PERIPHERAL NEUROPATHY IN HYPERTENSION

by

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ABSTRACT

Patients with essential hypertension have higher pain thresholds than individuals with normal blood pressure and may show evidence of subclinical peripheral neuropathy. Hypertension is strongly associated with diabetic neuropathy and the observed sensory loss may be aggravated by hypertension-induced nerve ischaemia and hypoxia. Two studies are presented in this thesis. First, 20 hypertensives and 25 normotensives had vibration, cooling, warming and heat-pain thresholds measured using the "CASE IV" system to assess evidence of subclinical peripheral neuropathy. Higher vibration thresholds were demonstrated in the feet of the hypertensives, which were significantly correlated with SBP and DBP. Conversely, a significant negative correlation between SBP and DBP with cooling and warming thresholds in the hand was found. Second, in a separate database analysis, cardiovascular risk, including metabolic profile and ambulatory arterial stiffness index, was compared in 83 confirmed and 154 borderline hypertensives. Cardiovascular risk factors of the borderline group suggested that these patients necessitate intervention with lifestyle measures at the very least. Further studies are needed to prove causality between hypertension and subclinical peripheral neuropathy. If such an association is found across all grades of hypertension, earlier intervention with antihypertensive medication might be appropriate, even in patients with low cardiovascular risk.

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ABBREVIATIONS

AASI:	Ambulatory Arterial Stiffness Index
ABCD:	The Appropriate Blood Pressure Control in Diabetes Trial
ABPM:	Ambulatory Blood Pressure Monitoring
ACC:	Associated Clinical Condition
ACE:	Angiotensin Converting Enzyme
AGEs:	Advanced Glycation End products
AIx:	Augmentation Index
ANCOVA:	Analysis of Co-Variance
ANOVA:	Analysis of Variance
ARBs:	Angiotensin II Receptor Antagonists
BHS:	British Hypertension Society
BMI:	Body Mass Index
BNF:	British National Formulary
BP:	Blood Pressure
CASE IV:	Computer Aided Sensory Evaluator IV
CHD:	Chronic Heart Disease
CI:	Confidence Interval
CIAP:	Chronic Idiopathic Axonal Polyneuropathy
cm:	centimetres
CNS:	Central Nervous System
CPT:	Current Perception Threshold
CSP:	Chronic Symmetric Polyneuropathy
CVD:	Cardiovascular Disease
DASH:	Dietary Approaches to Stop Hypertension
DBP:	Diastolic Blood Pressure
df:	degrees of freedom
dl:	decilitres
DSSP:	Distal Symmetrical Sensory Polyneuropathy
ECG:	Electrocardiogram
ESC:	European Society of Cardiology
ESH:	European Society of Hypertension
ESHC:	European Society of Hypertension-European Society of Cardiology
FPG:	Fasting Plasma Glucose
g:	grams
HDL:	High-Density Lipoprotein
HIV:	Human Immunodeficiency Virus
HP:	Heat-Pain
HT:	Hypertension
I-CAM:	Inter-Cellular Adhesion Molecule
IDF:	International Diabetes Federation
ISH:	International Society of Hypertension

JNC 7:	Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
JNDs:	Just Noticeable Differences
kg:	Kilograms
L:	Litres
LVH:	Left Ventricular Hypertrophy
m:	Meters
mg:	milligrams
mmHg:	Millimeters of mercury
mmol:	millimoles
MMP:	matrix metalloprotease
MΦ:	MMP-7
NaCl:	Sodium Chloride
NCEP:	National Cholesterol Education Program
NHANES:	National Health and Nutrition Examination Survey
NT:	Normotensive
OR:	Odds Ratio
oz:	ounces
PHARAO:	The Prevention of Hypertension with ACE-inhibitor Ramipril in Patients with High Normal Blood Pressure trial
PNS:	Peripheral Nervous System
PSN:	Peripheral Sensory Neuropathy
PVD:	Peripheral Vascular Disease
PWV:	Pulse Wave Velocity
QEH:	Queen Elizabeth Hospital
QST:	Qualitative Sensory Testing
ROS:	Reactive Oxygen Species
SBP:	Systolic Blood Pressure
SD:	Standard Deviation
SHR:	Spontaneously Hypertensive Rats
SMPN:	Sensorymotor Peripheral Neuropathy
TGF-β:	Transforming Growth Factor
TOD:	Target Organ Damage
TROPHY:	Trial of Preventing Hypertension study
US:	United States
VSMC:	Vascular Smooth Muscle Cell
WHO:	World Health Organisation
WTCRF:	Wellcome Trust Clinical Research Facility

CHAPTER 1: INTRODUCTION AND AIMS

1.1 Hypertension

1.1.1 Background and Epidemiology

Hypertension is defined as repeatedly raised clinic blood pressure (BP) ≥140/90 mmHg (Table 1).[1-3] Nearly 1 in 3 adults has high blood pressure and the prevalence of high blood pressure in England in 2007 was 31% among men and 29% among women.[4;5] Hypertension is a highly prevalent risk factor for cardiovascular disease (CVD) throughout the developed world.[6] Cardiovascular disease [including coronary heart disease (CHD) and stroke] has been the most common cause of death in England and Wales for nearly a century in both males and females.[7] For example, in 2003 CHD caused 21.6% and 15.8% respectively of all male and female deaths; cerebrovascular disease was a close second.[8] The World Health Organisation (WHO) estimates that high blood pressure related illness causes 1 in 8 deaths making hypertension the third leading killer in the world.[9] Hypertension is therefore an important area of medical research.

1.1.2 Pathophysiology of Hypertension

The pathophysiology of hypertension remains uncertain. Two to five percent of hypertensives have an underlying renal or adrenal cause for their raised blood pressure, known as secondary hypertension. However, the majority have no single identifiable cause and their condition is termed essential hypertension.[10] Even though there are no direct causes for essential hypertension there are many risk factors that contribute towards its development the most important of which are: excess body weight, excess dietary sodium intake, reduced physical activity, inadequate intake of fruits and vegetables, and excess alcohol.[2]

1.1.3 Grades of Hypertension

Two major guidelines for the assessment and treatment of hypertension were published in 2003, from the European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension (ESHC) and the Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).[1;2] The recommendations in the two guidelines are similar in some ways. However, the two guidelines classify blood pressure differently (Table 1). The ESHC guidelines grade hypertension as mild, moderate or severe, whereas the JNC 7 guidelines grade hypertension as either Stage 1 or Stage 2.

Table 1. Classification of BP Levels (mmHg) in the ESHC and JNC 7 2003 Guidelines[1;2]

ESHC			JNC 7		
Category	**Systolic**	**Diastolic**	**Category**	**Systolic**	**Diastolic**
Optimal	<120	<80	**Normal**	<120	<80
Normal	120-129	81-84	**Prehypertension**	120-139	80-89
High-Normal	130-139	85-89			
Grade 1 HT (mild)	140-159	90-99	**Stage 1 HT**	140-159	90-99
Grade 2 HT (moderate)	160-179	100-109	**Stage 2 HT**	≥160	≥100
Grade 3 HT (severe)	≥180	≥110			

HT, Hypertension

The British Hypertension Society (BHS) IV classification of blood pressure equates to that of the ESHC and that of The World Health Organization/International Society of Hypertension (WHO/ISH),[11] and is based on clinic blood pressure values [ABPM readings should be adjusted upwards (e.g. by 10/5mmHg)]. If systolic blood pressure and diastolic blood pressure fall into different categories, the higher value should be taken for classification.[1;3;11]

1.1.4 Hypertension and Cardiovascular Disease Risk

Increasing blood pressure has been shown to be positively correlated with risk of coronary heart disease (CHD) and stroke, together termed cardiovascular disease (CVD).[12] However, the coexistence of other risk factors such as age, smoking and cholesterol have been shown to result in dramatic increases in CVD risk associated with any blood pressure level.[13;14] Estimates of cardiovascular and/or coronary risk can be easily calculated using computer programmes or charts such as the risk prediction charts, which are located at the back of the British National Formulary (BNF). Some of these computer programmes/charts are derived from complex equations based on data from the Framingham Heart Study.[15] The so-called, 'Framingham

equation' has been shown to apply to Northern European populations including Britain, although it may not be applicable to all patient populations.[13;16] The BHS IV guidelines base risk prediction on the Framingham equation as it is the only method of estimating the risk of cardiovascular morbidity and mortality in both men and women, which includes most of the risk factors routinely available to the clinician, including:

- Age (years)
- Female (1, woman; 0, man)
- Systolic blood pressure (SBP) [average of 2 office measurements (mmHg)]
- Diastolic blood pressure (DBP) [average of 2 office measurements (mmHg)]
- Cholesterol [total serum cholesterol measured by the Abell-Kendall method (mg/dl)]
- HDL cholesterol [determined after heparin-manganese precipitation (mg/dl)]
- Smoking (1, cigarette smoking or quit within past year; 0, otherwise)
- Diabetes [1, diabetes; 0, otherwise (conservative definition is treatment with insulin or oral agents or having a fasting glucose of 7.7mmol/L or above)]
- ECG-LVH (1, definite; 0, otherwise).[3;15]

1.1.5 Hypertension Management

The ESHC 2003, ESHC 2007, JNC 7 and BHS IV guidelines differ slightly when it comes to the management of hypertension.

1.1.5.1 ESHC 2003

The ESHC 2003 guidelines base the decision to initiate antihypertensive medication on the total level of cardiovascular risk (Table 2) as well as the level of systolic and diastolic blood pressure (Table 1).[1]

Table 2. ESHC 2003 Guidelines for the Stratification of Cardiovascular Risk in Order to Quantify Prognosis[1]

Other risk factors and disease history	Blood pressure (mmHg)				
	Normal SBP 120-129 or DBP 80-84	High normal SBP 130-139 or DBP 85-89	Grade 1 HT SBP 140-159 or DBP 90-99	Grade 2 HT SBP 160-179 or DBP 100-109	Grade 3 HT SBP ≥180 or DBP ≥110
No other risk factors	Average risk	Average risk	Low added risk	Moderate added risk	High added risk
1-2 risk factors	Low added risk	Low added risk	Moderate added risk	Moderate added risk	Very high added risk
≥3 risk factors or TOD or diabetes	Moderate added risk	High added risk	High added risk	High added risk	Very high added risk
ACC	High added risk	Very high added risk	Very high added risk	Very high added risk	Very high added risk

ACC, associated clinical conditions; TOD, target organ damage

According to the ESHC 2003 guidelines, if an individual has Grade 1 or 2 hypertension clinicians should: (i) assess other risk factors, target organ damage (TOD), diabetes, and associated clinical conditions (ACC), (ii) initiate lifestyle measures and correction of other risk factors or disease and (iii) stratify absolute risk (Table 2). If absolute risk is 'very high' or 'high' begin drug treatment promptly, if 'moderate' monitor BP and other risk factors for at least 3 months. If after 3 months SBP ≥140 mmHg or DBP ≥90 mmHg then begin drug treatment but if after 3 months SBP <140 mmHg and DBP <90 mmHg then continue to monitor. If absolute risk is 'low' monitor BP and other risk factors for 3-12 months. If after 3-12 months SBP ≥140-159 mmHg or DBP ≥90-99 mmHg then consider drug treatment and elicit patient's preference but if after 3-12 months SBP <140 mmHg and DBP <90 mmHg then continue to monitor.[1]

If an individual has Grade 3 hypertension clinicians should: (i) begin drug treatment immediately, (ii) assess other risk factors, TOD, diabetes and ACC and (iii) add lifestyle measures and correction of other risk factors or disease.[1]

1.1.5.2 ESHC 2007

The ESHC 2007 guidelines are very similar to the 2003 guidelines with respect to the management of Grade 1-3 hypertension. The differences in the most recent guidelines are: (i) individuals with Grade 1 hypertension with no other risk factors (low added risk) should practice lifestyle changes for several months (as opposed to 3-12 months) and then start drug treatment if BP remains uncontrolled, (ii) individuals with Grade 1 hypertension with 1-2 risk factors and individuals with Grade 2 hypertension with no other risk factors or 1-2 risk factors (moderate added risk) should practice lifestyle changes for several weeks (as opposed to at least 3 months) and then start drug treatment if BP remains uncontrolled.[17]

1.1.5.3 JNC 7

In contrast to the ESHC guidelines, the JNC 7 guidelines do not take cardiovascular risk or target organ damage into account when making treatment decisions. Rather, all individuals with Stage 1 hypertension (SBP 140-159 mmHg or DBP 90-99 mmHg) or Stage 2 hypertension (SBP ≥160 mmHg or DBP ≥100 mmHg) should initiate lifestyle modification and drug therapy.[2]

1.1.5.4 BHS IV

The BHS IV guidelines recommend the following management of hypertension:

- Provide lifestyle advice for all people with high BP and those with borderline (Grade 1) or high-normal BP.
- Start drug therapy in all patients with sustained SBP ≥160 mmHg or sustained DBP ≥100 mmHg despite non-pharmacological measures.
- Drug treatment is also indicated in patients with sustained SBP 140-159 mmHg or DBP 90-99 mmHg if TOD is present, or there is evidence of established CVD, or diabetes, or the 10-year CVD risk is ≥20%, according to the Joint British Societies CVD risk assessment programme/risk chart.[3]

1.2 High-Normal Blood Pressure/Prehypertension

Normal blood pressure is 120-129/81-89 mmHg according to the 2003 ESHC guidelines or ≤120/80 mmHg according to the JNC 7 guidelines (Table 1).[1;2] Hypertension is defined as BP ≥140/90 mmHg.[1-3] There is a grey area in between these two thresholds where BP cannot be regarded as either normal or hypertensive. Such BP levels are now regarded as 'high-normal' in Europe (130-139/85-89 mmHg) or 'prehypertension' in the United States (US) (120-139/80-89 mmHg) (Table 1).[1;2] The latter has raised some controversy as it means that many people previously considered entirely normal according to ESHC guidelines will now be labeled with a medical condition.

1.2.1 Prevalence of High-Normal BP/Prehypertension

Data from the 1999 and 2000 National Health and Nutrition Examination Survey (NHANES III) estimated that the prevalence of prehypertension among adults in the US was approximately 31%.[18;19] The prevalence was higher among men than women (39% and 23% respectively) and higher in obese than normal weight individuals.[19;20] NHANES 2005-2006 reported that an estimated 25% of the US population aged 20 years or older has prehypertension, including over 32 million men and 21 million women.[21;22]

1.2.2 High Normal BP/Prehypertension and Rate of Progression to Hypertension

Prehypertension is likely to progress to hypertension fairly rapidly in the absence of lifestyle changes, particularly in the elderly and in individuals whose blood pressures lie in the upper portion of the prehypertensive range i.e. 130-139/85-89 mmHg.[13] Vasan RS *et al.,* 2001 assessed the development of hypertension in normotensive participants in the Framingham Heart Study and found a stepwise increase across three non-hypertensive BP categories (optimal, normal and high-normal BP) over a period of four years (Table 3). This increase was more prominent in individuals aged 65-94 years compared to those aged 35-64 years.[23] Similar findings were reported in the Trial of Preventing Hypertension (TROPHY) study where 63% of prehypertensive individuals (BP 130-139/85-89 mmHg) aged 30-65 years, receiving a placebo, developed hypertension over four years of follow-up.[24] In 2008 Falkner B *et al.,* found that among adolescents aged 13-17 years the progression from prehypertension to hypertension was significantly greater than the progression from normotension to hypertension. 15% of boys and 14% of girls with prehypertension had hypertension four years later whereas only 5% of boys and 6% of girls with normotension had hypertension four years later.[20]

Table 3. Rate of Progression of Optimal, Normal and High-Normal BP to Hypertension (≥140/90 mmHg) in Individuals Aged 35-64 Years and Those Aged 65-94 Years[23]

	Optimal BP (<120/80 mmHg)	**Normal BP (120-129/80-84 mmHg)**	**High-Normal BP (130-139/85-89 mmHg)**
35-64 years old	5.3%	17.6%	37.3%
65-94 years old	16%	25.5%	49.5%

1.2.3 Association of High-Normal BP/Prehypertension with Other CVD Risk Factors

In a study of the 1999 to 2000 National Health and Nutrition Examination Survey (NHANES) III data, Greenlund *et al.*, 2004 found that occurrence of CVD risk factors including: above-normal cholesterol levels, excess weight/obesity, and diabetes mellitus, were greater in individuals with prehypertension after adjusting for age, sex and ethnicity. Surprisingly, these individuals were less likely to smoke than their normotensive counterparts.[19] Prehypertensives were 1.65 times more likely to have at least 1 other CVD risk factor than normotensives.[19] Similarly, in the Women's Health Initiative, age, body mass index (BMI), and prevalence of diabetes mellitus and hypercholesterolaemia increased across blood pressure categories (normal, prehypertension and hypertension), whereas smoking decreased.[25] In a study of the NHANES II and NHANES II Mortality Study, Mainous III *et al.,* found that 90% of patients with prehypertension had other CVD risk factors.[26]

Risk ratios for specific risk factors including obesity, dyslipidaemia, insulin resistance, metabolic syndrome, and diabetes are all greater in prehypertensives than normotensives but fewer in prehypertensives than hypertensives.[13;27-30] Furthermore, microalbuminuria is more common in prehypertensives than normotensives as are abnormalities in circulating markers of inflammation, such as C-reactive proteins, interleukin 6, and tumour necrosis factor-α.[13;31-33]

In the studies referenced above, blood pressure was categorised according to the JNC 7 guidelines i.e. individuals were classed as having prehypertension if they were not taking antihypertensive medication and if they had SBP 120-139 mmHg or DBP 80-89 mmHg.[2]

1.2.4 High-Normal BP/Prehypertension and Risk of Mortality or Developing CVD

Prehypertension appears to be associated with an increased incidence of CVD, especially in those individuals with BP between 130-139/85-89 mmHg and/or a history of diabetes or glucose intolerance.[30;34-36] Qureshi *et al*., 2005 examined existing data from the Framingham study and found that a prehypertensive person is more than 3 times more likely to have a myocardial infarction and 1.7 times more likely to have heart disease than a person with normal BP: the risk of stroke was not increased.[37] Similarly, in a different study, mortality from CVD was found to be significantly greater in prehypertensive than normotensive individuals but the differences disappeared once adjustments were made for presence of other CVD risk factors.[26]

In a longitudinal population-based, US cohort, prehypertension was associated with increased risk of major cardiovascular events (including stroke, myocardial infarction and heart failure) independently of other cardiovascular risk factors.[35] These findings, along with the presence of cardiovascular risk factors in 93% of the participant sample, support recommendations for clinicians to actively target lifestyle modifications and multiple risk factor reduction in their prehypertensive patients.[35]

1.2.5 Treating High-Normal BP/Prehypertension with Lifestyle Approaches to Prevent Hypertension or CVD

Dietary approaches, either alone or with other lifestyle modifications, have been shown to reduce blood pressure in prehypertensives as well as hypertensives.[38-42] The results of the DASH (Dietary Approaches to Stop Hypertension) study in 1997 revealed that a diet rich in fruits, vegetables, and low-fat dairy foods and with reduced saturated and total fat can substantially lower blood pressure, especially when implemented in conjunction with weight control (BMI 20-25kg/m^2), adequate potassium intake [>90mmol (2500mg) per day], low salt diet (<100mmol/day or <6g sodium/day or <2.4g sodium chloride/day) and limited alcohol consumption (no more than 3 units/day in men and no more than 2 units/day in women).[3;38;39;43] Since 1997 it has become evident that a diet higher in protein and unsaturated fat and lower in carbohydrate can reduce blood pressure even further than the DASH diet alone.[3;40] In addition, physical activity (30 minutes 3-5 times each week) is known to reduce blood pressure further still.[3;44;45] Interestingly, cigarette smoking, (except when chronic and heavy) does not appear to be associated with hypertension.[46;47] However, smoking does increase risk of cardiovascular disease considerably, therefore smoking cessation is recommended.[3]

Lifestyle changes *can* lower blood pressure in prehypertensives and thus reduce the likelihood of developing hypertension with its associated CVD risk (Table 4).[2;48] Unfortunately, in order for lifestyle modifications to substantially lower blood pressure they need to be adhered to and sustained, which many people find difficult.[1]

Table 4. Lifestyle Interventions for Blood Pressure Reduction in Prehypertensives[48]

Strategy	Recommendation	SBP Effect in Prehypertension	Effect on Incidence of Prevalence of Hypertension
DASH dietary pattern	4-5 fruits/day 4-5 vegetables/day 2-3 low-fat dairy/day <25% fat	3.5 mmHg	Decreased by 62% (prevalence)
Weight loss	Effective BP lowering even without attaining normal BMI	1 mmHg/kg weight loss	Decreased by 42% (incidence)
Reduced sodium intake	<2400 mg/day	2 mmHg per 76 mmol/L per day decrease	Decreased by 38% (incidence)
Physical activity	Moderate exercise ≥30 minutes most days	3-4 mmHg	Not Available
Moderation of alcohol intake	≤2 oz/day (men); ≤1 oz/day (women)	3.5 mmHg	Not Available

In order to prevent or delay the onset of hypertension in the population as a whole, a public health strategy that complements the hypertension treatment strategy is warranted.[2] Such a strategy would require a multipronged approach directed to communities, schools, worksites, and the food industry.[2] For example, in 2002 the American Public Health Association and the National High Blood Pressure Education Program Coordinating Committee recommended that the food industry, including manufacturers and restaurants, reduce sodium in the food supply by 50% over the next 10 years.[2] More public health initiatives like this need to be implemented in order to reduce BP in the general population.[2]

As mentioned previously, Qureshi *et al.,* 2005 found that prehypertension is associated with increased risk of myocardial infarction and coronary artery disease. This raises the question of whether we should treat prehypertensive patients more aggressively i.e. with antihypertensive medication.[37]

1.2.6 Management of High-Normal BP/Prehypertension

Unsurprisingly, there is much controversy about how individuals with high-normal BP/prehypertension should be managed.

The JNC 7 guidelines focus on BP values as the main variables determining the need for treatment and the type of treatment.[17] JNC 7 recommends that individuals with prehypertension (BP in the range 120-139/80-89 mmHg) should be firmly advised to practice lifestyle modification and those who also have diabetes or kidney disease should be considered for appropriate drug therapy if lifestyle modification fails to reduce their BP to 130/80 mmHg or less.[2]

The ESHC guidelines emphasise the importance of overall (or global) cardiovascular risk assessment, rather than a strict focus on hypertension, when making treatment decisions.[1;17] The ESHC guidelines recommend that people with high-normal BP (130-139/85-89 mmHg) that have average (no other risk factors) or low added (1-2 risk factors) cardiovascular risk should have no BP intervention or lifestyle changes, respectively.[1;17] Individuals with high added cardiovascular risk (3 or more risk factors, metabolic syndrome, subclinical organ damage or diabetes) should initiate lifestyle changes and either consider drug treatment (if not diabetic) or start drug

treatment (if diabetic) and those with very high added cardiovascular risk (established cardiovascular or renal disease) should initiate lifestyle changes and start immediate drug treatment.[17] The BHS IV guidelines differ from both JNC 7 and the European guidelines in that they do not mention intervention with antihypertensive medication at blood pressures <140/90mmHg.[3]

1.2.7 Treating High-Normal BP/Prehypertension with Antihypertensive Medication to Prevent Hypertension or CVD

A handful of trials have investigated the effects of antihypertensive medication in individuals with prehypertension/high normal blood pressure.

The TROPHY trial was a large trial to assess the feasibility of treating prehypertension (130-139/85-89 mmHg) with the angiotensin-receptor blocker candesartan in order to prevent or delay the onset of hypertension.[24] Participants were randomised to receive two years of candesartan or placebo, followed by two years of placebo for both groups. After two years the blood pressure in the candesartan group decreased more rapidly than in the placebo group resulting in a relative risk reduction in the risk of new-onset hypertension of 66.3% in the candesartan group. After four years (two years after discontinuation of candesartan) blood pressure rose more rapidly in the candesartan group resulting in a relative risk reduction in the candesartan group of 15.6%. Thus, candesartan reduced blood pressure and delayed the onset of hypertension *during* treatment but the benefits were not sustained once treatment was withdrawn. Notably, rates of serious adverse events during candesartan treatment were low and were similar in the two groups.[24]

The Prevention of Hypertension with ACE-inhibitor Ramipril in Patients with High Normal Blood Pressure (PHARAO) trial investigated the capacity of ramipril (an angiotensin-converting enzyme inhibitor) to prevent or delay the onset of hypertension in patients with high-normal blood pressure.[49] Hypertension and high-normal blood pressure were defined in accordance with JNC 7/ESHC.[1;2] Participants were randomised to the ramipril treatment group or the control group. 30.7% of subjects in the ramipril group, and 42.9% in the control group developed hypertension over a period of three years. Treatment with ramipril significantly reduced the progression to hypertension by 34.4% compared with the control group. These results are in line with those of the TROPHY trial.[49] Interestingly, multivariate analysis revealed that the risk of progression to hypertension rises by 5% with each mmHg of office SBP. Although a clear-cut fall in BP was observed in the initial treatment phase with ramipril, the subsequent BP difference between the two groups was minimal (SBP 1-2 mmHg lower in the ramipril group), suggesting that treatment of high-normal blood pressure with ramipril may not be beneficial in the long-term. There was no difference in the frequency and type of adverse events between the treatment and control group. However, there was a higher incidence of cough in the ramipril group.[49]

In 2007, Skov *et al.,* performed a small study to investigating whether early treatment with an candesartan in young (18-20 years old) normotensive offspring of hypertensive parents persistently lowered blood pressure after treatment withdrawal. Participants were randomised to receive either candesartan or placebo. The intervention period was 12 months, with 24 months of follow-up. Findings of this study showed that although candesartan significantly reduced blood pressure, renal vascular resistance and left ventricular mass *during* treatment, at 12 and 24 months follow-up mean ambulatory blood pressure monitoring (ABPM) was no different

between subjects who received candesartan and those who received a placebo. Thus, temporary treatment of subjects at high familial risk of future hypertension with an angiotensin receptor blocker is feasible, but the treatment had no persistent effect on blood pressure when treatment was withdrawn. There were no significant differences in adverse events between the two groups.[50]

It might be difficult to know if long term antihypertensive therapy in pre-hypertensive patients has any effect. In addition, it is difficult to justify treatment with antihypertensive drugs that may result in adverse events and will have considerable cost implications. Further outcome trials are needed to determine i) the effects of pharmacological treatment on target organ damage and cardiovascular morbidity and mortality ii) whether they are safe when administered over many decades, as would be required to treat young individuals with prehypertension and iii) whether they are cost-effective for the treatment of patients with such low absolute risk of CVD.[22] In the meantime a more attractive strategy would be to improve lifestyle in the population for example by reducing "hidden" salts in processed foods such as bread (1 slice contains 0.5g of salt), breakfast cereals, ready-made meals and flavour enhancers such as stock cubes or manufactured sauces to reduce daily intake to <6 g.[3] Other population-based strategies might be to increase attention to health education by general practitioners, increase access to places to engage in physical activity, reduce servings of food in restaurants, increase availability of healthy food choices in schools, worksites and restaurants, increase exercise programs in schools and decrease the cost of low-sodium/low calorie food products.[2]

In summary, available guidelines recognise that high-normal or pre-hypertension may progress to established hypertension and is associated with an increased risk of cardiovascular disease. The management advice differs depending on the guidelines used but mostly suggests that lifestyle intervention is appropriate to reduce blood pressure. More accurate ways of establishing which individuals are at risk of developing cardiovascular disease include standard risk assessment as well as novel methods outlined in the next section.

1.3 Additional Ways to Assess Cardiovascular Risk

1.3.1 Arterial Stiffness

1.3.1.1 Definition and Mechanisms Underlying Arterial Stiffness

Arterial stiffness (arteriosclerosis) refers to stiffening of the arteries, particularly the more compliant and distensible central arteries e.g. the aorta and its branches.[51] Arterial stiffening occurs due to a complex interaction between stable and dynamic changes involving structural (e.g. collagen and elastin) and cellular elements (e.g. endothelial cell signalling and vascular smooth muscle cell tone) of the vessel wall.[52] For example, stimulation of an inflammatory environment causes overproduction of abnormal, cross-linked collagen and diminished quantities of normal elastin in the vessel wall, increasing arterial stiffness. These vascular alterations i.e. changes in the extracellular matrix and endothelial cell dysfunction, are influenced by haemodynamic forces such as shear stress and mechanical stretch as well as by "extrinsic factors" such as hormones (e.g. angiotensin II and aldosterone), advanced glycation end products (AGEs), chronic inflammation, vascular calcification, high sodium intake, and glucose regulation (Figure 1).[52;53] Aging is the dominant process altering vascular stiffness.[51] However, diseases such as hypertension, diabetes mellitus and chronic kidney disease amplify the vascular changes that result in arterial stiffening.[52;53]

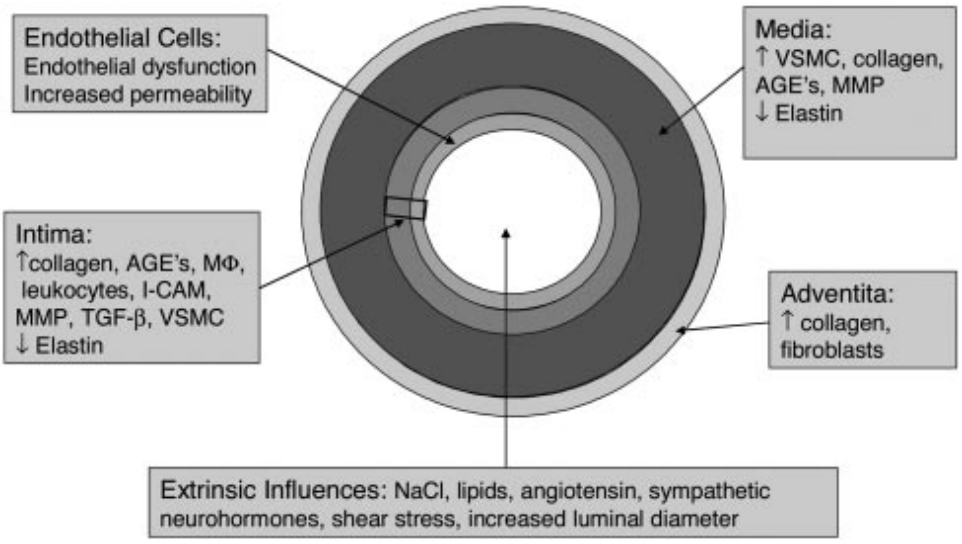

Figure 1. Summary of the Multiple Causes and Locations of Arterial Stiffness[52]

AGE's, advanced glycation end products; MΦ, MMP-7 (an elastase); I-CAM, Inter-Cellular Adhesion Molecule; MMP, matrix metalloproteases; TGF-β, transforming growth factor; VSMC, vascular smooth muscle cell; NaCl, sodium chloride

1.3.1.2 Arterial Stiffness and Cardiovascular Risk

Arterial stiffening increases systolic blood pressure and pulse pressure causing myocyte hypertrophy, increased left ventricular after load and reduced coronary perfusion, resulting in diastolic and systolic dysfunction and ultimately congestive heart failure.[53] Raised systolic and pulse pressures also promote further vascular damage increasing risk of stroke and progressive loss of kidney function (Figure 2).[53]

It is now well accepted that aortic stiffness is an intermediate endpoint for cardiovascular events, either fatal or non-fatal.[54] In addition, large-artery stiffness has proved to be an independent predictor of adverse cardiovascular outcomes in the general population as well as in patients with

primary hypertension, diabetes, or end-stage renal disease.[55] Its prognostic value has been shown to extend even beyond classic cardiovascular risk factors entering various types of risk score.[54;55] Arterial stiffness is therefore important, not only for assessing cardiovascular risk but also for predicting cardiovascular outcomes.[54] Arterial stiffening also provides direct evidence of target organ damage, which is of major importance in determining the overall cardiovascular risk of hypertensive patients.[54] Arterial stiffness should therefore be considered as a recommended test for the evaluation of cardiovascular risk, particularly in patients in whom target organ damage is not discovered by routine investigations.[54] With this in mind it is important to be able to measure arterial stiffness accurately and easily in order to predict an individual's cardiovascular risk. However, the widespread measurement of arterial stiffness in clinical practice is limited by the need for specific technical equipment and trained personnel.[55;56]

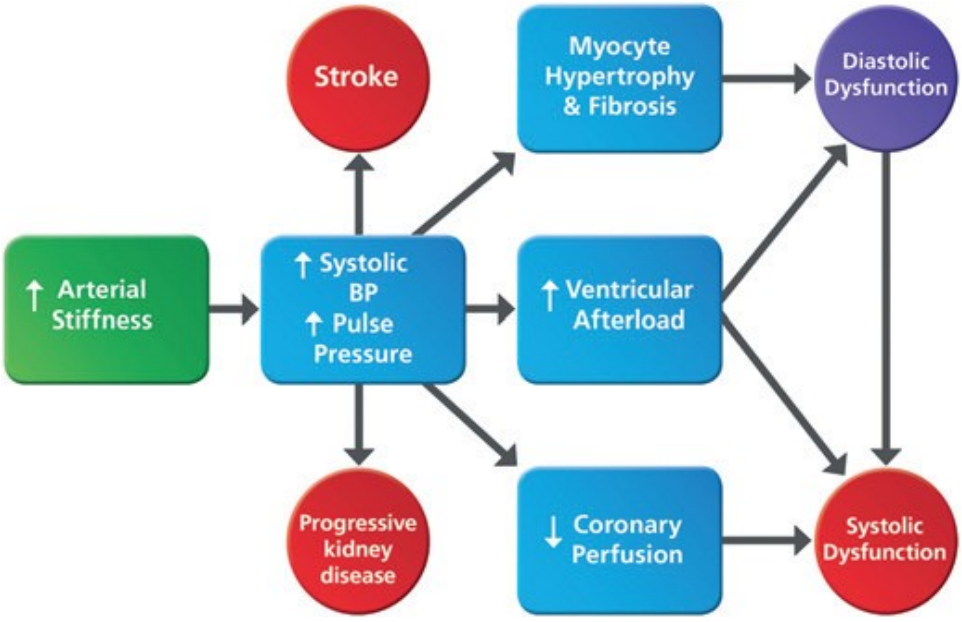

Figure 2. Consequences of Increased Arterial Stiffness[53]

1.3.1.3 Methods of Measuring Arterial Stiffness

Non-invasive measures of arterial stiffness fall into three broad groups: i) measuring pulse wave velocity (PWV), ii) using ultrasound to relate the change in diameter (or area) of an artery to distending pressure in order to measure arterial distensibility/compliance, and iii) assessing peripheral arterial pressure waveforms (e.g. augmentation index [AIx]) by applanation tonometry.[57;58]

The most common method for evaluating arterial stiffness is based on the study of PWV i.e. the velocity of the BP propagation wave along a given conduit artery, for example, the aorta.[59] Measurements involve applanation tonometry, mechanotransducer or Doppler probes and are regarded as the gold standard for determining arterial stiffness, independent of wave activity: the higher PWV, the higher arterial stiffness.[59] In parallel, the technique of applanation tonometry is widely used to evaluate central pressure and mostly wave reflections through the non-invasive use of the parameter called augmentation index (AIx). AIx is an indirect measure of carotid stiffness, but mainly a direct measure of central wave reflection.[59]

There are many commercial devices available that use different methods to measure arterial stiffness.[57] Unfortunately, these devices can be expensive and are often complicated to use and are thus operator-dependent. This means measurement of arterial stiffness cannot be performed easily in daily clinical practice. Consequently, there is a need for a more simple measure of arterial stiffness, which may have recently been identified in the form of the ambulatory arterial stiffness index (AASI).

1.3.1.3.1 Ambulatory Arterial Stiffness Index (AASI)

The Ambulatory Arterial Stiffness Index (AASI) is a new, easy-to-obtain index of arterial stiffness.[55;56] Ambulatory arterial stiffness index has been shown to strongly correlate with classic measures of arterial stiffness, such as PWV and AIx, and has been identified as an independent marker for target organ damage, cognitive function and cardiovascular and renal outcomes.[60]

AASI is derived from 24-hour ABPM recordings. The theory behind this method is that the dynamic relation between diastolic and systolic blood pressure over 24 hours provides insight into the stiffness of the arterial wall.[61] The regression slope of diastolic against systolic blood pressure is calculated and AASI is defined as 1 minus the regression slope (Figure 3). The stiffer the arterial tree, the closer the regression slope and AASI are to 0 and 1 respectively.[61]

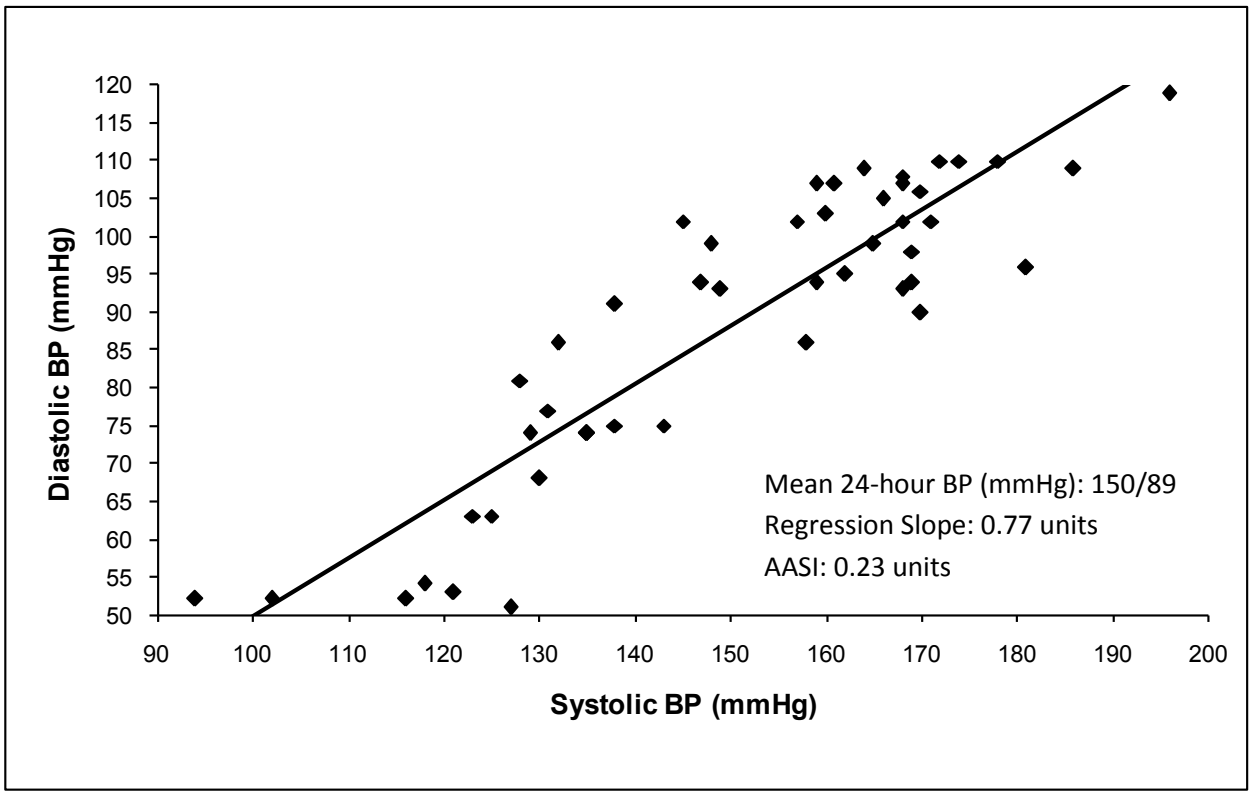

Figure 3. Scatter Graph Showing 24-hour SBP Readings (x) Plotted Against 24-hour DBP Readings (y). The regression slope of diastolic against systolic blood pressure is calculated and AASI is defined as 1 minus the regression slope.

1.3.2 Metabolic Syndrome

Metabolic syndrome is the name given to a cluster of closely related cardiovascular risk factors namely: visceral obesity, dyslipidaemia, hyperglycaemia, and hypertension.[62] The most recent diagnostic criteria for metabolic syndrome, from the International Diabetes Federation (IDF) is: central obesity (waist circumference ≥94 cm in males, ≥80cm in females or BMI >30 kg/m^2) plus any two of the following four factors: raised triglycerides (≥1.7 mmol/L or specific treatment for this lipid abnormality), reduced HDL cholesterol (<1.03 mmol/L in males, <1.29 mmol/L in females or specific treatment for this lipid abnormality), raised blood pressure (SBP ≥130 mmHg or DBP ≥85 mmHg or treatment of previously diagnosed hypertension) and/or raised fasting plasma glucose (FPG ≥5.6 mmol/L, or previously diagnosed type II diabetes).[62]

The cause of metabolic syndrome is unknown, which leads some people to question whether it is a syndrome at all. However, it remains useful to be able to identify individuals with metabolic syndrome because it is a risk factor for both cardiovascular disease and diabetes mellitus. In 2005, the population-attributable fraction due to the metabolic syndrome, [as defined by the National Cholesterol Education Program (NCEP) and WHO] was found to be ~12-17% for CVD and ~30-52% for diabetes.[63] More recently, Lorenzo *et al.,* 2007 found that the IDF definition of metabolic syndrome predicted incident CVD risk (OR 1.69 [95% CI 1.13-2.54]) and diabetes risk (OR 5.76 [95% CI 4.11-9.07]) independently of other risk factors.[64]

1.4 Peripheral Neuropathy

Hypertension is associated with reduced ability to feel pain, and there is some evidence that hypertensive individuals may have peripheral neuropathy.[65-71]

Peripheral neuropathy infers damage or central inhibition of impulses to nerves of the peripheral nervous system (PNS). The PNS consists of the nerves that lie outside the central nervous system (the brain and spinal cord) and that innervate the limbs and organs. The PNS is divided into the somatic nervous system (voluntary) and the autonomic nervous system (involuntary). Population prevalence of peripheral neuropathy is around 2.4%, rising with age to 8%.[72] There are many causes of peripheral neuropathy. The most common causes are diabetes mellitus, alcohol, certain drugs, and infections such as HIV (human immunodeficiency virus). Diabetes mellitus is the most common cause of peripheral neuropathy in the western world with a prevalence of 22.7% in Type I diabetics and 32% in Type II diabetics in the UK.[73;74] Patients with peripheral neuropathy may experience altered sensation, pain, weakness or autonomic symptoms.[72] Clinical features vary widely depending on the nerves affected, class and level of axon affected, age of onset, rate, course and severity of the pathologic process and degree of regeneration and sprouting, among other factors.[75] The symptoms typically begin distally in the toes and fingers. They commonly appear in the toes before the fingers and spread proximally.[72] Peripheral neuropathy is a long-term, degenerative, disabling condition for which there is currently no treatment other than symptomatic relief, therefore research in this area is paramount.

It is known that hypertension is strongly associated with the development of diabetic neuropathy (a form of peripheral neuropathy specific to diabetes) and that unmedicated hypertensives have higher pain thresholds than normotensives.[65;66;76-84] These findings lead us to hypothesise that hypertension may be associated with peripheral neuropathy i.e. increased thresholds of other sensory modalities as well as pain, and that this sensory loss may be a direct result of hypertension even when diabetes is not present.

1.5 Hypertension and Diabetic Neuropathy

A link between peripheral neuropathy and hypertension can be seen in diabetes mellitus where many studies have found hypertension to be strongly associated with the development of diabetic neuropathy. Diabetic neuropathy is a multifactorial process but hypertension has been found to be the most important predisposing cardiovascular risk factor.[76-81] Harris *et al.,* 1993 in a cross-sectional study, concluded that hypertension and hyperglycaemia predispose to symptoms of sensory neuropathy. In logistic regression, factors independently related to symptoms of sensory neuropathy in people with Type II diabetes included duration of diabetes, hypertension, hyperglycaemia and glycosuria. In fact, hypertension was associated with a 60% higher likelihood of symptoms.[76] Forrest *et al.,* 1997 in a prospective cohort study, found that hypertension had the greatest impact on the development of distal symmetrical sensory polyneuropathy (DSSP) (the most common type of diabetic neuropathy) in individuals with both short or long-term Type I diabetes.[77] Cohen *et al.,* 1998 analysed data from The Appropriate Blood Pressure Control in Diabetes (ABCD) Trial (a cross-sectional study) and found that diabetic sensory peripheral neuropathy is independently associated with diabetes duration, body weight, age, retinopathy, overt albuminuria, height, duration of hypertension, insulin use, and race/ethnicity.[78] More recently Tesfaye *et al.,* 2005 in a prospective cohort study, demonstrated that in addition to glycaemic control, the incidence of neuropathy is associated with potentially modifiable cardiovascular risk factors, including raised triglyceride levels, body mass index, smoking and hypertension.[79] Jarmuzewska *et al.,* 2005 in a cross-sectional study, concluded that pulse pressure (an indicator of arterial stiffness) is independently and negatively associated with nerve function i.e. as pulse pressure increases, nerve function decreases, and in a subsequent

cross-sectional study in 2007 concluded that there is a strong association between hypertension and development of SMPN (sensorimotor peripheral neuropathy) in patients with relatively short-term Type II diabetes.[80;81] More recently, Balducci *et al.,* 2006 found that long-term aerobic exercise training can prevent or modify the natural history of diabetic peripheral neuropathy. Interestingly, systolic and diastolic blood pressure in the exercise group decreased by 4 mmHg and 5 mmHg respectively over four years, potentially providing further evidence for a role of blood pressure in diabetic neuropathy.[82]

The foregoing studies indicate that hypertension is a risk factor for diabetic neuropathy. The precise mechanisms underlying the development of diabetic neuropathy are unknown. Studies have found reduced nerve oxygenation and impaired blood flow in human diabetic neuropathy suggesting a key role for microvascular disease in its pathogenesis.[85;86] There is evidence that diabetes-induced metabolic and vascular disturbances lead to abnormalities in the peripheral nerve microvasculature.[87] These abnormalities are thought to cause ischaemia and hypoxia in peripheral nerves, causing peripheral nerve damage, resulting in peripheral neuropathy.[88] Ischaemia from peripheral vascular disease (PVD), (for which hypertension is a risk factor) brings about oxidative stress and injury to nerves via increased production of reactive oxygen species (ROS) as reinforced by the finding that glutathione (a free radical scavenger) can be partially effective at preventing experimental diabetic neuropathy, possibly by improving oxygenation.[89-91] In this context, hypertension may contribute to the peripheral nerve damage seen in diabetic neuropathy. Interestingly, there is evidence of greater degrees of neuronal function impairment in painless than in painful neuropathy, with nerve blood flow impaired to a greater extent and oxygen saturation lower in individuals with painless than those with painful

neuropathy.[92;93] Acute painful neuropathy occurring in the setting of normalisation of glycaemia may be associated with new vessel formation on the surface of the nerve, further suggesting important differences between painful and painless neuropathy.[92]

In experimental studies on diabetic rats, antihypertensive agents have been shown to improve oxygenation, nerve blood flow, nerve abnormalities and nerve conduction as well as preventing nerve damage and dysfunction.[94-98] Similarly, in studies of human diabetes, antihypertensives have been found to significantly improve motor and sensory nerve conduction velocity, warming and vibratory detection thresholds and diabetic neuropathy.[99;100] These findings reinforce the possible deleterious effects of hypertension on peripheral nerve function in diabetics.

Hypertension may be a risk factor for peripheral neuropathy, even in the absence of diabetes, a postulation that has been supported in a number of studies on spontaneously hypertensive rats (SHR). These studies demonstrated impaired vascular supply to peripheral nerves and morphological changes and decreased nerve function in the sciatic nerve, all of which were improved with antihypertensive medication.[101-103]

1.6 Hypertension and Hypoalgesia

For nearly 30 years researchers have demonstrated that there is an association between decreased perception of pain (hypoalgesia) and hypertension. However, the mechanisms involved in this relationship are poorly understood.[104] There is still doubt as to whether, in human essential hypertension, hypoalgesia is secondary to raised blood pressure or whether both depend on some common mechanism.[105] Another uncertainty is whether there is a central cause for the hypoalgesia or damage to the peripheral nerves themselves. Based on the existing evidence, the baroreceptor reflex may coordinate cardiovascular and pain regulatory responses and opioid peptides may also be implicated.

Activation of baroreceptor afferents via increases in blood pressure may play an important part in hypertension-associated hypoalgesia. This relationship can be observed in numerous experimental studies on hypertensive rats, in which hypoalgesia is brought about by baroreceptor activation and can be alleviated by carotid sinus baroreceptor denervation or by decreasing the cardiopulmonary baroreceptor afferent input.[106] In addition, resection of the right cervical vagus, which reduces cardiopulmonary vasoreceptor afferent input, has been shown to markedly reduce hypoalgesic behaviour in SHR.[66] It has been postulated that cardiovascular and pain regulatory responses may be coordinated as part of an adaptive mechanism, helping the body to face stressful events.[83;107]

A role for endogenous opioids in hypertension-associated hypoalgesia is suggested as a result of the finding that, in rats, this type of hypoalgesia is successfully suppressed by the opiate

antagonist naloxone. Furthermore, hypertensive rats were found to have increased opioid activity in several areas of the central nervous system (CNS).[66;105] Interestingly, in a study on humans Ring *et al.,* 2008 observed that hypertensives experienced less pain than normotensives during their assessment of pain tolerance but that this manifestation of hypertensive hypoalgesia was *not* moderated by naltrexone.[108]

The existence of an association between increased arterial blood pressure and hypoalgesia has been repeatedly shown in experimental hypertension and confirmed in humans using various form of noxious stimuli, including electrical tooth pulp, thermal and electrocutaneous stimulation. Induction of hypertension in rats by different methods (mineralocorticoid treatment, phenylephrine administration, occlusion of abdominal aorta, renal artery clipping, deoxycorticoesterone acetate salt administration, social deprivation and hypothalamic grafts from SHR) is associated with reduced responsiveness to noxious stimuli (hot-plate, electric shock and mechanical force applied to a limb).[66;105;107] The interaction between hypertension and pain perception has also been supported by the finding that experimental interventions acting to lower blood pressure also reduce hypoalgesia.[107]

Increased tolerance to pain has also been observed in hypertensive humans. The first to report on hypertension-associated hypoalgesia in humans were Zamir and Shuber, 1980 who found a higher pain threshold during non-invasive tooth pulp stimulation in unmedicated essential hypertensives compared with normotensives.[65] These results were confirmed using the same technique by Ghione *et al.,* in 1988.[109] Sheps *et al.*, 1992 found that the average mean arterial

pressure in all participants (hypertensives and normotensives) was significantly related to both thermal pain threshold and tolerance.[84]

More recently, Rosa *et al.,* 1994 found significantly higher electrocutaneous perceptive, pain and tolerance thresholds in hypertensives compared with normotensives as well as significantly higher tooth pulp pain and tolerance thresholds.[66] In addition, the thresholds of two components (R2 and R3) of the blink reflex to electrical stimulation of the supraorbitalis nerve were significantly higher in hypertensives and a significant correlation was found between R3 threshold (nociceptive component of the blink reflex) and diastolic pressure. Together, these results confirm that hypertension is associated with hypoalgesia in humans.[66] Even among normotensive humans, resting systolic blood pressure has been found to correlate negatively with pain ratings and in addition, normotensives at risk for developing hypertension have demonstrated decreased pain sensitivity compared with low risk individuals.[110]

From a clinical perspective, hypertension-associated hypoalgesia is important since there is evidence that asymptomatic (silent) myocardial ischemia and unrecognised, presumably painless, myocardial infarction are more common in people with elevated blood pressure.[66;105;111]

1.7 Hypertension and Peripheral Neuropathy

There have been few studies investigating the relationship between hypertension and peripheral neuropathy. The results of these have been inconclusive but some have shown a possible positive association between the two variables.

Zamir and Shuber, 1980 found that sensory threshold as well as pain thresholds differed between normotensive and hypertensive subjects.[65] Rosa *et al.,* 1994 observed that other thresholds, which cannot easily be related to pain sensation (i.e. perceptive cutaneous threshold and the R2 component of the blink reflex), are significantly increased in human hypertension. These findings may suggest that other sensations besides pain are reduced in arterial hypertension, but this proposal warrants further investigation.[66] Zarrelli *et al.,* 2001 concluded that arterial hypertension might be an independent risk factor for chronic symmetric polyneuropathy (CSP) in the elderly.[67] Legrady *et al.,* 2006 found higher current perception threshold (CPT) values in the peroneal nerve of both diabetic and non-diabetic hypertensives compared to controls, indicating sensory loss. Duration of hypertension and diabetes mellitus both correlated positively with CPT's measured on the lower extremities in the diabetic hypertensive group. This study concluded that the severity of peripheral sensory neuropathy (PSN) was similar in non-diabetic and diabetic hypertensive patients although, in the latter, the PSN can involve more types of nerve fibres. Hypertension should thus be considered in the pathogenesis of peripheral nerve dysfunction.[68]

Rivero Luis *et al.,* 2006 found that peripheral and autonomic nerve function are often altered in obese people without diabetes and thus hypertension might contribute to peripheral nerve

dysfunction.[69] Edwards *et al.,* 2008 found that although hypertension did not affect sensory nerve conduction velocity, unmedicated essential hypertensives demonstrated sensory loss and significantly lower amplitude sensory nerve action potentials compared to normotensives.[70] These preliminary findings suggest that unmedicated hypertensives may have a reduced number of active nerve fibres, possibly resulting in mild subclinical peripheral neuropathy.[70] In a subsequent study investigating the effects of essential hypertension on short latency human somatosensory-evoked potentials, Edwards *et al.,* 2010 found unaltered peripheral nerve conduction velocities but reduced amplitude sensory nerve action potentials in unmedicated hypertensives compared to nomortensives, thus reinforcing their earlier findings and demonstrating that hypertension may affect the peripheral nervous system by causing axonal loss without affecting myelination.[71] Results from these two recent studies oppose those of Viskoper *et al.,* 1971 who showed reduced motor nerve conduction velocities in the upper extremities of medicated hypertensives compared to normotensives and that conduction velocities were inversely related to diastolic blood pressure.[112]

The foregoing studies suggest that essential hypertension is characterised by sensory loss. However, the mechanisms underlying these sensory deficits remain unclear. The pathological process may be similar to that in diabetic neuropathy, whereby metabolic and vascular disturbances lead to structural and functional changes in blood vessels supplying the peripheral nerves, resulting in ischaemia and hypoxia and ultimately peripheral neuropathy.[87;88;101-103] This is feasible since hypertension is a risk factor for peripheral vascular disease, which causes ischaemia leading to oxidative stress and injury to nerves via increase production of reactive oxygen species.[89-91] In fact, studies on non-diabetic spontaneously hypertensive rats have

demonstrated impaired vascular supply to peripheral nerves and morphological changes and decreased nerve function in the sciatic nerve, all of which improved with antihypertensive medication.[101-103] Furthermore, peripheral nerves are susceptible to ischaemia and hypoxia because they lack the ability to autoregulate (maintain a constant blood flow despite changes in perfusion pressure). Thus, they rely on a profuse blood supply to maintain oxygenation.[113]

In sum, hypertension is a known risk factor for diabetic neuropathy and unmedicated essential hypertensives have a reduced ability to feel pain compared to normotensives. There is also some evidence that hypertension may cause peripheral neuropathy. However, the mechanisms underlying these associations remain unclear.

1.8 Hypotheses

Two studies are presented in this thesis:

1.8.1 Study One: Peripheral Neuropathy in Hypertension

The evidence suggests an association between hypertension and peripheral neuropathy**.** It was therefore hypothesised that individuals with newly diagnosed unmedicated essential hypertension would show more sensory deficits in response to peripheral nerve stimulation than individuals with normal blood pressure.

1.8.2 Study Two: Retrospective Database Analysis of Cardiovascular Risk in Confirmed and Borderline Hypertensive Patients

Patients with hypertension often have other risk factors for cardiovascular disease which help clinicians decide whether to treat them with antihypertensive medications and/or lifestyle interventions. Cardiovascular risk profiles of patients with unmedicated confirmed hypertension or unmedicated borderline hypertension, selected from our Hypertension Database, were determined. It was hypothesised that the borderline hypertensives, who would not normally be started on antihypertensive medication in the absence of other risk factors, would have similar cardiovascular risk profiles to the confirmed hypertensives.

1.9 Aims

1.9.1 Study One: Peripheral Neuropathy in Hypertension

The primary aim of this thesis was to look at the influence of unmedicated essential hypertension (SBP ≥140 or DBP ≥90 mmHg) on a range of sensory thresholds to investigate the possibility that hypertension may cause subclinical peripheral neuropathy.

1.9.2 Study Two: Retrospective Database Analysis of Cardiovascular Risk in Confirmed and Borderline Hypertensive Patients

The secondary aim of this thesis was to compare cardiovascular risk factors, including ambulatory arterial stiffness index (AASI) and features consistent with the metabolic syndrome, in a larger number of patients with unmedicated confirmed hypertension (SBP ≥160 mmHg or DBP ≥100 mmHg) or unmedicated borderline hypertension (SBP 140-159 mmHg or DBP 90-99 mmHg), selected from our Hypertension Database.

CHAPTER 2: STUDY ONE - PERIPHERAL NEUROPATHY IN HYPERTENSION

2.1 Introduction and Aims

It is known that hypertension is strongly associated with the development of diabetic neuropathy and that unmedicated hypertensives have higher pain thresholds than normotensives. There is also some evidence that hypertension may be positively associated with peripheral neuropathy. The present study will look at the influence of newly diagnosed unmedicated essential hypertension on a *range* of sensory thresholds (not just heat-pain) to investigate the possibility that hypertension may cause subclinical peripheral neuropathy across an array of sensory modalities.

To our knowledge, no previous study has examined peripheral nerve function using a range of quantitative sensory tests, to assess a range of sensory nerve fibres and modalities in the upper and lower limbs of newly diagnosed unmedicated essential hypertensives.

2.2 Methods and Analysis

2.2.1 Participants

20 newly diagnosed unmedicated essential hypertensives (12 men, 8 women) and 25 normotensive controls (9 men, 16 women) were investigated for subclinical peripheral neuropathy. Table 5 and 6 show the characteristics of the participants in the hypertensive and normotensive groups. Newly diagnosed unmedicated essential hypertensives (SBP ≥140 or DBP ≥90 mmHg) were recruited from a hypertension clinic at the Wellcome Trust Clinical Research Facility (WTCRF), Queen Elizabeth Hospital (QEH), Birmingham, United Kingdom. Normotensive controls were recruited via email-sent out to all Queen Elizabeth and Selly Oak Hospital staff. Controls were paid £15 for their time. Participants gave written consent after reading an information sheet explaining the procedures involved. The local ethics advisory committee approved this study.

2.2.2 Establishment of Blood Pressure Status

Patients with newly diagnosed unmedicated essential hypertension were recruited from a hypertension clinic. All patients underwent 24-hour ambulatory blood pressure monitoring (ABPM) (SpaceLabs, Model 90217) and 3 clinic BP measurements taken with an OMRON Mi-5. These monitors have been validated according to the protocols of the Association for the Advancement of Medical Instrumentation and the British Hypertension Society. Account was taken of the differences between ABPM measurements and clinic measurements as per BHS IV guidelines.[3] **Hypertension** was defined as sustained systolic blood pressure at referral of ≥140mmHg or a diastolic blood pressure of ≥90mmHg, which was confirmed at clinic and on ABPM. **Normal blood pressure** was defined as mean clinic blood pressure, on the day of testing, of <140mmHg systolic and <90mmHg diastolic.

2.2.3 Exclusion Criteria

Participants were excluded for the following:

- Age younger than 18 years or older than 50 years (the latter were excluded because there is evidence that risk of peripheral neuropathy increases with age).[114;115]
- BP >180/110mmHg confirmed on ABPM with or without evidence of accelerated phase hypertension (blood pressure must be treated without delay)
- Pregnancy or menstrual period over 1 month ago
- Major psychiatric disorder
- Current use of prescription medication (excluding contraceptives)
- Excess alcohol intake (>21 units/week in men, or >14 units/week in women)
- Thyroid disease
- Chronic liver disease
- Cerebrovascular disease
- Angina
- Myocardial infarction
- Peripheral vascular disease
- Chronic neurological disease
- Rheumatoid or osteoarthritis
- Acromegaly or gout
- Any chronic disease or any condition predisposing to carpal tunnel syndrome or peripheral neuropathy including diabetes mellitus (either pre-existing or diagnosed on random blood sugar sample)

- Other causes of peripheral neuropathy (hereditary neuropathy, B12 deficiency, cryoglobulinaemia etc.)
- Neck or back surgery
- Cardiac pacemaker
- Consumption of alcoholic or caffeinated drinks 2 hours before the start of the study.

If clinically indicated the patients in the hypertensive group had appropriate investigations to exclude secondary causes of hypertension before taking part in the study. On the basis of these investigations patients were excluded if there was evidence of underlying renal or adrenal pathology including acute or chronic renal failure, renal artery stenosis, glomerulonephritis, pyelonephritis, Conn's syndrome, or phaeochromocytoma. Any person with a poor understanding of English was also excluded from the study to ensure that informed consent was obtained and that tasks were followed correctly.

2.2.4 Apparatus and Physiological Measurements

Height (m), weight (kg), waist (cm) and hip (cm) measurements were determined using standard methods. Blood pressure measurements were taken at rest in an upright sitting position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained using an oscillometric sphygmomanometer and brachial cuff (Omron IC, Omron Health Care Ltd.) attached to the participant's upper right arm (the right arm was used for logistical reasons). Three consecutive readings were obtained before starting the sensory tests. These three readings were averaged to give measures of mean resting systolic and diastolic pressure. The following sensory nerve function tests and database analyses then commenced.

2.2.5 Computer Aided Sensory Evaluator (CASE) IV

The CASE IV system (WR Medical Electronic Co., USA) attached to a laptop (Toshiba) was used to administer stimuli and record threshold data. This system has been shown to be useful in conjunction with clinical examination for diagnosing diabetic neuropathy.[116]

For vibration, cooling, warming and heat-pain detection thresholds, a cue device (WR Medical Electronics Co., USA) was used to denote the time period in which the stimuli were administered. For all these tests, except heat-pain, a response device (WR Medical Electronics Co., USA) with buttons marked 'yes' and 'no' was used to register the participant's response.

Vibration detection threshold was measured using a vibration stimulator (WR Medical Electronics Co., USA). The vibration stimulator was placed on the participant's right index finger or big toe, in the midline between the base of the nail and the most distal knuckle (Figure 4).

Vibration was delivered at 125 cycles per second and was variable between 0 and 576 micrometers.

Thermal sensation and heat-pain detection thresholds were measured using a thermal stimulator (WR Medical Electronics Co., USA). The thermal stimulator was strapped to the dorsal surface of the participant's right hand or foot (Figure 5). During operation the thermal stimulator produced a specified temperature on a 9.0-square-centimeter stimulating surface. The stimulating surface temperature could be varied from 8.0°C to 50.0°C with accuracy of 1.25°C to 0.25°C depending on the temperature.

Participants sat upright in a chair. During the sensory threshold assessment on the hand the right hand rested, palm down on the vibration board, on a table (Figure 4). During the sensory threshold assessment on the foot the right foot was rested, sole down, on the vibration board on the floor (Figure 5). The vibration board was a ceramic board with a foam base designed to dampen any unwanted vibrations transmitted through the table or the floor. Skin surface temperature was measured prior to the thermal test on the hand and foot using an infrared thermometer (C-500 C, Linear Laboratories, CA).

Units of stimulus intensity used were JND's (Just Noticeable Differences). Vibration, cooling and warming stimuli were increased and decreased using the 4, 2 and 1 stepping method devised by Dyck.[117] This method was chosen for its accuracy and speed. Heat-pain was increased and decreased using the heat pain non-repeating ascending with null stimuli method.[118] Using this

method it was possible to compare the heat-pain detection threshold (HP:0.5) and the intermediate heat-pain response (HP:5.0).[118]

2.2.6 Procedure

Testing was carried out in the Wellcome Trust Clinical Research Facility at the Queen Elizabeth Hospital, Birmingham, United Kingdom. The testing room was quiet, had sufficient light and air circulation and was at a comfortable temperature, free from drafts. Each participant attended a single 1 hour session.

On arrival at the clinic, participants were introduced and briefed on the experiment before signing a consent form. Their height (m), weight (kg), waist (cm) and hips (cm) were then measured. Participants sat comfortably in a chair next to a table and any questions they had were answered. Participant's risk factors and family history were then obtained by means of a questionnaire. This included questions about ethnicity, smoking, drinking habits, salt intake, exercise and family history of heart disease, diabetes and hypertension**.** Three resting blood pressure measurements were then obtained from the right upper arm before commencing the sensory tests.

During the session vibration, cooling, warming and heat-pain thresholds of the right hand and foot were determined using the CASE IV system (WR Medical Electronics Co., USA).

2.2.6.1 Quantitative Sensory Testing (QST) of Vibratory Perception

Vibration sensation assesses myelinated Aβ fibre function. Vibratory perception was measured on the dorsal surface of the right index finger followed by the right big toe, in the midline between the nail bed and the most distal knuckle. Normative data has been obtained at these body sites in healthy individuals (aged 3-79 years).[119]

2.2.6.1.1 Vibratory Testing in the Hand and Foot

Participants placed their right hand/foot (plantar surface down) onto the vibration board. The vibration stimulator was placed on the participant's right index finger/big toe in the midline between the base of the nail and the most distal knuckle. The stimulator was adjusted so that its body was horizontal using a knob, checking the level bubble. Instructions for the vibration test were read out and any questions were answered. Instructions were read from a pre-prepared sheet to ensure that all participants were given identical information. The need for the participants to wear headphones for this test was explained (the headphones played noise to block out any external noises) and the headphones were placed on the participant. Participants were required to press 'yes' or 'no' on the response device as to whether they felt a stimulus during the display of the number '1' on the cue device. Practice tests were performed to estimate the threshold then the vibration test was performed. If two positive responses were given to null stimuli the test was repeated.

2.2.6.2 QST of Cooling, Warming and Heat-Pain Perception

Cooling sensation assesses thinly myelinated Aδ fibre function and warming and heat-pain sensations assess unmyelinated C fibres. Thermal and heat-pain perception were measured on the dorsal surface of the participant's right foot followed by the right hand.

2.2.6.2.1 Cooling, Warming and Heat-Pain Testing of the Right Foot

Participants put a sock on into which a hole had been cut. The purpose of the sock was to insulate the foot. The temperature of the participant's foot was measured. The thermal stimulator was placed on the dorsal surface of the participant's right foot through the hole, ensuring the surface was flat against the skin and was in contact with the skin at all four corners. Instructions were read out for the cooling test and any questions were answered. Participants were required to respond 'yes' or 'no' on the response device as to whether they felt a stimulus during the display of the number '1' on the cue device. Practice tests were performed to estimate the threshold and the cooling test was performed. If two positive responses were given to null stimuli the test was repeated.

Instructions for the warming test were read out and any questions were answered. Participants were required to respond 'yes' or 'no' on the response device as to whether they felt a stimulus during the display of the number '1' on the cue-device. Practice tests were performed to estimate the threshold and then the warming test was performed. If two positive responses were given to null stimuli the test was repeated.

Instructions for the heat-pain test were read out and any questions were answered. Participants were reassured that we would be testing them only at low discomfort and pain levels and were told that they should inform us if the stimuli got too high and they did not wish to continue. Participants were required to verbally rate any discomfort or pain felt during the display of the number '1' on the patient cue device on a scale from 0-10 (0=nothing or warm or hot, 1=lowest level of discomfort or pain, 10=most severe pain). These responses were typed into the laptop. As soon as participants rated the pain as 5 or above the test stopped. A pain rating of '1' corresponded to the heat-pain detection threshold (HP: 0.5) and a rating of 5 or above corresponded to the intermediate heat pain response (HP: 5.0). It was explained to the participants that there was no practice for this test. Then the heat-pain test was performed. If a positive result was given to null stimuli the test was repeated.

2.2.6.2.2 Cooling, Warming and Heat-Pain Testing of the Right Hand

The same thermal and heat pain tests were performed on the participant's right hand.

The temperature of the participant's hand was measured. The thermal stimulator was positioned on the dorsal surface of the participant's right hand ensuring all four corners were in contact with the skin. Cooling, warming and heat-pain tests were performed as on the foot.

In several instances the participants did not report notable pain or warming even at the highest stimulus intensity of the instrument. For these participants in whom the pain and/warming threshold was not measurable since it was above the upper range of stimulation, the highest available value of 25 JND (Just Noticeable Difference) was assigned.

Participants were free to withdraw at any time. All information obtained from the study was kept confidential and anonymous and participants were identified by subject number only.

Figure 4. Measurement of Vibration Threshold in the Right Hand. The right hand rested on the vibration board and the vibration stimulator was placed on the index finger, in the midline between the base of the nail and the most distal knuckle.

Figure 5. Measurement of Thermal and Heat-Pain Thresholds in the Right Foot. Participants wore a sock with a hole in its dorsal surface. The thermal stimulator was strapped onto the dorsal surface of the foot, through the hole, so that all four corners of the stimulator were in contact with the skin.

2.2.7 Statistical Analysis

Results were analysed using statistical software (PASW Statistics 18, SPSS Inc., Chicago). The two groups (hypertensive and normotensive) were compared using various statistical tests. First, an independent-samples T Test was used to determine differences in continuous variables between the hypertensive males and normotensive males, and between the hypertensive females and normotensive females. Second, Chi-square analysis was used to determine differences in categorical variables between the hypertensive and normotensive group. Third, the sensory thresholds of the hypertensive and normotensive group were compared using one-way analyses of variance (ANOVA). Fourth, the sensory thresholds of the hypertensive and normotensive group were compared using univariate analysis of covariance (ANCOVA), which allowed adjustment for the influence of potential confounders such as age, sex and BMI. This was necessary because age, sex and BMI are known to influence peripheral nerve dysfunction.[114;115;120;121] Finally, Spearman's correlation coefficients were calculated for all sensory thresholds against mean systolic and diastolic blood pressure measurements. A significance level of .05 was adopted for all the above analyses.

2.2.8 Power Calculations

We assessed each individual's peripheral nerve function using a battery of sensory tests. In the initial analyses, for each outcome measure yielded by these tests, we compared the scores among the hypertensives and normotensive group. With alpha at 0.05 and a total sample size of 45, the study is powered at 0.80 to detect a group difference by ANCOVA corresponding to an effect size of f=0.43, which corresponds to a large effect size.[122]

2.3 Results and Brief Discussion

2.3.1 Group Characteristics and Risk Factors of Each Blood Pressure Group

Continuous variables of the hypertensive and normotensive males are presented in Table 5 and Figure 6. An independent-sample T Test confirmed that, compared to the normotensive males the hypertensive males exhibited significantly *higher* SBP, DBP, age, weight and BMI but the groups did not differ in terms of height or alcohol consumption (Table 5).

Continuous variables of hypertensive and normotensive females are presented in Table 6 and Figure 7. An independent-sample T Test confirmed that, compared to the normotensive females the hypertensive females exhibited significantly *higher* SBP and DBP but the groups did not differ in terms of age, height, weight, BMI or alcohol consumption (Table 6).

Table 5. Unadjusted Mean [Standard Deviation (SD)], Blood Pressures, Age, Height, Weight, BMI and Alcohol Consumption of the Hypertensive and Normotensive Males as well as the Degrees of Freedom, t-Values and Statistical Significance Level of the Group Effects

Variable	Mean (SD) Hypertensive Males n=12	Mean (SD) Normotensive Males n=9	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Standard Error Difference	95% Confidence Interval of the Difference	
										Lower	Upper
SBP (mmHg)	156.25 (16.64)	122.89 (11.92)	0.60	0.45	5.10	19	<0.001**	33.36	6.54	19.67	47.06
DBP (mmHg)	98.83 (11.04)	73.78 (6.40)	2.81	0.11	6.06	19	<0.001**	25.06	4.13	16.40	33.71
Age (years)	36.00 (7.70)	28 (4.18)	2.00	0.17	2.81	19	<0.05*	8.00	2.85	2.04	13.96
Height (m)	1.81 (0.10)	1.79 (0.06)	2.75	0.11	0.57	19	0.57	0.02	0.04	-0.06	0.10
Weight (kg)	99.80 (18.42)	73.24 (13.05)	0.85	0.37	3.68	19	<0.05*	26.56	7.22	11.44	41.67
BMI (kg/m^2)	30.58 (4.56)	22.89 (3.30)	1.25	0.28	4.28	19	<0.001**	7.69	1.80	3.93	11.46
Alcohol (units/week)	6.63 (7.00)	3.03 (4.84)	3.34	0.08	1.32	19	0.20	3.60	2.73	-2.11	9.30

*= significant group difference at 0.05 level **=significant group difference at 0.001 level

Table 6. Unadjusted Mean (SD), Blood Pressures, Age, Height, Weight, BMI and Alcohol Consumption of the Hypertensive and Normotensive Males as well as the Degrees of Freedom, t-Values and Statistical Significance Level of the Group Effects

Variable	Mean (SD) Hypertensive Females n=8	Mean (SD) Normotensive Females n=16	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Standard Error Difference	95% Confidence Interval of the Difference	
										Lower	Upper
SBP (mmHg)	148.38 (8.45)	113 (15.44)	1.48	0.24	6.00	22	<0.001**	35.38	5.89	23.15	47.60
DBP (mmHg)	96.50 (6.41)	71.81 (11.31)	2.22	0.15	5.69	22	<0.001**	24.69	4.34	15.69	33.68
Age (years)	36.38 (10.62)	34.31 (10.35)	0.14	0.71	0.46	22	0.65	2.06	4.52	-7.31	11.44
Height (m)	1.66 (0.06)	1.63 (0.06)	0.00	0.97	1.09	22	0.29	0.03	0.03	-0.03	0.08
Weight (kg)	73.28 (18.52)	63.22 (12.11)	2.50	0.13	1.61	22	0.12	10.06	6.26	-2.93	23.04
BMI (kg/m^2)	26.75 (5.65)	23.88 (4.21)	1.18	0.29	1.41	22	0.17	2.88	2.04	-1.36	7.11
Alcohol (units/week)	3.75 (3.81)	2.58 (4.20)	0.30	0.59	0.66	22	0.51	1.17	1.77	-2.49	4.84

*= significant group difference at 0.05 level **=significant group difference at 0.001 level

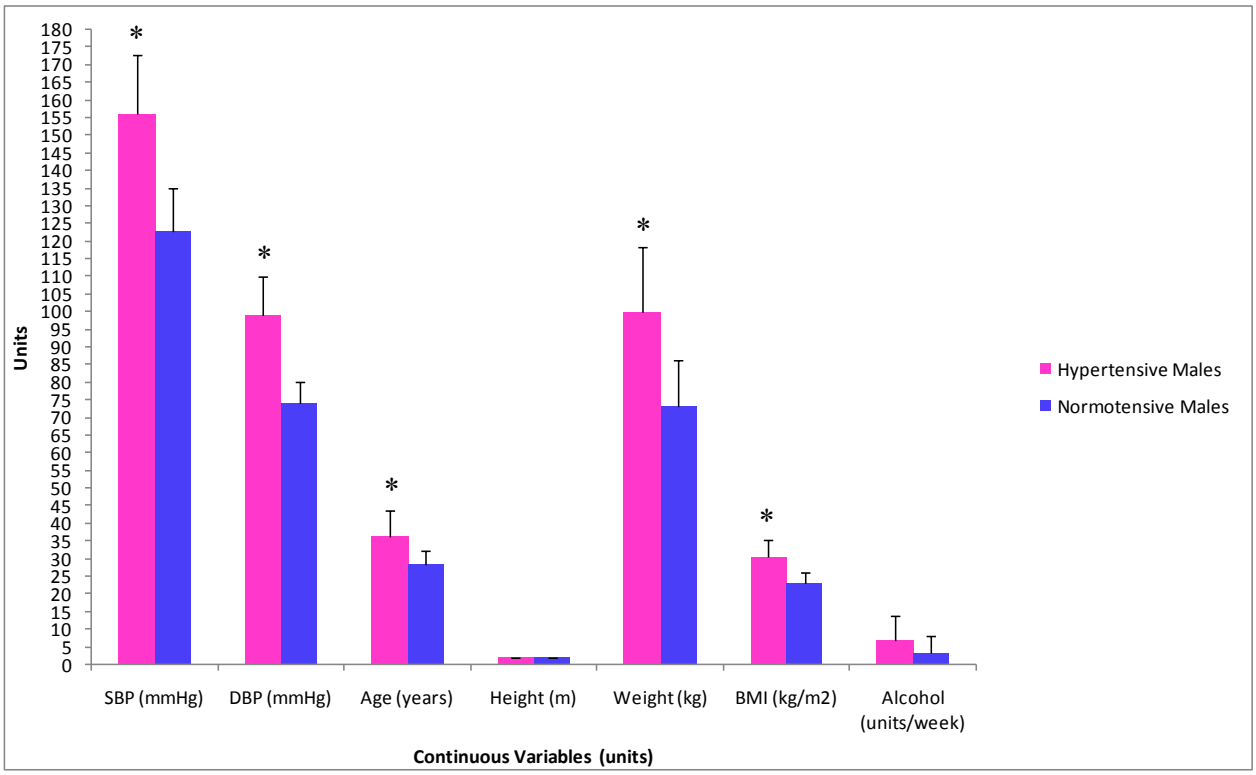

Figure 6. Unadjusted Mean and Standard Deviation of all Continuous Variables of the Hypertensive Males and the Normotensive Males * Denotes a Significant Difference at the 0.05 Level.

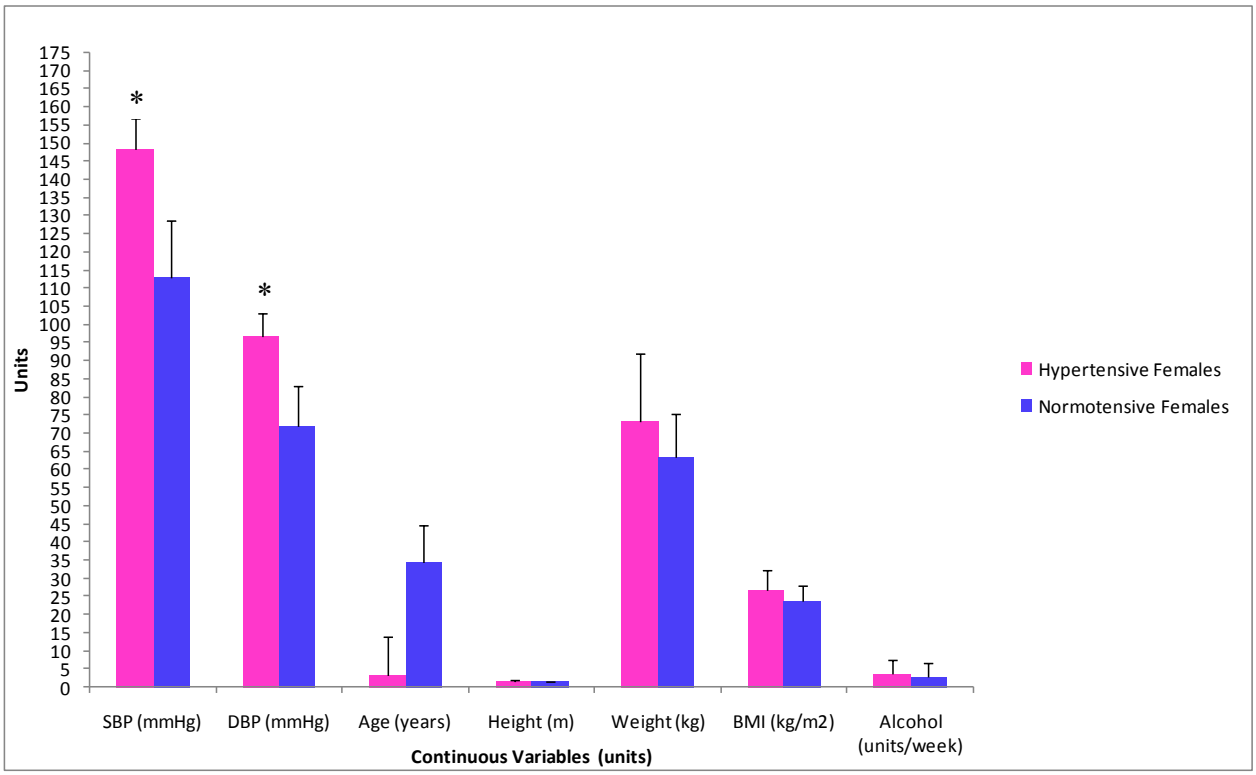

Figure 7. Unadjusted Mean and Standard Deviation of all Continuous Variables of the Hypertensive Females and the Normotensive Females * Denotes a Significant Difference at the 0.05 Level.

Categorical variables are presented in Table 7. Chi-square analysis revealed that the hypertensive and normotensive group did not differ significantly in terms of sex, smoking status, exercise or salt intake. However, there was a significantly *higher* incidence of family history of hypertension in the hypertensive group.

Table 7. Number of Males and Females and Number and Percentage of Cardiovascular Risk Factors in the Hypertensive and Normotensive Group as well as the Degrees of Freedom, Chi-square Values and Statistical Significance Levels of the Group Effects

		n=20 Hypertensives		n=25 Normotensives		df	χ^2	*p*
		Number of participants	%	Number of participants	%			
Sex	**Male**	12		9		1	2.57	0.11
	Female	8		16				
Family history of HT	**Yes**	14	74	6	25	1	10.10	<0.001**
	No	5		18				
Smoker/Ex-smoker	**Yes**	8	40	4	16	1	3.27	0.07
	No	12		21				
Exercise <5x30mins/week	**Yes**	7	47	14	58	1	0.04	0.84
	No	8		10				
Excess salt	**Yes**	7	35	12	48	1	1.97	0.16
	No	13		13				
Features consistent with the metabolic syndrome	**Yes**	11	65	NK	NK			
	No	6						

*= significant group difference at 0.05 level **=significant group difference at 0.001 level NK, Not Known

2.3.2 Analysis of Co-Variance and Spearman's Correlation Coefficients on Sensory Threshold Data Obtained from CASE IV

Because age, sex and BMI are known to influence peripheral nerve function and were different between blood pressure groups, these variables were adjusted for in the univariate analysis of co-variance (ANCOVAs) that follow.[114;115;120;121]

A series of one-way analysis of variance (ANOVAs) were performed on vibration, cooling, warming and heat-pain thresholds in the right hand and foot (Table 8). These analyses revealed a significant *group* difference for vibration threshold in the foot, cooling threshold in the hand, and vibration threshold in the hand. The hypertensive group exhibited *higher* vibration thresholds in the hand and foot than the normotensive group but *lower* cooling thresholds in the hand.

A series of univariate analysis of co-variance (ANCOVAs), adjusted for age, sex and BMI were performed on vibration, cooling, warming and heat-pain thresholds in the right hand and foot (Table 9). These analyses revealed a significant *group* difference for vibration threshold in the foot. The hypertensive group exhibited *higher* vibration thresholds in the foot than the normotensive group. Unadjusted mean sensory thresholds of the hypertensive and normotensive group are presented in Figure 8.

Table 8. ANOVA. Unadjusted Mead (SD) Sensory Thresholds of the Hypertensive and Normotensive Group, as well as the Degrees of Freedom, F Values, Statistical Significance Level of the Group Effects and Associated Effect Size

Variable	Hypertensives	Normotensives	df	F	*p*	η^2
Cooling Threshold Foot	8.87 (4.23)	9.83 (3.92)	1	0.63	0.43	0.01
Warming Threshold Foot	14.72 (5.66)	14.70 (4.66)	1	0.00	0.99	0.00
Vibration Threshold Foot	16.50 (2.50)	13.91 (1.64)	1	17.55	<0.001**	0.29
Heat-Pain 5.0 Foot	22.80 (2.21)	22.54 (2.67)	1	0.12	0.73	0.00
Heat-Pain 0.5 Foot	19.72 (2.02)	18.71 (2.62)	1	2.01	0.16	0.05
Cooling Threshold Hand	8.02 (2.25)	9.55 (2.54)	1	4.50	0.04*	0.10
Warming Threshold Hand	9.85 (2.53)	11.68 (3.32)	1	4.16	0.05	0.09
Vibration Threshold Hand	11.15 (2.13)	9.77 (1.32)	1	7.10	0.01*	0.14
Heat-Pain 5.0 Hand	22.45 (2.08)	21.85 (2.36)	1	0.79	0.38	0.02
Heat-Pain 0.5 Hand	19.10 (2.14)	18.08 (2.58)	1	2.03	0.16	0.05

*= significant group difference at 0.05 level **=significant group difference at 0.001 level

Table 9. ANCOVA. Unadjusted Mean (SD) Sensory Thresholds of the Hypertensive and Normotensive Group, as well as the Degrees of Freedom, F Values, Statistical Significance Level of the Group Effects (Adjusted for Age, Sex and BMI) and Associated Effect Size

Variable	Hypertensives	Normotensives	df	F	*p*	η^2
Cooling Threshold Foot	8.87 (4.23)	9.83 (3.92)	1	0.36	0.56	0.01
Warming Threshold Foot	14.72 (5.66)	14.70 (4.66)	1	1.25	0.27	0.03
Vibration Threshold Foot	16.51 (2.50)	13.91 (1.64)	1	8.98	0.01*	0.19
Heat-Pain 5.0 Foot	22.80 (2.21)	22.54 (2.67)	1	0.04	0.84	0.00
Heat-Pain 0.5 Foot	19.72 (2.02)	18.71 (2.62)	1	0.49	0.49	0.01
Cooling Threshold Hand	8.02 (2.25)	9.55 (2.54)	1	1.51	0.23	0.04
Warming Threshold Hand	9.85 (2.53)	11.68 (3.32)	1	3.02	0.09	0.07
Vibration Threshold Hand	11.15 (2.13)	9.77 (1.32)	1	4.11	0.05	0.10
Heat-Pain 5.0 Hand	22.45 (2.08)	21.85 (2.36)	1	0.20	0.66	0.01
Heat-Pain 0.5 Hand	19.10 (2.14)	18.08 (2.58)	1	1.173	0.29	0.03

*= significant group difference at 0.05 level **=significant group difference at 0.01 level

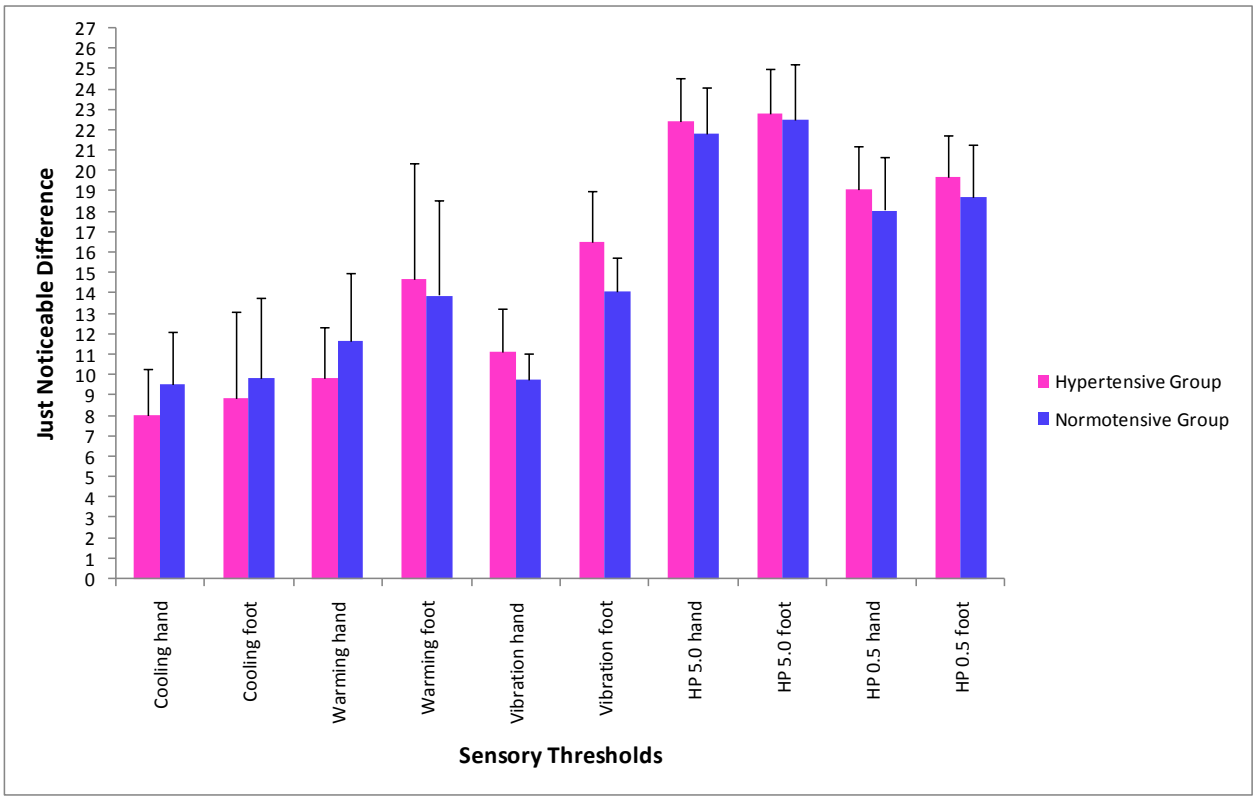

Figure 8. Unadjusted Mean and Standard Deviation of all Sensory Thresholds of the Hypertensive and Normotensive Groups * Denotes a Significant Group Difference at the 0.05 Level after Adjusting for Age, Sex and BMI

Spearman's correlation coefficients were calculated for all sensory thresholds against mean systolic and diastolic blood pressure measurements (Table 10). These analyses revealed a significant *positive* correlation between systolic and diastolic blood pressure and vibration threshold in the foot (Figure 9). Interestingly, there was a significant *negative* correlation between systolic and diastolic BP and cooling in the hand (Figure 10), and between systolic and diastolic blood pressure and warming in the hand (Figure 11).

Table 10. Spearman's Correlation Coefficients for all Sensory Thresholds against Mean Systolic and Diastolic Blood Pressure Measurements

Threshold	Systolic BP		Diastolic BP	
Cooling Threshold Foot	Correlation Coefficient	-0.16	Correlation Coefficient	-0.20
	Sig. (2-tailed)	0.30	Sig. (2-tailed)	0.19
Warming Threshold Foot	Correlation Coefficient	0.03	Correlation Coefficient	0.06
	Sig. (2-tailed)	0.84	Sig. (2-tailed)	0.68
Vibration Threshold Foot	Correlation Coefficient	0.47	Correlation Coefficient	0.38
	Sig.(2-tailed)	<0.001**	Sig. (2-tailed)	0.01*
Heat-Pain 5.0 Foot	Correlation Coefficient	0.04	Correlation Coefficient	0.02
	Sig. (2-tailed)	0.82	Sig. (2-tailed)	0.91
Heat-Pain 0.5 Foot	Correlation Coefficient	0.23	Correlation Coefficient	0.24
	Sig. (2-tailed)	0.14	Sig. (2-tailed)	0.11
Cooling Threshold Hand	Correlation Coefficient	-0.42	Correlation Coefficient	-0.37
	Sig. (2-tailed)	<0.001*	Sig. (2-tailed)	0.01*
Warming Threshold Hand	Correlation Coefficient	-0.39	Correlation Coefficient	-0.38
	Sig. (2-tailed)	0.01*	Sig. (2-tailed)	0.01**
Vibration Threshold Hand	Correlation Coefficient	0.19	Correlation Coefficient	0.25
	Sig. (2-tailed)	0.20	Sig. (2-tailed)	0.10
Heat-Pain 5.0 Hand	Correlation Coefficient	0.07	Correlation Coefficient	0.09
	Sig. (2-tailed)	0.66	Sig. (2-tailed)	0.55
Heat-Pain 0.5 Hand	Correlation Coefficient	0.09	Correlation Coefficient	0.11
	Sig. (2-tailed)	0.56	Sig. (2-tailed)	0.49

*= significant group difference at 0.05 level **=significant group difference at 0.001 level

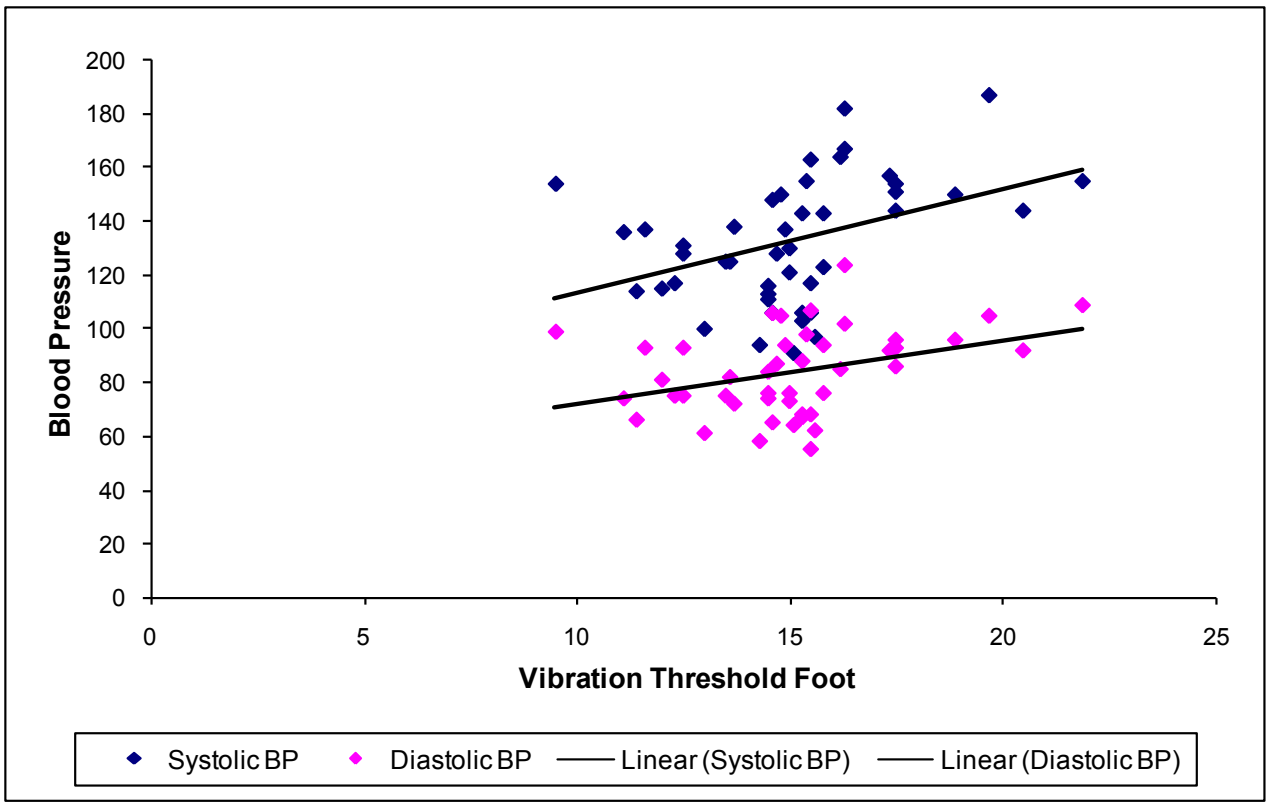

Figure 9. Scatter Graph Showing Vibration Threshold in the Foot (x) against Systolic and Diastolic Blood Pressure (y)

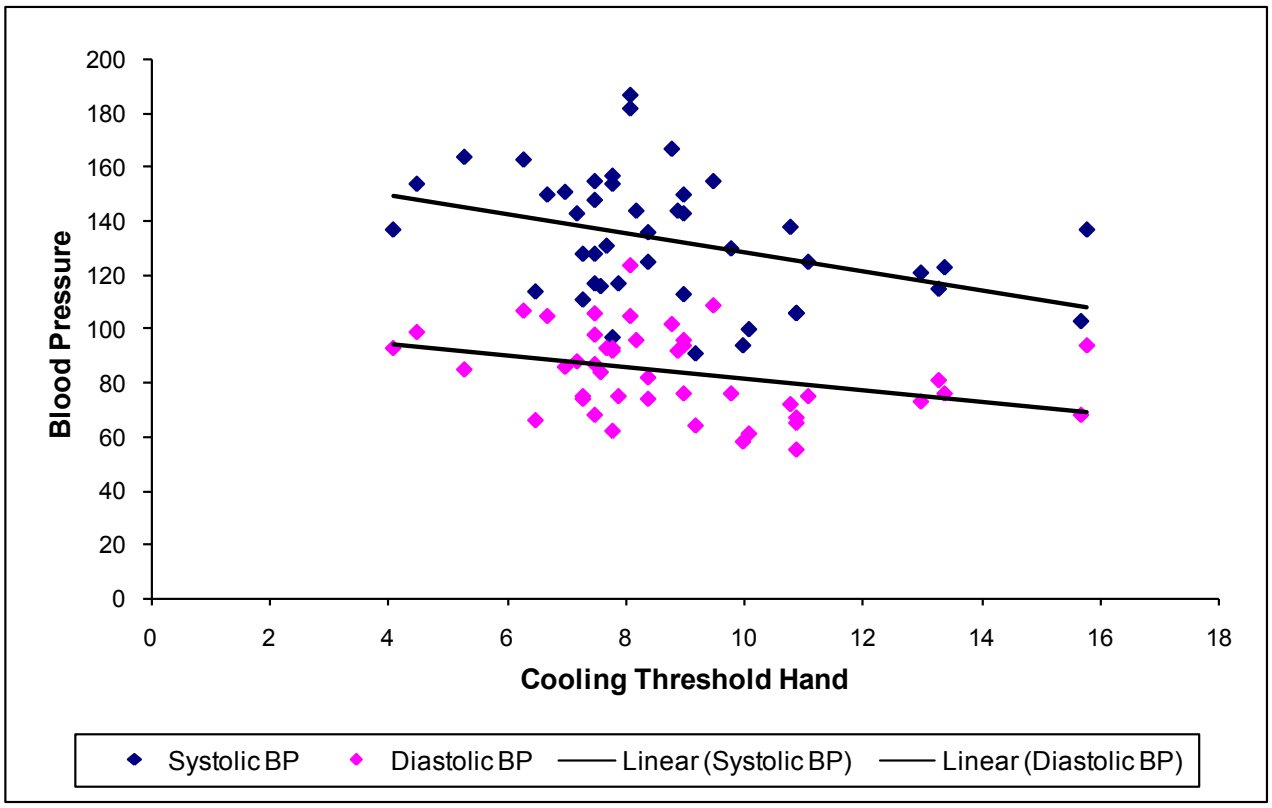

Figure 10. Scatter Graph Showing Cooling Threshold in the Hand (x) against Systolic and Diastolic Blood Pressure (y)

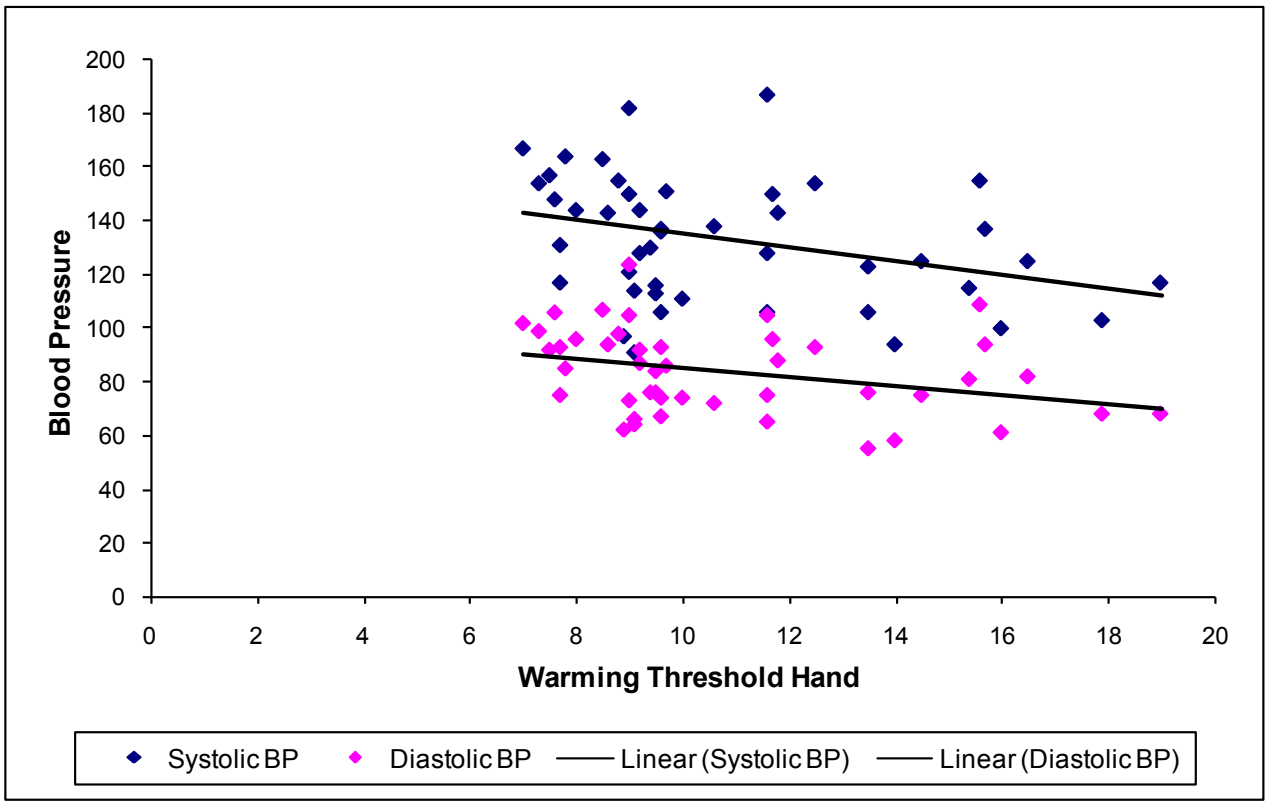

Figure 11. Scatter Graph Showing Warming Threshold in the Hand (x) against Systolic and Diastolic Blood Pressure (y)

2.3.3 Brief Discussion

To our knowledge, the current study was the first to use quantitative sensory testing (QST) to investigate different sensory thresholds in order to assess different nerve fibres (small as well as large) in a group of newly diagnosed unmedicated essential hypertensives as well as in a group of normotensive controls to investigate whether hypertensives have subclinical peripheral neuropathy. In previous studies cutaneous detection thresholds, current perception thresholds, sensory nerve conduction velocities and sensory action potentials were used to measure nerve function in general.[65;66;68;70] Earlier studies investigating peripheral nerve function in hypertensives did not measure specific senses, and measured pain by other means but not by the heat-pain method used in this study.[65-70;83;84]

The hypertensive males exhibited more risk factors for hypertension and cardiovascular disease than the normotensive males including significantly higher SBP, DBP, weight and BMI. The hypertensive females had significantly higher SBP and DBP than the normotensive females. The hypertensive group had significantly higher incidence of family history of hypertension than the normotensive group. It is important to note that the significantly higher weight and BMI of the hypertensive males may be confounding variables in this study. In addition, 65% of the hypertensive group had features consistent with the metabolic syndrome, which may be contributing to peripheral neuropathy rather than hypertension alone. Other possible confounders such as age, sex and alcohol consumption, however, were not significantly different between the hypertensive and normotensive group.

The major findings of this study were as follows. 1) The hypertensive group had significantly *higher v*ibration thresholds in the foot than the normotensive group, even after accounting for age, sex and BMI. 2) There was a significant *positive* correlation between SBP and DBP with vibration threshold in the foot. 3) There was a significant *negative* correlation between SBP and DBP with both cooling and warming thresholds in the hand.

The findings of increased vibration thresholds in the foot in the hypertensive group and of a positive correlation between SBP and DBP with vibration threshold support our hypothesis and some previous evidence of peripheral neuropathy in hypertension.

It is worth mentioning that vibration threshold in the hand was significantly *higher* in the hypertensive group before age, sex and BMI were accounted for. However, after accounting for these confounding variables the significance diminished, indicating that age, sex and/or BMI were contributing to the significant difference between groups rather than blood pressure alone. This supports evidence that age, sex and BMI influence peripheral nerve function.[114;115;120;121] Similarly, the significant group difference for cooling threshold in the hand diminished once age, sex and BMI were accounted for. With this in mind, it is possible that the significant negative correlation between SBP and DBP with cooling threshold in the hand may also disappear once these confounding factors are accounted for. This may also be the case for the significant negative correlation for warming in the hand. However, hierarchical linear regression analyses would have to be performed to investigate this.

Notably, all warming thresholds in the foot and heat-pain thresholds were higher in the hypertensive group even after adjusting for age, sex and BMI but not to a significant degree. However, these may become more significant with a larger sample size.

CHAPTER 3: STUDY TWO - RETROSPECTIVE DATABASE ANALYSIS OF CARDIOVASCULAR RISK IN CONFIRMED AND BORDERLINE HYPERTENSIVE PATIENTS

3.1 Introduction

Chapter two demonstrated some evidence of subclinical peripheral neuropathy, which may suggest damage to peripheral nerve vasculature in unmedicated hypertensives. The sample size was not large enough to determine whether these changes were happening in all grades of hypertension. However, vibration threshold was particularly affected and this appeared to be correlated with the level of blood pressure. This chapter will use information from our Hypertension Database to compare cardiovascular risk factors including features consistent with the metabolic syndrome and ambulatory arterial stiffness index (AASI) in a much larger group of confirmed hypertensives (n=83) and borderline hypertensives (n=154).

3.1.1 The Hypertension Database

The Hypertension Database (Microsoft Access 2002) contains extensive information on all new referrals to the Hypertension Clinic since May 2006. The database is updated every week at clinic from the patient's medical notes. Recorded information includes: hospital ID, initials, gender, date of birth, ethnicity, family history of hypertension, gestational hypertension, co-morbidities, current medications, risk factors (smoking, salt, exercise and alcohol consumption), reason for referral, clinic variables including waist, height, weight, BMI, mean clinic blood pressures, urinalysis results, fasting blood results including sugar, electrolytes, renal function, lipid profiles etc., electrocardiogram findings, ABPM findings including mean daytime and mean night time blood pressures, dipper status (i.e. whether or not the blood pressure is lower when the patient is asleep), clinical diagnosis based on ABPM results, record of any secondary investigations undertaken e.g. renal ultrasound, renin:aldosterone ratio etc., any changes to medications recommended to the general practitioner and further management plans e.g. lifestyle advice, repeat ABPM, discharge.

3.2 Methods

3.2.1 Establishment of Blood Pressure Status

Patients were categorised as having either confirmed or borderline hypertension depending on their mean clinic blood pressure readings and their mean daytime ABPM reading, which were available from the Hypertension Database. Account was taken of the 10/5 mmHg adjustment of ambulatory readings recommended by the BHS.[3] However, it is worth mentioning that the use of this adjustment is controversial and is not uniform across blood pressure levels. **Confirmed hypertension** was defined as sustained systolic blood pressure at referral of ≥160 mmHg or a diastolic blood pressure of ≥100mmHg, which was confirmed at clinic and on ABPM. **Borderline hypertension** was defined as sustained systolic blood pressure at referral of 140-159 mmHg or a diastolic blood pressure of 90-99 mmHg, which was confirmed at clinic and on ABPM.

3.2.2 Exclusion Criteria

Patients were excluded if they were taking any prescription medications, if there was evidence of secondary hypertension (renal or adrenal causes) or if they had any disease/disorder other than hypertension.

3.2.3 Database Analysis

1. Patient demographics (age, height, weight and BMI) and blood pressures (clinic and ABPM) were compared between the confirmed hypertensive group and the borderline hypertensive group.
2. Cardiovascular and/or hypertension risk factor profiles (sex, family history of hypertension, smoking, salt intake, exercise, alcohol intake and electrolytes) of the two groups were then compared. These analyses included a calculation of the 10 year coronary and CVD risk score in both groups calculated using the Framingham equation, which takes into account age, sex, SBP, DBP, cholesterol, HDL cholesterol, smoking, diabetes, and ECG-LVH.[15]
3. Ambulatory Arterial Stiffness Index (AASI) for each individual was calculated from their ABPM readings. The mean AASI of each group was compared. AASI was defined as 1 minus the regression slope of diastolic over systolic blood pressure readings obtained from 24-hour ABPM recordings. AASI gives a measure of arterial stiffness and thus cardiovascular risk.[55] The closer the AASI to 1, the stiffer the arteries.[61]
4. Body Mass Index (BMI), triglycerides, HDL cholesterol, blood pressure and fasting plasma glucose were used to determine the number of patients with features consistent with the metabolic syndrome.[62] Please note that waist measurements were not available for all patients so BMI was used to calculate metabolic syndrome. According to Alberti *et al.,* 2005, 'if body-mass index is over 30 kg/m^2, central obesity can be assumed and waist circumference does not need to be measured.'[62]

3.2.4 Statistical Analysis

Results were analysed using statistical software (PASW Statistics 18, SPSS Inc., Chicago). The two groups (confirmed hypertensive and borderline hypertensive) were compared using various statistical tests. First, an independent-samples T Test was used to determine differences in continuous variables between the confirmed hypertensive and borderline hypertensive group. Second, Chi-square analysis was used to determine differences in categorical variables between the confirmed hypertensive and borderline hypertensive group. Finally, the continuous variables were compared using univariate analysis of covariance (ANCOVA), which allowed adjustment for the influence of potential confounders such as age and sex. This was necessary because age and sex are known cardiovascular risk factors. A significance level of 0.05 was adopted for all the above analyses.

This was a retrospective analysis of a large database. It was not a prospective study so did not require a power calculation. The maximum number of patients that fulfilled the study criteria were pulled from the database for analysis.

3.3 Results and Brief Discussion

3.3.1 Group Characteristics and Cardiovascular Risk Factors of Each Blood Pressure Group

Group blood pressures and participant characteristics are presented in Table 11. An independent-sample T Test was performed on the patient demographics and blood pressures (Table 11). These analyses confirmed that, compared to the borderline group the confirmed hypertensive group exhibited significantly *higher* mean clinic SBP in the right arm, mean clinic DBP in the right arm, mean clinic SBP in the left arm, mean clinic DBP in the left arm, ABPM daytime SBP, ABPM daytime DBP, ABPM nighttime SBP, ABPM nighttime DBP and age. However, the groups did not differ in terms of height, weight or BMI (Table 11).

Table 11. Independent-Samples T Test. Unadjusted Mean (SD), Blood Pressures and Demographics of the Confirmed Hypertensive and Borderline Hypertensive Group as well as the Degrees of Freedom, t-Values and Statistical Significance Level of the Group Effects

Variable	Mean (SD) Confirmed HT's	Mean (SD) Borderline HT's	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Standard Error Difference	95% Confidence Interval of the Difference	
										Lower	Upper
Mean Clinic SBP right arm (mmHg)	170.31 (19.06)	153.39 (13.90)	9.45	<0.001**	-7.15	132.97	<0.001**	-16.92	2.37	-21.60	-12.24
Mean Clinic DBP right arm (mmHg)	96.83 (13.20)	90.89 (9.81)	5.58	0.02*	-3.61	135.08	<0.001**	-5.95	1.65	-9.21	-2.69
Mean Clinic SBP left arm (mmHg)	170.08 (19.57)	151.89 (15.56)	4.83	0.03*	-7.30	139.72	<0.001**	-18.19	2.49	-23.12	-13.26
Mean Clinic DBP left arm (mmHg)	95.66 (11.22)	89.81 (10.33)	0.57	0.45	-4.02	232	<0.001**	-5.86	1.46	-8.72	-2.99
ABPM daytime SBP (mmHg)	154.80 (10.23)	138.61 (6.27)	19.91	<0.001**	-13.20	118.02	<0.001**	-16.18	1.23	-18.61	-13.76
ABPM daytime DBP (mmHg)	95.29 (9.99)	88.44 (6.48)	17.51	<0.001**	-5.66	122.25	<0.001**	-6.84	1.21	-9.24	-4.45
ABPM nighttime SBP (mmHg)	136.46 (16.31)	121.57 (9.60)	6.62	0.01*	-7.66	115.69	<0.001**	-14.90	1.94	-18.75	-11.04
ABPM nighttime DBP (mmHg)	80.48 (12.53)	73.07 (8.69)	11.20	<0.001**	-4.82	128.31	<0.001**	-7.41	1.54	-10.46	-4.37
Age (years)	51.84 (15.79)	40.79 (12.53)	4.76	0.03*	-5.56	143.02	<0.001**	-11.05	1.99	-14.98	-7.12
Height (m)	1.69 (.11)	1.70 (.10)	0.06	0.81	0.43	232	0.67	0.006	0.01	-0.02	0.03
Weight (kg)	81.32 (17.47)	81.84 (15.22)	0.35	0.56	0.22	235	0.83	0.51	2.34	-4.11	5.13
BMI (kg/m^2)	28.42 (5.22)	28.45 (5.49)	1.45	0.23	0.04	232	0.97	0.03	0.74	-1.42	1.48

*=significant group difference at 0.05 level **=significant difference at 0.001 level

ANCOVA demonstrated that all blood pressures (mean clinic SBP in the right arm, mean clinic DBP in the right arm, mean clinic SBP in the left arm, mean clinic DBP in the left arm, ABPM daytime SBP, ABPM daytime DBP, ABPM nighttime SBP and ABPM nighttime DBP) remained significantly higher in the confirmed hypertensive group even after accounting for age and sex, F (1) = 36.39, p = <0.001, F (1) = 18.24, p = <0.001, F (1) = 37.77, p = <0.001, F (1) = 17.82, p = <0.001, F (1) = 180.49, p = <0.001, F (1) = 52.74, p = <0.001, F (1) = 64.90, p = <0.001 and F (1) = 30.04, p = <0.001 respectively.

Similarly, height, weight and BMI remained insignificantly different between groups once age and sex were accounted for, F (1) = 0.95, p = 0.33, F (1) = 0.01, p = 0.93 and F (1) = 0.04, p = 0.84 respectively.

Patient cardiovascular risk factors are presented in Table 12. The confirmed hypertensive group had more male patients (46% vs 42%, p<0.03). Most patients had a positive family history of hypertension (65% of the hypertensive group vs 69% of the borderline group), about a quarter of both groups were smokers/ex-smokers (24% vs 26%), over a third had self reported excess salt in their diet (39% vs 44%), over 50% in each group did less than the recommended amount of exercise each week and over 15% of each group drank in excess of the recommended weekly allowance of alcohol. Finally, over a quarter of patients in each group had a profile compatible with the metabolic syndrome. There were statistically significant differences between the two groups: the borderline group was more likely to have a family history of hypertension, smoke, take excess salt, drink over the recommended limit and were less likely to have features consistent with the metabolic syndrome. It was not clear why the two groups had different risk

factors. Some of the differences might suggest that the borderline patients were less aware of modifiable risks and lifestyle interventions, possibly because their diagnosis was more recent. Clinically, however, both groups presented with a similar profile of modifiable and non-modifiable risk factors.

Table 12. Number of Males and Females and Number and Percentage of Cardiovascular Risk Factors in the Confirmed and Borderline Hypertensive Groups as well as Degrees of Freedom, Chi-Square and Statistical Significance Levels of the Group Effects

		n=83 Confirmed Hypertensives		n=154 Borderline Hypertensives		df	χ^2	*p*
		Number of particpants	%	Number of particpants	%			
Sex	**Male**	38	44	65	42	1	4.56	0.03*
	Female	45	54	89	58			
Family History of HT	**Yes**	55	65	106	69	1	29.65	<0.001**
	No	30		47				
Smoker/Ex smoker<5 years	**Yes**	20	24	40	26	1	294.13	<0.001**
	No	64		114				
Excess Salt	**Yes**	33	39	67	44	1	6.07	0.01*
	No	51		87				
Exercise <5x30mins/week	**Yes**	47	56	87	57	1	3.78	0.05
	No	37		67				
Drink > recommended	**Yes**	14	17	29	19	1	96.21	<0.001**
weekly allowance of alcohol	**No**	69		125				
Features consistent with the	**Yes**	25	29	38	25	1	53.43	<0.001**
metabolic syndrome	**No**	60		116				

*=significant group difference at 0.05 level **=significant difference at 0.001 level

Patient biochemical values are presented in Table 13. An independent-sample T Test was performed on electrolyte values (Appendix A). These analyses confirmed that, compared to the borderline group the confirmed hypertensive group exhibited significantly higher urea and blood glucose. These differences were not clinically significant.

Table 13. Unadjusted Mean (SD), Biochemical Values of the Confirmed Hypertensive and Borderline Hypertensive Group as well as Degrees of Freedom, t Values and Statistical Significance Levels of the Groups Effects

Electrolyte	Confirmed Hypertensives	Borderline Hypertensives	df	t	*p*
Sodium	141.24 (2.53)	140.40 (5.38)	231	-1.33	0.18
Potassium	4.24 (0.35)	4.20 (0.34)	231	-0.99	0.32
Urea	5.25 (1.23)	4.70 (0.96)	140.84	-3.51	<0.001**
Creatinine	94.13 (13.63)	93.15 (15.13)	231	-0.49	0.63
Total Cholesterol	5.19 (1.06)	5.20 (1.00)	234	0.11	0.91
HDL Cholesterol	1.55 (0.51)	1.53 (0.45)	224	-0.34	0.73
Triglycerides	1.69 (0.96)	1.52 (1.13)	231	-1.15	0.25
GGT	34.78 (26.89)	37.67 (41.01)	228	0.58	0.57
Calcium	2.31 (0.10)	2.32 (0.10)	229	0.07	0.95
Glucose	5.44 (1.28)	5.07 (0.89)	126.16	-2.35	0.02*
Urate	322.68 (81.54)	324.80 (92.79)	227	0.17	0.86

*=significant group difference at 0.05 level **=significant difference at 0.001 level

ANCOVA demonstrated that urea remained significantly higher in the confirmed hypertensive group even after accounting for age and sex, F (1) = 4.46, p = 0.04 but this is of no clinical significance. However, the small difference in blood glucose was no longer significantly different between groups once age and sex were accounted for, F (1) = 1.88, p = 0.17. Interestingly, once age and sex were accounted for a group difference became apparent for total cholesterol, F (1) = 4.37, p = 0.04, with total cholesterol being significantly higher in the confirmed hypertensive group. However, this difference was not clinically significant.

Group AASI, 10 year coronary risk and 10 year CVD risk are presented in Table 14. An independent-sample T Test was performed on AASI, 10 year coronary risk and 10 year CVD risk (Appendix B). These analyses confirmed that, compared to the borderline group the confirmed

hypertensive group exhibited significantly higher AASI, significantly higher 10 year coronary risk, and significantly higher 10 year CVD risk.

Table 14. Unadjusted Mean (SD), AASI, 10 Year Coronary Risk and 10 Year CVD Risk for the Confirmed Hypertensive and Borderline Hypertensive Group as well as Degrees of Freedom, t Values and Statistical Significance Level of the Group Effects

	Confirmed Hypertensives	**Borderline Hypertensives**	**df**	**t**	***p***
AASI	0.39 (0.16)	0.31 (0.16)	214	-3.85	<0.001**
10 Year Coronary Risk	9.35 (8.14)	5.4 (6.21)	222	-4.04	<0.001**
10 Year CVD Risk	17.8 (13.15)	9.38 (10.19)	134.91	-5.01	<0.001**

*=significant group difference at 0.05 level **=significant difference at 0.001 level

ANCOVA demonstrated that the group difference for AASI remained significant even after accounting for age and sex, F (1) = 5.41, p = 0.02. However, 10 year coronary and Cardiovascular risk were no longer significantly different between groups once age and sex were accounted for, F (1) = 0.09, p = 0.76 and F (1) = 1.07, p = 0.30 respectively.

3.3.2 Brief Discussion

The confirmed hypertensives were significantly older and were more likely to have features consistent with the metabolic syndrome and stiffer arteries (as measured by AASI) than the borderline hypertensives. However, the borderline hypertensives had similar height, weight, BMI, amount of exercise, biochemical profile and 10 year coronary and CVD risk (once age and sex were accounted for) as the confirmed hypertensives. Both groups had a clinically similar pattern of smoking, excess salt and higher than recommended amounts of alcohol and had a positive family history of hypertension in over 60% of cases. It is therefore apparent that all of these patients, including the borderline group, require intensive lifestyle advice at the very least.

Metabolic syndrome and arterial stiffness are less standard cardiovascular risk factors but are important to take into consideration when assessing cardiovascular risk since individuals with metabolic syndrome are 1.69 times more likely to develop CVD than those without, and arterial stiffness is important for assessing cardiovascular risk as well as for predicting cardiovascular outcomes.[54;64] One quarter of the borderline group had features consistent with the metabolic syndrome, which again suggests that these individuals should have aggressive lifestyle intervention. Although the AASI was significantly higher in the confirmed hypertensive group the values were still within the normal range possibly because the mean age in this group was fairly young.

CHAPTER 4: OVERALL DISCUSSION AND CONCLUSIONS

4.1 Overall Discussion

4.1.1 Summary of Main Findings

Study One examined vibratory, cooling, warming and heat-pain thresholds in the right hand and foot of a group of newly diagnosed unmedicated essential hypertensives (BP ≥140/90 mmHg) as well as a group of normotensive controls, who were free from pre-existing peripheral neuropathy and associated symptoms. Study Two compared cardiovascular risk factors in a larger group of confirmed and borderline hypertensive patients selected from our Hypertension Database.

The major findings from Study One were as follows. 1) The hypertensive group had significantly *higher v*ibration thresholds in the foot than the normotensive group, even after accounting for age, sex and BMI. 2) There was a significant *positive* correlation between SBP and DBP with vibration threshold in the foot. 3) There was a significant *negative* correlation between systolic and diastolic blood pressure with both cooling and warming thresholds in the hand.

The major finding from Study Two was that the borderline hypertensives had similar modifiable cardiovascular risk factors as the confirmed hypertensives, suggesting that lifestyle intervention is appropriate for reducing blood pressure and improving health in all grades of hypertension.

4.1.2 Detailed Discussion of Main Findings

4.1.2.1 Study One

Vibration threshold in the foot was significantly *higher* in the hypertensive group when compared to the normotensive group even after accounting for age, sex and BMI and there was a significant *positive* correlation between both systolic and diastolic BP and vibration threshold in the foot, supporting some existing evidence for peripheral neuropathy in hypertension.[65-70]

Conversely, there was a significant *negative* correlation between systolic and diastolic BP and both cooling and warming thresholds in the hand. These unexpected contradictory findings oppose our hypothesis and some previous research but support other counter evidence suggesting that hypertension is in fact protective against peripheral neuropathy.[123;124] The reasons for a possible negative association between hypertension and peripheral neuropathy remain unexplained.[123] These significant negative correlations should be interpreted with some caution since we cannot exclude the possibility that confounding variables such as age, sex and BMI may be playing a part. It may be that when age, sex and BMI are accounted for using hierarchical linear regression analysis that the significance diminishes.

The findings of significantly *higher* vibration thresholds in the foot of the hypertensive group and a significant *positive* correlation between both systolic and diastolic BP and vibration threshold in the foot supports some existing evidence for peripheral neuropathy in hypertension using a novel form of stimulation (QST). These findings suggests that peripheral nerve fibres, specifically large

myelinated Aβ fibres that conduct vibration sensation, may be preferentially affected in hypertensives, particularly in their feet.

Myelin degeneration might explain why vibration sensation (myelinated Aβ fibres) rather than cooling (thinly myelinated Aδ fibres), warming or heat-pain (unmyelinated C fibres) sensations were preferentially affected. However, nerve conduction studies and/or nerve biopsies would have to be performed to confirm this. Still, if found to be true, this would support findings of Viskoper *et al.,* 1971 who found a reduction in motor nerve conduction velocities in the upper extremities in hypertensives compared to normotensives as well as an inverse relationship between nerve conduction velocity and DBP.[112] This theory is also supported, albeit indirectly, by the finding that in patients with vasculitic neuropathy, myelinated fibres are more vulnerable to ischaemia than unmyelinated fibres and large myelinated axons are affected before smaller ones.[125] In contrast, Edwards *et al.,* 2008 found that although cutaneous sensory thresholds were ~30% higher and sensory action potentials were ~20% lower in hypertensives than normotensives, sensory nerve conduction velocity did not differ between groups suggesting that hypertension may cause axonal rather than demyelinating neuropathy.[70] Also in contrast, in most neuropathies, including the most common type of diabetic neuropathy [Distal Symmetrical Sensory Polyneuropathy (DSSP)], it is the small nerve fibres (myelinated and unmyelinated) that detect temperature that are affected first followed by the large nerve fibres (myelinated) that detect vibration.[126;127] Having said this, DSSP can be categorised into sub-types: small-fibre type, large-fibre type and mixed. The mixed sub-type is the most common but the small-fibre and

large-fibre types can occur independently. Large-fibre DSSP was often seen in the past when there was no treatment for diabetes, but is less common today.[127]

The mechanisms underlying a possible hypertension-related axonal or demyelinating neuropathy are not known.[65-70;107] Evidence suggests that the pathological process may be similar to that in diabetic neuropathy, whereby metabolic and vascular disturbances lead to structural and functional changes in blood vessels supplying peripheral nerves, resulting in ischaemia and hypoxia, which induces oxidative stress and injury to peripheral nerves possibly via an increase in production of reactive oxygen species (ROS).[87-91;101-103]

Reactive oxygen species can damage the lipids, proteins, nucleic acids and mitochondria of cells, potentially causing cell death.[128] Oligodendrocytes (brain cells that produce myelin) are more sensitive to oxidative stress *in vitro* than astrocytes and microglia, possibly due to their reduced glutathione (antioxidant) content, their higher iron content and their higher dependency on oxidative phosphorylation.[128] Oxidative stress might therefore result *in vivo* in selective oligodendrocyte death and consequent demyelination. The ROS may also damage the myelin sheath, promoting its attack by macrophages.[128] Schwann cells are the peripheral nervous system equivalent of oligodendrocytes, thus it seems reasonable to assume that Schwann cells may be similarly affected when under oxidative stress i.e. in preference to other nerve cells. In fact, Iida *et al.,* 2004 found that Schwann cells are a specific target of oxidative injury and that there is some susceptibility of Schwann cells to even mild degrees of ischaemia.[129] Furthermore, ischaemia can affect Schwann cells when not intense enough to cause axonal degeneration and chronic ischaemia is known to induce demyelination in addition to axonal loss.[130-133] This would

perhaps go some way to explaining why loss of vibration sensation (myelinated Aβ fibres) preceded loss of warming and heat-pain (small unmyelinated C-fibres) in the present study. However, at this stage this theory is somewhat fabricated and is based on rather scanty evidence. If the findings of the present study were replicated and causality was proven, laboratory studies would then be necessary in order to investigate the pathological mechanisms involved.

The group difference for vibration threshold was significant in the foot (p =0.01) but not quite in the hand (p =0.05). This may be due to the fact that the hands are generally more sensitive than the feet because of their larger distribution on the homunculus. Consequently, the hands may be more effective than the feet at discriminating between vibration and non-vibration stimuli leading to a less significant difference between groups. Another, more simplistic explanation is that circulation is often better in the hands than the feet. Therefore, if poor blood supply to peripheral nerves does indeed contribute towards peripheral neuropathy, then the feet would be more noticeably affected. In addition, peripheral neuropathy usually occurs in a length dependent fashion i.e. the longest nerves (to the feet) are affected first.[126] In fact, vibration threshold increases with age and is often first lost in the feet.[134]

Only vibration threshold in the foot demonstrated a significant group difference once age, sex and BMI were accounted for. However, it is useful to note the trend between groups of all other sensory thresholds, despite them not reaching significance. Vibration threshold in the hand, warming threshold in the foot, heat-pain 5.0 in the hand and foot and heat-pain 0.5 in the hand and foot were *higher* in the hypertensive group after accounting for age, sex and BMI.

Conversely, cooling threshold in the hand and foot and warming threshold in the foot were *lower* in the hypertensive group after accounting for age, sex and BMI.

Heat-pain and warming are both conducted by the same nerve fibres (unmyelinated C-fibres). Therefore, one might expect warming thresholds to also be higher in the hypertensive group. However, this was only true for warming threshold in the foot. These discrepancies between sensory thresholds introduce the prospect that hypertension may affect different peripheral nerve fibres differently; a possibility that warrants further investigation but that could be particularly true for heat-pain since there is evidence that hypertension-associated hypoalgesia may be caused by a central rather than peripheral mechanism, which may explain the inverse group difference for heat-pain and warming hand. However, in the present study it is more than likely that these discrepancies are due to the small sample size and that with a larger sample the results may have been more consistent and significant. In such a small sample the opposing results for cooling threshold in the hand and foot and warming threshold in the hand might reflect the large variation in heat sensitivity among individuals, which QST is not very effective at accounting for.[116] Similarly, the insignificant group difference for heat-pain thresholds might reflect the subjectivity of the heat-pain methodology and the large variation in pain thresholds among individuals. Hence, QST may not be the best methodology for detecting group differences in thermal and heat-pain thresholds.

The findings of this small preliminary study indicate that newly diagnosed unmedicated essential hypertensives may have subclinical peripheral neuropathy in the form of reduced sensibility to vibration in their feet. However, this finding was not consistent across other sensory modalities or

in the upper limb. Due to the small sample and many other limitations of this study further studies with much larger sample sizes are needed in order to prove causality across all grades of hypertension and to look into the pathogenesis.

4.2.1.2 Study Two

Findings from the retrospective database analysis of cardiovascular risk demonstrated that unmedicated borderline hypertensives with no other medical conditions had similar cardiovascular risk factors and profiles as their confirmed hypertensive counterparts, suggesting that borderline hypertensives necessitate lifestyle advice at the very least. Most patients had a positive family history of hypertension (65% of the hypertensive group vs 69% of the borderline group), about a quarter of both groups were smokers/ex-smokers (24% vs 26%), over a third had self reported excess salt in their diet (39% vs 44%), over 50% in each group did less than the recommended amount of exercise each week and over 15% of each group drank in excess of the recommended weekly allowance of alcohol. Finally, over a quarter of patients in each group had a profile compatible with the metabolic syndrome, which would improve with lifestyle intervention. In conclusion, both groups had a similar pattern of modifiable risk factors suggesting that intensive lifestyle intervention is appropriate and necessary in all hypertensives even if medication is not indicated immediately.

The two studies were done separately and the small numbers, particularly in Study One make it difficult to link the findings. Nonetheless, the borderline hypertensives had similar cardiovascular risk profiles and risk factors to the confirmed hypertensives (Study Two) and there was some evidence of vascular damage in the form of subclinical peripheral neuropathy (Study One) in the

newly diagnosed unmedicated essential hypertensives, many of whom (n=14) had a mean blood pressure in the borderline range (140-159/90-99 mmHg). Taken together, this may suggest that earlier initiation of antihypertensive medications in borderline patients with low cardiovascular risk might be appropriate, especially if these patients are showing signs of early vascular damage in the form of subclinical peripheral neuropathy (a potential surrogate of TOD). However, due to the small sample size of this preliminary study, it is not possible to determine whether subclinical peripheral neuropathy was present across all grades of hypertension. Much larger studies would be needed to establish this.

4.1.3 Study One-Limitations

With regard to peripheral neuropathy in hypertension, the results (at least for vibration sensation in the feet), agree with a small number of previous studies. However, there are some limitations that should be considered. These limitations may be contributing to the lack of statistical significance between groups and the opposing findings for cooling thresholds in the hand and foot and warming thresholds in the hand.

The main limitation of this study was the small sample size, which reduced the power to detect differences in thresholds between the two groups. ANCOVA was only powered to detect a large effect size and for all sensory thresholds the effect size was either small or medium. Consequently, the results can only be regarded as preliminary. Furthermore, the hypertensive and normotensive groups were not matched for confounding variables such as age, sex, weight or BMI. The mean age of the hypertensive and normotensive groups were 36 years and 32 years respectively. There were more men in the hypertensive group than women (12, 8) and more

women in the normotensive group than men (16, 9), the mean weight of the hypertensive and normotensive groups were 69kg and 67kg respectively and the mean BMI of the hypertensive and normotensive groups were 29kg/m^2 and 23.5 kg/m^2 respectively. Although differences in age, sex and BMI were accounted for in the analyses and group differences for age and sex were not significant, statistical adjustment may not fully address the potential confounding effects of these variables in this study, thus it is always preferable to match study groups for confounding variables when possible.[135]

Patients had varying durations of hypertension, which may be having an effect on presence or severity of neuropathy. Diabetic sensory neuropathy has been found to be independently associated with duration of hypertension.[78] Conversely, hypoalgesia is evident even after acute increases in blood pressure in spontaneously hypertensive rats.[107]

Over half (65%) of the hypertensive patients had a cardiovascular risk profile compatible with metabolic syndrome i.e. BMI >30 kg/m^2 plus raised triglycerides, reduced HDL cholesterol and/or raised fasting plasma glucose. It is therefore difficult to ascertain, which risk factor(s) (if any) contributed to the sensory loss observed in the hypertensive group.

Many factors influence sensory detection thresholds. As well as sex, age and body location and possibly blood pressure, variability of threshold may be explained by differences in thickness of tissue overlying receptor, skin temperature, spacial distribution of receptors or impulse transmission, none of which were accounted for in the current study.[117] Central processing can also influence sensory detection threshold. Quantitative sensory testing cannot distinguish

between peripheral nerve damage and central nervous system (CNS) damage therefore this is another limitation. However, participants were not selected to take part if they had any condition predisposing to peripheral neuropathy, including CNS damage. Another limitation of QST is that it is not very effective at accounting for large variations in heat and pain sensitivity among individuals and although it can detect changes in sensory thresholds it does not provide any clues to the reasons for these changes such as axonal loss/demyelination. Sensory action potential amplitudes and sensory nerve conduction velocities would provide this additional information.

Finally, although Spearman's correlation showed significant differences, this statistical test is not as accurate hierarchical linear regression analysis, which can account for confounding variables. Overall, concrete conclusions regarding hypertension and peripheral neuropathy cannot be drawn from this study. Further studies are needed in order to expand on these exciting but preliminary findings.

4.1.4 Study One-Recommendations for Future Research

In light of these limitations, future studies would recruit more participants in order to achieve more significant results. Hypertensives would be categorised into those with Grade 1 (borderline) hypertension and those with Grade 2 hypertension, in order to see whether subclinical peripheral neuropathy is present across all grades of hypertension. The hypertensive and normotensive groups would be matched for age, sex, weight and BMI. If future research finds more established differences between hypertensives and normotensives, subsequent research might involve investigating mechanisms underlying these differences, using skin biopsies. Further studies might follow-up the hypertensive group, after the initiation of antihypertensive medication in any confirmed hypertensives (BP $\geq$160/100 mmHg), to see whether antihypertensive medications delay or prevent the onset of subclinical peripheral neuropathy or indeed prevent or delay its progression. Further studies might also follow up any borderline hypertensives (SBP 140-159 and/or DBP 90-99 mmHg) to observe whether peripheral neuropathy worsens as their blood pressure rises. The management of hypertension might then be tailored to prevent or treat subclinical peripheral neuropathy as well as hypertension. In addition, future findings may be used to validate the use of antihypertensive drugs to prevent or treat other forms of neuropathy including diabetic neuropathy. Future studies should include metabolic syndrome in the exclusion criteria and carry out ambulatory blood pressure measurements on all participants, as well as sensory nerve conduction velocities and sensory action potentials to investigate the type of peripheral nerve damage e.g. demyelination and/or axonal damage. Future studies should also endeavor to adequately control for variations in skin temperature and perform hierarchical linear regression analyses in addition to Spearman's correlation in order to account for confounding variables.

4.1.5 Study Two-Limitations and Recommended Future Directions

The main limitations of this study were the unbalanced numbers of patients in the confirmed and borderline group and the fact that outcome data was not available from the database. Future research might therefore follow-up all confirmed hypertensive and borderline hypertensive patients to investigate outcomes, in terms of blood pressure and cardiovascular events, using medical notes.

4.2 Conclusions

The results of Study One demonstrated some evidence of subclinical peripheral neuropathy, in the form of reduced vibration sensation in the feet of patients with newly diagnosed unmedicated essential hypertension. However, due to the small sample size it was not possible to determine whether this finding was true across all grades of hypertension. Interrogation of the Hypertension Database revealed that unmedicated borderline hypertensives have similar cardiovascular risk profiles and similar modifiable cardiovascular risk factors to their confirmed hypertensive counterparts, suggesting that borderline patients necessitate intensive lifestyle advice at the very least.

If the preliminary findings of Study One were replicated across all grades of hypertension in a much larger sample, this, in conjunction with the findings of Study Two may indicate the need for antihypertensive medications in borderline hypertensives, at otherwise low cardiovascular risk, who currently only receive lifestyle advice.

If we assume the hypertensive patients from Study One had subclinical peripheral neuropathy, this might be due to metabolic syndrome, differences in age, sex, weight and/or BMI rather than BP alone. As a result of this and because of the many limitations in this preliminary study much further research is needed to expand on these promising early findings, prove their significance across all grades of hypertension, and determine underlying mechanisms before any definitive conclusions are reached, which may then ultimately be applied to clinical medicine.

APPENDICES

Appendix A. Independent-Samples T Test. Unadjusted Mean (SD), Electrolyte Values of the Confirmed Hypertensive and Borderline Hypertensive Group as well as the Degrees of Freedom, t-Values and Statistical Significance Level of the Group Effects

Variable	Mean (SD) Confirmed HT's	Mean (SD) Borderline HT's	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Standard Error Difference	95% Confidence Interval of the Difference	
										Lower	Upper
Sodium	141.24 (2.53)	140.40 (5.38)	0.04	0.84	-1.33	231	0.18	-0.83	0.62	-2.06	0.40
Potassium	4.24 (.35)	4.20 (.34)	0.08	0.78	-0.99	231	0.32	-0.05	0.05	-0.14	0.05
Urea	5.25 (1.23)	4.70 (.96)	4.73	0.03*	-3.51	140.84	<0.001**	-0.54	0.16	-0.85	-0.24
Creatinine	94.13 (13.63)	93.15 (15.13)	1.38	0.24	-0.49	231	0.63	-0.98	2.0	-4.92	2.96
Total Cholesterol	5.19 (1.06)	5.20 (1.00)	0.12	0.12	0.11	234	0.91	0.02	0.14	-0.26	0.29
HDL Cholesterol	1.55 (.51)	1.53 (.45)	1.0	0.99	-0.34	224	0.73	-0.02	0.07	-0.15	0.11
Triglycerides	1.69 (.96)	1.52 (1.13)	0.03	0.03*	-1.15	231	0.25	-0.17	0.15	-0.46	0.12
GGT	34.78 (26.89)	37.67 (41.01)	2.25	2.25	0.58	228	0.57	2.89	5.02	-7.00	12.78
Calcium	2.31 (.10)	2.32 (.10)	0.44	0.44	0.07	229	0.95	0.00	0.01	-0.03	0.03
Glucose	5.44 (1.28)	5.07 (.89)	5.47	5.47	-2.35	126.16	0.02*	-0.37	0.16	-0.68	-0.06
Urate	322.68 (81.54)	324.80 (92.79)	2.25	2.25	0.17	227	0.86	2.12	12.26	-22.04	26.28

*=significant group difference at 0.05 level **=significant difference at 0.001 level

Appendix B. Independent-Samples T Test. Unadjusted Mean (SD), AASI, 10 year Coronary Risk and 10 year CVD Risk for the Confirmed Hypertensive and Borderline Hypertensive Group as well as the Degrees of Freedom, t-Values and Statistical Significance Levels of the Group Effects

Variable	Mean (SD) Confirmed HT's	Mean (SD) Borderline HT's	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	SE Difference	95% Confidence Interval of the Difference	
										Lower	Upper
AASI	0.39 (0.16)	0.31 (0.16)	0.51	0.48	-3.85	214	<0.001**	-.086	0.02	-0.13	-0.04
10 Year Coronary Risk	9.35 (8.14)	5.4 (6.21)	2.29	0.13	-4.04	222	<0.001**	-3.91	0.969	-5.82	-2.00
10 Year CDV Risk	17.8 (13.15)	9.38 (10.19)	4.42	0.04*	-5.01	134.91	<0.001**	-8.47	1.69	-11.82	-5.12

*=significant group difference at 0.05 level **=significant difference at 0.001 level

Appendix C. Presentations, Posters and Abstracts Arising From This Thesis

Presentations

Branch RL. Peripheral Neuropathy In Hypertension. *Clinical Pharmacology Colloquium.* 14th Nov 2009

Posters

Branch RL. Evidence of Peripheral Neuropathy in Unmedicated Hypertensives. *British Pharmacological Society Winter Meeting.* 2009

Abstracts

Branch R, Ali S, Ring C, Winer J, Martin U. Evidence of Peripheral Neuropathy in Unmedicated Hypertensives. *Br J Clin Pharmacol.* 2010; 70(2): 293

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