstrating efficacy in individuals predominantly with grade 1 hypertension. Given that both previous studies measured their primary outcome after 6 months, our 18-week protocol proves that a more rapid treatment protocol is feasible. Given the recent ESC guidelines recommending BP control within 3 months for patients with grade II and grade III hypertension (Williams et al., 2018), the difference in timeframe for the study protocols is certainly relevant. Moreover, we demonstrate that this model of care is applicable to the UK healthcare system, with STITCH-Care taking place in Canada and VIPER-BP in Australia. The limiting factor of our study in comparison with STITCH-Care and VIPER-BP is the absence of a control group to prove efficacy. This could be addressed in the future by a randomised double blind control trial now that our study has shown feasibility, safety and reductions in BP compared to before the intervention.

Therefore, the present study demonstrates, in newly-diagnosed grade II/III hypertensive patients, that rapid reduction of BP over 18 weeks is feasible. Achieving target rapidly could confer benefits above and beyond BP control as demonstrated in a retrospective analysis of the VALUE study (Julius et al., 2004) which showed that patients who reach target BP after 6 months' treatment have a legacy benefit of improved cardiovascular outcomes up to 6 years later (Weber et al., 2004). Furthermore, an initial response to antihypertensive treatment (within 1 month) also conferred a prognostic advantage in VALUE.

A similar effect has been noted in retrospective analysis of the Syst-Eur trial (Staessen et al., 1997), during which a control group were left untreated for hypertension for 6 months, which appears to have conferred an increase in cardiovascular event rate in this group during open-label follow-up for a median period of 6 years (Staessen et al., 2004).

Although these retrospective analyses can be criticised for employing post-hoc interpretations of studies designed for another purpose, potentially biasing the results, this flaw has been addressed by two subsequent studies specifically designed to explore the effect of delayed treatment on cardiovascular outcomes. Firstly, it has been shown that patients who suffer a cardiac event are consistently less likely to be at BP target, as determined by retrospective analysis of over 3000 sets of primary care electronic notes (Gradman et al., 2013). Furthermore, a delay in intensifying treatment in response to above-target BP measurements confers a significantly increased risk of subsequent cardiovascular events or all-cause mortality, even when this delay is only 18 weeks, as shown by retrospective analysis of over 88,000 primary care case notes (Xu et al., 2015).

Given the substantial BP reductions seen here over an 18-week period, it is notable that the protocol was remarkably well-tolerated by participants. This was a highly selected group. Nevertheless, only one participant withdrew from study participation (1.9%), which compares favourably with the dropout rate seen in similar studies of BP treatment protocols, such as the VIPER-BP study (Stewart et al., 2012) (5.0% dropout after randomisation in intervention group) and the STITCH-care protocol (Feldman et al., 2009) (2.9% dropout rate in intervention group). Our protocol involved more frequent visits than either the VIPER-BP study or STITCH-Care protocol. Given the low dropout out rates from our study in comparison, we conclude that the higher frequency of visits was acceptable to our participants, though of course other participants may have declined to join the study due to the number of visits involved.

The study protocol was designed to minimise drug side effects. By intervening in patients at the earliest possible time-point in their hypertensive disease process, before aortic stiffness and clinically-important BP variability become more prevalent, we theorised that tolerability to treatment may be improved. This theory requires testing in a larger trial.

Whilst 10 patients underwent discontinuation of medications due to lightheadedness, no syncopal events were reported throughout the study period. This number reflects the low threshold for changing medication within the study. The study team were instructed to ask directly at each appointment for these symptoms and to switch medications accordingly. This approach aimed to prevent the association of antihypertensive medication with side effects, thereby improving adherence to treatment.

Despite the rapid BP reductions observed, any episodes of altered renal function resolved following medication de-escalation. There was also no change in glucose handling using the rapid treatment protocol in our study.

3.6.3 Non-adherence

In the present study, we were able to demonstrate adherence in 44 out of 48 subjects (92%) on urine drug testing using samples which were taken on arrival for their week 18 visit. The patients were not pre-warned that they would be undergoing urinary metabolite testing before the sample was obtained.

Our adherence rates are outstanding when compared with a prior study of patients with uncontrolled hypertension attending a specialist clinic who were assessed by simultaneous DOT and ABPM. This process demonstrated a 50% non-adherence rate (Hameed et al., 2016), in keeping with retrospective observational studies of self-reported adherence in hypertensive patients, which have found an approximately 40% rate of medication discontinuation (Mazzaglia et al., 2005, Van Wijk et al., 2005).

Other studies have used liquid chromatography urine analysis to determine the presence of antihypertensive agents and their metabolites, as also employed in the present study. This technique has previously indicated a non-adherence rate of 53% in patients with resistant hypertension seen in a specialist clinic (Jung et al., 2013). A further study used the same method to determine an adherence rate of 75% in patients referred to a secondary care hypertension clinic, though the sample was a heterogeneous group of participants, including new referrals from primary care together with some patients with resistant hypertension under consideration for renal denervation (Tomaszewski et al., 2014).

Our excellent adherence rates may be due to the short duration of our study in comparison to the long durations of antihypertensive treatment in the observational studies. Furthermore, through participation in an interventional study, including frequent follow-up with members of the study team, we may have substantially increased adherence in our sample. The potential selection bias of highly-motivated participants willing to participate in a study of antihypertensive treatment will also have affected adherence, as will the selection bias inherent in referral to a specialist hypertension clinic in the comparator studies described.

Despite these caveats, the finding of 92% antihypertensive medication adherence on urinary testing in the present study is an interesting finding in a study where the protocol was designed to minimise drug side effects. The experience of side effects with anti-hypertensive medications is a factor known to increase non-adherence.

Furthermore, we theorised that tolerability would be improved by treating patients at the first possible point in the disease process (within days of first diagnosis of hypertension), before advanced hypertensive vascular disease can develop.

Whether the excellent adherence rate described is due to the protocol or other factors cannot be determined given the limitations of the study design and lack of control group, though this possibility could be explored in a future randomised controlled study.

3.6.4 Cognitive function

The study found no significant change in cognitive function over the 18-week treatment programme, though there was a trend towards an improvement in fluency, visuospatial ability, total ACE-R score and trail times. This trend towards an improvement in cognitive function is in-keeping with previous studies, which have highlighted an inverse relationship between BP and cognitive function (Elias et al., 1993, Kilander et al., 1998, Skoog et al., 1996). These previous studies were each conducted over 15-20 years and therefore our relative short follow-up period may not have allowed enough time for significant improvements in cognitive function to become apparent.

However, the association between cognitive function and BP control does not imply causation, determination of which would require a randomised controlled trial of treatment, with a duration sufficient enough to capture significant changes in cognitive function (and ideally the development of dementia as an additional outcome measure). This has previously been explored by analysis of the Syst-Eur trial (Staessen et al., 1997), which found that immediate versus delayed treatment with nitrendipine (a dihydropyridine calcium channel blocker) in elderly patients with isolated systolic hypertension reduced the incidence of dementia by 55% when follow-up was increased to a median of 3.9 years (Forette et al., 2002). Whether this relates to the use of nitrendipine or BP lowering per se is unclear, particularly as calcium ions and their modulation by calcium channel blockers

have been postulated to play an important role in dementia (Nimmrich and Eckert, 2013). Furthermore, other placebo-controlled studies of the effect of antihypertensive treatment on cognitive function have produced conflicting results (Tzourio et al., 2003, Applegate et al., 1994, McCorvey et al., 1993, Denolle et al., 2002), likely due to heterogeneities in treatment and cognitive assessment methods, with a Cochrane review of these reporting that further randomised controlled trials were warranted (Birns and Kalra, 2009).

Certainly, there is not enough evidence to conclude that rapid treatment of hypertension confers an advantage in terms of long-term cognitive function and prevention of dementia. However, given the encouraging trends found in the present study, the effect of this treatment strategy on cognitive function merits inclusion within a future larger study, which will need to be sufficiently powered to explore this further and over a longer timeframe than the present study.

In the context of the wider study, the results of cognitive function testing indicate that there is no evidence that rapid treatment of grade II-III hypertension results in short-term deterioration in cognition, lending further support to this treatment strategy.

3.6.5 Lifestyle measures

The present study explored the short-term impact of lifestyle measures and patient education, using methods which were designed to mimic usual care but were delivered more frequently. Despite being delivered 2-4 weekly over 18 weeks, our lifestyle intervention had no impact on participant anthropometry, smoking status, alcohol intake or exercise habits. This is despite the recruitment of a sample of patients likely to be highly motivated, having agreed to participation in an intensive research programme for treatment of hypertension and in whom 92% adherence to medication was demonstrated.

3.6.6 Secondary hypertension

Through systematic investigation of all patients with resistant hypertension and those aged under 45 years at enrolment, we determined a 3.7% prevalence of secondary hypertension in our cohort overall.

This finding is in keeping with previous studies (Berglund et al., 1976, Omura et al., 2004, Persu et al., 2014, Sinclair et al., 1987), though in our study, patients with significantly impaired renal function were excluded.

3.6.7 Study limitations

The before and after study design limits the conclusions in terms of attributing the remarkable control rates of moderate-severe hypertension to the protocol itself rather than other factors acting upon the single treatment group. Nevertheless, the data presented affirm that a rapid, protocol-directed treatment approach for the treatment of moderate-severe hypertension is feasible and could be implemented in routine practice or within a larger multi-centre randomised controlled study in order to prove effectiveness over standard care.

The protocol presented is intensive, including a large number of visits within a short period of time. The cost-effectiveness of such a strategy can therefore be questioned, though it is argued that the study aims to perform the usual number of visits for which patients with newly-diagnosed hypertension would expect to receive, though just in a shorter period of time. By using protocol-directed treatment, it is hoped that consultations within the protocol could be provided by allied healthcare professionals, rather than primary care physicians, providing further cost savings. Such a strategy could also potentially enhance the effectiveness of the treatment (Clark et al., 2010). Furthermore, by providing a putative benefit in terms of cardiovascular outcomes, it is possible that additional cost savings could be made. In view of these unknown factors, it would be reasonable to suggest that a cost-effectiveness analysis should be performed alongside a future randomised controlled trial of this treatment protocol before it can be recommended as the standard of care in the UK.

The single centre design of the study also limits the generalisation of its conclusions to the wider population, though we hope that this could be addressed by the proposed future study.

3.6.8 Conclusion

This study shows for the first time that the rapid treatment of moderate-severe hypertension using a protocol-directed regimen, designed to minimise drug side effects and improve tolerability, can be implemented in usual care with no evidence of harm. BP reductions occurring as a consequence of this treatment were remarkably well-tolerated, with urine drug testing demonstrating 92% adherence to medication within the study.

Earlier BP control in the newly diagnosed grade II/III hypertensive population could plausibly deliver gains in terms of medication adherence, BP control and even offer the potential for improved cardiovascular outcomes. Further randomised large scale studies of this concept are required.

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3.6.11 Conflicts of Interest / Disclosures

The authors have no conflicts of interest to declare.

Chapter 4 Rapid treatment of grade II-III hypertension in previously untreated patients is accompanied by an increase in total and perfused skin capillary density

4.1 Abstract and Keywords

Objective: Reduced capillary density (rarefaction) occurs in patients with, or at risk of, hypertension. This can be ameliorated within 6 months' treatment in grade I hypertension. We sought to determine whether rarefaction is reversible in grade II-III hypertension and whether this occurs within 18 weeks of intensive treatment.
Methods: We recruited participants with never-treated grade II or III hypertension, initiating a treatment protocol aiming to achieve target BP within 18 weeks. Before treatment and at 18 weeks, dorsal finger cutaneous capillary density was measured using intravital capillaroscopy at rest and following venous occlusion. In addition the sublingual microvasculature was assessed using sidetream dark field imaging (Microscan) before and after treatment.
Results: 53 of the 55 participants completed the protocol with sufficient data quality for analysis. Following treatment, capillary density during venous occlusion increased (104.4 ± 23.8caps/mm^2 to 111.5 ± 23.3caps/mm^2 (p=0.0032)) as did resting capillary density (83.8 ± 24.1caps/mm^2 to 92.2 ± 23.6caps/mm^2 (p=0.0037)). There was no significant change from pre- to post-treatment in the percentage increase from resting capillary density to capillary density during venous occlusion (27.3 ± 21.4% versus 23.6 ± 18.0% (p=0.291)) and no association between pre-treatment capillary density and ambulatory BP response to treatment. Treatment of the cohort conferred no significant change to microvascular parameters derived from sublingual imaging.
Conclusions: Rarefaction in moderate-severe hypertension improves within 18 weeks' intensive treatment due to neovascularization. This supports guidelines recommending intensive treatment for patients with grade II-III hypertension.

Keywords: Hypertension, rarefaction, capillaroscopy, rapid treatment

4.2 Summary Table

What is known about the topic?	• Hypertension is associated with reduced capillary density (rarefaction). • Cutaneous capillary density increases after treatment of grade I hypertension over 6 months.
What this study adds	• Cutaneous capillary density increases after only 18 weeks of antihypertensive treatment in grade II-III hypertension. • The increase in cutaneous capillary density seen with treatment in these conditions is underpinned by neovascularisation. • This phenomenon is accompanied by a reduction in urinary albumin excretion and a trend towards an increase in sublingual capillary density.

4.3 Introduction

Hypertension, the leading contributory cause of death worldwide (WHO, 2009), is characterized by structural and functional changes throughout the vasculature. These include a reduction in capillary and terminal arteriole density (rarefaction), a phenomenon first described in cadaveric conjunctival tissue (Ruedemann, 1933), though later found in other human microvascular beds (Gasser and Buhler, 1992, Henrich et al., 1988, Hughes et al., 2006) and in the mesentery of the spontaneously hypertensive rat (SHR) model (Henrich et al., 1978), though not the SHR brain (Lin et al., 1990).

Since these early studies, further investigation has raised the possibility that rarefaction may precede the development of clinical hypertension, given its finding in individuals with high-normal blood pressure (BP) (Antonios et al., 1999b) and normotensive offspring of hypertensive parents (Noon et al., 1997, Antonios et al., 2003, Antonios et al., 2012). The observation that anti-angiogenic chemotherapy agents commonly trigger hypertension (Mourad and Levy, 2011) is additional evidence supporting a primary role for capillary rarefaction in the pathogenesis of essential hypertension.

In established hypertension, the observed rarefaction appears to be due to a combination of a reduction in total vessel density (structural rarefaction) and perfusion of a lower fraction of capillaries at rest (functional rarefaction) (Prewitt et al., 1982, Antonios et al., 1999c). Exploration of whether structural rarefaction antedates functional rarefaction has found only structural rarefaction in young adults and infants pre-disposed to hypertension (Noon et al., 1997, Antonios et al., 2012), though studies of older adults early in the hypertensive disease process have determined both structural and functional rarefaction in this context (Antonios et al., 1999b, Antonios et al., 2003).

Investigations with the aim of determining whether the rarefaction of hypertension is reversible have produced conflicting results in animal models (Turek et al., 1987, Unger et al., 1992, Rakusan et al., 1994, Rakusan et al., 2000, Kobayashi et al., 1999, Pu et al., 2003, Sabino et al., 2008, Nascimento et al., 2010), limited by heterogeneity in the antihypertensive agents used, variation in methodologies

employed to assess capillary density and the limitations inherent to using animal models.

In human cross-sectional studies, rarefaction of nailfold capillaries is persistently present in patients receiving antihypertensive treatment (Cheng et al., 2008a, Penna et al., 2008). However in both of these studies, those treated with hypertension had significantly higher office BP than the comparator normotensive groups. Conversely, other studies have found an increase in cutaneous capillary density in individuals treated for hypertension versus normotensive controls (Debbabi et al., 2006), particularly in those treated with a combination of angiotensin converting enzyme inhibitors (ACEi) and thiazide diuretics (Debbabi et al., 2010).

Two linear studies in humans have helped to clarify matters. The first of these found an increase in retinal microvascular density in never-treated hypertensive patients after receiving either amlodipine or lisinopril for 52 weeks (Hughes et al., 2008). A further interventional study found an increase in cutaneous capillary density following 26 weeks of treatment with olmesartan or metoprolol (Kaiser et al., 2013), with a similar increase in capillary density at rest and after post-occlusive reactive hyperemia (PORH) indicating an improvement in structural rarefaction, rather than functional rarefaction, with treatment.

However, all studies concerning the reversibility of rarefaction in hypertension have enrolled patients with grade I or II hypertension. Whether capillary rarefaction is reversible in more advanced hypertension is unknown. Even if rarefaction is not the primary physiological basis of essential hypertension, its presence in established hypertension increases peripheral resistance (Greene et al., 1989), thereby perpetuating the hypertensive state. Therefore, if rarefaction is irreversible in grade II-III hypertension, this will provide a physiological basis for treatment-resistance in this group. Furthermore, rarefaction is associated with increased capillary pressure (Tooke et al., 1991) and the direct consequences of this process are to disrupt oxygen and metabolite exchange between the vasculature and tissue fluid, resulting in the target organ damage associated with uncontrolled hypertension. Rarefaction thus represents an important therapeutic target for hypertension treatment.

The aim of this study was to determine whether capillary density increases after only 18 weeks in treatment-naïve grade II-III hypertension. Reversal of rarefaction has never previously been demonstrated over such a short timeframe or in those with moderate or severe hypertension. Furthermore, the study aimed to determine whether reversal of rarefaction under these circumstances is driven by neovascularization or by an increase in the proportion of capillaries perfused at rest. As such, the study will explore the physiological basis of treatment-resistance in established hypertension, which may help guide therapeutic strategies for these individuals in the future.

4.4 Methods

Treatment-naïve individuals presenting with office systolic BP ≥170mmHg and aged 18-79 years were identified, with ambulatory monitoring then used to confirm daytime average systolic BP ≥150mmHg prior to enrolment.

Exclusion criteria were: renal impairment (GFR <60 ml/min/1.73m^2), Haemoglobin <10 g/dl, platelet count <100 x10^9/l or bleeding diathesis, pregnancy or breastfeeding, inability to provide informed consent, hypertension-related event (including stroke or acute kidney injury) within the preceding 3 months, or any condition, including hypertensive urgency, requiring immediate BP lowering or tailored antihypertensive strategy. These exclusion criteria were chosen for the clinical study, to enable renal denervation, should this become indicated within the treatment protocol as a consequence of proven resistance to pharmacological antihypertensive treatment.

Participants underwent treatment with fortnightly consultations mandating stepwise intensification of antihypertensive treatment as required (Jordan et al., 2020). Treatment targets and antihypertensive medication followed national and international consensus guidelines (NICE, 2011, Mancia et al., 2013), with initial treatment consisting of an angiotensin receptor blocker (ARB) and calcium channel blocker (CCB). At every visit, participants received lifestyle advice in line with British Heart Foundation guidance. Office and ambulatory BP were measured in accordance with established guidance (NICE, 2011), using an Omron M6 device for office BP (Omron Healthcare Europe B.V. Hoofddorp, Netherlands) and A&D TM-2430 system for ambulatory BP (A&D Instruments Limited, Abingdon, UK). Given the association between microalbuminuria and microvascular disease (Futrakul et al., 2009), overnight urine collections were undertaken before and after treatment to determine urine albumin/creatinine ratio (ACR) and albumin excretion rate (AER), using the COBAS method (Roche Diagnostics Limited, Burgess Hill, UK).

4.4.1 Capillaroscopy

Cutaneous capillary density was determined before and after 18 weeks of antihypertensive treatment using intravital capillary video microscopy (capillaroscopy). This was undertaken through microscopic examination of dorsal digital skin illuminated by a light source under which hemoglobin appears black, thereby revealing perfused capillaries. Measurements of capillary number were made in a temperature-controlled environment, whilst the participant was supine and with their hand at heart level, and averaged from six sequential microscopy fields. From these measurements, cutaneous capillary density was derived from the known area of analysis.

The protocol was undertaken at rest to determine functional cutaneous capillary density and then after 10 minutes of venous occlusion using a digital cuff at 50mmHg in order to assess total (structural) cutaneous capillary density. Venous occlusion was selected, rather than post-occlusive reactive hyperaemia, due to its greater accuracy in determining structural capillary density (Antonios et al., 1999a, Serne et al., 2001).

4.4.2 Sidestream dark field imaging

In addition to cutaneous capillaries, the sublingual microcirculation of participants was examined using sidestream dark field (SDF) imaging with a Microscan device (MicroVision Medical, Wallingford, USA). The imaging protocol followed established practice within the department (Hubble et al., 2009), with 5 videos of 15 seconds each acquired before treatment and after 18 weeks of treatment for each participant. These were analysed unblinded offline using dedicated software (AVA 3.0, Academic Medical Center, University of Amsterdam, Netherlands) to determine total vessel density, perfused vessel density, the proportion of perfused vessels, de Backer score and microvascular flow index in accordance with accepted standards (Spronk et al., 2002, Boerma et al., 2005, De Backer et al., 2002, De Backer et al., 2007).

4.4.3 Study endpoints

The primary endpoint for the study was the change in structural cutaneous capillary density following 18 weeks of antihypertensive treatment.

Key secondary endpoints were the change in functional capillary density following treatment, the proportion of capillaries recruited at rest (before and after treatment), the association of pre-treatment capillary density with BP response to treatment and the effect of treatment on markers of metabolic syndrome and renal protein excretion.

4.4.4 Sample size and statistical analysis

The only previous linear study of the effect of antihypertensive treatment on cutaneous structural capillary density found a mean increase from 73.3 $\pm$ 2.3 capillaries/mm^2 to 77.2 $\pm$ 1.6 capillaries/mm^2 in the group treated with an ARB (Kaiser et al., 2013). We anticipated a similar 1.7 standard deviation increase in cutaneous structural capillary density in the present study as, despite being undertaken over a shorter timeframe, participants received a greater intensity of antihypertensive treatment within our protocol.

We planned to enroll 50 participants into a clinical study of the feasibility and safety of rapid treatment of grade II/III hypertension (Jordan et al., 2020). Of these, 90% recruitment to paired capillaroscopy studies was anticipated. As such, using a two-tailed paired t-test (α = 0.05), 45 participants was calculated to provide a power of 0.83 for the primary endpoint.

Statistical analysis was performed using STATA v16.0 (StataCorp, College Station, Texas, USA). Parametric data were analyzed using a paired t-test, non-parametric data were analyzed using Wilcoxon's signed ranks test and proportions using a one-sample test of proportions. A two-sided *P* value threshold <0.05 was considered statistically significant. Univariate linear regression models were employed to determine the relationship between BP response during the study and measures of capillary density as outcome variables.

4.5 Results

Of the 55 participants recruited to the study, 1 paired set of capillaroscopy data was of insufficient quality for analysis and 1 participant did not complete the treatment protocol. The following results therefore refer to the remaining 53 participants.

The median age of the analysis group was 61 years, 58% were male and there were no diabetic subjects. Baseline demographic and clinical characteristics of this cohort before and after 18 weeks of antihypertensive treatment are summarized (Table 4.1).

Table 4.1: Characteristics of participants undergoing capillaroscopy before and after 18 weeks of antihypertensive treatment in 53 individuals

Variable	Before treatment	After treatment	P value
Office systolic BP (mmHg)	175 ± 16	132 ± 12	<0.0001
Office diastolic BP (mmHg)	103 ± 11	80 ± 8	<0.0001
Daytime average systolic BP (mmHg)	164 ± 12	135 ± 10	<0.0001
Daytime average diastolic BP (mmHg)	93 ± 10	78 ± 7	<0.0001
Heart rate (bpm)	70 ± 11	66 ± 9	0.007*
BMI (kg/m^2)	29.8 ± 5.6	29.8 ± 5.4	0.92
Waist circumference (cm)	103 ± 13	103 ± 13	0.85
% body fat	34.5 ± 8.5	34.3 ± 8.2	0.63
Current smoker (n;%)	6 (11)	6 (11)	1.00#
Alcohol (units/week)	6 (1-15)	4 (1-10)	0.59*
Fasting total cholesterol (mmol/L)	5.6 ± 1.1	5.5 ± 1.2	0.54
Fasting glucose (mmol/L)	5.5 ± 0.6	5.5 ± 0.6	0.70
HbA1c (mmol/mol)	38 ± 3.5	38 ± 3.7	0.41
Creatinine (μmol/L)	74 ± 13	77 ± 14	0.054
Urine albumin/creatinine ratio	0.8 (0.5-2.7)	0.7 (0.5-1.4)	0.0034

Urine albumin excretion rate (μg/minute)	6 (3-11)	4 (3-10)	0.010
Angiotensin receptor blocker (n;%)	0	46 (87)	n/a
Calcium channel blocker (n;%)	0	52 (98)	n/a
Thiazide diuretic (n;%)	0	30 (57)	n/a
Aldosterone antagonist (n;%)	0	10 (19)	n/a
α-blocker (n;%)	0	3 (6)	n/a
β-blocker (n;%)	0	3 (6)	n/a

Expressed as mean ± standard deviation or median and interquartile range; P values determined between groups using a paired t test unless otherwise indicated. With Bonferroni correction, $p<0.0023$ considered significant.
*Wilcoxon's signed ranks test
#one-sample test of proportions

4.5.1 BP reduction with treatment

Office systolic BP markedly fell from 175 ± 16mmHg to 132 ± 12mmHg with treatment ($p<0.001$), associated with a reduction in office diastolic BP from 103 ± 11mmHg to 80 ± 8mmHg ($p<0.001$). In tandem, daytime average systolic BP fell from 164 ± 12mmHg to 135 ± 10mmHg ($p<0.0001$) and daytime average diastolic BP reduced from 93 ± 10mmHg to 78 ± 7mmHg ($p<0.0001$) (Table 4.1).

The protocol was well-tolerated, with only one participant not completing treatment as described. 6 adverse events were reported during the study, all of which pertained to a transient increase in serum creatinine >30% from baseline. Of these, 5 occurrences were in response to initiation of candesartan and 1 followed the addition of indapamide. In every case, the causative medication was withdrawn and renal function returned to baseline within 2 weeks.

Full details of the clinical response to the protocol in the entire treatment cohort are reported elsewhere (Jordan et al., 2020).

4.5.2 Metabolic changes with treatment

From study enrollment to week 18, there was no significant change in BMI (29.8 ± 5.6kg/m^2 versus 29.8 ± 5.4kg/m^2; p=0.92), waist circumference (103 ± 13cm versus 103 ± 13cm; p=0.85), fasting total cholesterol (5.6 ± 1.1mmol/L versus 5.5 ± 1.2mmol/L; p=0.54), fasting glucose (5.5 ± 0.6mmol/L versus 5.5 ± 0.6mmol/L; p=0.70), or HbA1c (38 ± 3.5 versus 38 ± 3.7; p=0.41) (Table 4.1).

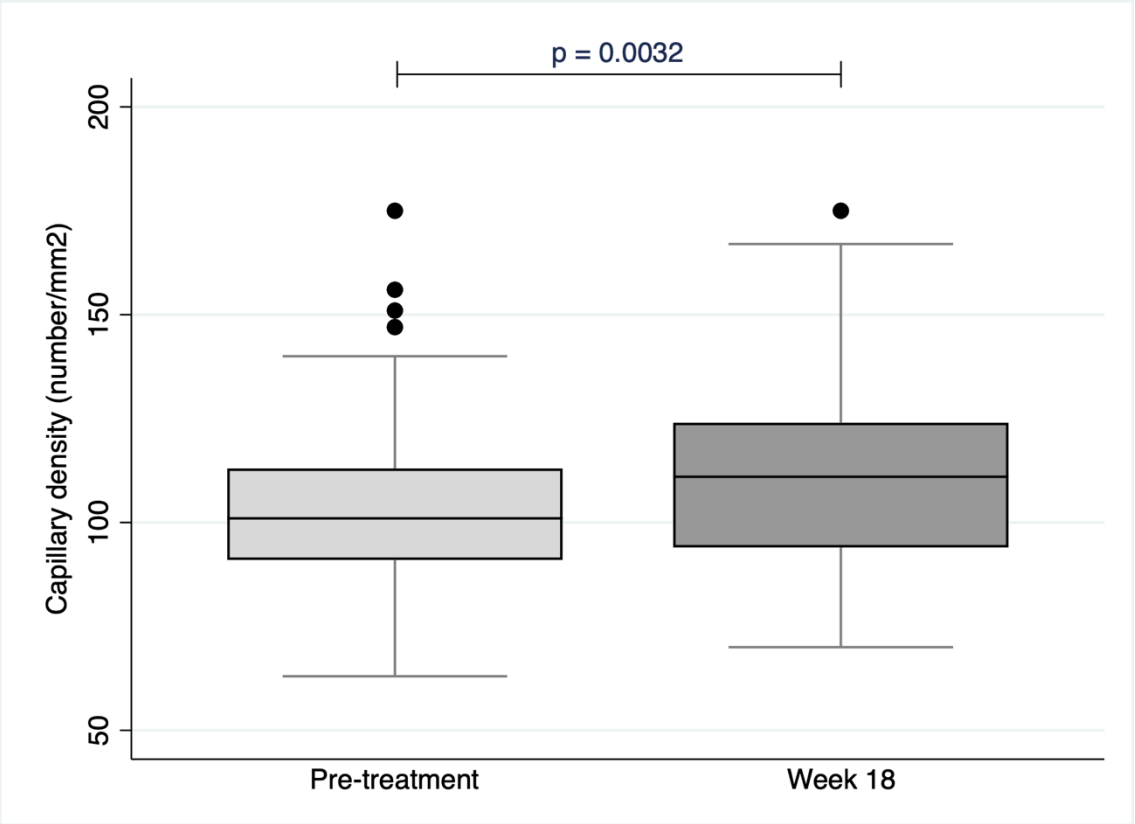
4.5.3 Microalbuminuria

Antihypertensive treatment within the protocol conferred a reduction in urinary ACR from 0.8 (0.5-2.7) to 0.7 (0.5-1.4) (p=0.0034). In addition, AER reduced significantly with treatment from 6µg/min (3-11µg/min) to 4µg/min (3-10µg/min) (p=0.010). Within these measurements, we diagnosed 5 participants with microalbuminuria (AER=30-300mg/day) at enrolment, of which only 1 failed to resolve by week 18.

4.5.4 Cutaneous capillary density

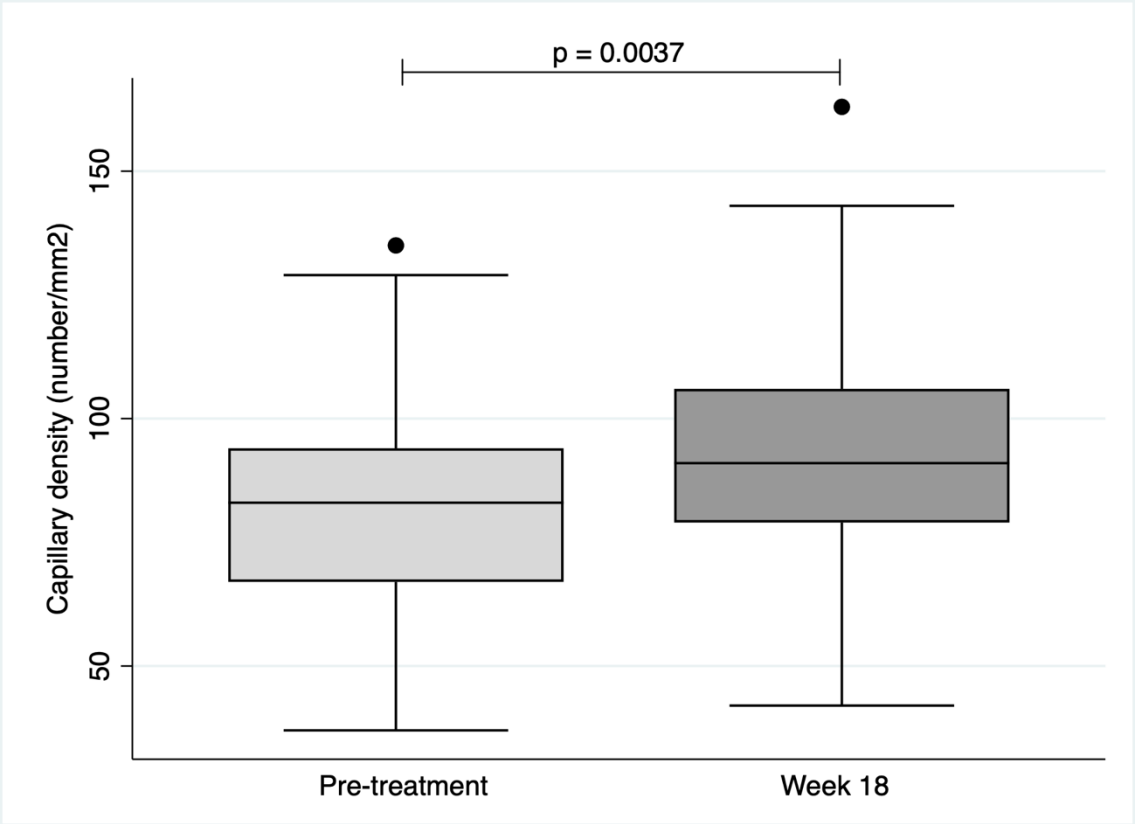
Cutaneous structural capillary density (measured during venous occlusion) increased significantly from 104.4 ± 23.8caps/mm^2 to 111.5 ± 23.3caps/mm^2 with treatment (p=0.0032) (Figure 4.1).

Figure 4.1: Cutaneous structural capillary density before and after 18 weeks of intensive antihypertensive treatment in 53 individuals



This was accompanied by an increase in cutaneous functional capillary density (measured during resting conditions) from 83.8 ± 24.1caps/mm^2 to 92.2 ± 23.6caps/mm^2 (p=0.0037) (Figure 4.2).

Figure 4.2: Cutaneous functional capillary density before and after 18 weeks of intensive antihypertensive treatment in 53 individuals



However, antihypertensive treatment conferred no significant change in the percentage increase in capillary density during venous occlusion from capillary density at rest (27.3 ± 21.4% before treatment versus 23.6 ± 18.0% after treatment (p=0.291)) (Figure 4.3) indicating that the improvement in rarefaction found following treatment of hypertension was due to neovascularization, rather than a change in capillary reserve. These results are summarized in Table 4.2.

Figure 4.3: Percentage change in cutaneous capillary density from resting state to during venous occlusion, before and after 18 weeks of intensive antihypertensive treatment in 53 individuals

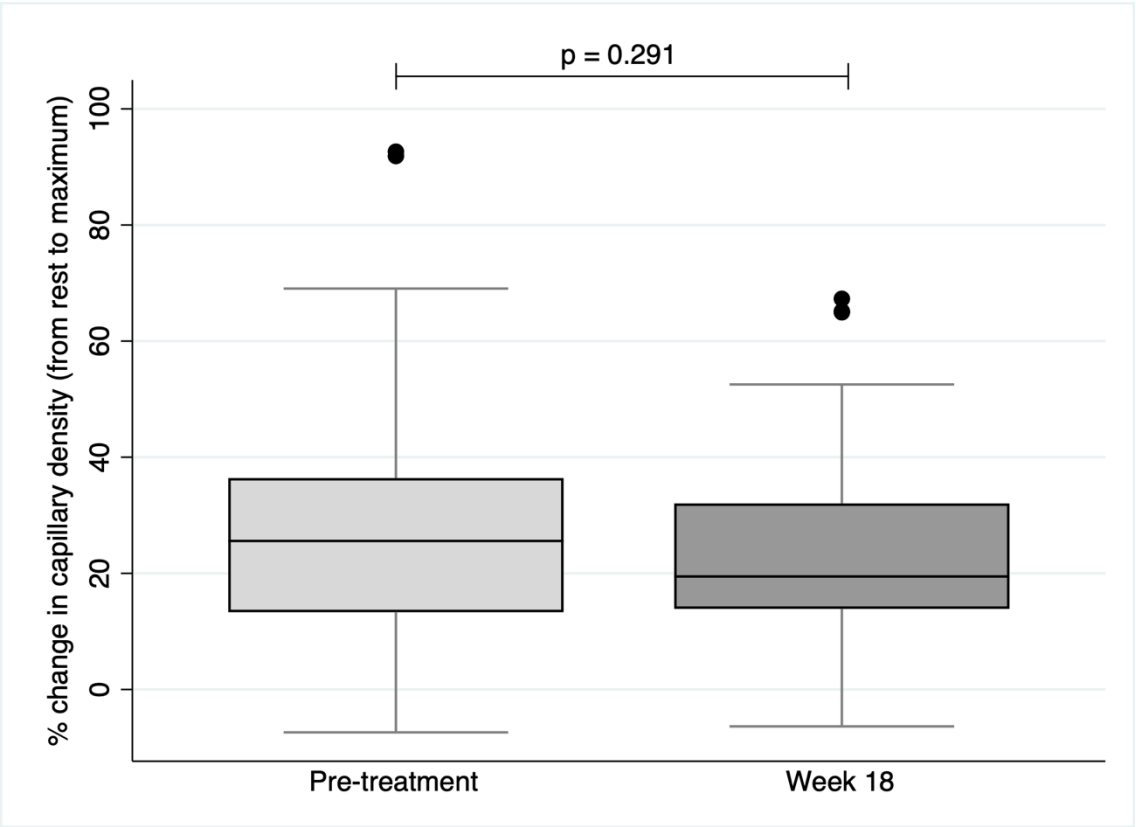


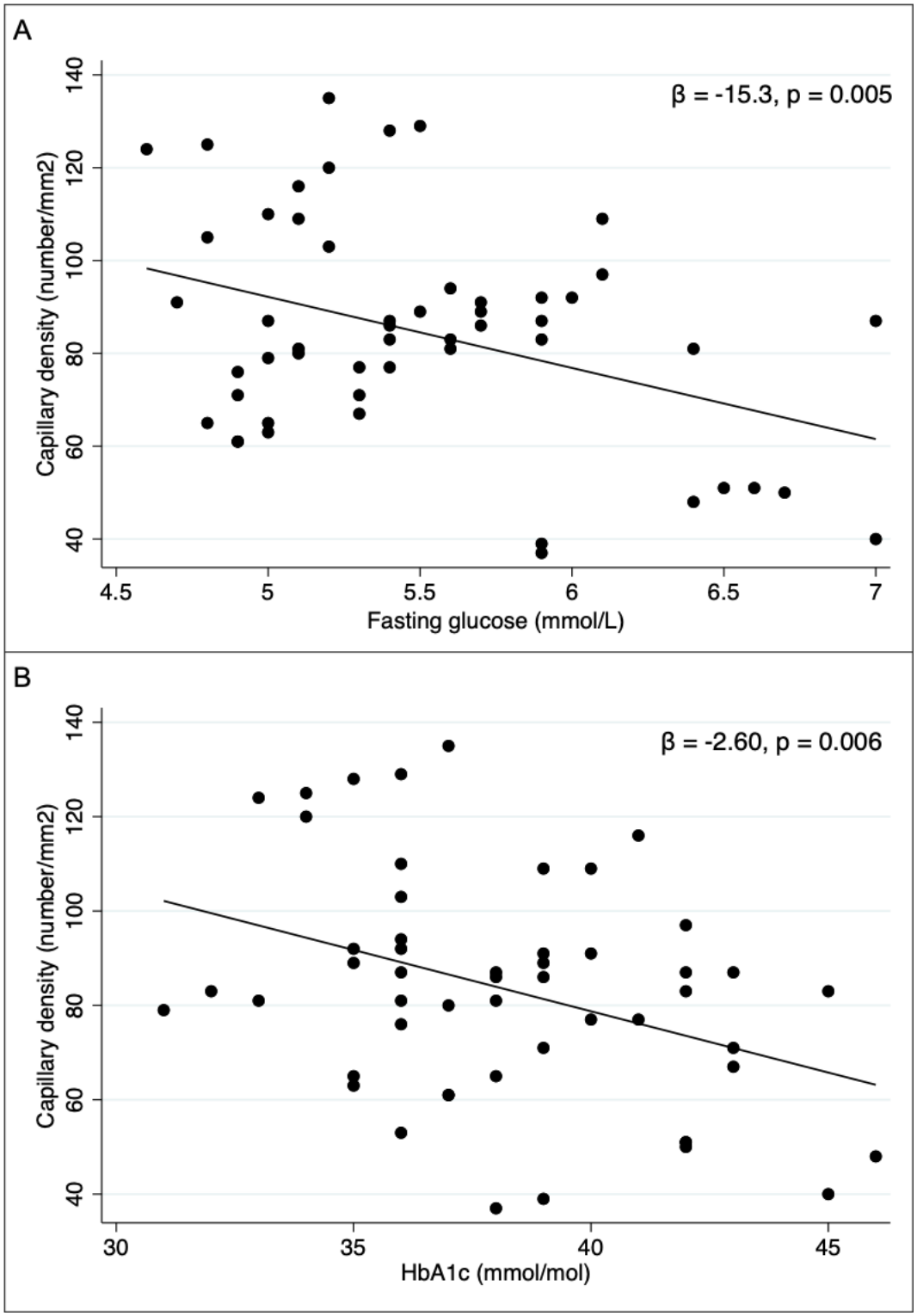
Table 4.2: Cutaneous capillary number and capillary density (number per mm^2) at rest and after venous occlusion, before and after 18 weeks of intensive antihypertensive treatment in 53 individuals. Expressed as mean ± standard deviation; P values determined between groups using a paired t test

Variable	Before treatment	After treatment	P value
Capillary Number (resting)	35.8 ± 9.9	39.2 ± 10.0	0.006
Capillary Number (venous occlusion)	44.3 ± 10.1	47.3 ± 9.9	0.0025
Capillary Density (resting) (caps/mm^2)	83.8 ± 24.1	92.2 ± 23.6	0.0037
Capillary Density (venous occlusion) (caps/mm^2)	104.4 ± 23.8	111.5 ± 23.3	0.0032
% change in capillary density from resting state to venous occlusion	27.3 ± 21.4	23.6 ± 18.0	0.291

4.5.5 Associations of cutaneous capillary rarefaction

At study enrolment, both fasting glucose and HbA1c were associated with cutaneous functional capillary density (β = -15.3, p = 0.005; β = -2.60, p = 0.006) (Figure 4.4).

Figure 4.4: Associations between baseline blood glucose and cutaneous functional capillary density (A) and HbA1c and cutaneous functional capillary density (B) before treatment in 53 individuals using univariate linear regression



There was no association between pre-treatment cutaneous functional capillary density and ambulatory BP response to treatment, either systolic daytime average BP (p=0.987) or diastolic daytime average BP (p=0.443). Similarly, no associations were found between pre-treatment cutaneous structural capillary density and systolic daytime average BP change with treatment (p=0.642) or diastolic daytime average BP change with treatment (p=0.764).

4.5.6 Sidestream dark field imaging

55 participants were enrolled in the clinical study. 1 individual withdrew from participation before completion. Sublingual imaging of insufficient quality for analysis was acquired for 13 participants on at least one visit, predominantly due to movement artefact.

Concerning the remaining 41 participants, no significant change was found in measured parameters of vessel density from baseline to week 18, though a numerical (non-significant) increase in mean grid crossings, total vessel density, perfused vessel density and proportion of perfused vessels from baseline to week 18 was noted (Table 4.3).

For all pooled data (pre- and post-treatment) there were significant associations between equivalent parameters measured on the same day with sublingual SDF imaging and those derived from cutaneous capillaroscopy, specifically sublingual total small vessel density and cutaneous structural capillary density ($\beta = 0.043$, $p = 0.024$) (Figure 4.5A), sublingual perfused small vessel density and cutaneous functional capillary density ($\beta = 0.761$, $p < 0.001$) (Figure 4.5B) and sublingual proportion of perfused vessels and cutaneous percentage change in capillary density with venous occlusion ($\beta = -0.133$, $p = 0.020$).

Table 4.3: Parameters measured using sublingual sidestream dark field imaging in grade II-III hypertension before and after 18 weeks' intensive antihypertensive treatment

Parameter		Baseline	Week 18	P
Mean grid crossings (n)		70 ± 11.2	74 ± 12.0	0.05
Total grid length (mm)		4.8 ± 0.2	4.8 ± 0.2	0.52
De Backer score (1/mm)		14.5 ± 2.2	14.4 ± 2.5	0.95
TVD (mm/mm^2)	**Small vessels**	21.9 ± 3.9	22.5 ± 4.2	0.39
	All vessels	25.1 ± 3.7	25.3 ± 4.2	0.68
PVD (mm/mm^2)	**Small vessels**	20.6 ± 4.9	21.6 ± 4.6	0.18
	All vessels	23.5 ± 4.9	24.3 ± 4.8	0.34
PPV (%)	**Small vessels**	97.8 (93.4-99.4)	98.8 (96.2-100.0)	0.32*
	All vessels	98.0 (94.3-99.2)	98.7 (95.8-99.7)	0.30*
MFI	**Small vessels**	3.0 (2.8-3.0)	3.0 (2.9-3.0)	0.24*
	All vessels	3.0 (2.8-3.0)	2.9 (2.8-3.0)	0.33*

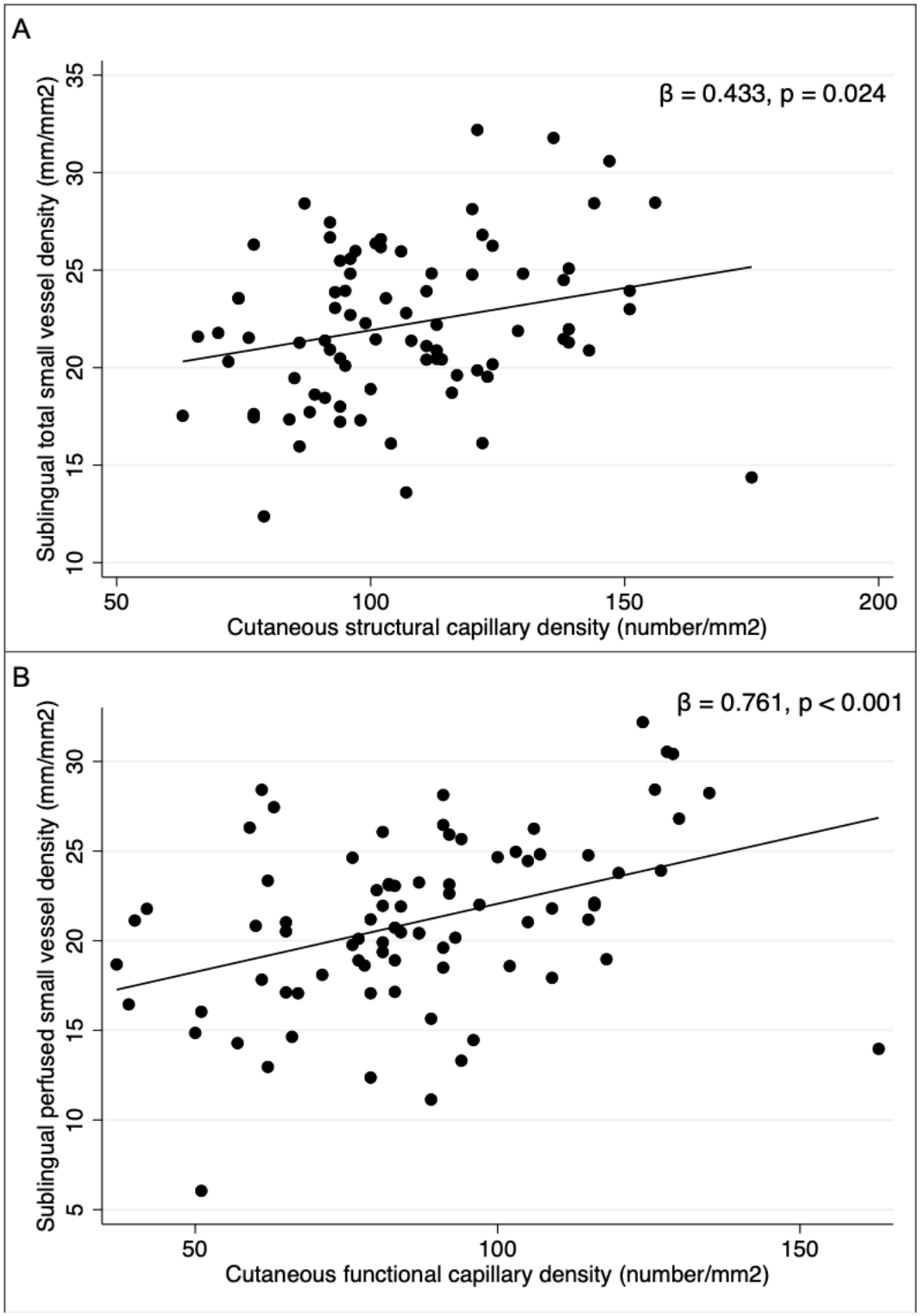
N=41; expressed as mean ± standard deviation or median (interquartile range)

*Wilcoxon's signed ranks test.

Small vessels defined as 1-10μm diameter.

TVD: total vessel density; PVD: perfused vessel density; PPV: proportion of perfused vessels; MFI: microvascular flow index

Figure 4.5: Associations between sublingual total small vessel density and cutaneous structural capillary density (A) and sublingual perfused small vessel density and cutaneous functional capillary density (B) in 41 participants (measured both before and after antihypertensive treatment) using univariate linear regression



4.6 Discussion

This is the first study to demonstrate that cutaneous capillary density in individuals with moderate-severe hypertension increases within 18 weeks of intensive treatment and that this change is underpinned by neovascularization. This finding is in keeping with previous linear human studies of hypertension treatment (Hughes et al., 2008, Kaiser et al., 2013), which have investigated the reversibility of capillary rarefaction in patients with mild hypertension. However, the expeditious timing of neovascularization during intensive antihypertensive treatment and the observation of this process even in moderate and severe hypertensive disease are unique to this study.

To the authors' knowledge, this is the first examination of the sublingual microvascular circulation in individuals with hypertension, with the findings of nominal increases in total vessel density, perfused vessel density and the proportion of perfused vessels following treatment in-keeping with the observations within the cutaneous microcirculation, though not reaching statistical significance. Notwithstanding this, our study found significant associations between parameters examining capillary density in the cutaneous microvasculature and the vessel density in the sublingual microvasculature, suggesting commonality between factors governing vessel density in these separate microvascular beds.

4.6.1 Reversal of rarefaction

The evaluation of rarefaction as a dynamic phenomenon is supported by experimental models, particularly normotensive Sprague-Dawley rats, in which iatrogenic hypertension due to surgically-induced aortic coarctation induces rarefaction in the downstream cremaster muscle capillaries exposed to normotension. This experimental finding also implies that rarefaction is not simply a reactive phenomenon to raised microvasculature perfusion pressure. A putative alternative mechanism for the rarefaction of hypertension can be provided by the activity of bone-marrow-derived endothelial progenitor cells (EPCs), first described in 1997 (Asahara et al., 1997) and thought to play a key role in vascular

repair and angiogenesis (Hristov et al., 2003b) as senescence of these cells inhibits angiogenesis, promoting rarefaction.

In accordance with this hypothesis, EPC senescence is increased in rat models of hypertension and in hypertensive human subjects, when compared with normotensive controls (Imanishi et al., 2005). Impaired in vitro function has also been demonstrated in EPCs isolated from subjects with pre-hypertension (defined as office systolic BP = 130-139mmHg or office diastolic BP = 85-89mmHg). Although it should be noted that this definition of pre-hypertension differs from the *a priori* definition, the authors' post-hoc definition of pre-hypertension is in-keeping with that found in international consensus guidelines (Williams et al., 2018). In addition, reduced EPC function has been found in human subjects at risk of hypertension as a consequence of being born of hypertensive pregnancies (Yu et al., 2016), raising the possibility that increased EPC senescence may promote rarefaction as a primary mechanism in the pathogenesis of essential hypertension.

Furthermore, losartan (an angiotensin receptor blocker) has been shown to reduce EPC senescence in the SHR model (Yao et al., 2007) and the dihydropyridine calcium channel blockers nifedipine and barnidipine have been shown to reduce EPC senescence in human subjects with mild hypertension (Sugiura et al., 2008, de Ciuceis et al., 2011). As the treatment protocol in the present study mandates both an angiotensin receptor blocker (candesartan) and dihydropyridine calcium channel blocker (amlodipine) as initial treatment, it is reasonable to conclude that reduced EPC senescence may have underpinned the finding of neovascularization with antihypertensive treatment in our sample.

The present findings suggest that rarefaction can be ameliorated with antihypertensive treatment, even in advanced disease, through neovascularization, which is likely to be driven by increased EPC activity. Given this demonstrable reduction of capillary rarefaction, even in grade II or III hypertension, the promotion of neovascularization and reduction of rarefaction is a potential therapeutic target in the future for the treatment of established hypertensive disease. Such a strategy could involve the reversal of EPC senescence, as observed with existing antihypertensive agents (Yao et al., 2007,

Sugiura et al., 2008, de Ciuceis et al., 2011), and therefore further research into the molecular mechanisms underpinning this process should be a priority.

Conversely, an increase in vessel density with antihypertensive treatment was not observed within the sublingual microvasculature in the present study. One reason for this may be the fact that SDF imaging visualizes all vessels within the microvasculature, including capillaries, arterioles and venules, whereas cutaneous capillaroscopy visualizes only capillaries. In addition, the longer time for data collection with cutaneous capillaroscopy, versus the 15-second data collection time for SDF imaging, allows counting of more vessels which are only transiently perfused. Furthermore, cutaneous capillaroscopy is able to detect a greater number of vessels per unit area, which may enable a higher sensitivity for detection of small differences in vessel density, when compared with SDF imaging. The fact that a trend to improvement in rarefaction with antihypertensive treatment in the sublingual microvascular bed was observed, though this did not reach statistical significance, suggests that the reason for the discrepancy of findings between the cutaneous and sublingual microvascular beds in this study is less likely to be due to physiological variation between the two tissues, though this remains a possibility.

4.6.2 Pathophysiology of resistant hypertension

The present study also found that the degree of rarefaction does not predict BP response to treatment. Taken in tandem with the finding that rarefaction is reversible, at least in part, in advanced hypertensive disease, it may be postulated that rarefaction may not underpin treatment resistance in hypertension.

An alternative explanation for the pathophysiology of resistant hypertension is provided by structural changes within larger arteries, rather than capillaries. As a consequence of ageing and accelerated by conditions such as hypertension, elastin within the tunica media of larger arteries degrades, becomes cross-linked and calcified. Consequently the mechanical load of the pulse pressure is transferred to collagen, which itself becomes cross-linked (Greenwald, 2007). This stiffens arteries, a process which can be quantified by measuring pulse wave

velocity (PWV) (Nichols, 2005). Increased arterial stiffness, as measured by PWV, has been shown to be associated with treatment-resistance in hypertension in several studies (Chung et al., 2014, Pabuccu et al., 2012), leading to the conclusion that increased arterial stiffness is responsible for the resistant hypertension phenotype (Alsharari et al., 2020). However, the cross-sectional design of these studies does not allow a conclusion of causality from this association. In addition, PWV is known to correlate with brachial BP (Gribbin et al., 1976). Given that office BP was significantly higher in the resistant hypertension groups compared to the control groups in all of these studies, it must be concluded that this is a significant confounding variable. A further study of PWV in treatment-naïve subjects compared with those diagnosed with resistant hypertension (whilst controlling for BP) would therefore provide a valuable insight.

4.6.3 Benefits of rapid hypertension treatment

Our study has determined that rapid, intense pharmacological antihypertensive treatment has similarly rapid effects on the microvasculature. The neovascularization demonstrated in this study within 18 weeks would reduce capillary pressure and improve exchange between plasma and tissue fluid, thereby attenuating the end organ damage associated with hypertension.

Furthermore, we found a significant improvement in urinary albumin excretion following treatment. It is known that there is a strong relationship between albumin excretion rate and other measures of microvascular dysfunction (Strain et al., 2005, Chapman, 2017), therefore suggesting that the improvements in microvascular structure and function seen in the present study occur in multiple microvascular beds. Moreover, as elevated albumin exertion rate is associated with raised cardiovascular risk (Mattock et al., 1998, Ljungman et al., 1996), including into the normal range (Yudkin et al., 1988, Borch-Johnsen et al., 1999, Gerstein et al., 2001), our finding of a significant improvement in albumin excretion rate with rapid treatment of moderate-severe hypertension, even within the normal range for this parameter, lends support to the proposal that this strategy may be beneficial in terms of cardiovascular outcomes.

Accordingly, this study provides evidence to support recent guidance suggesting that moderate-severe hypertension be treated rapidly, aiming for normotension within 3 months (Williams et al., 2018). Such an approach would quickly attenuate the physiological basis of end-organ damage in hypertension, providing rapid benefits and potentially preventing adverse long-term sequelae.

The postulated legacy effect of rapid hypertension treatment is supported by retrospective studies showing an ongoing benefit from rapid treatment, even after normotension is achieved. In particular, a retrospective analysis of the VALUE study (Julius et al., 2004) has found that delaying reaching BP treatment targets by 6 months confers a significantly greater risk of adverse cardiovascular outcomes up to 6 years thereafter (Weber et al., 2004). Moreover, a 6-month delay in starting pharmacological antihypertensive treatment in the Syst-Eur trial (Staessen et al., 1997), was found to increase cardiovascular events during a median follow-up of 6 years (Staessen et al., 2004).

Both these studies were, however, post-hoc analyses and therefore subject to the bias of selecting the outcome after the results of the study are known. This criticism can be countered by two studies investigating the effect of delayed antihypertensive treatment a priori (Gradman et al., 2013, Xu et al., 2015). Both of these retrospective analyses of primary care medical records found a prognostic benefit from avoiding delay in intensification of pharmacological treatment when needed in the management of hypertension, adding further credence to the conclusion that prompt treatment of hypertension has a prognostic benefit.

4.6.4 Limitations of the study

The conclusions drawn from this study are limited by the heterogeneity of antihypertensive agents used in the treatment protocol. As such, it is not possible to assess whether the reversal of rarefaction demonstrated is limited to a specific class of antihypertensive agent or is a product of BP reduction itself. However, the use of a pragmatic protocol which mirrors treatment regimens recommended in consensus guidelines allows the findings to be related to current clinical

dogma, facilitating the applicability of these positive results to the recent iteration of European treatment guidelines and to the clinical care of patients.

A further limitation of this study is the absence of a control group receiving standard care over a longer time-frame. A future study with this design would be helpful in determining whether the improvement in rarefaction occurs commensurately to the rate of BP reduction or whether a similar rate of increase in capillary density can be achieved with a less intense treatment regimen.

In addition, our study employed a single center design with all but one patient being of White British ethnicity. Therefore it is not known whether the rapid reversal of rarefaction observed also occurs in other ethnic groups, a question which should be addressed by a future multi-center study.

4.6.5 Conclusions

In summary, our results indicate a rapid reversal of capillary rarefaction with treatment in moderate-severe hypertension, a process which is underpinned by neovascularization. The extent of rarefaction pre-treatment does not predict BP response to treatment and these findings therefore support the assertion that rarefaction may not be the basis of treatment-resistance in hypertension, though does suggest the reversal of rarefaction as a potential therapeutic target. Furthermore, the finding of such rapid changes in the cutaneous microvasculature with treatment, coupled with the detection of an improvement in albumin excretion rate, support the recent clinical recommendation for intensive treatment to target BP within 3 months in patients presenting with grade II or III hypertension.

4.6.6 Perspectives

This study, for the first time, demonstrates that the rarefaction of moderate-severe hypertension can be ameliorated. Moreover, neovascularization underpins this reversibility, which occurs within 18 weeks of intensive pharmacological treatment, potentially attenuating end organ damage and improving prognosis.

This provides a physiological basis for the new and recent recommendation in European consensus guidelines to aim for rapid control of BP in subjects presenting with grade II or III hypertension.

Chapter 5 Morphological and functional cardiac consequences of rapid hypertension treatment: a cohort study

5.1 Abstract

Background: Left ventricular hypertrophy (LVH) in uncontrolled hypertension is an independent predictor of mortality, though its regression with treatment improves outcomes. Retrospective data suggest that early control of hypertension provides a prognostic advantage and this strategy is included in the 2018 European guidelines, which recommend treating grade II/III hypertension to target blood pressure (BP) within 3 months. The earliest LVH regression to date was demonstrated by echocardiography at 24 weeks. The effect of a rapid guideline-based treatment protocol on left ventricular (LV) remodelling, with very early BP control by 18 weeks remains controversial and previously unreported. We aimed to determine whether such rapid hypertension treatment is associated with improvements in LV structure and function through paired cardiac MRI scanning at baseline and 18 weeks, utilising MRI mass and feature tracking analysis.

Methods: We recruited participants with never-treated grade II/III hypertension, initiating a guideline-based treatment protocol which aimed to achieve BP control within 18 weeks. Cardiac MRI and feature tracking were used to assess myocardial morphology and function immediately before and after treatment.

Results: We acquired complete pre- and 18-week post-treatment data for 41 participants. Expressed as mean ± standard deviation, LV mass index reduced significantly (43.5 ± 9.8 to 37.6 ± 8.3g/m2, p<0.0001) following treatment, accompanied by reductions in LV ejection fraction (65.6 ± 6.8 to 63.4 ± 7.1, p=0.03), radial strain (46.1 ± 9.7 to 39.1 ± 10.9, p<0.0001), mid-circumferential strain (-20.8 ± 4.9 to -19.1 ± 3.7, p=0.02), apical circumferential strain (-26.0 ± 5.3 to -23.4 ± 4.2, p=0.003) and apical rotation (9.8 ± 5.0 to 7.5 ± 4.5, p=0.003).

Conclusions: LVH regresses following just 18 weeks of intensive antihypertensive treatment in newly-diagnosed subjects with grade II/III hypertension. This is accompanied by potentially advantageous functional changes within the

myocardium and supports the hypothesis that rapid treatment of hypertension could improve clinical outcomes.

Key words:
Left ventricular hypertrophy, feature tracking, strain, torsion, rapid treatment, hypertension

5.2 Summary Table

What is known about the topic?	• LVH is a predictor of adverse outcomes, which can be ameliorated through its regression with treatment. • Regression of LVH in grade I-II hypertension has been demonstrated after 24 weeks of antihypertensive treatment. • Previous longitudinal and cross-sectional studies investigating the change in left ventricular mechanics following treatment of hypertension have produced conflicting results.
What this study adds	• LVH associated with grade II-III hypertension regresses after only 18 weeks of intensive antihypertensive treatment. • Left ventricular ejection fraction reduces from supra-normal values after rapid treatment of grade II-III hypertension, accompanied by a reduction in left ventricular radial strain, mid and apical circumferential strain and apical rotation.

5.3 Background

Hypertension is associated with disruption of the structure and function of the left ventricular (LV) myocardium. Foremost amongst these changes is LV hypertrophy (LVH), quantified as LV mass and normalised to body surface area (BSA) as LV mass index.

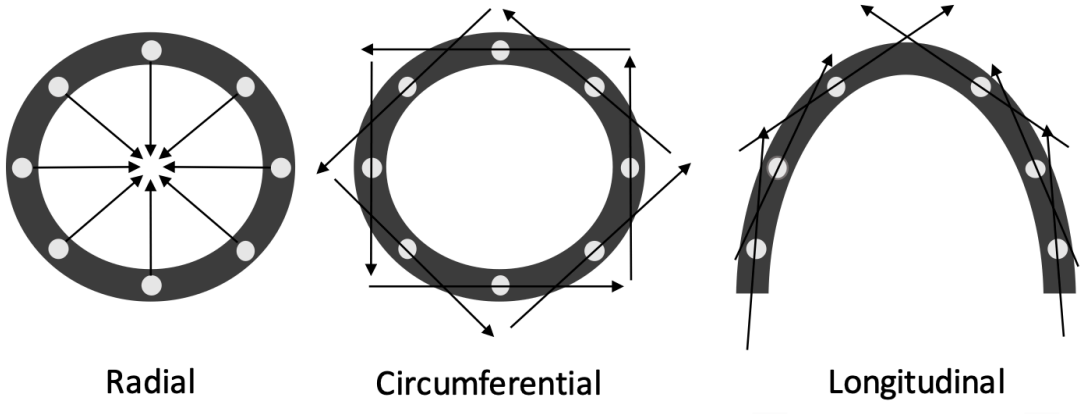
Increased LV mass has been shown to be an independent predictor of mortality in the Framingham cohort (Levy et al., 1990). Further cohort studies have demonstrated that LV mass also stratifies risk in subjects with and without coronary artery disease (Ghali et al., 1992) and specifically in the hypertensive population (Koren et al., 1991).

Following treatment of hypertension, persistent LVH is an indicator of poor prognosis, whereas complete regression almost normalises cardiovascular risk (Muiesan et al., 1995). The association of treatment-induced LV mass regression with favourable prognosis is independent of baseline LV mass and degree of blood pressure (BP) reduction (Verdecchia et al., 1998). As a measure of end-organ damage, LV mass may be more closely linked to prognosis than office BP reductions (Koren et al., 2002), though low event rates in cohort studies of uncomplicated essential hypertension hamper definitive conclusions as to the relative weighting of these associations (Muiesan et al., 1995, Verdecchia et al., 1998, Koren et al., 2002, Cipriano et al., 2001). The timing of LVH regression is also unclear, with the most rapid improvement demonstrated in a longitudinal study of 24 weeks' duration using echocardiography to quantify LV mass before and after treatment for patients enrolled with grade I or II hypertension (mean baseline office BP: 164/93mmHg) and receiving either standard BP management or intensive management within the study (Cheng et al., 2014).

LVH in hypertension allows the heart to maintain a normal, or even supra-normal LV ejection fraction (LVEF) in the face of increased afterload (Grossman et al., 1975), at least in the early stages of the disease. However, despite a normal LVEF, systolic function is demonstrably not normal in hearts of hypertensive patients. Cross-sectional studies using speckle tracking, an echocardiography technology, have shown a reduction in measures of myocardial strain

(deformation per unit length) in hypertensive individuals versus normotensive control subjects (Celic et al., 2014, Galderisi et al., 2012, Kao et al., 2013), which can be described according to the direction of deformation relative to the cardiac axis (Figure 5.1).

Figure 5.1: Schematic representation of strain parameters in relation to the LV myocardium



In individuals with hypertension, speckle tracking has demonstrated a reduction in myocardial global longitudinal strain and global radial strain (Celic et al., 2014, Galderisi et al., 2012). Analysis of circumferential strain magnitude in hypertensive subjects have been inconsistent, with some studies suggesting a reduction in global circumferential strain in hypertension (Celic et al., 2014) and others determining no significant difference compared with normotensive controls (Galderisi et al., 2012, Kao et al., 2013).

LV torsion, defined as the relative systolic twisting motion as a consequence of basal clockwise rotation and apical anticlockwise rotation normalised to LV length (Russel et al., 2009), has been shown to increase in amplitude in hypertensive subjects when measured using echocardiographic speckle tracking (Celic et al., 2014) and cardiac magnetic resonance (CMR) imaging (Ahmed et al., 2012). This may, at least in part, facilitate the preservation of LVEF seen in hypertensive subjects despite the reduction in longitudinal and radial strain (Ahmed et al., 2012).

Resolution of LV systolic dysfunction, defined according to global longitudinal, global circumferential and global radial strain, in subjects with treated hypertension when compared with untreated hypertension and normotensive controls has been demonstrated in a cross-sectional study (Celic et al., 2014). Longitudinal studies investigating the effect of antihypertensive treatment on LV myocardial mechanics have concentrated on circumferential and longitudinal strain (Alam et al., 2013, Mizuguchi et al., 2009) or longitudinal strain alone (Cheng et al., 2014). The effects of antihypertensive treatment on radial strain and LV torsion have not been reported in longitudinal studies. Furthermore, the shortest duration of antihypertensive treatment shown to improve longitudinal and circumferential strain is 24 weeks, within the study design described above and including a heterogenous cohort of participants treated either with intensive management or standard care (Cheng et al., 2014).

CMR has been shown to have greater accuracy in the measurement of LV mass when compared with 2D echocardiography (Grothues et al., 2002). CMR feature tracking for strain assessment, which uses post-processing technology to track intramyocardial features throughout the cardiac cycle (Schuster et al., 2016), has been shown to be more reproducible than older CMR techniques (Singh et al., 2015).

This study therefore aims to use CMR to establish for the first time whether LVH regression occurs within 18 weeks of commencing antihypertensive treatment in individuals with never-treated essential hypertension. Furthermore, CMR feature tracking will supplement this through a comprehensive assessment of myocardial strain in all planes relative to the cardiac axis: the first time that this technology has been applied in a longitudinal study, determining the cardiac effects of antihypertensive treatment with greater accuracy than previous studies using echocardiography.

5.4 Methods

Potential participants aged 18-79 years and never treated for hypertension were identified by their usual care clinician following an office systolic BP of ≥170mmHg. Ambulatory monitoring was then used to confirm at least grade II hypertension (daytime average systolic BP ≥150mmHg) prior to enrolment, using an A&D TM-2430 ambulatory monitoring system (A&D Instruments Limited, Abingdon, UK).

Exclusion criteria were: renal impairment (GFR <60 ml/min/1.73m^2, Hb <10 g/dl, platelet count <100 x10^9/l or bleeding diathesis, pregnancy or breastfeeding, inability to provide informed consent, hypertension-related event (including stroke or acute kidney injury) within the preceding 3 months, or any condition, including hypertensive urgency, requiring immediate BP lowering or tailored antihypertensive strategy. The criteria were selected during planning for the clinical study (Jordan et al., 2020), to enable renal denervation, should this become indicated within the a priori treatment protocol.

Participants underwent a treatment programme involving fortnightly nurse-led consultations. At each consultation stepwise intensification of antihypertensive treatment or further investigation was mandated for those not at office BP target according to a pre-defined protocol, as detailed previously (Jordan et al., 2020). Treatment targets and antihypertensive medication followed national and international consensus guidelines (NICE, 2011, Mancia et al., 2013).

CMR studies were undertaken immediately before and after 18 weeks' antihypertensive treatment. Imaging was performed at the Exeter Magnetic Resonance Research Centre, St Luke's Campus, University of Exeter at 1.5T (Philips Intera, Philips Healthcare, Best, Netherlands) using a 5-channel surface phased array coil. Our standard clinical hypertension CMR protocol was undertaken which includes 4-chamber, 2-chamber, 3-chamber and short axis stack steady-state free precession cine imaging (balanced fast field gradient echo sequences, repetition time 3.2ms, echo time 1.6ms, 20-30 phases, slice thickness 8mm, 1.2x1.2mm spatial resolution). T1-weighted gradient echo sequences were used to determine renal and adrenal anatomy, followed by aortic

and renal artery delineation using early gadolinium enhancement (0.15mmol/kg gadopentate dimeglumine, Gadovist®, Bayer Healthcare Pharmaceuticals, Wayne NJ, USA), (repetition time 5.2ms, echo time 1.5ms, 40 slices, 8mm slice thickness, 0.7x0.7mm spatial resolution). Late gadolinium enhancement gradient echo phase-sensitive inversion recovery sequences performed 8-10 minutes after gadolinium administration were acquired in three short axis slices and a long axis plane to assess for myocardial fibrosis or infiltration before and after antihypertensive treatment (repetition time 5.4ms, echo time 2.6ms, 8mm slice thickness, 1.2x1.2mm spatial resolution). This ensured that it would be possible to exclude other causes of LVH, such as Fabry disease and amyloidosis, together with prior myocardial infarction, which could affect the volumetric and feature tracking analysis.

Post-hoc MRI data analysis was conducted by a single operator. LV volumes and mass were calculated through planimetry of end-diastolic epicardial areas and end-diastolic and end-systolic endocardial areas for each short axis slice covering the entire left ventricle from mitral valve to apex, in line with standard protocols (Kramer et al., 2013) and using commercially-available software (Extended MR WorkSpace, Philips Healthcare, Best, Netherlands). Left atrial (LA) volume was determined using the biplane area length method (Jiamsripong et al., 2008). LV long-axis length was assessed in a 4-chamber view by measuring the distance from the middle of the atrioventricular ring to the apex at end-diastole. Short-axis length was assessed in the same view at the papillary muscle insertion point. LV sphericity index was defined as the ratio of LV short axis length to long axis length and LV thickness was measured in the short axis at papillary muscle level in end-diastole.

Feature tracking analysis required dedicated software (TomTec Imaging Systems, 2-dimensional CPA MR, Cardiac Performance Analysis, Unterschleissheim, Germany). Short axis basal, mid-ventricular and apical image plane selection was determined as previously described (Kowallick et al., 2016). Left ventricular endocardial and epicardial borders were manually traced with the initial contour set in end-diastole. The software then automatically traced the tissue voxels throughout the cardiac cycle, which was reviewed and repeated if myocardial tracking was not adequate visually. These were calculated as

endocardial strain and mean strain (average between endocardial and epicardial strain values).

Basal clockwise rotation (ϕ_{base}), apical anticlockwise rotation (ϕ_{apex}) and the distance between the basal and apical imaging planes (D) were used to derive twist and torsion:

$$Twist = \phi_{apex} - \phi_{base}$$

$$Torsion = \frac{\phi_{apex} - \phi_{base}}{D}$$

5.4.1 Study endpoints

The aim of the MRI investigation was to determine whether LV mass index changed following 18 weeks of antihypertensive treatment.

Key further endpoints were the change in LV volume, LVEF, sphericity index, LA volume and strain parameters with treatment. The presence and distribution of late gadolinium enhancement in our cohort was also evaluated, together with the incidence of detection of secondary causes of hypertension.

5.4.2 Sample size and statistical analysis

A previous study assessing the change in CMR-defined LV mass index with antihypertensive treatment found a mean reduction from 80.3 ± 15.7g/m^2 to 70.1 ± 16.7g/m^2 after 52 weeks of treatment with a combination of an angiotensin converting enzyme inhibitor and calcium channel blocker (Reichek et al., 2009). An effect size of at least a 0.65 standard deviation difference in LV mass index before and after antihypertensive treatment was therefore used to inform our study sample size given our study has a shorter intervention period but uses a technique better able to detect smaller changes in LV mass.

We planned to enrol 50 participants into a clinical study of the feasibility and safety of rapid treatment of grade II/III hypertension (Jordan et al., 2020). Of these, 75% recruitment to paired MRI studies was anticipated. Using a two-tailed paired t-test (α = 0.05) comparison, 38 participants would enable the detection of a 0.55 SD with 90% power at a level of significance of 5% .

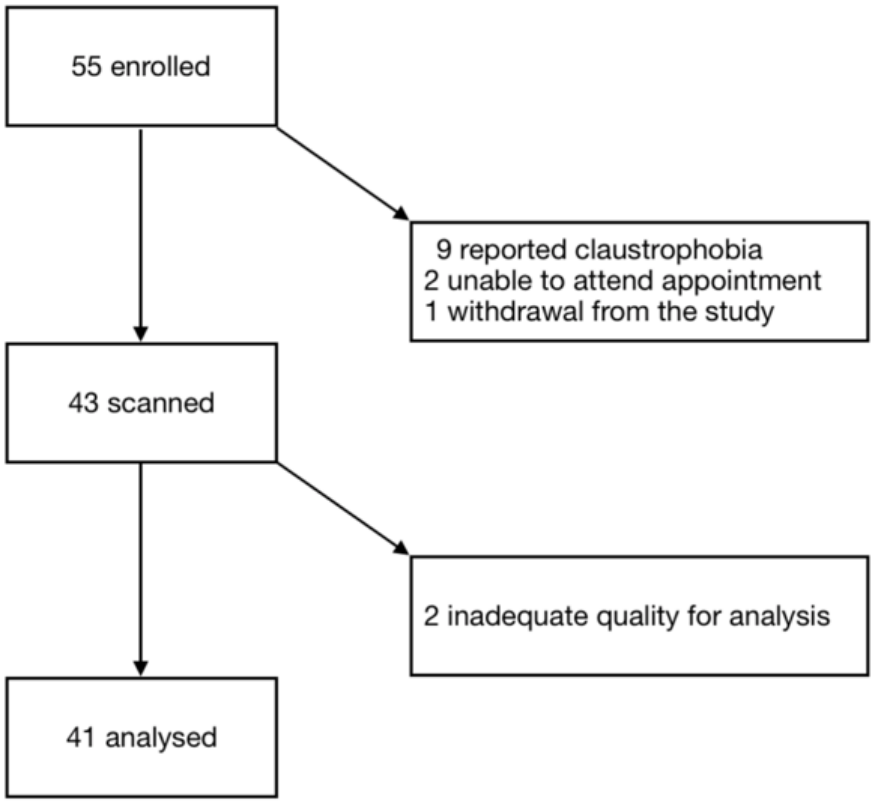
Statistical analysis was performed using STATA v14.1 (StataCorp, College Station, Texas, USA). Baseline and outcome data are presented as means ($\pm$ standard deviation) or medians (interquartile range) for continuous data depending on the normality of the data and counts (percentages) for categorical and binary variables.

Parametric data were analysed using a paired t-test, non-parametric data were analysed using Wilcoxon's signed ranks test and proportions using a one-sample test of proportions. A two-sided *P* value threshold <0.05 was considered statistically significant. Univariate linear regression models were employed to determine the relationship between BP response during the study and markers of change in LV structure and function as outcome variables. Beta values were thereby calculated as the coefficient describing the degree of change in the outcome variable for every 1 unit change in the predictor variable.

5.5 Results

Of the 55 participants recruited to the study, 1 did not complete the treatment protocol and 9 did not complete 2 MRI studies due to claustrophobia. In a further 2 subjects, MRI data were subject to artefact and 2 patients were unable to attend their final appointments. The following results therefore refer to the remaining 41 participants (Figure 5.2).

Figure 5.2: Number of enrolled participants in the clinical study of rapid hypertension treatment with MR imaging appropriate for analysis



Median age of the analysis group was 59 years, 61% were male, there were no diabetic subjects and mean office BP reduced from 175/103mmHg to 132/80mmHg with treatment. Further characteristics prior to and following the treatment programme are summarised (Table 5.1).

Table 5.1: Characteristics of participants before and after 18 weeks' antihypertensive treatment in 41 individuals. Expressed as mean ± standard deviation or median (interquartile range); P values determined between groups using a paired t test (substituted for Wilcoxon's signed ranks test* or one-sample test of proportions# where indicated).

Variable	Before treatment	After treatment	P value
Office systolic BP (mmHg)	174 ± 15.7	132 ± 11.9	<0.0001
Office diastolic BP (mmHg)	103 ± 9.3	80 ± 8.4	<0.0001
Daytime average systolic BP (mmHg)	163 ± 10.4	134 ± 10.4	<0.0001
Daytime average diastolic BP (mmHg)	93 ± 8.9	78 ± 6.8	<0.0001
Heart rate (bpm)	71 ± 12.0	64 ± 10.1	<0.0001*
BMI (kg/m^2)	29.7 ± 5.3	29.7 ± 4.9	0.9
Current smoker (n)	5 (12%)	5 (12%)	1.0#
Alcohol (units/week)	8 (1-18)	9 (1-15)	0.8*
Fasting total cholesterol (mmol/L)	5.6 ± 1.2	5.6 ± 1.3	0.7
Creatinine (μmol/L)	75 ± 13.0	78 ± 13.4	0.3
HbA1c (mmol/mol)	38 ± 0.5	38 ± 0.6	0.6
Angiotensin receptor blocker (n)	0	37 (90%)	n/a
Calcium channel blocker (n)	0	40 (98%)	n/a
Thiazide diuretic (n)	0	24 (59%)	n/a
Aldosterone antagonist (n)	0	9 (21%)	n/a
α-blocker (n)	0	1 (2%)	n/a
β-blocker (n)	0	3 (7%)	n/a
Number of anti-hypertensives (n)	0	3 (2-3)	n/a

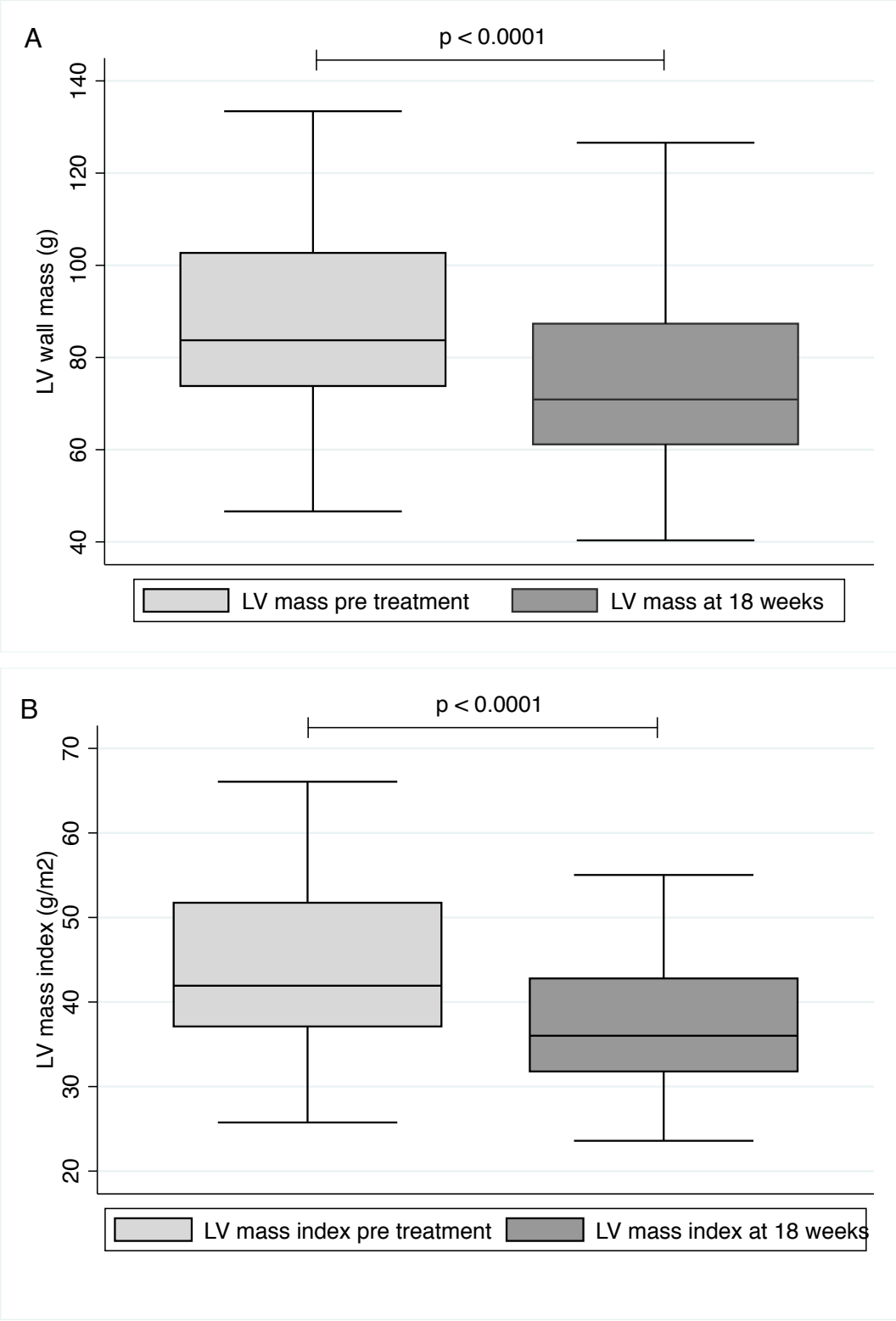
With Bonferroni correction, p<0.0045 considered significant.

5.5.1 Left ventricular mass and mass index

LV mass index reduced significantly after 18 weeks of antihypertensive treatment (43.5 ± 9.8 to 37.6 ± 8.3g/m2, p<0.0001). This marked reduction was also

observed in non-indexed LV mass (88.9 ± 24.5 to 76.8 ± 21.7g, p<0.0001) (Figure 5.3A) and left ventricular diastolic thickness (12.2 ±2.0 to 10.5 ±1.6mm, p<0.0001 Figure 5.3B).

Figure 5.3: Left ventricular (LV) mass (A) and mass index (B) before and after 18 weeks' antihypertensive treatment



Change in LV mass index was associated with diastolic BP change (β = 0.4, p = 0.01), and with systolic BP change (β = 0.3, p = 0.04). Likewise, non-indexed LV mass regression was associated with office diastolic BP change after 18 weeks of antihypertensive treatment (β = 0.4, p = 0.01). Non-indexed LV mass regression was also associated with office systolic BP change over 18 weeks (β = 0.3, p = 0.04).

5.5.2 LV and left atrial volumes

LV and LA volumes and derived parameters are given in Table 5.2.

Table 5.2: Left ventricular (LV) and left atrial (LA) dimensions and derived parameters determined by CMR imaging before and after 18 weeks' antihypertensive treatment in 41 individuals. Expressed as mean ± standard deviation; P value from paired t-test (substituted for Wilcoxon's signed ranks test where indicated*)

Variable	Before treatment	After treatment	P value
LV end-diastolic volume (ml)	130.7 ± 28.0	125.4 ± 25.7	<0.0001
LV end diastolic volume index (ml/m2)	64.4 ± 12.0	61.8 ± 10.8	<0.0001
LV end-systolic volume (ml)	45.8 ± 16.6	46.7 ± 15.9	0.04
LV end-systolic volume index (ml/m2)	22.6 ± 8.0	23.1 ± 7.7	0.04
LV stroke volume (ml)	84.9 ± 16.5	78.6 ± 14.4	0.0008
LV ejection fraction (%)	65.6 ± 6.8	63.4 ± 7.1	0.03
LV sphericity index	0.6 ± 0.05	0.6 ± 0.06	0.2
LA volume (ml)	73.3 ± 19.9	68.9 ± 18.5	0.2*

Over 18 weeks, LV end-diastolic volume (LVEDV) and LVEDV index (LVEDVi) reduced significantly whereas LV end-systolic volume (LVESV) and LVESV index (LVESVi) increased significantly following antihypertensive treatment. In accordance with this, stroke volume reduced over the study period, as did LVEF. There was no significant change in LA volume.

When participants prescribed β-blockers were excluded from the analysis, changes in LVESV and LVESVi were no longer significant, though the finding of significant reductions in LVEDV, LVEDVi, LVEF and stroke volume persisted (Table 5.3). In addition, sub-group analyses were conducted for each class of medication, with no new significant results found.

Table 5.3: Left ventricular (LV) and left atrial (LA) dimensions and derived parameters determined by CMR imaging before and after 18 weeks' antihypertensive treatment in 38 individuals (those on β-blockers excluded). Expressed as mean ± standard deviation; P value from paired t-test (substituted for Wilcoxon's singed rank test where indicated*)

Variable	Before treatment	After treatment	P value
LV end-diastolic volume (ml)	129.9 ± 28.2	122.5 ± 23.4	0.002
LV end diastolic volume index (ml/m2)	64.2 ± 12.2	60.6 ± 10.8	0.002
LV end-systolic volume (ml)	45.0 ± 16.5	45.4 ± 15.4	0.84
LV end-systolic volume index (ml/m2)	22.3 ± 8.1	22.5 ± 7.6	0.82
LV stroke volume (ml)	84.9 ± 16.4	77.1 ± 13.1	<0.0001
LV ejection fraction (%)	66.0 ± 6.6	63.6 ± 7.3	0.03
LV sphericity index	0.6 ± 0.05	0.6 ± 0.06	0.12
LA volume (ml)	74.3 ± 20.3	68.3 ± 18.6	0.05*

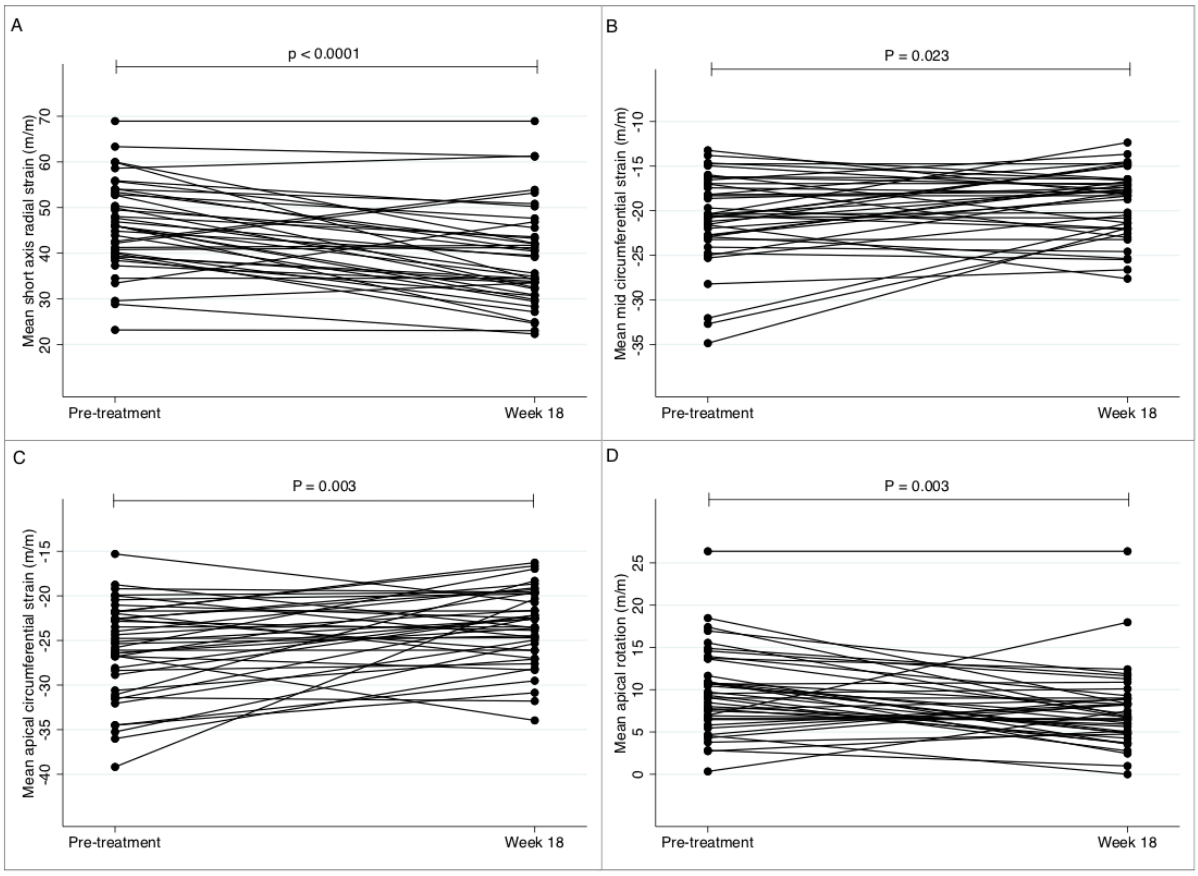
5.5.3 Left ventricular strain before and after antihypertensive treatment

Feature tracking analysis of all MRI study participants revealed the reduction in global LVEF and stroke volume following antihypertensive treatment was predominantly linked to a significant reduction in radial strain (measured in the short axis view), a reduction in mid to apical circumferential strain and a reduction in apical rotation (Table 5.4, Figure 5.4). Longitudinal strain trended towards increasing with treatment, whereas torsion tended to decrease with treatment, though these changes were not statistically significant.

Table 5.4: LV endocardial and mean (average of endocardial and epicardial) strain before and after 18 weeks' antihypertensive treatment in 41 individuals. Mean ± standard deviation; P value from paired t-test.

Strain parameter (m/m)		Before treatment	After treatment	P value
Radial strain (short axis)	Endocardial	48.5 ± 18.2	41.7 ± 19.7	<0.0001
	Mean	46.1 ± 9.7	39.1 ± 10.9	<0.0001
Radial strain (long axis)	Endocardial	29.0 ± 9.2	27.4 ± 9.6	0.4
	Mean	29.0 ± 9.2	27.4 ± 9.6	0.4
Longitudinal strain	Endocardial	-20.3 ± 5.3	-21.0 ± 6.0	0.4
	Mean	-19.1 ± 4.7	-19.4 ± 5.4	0.6
Basal circumferential strain	Endocardial	-28.7 ± 4.7	-27.6 ± 4.5	0.1
	Mean	-20.4 ± 3.9	-19.9 ± 3.4	0.4
Mid circumferential strain	Endocardial	-29.9 ± 6.5	-27.0 ± 5.2	0.003
	Mean	-20.8 ± 4.9	-19.1 ± 3.7	0.02
Apical circumferential strain	Endocardial	-35.6 ± 6.9	-32.0 ± 5.9	0.001
	Mean	-26.0 ± 5.3	-23.4 ± 4.2	0.003
Rotation (apical)	Endocardial	11.9 ± 6.7	9.4 ± 6.0	0.02
	Mean	9.8 ± 5.0	7.5 ± 4.5	0.003
Rotation (basal)	Endocardial	3.9 ± 3.7	3.8 ± 2.9	0.9
	Mean	3.1 ± 2.6	2.8 ± 2.0	0.5
Twist	Endocardial	15.7 ± 7.6	13.2 ± 6.3	0.03
	Mean	12.8 ± 5.9	10.4 ± 4.6	0.006
Torsion	Endocardial	5.4 ± 3.0	4.9 ± 2.8	0.3
	Mean	4.4 ± 2.4	3.8 ± 2.0	0.2

Figure 5.4: Radial strain (measured in the short axis) (A), mid-circumferential strain (B), apical circumferential strain (C) and apical rotation (D) before and after 18 weeks of antihypertensive treatment



Change in mean radial strain measured in the short axis was not significantly associated with change in systolic BP (β = 0.05, p = 0.8) or diastolic BP (β = 0.2, p = 0.2) over the 18-week study period.

5.5.4 Late gadolinium enhancement in treatment-naïve moderate-severe hypertension

30 participants underwent late gadolinium enhancement imaging at baseline, the remaining 11 MRI participants either declining this aspect of the imaging protocol or not receiving contrast due to equipment failure. 7 (23%) were found to have myocardial late gadolinium enhancement. The most frequent segment affected was the basal inferoseptum (71%), with the remainder affecting exclusively the basal inferior or basal inferolateral segments. In those with basal inferoseptal or

basal inferior late enhancement, Fabry disease was excluded through measurement of serum α-galactosidase.

All enhancement was subepicardial in distribution and therefore not indicative of underlying coronary artery disease. There were no significant changes in late gadolinium enhancement pattern in any participants after 18 weeks.

5.6 Discussion

This is the first study to examine the effect on myocardial structure and function of a rapid, 18-week, intensive antihypertensive treatment strategy. Using CMR, our study demonstrates that LVH regresses rapidly after only 18 weeks intensive antihypertensive treatment and that this is associated with further, potentially beneficial, alterations in cardiac physiology.

Prior to the current study, the most rapid observed regression of LVH during hypertension treatment was measured at 24 weeks (Cheng et al., 2014) and this study therefore adds credence to the proposal in recent consensus guidelines that benefits can be gained from aiming to control BP in a shorter timeframe than 24 weeks for grade II-III hypertension (Williams et al., 2018). Furthermore, the novel use of CMR feature tracking in assessing all directional components of myocardial deformation in a longitudinal study before and after the introduction of antihypertensive agents has for the first time found that radial strain reduces with treatment, whilst also confirming the previously described higher longitudinal strain, lower mid and apical circumferential strain and lower apical torsion in treated hypertension compared with untreated hypertension (Cheng et al., 2014, Celic et al., 2014, Alam et al., 2013, Mizuguchi et al., 2009).

The 2018 European guidelines for the treatment of hypertension recommend that patients with grade II-III hypertension are treated to target within 3 months in order to improve prognosis (Williams et al., 2018). The proposed prognostic benefit for rapid treatment of hypertension is supported by observational evidence from clinical records demonstrating that a delay in achieving target BP is associated with adverse outcomes (Gradman et al., 2013, Xu et al., 2015). Additionally, retrospective analyses of major hypertension trials have revealed that early and effective BP treatment reduces subsequent adverse events with an apparent legacy effect beyond the period of treatment delay (Weber et al., 2004, Staessen et al., 2004). In the present study, a 14% reduction in LV mass index was observed, as compared with a 17% reduction in LV mass index found in the losartan treatment arm of the LIFE study (Devereux et al., 2004) and the 8% reduction in LV mass index detected in a study of intensive antihypertensive treatment delivered over 24 weeks (Cheng et al., 2014). The rapid reversal of

LVH demonstrated by this study may suggest one of the mechanisms by which improved prognosis could be gained and indicate that an appropriately-designed randomised controlled trial should be considered to examine this.

The current study also explores functional changes in the myocardium following antihypertensive treatment, demonstrating that supra-normal LVEF is reduced by treatment, predominantly as a result of a reduction in LVEDV. Although it could be argued that the negative inotropic effect of β-blockade could influence these results, it was noted that the statistically significant observation persists when participants receiving β-blockers at week 18 are excluded from the analysis. Left ventricular stroke volume was also found to be lower following antihypertensive treatment in the present study, despite the fact that untreated hypertension has been found to be associated with a lower stroke volume when compared with normotensive control subjects (Finkielman et al., 1965). We propose that this finding is related to the pharmacological effects of antihypertensive agents used within the treatment protocol, with both angiotensin receptor blockers and diuretics likely to confer a reduction in stroke volume.

This study also shows a reduction in radial strain with treatment (when measured in the short axis), a finding which has not previously been demonstrated. This reduction in radial strain is in keeping with the demonstrated reduction in LVEDV with treatment and Starling's Law, though discrepant with previous cross-sectional data using echocardiographic techniques, which suggested a lower radial strain in untreated hypertension when compared with normotensive controls (Celic et al., 2014, Galderisi et al., 2012) and subjects with treated hypertension (Celic et al., 2014). This difference compared with previous studies may relate to the variance in imaging modalities used to assess radial strain, with the MRI-based technique used in our study known to be more accurate than the echocardiography techniques used in comparable studies. Moreover, the cross-sectional study designs used by previous studies are susceptible to inter-group confounding variables, present to a lesser extent in our longitudinal study design.

Our finding of reduced radial strain following antihypertensive treatment may relate to the concurrent reduction in LV radial thickness also observed, as left ventricular hypertrophy has previous been demonstrated to be associated with

increased radial strain (Saltijeral et al., 2010). Alternatively, this finding may be a product of the unique population studied compared with previous investigations (treatment-naïve grade II-III hypertension) or an early response to treatment, which then reverses. To study the possibility of the latter explanation, additional imaging of participants at a later timepoint in their treatment would be informative.

Longitudinal strain tended to increase with treatment of hypertension, which is in keeping with previous cross-sectional and longitudinal studies (Cheng et al., 2014). In our study, this increase in longitudinal strain did not reach statistical significance, though this is likely to be due to the fact that we found this measurement to be highly variable in untreated hypertension.

We also demonstrated a reduction in circumferential strain in the mid and apical segments in response to antihypertensive treatment. As with previous studies, functional disruption of the heart when exposed to increased afterload appears to affect apical segments to a greater extent than basal segments (Celic et al., 2014). This was also the case in the analysis of LV rotation, with increased apical torsion in untreated hypertension compared with treated hypertension in keeping with a previous cross-sectional study (Celic et al., 2014). As expected, this translated to increased LV twist in untreated hypertension, though the difference became statistically insignificant when corrected for LV length. This may be due to the reduction in LV length in treated hypertension, as shown by the reduction in LVEDV, which would tend to reduce the torsion measurement.

In addition to myocardial changes with treatment, our protocol aimed to characterise the presence of focal fibrosis within the myocardium in hypertensive heart disease, as determined using late gadolinium enhancement sequences. This identified non-ischaemic focal fibrosis in the myocardium of 23% participants, with predilection for affecting the basal infero-septal segment, in agreement with previous observational studies of similar patient groups (Rudolph et al., 2009, Treibel et al., 2015, Krittayaphong et al., 2010).

5.6.1 Limitations of this study

The observations in this study are based on a broad range of antihypertensive medication, as dictated by the treatment protocol and reported in Table 5.1. Although a meta-analysis has shown some variability in effect on LV mass index between different classes of antihypertensive agents (Klingbeil et al., 2003), these heterogeneities appear to be relatively small when only the classes of agents recommended in the most recent consensus guideline iterations are considered. It is unknown, however, if there is heterogeneity in the functional response of the myocardium to different antihypertensive agents, though the majority of participants in our study received the standard combination of renin-angiotensin axis blockade and calcium-channel blocker with or without the addition of a thiazide diuretic, ensuring that our results are translatable to clinical patients.

In terms of late gadolinium enhancement, this relies on visual determination of the inversion time based on nulling of the “normal” myocardium. If diffuse myocardial fibrosis occurs in hypertension, this would not be visualised if the process homogeneously affects the whole heart and therefore would not have been detected by our MRI protocol, a limitation of the study. However, diffuse fibrosis can be detected using T1 mapping and this has previously been utilised in participants with essential hypertension (Treibel et al., 2015), revealing no significant myocardial fibrosis between the groups of treated hypertensive patients (mean office BP: 152/88mmHg) versus normotensive controls (mean office BP: 123/74mmHg). However, this study found a significant degree of diffuse fibrosis in the subgroup of patients with LVH. As LVH regresses with antihypertensive treatment, it can be postulated that this group of patients reflects those with poorly-controlled hypertension. As such, diffuse fibrosis may be a feature of uncontrolled hypertension, which would have not been evident in our study in the absence of T1 mapping. Further investigation for diffuse fibrosis using T1 mapping in grade II/III uncontrolled hypertension may therefore be warranted.

It is also noted that 16% subjects enrolled in the treatment programme could not undergo MRI testing as a consequence of claustrophobia, which is a higher proportion than would be expected. This may relate to the larger body habitus of

participants compared to the population average, particularly when considered alongside the 60cm bore size of the MRI hardware. In addition, the study protocol dictated that MRI examinations were performed after a significant number of microvascular investigations, which may have led to fatigue amongst participants, contributing to aborted examinations.

5.6.2 Conclusions

The present study demonstrates rapid improvement in LVH as a consequence of implementation of an intensive antihypertensive treatment protocol. These structural changes are accompanied by functional changes in the heart which include a reduction in LVEDV, radial strain, mid and apical circumferential strain and apical rotation.

Given the improved structural and functional parameters seen on CMR following a relatively short period of antihypertensive treatment for newly diagnosed hypertension, early and aggressive treatment of hypertension could plausibly lead to improved clinical outcomes. In the absence of a dedicated randomised clinical trial, these data support a strategy of early blood pressure control, as recommended in the latest European Society of Hypertension guidelines (Williams et al., 2018).

5.6.3 Declarations

Ethics Approval and Consent to Participate

Ethical approvement for this study was agreed prospectively (NRES Committee South West ref. 15/SW0077). All participants gave voluntary informed consent in accordance with the Declaration of Helsinki.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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The design of the study and collection, analysis and interpretation of the data was free from influence from the funding organisations.

Authors' contributions

Study concept and design: ANJ, JF, CEC, ACS, ASPS, NGB. Acquisition of the data: ANJ, JF, KG, CA, LW, CB, NP, DM. Analysis and interpretation of the data: ANJ, CEC, ACS, ASPS, NGB. Initial draft of the manuscript: ANJ. Study supervision: ACS, ASPS, NGB. All authors read and approved the final manuscript.

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Chapter 6 Cross-cultural adaptation of the Spanish MINICHAL instrument into English for use in the United Kingdom

6.1 Abstract

Background: Hypertension is a highly prevalent condition, with optimal treatment to BP targets conferring significant gains in terms of cardiovascular outcomes. Understanding why some patients do not achieve BP targets would be enhanced through greater understanding of their health-related quality of life (HRQoL). However, the only English language disease-specific instruments for measurement of HRQoL in hypertension have not been validated in accordance with accepted standards. It is proposed that the Spanish MINICHAL instrument for the assessment of HRQoL in hypertension could be translated, adapted and validated for use in the United Kingdom. The aim of the study was therefore to complete this process, using a cohort of patients enrolled in an 18-week programme for the treatment of grade II-III hypertension.

Methods: The MINICHAL authors were contacted and the original instrument obtained. This was then translated into English by two independent English-speakers, with these versions then reconciled, before back-translation and subsequent production of a 2nd reconciled version. Thereafter, a final version was produced after cognitive debriefing, for administration and psychometric analysis in the target population.

Results: The final version of the instrument was administered to 30 individuals with grade II/III hypertension before and after 18 weeks' intensive treatment. Psychometric analysis demonstrated a floor effect, though no ceiling effect. Internal consistency for both state of mind (StM) and somatic manifestations (SM) dimensions of the instrument were acceptable (Cronbach's alpha = 0.81 and 0.75), as was test-retest reliability (ICC=0.717 and 0.961) and construct validity, which was measured through co-administration with the EQ5D5L and Bulpitt-Fletcher instruments. No significant associations were found between scores and patient characteristics known to affect HRQoL. The EQ5D5L instrument found an improvement in HRQoL following treatment, with the StM

and SM dimensions of the English language MINICHAL trending to support this (d=0.32 and 0.02 respectively).
Conclusions: The present study details the successful English translation and validation of the MINICHAL instrument for use in individuals with hypertension. The data reported also supports an improvement in HRQoL with rapid treatment of grade II/III hypertension, a strategy which has been recommended by contemporaneous European guidelines.

Keywords: hypertension, adaptation, validation, MINICHAL

6.2 Summary Table

What is known about the topic?	• No disease-specific English language HRQoL instrument has previously been validated for use in individuals with hypertension, in accordance with modern standards. • HRQoL is reduced in hypertension and improves with treatment. • Current European guidelines suggest rapid treatment of grade II-III hypertension. The acceptability to patients of this approach is unknown.
What this study adds	• Successful translation and validation of the MINICHAL disease-specific instrument for measurement of HRQoL in hypertension. • HRQoL, as measured using the EQ5D5L visual analogue scale, improves with rapid treatment of grade II-III hypertension.

6.3 Background

Hypertension affected approximately 1 billion people worldwide in the year 2000, a number which is expected to rise to 1.5 billion by 2025 (Kearney et al., 2005). The benefits of blood pressure (BP) control are well-established, with a halving of cardiovascular risk for each incremental reduction of 20/10mmHg down to 115/80mmHg (Lewington et al., 2002). Despite these clear benefits of effective antihypertensive therapy, BP control is achieved in only 63% of patients with treated hypertension in England (Falaschetti et al., 2014).

Non-adherence to medical therapy is an important contributor to apparent treatment-resistant hypertension, as shown by the incorporation of drug level assays and directly-observed therapy into specialist hypertension clinics (Tomaszewski et al., 2014, Hameed et al., 2016). Poor adherence to medication regimens is particularly understandable for a condition in which the majority of patients are asymptomatic before treatment, considering that diagnosis and treatment have the potential to negatively impact health-related quality of life (HRQoL). It is therefore clear that addressing the HRQoL of patients with hypertension should form part of the holistic approach to care for these individuals, particularly given the association between impaired subjective wellbeing and cardiovascular events (Svardsudd and Tibblin, 1990, Moller et al., 1996, Waller et al., 2015).

Previous studies have shown HRQoL to be reduced in those with a diagnosis of hypertension compared with control subjects (Erickson et al., 2001, Bardage and Isacson, 2001). A cross-sectional study has demonstrated that this may, at least in part, be owing to patients' awareness of their diagnosis (Mena-Martin et al., 2003), with higher perception of well-being found in those who were incidentally hypertensive but not treated, as compared to those with a known diagnosis of hypertension. Additionally, comorbid disease, medication side effects or under-reported symptoms attributable to hypertension (such as mood change, headache or dizziness) may adversely affect HRQoL. Treatment of hypertension, for example with 1 month of angiotensin receptor blockade (Roca-Cusachs et al., 2001), improves HRQoL in longitudinal studies. BP reduction and achievement of target BP following

combination therapy have also been shown to be positive influencers on perceived well-being (Marques da Silva et al., 2015). Monitoring of HRQoL during treatment may therefore provide a useful tool in determining those participants at higher risk of adverse events or non-adherence.

To date, studies exploring HRQoL in hypertensive subjects have employed generic instruments alone (such as the EuroQol-5D or SF-36) or in combination with disease-specific instruments (Erickson et al., 2001, Bardage and Isacson, 2001, Mena-Martin et al., 2003, Roca-Cusachs et al., 2001). Disease-specific instruments are valued as they are felt to be more responsive to change; it is unlikely that generic instruments are able to adequately capture HRQoL in all populations suffering from all types of conditions (Bulpitt, 1997). Although disease-specific instruments for hypertension have been validated in Spanish (Badia et al., 2002) and Brazilian Portuguese (Schulz et al., 2008), those in English, such as the Bulpitt-Fletcher questionnaire (Bulpitt and Fletcher, 1990), have not undergone appropriate validation according to current standards (Valderas et al., 2008, Wild et al., 2005).

Considering the Bulpitt-Fletcher instrument in detail, a degree of redundancy can be demonstrated, with 11 of 46 questions not contributing to the overall score as per the scoring methods proposed by the authors (Bulpitt and Fletcher, 1990). The Bulpitt-Fletcher instrument has a not trivial administrative burden, with an estimated administration time of 20-40 minutes (Bulpitt and Fletcher, 1990), which is incongruent with the notion that questionnaires should be kept short and simple to minimize measurement error (Hunt, 1997) and further limiting its widespread adoption. In addition, not all questions within the Bulpitt-Fletcher instrument can be applied to all participants, with question 35 applying to men only and question 37 to only those in paid employment. This will inevitably lead to missing data when the instrument is administered, impacting on its performance. Further, the Bulpitt-Fletcher instrument includes several items (11, 14, 32, 37) that place it overall among clinimetric measures rather than psychometric measures and hence better placed to estimate disease severity than measuring HRQoL (Valderas and Alonso, 2008, de Vet et al., 2003). Finally, given that both treatment and

general patient perceptions and expectations have changed markedly since the inception of the Bulpitt-Fletcher instrument, it may no longer capture the somatic manifestations of side effects from first-line medications or up-to-date cultural values affecting HRQoL. For example, questions 21, 25 and 35 aim to establish common side effects of beta-blockers, which are no longer considered a mainstay of treatment for hypertension (Williams et al., 2018) and questions related to sexual activity (questions 31-35) may raise more doubts regarding the safe storage of sensitive personal data in the digital age.

Adapting and validating the readily available Spanish MINICHAL hypertension disease-specific instrument in English offers therefore obvious advantages and may be an efficient alternative to the development of a new instrument. The MINICHAL instrument, originally conceived and validated in Spanish (Badia et al., 2002) (Figure 6.1), has an average administration time of just over 7 minutes. Although 17 questions are described, the final question pertains to the subject's overall perception of their own health; it is not included in the scoring (or the validation) of the original instrument. Within the remaining 16 questions, 2 domains have been determined: State of Mind (StM) and Somatic Manifestations (SM).

Total scores are summated from these responses and range from 0 to 30 for StM and from 0 to 18 for SM; lower scores reflect higher HRQoL (Badia et al., 2002). Psychometric evaluation (Badia et al., 2002, Roca-Cusachs et al., 2003) has shown that it meets current standards for internal consistency, test-retest reliability and responsiveness to change. Validation of the MINICHAL instrument has been confirmed through co-administration with 2 generic instruments and responsiveness to change evaluated following 6 months of antihypertensive treatment intensification, finding a positive correlation between degree of BP (and heart rate) reduction and improvement in the MINICHAL score, especially the StM domain (Roca-Cusachs et al., 2003).

The aim of this study was therefore to translate, adapt and evaluate the psychometric performance of the existing MINICHAL for its use in the United Kingdom. Within this, we aimed to test responsiveness to change of the instrument through administration before and after an 18-week intensive

treatment programme for subjects with newly diagnosed grade II-III hypertension (Jordan et al., 2020).

Figure 6.1: Original MINICHAL instrument (Roca-Cusachs et al., 2003)

Cuestionario de Calidad de Vida de la Hipertensión Arterial (MINICHAL)

	Marque una cruz en la casilla que elija, sólo una por línea			
¿En los últimos 7 días…	**No, en absoluto**	**Sí, algo**	**Sí, bastante**	**Sí, mucho**
1. ha tenido dificultades para conciliar el sueño?				
2. ha tenido dificultades para continuar con sus relaciones sociales habituales?				
3. le ha resultado difícil entenderse con la gente?				
4. siente que *no* está jugando un papel útil en la vida?				
5. se siente incapaz de tomar decisiones y empezar nuevas cosas?				
6. se ha notado constantemente agobiado y en tensión?				
7. tiene la sensación de que la vida es una lucha continua?				
8. se siente incapaz de disfrutar sus actividades habituales de cada día?				
9. se ha sentido agotado y sin fuerzas?				
10. ha tenido la sensación de que estaba enfermo?				
11. ha notado dificultades al respirar o sensación de falta de aire sin causa aparente?				
12. se le han hinchado los tobillos?				
13. ha notado que orina más a menudo?				
14. ha notado sequedad de boca?				
15. ha notado dolor en el pecho sin hacer ningún esfuerzo?				
16. ha notado una sensación de entumecimiento u hormigueo en alguna parte del cuerpo?				
¿Diría usted que su hipertensión y el tratamiento de la misma afecta a su calidad de vida?				

6.4 Methodology

6.4.1 Translation and cross-cultural adaptation

Adaptation of the MINICHAL instrument into English followed the guidelines set out by the International Society for Quality of Life Research (ISOQOL) (Reeve et al., 2013), International Society for Pharmacoeconomics and Outcomes Research (ISPOR) (Wild et al., 2005) and the Evaluating the Measurement of Patient-Reported Outcomes (EMPRO) tool (Valderas et al., 2008).

The MINICHAL instrument was obtained from its original publication in Spanish (Roca-Cusachs et al., 2003) and permission for adaptation secured through contact with authors of the original publication.

Forward translation was provided by 2 independent native English-speakers. This version was discussed by a panel of researchers with experience of cross-cultural adaptation, including 1 of the aforementioned translators, producing a consensus for each of the 16 questions in turn. This process produced the first reconciled version of the English instrument.

The first reconciled version was then back-translated by a third independent native Spanish-speaking professional translator. To ensure that language equivalence between questionnaires had been achieved, this version was compared with the original Spanish MINICHAL to highlight discrepancies, allowing the panel to produce a second reconciled version of the questionnaire.

Following this, the second reconciled version underwent cognitive debriefing (pilot testing using the techniques “thinking aloud”, probing and debriefing) with 8 participants from the typical demographics of the target population with hypertension, all of whom were native English-speakers and included an appropriate range of educational backgrounds. Harmonization with the previous Brazilian Portuguese translation of the instrument was also

completed at this stage (Schulz et al., 2008). The results informed the production of the final version of the adapted instrument.

6.4.2 Evaluation of psychometric properties

Accepted standards for psychometric evaluation of the instrument were followed as per ISOQOL (Reeve et al., 2013), ISPOR (Wild et al., 2005) and EMPRO (Valderas et al., 2008). The metric qualities were determined through administration at weeks 0, 8, 10 and 18 of an 18-week treatment programme for patients with newly-diagnosed grade II and grade III hypertension. This treatment programme aimed to enrol 50 participants and it was envisaged that the new instrument be applied to all participants enrolled subsequent to completion of the necessary translation and cross-cultural steps.

Each of the 16 questions was scored, receiving a numerical value based on the response, as in the original Spanish instrument: “No, en absolute” = 0, “Si, algo” = 1, “Si, bastante = 2, “Si, mucho” = 3. The scores were summated within the previously defined domains with lower scores indicating a higher HRQoL. As in the original validation and subsequent cross-cultural adaptation, the StM and SM domains were reported separately and the final question of the instrument reflecting the subjects overall assessment of their HRQoL was not used in the analysis (Roca-Cusachs et al., 2003, Badia et al., 2002, Schulz et al., 2008). Internal consistency was determined by calculating pairwise correlations between items and Cronbach’s alpha. Test-retest reliability was evaluated through administration of the instrument twice, 2 weeks apart, after no change in medication at weeks 8 and 10 after enrolment (intraclass correlation coefficient). Given the reliability of 0.80 in the original MINICHAL instrument evaluation (Badia et al., 2002), a 2-tailed intraclass correlation coefficient was expected to be >0.70 (α=0.05).

Construct validity was assessed through co-administration with a generic questionnaire (EQ-5D-5L) and the hypertension-specific Bulpitt-Fletcher instrument (Spearman correlation), predicting a moderate to low correlation with the EQ-5D-5L instrument and moderate to high correlation with the

Bulpitt-Fletcher instrument. For both the Bulpitt-Fletcher and EQ-5D-5L instruments, higher scores indicate higher HRQoL.

Construct validity was further evaluated through association with variables known to affect quality of life measures in hypertensive patients, including heart rate, body mass index (BMI), number of antihypertensive agent, BP and female gender (Roca-Cusachs et al., 2001, Zygmuntowicz et al., 2013)(Mann-Whitney test and Pearson's correlation coefficient). As reported in the original evaluation of the MINICHAL instrument, it was predicted that female gender would be associated with an increased score in the StM domain, increased age and increased BMI would be associated with an increased score in the SM domain and raised BP and number of co-morbidities would correlate with an increased score in both domains (Badia et al., 2002).

Responsiveness to change was evaluated through comparison of the MINICHAL scores before and after 18 weeks' intensive antihypertensive treatment (paired t-test, following test of normality, and effect size). It was anticipated that this intervention would affect HRQoL as a previous meta-analysis had found a small but significant improvement in general well-being following active treatment of hypertension (d = 0.139) (Beto and Bansal, 1992), including whilst using the same pharmacological groups employed in the treatment protocol, with the potential for accelerated treatment in our protocol to accentuate HRQoL gains.

In the original evaluation of the Spanish MINICHAL instrument, re-testing 6 months after treatment intensification was found to improve HRQoL, with an effect size of 0.55 and 0.46 for the StM and SM domains respectively. Moreover, there was a significant correlation between improvement in HRQoL and BP reduction (Badia et al., 2002). In the present study, univariate linear regression models were used to determine the relationship between BP response during the study and instrument scores as outcome variables, with the hypothesis that change in HRQoL would be related to degree of BP reduction, particularly the StM domain of the MINICHAL instrument, as previously reported.

6.5 Results

6.5.1 Translation

Differences between the forward translations were noted, in particular the grading of responses to each question being translated as “not at all, yes occasionally, yes quite often and yes often” compared with “no not at all, yes a little, yes a fair amount and yes a lot”. Reconciliation was achieved through a meeting between the investigators and one of the forward translators, with phraseology chosen to provide a more distinct gradient of response in the final reconciled version: “no not at all, yes a little, yes a moderate amount and yes a lot”. In addition, the two forward translations for question 3 were markedly different, focusing on “being understood by people” versus “getting on with other people”, requiring reconciliation to “Have you had difficulties communicating with other people?”, in order to combine both interpretations of the question. Question 8 produced differing translations: “everyday activities” versus “normal routine”, with “usual activities” chosen for the reconciled instrument as this reflected the senses of both translations. For question 11, “shortness of breath” was felt to be easier to understand than the alternative translation of “feeling of suffocation”. In question 15, “exertion” was selected over “straining”, as it was concluded that the authors of the instrument were seeking to elicit a symptom of ischaemic heart disease rather than muscular chest wall pain on straining.

The reconciled version of the instrument was then back-translated by a native Spanish speaker. This highlighted a difference in question 15 compared with the original Spanish version, with “without having exerted yourself” rephrased to “without physical exertion” as a consequence. The overall grading of response also differed in the Brazilian Portuguese translation when compared with the English back translation, though it was felt that the Portuguese translation lacked sufficient gradient of response between “yes a lot” and “yes very much” and therefore the second reconciled version was preferred.

Cognitive debriefing took place with 8 individuals: the median age was 60 years, 50% were educated to undergraduate degree level or higher and 50%

were female. All participants in the cognitive debriefing process reported no difficulties in understanding the questions as drafted in the second reconciled version. No consistent changes to the instrument were suggested and, in light of these findings, a final version of the instrument was approved for evaluation (Figure 6.2).

6.5.2 Instrument evaluation

The final version of the instrument was administered to 30 native English speakers before and after antihypertensive treatment. Of these, 53% were male, median age was 58.5 years and mean pre-treatment office BP measured 171/101mmHg, falling to 130/80mmHg after 18 weeks of treatment. The characteristics of these participants are given in Table 6.1.

Figure 6.2: English-language MINICHAL instrument

High Blood Pressure Quality of Life Questionnaire (HBP-QLQ) [UK version of the Arterial Hypertension Quality of Life Questionnaire (MINICHAL)]	Please, put an cross in ONE BOX for each question			
In the last 7 days…	No, not at all	Yes, a little	Yes, a moderate amount	Yes, a lot
1. Have you had difficulties falling asleep?				
2. Have you had difficulties in your social relationships?				
3. Have you had difficulties communicating with other people?				
4. Have you felt that you're not playing a useful role in life?				
5. Have you felt unable to make decisions and start new things?				
6. Have you felt constantly overwhelmed or stressed?				
7. Have you felt that your life is a constant struggle?				
8. Have you felt unable to enjoy your usual activities?				
9. Have you felt exhausted or weak?				
10. Have you felt ill?				
11. Have you experienced any breathing difficulties or shortness of breath for no reason?				
12. Have your ankles become swollen?				
13. Have you passed water more often?				
14. Have you had a dry mouth?				
15. Have you experienced any pain in your chest without physical exertion?				
16. Have you experienced pins & needles or any numbness in any part of your body?				

Table 6.1: Characteristics of 30 participants administered the final English language version of the MINICHAL instrument, before and after 18 weeks of intensive antihypertensive treatment. Expressed as mean ± standard deviation or median (interquartile range); P values determined between groups using a paired t test (except where indicated)

Variable	Before intervention	After intervention	P value
Office systolic BP (mmHg)	171 ± 15.8	130 ± 10.6	<0.0001
Office diastolic BP (mmHg)	101 ± 11.5	80 ± 8.7	<0.0001
Daytime average systolic BP (mmHg)	164 ± 12.2	134 ± 10.8	<0.0001
Daytime average diastolic BP (mmHg)	93 ± 10.1	78 ± 6.8	<0.0001
Heart rate (bpm)	69 ± 10.9	66 ± 9.6	0.024
BMI (kg/m^2)	30.0 ± 5.9	29.9 ± 5.5	0.79
Current smoker (n)	2 (7%)	2 (7%)	1.00[#]
Alcohol (units/week)	7 (1-15)	2 (1-10)	0.36*
Angiotensin receptor blocker (n)	0	25 (83%)	n/a
Calcium channel blocker (n)	0	29 (97%)	n/a
Thiazide diuretic (n)	0	15 (50%)	n/a
Aldosterone antagonist (n)	0	4 (13%)	n/a
α-blocker (n)	0	1 (3%)	n/a
β-blocker (n)	0	1 (3%)	n/a
Number of anti-hypertensives (n)	0	2.5 (2-3)	n/a
Number of other medications (n)	0 (0-1)	0 (0-1)	0.32*
Number of co-morbidities (n)	1.0 ± 0.9	1.0 ± 0.9	1.00

*Wilcoxon's signed ranks test

[#]one-sample test of proportions

Floor and ceiling effects

For each item, the minimum score was returned in 67-97% responses, with the greatest floor effect seen in item 3: “Have you had difficulties communicating with other people?”. The maximum score for an item was returned in 0-3% responses, indicating no ceiling effect, as previously reported for the Spanish version of the instrument (Badia et al., 2002).

Reliability

Reliability (internal consistency) was acceptable for both the StM and SM domains: Cronbach’s alpha 0.81 and 0.75 respectively. As 1 participant did not return completed questionnaires for the evaluation of test-retest reliability, the intraclass correlation coefficient (ICC) between the scores derived for the remaining 29 participants who underwent test-retest data acquisition between weeks 8 and 10 of treatment was calculated, with no change in medication undertaken between these two appointments. This determined acceptable test-retest reliability for both domains: StM ICC=0.717 (95% CI: 0.378-0.913); SM ICC=0.961 (95% CI: 0.876-0.988).

Validity

Both StM and SM domains significantly correlated with the EQ-5D-5L summary index, EQ-5D-5L linear scale and the Bulpitt-Fletcher instrument (Table 6.2).

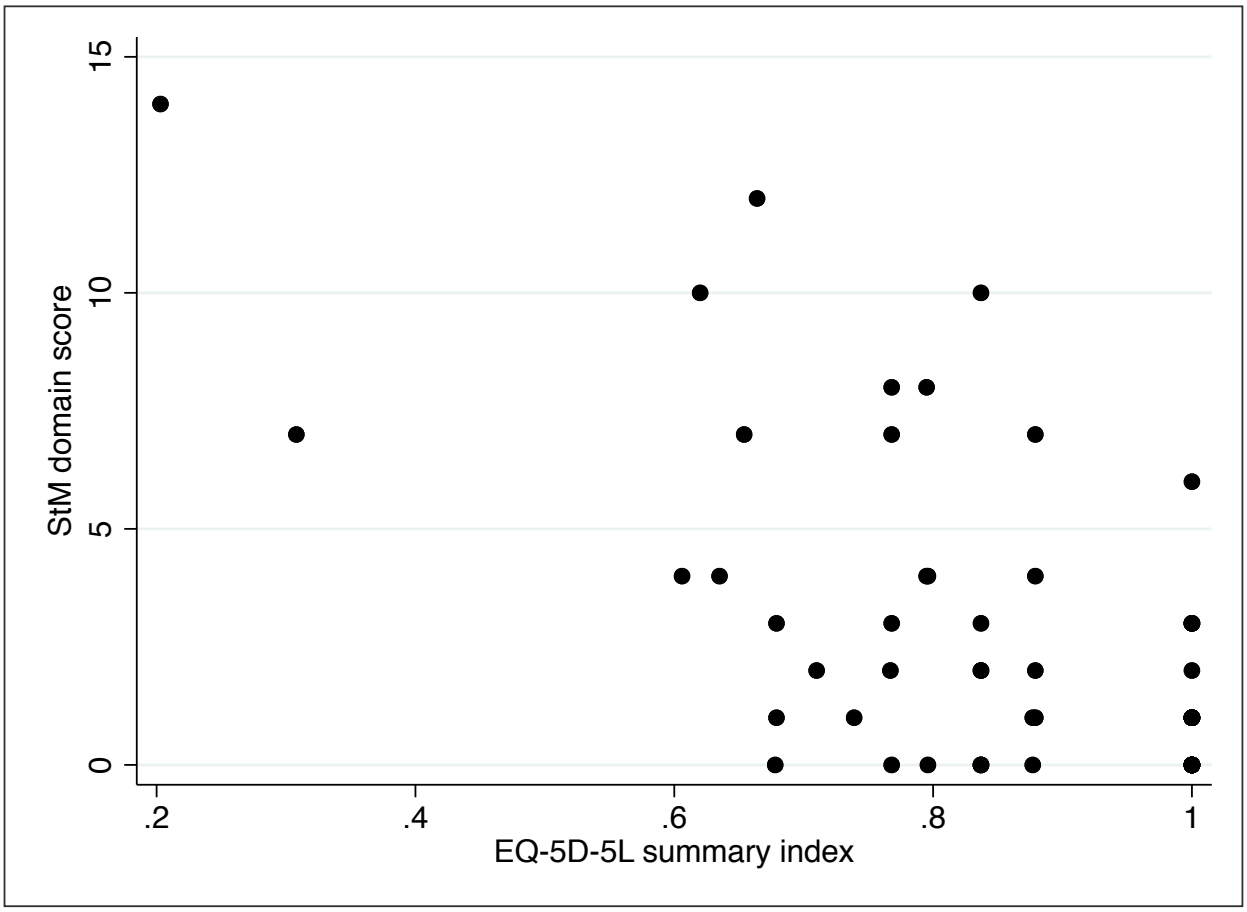
Table 6.2: Correlations between MINICHAL domains and other HRQoL measurements in 30 participants administered the instrument before and after 18 weeks of intensive antihypertensive treatment

Instrument/dimension	StM		SM	
	r_s	P	r_s	P
EQ5D5L linear scale	-0.394	0.0019	-0.362	0.0045
EQ5D5L summary index (UK)	-0.500	<0.0001	-0.491	0.0001
Bulpitt-Fletcher	-0.472	0.0001	-0.291	0.0243

StM: State of Mind, SM: Somatic Manifestations
Spearman's correlation coefficient (r_s)

All correlations were moderate, with the strongest correlation found between the StM domain and the EQ-5D-5L summary index (UK values) (Figure 6.3). The StM domain showed a higher correlation with the Bulpitt-Fletcher questionnaire than the SM domain. Within the same instruments, correlations were determined between the StM and SM dimensions of the English MINICHAL (r_s=0.5257; p<0.0001) and between the EQ-5D-5L summary index and EQ-5D-5L linear scale (r_s=0.3725; p=0.0034).

Figure 6.3: Correlation between the MINICHAL StM domain and EQ-5D-5L summary index



R_s = -0.500, p<0.0001

No significant difference was found between genders for either the StM domain (female: 2 (1-5); male: 2 (1-4.5); p=0.164) or SM domain (female: 1 (0-2); male: 1 (0-3); p=0.901). Pearson's correlation was used to explore relationships between scores and patient characteristics. Although most of the associations were in the direction predicted by our hypotheses, none of them were found to be statistically significant (Table 6.3).

Table 6.3: Correlations between MINICHAL scores and subject demographic and clinical characteristics administered to 30 participants before and after 18 weeks of intensive antihypertensive treatment

Variable	StM		SM	
	r	P	r	P
Age	-0.068	0.608	0.128	0.331
BMI (kg/m2)	0.024	0.858	0.105	0.426
Office sBP	-0.044	0.737	-0.004	0.975
Office dBP	-0.034	0.796	-0.181	0.166
Daytime average sBP	0.079	0.551	0.034	0.798
Daytime average dBP	-0.001	0.993	-0.187	0.152
Heart rate	-0.117	0.372	-0.194	0.138
No. of antihypertensive medications	-0.198	0.129	-0.025	0.851

StM: State of Mind, SM: Somatic Manifestations, sBP: systolic blood pressure, dBP: diastolic blood pressure
Pearson's correlation coefficient (r)

Responsiveness

Results from the application of the patient-reported quality of life instruments before and after 18 weeks of intensive antihypertensive treatment are summarized in Table 6.4.

Table 6.4: Change in patient-reported quality of life in 30 participants after 18 weeks' intensive antihypertensive treatment Expressed as mean ± standard deviation or median (interquartile range); P values determined between groups using a paired t test

	Pre-treatment score	Week 18 score	P	Effect size (d)
EQ5D5L linear scale	79.6 ± 13.54	88.8 ± 8.11	0.0001	0.82
EQ5D5L summary index (UK)	0.84 ± 0.18	0.87 ± 0.17	0.05	0.17
Bulpitt-Fletcher	0.88 ± 0.017	0.91 ± 0.017	0.049	1.76
MINICHAL StM	3.23 ± 3.52	2.17 ± 3.03	0.076	0.32
MINICHAL SM	1.33 ± 1.86	1.30 ± 2.11	0.932	0.02

StM: State of Mind, SM: Somatic Manifestations
d: Cohen's

Following 18 weeks of intensive hypertension treatment there was a significant improvement in HRQoL as measured by the EQ-5D-5L, in particular the linear scale (d=0.82). Application of the MINICHAL instrument produced results in agreement with this, with a greater responsiveness of the StM domain (d=0.32) when compared with the SM domain (d=0.02), though these did not reach statistical significance. On the contrary, the Bulpitt-Fletcher instrument found a significant reduction in HRQoL following treatment.

Office systolic BP response to treatment was not associated with a change in any of the measures of HRQoL (MINICHAL StM domain (p=0.342), MINICHAL SM domain (p=0.406), EQ-5D-5L linear scale (p=0.532), EQ-5D-5L summary index (p=0.740) or Bulpitt-Fletcher scores (p=0.553)). Neither was the case for daytime average systolic BP measured with ambulatory BP monitoring (data not shown).

6.6 Discussion

We report the first validation of a disease-specific English-language patient-reported outcome instrument for use in hypertension. The successful translation and validation of the instrument was completed in accordance with accepted standards (Valderas et al., 2008, Wild et al., 2005, Reeve et al., 2013).

Evaluation of the instrument demonstrated an important floor effect though no ceiling effect. This is in-keeping with the initial evaluation of the Spanish MINICHAL instrument (Badia et al., 2002) and reflects a relatively low symptom burden for the majority of subjects with hypertension. Internal consistency for the English MINICHAL instrument comfortably met current standards for use at group level, with dimension analysis for StM and SM finding similar values to those reported for the Spanish iteration of the instrument (0.81 and 0.75 versus 0.87 and 0.75 respectively) (Badia et al., 2002). A similar situation was observed for test-rest reliability and, in this case, the English version of the instrument also compared favourably with the Spanish instrument.

Construct validity was confirmed through the instrument's correlation with generic instruments (EQ-5D-5L summary index and EQ-5D-5L linear scale) and the disease-specific Bulpitt-Fletcher instrument. In terms of strength of association, this was greatest with the EQ-5D-5L summary index and weakest with the Bulpitt-Fletcher instrument. This is not completely surprising considering that the Bulpitt-Fletcher instrument, though developed specifically for hypertension, is not an ideal instrument for measuring quality of life because of its mixed clinimetric-psychometric approach. A higher correlation between two instruments whose main focus is HRQoL can therefore be expected.

Responsiveness was tested through administration of the instrument before and after 18 weeks' intensive treatment of hypertension, using medications and medication combinations recommended in current international guidelines, though over an accelerated timeframe (Jordan et al., 2020), an

intervention that seemed to have a measurable significant impact on generic quality of life as measured through the visual analogue scale of the EQ-5D-5L (large effect size), but not on the EQ-5D-5L index (small effect size). The effect size for the MINICHAL StM scale was larger than for the EQ-5D-5L index, but not statistically significant due to a high dispersion of scores, and was negligible for the MINICHAL SM scale. It must be noted that scores were already very low for the latter scale, suggesting that the floor effect observed in this group of patients may have limited our ability to detect improvement.

A meta-analysis of cross-sectional studies has found that HRQoL is impaired across all eight domains of the SF-36 and SF-12 instruments in those with hypertension, when compared with normotensive individuals (Trevisol et al., 2011). Subsequent investigation has found reduced HRQoL in patients treated for hypertension, when compared with untreated hypertensive subjects (Trevisol et al., 2012), which may be related in large part to the subjects' awareness of their diagnosis (Korhonen et al., 2011). However, these conclusions are limited by the inherent bias imparted by the cross-sectional nature of their design. In terms of longitudinal studies, which enable subjects to act as their own control group thereby minimizing confounding factors, improvement in HRQoL following treatment of hypertension has been demonstrated in a meta-analysis (Beto and Bansal, 1992). This observation can be found with a variety of antihypertensive agents (Grimm et al., 1997). Our report of improved HRQoL following treatment using the EQ-5D-5L instrument is therefore in-keeping with previous longitudinal data. Moreover, given that visits were predominantly delivered by allied healthcare professionals within the clinical study, our finding mirrors that of a recent Cochrane review, which concluded that HRQoL, in particular the physical functioning domain, improves with treatment of hypertension delivered by allied healthcare professionals (Clark et al., 2021). This would be also in line with the lack of responsiveness observed in this study for the SM scale.

In addition, our intervention was delivered in an accelerated timeframe, a manner of treatment delivery which is known to improve HRQoL in other fields of medicine, such as hip arthroplasty (Larsen et al., 2008) and radiotherapy in the treatment of breast cancer (Van Hulle et al., 2020). It would therefore

be reasonable to propose that the improvement in HRQoL conferred through treatment of hypertension will have been accentuated by the rapid treatment protocol employed in this study.

Conversely, administration of the Bulpitt-Fletcher instrument within the protocol found a significant reduction in HRQoL following hypertension treatment. Notably, psychometric evaluation with measurement of internal consistency, floor effect, ceiling effect and construct validity for the instrument has not been reported. Additionally, test-retest reliability has only been examined for selected concepts within the questionnaire, rather than the instrument itself (Bulpitt et al., 1976). However, responsiveness to change of the Bulpitt-Fletcher questionnaire has previously been reported through administration of the instrument within clinical studies, such as a trial comparing hypertension treatment with verapamil versus propranolol (Fletcher et al., 1989) and a further study comparing captopril with atenolol (Fletcher et al., 1990). Although generic instruments were co-administered with the Bulpitt-Fletcher instrument within these studies, no direct statistical comparison was conducted and therefore construct validity was not evaluated.

Dimensions analysis of the MINICHAL instrument results revealed a nominally greater responsiveness within the StM dimension compared to the SM dimension. Different weighting between the EQ-5D-5L and the Bulpitt-Fletcher instruments in terms of somatic symptoms versus psychological well-being may also therefore explain the discrepant results between these two instruments when applied to subjects within the study. The responsiveness to change analysis may also have been affected by a high variability in responses within the MINICHAL instrument domains, as indicated by the relatively high standard deviations of results pertaining to this instrument.

6.6.1 Study limitations

The conclusions drawn from the study are limited by the relatively low number of subjects enrolled, a drawback which could be addressed through further deployment of the translated English language MINICHAL instrument in future

studies of hypertension treatment. Furthermore, as this was a before-and-after study, the effects of time, rather than treatment, on HRQoL cannot be discounted from the analysis, though this is limited by the relatively short 18-week treatment phase.

In addition, it is acknowledged that our study cohort was geographically limited to south-west England. Nevertheless, region-specific language is not used within the translated instrument and no difficulties with comprehension or cultural applicability are envisaged should the instrument be used across the United Kingdom.

The evaluation of the MINICHAL instrument alongside the EQ-5D-5L linear scale has demonstrated the latter, generic instrument to be more responsive to change than our disease-specific instrument, the converse to the expected. The reason for this is unclear, though may relate to the greater range of responses afforded by the EQ-5D-5L analogue scale in comparison with the MINICHAL's 4 response options. Furthermore, given the brevity of the MINICHAL instrument and specific nature of the questions, some subtle features of wellbeing may not be captured with this instrument, compared to a generic assessment of HRQoL. This is particularly pertinent given the broad range of medication side effects which can result from pharmacological antihypertensive treatment, which would be difficult to capture entirely with a specific questionnaire short enough to be an acceptable burden to patients. Analysis of the components of the EQ-5D-5L summary index which changed most during treatment determined that the pain/discomfort and anxiety/depression items returned differing scores most frequently. Although anxiety/depression is covered well by the MINICHAL questions, only one question pertains to pain ("Have you experienced any pain in your chest without physical exertion?"). Therefore, a relative deficiency of the MINICHAL instrument in exploring this aspect of symptoms, together with the limited sample size in the present study, may in part explain this discrepancy in responsiveness to change.

In light of these findings, we recommend that future studies of hypertension should consider using both the MINICHAL instrument and EQ-5D-5L in tandem for the assessment of HRQoL.

6.6.2 Future implications

The availability of an English language, short, validated, disease-specific instrument for the evaluation or HRQoL in hypertension is of value, particularly given the high prevalence of this condition and therefore its wide applicability to patients. Non-adherence to treatment is a crucial element of apparent treatment-resistance in hypertension (Hyman and Pavlik, 2015) and therefore the ability to monitor the impact of hypertension and its treatment for patients could help address this important limiter to successful treatment. Furthermore, it is anticipated that the newly-adapted and validated English language instrument will be used in future research practice to ensure that new treatment strategies for hypertension positively impact HRQoL.

6.6.3 Conclusions

The study describes the first validation of an English-language disease-specific instrument for use in the assessment of HRQoL in subjects with hypertension. Furthermore, evidence of acceptability for patients in the rapid treatment of moderate and severe hypertension is reported, a treatment strategy which is recommended in the most recent European guidelines (Williams et al., 2018), though previously without evidence of acceptability for patients.

6.6.4 Declarations

Ethics approval and consent to participate

Ethical approvement for this study was agreed prospectively (NRES Committee South West ref. 15/SW0077). All participants gave voluntary informed consent in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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The design of the study and collection, analysis and interpretation of the data was free from influence from the funding organizations.

Authors' contributions

Study concept and design: ANJ, JV, CEC, ACS, ASPS, NGB. Acquisition of the data: ANJ, CA, LW, CB, NP, DM. Analysis and interpretation of the data: ANJ, JV. Initial draft of the manuscript: ANJ. Study supervision: JV, ACS, ASPS, NGB. All authors read and approved the final manuscript.

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Chapter 7 Overall discussion

Contemporaneous international guidelines have recommended that grade II and III hypertension be treated to target within 3 months (Williams et al., 2018) though, prior to this research project, the feasibility and consequences of this approach were not well-understood.

The benefits of achieving target BP within an accelerated timeframe for those with moderate or severe hypertension can be proposed following retrospective analyses of major trials of antihypertensive treatment, which have found an advantageous prognostic legacy benefit in those for whom treatment was not delayed within the studies, compared with those patients with a 6-month treatment delay (Weber et al., 2004, Staessen et al., 2004). Systematic reviews of clinical case notes have found that patients who suffer from a cardiac event are less likely to be at BP target than those who do not suffer such an event (Gradman et al., 2013) and that delays in intensifying BP treatment in those above target confer an increased risk of cardiovascular events and all-cause mortality (Xu et al., 2015).

Thus, the first aim of the research project was to determine whether rapid treatment of grade II and III hypertension was feasible, using an 18-week treatment programme employing guideline-based treatment delivered stepwise over an accelerated timeframe. A nested experimental medicine component of the project was incorporated in order to increase the understanding of the physiological basis of treatment-response, exploring both the microvascular and cardiac changes conferred by rapid treatment of moderate and severe hypertension.

During the clinical study, the treatment protocol reduced office BP by a mean of 43/23mHg and daytime average BP by 28/15mmHg. Target office BP was achieved in 69% participants at week 18. This compares favourably with Health Survey for England data, which suggests that only 63% patients with treated hypertension are at target with usual care (Falaschetti et al., 2014). Our data also compares favourably with previous randomised controlled studies of protocol-

directed therapy for the treatment of hypertension, including the STITCH-care protocol, which achieved BP control in 64.7% patients following treatment over 6 months, though predominantly enrolled participants with mild hypertension (Feldman et al., 2009). In addition, the VIPER-BP study of protocol-directed antihypertensive treatment for mild hypertension in Australia achieved an office BP ≤140/90mmHg in 63.5% participants in the intervention group after 6 months of treatment (Stewart et al., 2012). It should be emphasised that the proportion of subjects achieving target BP in our study was superior to these comparable studies despite recruiting a cohort of participants with a markedly higher initial BP and providing the intervention over a shorter timeframe.

The rapid timescale of neovascularisation during treatment for hypertension and the improvement in capillary rarefaction in moderate-severe hypertension are novel findings for this work. Given the finding of reduced capillary density (rarefaction) early in the hypertensive disease process (Antonios et al., 1999b, Antonios et al., 2003, Antonios et al., 2012, Noon et al., 1997) and its potential contribution to an increase in peripheral resistance, which itself would act to perpetuate the hypertensive state, the clinical study was accompanied by a nested experimental medicine study with the aim of determining whether capillary rarefaction is reversible within 18 weeks' treatment of moderate and severe hypertension. This determined an increase in cutaneous nailfold capillary density following treatment, which was found to be due to neovascularisation, rather than an increase in the proportion of capillaries perfused at rest. This work adds to previous findings in before-and-after cohort studies of capillary density, which investigated the reversibility of rarefaction in mild hypertension (Hughes et al., 2008, Kaiser et al., 2013), with the new finding of the study presented being its recruitment of patients with moderate and severe hypertension.

The cellular and biochemical mechanisms underpinning this reversal in rarefaction are unclear. As detailed in section 1.5.7, evidence suggests that EPC senescence may be responsible for the development of rarefaction in hypertension, given that EPCs isolated from subjects with established hypertension, pre-hypertension and those at greater risk of hypertension later in life show enhanced senescence in vitro (Imanishi et al., 2005, MacEneaney et al., 2011, Yu et al., 2016). It can therefore be proposed that a reversal of this

process, reduced EPC senescence, may be responsible for the neovascularisation observed in this study.

On the contrary, the reversal of rarefaction observed in the present study may be due to the proliferation of co-located endothelial cells (ECs). This was previously felt to be the predominant mechanism of neovascularisation until 1963, when it was shown that surgically implanted 5cm-long Dacron grafts become endothelialised independent of the anastomotic sites, strongly suggesting seeding from circulating cells (Stump et al., 1963). Since then, circulating EPCs, derived from bone marrow, have been widely studied and characterised, demonstrating a key role in human health and disease (Yoder, 2012).

Should an alteration in EPC senescence be responsible for the neovascularisation observed in this study, it can be postulated that this, at least in part, is influenced by vascular endothelial growth factor (VEGF). This cytokine has been shown to mobilise and recruit EPCs in humans (Asahara et al., 1999, Kalka et al., 2000, Li et al., 2006) and is strongly mitogenic (Leung et al., 1989). VEGF thereby plays a crucial role in regulating endothelial development, as evidenced by the fact that inactivation of the VEGF gene results in defective angiogenesis and early embryonic death (Ferrara et al., 1996, Carmeliet et al., 1996). Analysing blood samples before and after treatment within the programme for markers of EPC senescence, including VEGF, could therefore provide an insight into the mechanistic processes responsible for the neovascularisation observed after 18 weeks' intensive antihypertensive treatment. Blood samples from participants before and after treatment were obtained and stored with prior ethical approval for biomarker testing and therefore the means to explore this further are available.

The novel findings of the MRI component of this work were the rapid myocardial response to treatment in moderate and severe hypertension, both in terms of myocardial structure (specifically a reduction in LV mass) and function (as defined by feature tracking). Furthermore, the application of feature tracking in a before-and-after study of hypertension treatment allowed a comprehensive exploration of myocardial mechanics in terms of treatment response.

The determination of a significant reduction in LV mass (and indexed LV mass) following treatment is in agreement with previous studies, though in a shorter timeframe than had previously been demonstrated (Cheng et al., 2014). Given the association of raised LV mass with poor prognosis (Levy et al., 1990, Ghali et al., 1992), its reversal within a rapid timeframe of treatment lends support to the aforementioned guidance proposing rapid treatment of grade II and III hypertension. Accompanied by the regression in LV mass were changes in the functional characteristics of the myocardium, as characterised by magnitude changes in LV strain, twist and torsion, measured using feature tracking software. In particular, the finding of a reduction in radial strain with treatment of hypertension is discrepant with previous cross-sectional studies using echocardiographic techniques (Celic et al., 2014, Galderisi et al., 2012). This may relate to the greater reproducibility of the MRI technique used in the present study (compared with echocardiography), the linear nature of the study design, the rapid timeframe of treatment and observation of its effects or may be a by-product of the unique group studied, in terms of its overall severity of hypertension and treatment-naivety. Nevertheless, this new finding merits further investigation within future studies.

The clinical study results determined the treatment protocol to be well-tolerated, with only 1 participant withdrawing from the study before completion. Furthermore, no episodes of syncope were reported, there were 10 incidences of medication discontinuation due to lightheadedness and all disturbances of kidney function resolved within 2 weeks of detection and after antihypertensive medication de-escalation. During the study, it was also felt that a comprehensive examination of HRQoL would be informative, through co-administration of generic and disease-specific instruments 4 times during the 18 weeks of treatment. This process determined an improvement in HRQoL following the rapid treatment protocol and facilitated the first validation of an English language disease-specific instrument for hypertension, according to modern standards (Valderas et al., 2008, Wild et al., 2005).

The translation into English of the MINICHAL instrument and its validation, as above, have been published in a peer-review journal (Jordan et al., 2022) and it is therefore envisaged that this instrument will become widely used to monitor

HRQoL in future studies of hypertension and its treatment. Further studies could also investigate whether the HRQoL gains conferred by rapid treatment of hypertension correlate with changes in left ventricular mass or cutaneous capillary density. Such an analysis would thereby explore whether individuals with a greater self-reported sense of wellbeing following treatment are also those in whom a greater physiological response to treatment can be detected, beyond the analysis limited to just office and ambulatory BP response described above.

Overall the project's findings provide evidence for the clinical safety and feasibility of rapid treatment of moderate and severe hypertension, together with outlining the microvascular and myocardial consequences of the treatment regimen. This provides a framework for understanding the physiological basis of treatment-response, which may inform the proposed prognostic benefits of such an approach. The project as a whole supports the guideline recommendation of rapid treatment for grade II and III hypertension, though falls short of allowing a conclusion in terms of a prognostic benefit of this approach. This should be addressed in future studies, designed *a priori* to explore the clinical impact of treatment of moderate and severe hypertension within an accelerated timeframe.

7.1 Proposal for a randomised controlled trial

To determine the efficacy and potential prognostic benefit of rapid treatment of grade II/III hypertension, a randomised controlled study would be required, comparing two treatment strategy arms: rapid treatment and standard care. The proposed study would need to be of sufficient size and duration to ensure statistical power to inform a primary composite endpoint of cardiovascular outcomes. Unfortunately, blinding of participants and those delivering the treatment to the format of care received would not be possible, though it would certainly be feasible and advantageous to blind those performing the enrolment and final visit investigations to the treatment received by the participants, removing potential bias from the analysis of the data acquired during these visits. In addition, the option of randomising unequally to rapid treatment versus standard care (e.g. 2:1 in favour of rapid treatment) should be considered, as such a protocol would mitigate a potential loss in power from cross-over and drop-out from the higher intensity treatment arm, whilst also facilitating a greater

understanding of the consequences of rapid treatment in a larger cohort, beyond those described in the present study.

In planning this future randomised controlled trial, the ethical implications and acceptability to patients of conducting a study with a control group of usual care would need to be considered, given that it could be proposed that there is already a reasonable weight of evidence to suggest that rapid treatment of moderate and severe hypertension is beneficial in terms of cardiovascular outcomes and HRQoL. The study team should also consider reducing the costs of the trial, by allowing usual care to be delivered by patients' primary care physicians, rather than the study team. On the contrary, by also delivering the usual care arm visits within the same environment and by the same personnel as those used for the rapid treatment arm of the study, potential confounders could be mitigated. The feasibility of delivering rapid treatment with the majority of follow-up visits being conducted by appropriately trained nursing staff has been proven by the present study and therefore standardising these visits to be conducted by nursing staff (rather than physicians) should be considered for the future randomised controlled trial. As the use of nurse-led follow-up visits are known to improve BP control versus usual care (Clark et al., 2010), it would be important to standardise the profession and training of the healthcare professionals performing follow-up visits within the trial, a practice which could only be enabled by the research team also delivering visits within the usual care arm.

The implications of conducting the proposed trial in the post-COVID-19 era must also be considered, particularly given that frequent face-to-face interactions with healthcare professionals form the basis of the treatment protocol. Another method for delivering visits could be with virtual consultations using home BP measurements, which could replace all or some of the in-person appointments. Alternatively, a smartphone application and network, such as BP@Home, could be employed. As the efficacy of virtual appointments is of particular interest at present, this could be integrated into the proposed study design. Utilising the suggested 2:1 randomisation in favour of rapid treatment, it is proposed that half of those randomised to rapid treatment receive this remotely, either via video consultation or using a commercially available smartphone application.

A further consideration for a future randomised controlled trial of rapid treatment of moderate-severe hypertension is whether this should be a multi-centre or single centre study. Although the research facilities at the NIHR Exeter Clinical Research Facility are sufficient to conduct studies on a large-scale, by planning the trial as a multi-centre study, the scale could be increased as necessary to enable sufficient power to detect a suitable primary outcome to prove that rapid treatment confers a prognostic benefit, such a composite outcome of cardiovascular death, myocardial infarction (or other acute coronary syndromes), heart failure and stroke. The frequency of this outcome in the SPRINT study of intensive BP treatment in high risk individuals was 2.19% per year in the standard care arm (Wright et al., 2015). Analysis of the Syst-Eur study suggested that timely treatment reduced cardiovascular complications by 15% (Staessen et al., 2004). Therefore, in order to detect this difference over 5 years, using a two-tailed two-sample test of proportions (α = 0.05), at least 5243 participants would need to be recruited, to achieve a power > 0.80. On a practical basis, this strongly suggests a multicentre study would be needed and, if necessary, the role of remote follow-up expanded. Accordingly, the proposed structure of a future randomised controlled trial is provided in Figure 7.1.

Conversely, if such a large trial is considered impractical, a further study could examine whether intensive treatment is efficacious versus usual care in terms of reducing BP at 18 weeks. If the primary endpoint for this study is considered the proportion of participants at office target BP after 18 weeks, using the present study as pilot data and assuming usual care provided by Health Survey for England data (Falaschetti et al., 2014), 975 participants would be needed for this study to inform the primary endpoint (α = 0.05; 1-β = 0.80). However, it should be noted that the usual care data reported in this case refers to BP control to target values for all individuals with hypertension; it is anticipated that the proportion with treatment-naïve grade II/III hypertension at target BP after only 18 weeks of usual care treatment would be significantly lower than this.

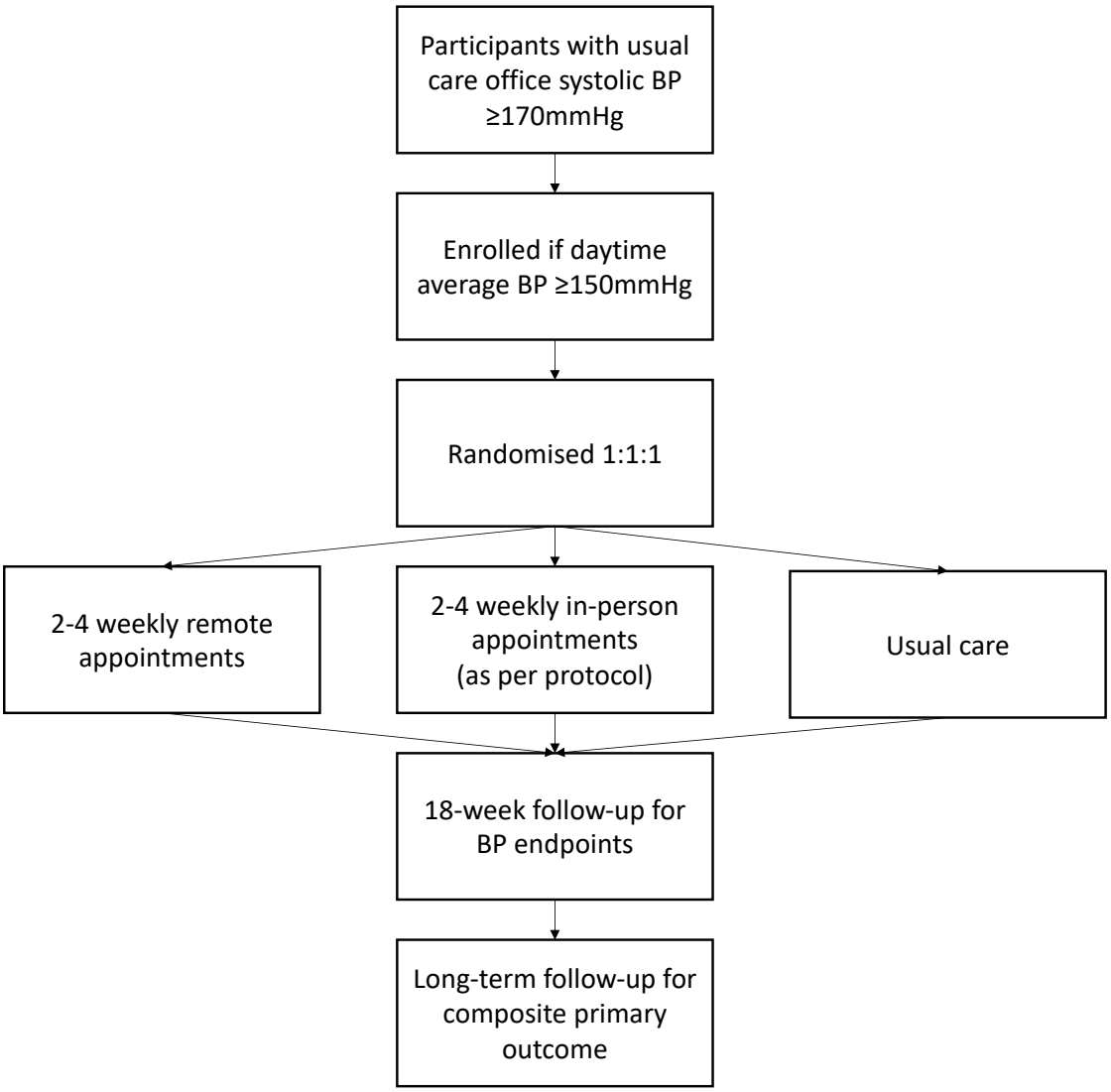
As part of this project, a health economics analysis could be conducted to assess the feasibility of initial rapid and intensive treatment of grade II and III hypertension from a financial standpoint, as this would help inform whether such a strategy could also be promoted along purely economic grounds, in addition to

clinical outcomes. Should a significant proportion of visits be undertaken remotely, this would further strengthen the economic case for employing a rapid treatment strategy in this context.

An exploration of the adherence of individuals to the regimen at a later point in time would also help determine the long-term feasibility and lasting impact of the treatment programme. Although this should be added to a future randomised controlled trial described above, in the shorter term, a recall of participants from the present study should be contemplated. Now that sufficient time has elapsed after the completion of this study, an audit of previous participants' current prescriptions using primary care data, supplemented by liquid chromatography testing for urinary metabolites of antihypertensive medications (with the appropriate consent and ethical approval) would help inform whether participation in the programme affected long-term adherence.

In addition, following the experiences and evolution of the project presented, it is likely that a future trial would remove renal denervation from the protocol, given that this was not deployed for practical reasons within the present study. This would allow for a rationalisation of the study exclusion criteria. Furthermore, noting that lightheadedness necessitated medication discontinuation on 10 occasions within the present study, a more detailed assessment of these subjects would be proposed, including an assessment of orthostatic hypotension. Nevertheless, it should be noted that asymptomatic orthostatic hypotension should not be used as a guide to de-escalate antihypertensive treatment within a future protocol, given that this phenomenon was not associated with adverse cardiovascular outcomes during intensive BP treatment within the SPRINT study (Juraschek et al., 2020).

Figure 7.1: Proposed structure for a randomised controlled trial for efficacy of rapid treatment of grade II/III hypertension



7.2 Future cardiac and microvascular study

In addition to the randomised controlled trial described above, further areas of research could involve newer MR techniques, including T1 mapping, to determine whether diffuse fibrosis occurs in grade II and III hypertension and whether this improves with treatment. Furthermore, contemporaneous MRI microvascular assessment techniques, such as myocardial perfusion reserve index and myocardial blood flow measurement, could be used to assess the response of myocardial microvascular function to rapid treatment of grade 2-3 hypertension. Retinal data could also be gathered and analysed to explore whether the

rarefaction and its reversal with treatment determined in nailfold cutaneous microvascular beds, are mirrored within the eyes of hypertensive patients. This could then also be compared to Microscan data acquired form the sublingual microvasculature of participants, using univariate regression to determine whether measurements of sublingual functional capillary density correspond with retinal capillary density determined using retinal photography. Such a protocol would be enhanced through the use of optical coherence tomography (OCT), to determine whether the previous findings of reduced retinal capillary density and an increased foveal avascular zone previously determined using this technique in hypertensive patients (Sun et al., 2020) reverse rapidly with intensive antihypertensive treatment. Furthermore, OCT could be used to assess for a change in macular thickness with intensive treatment of moderate-severe hypertension, given the relationship of this measurement with capillary pressure (Gooding et al., 2010), a key component of microvascular function.

A greater phenotyping of patients from a microvascular perspective before treatment in a larger cohort may also provide insights into the predictors of response to treatment and the physiological basis of resistant hypertension within those individuals who initially present with moderate and severe hypertensive disease. This could include an assessment of endothelium-dependent vasodilatation, with has been found to be impaired in hypertensive subjects using assessment with local warming and acetylcholine delivered transcutaneously using iontophoresis (Lindstedt et al., 2006). Furthermore Pulse Amplitude Tomography (PAT) could be employed to measure non-invasive digital pulse wave amplitude in participants prior to treatment, noting that this measurement has been found to be related to endothelial function (Nohria et al., 2006). Should this method for the assessment of endothelial function be chosen, this could also be used to measure Reactive Hyperaemic Index (RHI), calculated as a ratio of signal measured by PAT in a limb before and after 5-minute arterial occlusion, and known to correlate inversely with coronary endothelial function measured invasively (Bonetti et al., 2004).

Finally, it would also be informative to determine whether these indicators of microvascular dysfunction correlate with analogous parameters of microvascular function determined using MR imaging techniques. The value of this would be to

attempt to validate the cardiac MR measurements, used in clinical practice, as measures of systemic microvascular function, which would have utility in assessing patients with systemic manifestations of microvascular disease.

7.3 Conclusion

Overall, the body of work presented brings together evidence supporting the feasibility of rapid treatment of moderate-severe hypertension and gains from this approach in terms of the microvasculature, HRQoL and cardiac structure and function. This support the present guidelines, which recommend treatment of grade II and grade III hypertension within a shortened timeframe, when previously the evidence supporting such an approach was limited.

Chapter 8 Appendix

Appendix A: Standard Operating Procedure for measurement of blood pressure (initial and final visit)

1. BACKGROUND:

Blood pressure (BP) may be defined as the force exerted by blood against the walls of the vessel in which it is contained. Differences in blood pressure between different areas of the circulation provide the driving force that keeps blood moving through the body. Blood pressure is usually expressed in terms of millimetres of mercury (mmHg).

The systolic pressure is the maximum pressure of the blood against the wall of the vessel following ventricular contraction. The diastolic pressure is the minimum pressure of the blood against the wall of the vessel during ventricular relaxation and is related to blood vessel resistance.

Blood pressure measures are common physiological parameters. They are used for clinical, diagnostic and research purposes. High blood pressure is a common risk factor for coronary artery disease, stroke and chronic kidney disease. A significant difference between the blood pressures measured simultaneously in each arm has been shown to correlate with cardiovascular risk over and above the absolute blood pressure level, though the reasons for this association remain unclear.

2. SCOPE:

This SOP is specifically for use in the DASHER study (CRF186).

3. PURPOSE:

The purpose of this SOP is to ensure uniformity in the method for measuring bilateral blood pressure in the DASHER study.

4. DEFINITIONS AND ABBREVIATIONS

The headings below contain the definition of terms and meanings of abbreviations used within this document.

BP: Blood pressure

DCF: Data collection form

5. ROLES AND RESPONSIBILITIES:

It is the responsibility of staff undertaking clinical research to read and use this SOP when collecting bilateral BP data for the DASHER study.

Moreover, staff must ensure use of 2 checked Omron blood pressure devices for which the batteries are changed every 3 months.

Staff must use the same type/model of device throughout the study.

6. SKILL LEVEL:

This procedure should only be carried out by personnel who have undergone the appropriate departmental training for measuring blood pressure.

7. EQUIPMENT:

Chair

Two Omron automated BP monitors.

8. PROCEDURE:

In quiet seated area:

1) Check the patient is seated comfortably for 5 minutes before the test. Keep the patient seated with both arms supported. The antecubital fossa should be at the level of the heart (1, 3-6).

   Subjects should be encouraged to relax their arm muscles. They should be asked to remain still and silent during the BP measurement (4) and legs should not be crossed (4). You will advise them when you have completed the measurements.

Use standard cuff size for arms between 22-32cm, large cuff for arms larger than 32cm.

Record mid arm circumference for both arms: record on data collection form (DCF).

2) Remove tight or restrictive clothing from the arm (3-4).

3) Randomise cuff allocation to patients' arms using number table provided.

4) Position the cuffs from the OMRON machines on the upper arm on the left and right side. Cuffs should be placed two to three centimetres above the elbow joint with the indicator mark on the cuff over the brachial artery (MHRA, top 10 hints in measuring blood pressure www.mhra.gov.uk).

5) Carry out three pairs of simultaneous BP measurements: record on DCF. *Indicate machine A or B next to each set of recordings.*

6) Swap cuffs over (do not disconnect cuffs from BP machine) and obtain three further pairs of simultaneous readings: record on DCF.

7) All blood pressures will be recorded in the CRF. The mean of all blood pressures, excluding the first reading taken with each machine on each arm, will also be calculated to determine the arm with the highest blood pressure if this needs to be defined for immediate decision-making (6).

Exclusion Criteria:
Missing/deform arm or markedly visually different (e.g. lymphoedema).
Haemodialysis / dialysis with arterial-venous shunt in forearm.
Previous breast surgery including axillary lymph node clearance.

9. DESIRED OUTCOME:

Three blood pressure measurements recorded from each arm with each machine recorded on the DCF. The first measurement on each arm with each

machine will be discarded and the means of the remaining blood pressures in each arm calculated.

10. REFERENCES:

1. Excellence NIfHaC. Hypertension: Clinical Management of Primary Hypertension in Adults (Update). NICE, 2011.
2. British, H.S., *Blood Pressure Management Fact File.* British Hypertension Society, 2006.
3. O'Brien, E., et al., *European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement.* J Hypertens, 2003. **21**(5): p. 821-48.
4. Pickering, T.G., et al., *Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research.* Circulation, 2005. **111**(5): p. 697-716.
5. Williams, B., et al., *Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV.* J Hum Hypertens, 2004. **18**(3): p. 139-85.
6. UKPDS, *Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group.* BMJ, 1998. **317**: p. 703-713.

Appendix B: Standard Operating Procedure for measurement of blood pressure (follow-up visits)

1. BACKGROUND:

Blood pressure (BP) may be defined as the force exerted by blood against the walls of the vessel in which it is contained. Differences in blood pressure between different areas of the circulation provide the driving force that keeps blood moving through the body. Blood pressure is usually expressed in terms of millimetres of mercury (mmHg).

The systolic pressure is the maximum pressure of the blood against the wall of the vessel following ventricular contraction. The diastolic pressure is the minimum pressure of the blood against the wall of the vessel during ventricular relaxation and is related to blood vessel resistance.

Blood pressure measures are common physiological parameters. They are used for clinical, diagnostic and research purposes. High blood pressure is a common risk factor for coronary artery disease, stroke and chronic kidney disease.

2. SCOPE:

This SOP is specifically for use in the DASHER study (CRF186).

3. PURPOSE:

The purpose of this SOP is to ensure uniformity in the method for measuring blood pressure in the DASHER study. For bilateral blood pressure measurement, the separate SOP for bilateral blood pressure measurement should be used.

4. DEFINITIONS AND ABBREVIATIONS

The headings below contain the definition of terms and meanings of abbreviations used within this document.

BP: Blood pressure

DCF: Data collection form

5. ROLES AND RESPONSIBILITIES:

It is the responsibility of staff undertaking clinical research to read and use this SOP when collecting BP data for the DASHER study,

excluding bilateral blood pressure measurement, for which a distinct SOP is provided.

Moreover, staff must ensure use of a checked Omron blood pressure devices for which the batteries are changed every 3 months.

Staff must use the same type/model of device throughout the study.

6. SKILL LEVEL:

This procedure should only be carried out by personnel who have undergone the appropriate departmental training for measuring blood pressure.

7. EQUIPMENT:

Chair

Omron automated BP monitor.

8. PROCEDURE:

In quiet seated area:

1. Patients will be asked to take their usual medications on the day of each study visit.

2. They will be asked not to consume caffeine or over the counter medicines within 12 hours of their visit

3. Check the patient is seated comfortably for 5 minutes before the test. Keep the patient seated with both arms supported. The antecubital fossa should be at the level of the heart (1, 3-6).

   Subjects should be encouraged to relax their arm muscles. They should be asked to remain still and silent during the BP measurement and legs should not be crossed (4). You will advise them when you have completed the measurements.

Use standard cuff size for arms between 22-32cm, large cuff for arms larger than 32cm.

Record mid arm circumference for both arms: record on data collection form (DCF).

4. Remove tight or restrictive clothing from the arm (3-4).

5. BP and heart rate (HR) will be recorded four times, using the same automated blood pressure device, from the same arm, at each visit following screening. This will be the arm that records the higher average systolic BP upon previous completion of the bilateral BP SOP.

6. Appropriate cuff size will be determined at screening and the same cuff size will be used subsequently for all visits.

7. The mean of all supine blood pressures, excluding the first reading, will be calculated and recorded (6).

8. During all visits, further BP and HR measurements will be performed after standing for 30 seconds, 1 minute and 5 minutes.

9. DESIRED OUTCOME:

Three blood pressure measurements recorded from the same arm will be recorded on the DCF. The first measurement on each arm with each machine will be discarded and the means of the remaining blood pressures in each arm calculated and additionally recorded.

10. REFERENCES:

1. Excellence NIfHaC. Hypertension: Clinical Management of Primary Hypertension in Adults (Update). NICE, 2011.
2. British, H.S., *Blood Pressure Management Fact File.* British Hypertension Society, 2006.

3. O'Brien, E., et al., *European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement.* J Hypertens, 2003. **21**(5): p. 821-48.
4. Pickering, T.G., et al., *Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research.* Circulation, 2005. **111**(5): p. 697-716.
5. Williams, B., et al., *Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV.* J Hum Hypertens, 2004. **18**(3): p. 139-85.
6. UKPDS, *Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group.* BMJ, 1998. **317**: p. 703-713.

Appendix C: Standard Operating Procedure for measurement of ambulatory blood pressure

1. BACKGROUND:

High blood pressure is a major cardiovascular risk factor. Its contribution to cardiovascular risk is even greater in the diabetic population. Consequently target blood pressure is lower for the diabetic population than for the general population. Isolated readings, particularly in the clinical setting, do not provide a reliable guide to an individual's typical blood pressure. Repeated measurements in an ambulatory setting (i.e. the participant's home, place of work etc) provide a much more accurate guide as to the individual's typical blood pressure profile.

The efficacy of dietary nitrate in lowering blood pressure in healthy people has previously been demonstrated.

2. RATIONALE:

Ambulatory blood pressure monitoring will be used to assess whether dietary nitrate has a blood pressure lowering effect in people with Type 2 diabetes and high blood pressure.

3. LOCATION:

The setting up of the ambulatory blood pressure recorder will take place at the participant's home. The cuff and recorder will be fitted by a member of either the nursing or medical team.

4. TIMING OF PROCEDURE:

For the study the participant has vascular testing on two separate occasions. The 24 hour ambulatory blood pressure monitoring will be started the day before these tests.

The monitoring period will finish on the morning of the vascular tests.

5. PERSONAL REQUIRED:

One

6. EQUIPMENT REQUIRED:

An Ambulatory Blood Pressure Monitor (TM-2430, located in Harvey Room, DVRC)

3 x LR5 alkaline batteries

Laptop Computer, with analysis software installed and RS-232C cable (located in Willocks Room, DVRC and clearly labelled).

Personal Safety Equipment Required:

None.

Emergency Equipment Required:

This procedure involves the application of a 24 hour blood pressure monitor. This is a routine procedure that will not induce an emergency situation. However, if the subject should become unwell for any other reason:

- If the procedure is taking place at the participant's home, dial 999 if an ambulance is required.

- If the procedure is taking place at the DVRC, there is a resuscitation trolley located outside Starling Room. Dial 2222 if an ambulance is required.

7. PREPARATION:

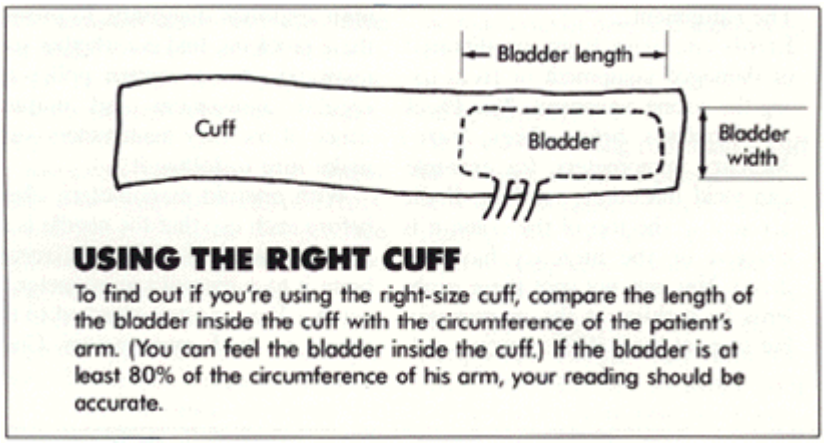
Preparation of the participant:

Explain the procedure and rationale to the participant (detailed below). Advise him/her that they should relax when the cuff begins to inflate, and also minimise noise and movement during the measurement. The maximum measurement time is 90 seconds; one minute after the reading has been taken. Should the participant experience extreme pain during the reading, he/she should stop the procedure by pressing the START STOP key. The recorder will emit a beep, the cuff will deflate and an error code will be displayed. The recorder will then continue with the Automatic Measurement sequence at the next pre-set time interval.

The monitor is only in situ for 24 hours. Advise the participant not to have either a bath or shower for this period as neither the recorder nor cuff are water resistant.

Ensure that the appropriate sized cuff is used. Incorrect cuff size is a major source of equipment related errors. A cuff that is too small will produce a falsely high reading: one that is too large will produce a falsely low reading. To ensure that the correct sized cuff is being used, wrap the cuff circumferentially around the upper arm, the length of the bladder should be 80% of the arm's circumference (see Figure 1).

Figure 1 Using the right cuff



The correct size can also be checked by ensuring that once the cuff is around the arm, the end of the cuff is within the given range displayed on the cuff.

Preparation of the Equipment (to be undertaken prior to the visit to the participant's home):

Ensure that all necessary components are clean and ready for use.

Ensure that the blood pressure recorder has new LR6 alkaline batteries (these need to be changed for each new participant.)

Using the ON/OFF switch (located within the battery chamber of the recorder), turn on the recorder.

If the buzzer sounds once and the current time displayed, the recorder is ready to use.

If the display is flashing, the buzzer sounds four times and the error code (E00) displayed, the parameters for Display and Clock and Automatic Measurement will need setting up. See below.

Delete the old data stored in the recorder by pressing and holding the START/STOP key for approximately 9 seconds. The letters CL will be

displayed. Press and hold the START/STOP key until the beep stops. The time will then be displayed.

Fit the protective cover to the cuff using the Velcro straps.

8. PROCEDURE:

Attach the cuff to the participant's non-dominant arm in the following way:

Locate the brachial artery using palpation. Place the cuff against the skin so that the yellow marker is directly over the brachial artery and space it 2.5cm above the inside of the elbow and the lower edge of the cuff.

Wrap the cuff so that the ring is inside the slide range, is flat and does not slip.

Position the tubing around the back of the neck and over the opposite shoulder. Secure with adhesive tape if required (and appropriate).

Once the cuff is correctly positioned check that the participant is comfortable.

Attach the carrying case to the belt by threading the belt through the slot of the case.

Attach the carrying case to the participant using the belt.

Connect the cuff tubing to the recorder socket and insert the recorder into the carrying case.

Set the Automatic Measurement by pressing and holding the AUTO ON/OFF key. The letter **A** will be displayed in the left hand corner of the screen.

Check the recorder by taking an initial manual measurement (press the START/STOP key). After this manual measurement the machine will automatically start its 24 hr sequence

Blood pressure readings will be taken at the timed intervals previously set by the Research Team. For this study MODE 1 will be selected (see Appendix A).

The participant will attend the DVRC the following day, with the 24 hour blood pressure monitoring equipment still attached.

Stop the Automatic Measurement sequence by pressing and holding the **A** button for approximately 3 seconds. The letter **A** will no longer be displayed.

Remove the cuff and recorder from the participant.

Transfer the participant's data to the laptop computer via the RS-232C terminal/cable and using the analysis software – see below.

Do not switch off the recorder until all the data has been transferred.

Remove batteries and discard/recycle

Clean the cuff, its protective cover and, along with the recorder, return to the storage case. Use warm water and detergent, not alcohol/antiseptic solutions, etc.

8. REFERENCES:

A&D Company Ltd, Draycott Business Centre, Draycott, Moreton-in-Marsh, Gloucestershire GL56 9JY TM-2430 Recorder for Ambulatory Blood Pressure Monitor Instruction Manual

9. Setting the Display and Clock and Automatic Measurement Mode:

Switch on the computer

Connect the recorder to the laptop computer using the RS-232C cable.

Click on the AND TM-2430-13 icon

Click on RECORDER

Click on CLEAR DATA - “This will clear all data in the recorder” (if not done manually on the recorder)

Click on OK
Click on SETUP
Input Recorder ID (the recorder number is written on the outer casing) Check date and time are correct
Recorder Display ON (this is a default setting)

Select Mode:

There are three different modes, allowing a variety of measurement intervals to be used.

Mode I Mode II

Mode III

0700 – 2159 the measurement occurs every 15 minutes 2200 – 0659 the measurement occurs every half hour

The measurement intervals are changed for the overnight period, i.e. during sleep

The measurement interval can be set to change up to 6 times within the 24 hour period.

For the purpose of this study, Mode I will be used.

10. Data Retrieval

Switch on the computer
Connect the recorder to the laptop computer using the RS-232C cable. Click on the AND TM-2430-13 icon

Click on RECORDER
Click on RETRIEVE DATA
Input Participant’s Name

Ensure the box for “Convert Data to CVS File” is ticked (this is a default setting)
Save in: “Dietary Nitrate Folder”
File Name: “Participant’s Initials/Study Number/Date of Visit”
SAVE
Patient Information Screen : Participant’s Name

Comments

- Visit 1 or 2

Click on SAVE
Click on FILE (at top of Toolbar)
Click on OPEN
Click on participant’s file
Click on VIEW (at top of Toolbar)
Click on “Summary Data”
This will show the data retrieved from the recorder. Click on “Print” (at left of screen) if printout required Click on CLOSE

Appendix D: Standard Operating Procedure for measurement of height

1. BACKGROUND:

Height measurement can be used in the assessment of body composition, and is essential in determining individuals' Body Mass Index (BMI). It can be affected by posture, foot wear, and the positioning of head and feet.

2. SCOPE:

This SOP applies generically to clinical trials and research projects, clinical and health research in Exeter, unless a trial agreement specifically indicates that another organisation's SOP should be used.

3. PURPOSE:

To ensure the correct and uniform measurement of the height of research participants

4. DEFINITIONS AND ABBREVIATIONS:

SOP
BMI Stadiometer Frankfurt Plane

Standard Operating Procedures.
Body Mass Index
Height measuring device (portable or fixed)
An imaginary, horizontal line between the external ear canal and the lower border of the orbit of the eye, achieved when the chin is lowered.

5. ROLES AND RESPONSIBILITIES:

It is the responsibility of staff undertaking clinical research, to read and use this SOP when measuring height.

6. SKILL LEVEL:

This procedure should only be carried out by personnel who have undergone the appropriate departmental training.

7. EQUIPMENT:

Portable Harpenden pocket stadiometer, or study-specific height measuring device.

8. PROCEDURE:

The measurement is performed in the same way for both males and females.

Three readings should be taken for each individual (or as needed according to study requirements).

1. Explain procedure to the patient/volunteer.
2. Ask the patient/volunteer to remove their shoes and stand on the base plate of the equipment, with their back to the height scale.
3. The patient/volunteer should stand as tall and straight as possible with feet together, arms held loosely at the side, shoulders relaxed and chin lowered.
4. Adjust the scale so that the head plate rests on the patient's/volunteer's head. The head should be placed in the Frankfurt Plane, so that an imaginary line joining the upper margin of the external auditory meatus and the lower border of the orbit of the eye is horizontal.

5. Measurers should aim to read the scale from as level a position as possible. If a short person measures a tall person, or vice versa, considerable error can be introduced.
6. Measure to the nearest 0.1cm.
7. Record the measurements on the appropriate data collection sheet.

9. DESIRED OUTCOME:

To accurately measure and record the participant's height.

10. REFERENCES:

1. National Health and Nutrition Examination Survey (NHANES) Anthropometry Procedure Manual 2007.
http://www.cdc.gov/nchs/data/nhanes_07_08/manual_an.pdf

Appendix E: Standard Operating Procedure for measurement of body composition

1. BACKGROUND:

The Tanita Body Composition Analyzer (BCA) uses Bioelectrical Impedance Analysis (BIA) to help calculate results. BIA helps to calculate body composition by sending a low, safe electrical signal through the body. The signal passes freely through fluids contained in lean tissue, such as muscle and blood, but meets resistance passing through fat tissue. The monitor accurately measures this resistance and uses it to calculate elements of body composition.

When set against gender, height, weight and body type, the monitors can calculate body fat percentage.

2. SCOPE:

This SOP applies generically to clinical trials and research projects, clinical and health research in Exeter, unless a trial agreement specifically indicates that another organisations' SOP should be used.

3. PURPOSE:

Using the Tanita BCA helps to determine and record accurate measurements of the body.

4. DEFINITIONS AND ABBREVIATIONS:

The headings below contain the definition of terms and meanings of abbreviations used within this document.

Body Composition Analyzers (BCA)

Bioelectrical Impedance Analysis (BIA)

5. ROLES AND RESPONSIBILITIES:

This SOP applies to all the individuals taking Body composition measurements using the Tanita BCA in projects undertaken by the Clinical Research Facility.

6. SKILL LEVEL:

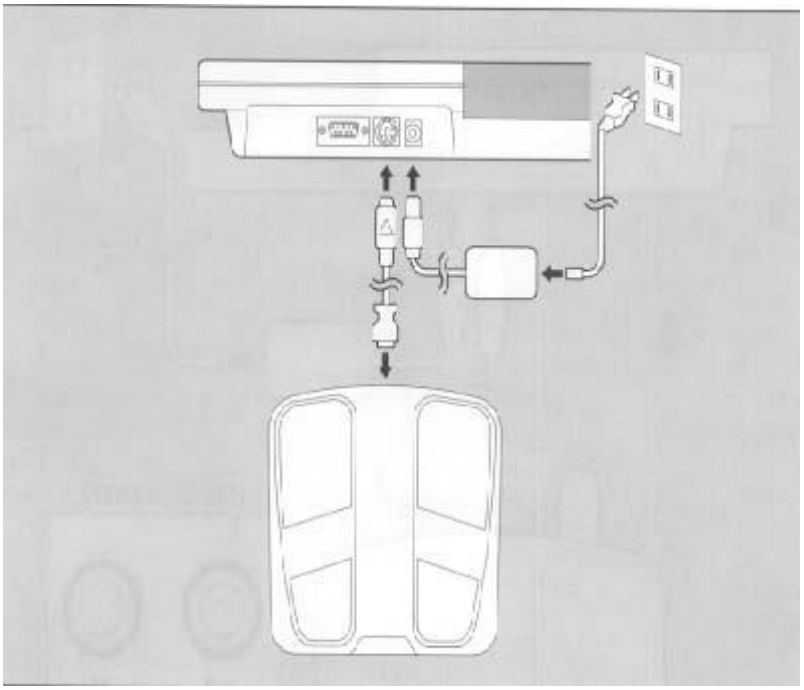
This procedure should only be carried out by personnel who have undergone the appropriate departmental training.

7. EQUIPMENT:

The Tanita BCA (BC-418MA) comprises of a weighing scale, power cable, AC Adapter, and Control Box.

8. PROCEDURE:

Set up- Connecting the weighing platform to the control box:



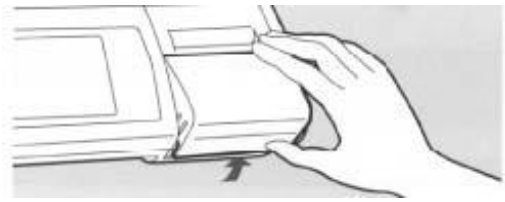
1. Connect the circular shaped plug of the connection cable to the jack located on the back of the control box. The ▲ on the plug should be facing up when inserted.
2. Connect the rectangular plug of the connection cable to the jack located on weighing platform.
3. Connect the plug of AC adapter to the DC jack located on the back of the control box.
4. Insert the power cord to the AC adapter, and plug it into the power outlet.

Loading the printer paper:

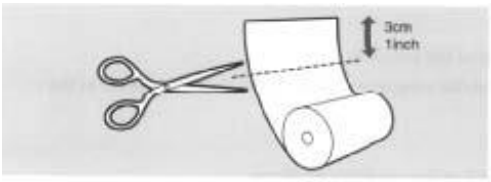
1. When there is no printer paper in the feeder. “P-End” will appear on the LCD:

2. If you don’t want to use printer paper, press the [CE] key to continue measurement with no printer paper. When there is no “P-End” message, but the printer fails to print, the chosen number of prints outs may be “0”. Select a number of print out greater than”0”.

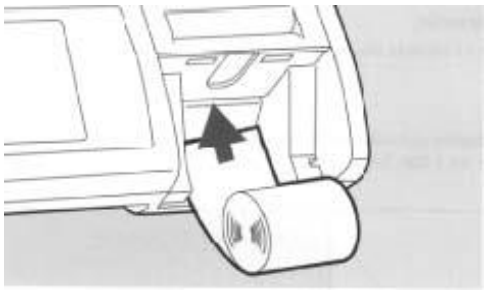
3. Remove the paper dispenser cover by lifting it up from the back:



4. In a straight line, cut approximately 1 inch (3cm) off the paper roll as this will ensure smooth feeding:



5. Insert the printer paper in the holder as displayed. Be sure to feed the printer paper straight into the automatic feeder. As the front edge of the printer paper enters the appropriate slot, it will automatically feed. Once the printer paper feeds, it will exit the printer paper feed slot located on the printer cover, and be cut. Remove printer paper from the printer cover.



6. Replace the paper dispenser cover as displayed:

Operating Instructions:

Tanita's BCA sends a weak electric signal through the body; **it is advised that the individuals who are pregnant or who have a pacemaker or other internal electric medical devices should not use this product,** as the weak electric signal may cause such internal devices to malfunction.

Do not step on the weighing platform until all data has been entered.

1. Press the ON/OFF key to turn on the power. The ◄ mark and "0.0" will appear on the LCD. If measuring units need to be changed, do so at this time by pressing the [kg/lb] key. An arrow on the LCD will follow the selection of weighing units. Throughout data entry mistakes may be corrected by pressing the [CE]. Follow the flashing arrow on the LCD for proper sequence.

2. Enter clothes weight which will subtract the chosen amount of clothes weight. Clothes weight can be entered by 0.1kg /0.2lb increments.

   e.g: 2.0kg- Press the [2][.][0] keys
   4.0lb – Press the [4][.][0] keys
   For e.g: Scrubs and cardigan up to 0.9kgs

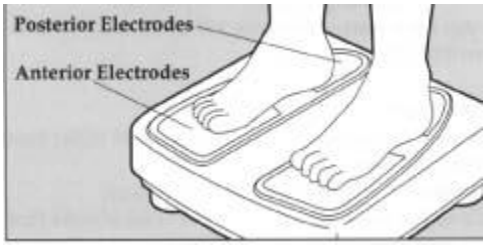
   Heavy clothing like Jeans and Jumpers up to 2.0kgs

3. Select Gender and 'Standard' for Body Type.

4. Enter age. After the age is entered the arrow will automatically flash at the Height on the LCD.

5. Enter the height in cms. The arrow will now flash at STEP ON.

6. Step on the weighing scales with bare feet. Poor contact between the feet and electrodes may produce an error message. Heels should be placed directly on top of the posterior electrodes, while the front part of the foot needs to be in contact with the anterior electrodes. See below. It is also important to make sure the soles of the feet are free of excess dirt, as this may act as a barrier to the mild current.



7. After the weight appears on the upper portion of the LCD, grasp and hold the handsets with arms relaxed by sides. The impedance measurement will now be taken. This is denoted by four "bubbles" *0 0 0 0* which appear on the bottom half of the LCD. As the measurement is being taken, the bubbles will disappear one by one and a beep will sound ***five times***. **Do not step off the weighing platform until the fifth beep, and the final bubble has disappeared, and the body fat percentage appears on the LCD.**

8. Measurement is now complete and detailed results will automatically print out. The results would include Body Mass Index (BMI), Basal Metabolic Rate (BMR), Impedance, Fat %, Fat Mass, Fat Free Mass (FFM), and Total Body Water (TBW) etc.

9. If all the measuring is complete, Press the [ON/OFF] key to turn off the power.

10. Please clean the weighing platform with appropriate mild disinfectant wipes after each use. Do not use strong chemicals to clean the platform.

Maintenance:

To reduce the risk of fire hazards or equipment damage, use only the original AC adapter provided by TANITA. To reduce the risk of electric shock or product damage, never insert or remove the power cord with wet hands. To reduce the risk of injury or equipment malfunction, always step on the weighing platform slowly. Unplug the unit from the wall outlet when it is not in use for longer periods. Always turn the equipment off before unplugging from a wall outlet.

Avoid using the equipment with constant vibration.

9. DESIRED OUTCOME:

The correct and safe technique in the use of the equipment, ensuring accurate data collection.

10. REFERENCES:

Tanita Body Composition Analyser (CC-418MA) Instruction Manual.

Appendix F: Standard Operating Procedure for measurement of Aldosterone:Renin ratio

1. BACKGROUND:

Participants enrolled in the DASHER study (CRF186) have a diagnosis of new severe hypertension. The study aims to determine whether a new protocol can be used successfully to treat these participants and achieve target blood pressure (<140/90mmHg) within 18 weeks.

As part of the project, participants undergo a full evaluation for secondary causes of hypertension, as these require specific further investigation and treatment. Missed diagnosis of secondary hypertension could lead to participants not being able to achieve the consensus guideline blood pressure target (the primary endpoint of the study), placing them at higher risk of cardiovascular complications in the future(Lewington et al., 2002).

Primary aldosteronism (Conn's syndrome) is one such secondary cause of hypertension. This syndrome is caused by overactive adrenal cortical tissue, producing inappropriately high plasma levels of the steroid hormone, aldosterone. This acts on the kidneys to increase sodium reabsorption and increase potassium excretion. Hence, hypernatraemia and hypokalaemia are typical hallmarks (but not ubiquitous features(Gordon, 1995)) of the disease. Enhanced sodium (and consequently water) retention by the kidney results in raised blood pressure.

Overactive adrenal cortex tissue can take the form of functional adrenal adenomas, which can often be visualised through non-invasive imaging such as MRI. However, not all adenomas detected on imaging produce aldosterone (i.e. they are non-functional) and 50% of Conn's syndromes are in fact caused by adrenal hyperplasia rather than an adenoma(Rossi et al., 2006). Thus, the ratio of plasma aldosterone to renin concentration (the aldosterone-renin ratio) provides the cornerstone of Conn's syndrome diagnosis.

Previous studies have shown that the aldosterone-renin assay is sensitive to patient posture, with measurement after 30 minutes in the sitting position

providing an acceptable and valid alternative to blood sampling following 4 hours' recumbency and an intravenous saline challenge(Tiu et al., 2005). This procedure has therefore been adopted by subsequent studies by cognoscenti incorporating aldosterone-renin ratio as an outcome(Hood et al., 2005).

Present consensus guidelines recommend screening for secondary hypertension causes in younger patients and those resistant to treatment, particularly those presenting with severe hypertension(Mancia et al., 2013, Excellence, 2011). Therefore, we propose measurement of aldosterone-renin ratio in all patients enrolled who are under the age of 45 years, and in those aged over 45 years in whom blood pressure targets are not met after the initiation of a third antihypertensive agent (visit 5, week 6). As plasma renin and aldosterone levels can be affected by antihypertensive agents(Mackenzie and Brown, 2009), samples should be taken on visit 2 (before the initiation of antihypertensive medication) and stored for further analysis at visit 5, regardless of patient age. Additionally, if under the age of 45 years, a second sample should be taken to the biochemistry lab immediately after being acquired on visit 2, as significant degredation in plasma renin concentration has been reported in samples not stored at 0-5°C within 30 minutes of blood sampling(Locsei et al., 2009).

2. SCOPE:

This SOP is specifically for use in the DASHER study.

3. PURPOSE:

The purpose of this SOP is to standardise the measurement of aldosterone-renin ratios within the DASHER project (CRF186).

4. ROLES AND RESPONSIBILITIES:

It is the responsibility of staff undertaking clinical research to read and use this SOP when measuring aldosterone-renin ratio within the DASHER study.

5. SKILL LEVEL:

This procedure should only be carried out by personnel who have undergone the appropriate NHS trust and departmental training in venepuncture and/or cannulation, sample spinning and storage. Personnel should read all relevant SOPs and adhere to the NHS trust infection control policy.

6. EQUIPMENT:

Disposable gloves
Arterial cannula or safetymultifly needle or safety-needle for s-monovette.
Multi-adaptor for s-monovette system.
One large (7.5ml) EDTA tube, with additional large EDTA tube if participant is over 45 years old.

7. PROCEDURE:

The procedure assumes that venous access has already been acquired.

In a clinically appropriate area for blood sampling:

1. Ensure the participant has been seated for 30 minutes prior to blood sampling and remains seated during the procedure. This may therefore be best performed after the informed consent process within the DASHER visit 2 protocol.

2. Withdraw blood into one large (7.5ml) Red Sarstedt Monovette EDTA blood tube; withdraw into an additional tube if the participant is <45 years old.

3. If blood samples for other investigations are taken at the same point in time, adhere to the DASHER spinning protocol for blood draw order.

4. If arterial cannula is in-situ, flush with 3-5ml 0.9% sodium chloride following blood sampling

5. The sample should be kept at 18°C and centrifuged within 30 minutes at 1500g for 15 minutes whilst still at 18°C. Following centrifugation, the sample should be immediately frozen to -20°C and transferred to -80°C within one week.

   If the participant is aged under 45 years, an additional large (7.5ml) Red Sarstedt Monovette EDTA tube should be taken (as above) and personally delivered to the biochemistry laboratory within 30 minutes (*not frozen and not centrifuged*). Samples must be transferred quickly due to degradation of renin at room temperature(Locsei et al., 2009). The biochemistry laboratory should be called prior to delivering the sample as a courtesy and to ensure that the sample can be processed urgently.

6. The frozen EDTA sample can be analysed at a later date, for example if a subject over 45 years old has not achieved target blood pressure at visit 5.

9. DESIRED OUTCOME:

Standardised acquisition of aldosterone-renin ratios during the DASHER project to facilitate diagnosis of secondary hypertensive causes and inter-participant comparison.

9. REFERENCES:

1. Lewington S, Clarke R, Qizilbash N, et al. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002;**360**(9349):1903-13.
2. Gordon RD. Primary aldosteronism. J Endocrinol Invest 1995;**18**(7):495-511.
3. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. Journal of the American College of Cardiology 2006;**48**(11):2293-300.
4. Tiu SC, Choi CH, Shek CC, et al. The use of aldosterone-renin ratio as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. J Clin Endocrinol Metab 2005;**90**(1):72-8.
5. Hood S, Cannon J, Foo R, et al. Prevalence of primary hyperaldosteronism assessed by aldosterone/renin ratio and spironolactone testing. Clin Med 2005;**5**(1):55-60.
6. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2013;**34**(28):2159-219.
7. Excellence NIfHaC. Hypertension: Clinical Management of Primary Hypertension in Adults (Update). NICE, 2011.
8. Mackenzie IS, Brown MJ. Molecular and clinical investigations in patients with low-renin hypertension. Clin Exp Nephrol 2009;**13**(1):1-8.
9. Locsei Z, Racz K, Patocs A, et al. Influence of sampling and storage conditions on plasma renin activity and plasma renin concentration. Clin Chim Acta 2009;**402**(1-2):203-5.

Appendix G: Data Collection Form for Determining Epworth Score

Name: _____

Date: _____

Your age: (Yr) _____ **Your sex**: ❑ Male ❑ Female

How likely are you to doze off or fall asleep in the situations described below, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the most appropriate number for each situation:-

0 = would never doze
1 = Slight chance of dozing
2 = Moderate chance of dozing
3 = High chance of dozing

Situation	**Chance of dozing**
Sitting and reading	
Watching TV	
Sitting, inactive in a public place (e.g. a theatre or a meeting)	
As a passenger in a car for an hour without a break	
Lying down to rest in the afternoon when circumstances permit	
Sitting and talking to someone	
Sitting quietly after a lunch without alcohol	
In a car, while stopped for a few minutes in the traffic	
Total	

Score:	
0-10	Normal range
10-12	Borderline
12-24	Abnormal

Appendix H: Standard Operating Procedure for feature tracking analysis of cardiac MR data

1. BACKGROUND:

Strain is a measurement of myocardial deformation during contraction and relaxation. It can be used to precisely quantify regional and overall heart function which, with a high degree of precision, can identify heart disease before this is apparent with other techniques.

As such, measurement of strain is becoming incorporated into clinical practice and has become a key outcome measure in cardiac research. Over 6000 articles in Medline-searchable journals have been published pertaining to strain imaging over the past 10 years, including in high impact journals such as *Circulation, JACC* and the *European Heart Journal*, with the publication rate still increasing.

The pioneering technique for measuring strain was “speckle tracking”, which uses ultrasound (echocardiography) to follow the movement of individual pixels produced in an image throughout a single heartbeat. The directions and velocities of a number of pixels are used to calculate the exact motion of each part of the heart in three dimensions.

For example, strain has been shown to be reduced in the radial and longitudinal directions relative to the cardiac axis in hypertensive subjects(Galderisi et al., 2012) whereas twisting of the heart during contraction appears to increase in amplitude as an early compensatory mechanism in hypertensive heart disease(Celic et al., 2014). These functional changes are less pronounced in treated hypertensive patients compared with an untreated hypertensive population(Celic et al., 2014). Longitudinal strain has been shown to improve following 24 weeks’ antihypertensive treatment(Cheng et al., 2014), though changes in other myocardial deformation parameters before and after treatment in the same study group have not been described.

Cardiac Magnetic Resonance (CMR) is a safe non-invasive technique which facilitates serial assessment of myocardial structure and function with high sensitivity and reproducibility. Building on echocardiographic speckle tracking, CMR techniques have been developed to measure myocardial strain in an analogous manner. Termed “feature tracking”, this post-processing (offline) method has been shown to have greater reproducibility than older techniques, such as myocardial tagging, for determining strain values(Singh et al., 2015).

MR imaging is presently undertaken at the Exeter NIHR Clinical Research Facility at the Exeter Magnetic Resonance Research Centre, St Luke’s Campus, University of Exeter. Imaging is performed at 1.5T (Magnetom Avanto, Siemens Healthcare Sector, Erlangen, Germany) using a 32-channel coil. Standard acquisition, such as in the DASHER study (CRF186), includes 4-chamber, 2-chamber, 3-chamber, outflow and short axis stack sequences using stead-state free precession cine imaging. However, it is possible to perform feature tracking analysis from any basic CMR study image set, including those acquired at 3T.

For feature tracking analysis, the University of Exeter has purchased a software package from TomTec Imaging Systems: Image Arena 4.6. This is installed on a dedicated platform in St Luke’s which allows viewing of the images at sufficient resolution to perform the analysis.

2. SCOPE:

This SOP is specifically for use in the DASHER study and any future studies using feature tracking analysis in the NIHR Exeter Clinical Research Facility.

3. PURPOSE:

The purpose of this SOP is to standardise the measurement of myocardial strain by feature tracking at the Exeter NIHR Clinical Research Facility.

4. ROLES AND RESPONSIBILITIES:

It is the responsibility of staff undertaking clinical research to read and use this SOP when determining myocardial strain using feature tracking in the Exeter NIHR clinical Research Facility.

5. SKILL LEVEL:

This procedure should only be carried out by personnel who have sufficient understanding of myocardial anatomy as viewed using CMR and are able to identify and interpret basic CMR image sets.

6. EQUIPMENT:

Basic set of SSFP cine images to include 4-chamber and short axis stack in DICOM format.

Access to TomTec Image Arena 4.6 software on the appropriate platform.

7. PROCEDURE:

The procedure assumes that adequate CMR images for the analysis have already been acquired.

Basic analysis, to include left ventricular ejection fraction, volumes and mass, should be performed using a separate software package (such as the Philips software installed at St Luke's). It is important to remember that ejection fraction and ventricular mass data provided by the TomTec software is calculated by fractional area change of single sequences rather than the entire short axis stack. These should therefore not be considered to be accurate and as such should not be included in any quantitative reporting.

7. Transfer images for analysis as single DICOM files to the dedicated computer at St Luke's campus.

NB. If DICOM files are grouped, the TomTec software may mis-handle different slices into the same short axis sequence thereby preventing appropriate tracking and analysis.

8. Log into computer as emrrc (password = emrrc).

9. Open TomTec “Image Arena” software (shortcut on desktop; username = tomtec, password = tomtec).

10. Select “Import” tab.

11. Select browse at top of the screen then import desired sequences by selecting one of the DICOM files within a study and click “open”.

12. Return to the “Studies List” tab. Left click on the imported study to select. To rename, right click on the study name and edit.

13. To start feature tracking analysis, right click on one of the images in the preview pane and select “CPA-MR”.

14. Select the appropriate CMR sequence for the desired parameter – double click on the sequence in the list.

    - 4-chamber view:
        - LV longitudinal strain (ELLLV).
        - RV longitudinal strain (ELLRV).
        - LV long axis radial (transverse) strain (ERRLAX).
    - Apical short axis:
        - Determine the most apical plane still showing the ventricular cavity in systole, then select the adjacent cine in the mid-ventricle direction for analysis as this has greater reproducibility(Kowallick et al., 2014).
        - Apical LV rotation (figure located in displacement analysis – in degrees).
        - Apical short axis circumferential strain (ECCSAX).

- Basal short axis:
    - Determine the most basal plane still showing a complete circumference of myocardium during the entire cardiac cycle, then select the adjacent cine in the mid-ventricle direction for analysis(Kowallick et al., 2014).
    - Basal LV rotation (figure located in displacement analysis – in degrees).
    - Basal short axis circumferential strain (ECCSAX).
- Mid-ventricle short axis:
    - Select a view showing both papillary muscles.
    - Short axis circumferential strain (ECCSAX).
    - Short axis radial strain (ERRSAX).

15. Select the corresponding view diagram for analysis in the top right of screen.

16. Check the box for 17-segment model analysis is selected; the standard model recommended for cardiac imaging analysis(Cerqueira et al., 2002).

17. Select the end-diastolic frame using the arrows.

18. Use left mouse clicks to trace around the endocardial border, with at least one marker for every myocardial segment. To complete the tracing, the final click is a right mouse click.

19. Select “start analysis” (to right of screen; coloured lines in box).

20. Check (in viewing window located in top left corner of screen) that the tracings track the myocardium precisely. If not, discard the tracking and repeat from step 10.

21. Review regional analysis: select “time-to-peak” analysis to view velocity, displacement, strain and strain rate. De-select segments with regional wall motion abnormalities.

22. Exit regional analysis and return to edit page: “edit trace”.

23. Select option for “endo+epi”; add a tracing for the epicardial border as for the endocardium above, reviewing the results to ensure that tracking is accurate.

24. If confident that tracking has been satisfactory, select “time-to-peak analysis” option.

25. In the analysis screen, select “strain” (or “displacement” for rotation values).

26. De-select segments with regional wall motion abnormalities.

27. Segmental and mean values are given on the left side of the analysis screen, with transverse values at the top of the pane and longitudinal values at the bottom. We use peak (systolic) values for reporting by convention.

    a. Record endocardial and epicardial strain separately by choosing each option in the “history” section on the right of the screen. Average strain should then be calculated manually and recorded.

28. When documentation is completed for all desired variables, save the analysis by exporting as text using the option in the upper left corner.

With the present version of the software, twist parameters must be calculated manually using previously verified methods(Kowallick et al., 2015):

Twist = apical anticlockwise rotation (φ_{apex}) – basal clockwise rotation (φ_{base})

Apical rotation is, by definition, positive. Net rotation defines twist.

Twist normalised to LV length ("normalised twist") is also referred to as "torsion" in the literature.

Using D as the distance between the apical and basal imaging planes (as calculated from adding together the total interslice gaps ad slice thicknesses):

$$\text{Torsion} = (\varphi_{apex} - \varphi_{base}) / D$$

Circumferential shear angle can also be calculated manually (though not in DASHER), using the apical radius (ρ_{apex}) and basal radius (ρ_{basal}), which are quantified using the area (endocardial or epicardial) within the tracked contour at each time frame.

$$\text{Circumferential shear angle} = [(\varphi_{apex} - \varphi_{base}).(\rho_{apex} + \rho_{base})] / 2D$$

9. DESIRED OUTCOME:

Standardised analysis of MR images to calculate myocardial strain parameters using feature tracking, increasing inter-study reproducibility to an acceptable level, as seen in previous publications (Kowallick JMRI 2015).

9. REFERENCES:

1. Galderisi M, Esposito R, Schiano-Lomoriello V, Santoro A, Ippolito R, Schiattarella P, et al. Correlates of global area strain in native hypertensive patients: a three-dimensional speckle-tracking echocardiography study. European heart journal cardiovascular Imaging. 2012;13(9):730-8.

2. Celic V, Tadic M, Suzic-Lazic J, Andric A, Majstorovic A, Ivanovic B, et al. Two- and three-dimensional speckle tracking analysis of the relation

between myocardial deformation and functional capacity in patients with systemic hypertension. Am J Cardiol. 2014;113(5):832-9.
3. Cheng S, Shah AM, Albisu JP, Desai AS, Hilkert RJ, Izzo J, et al. Reversibility of left ventricular mechanical dysfunction in patients with hypertensive heart disease. J Hypertens. 2014;32(12):2479-86; discussion 86-7.
4. Singh A, Steadman CD, Khan JN, Horsfield MA, Bekele S, Nazir SA, et al. Intertechnique agreement and interstudy reproducibility of strain and diastolic strain rate at 1.5 and 3 Tesla: a comparison of feature-tracking and tagging in patients with aortic stenosis. J Magn Reson Imaging. 2015;41(4):1129-37.
5. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. Circulation. 2002;105(4):539-42.
6. Kowallick JT, Lamata P, Hussain ST, Kutty S, Steinmetz M, Sohns JM, et al. Quantification of left ventricular torsion and diastolic recoil using cardiovascular magnetic resonance myocardial feature tracking. PLoS One. 2014;9(10):e109164.
7. Kowallick JT, Morton G, Lamata P, Jogiya R, Kutty S, Lotz J, et al. Inter-study reproducibility of left ventricular torsion and torsion rate quantification using MR myocardial feature tracking. J Magn Reson Imaging. 2015.

Abstracts and Publications

SHORE, A., SHARP, A., ANNING, C., PAMPHILON, N., BALL, C., BELLENGER, N., JORDAN, A.: Rapid treatment of grade 2-3 hypertension in previously untreated patients is accompanied by an increase in total and perfused skin capillary density.
British and Irish Hypertension Society Annual Scientific Meeting 2018
World Society for Microcirculation, Vancouver 2018

JORDAN, A. N., ANNING, C., BALL, C., PAMPHILON, N., CLARK, C. E., BELLENGER, N. G., SHORE, A. C. & SHARP, A. S. P.: Rapid treatment of moderate-to-severe hypertension using a novel nurse-directed protocol is safe and effective.
British and Irish Hypertension Society Annual Scientific Meeting 2018

JORDAN, A. N., ANNING, C., BALL., C., PAMPHILON, N., FULFORD, J., CLARK, C. E., SHORE, A. C., SHARP, A. S. P., BELLENGER, N.G.: Left ventricular hypertrophy in hypertensive heart disease: early myocardial response to treatment.
British and Irish Hypertension Society Annual Scientific Meeting 2018

JORDAN, A. N., ANNING, C., BALL, C., WILKES, L., PAMPHILON, N., BELLENGER, N. G., CLARK, C. E., SHORE, A. C., SHARP, A.: Rapid treatment of newly-diagnosed moderate-severe hypertension is safe and effective.
European Society of Hypertension 2017

(PAPER)

JORDAN, A. N., ANNING, C., WILKES, L., BALL, C., PAMPHILON, N., CLARK, C. E., BELLENGER, N. G., SHORE, A. C. & SHARP, A. S. P. 2020. Rapid treatment of moderate to severe hypertension using a novel protocol in a single-centre, before and after interventional study. *J Hum Hypertens* 34**,** 165-175.

(PAPER)

JORDAN, A. N., FULFORD, J., GOODING, K., ANNING, C., WILKES, L., BALL, C., PAMPHILON, N., CLARK, C. E., SHORE, A. C., SHARP, A. S. P., BELLENGER, N. G. 2021. Morphological and functional cardiac consequences of rapid hypertension treatment: a cohort study. *J Cardiovasc Magn Reson.* 23(1):122.

(PAPER)

JORDAN, A. N., ANNING, C., WILKES, L., BALL, C., PAMPHILON, N., CLARK, C. E., BELLENGER, N. G., SHORE, A. C., SHARP, A. S. P., VALDERAS, J. M. 2022. Cross-cultural adaptation of the Spanish MINICHAL instrument into English for use in the United Kingdom. *Health Quality Life Outcomes 20(1):39.*

References

AALKJAER, C., HEAGERTY, A. M., BAILEY, I., MULVANY, M. J. & SWALES, J. D. 1987a. Studies of isolated resistance vessels from offspring of essential hypertensive patients. *Hypertension,* 9**,** 155-8.

AALKJAER, C., HEAGERTY, A. M., PETERSEN, K. K., SWALES, J. D. & MULVANY, M. J. 1987b. Evidence for increased media thickness, increased neuronal amine uptake, and depressed excitation--contraction coupling in isolated resistance vessels from essential hypertensives. *Circ Res,* 61**,** 181-6.

ADAIR, J. G. 1984. The Hawthorne effect: A reconsideration of the methodological artifact. *Journal of applied psychology,* 69**,** 334.

AHMED, M. I., DESAI, R. V., GADDAM, K. K., VENKATESH, B. A., AGARWAL, S., INUSAH, S., LLOYD, S. G., DENNEY, T. S., JR., CALHOUN, D., DELL'ITALIA, L. J. & GUPTA, H. 2012. Relation of torsion and myocardial strains to LV ejection fraction in hypertension. *JACC Cardiovasc Imaging,* 5**,** 273-81.

ALAM, M., ZHANG, L., STAMPEHL, M., LAKKIS, N. & DOKAINISH, H. 2013. Usefulness of speckle tracking echocardiography in hypertensive crisis and the effect of medical treatment. *Am J Cardiol,* 112**,** 260-5.

ALSHARARI, R., LIP, G. Y. H. & SHANTSILA, A. 2020. Assessment of Arterial Stiffness in Patients With Resistant Hypertension: Additional Insights Into the Pathophysiology of This Condition? *Am J Hypertens,* 33**,** 107-115.

ANDERSSON, O. K., SANNERSTEDT, R., BECKMAN, M., DE LEEUW, P. & SIVERTSSON, R. 1983. Essential hypertension--implications for pathogenesis from repeated haemodynamic investigations in young men with elevated blood pressure. *J Hypertens Suppl,* 1**,** 91-3.

ANTONIOS, T. F., RAGHURAMAN, R. P., D'SOUZA, R., NATHAN, P., WANG, D. & MANYONDA, I. T. 2012. Capillary remodeling in infants born to hypertensive pregnancy: pilot study. *Am J Hypertens,* 25**,** 848-53.

ANTONIOS, T. F., RATTRAY, F. E., SINGER, D. R., MARKANDU, N. D., MORTIMER, P. S. & MACGREGOR, G. A. 1999a. Maximization of skin capillaries during intravital video-microscopy in essential hypertension:

comparison between venous congestion, reactive hyperaemia and core heat load tests. *Clin Sci (Lond),* 97**,** 523-8.

ANTONIOS, T. F., RATTRAY, F. M., SINGER, D. R., MARKANDU, N. D., MORTIMER, P. S. & MACGREGOR, G. A. 2003. Rarefaction of skin capillaries in normotensive offspring of individuals with essential hypertension. *Heart,* 89**,** 175-8.

ANTONIOS, T. F., SINGER, D. R., MARKANDU, N. D., MORTIMER, P. S. & MACGREGOR, G. A. 1999b. Rarefaction of skin capillaries in borderline essential hypertension suggests an early structural abnormality. *Hypertension,* 34**,** 655-8.

ANTONIOS, T. F., SINGER, D. R., MARKANDU, N. D., MORTIMER, P. S. & MACGREGOR, G. A. 1999c. Structural skin capillary rarefaction in essential hypertension. *Hypertension,* 33**,** 998-1001.

APPEL, L. J., CHAMPAGNE, C. M., HARSHA, D. W., COOPER, L. S., OBARZANEK, E., ELMER, P. J., STEVENS, V. J., VOLLMER, W. M., LIN, P. H., SVETKEY, L. P., STEDMAN, S. W. & YOUNG, D. R. 2003. Effects of comprehensive lifestyle modification on blood pressure control: main results of the PREMIER clinical trial. *Jama,* 289**,** 2083-93.

APPEL, L. J., MOORE, T. J., OBARZANEK, E., VOLLMER, W. M., SVETKEY, L. P., SACKS, F. M., BRAY, G. A., VOGT, T. M., CUTLER, J. A., WINDHAUSER, M. M., LIN, P. H. & KARANJA, N. 1997. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med,* 336**,** 1117-24.

APPLEGATE, W. B., PRESSEL, S., WITTES, J., LUHR, J., SHEKELLE, R. B., CAMEL, G. H., GREENLICK, M. R., HADLEY, E., MOYE, L., PERRY, H. M., JR. & ET AL. 1994. Impact of the treatment of isolated systolic hypertension on behavioral variables. Results from the systolic hypertension in the elderly program. *Arch Intern Med,* 154**,** 2154-60.

ARBUTHNOTT, K. & FRANK, J. 2000. Trail making test, part B as a measure of executive control: validation using a set-switching paradigm. *J Clin Exp Neuropsychol,* 22**,** 518-28.

ASAHARA, T., MUROHARA, T., SULLIVAN, A., SILVER, M., VAN DER ZEE, R., LI, T., WITZENBICHLER, B., SCHATTEMAN, G. & ISNER, J. M. 1997. Isolation of putative progenitor endothelial cells for angiogenesis. *Science,* 275**,** 964-7.

ASAHARA, T., TAKAHASHI, T., MASUDA, H., KALKA, C., CHEN, D., IWAGURO, H., INAI, Y., SILVER, M. & ISNER, J. M. 1999. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J,* 18**,** 3964-72.

BADIA, X., ROCA-CUSACHS, A., DALFO, A., GASCON, G., ABELLAN, J., LAHOZ, R., VARELA, C., VELASCO, O. & GROUP, M. 2002. Validation of the short form of the Spanish Hypertension Quality of Life Questionnaire (MINICHAL). *Clin Ther,* 24**,** 2137-54.

BAMFI, F., DE CARLI, G., ARPINELLI, F., RECCHIA, G., IRVINE, S. H. & APOLONE, G. 1998. Development of a new specific questionnaire for hypertensive patients. *Journal of Hypertension,* Supplement 16(9).

BANSCH, P., FLISBERG, P. & BENTZER, P. 2014. Changes in the sublingual microcirculation during major abdominal surgery and post-operative morbidity. *Acta anaesthesiologica Scandinavica,* 58**,** 89-97.

BARBARO, N. R., FONTANA, V., MODOLO, R., DE FARIA, A. P., SABBATINI, A. R., FONSECA, F. H., ANHE, G. F. & MORENO, H. 2015. Increased arterial stiffness in resistant hypertension is associated with inflammatory biomarkers. *Blood Press,* 24**,** 7-13.

BARDAGE, C. & ISACSON, D. G. 2001. Hypertension and health-related quality of life. an epidemiological study in Sweden. *J Clin Epidemiol,* 54**,** 172-81.

BECKETT, N. S., PETERS, R., FLETCHER, A. E., STAESSEN, J. A., LIU, L., DUMITRASCU, D., STOYANOVSKY, V., ANTIKAINEN, R. L., NIKITIN, Y., ANDERSON, C., BELHANI, A., FORETTE, F., RAJKUMAR, C., THIJS, L., BANYA, W. & BULPITT, C. J. 2008. Treatment of hypertension in patients 80 years of age or older. *N Engl J Med.,* 358**,** 1887-98.

BELLO, C. T., SEVY, R. W., HARAKAL, C. & HILLYER, P. N. 1967. Relationship between clinical severity of disease and hemodynamic patterns in essential hypertension. *Am J Med Sci,* 253**,** 194-208.

BENJAMIN, E. J., LARSON, M. G., KEYES, M. J., MITCHELL, G. F., VASAN, R. S., KEANEY, J. F., JR., LEHMAN, B. T., FAN, S., OSYPIUK, E. & VITA, J. A. 2004. Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. *Circulation,* 109**,** 613-9.

BERGLUND, G., ANDERSSON, O. & WILHELMSEN, L. 1976. Prevalence of primary and secondary hypertension: studies in a random population sample. *Bmj,* 2**,** 554-6.

BETO, J. A. & BANSAL, V. K. 1992. Quality of life in treatment of hypertension. A metaanalysis of clinical trials. *American journal of hypertension,* 5**,** 125-33.

BHATT, D. L., KANDZARI, D. E., O'NEILL, W. W., D'AGOSTINO, R., FLACK, J. M., KATZEN, B. T., LEON, M. B., LIU, M., MAURI, L., NEGOITA, M., COHEN, S. A., OPARIL, S., ROCHA-SINGH, K., TOWNSEND, R. R., BAKRIS, G. L. & INVESTIGATORS, S. H.-. 2014. A controlled trial of renal denervation for resistant hypertension. *N Engl J Med,* 370**,** 1393-401.

BIRNS, J. & KALRA, L. 2009. Cognitive function and hypertension. *J Hum Hypertens,* 23**,** 86-96.

BLAND, J. M. & ALTMAN, D. G. 1994. Regression towards the mean. *Bmj,* 308**,** 1499.

BLEAKLEY, C., HAMILTON, P. K., PUMB, R., HARBINSON, M. & MCVEIGH, G. E. 2015. Endothelial Function in Hypertension: Victim or Culprit? *The Journal of Clinical Hypertension,* 17**,** 651-654.

BOEGEHOLD, M. A., JOHNSON, M. D. & OVERBECK, H. W. 1991. Pressure-independent arteriolar rarefaction in hypertension. *Am J Physiol,* 261**,** H83-7.

BOERMA, E. C., MATHURA, K. R., VAN DER VOORT, P. H., SPRONK, P. E. & INCE, C. 2005. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Crit Care,* 9**,** R601-6.

BOHLEN, H. G. 1987. Enhanced cerebral vascular regulation occurs by age 4 to 5 weeks in spontaneously hypertensive rats. *Hypertension,* 9**,** 325-31.

BOHLEN, H. G. & LASH, J. M. 1994. Active and passive arteriolar regulation in spontaneously hypertensive rats. *Hypertension,* 23**,** 757-64.

BONETTI, P. O., PUMPER, G. M., HIGANO, S. T., HOLMES, D. R., JR., KUVIN, J. T. & LERMAN, A. 2004. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol,* 44**,** 2137-41.

BORCH-JOHNSEN, K., FELDT-RASMUSSEN, B., STRANDGAARD, S., SCHROLL, M. & JENSEN, J. S. 1999. Urinary albumin excretion. An independent predictor of ischemic heart disease. *Arterioscler Thromb Vasc Biol,* 19**,** 1992-7.

BOTS, M. L., REMME, W. J., LUSCHER, T. F., FOX, K. M., BERTRAND, M., FERRARI, R., SIMOONS, M. L. & GROBBEE, D. E. 2007. ACE inhibition and endothelial function: main findings of PERFECT, a sub-study of the EUROPA trial. *Cardiovasc Drugs Ther,* 21**,** 269-79.

BRIGHT, R. 1836. Tabular view of the morbid appearances in 100 cases connected with albuminous urine with observations. *Guys Hosp Rep,* 1**,** 380-400.

BULPITT, C. J. 1997. Quality of life as an outcome measure. *Postgrad Med J,* 73**,** 613-6.

BULPITT, C. J., DOLLERY, C. T. & CARNE, S. 1976. Change in symptoms of hypertensive patients after referral to hospital clinic. *British heart journal,* 38**,** 121-8.

BULPITT, C. J. & FLETCHER, A. E. 1990. The measurement of quality of life in hypertensive patients: a practical approach. *British Journal of Clinical Pharmacology,* 30**,** 353-364.

BUND, S. J., WEST, K. P. & HEAGERTY, A. M. 1991. Effects of protection from pressure on resistance artery morphology and reactivity in spontaneously hypertensive and Wistar-Kyoto rats. *Circ Res,* 68**,** 1230-40.

BURTON, J. 1743. *An Account of the Life and Writings of Herman Boerhaave*, Henry Lintot.

CALHOUN, D. A., JONES, D., TEXTOR, S., GOFF, D. C., MURPHY, T. P., TOTO, R. D., WHITE, A., CUSHMAN, W. C., WHITE, W., SICA, D., FERDINAND, K., GILES, T. D., FALKNER, B. & CAREY, R. M. 2008. Resistant Hypertension: Diagnosis, Evaluation, and Treatment. *A Scientific Statement From the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research,* 117**,** e510-e526.

CALVER, A., COLLIER, J., MONCADA, S. & VALLANCE, P. 1992. Effect of local intra-arterial NG-monomethyl-L-arginine in patients with hypertension: the nitric oxide dilator mechanism appears abnormal. *Journal of Hypertension,* 10**,** 1025-1032.

CARMELIET, P., FERREIRA, V., BREIER, G., POLLEFEYT, S., KIECKENS, L., GERTSENSTEIN, M., FAHRIG, M., VANDENHOECK, A., HARPAL, K., EBERHARDT, C., DECLERCQ, C., PAWLING, J., MOONS, L., COLLEN,

D., RISAU, W. & NAGY, A. 1996. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature,* 380**,** 435-9.

CELERMAJER, D. S., SORENSEN, K. E., GOOCH, V., SPIEGELHALTER, D., MILLER, O., SULLIVAN, I., LLOYD, J. & DEANFIELD, J. 1992. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet,* 340**,** 1111-1115.

CELERMAJER, D. S., SORENSEN, K. E., SPIEGELHALTER, D. J., GEORGAKOPOULOS, D., ROBINSON, J. & DEANFIELD, J. E. 1994. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol,* 24**,** 471-476.

CELIC, V., TADIC, M., SUZIC-LAZIC, J., ANDRIC, A., MAJSTOROVIC, A., IVANOVIC, B., STEVANOVIC, P., IRACEK, O. & SCEPANOVIC, R. 2014. Two- and three-dimensional speckle tracking analysis of the relation between myocardial deformation and functional capacity in patients with systemic hypertension. *Am J Cardiol,* 113**,** 832-9.

CERQUEIRA, M. D., WEISSMAN, N. J., DILSIZIAN, V., JACOBS, A. K., KAUL, S., LASKEY, W. K., PENNELL, D. J., RUMBERGER, J. A., RYAN, T. & VERANI, M. S. 2002. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. *Circulation,* 105**,** 539-42.

CHAMBERS, J. C., MCGREGOR, A., JEAN-MARIE, J., OBEID, O. A. & KOONER, J. S. 1999. Demonstration of Rapid Onset Vascular Endothelial Dysfunction After Hyperhomocysteinemia. An Effect Reversible With Vitamin C Therapy*. Circulation,* 99**,** 1156-1160.

CHAPMAN, D. 2017. *The relationship between low levels of urinary albumin excretion and microvacular function.* PhD in Medical Studies, University of Exeter. (Thesis).

CHEN, II, PREWITT, R. L. & DOWELL, R. F. 1981. Microvascular rarefaction in spontaneously hypertensive rat cremaster muscle. *Am J Physiol,* 241**,** H306-10.

CHENG, C., DASKALAKIS, C. & FALKNER, B. 2008a. Capillary rarefaction in treated and untreated hypertensive subjects. *Ther Adv Cardiovasc Dis,* 2**,** 79-88.

CHENG, C., DIAMOND, J. J. & FALKNER, B. 2008b. Functional capillary rarefaction in mild blood pressure elevation. *Clin Transl Sci,* 1**,** 75-9.

CHENG, S., SHAH, A. M., ALBISU, J. P., DESAI, A. S., HILKERT, R. J., IZZO, J., OPARIL, S., PITT, B. & SOLOMON, S. D. 2014. Reversibility of left ventricular mechanical dysfunction in patients with hypertensive heart disease. *Journal of Hypertension,* 32**,** 2479-86.

CHOWIENCZYK, P., WATTS, G., COCKCROFT, J. & RITTER, J. 1992. Impaired endothelium-dependent vasodilation of forearm resistance vessels in hypercholesterolaemia. *The Lancet,* 340**,** 1430-1432.

CHRISTENSEN, K. L. & MULVANY, M. J. 1993. Mesenteric arcade arteries contribute substantially to vascular resistance in conscious rats. *J Vasc Res,* 30**,** 73-9.

CHRISTENSEN, K. L. & MULVANY, M. J. 1994. Perindopril changes the mesenteric pressure profile of conscious hypertensive and normotensive rats. *Hypertension,* 23**,** 325-8.

CHRISTENSEN, K. L. & MULVANY, M. J. 2001. Location of resistance arteries. *J Vasc Res,* 38**,** 1-12.

CHUNG, C. M., CHENG, H. W., CHANG, J. J., LIN, Y. S., HSIAO, J. F., CHANG, S. T. & HSU, J. T. 2014. Relationship between resistant hypertension and arterial stiffness assessed by brachial-ankle pulse wave velocity in the older patient. *Clin Interv Aging,* 9**,** 1495-502.

CIPRIANO, C., GOSSE, P., BEMURAT, L., MAS, D., LEMETAYER, P., N'TELA, G. & CLEMENTY, J. 2001. Prognostic value of left ventricular mass and its evolution during treatment in the Bordeaux cohort of hypertensive patients. *Am J Hypertens,* 14**,** 524-9.

CLARK, C., SMITH, L., CLOUTIER, L., KONYA, J., TODKAR, S., MCDONAGH, S., CLARK, O., GLYNN, L., TAYLOR, R. & CAMPBELL, J. 2021. Allied health professional-led interventions for improving control of blood pressure in patients with hypertension (review). Cochrane Library (in press).

CLARK, C. E. & MCMANUS, R. 2012. The use of highly structured care to achieve blood pressure targets. *Bmj,* 345**,** e7777.

CLARK, C. E., SMITH, L. F., TAYLOR, R. S. & CAMPBELL, J. L. 2010. Nurse led interventions to improve control of blood pressure in people with hypertension: systematic review and meta-analysis. *Bmj,* 341**,** c3995.

CLARKSON, P., CELERMAJER, D. S., POWE, A. J., DONALD, A. E., HENRY, R. M. A. & DEANFIELD, J. E. 1997. Endothelium-Dependent Dilatation Is Impaired in Young Healthy Subjects With a Family History of Premature Coronary Disease. *Circulation,* 96**,** 3378-3383.

COLLINS, R., PETO, R., MACMAHON, S., HEBERT, P., FIEBACH, N. H., EBERLEIN, K. A., GODWIN, J., QIZILBASH, N., TAYLOR, J. O. & HENNEKENS, C. H. 1990. Blood pressure, stroke, and coronary heart disease. Part 2, Short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet,* 335**,** 827-38.

CONEN, D. & BAMBERG, F. 2008. Noninvasive 24-h ambulatory blood pressure and cardiovascular disease: a systematic review and meta-analysis. *Journal of Hypertension,* 26**,** 1290-9.

COOKE, J. P. & DZAU, V. J. 1997. Nitric oxide synthase: role in the genesis of vascular disease. *Annu Rev Med,* 48**,** 489-509.

CORDELLINI, S. 1999. Endothelial dysfunction in DOCA-salt hypertension Possible involvement of prostaglandin endoperoxides. *General Pharmacology: The Vascular System,* 32**,** 315-320.

CRAWFORD, S., WHITNALL, L., ROBERTSON, J. & EVANS, J. J. 2012. A systematic review of the accuracy and clinical utility of the Addenbrooke's Cognitive Examination and the Addenbrooke's Cognitive Examination-Revised in the diagnosis of dementia. *Int J Geriatr Psychiatry,* 27**,** 659-69.

CROWE, S. F. 1998. The differential contribution of mental tracking, cognitive flexibility, visual search, and motor speed to performance on parts A and B of the Trail Making Test. *J Clin Psychol,* 54**,** 585-91.

CUTOLO, M., PIZZORNI, C. & SULLI, A. 2005. Capillaroscopy. *Best practice & research Clinical rheumatology,* 19**,** 437-52.

DAHLOF, B., DEVEREUX, R. B., KJELDSEN, S. E., JULIUS, S., BEEVERS, G., DE FAIRE, U., FYHRQUIST, F., IBSEN, H., KRISTIANSSON, K., LEDERBALLE-PEDERSEN, O., LINDHOLM, L. H., NIEMINEN, M. S., OMVIK, P., OPARIL, S. & WEDEL, H. 2002. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet.,* 359**,** 995-1003.

DAHLOF, B., SEVER, P. S., POULTER, N. R., WEDEL, H., BEEVERS, D. G., CAULFIELD, M., COLLINS, R., KJELDSEN, S. E., KRISTINSSON, A., MCINNES, G. T., MEHLSEN, J., NIEMINEN, M., O'BRIEN, E. & OSTERGREN, J. 2005. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. *Lancet,* 366**,** 895-906.

DAUGHERTY, S. L., POWERS, J. D., MAGID, D. J., TAVEL, H. M., MASOUDI, F. A., MARGOLIS, K. L., O'CONNOR, P. J., SELBY, J. V. & HO, P. M. 2012. Incidence and prognosis of resistant hypertension in hypertensive patients. *Circulation,* 125**,** 1635-42.

DAVIS, M. J. & HILL, M. A. 1999. Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev,* 79**,** 387-423.

DE BACKER, D., CRETEUR, J., PREISER, J. C., DUBOIS, M. J. & VINCENT, J. L. 2002. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med,* 166**,** 98-104.

DE BACKER, D., HOLLENBERG, S., BOERMA, C., GOEDHART, P., BUCHELE, G., OSPINA-TASCON, G., DOBBE, I. & INCE, C. 2007. How to evaluate the microcirculation: report of a round table conference. *Crit Care,* 11**,** R101.

DE CIUCEIS, C., PILU, A., RIZZONI, D., PORTERI, E., MUIESAN, M. L., SALVETTI, M., PAINI, A., BELOTTI, E., ZANI, F., BOARI, G. E., ROSEI, C. A. & ROSEI, E. A. 2011. Effect of antihypertensive treatment on circulating endothelial progenitor cells in patients with mild essential hypertension. *Blood Press,* 20**,** 77-83.

DE VET, H. C. W., TERWEE, C. B. & BOUTER, L. M. 2003. Current challenges in clinimetrics. *Journal of clinical epidemiology,* 56**,** 1137-41.

DEBBABI, H., BONNIN, P. & LEVY, B. I. 2010. Effects of blood pressure control with perindopril/indapamide on the microcirculation in hypertensive patients. *Am J Hypertens,* 23**,** 1136-43.

DEBBABI, H., UZAN, L., MOURAD, J. J., SAFAR, M., LEVY, B. I. & TIBIRICA, E. 2006. Increased skin capillary density in treated essential hypertensive patients. *Am J Hypertens,* 19**,** 477-83.

DELANO, F. A., SCHMID-SCHONBEIN, G. W., SKALAK, T. C. & ZWEIFACH, B. W. 1991. Penetration of the systemic blood pressure into the microvasculature of rat skeletal muscle. *Microvasc Res,* 41**,** 92-110.

DENOLLE, T., SASSANO, P., ALLAIN, H., BENTUE-FERRER, D., BRETON, S., CIMAROSTI, I., OUATARA, B., MERIENNE, M. & GANDON, J. M. 2002. Effects of nicardipine and clonidine on cognitive functions and electroencephalography in hypertensive patients. *Fundam Clin Pharmacol,* 16**,** 527-35.

DEVEREUX, R. B., DAHLOF, B., GERDTS, E., BOMAN, K., NIEMINEN, M. S., PAPADEMETRIOU, V., ROKKEDAL, J., HARRIS, K. E., EDELMAN, J. M. & WACHTELL, K. 2004. Regression of hypertensive left ventricular hypertrophy by losartan compared with atenolol: the Losartan Intervention for Endpoint Reduction in Hypertension (LIFE) trial. *Circulation,* 110**,** 1456-62.

DONATI, A., DAMIANI, E., BOTTICELLI, L., ADRARIO, E., LOMBRANO, M. R., DOMIZI, R., MARINI, B., VAN TEEFFELEN, J. W., CARLETTI, P., GIRARDIS, M., PELAIA, P. & INCE, C. 2013. The aPC treatment improves microcirculation in severe sepsis/septic shock syndrome. *BMC Anesthesiol,* 13**,** 25.

DOUMAS, M., TSIOUFIS, C., FASELIS, C., LAZARIDIS, A., GRASSOS, H. & PAPADEMETRIOU, V. 2014. Non-interventional management of resistant hypertension. *World J Cardiol,* 6**,** 1080-90.

DREXLER, H., ZEIHER, A., MEINZER, K. & JUST, H. 1991. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *The Lancet,* 338**,** 1546-1550.

DULING, B. 1991. The role of the resistance arteries in the control of peripheral resistance. *Resistance Arteries: Structure and Function***,** 3-9.

EGASHIRA, K., SUZUKI, S., HIROOKA, Y., KAI, H., SUGIMACHI, M., IMAIZUMI, T. & TAKESHITA, A. 1995. Impaired endothelium-dependent vasodilation of large epicardial and resistance coronary arteries in patients with essential hypertension. *Hypertension,* 25**,** 201-206.

ELIAS, M. F., WOLF, P. A., D'AGOSTINO, R. B., COBB, J. & WHITE, L. R. 1993. Untreated blood pressure level is inversely related to cognitive functioning: the Framingham Study. *Am J Epidemiol,* 138**,** 353-64.

ENDEMANN, D. H. & SCHIFFRIN, E. L. 2004. Endothelial dysfunction. *J Am Soc Nephrol,* 15**,** 1983-92.

ERICKSON, S. R., WILLIAMS, B. C. & GRUPPEN, L. D. 2001. Perceived symptoms and health-related quality of life reported by uncomplicated hypertensive patients compared to normal controls. *J Hum Hypertens,* 15**,** 539-48.

ESCH, B. T. & WARBURTON, D. E. R. 2009. Left ventricular torsion and recoil: implications for exercise performance and cardiovascular disease. *Journal of Applied Physiology,* 106**,** 362-369.

NICE. 2011. Hypertension: Clinical Management of Primary Hypertension in Adults (Update).

FAGARD, R. H., CELIS, H., THIJS, L., STAESSEN, J. A., CLEMENT, D. L., DE BUYZERE, M. L. & DE BACQUER, D. A. 2008. Daytime and nighttime blood pressure as predictors of death and cause-specific cardiovascular events in hypertension. *Hypertension,* 51**,** 55-61.

FALASCHETTI, E., MINDELL, J., KNOTT, C. & POULTER, N. 2014. Hypertension management in England: a serial cross-sectional study from 1994 to 2011. *Lancet,* 383**,** 1912-9.

FARIA, C., WENZEL, M., LEE, K. W., CODERRE, K., NICHOLS, J. & BELLETTI, D. A. 2009. A narrative review of clinical inertia: focus on hypertension. *J Am Soc Hypertens,* 3**,** 267-76.

FEDOROVICH, A. A., DRAPKINA, O. M., PRONKO, K. N., SINOPALNIKOV, V. I. & ZEMSKOV, V. M. 2018. Telemonitoring of Capillary Blood Flow in the Human Skin: New Opportunities and Prospects. *Clinical Practice,* 15**,** 561-567.

FEIHL, F., LIAUDET, L., LEVY, B. I. & WAEBER, B. 2008. Hypertension and microvascular remodelling. *Cardiovasc Res,* 78**,** 274-85.

FEIHL, F., LIAUDET, L., WAEBER, B. & LEVY, B. I. 2006. Hypertension: a disease of the microcirculation? *Hypertension,* 48**,** 1012-7.

FELDMAN, R. D., ZOU, G. Y., VANDERVOORT, M. K., WONG, C. J., NELSON, S. A. & FEAGAN, B. G. 2009. A simplified approach to the treatment of uncomplicated hypertension: a cluster randomized, controlled trial. *Hypertension,* 53**,** 646-53.

FELETOU, M. & VANHOUTTE, P. M. 2006. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). *Am J Physiol Heart Circ Physiol,* 291**,** H985-1002.

FERRARA, N., CARVER-MOORE, K., CHEN, H., DOWD, M., LU, L., O'SHEA, K. S., POWELL-BRAXTON, L., HILLAN, K. J. & MOORE, M. W. 1996. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature,* 380**,** 439-42.

FILBIN, M. R., HOU, P. C., MASSEY, M., BARCHE, A., KAO, E., BRACEY, A., SKIBSTED, S., CHANG, Y. & SHAPIRO, N. I. 2014. The microcirculation is preserved in emergency department low-acuity sepsis patients without hypotension. *Academic emergency medicine,* 21**,** 154-62.

FINKIELMAN, S., WORCEL, M. & AGREST, A. 1965. Hemodynamic Patterns in Essential Hypertension. *Circulation,* 31**,** 356-68.

FITZPATRICK, D. F. & SZENTIVANYI, A. 1980. The relationship between increased myogenic tone and hyporesponsiveness in vascular smooth muscle of spontaneously hypertensive rats. *Clin Exp Hypertens,* 2**,** 1023-37.

FLETCHER, A. E., BULPITT, C. J., HAWKINS, C. M., HAVINGA, T. K., TEN BERGE, B. S., MAY, J. F., SCHUURMAN, F. H., VAN DER VEUR, E. & WESSELING, H. 1990. Quality of life on antihypertensive therapy: a randomized double-blind controlled trial of captopril and atenolol. *Journal of hypertension,* 8**,** 463-6.

FLETCHER, A. E., CHESTER, P. C., HAWKINS, C. M., LATHAM, A. N., PIKE, L. A. & BULPITT, C. J. 1989. The effects of verapamil and propranolol on quality of life in hypertension. *J Hum Hypertens,* 3**,** 125-30.

FORETTE, F., SEUX, M. L., STAESSEN, J. A., THIJS, L., BABARSKIENE, M. R., BABEANU, S., BOSSINI, A., FAGARD, R., GIL-EXTREMERA, B., LAKS, T., KOBALAVA, Z., SARTI, C., TUOMILEHTO, J., VANHANEN, H., WEBSTER, J., YODFAT, Y., BIRKENHAGER, W. H. & SYSTOLIC HYPERTENSION IN EUROPE, I. 2002. The prevention of dementia with antihypertensive treatment: new evidence from the Systolic Hypertension in Europe (Syst-Eur) study. *Arch Intern Med,* 162**,** 2046-52.

FRANKLIN, S. S., THIJS, L., LI, Y., HANSEN, T. W., BOGGIA, J., LIU, Y., ASAYAMA, K., BJORKLUND-BODEGARD, K., OHKUBO, T., JEPPESEN, J., TORP-PEDERSEN, C., DOLAN, E., KUZNETSOVA, T.,

STOLARZ-SKRZYPEK, K., TIKHONOFF, V., MALYUTINA, S., CASIGLIA, E., NIKITIN, Y., LIND, L., SANDOYA, E., KAWECKA-JASZCZ, K., FILIPOVSKY, J., IMAI, Y., WANG, J., IBSEN, H., O'BRIEN, E. & STAESSEN, J. A. 2013. Masked hypertension in diabetes mellitus: treatment implications for clinical practice. *Hypertension,* 61**,** 964-71.

FREIS, E. 1967. Effect of Treatment on Morbidity in Hypertension. *Jama,* 202**,** 1028-34.

FUCHS, F. D. & WHELTON, P. K. 2020. High Blood Pressure and Cardiovascular Disease. *Hypertension,* 75**,** 285-292.

FUKUTOMI, M., HOSHIDE, S., MIZUNO, H. & KARIO, K. 2014. Differential effects of aliskiren/amlodipine combination and high-dose amlodipine monotherapy on endothelial function in elderly hypertensive patients. *Am Journal of Hypertension,* 27**,** 14-20.

FURCHGOTT, R. F. & ZAWADZKI, J. V. 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature,* 288**,** 373-6.

FUTRAKUL, N., SRIDAMA, V. & FUTRAKUL, P. 2009. Microalbuminuria--a biomarker of renal microvascular disease. *Ren Fail,* 31**,** 140-3.

GALDERISI, M., ESPOSITO, R., SCHIANO-LOMORIELLO, V., SANTORO, A., IPPOLITO, R., SCHIATTARELLA, P., STRAZZULLO, P. & DE SIMONE, G. 2012. Correlates of global area strain in native hypertensive patients: a three-dimensional speckle-tracking echocardiography study. *Eur Heart J Cardiovasc Imaging,* 13**,** 730-8.

GAO, H., ALLAN, A., MCCOMB, C., LUO, X. & BERRY, C. 2014. Left ventricular strain and its pattern estimated from cine CMR and validation with DENSE. *Phys Med Biol,* 59**,** 3637-56.

GASSER, P. & BUHLER, F. R. 1992. Nailfold Microcirculation in Normotensive and Essential Hypertensive Subjects, as Assessed by Video-Microscopy. *Journal of Hypertension,* 10**,** 83-86.

GERSTEIN, H. C., MANN, J. F., YI, Q., ZINMAN, B., DINNEEN, S. F., HOOGWERF, B., HALLE, J. P., YOUNG, J., RASHKOW, A., JOYCE, C., NAWAZ, S., YUSUF, S. & INVESTIGATORS, H. S. 2001. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA,* 286**,** 421-6.

GHALI, J. K., LIAO, Y., SIMMONS, B., CASTANER, A., CAO, G. & COOPER, R. S. 1992. The prognostic role of left ventricular hypertrophy in patients with or without coronary artery disease. *Ann Intern Med,* 117**,** 831-6.

GILJE, O., O'LEARY, P. A. & BALDES, E. J. 1953. Capillary microscopic examination in skin diseases. *AMA archives of dermatology and syphilology,* 68**,** 136-47.

GOEDHART, P. T., KHALILZADA, M., BEZEMER, R., MERZA, J. & INCE, C. 2007. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. *Opt Express,* 15**,** 15101-14.

GOODING, K. M., TOOKE, J. E., VON LANY, H., MITRA, M., LING, R., BALL, C. I., MAWSON, D., SKINNER, K. & SHORE, A. C. 2010. Capillary pressure may predict preclinical changes in the eye. *Diabetologia,* 53**,** 2029-35.

GORDON, R. D. 1995. Primary aldosteronism. *J Endocrinol Invest,* 18**,** 495-511.

GRADMAN, A. H., PARISE, H., LEFEBVRE, P., FALVEY, H., LAFEUILLE, M. H. & DUH, M. S. 2013. Initial combination therapy reduces the risk of cardiovascular events in hypertensive patients: a matched cohort study. *Hypertension,* 61**,** 309-18.

GREENE, A. S., TONELLATO, P. J., LUI, J., LOMBARD, J. & COWLEY, A. 1989. Microvascular rarefaction and tissue vascular resistance in hypertension. *American Journal of Physiology-Heart and Circulatory Physiology,* 256**,** H126-H131.

GREENWALD, S. E. 2007. Ageing of the conduit arteries. *J Pathol,* 211**,** 157-72.

GRIBBIN, B., STEPTOE, A. & SLEIGHT, P. 1976. Pulse wave velocity as a measure of blood pressure change. *Psychophysiology,* 13**,** 86-90.

GRIMM, R. H., JR., GRANDITS, G. A., CUTLER, J. A., STEWART, A. L., MCDONALD, R. H., SVENDSEN, K., PRINEAS, R. J. & LIEBSON, P. R. 1997. Relationships of quality-of-life measures to long-term lifestyle and drug treatment in the Treatment of Mild Hypertension Study. *Archives of internal medicine,* 157**,** 638-48.

GRONER, W., WINKELMAN, J. W., HARRIS, A. G., INCE, C., BOUMA, G. J., MESSMER, K. & NADEAU, R. G. 1999. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. *Nat Med,* 5**,** 1209-12.

GROSSMAN, W., JONES, D. & MCLAURIN, L. P. 1975. Wall stress and patterns of hypertrophy in the human left ventricle. *J Clin Invest,* 56**,** 56-64.

GROTHUES, F., SMITH, G. C., MOON, J. C., BELLENGER, N. G., COLLINS, P., KLEIN, H. U. & PENNELL, D. J. 2002. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol,* 90**,** 29-34.

HAACK, D. W., SCHAFFER, J. J. & SIMPSON, J. G. 1980. Comparisons of cutaneous microvessels from spontaneously hypertensive, normotensive Wistar-Kyoto, and normal Wistar rats. *Proc Soc Exp Biol Med,* 164**,** 453-8.

HAMEED, M. A., TEBBIT, L., JACQUES, N., THOMAS, M. & DASGUPTA, I. 2016. Non-adherence to antihypertensive medication is very common among resistant hypertensives: results of a directly observed therapy clinic. *J Hum Hypertens,* 30**,** 83-9.

HARPER, S. L. & BOHLEN, H. G. 1984. Microvascular adaptation in the cerebral cortex of adult spontaneously hypertensive rats. *Hypertension,* 6**,** 408-19.

HE, F. J., LI, J. & MACGREGOR, G. A. 2013. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. *Bmj,* 346**,** f1325.

HE, F. J., MARCINIAK, M., MARKANDU, N. D., ANTONIOS, T. F. & MACGREGOR, G. A. 2010. Effect of modest salt reduction on skin capillary rarefaction in white, black, and Asian individuals with mild hypertension. *Hypertension,* 56**,** 253-9.

HE, Y., SI, D., YANG, C., NI, L., LI, B., DING, M. & YANG, P. 2014. The effects of amlodipine and S(-)-amlodipine on vascular endothelial function in patients with hypertension. *Am J Hypertens,* 27**,** 27-31.

HEITZER, T., YLÄ-HERTTUALA, S., LUOMA, J., KURZ, S., MÜNZEL, T., JUST, H., OLSCHEWSKI, M. & DREXLER, H. 1996. Cigarette Smoking Potentiates Endothelial Dysfunction of Forearm Resistance Vessels in Patients With Hypercholesterolemia. *Role of Oxidized LDL,* 93**,** 1346-1353.

HENRICH, H., HERTEL, R. & ASSMANN, R. 1978. Structural differences in the mesentery microcirculation between normotensive and spontaneously hypertensive rats. *Pflugers Arch,* 375**,** 153-9.

HENRICH, H. A., ROMEN, W., HEIMGARTNER, W., HARTUNG, E. & BAUMER, F. 1988. Capillary rarefaction characteristic of the skeletal muscle of hypertensive patients. *Klin Wochenschr,* 66**,** 54-60.

HIGASHI, Y., SASAKI, S., NAKAGAWA, K., UEDA, T., YOSHIMIZU, A., KURISU, S., MATSUURA, H., KAJIYAMA, G. & OSHIMA, T. 2000. A comparison of angiotensin-converting enzyme inhibitors, calcium antagonists, beta-blockers and diuretic agents on reactive hyperemia in patients with essential hypertension: a multicenter study. *J Am Coll Cardiol,* 35**,** 284-91.

HINK, U., LI, H., MOLLNAU, H., OELZE, M., MATHEIS, E., HARTMANN, M., SKATCHKOV, M., THAISS, F., STAHL, R. A. K., WARNHOLTZ, A., MEINERTZ, T., GRIENDLING, K., HARRISON, D. G., FORSTERMANN, U. & MUNZEL, T. 2001. Mechanisms Underlying Endothelial Dysfunction in Diabetes Mellitus. *Circulation Research,* 88**,** e14-e22.

HINOI, T., TOMOHIRO, Y., KAJIWARA, S., MATSUO, S., FUJIMOTO, Y., YAMAMOTO, S., SHICHIJO, T. & ONO, T. 2008. Telmisartan, an angiotensin II type 1 receptor blocker, improves coronary microcirculation and insulin resistance among essential hypertensive patients without left ventricular hypertrophy. *Hypertens Res,* 31**,** 615-22.

HOOD, S., CANNON, J., FOO, R. & BROWN, M. 2005. Prevalence of primary hyperaldosteronism assessed by aldosterone/renin ratio and spironolactone testing. *Clin Med,* 5**,** 55-60.

HOR, K. N., BAUMANN, R., PEDRIZZETTI, G., TONTI, G., GOTTLIEBSON, W. M., TAYLOR, M., BENSON, D. W. & MAZUR, W. 2011. Magnetic Resonance Derived Myocardial Strain Assessment Using Feature Tracking. *J Vis Exp,* 48, e2356.

HOR, K. N., GOTTLIEBSON, W. M., CARSON, C., WASH, E., CNOTA, J., FLECK, R., WANSAPURA, J., KLIMECZEK, P., AL-KHALIDI, H. R., CHUNG, E. S., BENSON, D. W. & MAZUR, W. 2010. Comparison of Magnetic Resonance Feature Tracking for Strain Calculation With Harmonic Phase Imaging Analysis. *Jacc-Cardiovascular Imaging,* 3**,** 144-151.

HRISTOV, M., ERL, W. & WEBER, P. C. 2003a. Endothelial progenitor cells. *Arteriosclerosis, thrombosis, and vascular biology,* 23**,** 1185-1189.

HRISTOV, M., ERL, W. & WEBER, P. C. 2003b. Endothelial progenitor cells: isolation and characterization. *Trends Cardiovasc Med,* 13**,** 201-6.

HUBBLE, S. M., KYTE, H. L., GOODING, K. & SHORE, A. C. 2009. Variability in sublingual microvessel density and flow measurements in healthy volunteers. *Microcirculation,* 16**,** 183-91.

HUGHES, A. D., MARTINEZ-PEREZ, E., JABBAR, A. S., HASSAN, A., WITT, N. W., MISTRY, P. D., CHAPMAN, N., STANTON, A. V., BEEVERS, G., PEDRINELLI, R., PARKER, K. H. & THOM, S. A. 2006. Quantification of topological changes in retinal vascular architecture in essential and malignant hypertension. *Journal of Hypertension,* 24**,** 889-94.

HUGHES, A. D., STANTON, A. V., JABBAR, A. S., CHAPMAN, N., MARTINEZ-PEREZ, M. E. & MC, G. T. S. A. 2008. Effect of antihypertensive treatment on retinal microvascular changes in hypertension. *Journal of Hypertension,* 26**,** 1703-7.

HUNT, S. M. 1997. Quality of life claims in trials of anti-hypertensive therapy. *Quality of Life Research,* 6**,** 185-191.

HYMAN, D. J. & PAVLIK, V. 2015. Medication adherence and resistant hypertension. *J Hum Hypertens,* 29**,** 213-8.

IMANISHI, T., MORIWAKI, C., HANO, T. & NISHIO, I. 2005. Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. *J Hypertens,* 23**,** 1831-7.

INCE, C. 2005. Sidestream dark field imaging: an improved technique to observe sublingual microcirculation. *Crit Care,* 9**,** 72. (Abstract).

IZZARD, A. S., BUND, S. J. & HEAGERTY, A. M. 1996. Increased wall-lumen ratio of mesenteric vessels from the spontaneously hypertensive rat is not associated with increased contractility under isobaric conditions. *Hypertension,* 28**,** 604-8.

JABLONSKI, K. L., GATES, P. E., PIERCE, G. L. & SEALS, D. R. 2009. Low dietary sodium intake is associated with enhanced vascular endothelial function in middle-aged and older adults with elevated systolic blood pressure. *Ther Adv Cardiovasc Dis,* 3**,** 347-56.

JAMERSON, K., WEBER, M. A., BAKRIS, G. L., DAHLOF, B., PITT, B., SHI, V., HESTER, A., GUPTE, J., GATLIN, M. & VELAZQUEZ, E. J. 2008. Benazepril plus amlodipine or hydrochlorothiazide for hypertension in high-risk patients. *N Engl J Med.,* 359**,** 2417-28.

JAMES, M. A., TULLETT, J., HEMSLEY, A. G. & SHORE, A. C. 2006. Effects of aging and hypertension on the microcirculation. *Hypertension,* 47**,** 968-74.

JANSSEN, M. F., PICKARD, A. S., GOLICKI, D., GUDEX, C., NIEWADA, M., SCALONE, L., SWINBURN, P. & BUSSCHBACH, J. 2013. Measurement properties of the EQ-5D-5L compared to the EQ-5D-3L across eight patient groups: a multi-country study. *Qual Life Res,* 22**,** 1717-27.

JIAMSRIPONG, P., HONDA, T., REUSS, C. S., HURST, R. T., CHALIKI, H. P., GRILL, D. E., SCHNECK, S. L., TYLER, R., KHANDHERIA, B. K. & LESTER, S. J. 2008. Three methods for evaluation of left atrial volume. *Eur J Echocardiogr,* 9**,** 351-5.

JOFFRES, M., FALASCHETTI, E., GILLESPIE, C., ROBITAILLE, C., LOUSTALOT, F., POULTER, N., MCALISTER, F. A., JOHANSEN, H., BACLIC, O. & CAMPBELL, N. 2013. Hypertension prevalence, awareness, treatment and control in national surveys from England, the USA and Canada, and correlation with stroke and ischaemic heart disease mortality: a cross-sectional study. *BMJ Open,* 3**,** e003423.

JOHNS, M. W. 1993. Daytime sleepiness, snoring, and obstructive sleep apnea. The Epworth Sleepiness Scale. *Chest,* 103**,** 30-6.

JOHNSON, A. G., NGUYEN, T. V. & DAY, R. O. 1994. Do Nonsteroidal Anti-inflammatory Drugs Affect Blood Pressure? A Meta-Analysis. *Annals of Internal Medicine,* 121**,** 289-300.

JORDAN, A. N., ANNING, C., WILKES, L., BALL, C., PAMPHILON, N., CLARK, C. E., BELLENGER, N. G., SHORE, A. C. & SHARP, A. S. P. 2020. Rapid treatment of moderate to severe hypertension using a novel protocol in a single-centre, before and after interventional study. *J Hum Hypertens,* 34**,** 165-175.

JORDAN, A. N., ANNING, C., WILKES, L., BALL, C., PAMPHILON, N., CLARK, C. E., BELLENGER, N. G., SHORE, A. C., SHARP, A. S. P. & VALDERAS, J. M. 2022. Cross-cultural adaptation of the Spanish MINICHAL instrument into English for use in the United Kingdom. *Health Qual Life Outcomes,* 20**,** 39.

JULIUS, S. 1991. Autonomic nervous system dysregulation in human hypertension. *Am J Cardiol,* 67**,** 3b-7b.

JULIUS, S., KJELDSEN, S. E., WEBER, M., BRUNNER, H. R., EKMAN, S., HANSSON, L., HUA, T., LARAGH, J., MCINNES, G. T. & MITCHELL, L.

2004. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. *The Lancet,* 363**,** 2022-2031.

JULIUS, S., PASCUAL, A. V., SANNERSTEDT, R. & MITCHELL, C. 1971. Relationship between cardiac output and peripheral resistance in borderline hypertension. *Circulation,* 43**,** 382-90.

JUNG, O., GECHTER, J. L., WUNDER, C., PAULKE, A., BARTEL, C., GEIGER, H. & TOENNES, S. W. 2013. Resistant hypertension? Assessment of adherence by toxicological urine analysis. *Journal of Hypertension,* 31**,** 766-74.

JURASCHEK, S. P., TAYLOR, A. A., WRIGHT, J. T., JR., EVANS, G. W., MILLER, E. R., 3RD, PLANTE, T. B., CUSHMAN, W. C., GURE, T. R., HALEY, W. E., MOINUDDIN, I., NORD, J., OPARIL, S., PEDLEY, C., ROUMIE, C. L., WHITTLE, J., WIGGERS, A., FINUCANE, C., ANNE KENNY, R., APPEL, L. J., TOWNSEND, R. R. & GROUP, S. R. 2020. Orthostatic Hypotension, Cardiovascular Outcomes, and Adverse Events: Results From SPRINT. *Hypertension,* 75**,** 660-667.

JURVA, J. W., PHILLIPS, S. A., SYED, A. Q., SYED, A. Y., PITT, S., WEAVER, A. & GUTTERMAN, D. D. 2006. The effect of exertional hypertension evoked by weight lifting on vascular endothelial function. *J Am Coll Cardiol,* 48**,** 588-9.

KAISER, S. E., SANJULIANI, A. F., ESTATO, V., GOMES, M. B. & TIBIRICA, E. 2013. Antihypertensive treatment improves microvascular rarefaction and reactivity in low-risk hypertensive individuals. *Microcirculation,* 20**,** 703-16.

KALKA, C., MASUDA, H., TAKAHASHI, T., GORDON, R., TEPPER, O., GRAVEREAUX, E., PIECZEK, A., IWAGURO, H., HAYASHI, S. I., ISNER, J. M. & ASAHARA, T. 2000. Vascular endothelial growth factor(165) gene transfer augments circulating endothelial progenitor cells in human subjects. *Circ Res,* 86**,** 1198-202.

KANATSUKA, H., LAMPING, K. G., EASTHAM, C. L., MARCUS, M. L. & DELLSPERGER, K. C. 1991. Coronary microvascular resistance in hypertensive cats. *Circ Res,* 68**,** 726-33.

KAO, Y. C., LEE, M. F., MAO, C. T., CHEN, W. S., YANG, N. I., CHERNG, W. J. & HUNG, M. J. 2013. Differences of left ventricular systolic deformation in

hypertensive patients with and without apical hypertrophic cardiomyopathy. *Cardiovasc Ultrasound,* 11**,** 40.

KEARNEY, P. M., WHELTON, M., REYNOLDS, K., MUNTNER, P., WHELTON, P. K. & HE, J. 2005. Global burden of hypertension: analysis of worldwide data. *Lancet,* 365**,** 217-23.

KELLY, A. S., GONZALEZ-CAMPOY, J. M., RUDSER, K. D., KATZ, H., METZIG, A. M., THALIN, M. & BANK, A. J. 2012. Carvedilol-lisinopril combination therapy and endothelial function in obese individuals with hypertension. *J Clin Hypertens (Greenwich),* 14**,** 85-91.

KILANDER, L., NYMAN, H., BOBERG, M., HANSSON, L. & LITHELL, H. 1998. Hypertension is related to cognitive impairment: a 20-year follow-up of 999 men. *Hypertension,* 31**,** 780-6.

KIM, K. H., JEONG, M. H., CHO, S. H., MOON, J. Y., HONG, Y. J., PARK, H. W., KIM, J. H., AHN, Y., CHO, J. G., PARK, J. C. & KANG, J. C. 2009. Clinical effects of calcium channel blocker and Angiotensin converting enzyme inhibitor on endothelial function and arterial stiffness in patients with angina pectoris. *J Korean Med Sci,* 24**,** 223-31.

KIM, R. J., SIMONETTI, O. P., WESTWOOD, M., KRAMER, C. M., NARANG, A., FRIEDRICH, M. G., POWELL, A. J., CARR, J. C., SCHULZ-MENGER, J., NAGEL, E., CHAN, W. S., BREMERICH, J., ORDOVAS, K. G., ROLLINGS, R. C., PATEL, A. R. & FERRARI, V. A. 2018. Guidelines for training in cardiovascular magnetic resonance (CMR). *J Cardiovasc Magn Reson,* 20**,** 57.

KLINGBEIL, A. U., SCHNEIDER, M., MARTUS, P., MESSERLI, F. H. & SCHMIEDER, R. E. 2003. A meta-analysis of the effects of treatment on left ventricular mass in essential hypertension. *Am J Med,* 115**,** 41-6.

KOBAYASHI, N., DELANO, F. A. & SCHMID-SCHONBEIN, G. W. 2005. Oxidative stress promotes endothelial cell apoptosis and loss of microvessels in the spontaneously hypertensive rats. *Arterioscler Thromb Vasc Biol,* 25**,** 2114-21.

KOBAYASHI, N., KOBAYASHI, K., HARA, K., HIGASHI, T., YANAKA, H., YAGI, S. & MATSUOKA, H. 1999. Benidipine stimulates nitric oxide synthase and improves coronary circulation in hypertensive rats. *Am J Hypertens,* 12**,** 483-91.

KOREN, M. J., DEVEREUX, R. B., CASALE, P. N., SAVAGE, D. D. & LARAGH, J. H. 1991. Relation of left ventricular mass and geometry to morbidity and mortality in uncomplicated essential hypertension. *Ann Intern Med,* 114**,** 345-52.

KOREN, M. J., ULIN, R. J., KOREN, A. T., LARAGH, J. H. & DEVEREUX, R. B. 2002. Left ventricular mass change during treatment and outcome in patients with essential hypertension. *Am J Hypertens,* 15**,** 1021-8.

KORHONEN, P. E., KIVELA, S.-L., KAUTIAINEN, H., JARVENPAA, S. & KANTOLA, I. 2011. Health-related quality of life and awareness of hypertension. *Journal of hypertension,* 29**,** 2070-4.

KORNER, P. I., BOBIK, A., JENNINGS, G. L., ANGUS, J. A. & ANDERSON, W. P. 1991. Significance of cardiovascular hypertrophy in the development and maintenance of hypertension. *J Cardiovasc Pharmacol,* 17 Suppl 2**,** S25-32.

KORTTE, K. B., HORNER, M. D. & WINDHAM, W. K. 2002. The trail making test, part B: cognitive flexibility or ability to maintain set? *Appl Neuropsychol,* 9**,** 106-9.

KOWALLICK, J. T., LAMATA, P., HUSSAIN, S. T., KUTTY, S., STEINMETZ, M., SOHNS, J. M., FASSHAUER, M., STAAB, W., UNTERBERG-BUCHWALD, C., BIGALKE, B., LOTZ, J., HASENFUSS, G. & SCHUSTER, A. 2014. Quantification of Left Ventricular Torsion and Diastolic Recoil Using Cardiovascular Magnetic Resonance Myocardial Feature Tracking. *Plos One,* 9**,** e109164.

KOWALLICK, J. T., MORTON, G., LAMATA, P., JOGIYA, R., KUTTY, S., LOTZ, J., HASENFUSS, G., NAGEL, E., CHIRIBIRI, A. & SCHUSTER, A. 2015. Inter-study reproducibility of left ventricular torsion and torsion rate quantification using MR myocardial feature tracking. *J Magn Reson Imaging*.

KOWALLICK, J. T., MORTON, G., LAMATA, P., JOGIYA, R., KUTTY, S., LOTZ, J., HASENFUSS, G., NAGEL, E., CHIRIBIRI, A. & SCHUSTER, A. 2016. Inter-study reproducibility of left ventricular torsion and torsion rate quantification using MR myocardial feature tracking. *J Magn Reson Imaging,* 43**,** 128-37.

KRAMER, C. M., BARKHAUSEN, J., FLAMM, S. D., KIM, R. J. & NAGEL, E. 2013. Standardized cardiovascular magnetic resonance (CMR) protocols 2013 update. *J Cardiovasc Magn Reson,* 15**,** 91.

KRITTAYAPHONG, R., BOONYASIRINANT, T., CHAITHIRAPHAN, V., MANEESAI, A., SAIVIROONPORN, P., NAKYEN, S., THANAPIBOONPOL, P., YINDEENGAM, A. & UDOMPANTURAK, S. 2010. Prognostic value of late gadolinium enhancement in hypertensive patients with known or suspected coronary artery disease. *Int J Cardiovasc Imaging,* 26 Suppl 1**,** 123-31.

KUO, P. H., KANAL, E., ABU-ALFA, A. K. & COWPER, S. E. 2007. Gadolinium-based MR contrast agents and nephrogenic systemic fibrosis. *Radiology,* 242**,** 647-9.

LANDMESSER, U., DIKALOV, S., PRICE, S. R., MCCANN, L., FUKAI, T., HOLLAND, S. M., MITCH, W. E. & HARRISON, D. G. 2003. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest,* 111**,** 1201-9.

LARSEN, K., HANSEN, T. B. & SOBALLE, K. 2008. Hip arthroplasty patients benefit from accelerated perioperative care and rehabilitation: a quasi-experimental study of 98 patients. *Acta orthopaedica,* 79**,** 624-30.

LAW, M. R., MORRIS, J. K. & WALD, N. J. 2009. Use of blood pressure lowering drugs in the prevention of cardiovascular disease: meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *Bmj,* 338**,** b1665.

LE NOBLE, J. L., TANGELDER, G. J., SLAAF, D. W., VAN ESSEN, H., RENEMAN, R. S. & STRUYKER-BOUDIER, H. A. 1990. A functional morphometric study of the cremaster muscle microcirculation in young spontaneously hypertensive rats. *Journal of Hypertension,* 8**,** 741-8.

LEE, J., CHOI, K. C., YEUM, C.-H., KIM, W., YOO, K., PARK, J. W. & YOON, P. J. 1995. Impairment of endothelium—dependent vasorelaxation in chronic two-kidney, one clip hypertensive rats. *Nephrology Dialysis Transplantation,* 10**,** 619-623.

LEISHMAN, A. 1959. Hypertension—treated and untreated. *Bmj,* 1**,** 1361.

LEUNG, D. W., CACHIANES, G., KUANG, W. J., GOEDDEL, D. V. & FERRARA, N. 1989. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science,* 246**,** 1306-9.

LEVY, B. I., DURIEZ, M. & SAMUEL, J. L. 2001. Coronary microvasculature alteration in hypertensive rats. Effect of treatment with a diuretic and an ACE inhibitor. *Am J Hypertens,* 14**,** 7-13.

LEVY, D., GARRISON, R. J., SAVAGE, D. D., KANNEL, W. B. & CASTELLI, W. P. 1990. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med,* 322**,** 1561-6.

LEWINGTON, S., CLARKE, R., QIZILBASH, N., PETO, R. & COLLINS, R. 2002. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet.,* 360**,** 1903-13.

LI, B., SHARPE, E. E., MAUPIN, A. B., TELERON, A. A., PYLE, A. L., CARMELIET, P. & YOUNG, P. P. 2006. VEGF and PlGF promote adult vasculogenesis by enhancing EPC recruitment and vessel formation at the site of tumor neovascularization. *FASEB J,* 20**,** 1495-7.

LIN, S. Z., SPOSITO, N., PETTERSEN, S., RYBACKI, L., MCKENNA, E., PETTIGREW, K. & FENSTERMACHER, J. 1990. Cerebral Capillary Bed Structure of Normotensive and Chronically Hypertensive Rats. *Microvascular Research,* 40**,** 341-357.

LINDER, L., KIOWSKI, W., BÜHLER, F. R. & LÜSCHER, T. F. 1990. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo. Blunted response in essential hypertension. *Circulation,* 81**,** 1762-1767.

LINDSTEDT, I. H., EDVINSSON, M. L. & EDVINSSON, L. 2006. Reduced responsiveness of cutaneous microcirculation in essential hypertension--a pilot study. *Blood Press,* 15**,** 275-80.

LITHELL, H., HANSSON, L., SKOOG, I., ELMFELDT, D., HOFMAN, A., OLOFSSON, B., TRENKWALDER, P. & ZANCHETTI, A. 2003. The Study on Cognition and Prognosis in the Elderly (SCOPE): principal results of a randomized double-blind intervention trial. *Journal of Hypertension,* 21**,** 875-86.

LIU, L., ZHANG, Y., LIU, G., LI, W., ZHANG, X. & ZANCHETTI, A. 2005. The Felodipine Event Reduction (FEVER) Study: a randomized long-term placebo-controlled trial in Chinese hypertensive patients. *Journal of Hypertension,* 23**,** 2157-72.

LJUNGMAN, S., WIKSTRAND, J., HARTFORD, M. & BERGLUND, G. 1996. Urinary albumin excretion--a predictor of risk of cardiovascular disease. A prospective 10-year follow-up of middle-aged nondiabetic normal and hypertensive men. *Am J Hypertens,* 9**,** 770-8.

LOCSEI, Z., RACZ, K., PATOCS, A., KOVACS, G. L. & TOLDY, E. 2009. Influence of sampling and storage conditions on plasma renin activity and plasma renin concentration. *Clin Chim Acta,* 402**,** 203-5.

LUND-JOHANSEN, P. 1991. Twenty-year follow-up of hemodynamics in essential hypertension during rest and exercise. *Hypertension,* 18**,** Iii54-61. (Abstract).

LUSCHER, T. F., PIEPER, M., TENDERA, M., VROLIX, M., RUTSCH, W., VAN DEN BRANDEN, F., GIL, R., BISCHOFF, K. O., HAUDE, M., FISCHER, D., MEINERTZ, T. & MUNZEL, T. 2009. A randomized placebo-controlled study on the effect of nifedipine on coronary endothelial function and plaque formation in patients with coronary artery disease: the ENCORE II study. *Eur Heart J,* 30**,** 1590-7.

MACEIRA, A. M., PRASAD, S. K., KHAN, M. & PENNELL, D. J. 2006. Normalized left ventricular systolic and diastolic function by steady state free precession cardiovascular magnetic resonance. *Journal of Cardiovascular Magnetic Resonance,* 8**,** 417-426.

MACENEANEY, O. J., DESOUZA, C. A., WEIL, B. R., KUSHNER, E. J., VAN GUILDER, G. P., MESTEK, M. L., GREINER, J. J. & STAUFFER, B. L. 2011. Prehypertension and endothelial progenitor cell function. *J Hum Hypertens,* 25**,** 57-62.

MACKENZIE, I. S. & BROWN, M. J. 2009. Molecular and clinical investigations in patients with low-renin hypertension. *Clin Exp Nephrol,* 13**,** 1-8.

MAGUIRE, S. M., NUGENT, A. G., MCGURK, C., JOHNSTON, G. D. & NICHOLLS, D. P. 1998. Abnormal vascular responses in human chronic cardiac failure are both endothelium dependent and endothelium independent. *Heart,* 80**,** 141-145.

MANCIA, G., FAGARD, R., NARKIEWICZ, K., REDON, J., ZANCHETTI, A., BOHM, M., CHRISTIAENS, T., CIFKOVA, R., DE BACKER, G., DOMINICZAK, A., GALDERISI, M., GROBBEE, D. E., JAARSMA, T., KIRCHHOF, P., KJELDSEN, S. E., LAURENT, S., MANOLIS, A. J., NILSSON, P. M., RUILOPE, L. M., SCHMIEDER, R. E., SIRNES, P. A.,

SLEIGHT, P., VIIGIMAA, M., WAEBER, B., ZANNAD, F., REDON, J., DOMINICZAK, A., NARKIEWICZ, K., NILSSON, P. M., BURNIER, M., VIIGIMAA, M., AMBROSIONI, E., CAUFIELD, M., COCA, A., OLSEN, M. H., SCHMIEDER, R. E., TSIOUFIS, C., VAN DE BORNE, P., ZAMORANO, J. L., ACHENBACH, S., BAUMGARTNER, H., BAX, J. J., BUENO, H., DEAN, V., DEATON, C., EROL, C., FAGARD, R., FERRARI, R., HASDAI, D., HOES, A. W., KIRCHHOF, P., KNUUTI, J., KOLH, P., LANCELLOTTI, P., LINHART, A., NIHOYANNOPOULOS, P., PIEPOLI, M. F., PONIKOWSKI, P., SIRNES, P. A., TAMARGO, J. L., TENDERA, M., TORBICKI, A., WIJNS, W., WINDECKER, S., CLEMENT, D. L., COCA, A., GILLEBERT, T. C., TENDERA, M., ROSEI, E. A., AMBROSIONI, E., ANKER, S. D., BAUERSACHS, J., HITIJ, J. B., CAULFIELD, M., DE BUYZERE, M., DE GEEST, S., DERUMEAUX, G. A., ERDINE, S., FARSANG, C., FUNCK-BRENTANO, C., GERC, V., GERMANO, G., GIELEN, S., HALLER, H., HOES, A. W., JORDAN, J., KAHAN, T., KOMAJDA, M., LOVIC, D., MAHRHOLDT, H., OLSEN, M. H., OSTERGREN, J., PARATI, G., PERK, J., POLONIA, J., POPESCU, B. A., REINER, Z., RYDEN, L., SIRENKO, Y., STANTON, A., et al. 2013. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J,* 34**,** 2159-219.

MANCINI, G. B., HENRY, G. C., MACAYA, C., O'NEILL, B. J., PUCILLO, A. L., CARERE, R. G., WARGOVICH, T. J., MUDRA, H., LUSCHER, T. F., KLIBANER, M. I., HABER, H. E., UPRICHARD, A. C., PEPINE, C. J. & PITT, B. 1996. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study. *Circulation,* 94**,** 258-65.

MARQUES DA SILVA, P., HAAG, U., GUEST, J. F., BRAZIER, J. E. & SORO, M. 2015. Health-related quality of life impact of a triple combination of olmesartan medoxomil, amlodipine besylate and hydrochlorotiazide in subjects with hypertension. *Health Qual Life Outcomes,* 13**,** 24.

MATTOCK, M. B., BARNES, D. J., VIBERTI, G., KEEN, H., BURT, D., HUGHES, J. M., FITZGERALD, A. P., SANDHU, B. & JACKSON, P. G. 1998.

Microalbuminuria and coronary heart disease in NIDDM: an incidence study. *Diabetes,* 47**,** 1786-92.

MAZZAGLIA, G., MANTOVANI, L. G., STURKENBOOM, M. C., FILIPPI, A., TRIFIRO, G., CRICELLI, C., BRIGNOLI, O. & CAPUTI, A. P. 2005. Patterns of persistence with antihypertensive medications in newly diagnosed hypertensive patients in Italy: a retrospective cohort study in primary care. *Journal of Hypertension,* 23**,** 2093-100.

MCALLISTER, A., ATKINSON, A., JOHNSTON, G., HADDEN, D., BELL, P. & MCCANCE, D. 1999. Basal nitric oxide production is impaired in offspring of patients with essential hypertension. *Clinical science,* 97**,** 141-147.

MCCALL, D. O., MCGARTLAND, C. P., MCKINLEY, M. C., PATTERSON, C. C., SHARPE, P., MCCANCE, D. R., YOUNG, I. S. & WOODSIDE, J. V. 2009. Dietary intake of fruits and vegetables improves microvascular function in hypertensive subjects in a dose-dependent manner. *Circulation,* 119**,** 2153-60.

MCCORVEY, E., JR., WRIGHT, J. T., JR., CULBERT, J. P., MCKENNEY, J. M., PROCTOR, J. D. & ANNETT, M. P. 1993. Effect of hydrochlorothiazide, enalapril, and propranolol on quality of life and cognitive and motor function in hypertensive patients. *Clin Pharm,* 12**,** 300-5.

MEININGER, G. A., FEHR, K. L., YATES, M. B., BORDERS, J. L. & GRANGER, H. J. 1986. Hemodynamic characteristics of the intestinal microcirculation in renal hypertension. *Hypertension,* 8**,** 66-75.

MENA-MARTIN, F. J., MARTIN-ESCUDERO, J. C., SIMAL-BLANCO, F., CARRETERO-ARES, J. L., ARZUA-MOURONTE, D. & HERREROS-FERNANDEZ, V. 2003. Health-related quality of life of subjects with known and unknown hypertension: results from the population-based Hortega study. *Journal of Hypertension,* 21**,** 1283-9.

MERVAALA, E. M., TERAVAINEN, T. L., MALMBERG, L., LAAKSO, J., VAPAATALO, H. & KARPPANEN, H. 1997. Cardiovascular effects of a low-dose combination of ramipril and felodipine in spontaneously hypertensive rats. *Br J Pharmacol,* 121**,** 503-10.

MICHEL, F., MAN, G., MAN, R. & VANHOUTTE, P. 2008. Hypertension and the absence of EDHF-mediated responses favour endothelium-dependent contractions in renal arteries of the rat. *British J Pharmacol,* 155**,** 217-226.

MILLGÅRD, J. & LIND, L. 1998. Acute hypertension impairs endothelium-dependent vasodilatation. *Clinical science,* 94**,** 601-607.

MIOSHI, E., DAWSON, K., MITCHELL, J., ARNOLD, R. & HODGES, J. R. 2006. The Addenbrooke's Cognitive Examination Revised (ACE-R): a brief cognitive test battery for dementia screening. *Int J Geriatr Psychiatry,* 21**,** 1078-85.

MIZUGUCHI, Y., OISHI, Y., MIYOSHI, H., IUCHI, A., NAGASE, N. & OKI, T. 2009. Beneficial effects of telmisartan on left ventricular structure and function in patients with hypertension determined by two-dimensional strain imaging. *Journal of Hypertension,* 27**,** 1892-9.

MOLLER, L., KRISTENSEN, T. S. & HOLLNAGEL, H. 1996. Self rated health as a predictor of coronary heart disease in Copenhagen, Denmark. *J Epidemiol Community Health,* 50**,** 423-8.

MOTZ, W. & STRAUER, B. E. 1996. Improvement of coronary flow reserve after long-term therapy with enalapril. *Hypertension,* 27**,** 1031-8.

MOURAD, J. J. & LEVY, B. I. 2011. Mechanisms of Antiangiogenic-Induced Arterial Hypertension. *Current Hypertension Reports,* 13**,** 289-293.

MUIESAN, M. L., SALVETTI, M., RIZZONI, D., CASTELLANO, M., DONATO, F. & AGABITI-ROSEI, E. 1995. Association of change in left ventricular mass with prognosis during long-term antihypertensive treatment. *Journal of Hypertension,* 13**,** 1091-5.

MULVANY, M. J. & AALKJAER, C. 1990. Structure and function of small arteries. *Physiol Rev,* 70**,** 921-61.

MULVANY, M. J., HANSEN, O. K. & AALKJAER, C. 1978. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. *Circ Res,* 43**,** 854-64.

NASCIMENTO, A. R., LESSA, M. A., SABINO, B., BOUSQUET, P. & TIBIRICA, E. 2010. Microvascular effects of centrally acting antihypertensive drugs in spontaneously hypertensive rats. *J Cardiovasc Pharmacol,* 55**,** 240-7.

NEGISHI, K., NEGISHI, T., HALUSKA, B. A., HARE, J. L., PLANA, J. C. & MARWICK, T. H. 2014. Use of speckle strain to assess left ventricular responses to cardiotoxic chemotherapy and cardioprotection. *Eur Heart J Cardiovasc Imaging,* 15**,** 324-331.

NEGISHI, K., NEGISHI, T., HARE, J. L., HALUSKA, B. A., PLANA, J. C. & MARWICK, T. H. 2013. Independent and Incremental Value of Deformation Indices for Prediction of Trastuzumab-Induced Cardiotoxicity. *Journal of the American Society of Echocardiography,* 26**,** 493-498.

NICE 2011. Hypertension: Clinical Management of Primary Hypertension in Adults (Update). NICE.

NICHOLS, W. W. 2005. Clinical measurement of arterial stiffness obtained from noninvasive pressure waveforms. *Am J Hypertens,* 18**,** 3S-10S.

NIMMRICH, V. & ECKERT, A. 2013. Calcium channel blockers and dementia. *Br J Pharmacol,* 169**,** 1203-10.

NISHIZAKA, M. K., ZAMAN, M. A., GREEN, S. A., RENFROE, K. Y. & CALHOUN, D. A. 2004. Impaired endothelium-dependent flow-mediated vasodilation in hypertensive subjects with hyperaldosteronism. *Circulation,* 109**,** 2857-61.

NOHRIA, A., GERHARD-HERMAN, M., CREAGER, M. A., HURLEY, S., MITRA, D. & GANZ, P. 2006. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. *J Appl Physiol (1985),* 101**,** 545-8.

NOON, J. P., WALKER, B. R., WEBB, D. J., SHORE, A. C., HOLTON, D. W., EDWARDS, H. V. & WATT, G. C. 1997. Impaired microvascular dilatation and capillary rarefaction in young adults with a predisposition to high blood pressure. *J Clin Invest,* 99**,** 1873-9.

O'BRIEN, E., ASMAR, R., BEILIN, L., IMAI, Y., MALLION, J. M., MANCIA, G., MENGDEN, T., MYERS, M., PADFIELD, P., PALATINI, P., PARATI, G., PICKERING, T., REDON, J., STAESSEN, J., STERGIOU, G., VERDECCHIA, P. & EUROPEAN SOCIETY OF HYPERTENSION WORKING GROUP ON BLOOD PRESSURE, M. 2003. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *Journal of Hypertension,* 21**,** 821-48.

OHIRA, T., TANIGAWA, T., TABATA, M., IMANO, H., KITAMURA, A., KIYAMA, M., SATO, S., OKAMURA, T., CUI, R., KOIKE, K. A., SHIMAMOTO, T. & ISO, H. 2009. Effects of Habitual Alcohol Intake on Ambulatory Blood Pressure, Heart Rate, and Its Variability Among Japanese Men. *Hypertension,* 53**,** 13-19.

OKONOFUA, E. C., SIMPSON, K. N., JESRI, A., REHMAN, S. U., DURKALSKI, V. L. & EGAN, B. M. 2006. Therapeutic inertia is an impediment to

achieving the Healthy People 2010 blood pressure control goals. *Hypertension,* 47**,** 345-51.

OMAR, H. R., KOMAROVA, I., EL-GHONEMI, M., FATHY, A., RASHAD, R., ABDELMALAK, H. D., YERRAMADHA, M. R., ALI, Y., HELAL, E. & CAMPORESI, E. M. 2012. Licorice abuse: time to send a warning message. *Ther Adv Endocrinol Metab,* 3**,** 125-38.

OMURA, M., SAITO, J., YAMAGUCHI, K., KAKUTA, Y. & NISHIKAWA, T. 2004. Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. *Hypertens Res,* 27**,** 193-202.

OSLER, W. 1912. An Address ON HIGH BLOOD PRESSURE: ITS ASSOCIATIONS, ADVANTAGES, AND DISADVANTAGES: Delivered at the Glasgow Southern Medical Society. *Bmj,* 2**,** 1173-7.

OSMAN, N. F., SAMPATH, S., ATALAR, E. & PRINCE, J. L. 2001. Imaging longitudinal cardiac strain on short-axis images using strain-encoded MRI. *Magn Reson Med,* 46**,** 324-34.

OSTERLOH, I. 1989. The safety of amlodipine. *Am Heart J,* 118**,** 1114-20.

PABUCCU, T., BARIS, N., OZPELIT, E., AKDENIZ, B. & GUNERI, S. 2012. The relationship between resistant hypertension and arterial stiffness. *Clin Exp Hypertens,* 34**,** 57-62.

PANZA, J. A., CASINO, P. R., KILCOYNE, C. M. & QUYYUMI, A. A. 1993. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. *Circulation,* 87**,** 1468-1474.

PANZA, J. A., QUYYUMI, A. A., BRUSH JR, J. E. & EPSTEIN, S. E. 1990. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *New Eng J Med,* 323**,** 22-27.

PARATI, G., POMIDOSSI, G., ALBINI, F., MALASPINA, D. & MANCIA, G. 1987. Relationship of 24-hour blood pressure mean and variability to severity of target-organ damage in hypertension. *Journal of Hypertension,* 5**,** 93-8.

PATRICK, D. L. & DEYO, R. A. 1989. Generic and disease-specific measures in assessing health status and quality of life. *Med Care,* 27**,** S217-32.

PENNA, G. L., GARBERO RDE, F., NEVES, M. F., OIGMAN, W., BOTTINO, D. A. & BOUSKELA, E. 2008. Treatment of essential hypertension does not normalize capillary rarefaction. *Clinics (Sao Paulo),* 63**,** 613-8.

PERSU, A., JIN, Y., BAELEN, M., VINK, E., VERLOOP, W. L., SCHMIDT, B., BLICHER, M. K., SEVERINO, F., WUERZNER, G., TAYLOR, A., PECHERE-BERTSCHI, A., JOKHAJI, F., FADL ELMULA, F. E., ROSA, J., CZARNECKA, D., EHRET, G., KAHAN, T., RENKIN, J., WIDIMSKY, J., JR., JACOBS, L., SPIERING, W., BURNIER, M., MARK, P. B., MENNE, J., OLSEN, M. H., BLANKESTIJN, P. J., KJELDSEN, S., BOTS, M. L. & STAESSEN, J. A. 2014. Eligibility for renal denervation: experience at 11 European expert centers. *Hypertension,* 63**,** 1319-25.

PERTICONE, F., CERAVOLO, R., PUJIA, A., VENTURA, G., IACOPINO, S., SCOZZAFAVA, A., FERRARO, A., CHELLO, M., MASTROROBERTO, P., VERDECCHIA, P. & SCHILLACI, G. 2001. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation,* 104**,** 191-6.

PHILLIPS, L. S., BRANCH, W. T., COOK, C. B., DOYLE, J. P., EL-KEBBI, I. M., GALLINA, D. L., MILLER, C. D., ZIEMER, D. C. & BARNES, C. S. 2001. Clinical inertia. *Ann Intern Med,* 135**,** 825-34.

PICKERING, T. G., HALL, J. E., APPEL, L. J., FALKNER, B. E., GRAVES, J., HILL, M. N., JONES, D. W., KURTZ, T., SHEPS, S. G. & ROCCELLA, E. J. 2005. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation,* 111**,** 697-716.

POULTER, N. R., BORGHI, C., PARATI, G., PATHAK, A., TOLI, D., WILLIAMS, B. & SCHMIEDER, R. E. 2020. Medication adherence in hypertension. *Journal of Hypertension,* 38**,** 579-587.

PRASAD, A., DUNNILL, G. S., MORTIMER, P. S. & MACGREGOR, G. A. 1995. Capillary rarefaction in the forearm skin in essential hypertension. *Journal of Hypertension,* 13**,** 265-8.

PRASAD, A., TUPAS-HABIB, T., SCHENKE, W. H., MINCEMOYER, R., PANZA, J. A., WACLAWIN, M. A., ELLAHHAM, S. & QUYYUMI, A. A. 2000. Acute and chronic angiotensin-1 receptor antagonism reverses endothelial dysfunction in atherosclerosis. *Circulation,* 101**,** 2349-54.

PREWITT, R. L., CHEN, II & DOWELL, R. 1982. Development of microvascular rarefaction in the spontaneously hypertensive rat. *Am J Physiol,* 243**,** H243-51.

PU, Q., LAROUCHE, I. & SCHIFFRIN, E. L. 2003. Effect of dual angiotensin converting enzyme/neutral endopeptidase inhibition, angiotensin converting enzyme inhibition, or AT1 antagonism on coronary microvasculature in spontaneously hypertensive rats. *Am J Hypertens,* 16**,** 931-7.

RAKUSAN, K., CICUTTI, N., KAZDA, S. & TUREK, Z. 1994. Effect of nifedipine on coronary capillary geometry in normotensive and hypertensive rats. *Hypertension,* 24**,** 205-11.

RAKUSAN, K., CICUTTI, N., MAURIN, A., GUEZ, D. & SCHIAVI, P. 2000. The effect of treatment with low dose ACE inhibitor and/or diuretic on coronary microvasculature in stroke-prone spontaneously hypertensive rats. *Microvasc Res,* 59**,** 243-54.

REEVE, B. B., WYRWICH, K. W., WU, A. W., VELIKOVA, G., TERWEE, C. B., SNYDER, C. F., SCHWARTZ, C., REVICKI, D. A., MOINPOUR, C. M., MCLEOD, L. D., LYONS, J. C., LENDERKING, W. R., HINDS, P. S., HAYS, R. D., GREENHALGH, J., GERSHON, R., FEENY, D., FAYERS, P. M., CELLA, D., BRUNDAGE, M., AHMED, S., AARONSON, N. K. & BUTT, Z. 2013. ISOQOL recommends minimum standards for patient-reported outcome measures used in patient-centered outcomes and comparative effectiveness research. *Qual Life Res,* 22**,** 1889-905.

REICHEK, N., DEVEREUX, R. B., ROCHA, R. A., HILKERT, R., HALL, D., PURKAYASTHA, D. & PITT, B. 2009. Magnetic resonance imaging left ventricular mass reduction with fixed-dose angiotensin-converting enzyme inhibitor-based regimens in patients with high-risk hypertension. *Hypertension,* 54**,** 731-7.

REITAN, R. M. 1958. Validity of the Trail Making test as an indicator of organic brain damage. *Perceptual and Motor Skills,* 8**,** 271-276.

REITAN, R. M. & WOLFSON, D. 2004. The Trail Making Test as an initial screening procedure for neuropsychological impairment in older children. *Arch Clin Neuropsychol,* 19**,** 281-8.

REYNOLDS, T., VIVIAN-SMITH, A., JHANJI, S. & PEARSE, R. M. 2013. Observational study of the effects of age, diabetes mellitus, cirrhosis and chronic kidney disease on sublingual microvascular flow. *Perioper Med (Lond),* 2**,** 7.

RITT, M., HARAZNY, J. M., OTT, C., SCHLAICH, M. P., SCHNEIDER, M. P., MICHELSON, G. & SCHMIEDER, R. E. 2008. Analysis of retinal arteriolar structure in never-treated patients with essential hypertension. *Journal of Hypertension,* 26**,** 1427-34.

RIZZONI, D., PORTERI, E., BOARI, G. E., DE CIUCEIS, C., SLEIMAN, I., MUIESAN, M. L., CASTELLANO, M., MICLINI, M. & AGABITI-ROSEI, E. 2003. Prognostic significance of small-artery structure in hypertension. *Circulation,* 108**,** 2230-5.

RIZZONI, D., PORTERI, E., CASTELLANO, M., BETTONI, G., MUIESAN, M. L., MUIESAN, P., GIULINI, S. M. & AGABITI-ROSEI, E. 1996. Vascular hypertrophy and remodeling in secondary hypertension. *Hypertension,* 28**,** 785-90.

RIZZONI, D., PORTERI, E., DUSE, S., DE CIUCEIS, C., ROSEI, C. A., LA BORIA, E., SEMERARO, F., COSTAGLIOLA, C., SEBASTIANI, A., DANZI, P., TIBERIO, G. A., GIULINI, S. M., DOCCHIO, F., SANSONI, G., SARKAR, A. & ROSEI, E. A. 2012. Relationship between media-to-lumen ratio of subcutaneous small arteries and wall-to-lumen ratio of retinal arterioles evaluated noninvasively by scanning laser Doppler flowmetry. *Journal of Hypertension,* 30**,** 1169-75.

ROBERT, N., WONG, G. W. & WRIGHT, J. M. 2010. Effect of cyclosporine on blood pressure. *Cochrane Database Syst Rev.***,** CD007893.

ROCA-CUSACHS, A., AMETLLA, J., CALERO, S., COMAS, O., FERNANDEZ, M., LOSPAUS, R., MONMANY, J., MORERA, R., MUÑOZ, M. & PERIS, A. 1992. Quality of life in arterial hypertension. *Medicina Clinica,* 98**,** 486-490.

ROCA-CUSACHS, A., BADIA, X., DALFO, A., GASCON, G., ABELLAN, J., LAHOZ, R., VARELA, C. & VELASCO, O. 2003. [Relationship between clinical and therapeutic variables and health-related quality of life in patients with hypertension. MINICHAL Study]. *Medicina clinica,* 121**,** 12-7.

ROCA-CUSACHS, A., DALFO, A., BADIA, X., ARISTEGUI, I. & ROSET, M. 2001. Relation between clinical and therapeutic variables and quality of life in hypertension. *Journal of Hypertension,* 19**,** 1913-9.

ROSANO, G. M. C., TAMARGO, J., KJELDSEN, K. P., LAINSCAK, M., AGEWALL, S., ANKER, S. D., CECONI, C., COATS, A. J. S., DREXEL, H., FILIPPATOS, G., KASKI, J. C., LUND, L., NIESSNER, A.,

PONIKOWSKI, P., SAVARESE, G., SCHMIDT, T. A., SEFEROVIC, P., WASSMANN, S., WALTHER, T. & LEWIS, B. S. 2018. Expert consensus document on the management of hyperkalaemia in patients with cardiovascular disease treated with renin angiotensin aldosterone system inhibitors: coordinated by the Working Group on Cardiovascular Pharmacotherapy of the European Society of Cardiology. *Eur Heart J Cardiovasc Pharmacother,* 4**,** 180-188.

ROSEI, E. A., RIZZONI, D., CASTELLANO, M., PORTERI, E., ZULLI, R., MUIESAN, M. L., BETTONI, G., SALVETTI, M., MUIESAN, P. & GIULINI, S. M. 1995. Media: lumen ratio in human small resistance arteries is related to forearm minimal vascular resistance. *Journal of Hypertension,* 13**,** 341-7.

ROSSI, E. & RASORI, G. 1955. Giovanni Rasori; 1766-1837 or Italian medicine in transition. *Bulletin of the history of medicine,* 29**,** 116-33.

ROSSI, G. P., BERNINI, G., CALIUMI, C., DESIDERI, G., FABRIS, B., FERRI, C., GANZAROLI, C., GIACCHETTI, G., LETIZIA, C., MACCARIO, M., MALLAMACI, F., MANNELLI, M., MATTARELLO, M. J., MORETTI, A., PALUMBO, G., PARENTI, G., PORTERI, E., SEMPLICINI, A., RIZZONI, D., ROSSI, E., BOSCARO, M., PESSINA, A. C. & MANTERO, F. 2006. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol,* 48**,** 2293-300.

ROY, J. W. & MAYROVITZ, H. N. 1984. Microvascular pressure, flow, and resistance in spontaneously hypertensive rats. *Hypertension,* 6**,** 877-86.

RUDOLPH, A., ABDEL-ATY, H., BOHL, S., BOYE, P., ZAGROSEK, A., DIETZ, R. & SCHULZ-MENGER, J. 2009. Noninvasive detection of fibrosis applying contrast-enhanced cardiac magnetic resonance in different forms of left ventricular hypertrophy relation to remodeling. *J Am Coll Cardiol,* 53**,** 284-91.

RUEDEMANN, A. 1933. Conjunctival vessels. *Jama,* 101**,** 1477-1481.

RUSSEL, I. K., GOTTE, M. J., BRONZWAER, J. G., KNAAPEN, P., PAULUS, W. J. & VAN ROSSUM, A. C. 2009. Left ventricular torsion: an expanding role in the analysis of myocardial dysfunction. *JACC Cardiovasc Imaging,* 2**,** 648-55.

SABINO, B., LESSA, M. A., NASCIMENTO, A. R., RODRIGUES, C. A., HENRIQUES, M., GARZONI, L. R., LEVY, B. I. & TIBIRICA, E. 2008.

Effects of antihypertensive drugs on capillary rarefaction in spontaneously hypertensive rats: intravital microscopy and histologic analysis. *J Cardiovasc Pharmacol,* 51**,** 402-9.

SABRI, A., SAMUEL, J. L., MAROTTE, F., POITEVIN, P., RAPPAPORT, L. & LEVY, B. I. 1998. Microvasculature in angiotensin II-dependent cardiac hypertrophy in the rat. *Hypertension,* 32**,** 371-5.

SALGUERO, G., AKIN, E., TEMPLIN, C., KOTLARZ, D., DOERRIES, C., LANDMESSER, U., GROTE, K. & SCHIEFFER, B. 2008. Renovascular hypertension by two-kidney one-clip enhances endothelial progenitor cell mobilization in a p47phox-dependent manner. *Journal of Hypertension,* 26**,** 257-68.

SALTIJERAL, A., PEREZ DE ISLA, L., VERAS, K., FERNANDEZ MDE, J., GORISSEN, W., REMENTERIA, J., ALMERIA, C., RODRIGO, J. L., FERNANDEZ-GOLFIN, C., MARCOS-ALBERCA, P., MACAYA, C. & ZAMORANO, J. 2010. Myocardial strain characterization in different left ventricular adaptative responses to high blood pressure: a study based on 3D-wall motion tracking analysis. *Echocardiography,* 27**,** 1238-46.

SANZ-ROSA, D., OUBINA, M. P., CEDIEL, E., DE LAS HERAS, N., ARAGONCILLO, P., BALFAGON, G., CACHOFEIRO, V. & LAHERA, V. 2005. Eplerenone reduces oxidative stress and enhances eNOS in SHR: vascular functional and structural consequences. *Antioxid Redox Signal,* 7**,** 1294-301.

SCATTEIA, A., BARITUSSIO, A. & BUCCIARELLI-DUCCI, C. 2017. Strain imaging using cardiac magnetic resonance. *Heart Failure Reviews,* 22**,** 465-476.

SCHIFFRIN, E. L. 1992. Reactivity of small blood vessels in hypertension: relation with structural changes. State of the art lecture. *Hypertension,* 19**,** Ii1-9.

SCHULZ, R. B., ROSSIGNOLI, P., CORRER, C. J., FERNANDEZ-LLIMOS, F. & TONI, P. M. 2008. Validation of the short form of the Spanish hypertension quality of life questionnaire (MINICHAL) for Portuguese (Brazil). *Arq Bras Cardiol,* 90**,** 127-31.

SCHUSTER, A., HOR, K. N., KOWALLICK, J. T., BEERBAUM, P. & KUTTY, S. 2016. Cardiovascular Magnetic Resonance Myocardial Feature Tracking:

Concepts and Clinical Applications. *Circulation-Cardiovascular Imaging,* 9(4):e004077.

SEKIGUCHI, F., NAKAHIRA, T., KAWATA, K. & SUNANO, S. 2002. Responses to endothelium-derived factors and their interaction in mesenteric arteries from Wistar-Kyoto and stroke-prone spontaneously hypertensive rats. *Clinical and experimental pharmacology and physiology,* 29**,** 1066-1074.

SEKURI, C., BAYTURAN, O., GOCER, H., TAVLI, T. & TEZCAN, U. K. 2003. Effects of low-dose combination therapy with an angiotensin-converting enzyme inhibitor and a diuretic on flow-mediated vasodilation in hypertensive patients: a 6-month, single-center study. *Curr Ther Res Clin Exp,* 64**,** 715-24.

SERNE, E. H., GANS, R. O., TER MAATEN, J. C., TANGELDER, G. J., DONKER, A. J. & STEHOUWER, C. D. 2001. Impaired skin capillary recruitment in essential hypertension is caused by both functional and structural capillary rarefaction. *Hypertension,* 38**,** 238-42.

SHARMAN, J. E., MARWICK, T. H., GILROY, D., OTAHAL, P., ABHAYARATNA, W. P., STOWASSER, M. & VALUE OF CENTRAL BLOOD PRESSURE FOR, G. M. O. H. S. I. 2013. Randomized trial of guiding hypertension management using central aortic blood pressure compared with best-practice care: principal findings of the BP GUIDE study. *Hypertension,* 62**,** 1138-45.

SHAW, R. J., MCDUFFIE, J. R., HENDRIX, C. C., EDIE, A., LINDSEY-DAVIS, L. & WILLIAMS, J. W. 2013. Effects of Nurse-Managed Protocols in the Outpatient Management of adults with Chronic Conditions. *VA Evidence-based Synthesis Program Reports.* Washington (DC): Department of Veternas Affairs.

SHIMBO, D., MUNTNER, P., MANN, D., VIERA, A. J., HOMMA, S., POLAK, J. F., BARR, R. G., HERRINGTON, D. & SHEA, S. 2010. Endothelial dysfunction and the risk of hypertension: the multi-ethnic study of atherosclerosis. *Hypertension,* 55**,** 1210-6.

SHORE, A. C. 2000. Capillaroscopy and the measurement of capillary pressure. *British journal of clinical pharmacology,* 50**,** 501-13.

SHORE, A. C., SANDEMAN, D. D. & TOOKE, J. E. 1993. Effect of an increase in systemic blood pressure on nailfold capillary pressure in humans. *Am J Physiol,* 265**,** H820-3.

SIGMUND, C. D., CAREY, R. M., APPEL, L. J., ARNETT, D. K., BOSWORTH, H. B., CUSHMAN, W. C., GALIS, Z. S., GREEN PARKER, M., HALL, J. E., HARRISON, D. G., MCDONOUGH, A. A., NICASTRO, H. L., OPARIL, S., OSBORN, J. W., RAIZADA, M. K., WRIGHT, J. D. & OH, Y. S. 2020. Report of the National Heart, Lung, and Blood Institute Working Group on Hypertension: Barriers to Translation. *Hypertension,* 75**,** 902-917.

SIM, J. J., HANDLER, J., JACOBSEN, S. J. & KANTER, M. H. 2014. Systemic implementation strategies to improve hypertension: the Kaiser Permanente Southern California experience. *Can J Cardiol,* 30**,** 544-52.

SIMPSON, R. M., KEEGAN, J. & FIRMIN, D. N. 2013. MR assessment of regional myocardial mechanics. *J Magn Reson Imaging,* 37**,** 576-99.

SINCLAIR, A. M., ISLES, C. G., BROWN, I., CAMERON, H., MURRAY, G. D. & ROBERTSON, J. W. 1987. Secondary hypertension in a blood pressure clinic. *Arch Intern Med,* 147**,** 1289-93.

SINGER, G. M., IZHAR, M. & BLACK, H. R. 2002. Goal-oriented hypertension management: translating clinical trials to practice. *Hypertension,* 40**,** 464-9.

SINGH, A., STEADMAN, C. D., KHAN, J. N., HORSFIELD, M. A., BEKELE, S., NAZIR, S. A., KANAGALA, P., MASCA, N. G., CLARYSSE, P. & MCCANN, G. P. 2015. Intertechnique agreement and interstudy reproducibility of strain and diastolic strain rate at 1.5 and 3 Tesla: a comparison of feature-tracking and tagging in patients with aortic stenosis. *J Magn Reson Imaging,* 41**,** 1129-37.

SKOOG, I., LERNFELT, B., LANDAHL, S., PALMERTZ, B., ANDREASSON, L. A., NILSSON, L., PERSSON, G., ODEN, A. & SVANBORG, A. 1996. 15-year longitudinal study of blood pressure and dementia. *Lancet,* 347**,** 1141-5.

SLAAF, D. W., TANGELDER, G. J., RENEMAN, R. S., JAGER, K. & BOLLINGER, A. 1987. A versatile incident illuminator for intravital microscopy. *Int J Microcirc Clin Exp,* 6**,** 391-7.

SPRONK, P. E., INCE, C., GARDIEN, M. J., MATHURA, K. R., OUDEMANS-VAN STRAATEN, H. M. & ZANDSTRA, D. F. 2002. Nitroglycerin in septic shock after intravascular volume resuscitation. *Lancet,* 360**,** 1395-6.

STAESSEN, J. A., FAGARD, R., THIJS, L., CELIS, H., ARABIDZE, G. G., BIRKENHAGER, W. H., BULPITT, C. J., DE LEEUW, P. W., DOLLERY,

C. T., FLETCHER, A. E., FORETTE, F., LEONETTI, G., NACHEV, C., O'BRIEN, E. T., ROSENFELD, J., RODICIO, J. L., TUOMILEHTO, J. & ZANCHETTI, A. 1997. Randomised double-blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. The Systolic Hypertension in Europe (Syst-Eur) Trial Investigators. *Lancet,* 350**,** 757-64.

STAESSEN, J. A., THIJISQ, L., FAGARD, R., CELIS, H., BIRKENHAGER, W. H., BULPITT, C. J., DE LEEUW, P. W., FLETCHER, A. E., FORETTE, F., LEONETTI, G., MCCORMACK, P., NACHEV, C., O'BRIEN, E., RODICIO, J. L., ROSENFELD, J., SARTI, C., TUOMILEHTO, J., WEBSTER, J., YODFAT, Y. & ZANCHETTI, A. 2004. Effects of immediate versus delayed antihypertensive therapy on outcome in the Systolic Hypertension in Europe Trial. *Journal of Hypertension,* 22**,** 847-57.

STANKEVICIUS, E., MARTINEZ, A. C., MULVANY, M. J. & SIMONSEN, U. 2002. Blunted acetylcholine relaxation and nitric oxide release in arteries from renal hypertensive rats. *Journal of hypertension,* 20**,** 1571-1579.

STANULOVIC, V., HODOLIC, M., MITSIKOSTAS, D. D. & PAPADOPOULOS, D. 2022. Drug tolerability: How much ambiguity can be tolerated? A systematic review of the assessment of tolerability in clinical studies. *Br J Clin Pharmacol,* 88**,** 551-565.

STEWART, S., CARRINGTON, M. J., SWEMMER, C. H., ANDERSON, C., KURSTJENS, N. P., AMERENA, J., BROWN, A., BURRELL, L. M., DE LOOZE, F. J., HARRIS, M., HUNG, J., KRUM, H., NELSON, M., SCHLAICH, M., STOCKS, N. P. & JENNINGS, G. L. 2012. Effect of intensive structured care on individual blood pressure targets in primary care: multicentre randomised controlled trial. *Bmj,* 345**,** e7156.

STRAIN, W. D., CHATURVEDI, N., BULPITT, C. J., RAJKUMAR, C. & SHORE, A. C. 2005. Albumin excretion rate and cardiovascular risk: could the association be explained by early microvascular dysfunction? *Diabetes,* 54**,** 1816-22.

STRUIJKER-BOUDIER, H. A., VAN ESSEN, H., FAZZI, G., DE MEY, J. G., QIU, H. Y. & LEVY, B. I. 1996. Disproportional arterial hypertrophy in hypertensive mRen-2 transgenic rats. *Hypertension,* 28**,** 779-84.

STUMP, M. M., JORDAN, G. L., JR., DEBAKEY, M. E. & HALPERT, B. 1963. Endothelium Grown from Circulating Blood on Isolated Intravascular Dacron Hub. *Am J Pathol,* 43**,** 361-7.

SUGIURA, T., KONDO, T., KUREISHI-BANDO, Y., NUMAGUCHI, Y., YOSHIDA, O., DOHI, Y., KIMURA, G., UEDA, R., RABELINK, T. J. & MUROHARA, T. 2008. Nifedipine improves endothelial function: role of endothelial progenitor cells. *Hypertension,* 52**,** 491-8.

SUN, C., LADORES, C., HONG, J., NGUYEN, D. Q., CHUA, J., TING, D., SCHMETTERER, L., WONG, T. Y., CHENG, C. Y. & TAN, A. C. S. 2020. Systemic hypertension associated retinal microvascular changes can be detected with optical coherence tomography angiography. *Sci Rep,* 10**,** 9580.

SVARDSUDD, K. & TIBBLIN, G. 1990. Is quality of life affecting survival? The study of men born in 1913. *Scand J Prim Health Care Suppl,* 1**,** 55-60.

TEDESCO, M. A., RATTI, G., MENNELLA, S., MANZO, G., GRIECO, M., RAINONE, A. C., IARUSSI, D. & IACONO, A. 1999. Comparison of losartan and hydrochlorothiazide on cognitive function and quality of life in hypertensive patients. *Am J Hypertens,* 12**,** 1130-4.

TERESA AGUILERA, M., DE LA SIERRA, A., COCA, A., ESTRUCH, R., FERNÁNDEZ-SOLÁ, J. & URBANO-MÁRQUEZ, A. 1999. Effect of Alcohol Abstinence on Blood Pressure: Assessment by 24-Hour Ambulatory Blood Pressure Monitoring. *Hypertension,* 33**,** 653-657.

THYBO, N. K., KORSGAARD, N., ERIKSEN, S., CHRISTENSEN, K. L. & MULVANY, M. J. 1994. Dose-dependent effects of perindopril on blood pressure and small-artery structure. *Hypertension,* 23**,** 659-66.

TIU, S. C., CHOI, C. H., SHEK, C. C., NG, Y. W., CHAN, F. K., NG, C. M. & KONG, A. P. 2005. The use of aldosterone-renin ratio as a diagnostic test for primary hyperaldosteronism and its test characteristics under different conditions of blood sampling. *J Clin Endocrinol Metab,* 90**,** 72-8.

TOMASZEWSKI, M., WHITE, C., PATEL, P., MASCA, N., DAMANI, R., HEPWORTH, J., SAMANI, N. J., GUPTA, P., MADIRA, W., STANLEY, A. & WILLIAMS, B. 2014. High rates of non-adherence to antihypertensive treatment revealed by high-performance liquid chromatography-tandem mass spectrometry (HP LC-MS/MS) urine analysis. *Heart,* 100**,** 855-61.

TOOKE, J. E., SANDEMAN, D. D. & SHORE, A. C. 1991. Microvascular hemodynamics in hypertension and diabetes. *J Cardiovasc Pharmacol,* 18 Suppl 2**,** S51-3.

TREASURE, C. B., KLEIN, J. L., VITA, J. A., MANOUKIAN, S. V., RENWICK, G. H., SELWYN, A. P., GANZ, P. & ALEXANDER, R. W. 1993. Hypertension and left ventricular hypertrophy are associated with impaired endothelium-mediated relaxation in human coronary resistance vessels. *Circulation,* 87**,** 86-93.

TREIBEL, T. A., ZEMRAK, F., SADO, D. M., BANYPERSAD, S. M., WHITE, S. K., MAESTRINI, V., BARISON, A., PATEL, V., HERREY, A. S., DAVIES, C., CAULFIELD, M. J., PETERSEN, S. E. & MOON, J. C. 2015. Extracellular volume quantification in isolated hypertension - changes at the detectable limits? *J Cardiovasc Magn Reson,* 17**,** 74.

TREU, C. M., LUPI, O., BOTTINO, D. A. & BOUSKELA, E. 2011. Sidestream dark field imaging: the evolution of real-time visualization of cutaneous microcirculation and its potential application in dermatology. *Arch Dermatol Res,* 303**,** 69-78.

TREVISOL, D. J., MOREIRA, L. B., FUCHS, F. D. & FUCHS, S. C. 2012. Health-related quality of life is worse in individuals with hypertension under drug treatment: results of population-based study. *Journal of human hypertension,* 26**,** 374-80.

TREVISOL, D. J., MOREIRA, L. B., KERKHOFF, A., FUCHS, S. C. & FUCHS, F. D. 2011. Health-related quality of life and hypertension: a systematic review and meta-analysis of observational studies. *Journal of hypertension,* 29**,** 179-88.

TRZECIAK, S., DELLINGER, R. P., PARRILLO, J. E., GUGLIELMI, M., BAJAJ, J., ABATE, N. L., ARNOLD, R. C., COLILLA, S., ZANOTTI, S., HOLLENBERG, S. M., MICROCIRCULATORY ALTERATIONS IN, R. & SHOCK, I. 2007. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Annals of emergency medicine,* 49**,** 88-98, 98.e1-2.

TUREK, Z., KUBAT, K., KAZDA, S., HOOFD, L. & RAKUSAN, K. 1987. Improved myocardial capillarisation in spontaneously hypertensive rats treated with nifedipine. *Cardiovasc Res,* 21**,** 725-9.

TZEMOS, N., LIM, P. O. & MACDONALD, T. M. 2001. Nebivolol reverses endothelial dysfunction in essential hypertension: a randomized, double-blind, crossover study. *Circulation,* 104**,** 511-4.

TZOURIO, C., ANDERSON, C., CHAPMAN, N., WOODWARD, M., NEAL, B., MACMAHON, S., CHALMERS, J. & GROUP, P. C. 2003. Effects of blood pressure lowering with perindopril and indapamide therapy on dementia and cognitive decline in patients with cerebrovascular disease. *Arch Intern Med,* 163**,** 1069-75.

UNGER, T., MATTFELDT, T., LAMBERTY, V., BOCK, P., MALL, G., LINZ, W., SCHOLKENS, B. A. & GOHLKE, P. 1992. Effect of early onset angiotensin converting enzyme inhibition on myocardial capillaries. *Hypertension,* 20**,** 478-82.

VALDERAS, J. M. & ALONSO, J. 2008. Patient reported outcome measures: a model-based classification system for research and clinical practice. *Quality of life research,* 17**,** 1125-35.

VALDERAS, J. M., FERRER, M., MENDIVIL, J., GARIN, O., RAJMIL, L., HERDMAN, M., ALONSO, J. & SCIENTIFIC COMMITTEE ON "PATIENT-REPORTED OUTCOMES" OF THE, I. N. 2008. Development of EMPRO: a tool for the standardized assessment of patient-reported outcome measures. *Value Health,* 11**,** 700-8.

VALLANCE, P. & CHAN, N. 2001. Endothelial function and nitric oxide: clinical relevance. *Heart,* 85**,** 342-350.

VALDERAS, J. M. & ALONSO, J. 2008. Patient reported outcome measures: a model-based classification system for research and clinical practice. *Quality of life research,* 17**,** 1125-35.

VAN WIJK, B. L., KLUNGEL, O. H., HEERDINK, E. R. & DE BOER, A. 2005. Rate and determinants of 10-year persistence with antihypertensive drugs. *Journal of Hypertension,* 23**,** 2101-7.

VERDECCHIA, P., SCHILLACI, G., BORGIONI, C., CIUCCI, A., GATTOBIGIO, R., ZAMPI, I., REBOLDI, G. & PORCELLATI, C. 1998. Prognostic significance of serial changes in left ventricular mass in essential hypertension. *Circulation,* 97**,** 48-54.

VOHRINGER, M., MAHRHOLDT, H., YILMAZ, A. & SECHTEM, U. 2007. Significance of late gadolinium enhancement in cardiovascular magnetic resonance imaging (CMR). *Herz,* 32**,** 129-37.

VOLPE, M., TOCCI, G., TRIMARCO, B., ROSEI, E. A., BORGHI, C., AMBROSIONI, E., MENOTTI, A., ZANCHETTI, A. & MANCIA, G. 2007. Blood pressure control in Italy: results of recent surveys on hypertension. *Journal of Hypertension,* 25**,** 1491-8.

WALLER, G., JANLERT, U., NORBERG, M., LUNDQVIST, R. & FORSSEN, A. 2015. Self-rated health and standard risk factors for myocardial infarction: a cohort study. *BMJ Open,* 5**,** e006589.

WEBER, M. A., JULIUS, S., KJELDSEN, S. E., BRUNNER, H. R., EKMAN, S., HANSSON, L., HUA, T., LARAGH, J. H., MCINNES, G. T., MITCHELL, L., PLAT, F., SCHORK, M. A., SMITH, B. & ZANCHETTI, A. 2004. Blood pressure dependent and independent effects of antihypertensive treatment on clinical events in the VALUE Trial. *Lancet,* 363**,** 2049-51.

WHELTON, P. K., CAREY, R. M., ARONOW, W. S., CASEY, D. E., COLLINS, K. J., DENNISON HIMMELFARB, C., DEPALMA, S. M., GIDDING, S., JAMERSON, K. A., JONES, D. W., MACLAUGHLIN, E. J., MUNTNER, P., OVBIAGELE, B., SMITH, S. C., SPENCER, C. C., STAFFORD, R. S., TALER, S. J., THOMAS, R. J., WILLIAMS, K. A., WILLIAMSON, J. D. & WRIGHT, J. T. 2017. 2017 ACC / AHA / AAPA / ABC / ACPM / AGS / APhA / ASH / ASPC / NMA / PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: Executive Summary. *J Am Coll Cardiol,* 71**,** e127-248.

WHITE, P. 1937. *Heart Disease.,* New York, McMillan Co.

WHO 2009. Global health risks: Mortality and burden of disease attributable to selected major risks. World Health Organisation. Geneva.

WIEDERHIELM, C. A., WOODBURY, J. W., KIRK, S. & RUSHMER, R. F. 1964. PULSATILE PRESSURES IN THE MICROCIRCULATION OF FROG'S MESENTERY. *Am J Physiol,* 207**,** 173-6.

WILD, D., GROVE, A., MARTIN, M., EREMENCO, S., MCELROY, S., VERJEE-LORENZ, A., ERIKSON, P., TRANSLATION, I. T. F. F. & CULTURAL, A. 2005. Principles of Good Practice for the Translation and Cultural Adaptation Process for Patient-Reported Outcomes (PRO) Measures: report of the ISPOR Task Force for Translation and Cultural Adaptation. *Value Health,* 8**,** 94-104.

WILLIAMS, B. 2005. Recent hypertension trials: implications and controversies. *J Am Coll Cardiol,* 45**,** 813-27.

WILLIAMS, B., MANCIA, G., SPIERING, W., AGABITI ROSEI, E., AZIZI, M., BURNIER, M., CLEMENT, D. L., COCA, A., DE SIMONE, G., DOMINICZAK, A., KAHAN, T., MAHFOUD, F., REDON, J., RUILOPE, L., ZANCHETTI, A., KERINS, M., KJELDSEN, S. E., KREUTZ, R., LAURENT, S., LIP, G. Y. H., MCMANUS, R., NARKIEWICZ, K., RUSCHITZKA, F., SCHMIEDER, R. E., SHLYAKHTO, E., TSIOUFIS, C., ABOYANS, V. & DESORMAIS, I. 2018. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur Heart J,* 39**,** 3021-3104.

WILLIAMS, S. A., BOOLELL, M., MACGREGOR, G. A., SMAJE, L. H., WASSERMAN, S. M. & TOOKE, J. E. 1990. Capillary hypertension and abnormal pressure dynamics in patients with essential hypertension. *Clin Sci (Lond),* 79**,** 5-8.

WING, L. M., REID, C. M., RYAN, P., BEILIN, L. J., BROWN, M. A., JENNINGS, G. L., JOHNSTON, C. I., MCNEIL, J. J., MACDONALD, G. J., MARLEY, J. E., MORGAN, T. O. & WEST, M. J. 2003. A comparison of outcomes with angiotensin-converting--enzyme inhibitors and diuretics for hypertension in the elderly. *N Engl J Med,* 348**,** 583-92.

WOLF-MAIER, K., COOPER, R. S., KRAMER, H., BANEGAS, J. R., GIAMPAOLI, S., JOFFRES, M. R., POULTER, N., PRIMATESTA, P., STEGMAYR, B. & THAMM, M. 2004. Hypertension treatment and control in five European countries, Canada, and the United States. *Hypertension,* 43**,** 10-7.

WRIGHT, J. T., JR., WILLIAMSON, J. D., WHELTON, P. K., SNYDER, J. K., SINK, K. M., ROCCO, M. V., REBOUSSIN, D. M., RAHMAN, M., OPARIL, S., LEWIS, C. E., KIMMEL, P. L., JOHNSON, K. C., GOFF, D. C., JR., FINE, L. J., CUTLER, J. A., CUSHMAN, W. C., CHEUNG, A. K. & AMBROSIUS, W. T. 2015. A Randomized Trial of Intensive versus Standard Blood-Pressure Control. *N Engl J Med,* 373**,** 2103-16.

WU, A. S., DODHIA, H., WHITNEY, D. & ASHWORTH, M. 2019. Is the rule of halves still relevant today? A cross-sectional analysis of hypertension detection, treatment and control in an urban community. *Journal of Hypertension,* 37**,** 2470-2480.

XU, W., GOLDBERG, S. I., SHUBINA, M. & TURCHIN, A. 2015. Optimal systolic blood pressure target, time to intensification, and time to follow-up in

treatment of hypertension: population based retrospective cohort study. *Bmj,* 350**,** h158.

YANG, D., GLUAIS, P., ZHANG, J. N., VANHOUTTE, P. M. & FÉLÉTOU, M. 2004. Endothelium-dependent contractions to acetylcholine, ATP and the calcium ionophore A 23187 in aortas from spontaneously hypertensive and normotensive rats. *Fundamental & clinical pharmacology,* 18**,** 321-326.

YAO, E. H., FUKUDA, N., MATSUMOTO, T., KOBAYASHI, N., KATAKAWA, M., YAMAMOTO, C., TSUNEMI, A., SUZUKI, R., UENO, T. & MATSUMOTO, K. 2007. Losartan improves the impaired function of endothelial progenitor cells in hypertension via an antioxidant effect. *Hypertens Res,* 30**,** 1119-28.

YODER, M. C. 2012. Human endothelial progenitor cells. *Cold Spring Harb Perspect Med,* 2(7)**,** a006692.

YU, G. Z., AYE, C. Y., LEWANDOWSKI, A. J., DAVIS, E. F., KHOO, C. P., NEWTON, L., YANG, C. T., AL HAJ ZEN, A., SIMPSON, L. J., O'BRIEN, K., COOK, D. A., GRANNE, I., KYRIAKOU, T., CHANNON, K. M., WATT, S. M. & LEESON, P. 2016. Association of Maternal Antiangiogenic Profile at Birth With Early Postnatal Loss of Microvascular Density in Offspring of Hypertensive Pregnancies. *Hypertension,* 68**,** 749-59.

YUDKIN, J. S., FORREST, R. D. & JACKSON, C. A. 1988. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. *Lancet,* 2**,** 530-3.

ZANCHETTI, A. 2009. Bottom blood pressure or bottom cardiovascular risk? How far can cardiovascular risk be reduced? *Journal of Hypertension,* 27**,** 1509-1520.

ZANCHETTI, A., THOMOPOULOS, C. & PARATI, G. 2015. Randomized Controlled Trials of Blood Pressure Lowering in Hypertension. *A Critical Reappraisal,* 116**,** 1058-1073.

ZEPEDA, R. J., CASTILLO, R., RODRIGO, R., PRIETO, J. C., ARAMBURU, I., BRUGERE, S., GALDAMES, K., NORIEGA, V. & MIRANDA, H. F. 2012. Effect of carvedilol and nebivolol on oxidative stress-related parameters and endothelial function in patients with essential hypertension. *Basic Clin Pharmacol Toxicol,* 111**,** 309-16.

ZHOU, M.-S., KOSAKA, H., TIAN, R.-X., ABE, Y., CHEN, Q.-H., YONEYAMA, H., YAMAMOTO, A. & ZHANG, L. 2001. L-Arginine improves endothelial function in renal artery of hypertensive Dahl rats. *Journal of hypertension,* 19**,** 421-429.

ZHOU, M.-S., NISHIDA, Y., CHEN, Q.-H. & KOSAKA, H. 1999. Endothelium-derived contracting factor in carotid artery of hypertensive Dahl rats. *Hypertension,* 34**,** 39-43.

ZHU, X. Y., URBIETA CACERES, V. H., FAVREAU, F. D., KRIER, J. D., LERMAN, A. & LERMAN, L. O. 2011. Enhanced endothelial progenitor cell angiogenic potency, present in early experimental renovascular hypertension, deteriorates with disease duration. *Journal of Hypertension,* 29**,** 1972-9.

ZYGMUNTOWICZ, M., OWCZAREK, A., ELIBOL, A., OLSZANECKA-GLINIANOWICZ, M. & CHUDEK, J. 2013. Blood pressure for optimal health-related quality of life in hypertensive patients. *Journal of Hypertension,* 31**,** 830-9.