BIOMARKERS IN WHITE-COAT HYPERTENSION

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Abstract

The introduction of ambulatory blood pressure monitoring in the 1960s provided new insights into the nature of high blood pressure disorders. Blood pressure is now categorised into four quadrants: normotension, masked hypertension, hypertension and white-coat hypertension. In white-coat hypertension blood pressure is elevated when taken at the doctor's office but normal if taken outside the doctor's office.

Several controversies are associated with white-coat hypertension, which are discussed in Chapter 1. Whether white-coat hypertension is a condition of increased cardiovascular risk is the most important current issue. The consensus from prospective studies has been that the cardiovascular risk in white-coat hypertension is similar to normotensives, but recent studies suggest that white-coat hypertension is associated with an increased risk for the development of hypertension, which may mean an increased cardiovascular risk is present but delayed in white-coat hypertension. Examining the literature (Chapter 2) at the time of the thesis commencement highlighted inconsistencies in the definition for white-coat hypertension, which makes it difficult to determine if white-coat hypertension is associated with biomarkers of increased cardiovascular risk.

This thesis by publication investigated whether white-coat hypertension is a condition of increased cardiovascular risk by determining: (1) if biomarkers of increased cardiovascular risk that are known to be present in essential hypertension are present in strictly defined white-coat hypertension based on a consensus definition for white-coat hypertension (Chapter 4); (2) if white-coat hypertension is associated with an increased morning blood pressure surge (Chapter 5); (3) if white-coat hypertension is associated with increased risk for the development of new-onset hypertension; and (4) if biomarkers measured at baseline predict the development of sustained hypertension in white-coat hypertension (Chapter 7).

Participants underwent measurement of artery stiffness, autonomic function, glucose and insulin status, circulating measures of inflammation and endothelial dysfunction, and twenty-four hour ambulatory blood pressure monitoring to confirm blood pressure status. White-coat hypertension participants required two twenty-four hour ambulatory blood pressure monitorings to confirm blood pressure status. Participants returned yearly for ambulatory blood pressure monitoring for three years. New-onset hypertension was defined based on elevated mean ambulatory blood pressure.

This research has identified that white-coat hypertension is associated with markers of increased cardiovascular risk, including an elevated baseline two-hour glucose post glucose load and increased progression to sustained hypertension compared to normotensives. Elevated baseline measures of waist circumference, artery stiffness and two-hour load blood glucose were elevated in white-coat hypertension who progressed to sustained hypertension.

White-coat hypertension was not associated with an increased morning blood pressure surge but univariate analysis found lipids were associated with the morning blood pressure surge (Chapter 5), which was confirmed in a larger study that included normotensive, treated and untreated hypertensive participants (Chapter 6).

White-coat hypertension should be considered a condition of increased cardiovascular risk. Based on the results of this thesis treating doctors are advised that they should not focus solely on the white-coat hypertensive subject's blood pressure but on their total cardiovascular risk and such subjects should be monitored for the development of both hypertension and type 2 diabetes.

Declaration

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes four original papers published in peer reviewed journals and two submitted papers. The core theme of the thesis is biomarkers in white-coat hypertension. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Medicine, Southern Clinical School under the supervision of Professor Barry McGrath and Professor James Cameron.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 2, 4, 5, 6, 7 and 8 my contribution to the work involved the following:

Thesis Chapter	Publication title	Publication status	Nature of candidate's contribution	Extent of candidate's contribution
Two	Circulating and mechanical biomarkers in isolated clinic hypertension. Clin Exp Pharmacol Physiol. 2008 Apr;35(4):402-8.	Published	Review of evidence, interpretation of evidence and writing of paper.	70%
Four	Two hour glucose post loading: a biomarker of cardiovascular risk in isolated clinic hypertension. J Hypertens. 2011 Apr;29(4):749-57.	Published	Study conception, data collection and analysis, writing of paper.	80%
Five	The morning blood pressure surge is related to serum cholesterol. J Hum Hypertens. 2013 May;27(5):315-20.	Published	Study conception, data collection and analysis, writing of paper.	80%
Six	Predictors of mean arterial pressure morning rate of rise and power function in subjects undergoing ambulatory blood pressure recording	Submitted	Data collection and review of paper.	25%
Seven	Biomarkers of future hypertension in white-coat hypertension	Submitted	Study conception, data collection and analysis, writing of paper.	80%
Eight	White-coat hypertension Clin Exp Pharmacol Physiol. 2013 May 18 [Epub ahead of print]	Published	Review of evidence, interpretation of evidence and writing of paper.	90%

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Signed: [redacted] Date: 18/9/13

Conference abstracts

1. Martin CA, Cameron JD, McGrath BP. Baseline predictors of future hypertension in white-coat hypertension. International Society of Hypertension Scientific Meeting. Published Journal of Hypertension. 2012. 30: e215
2. Martin CA, Cameron JD, Chen SS, McGrath BP. Two hour post glucose load: a biomarker of increased cardiovascular risk in patients with isolated clinic hypertension. International Society of Hypertension Scientific Meeting, 2010.
3. Head G, Andrianopoulos N, Reid C, McGrath B, Martin C, Carrington M, La Greca L, Chatzivlastou K. Predictors of mean arterial pressure morning rate of rise and power function in subjects undergoing ambulatory blood pressure recording. International Society of Hypertension Scientific Meeting, 2010.
4. Martin CA, Cameron JD, Chen SS, McGrath BP. Glucose intolerance, a cardiovascular risk biomarker in subjects with isolated clinic hypertension. High Blood Pressure Research Council of Australia Annual Meeting, 2010.
5. Martin CA, Cameron JD, Chen SS, McGrath BP. Bedside autonomic function normal values should be age standardised. High Blood Pressure Research Council of Australia Annual Meeting, 2010.
6. Martin CA, Cameron JD, Chen SS, McGrath BP. Insulin resistance, arterial stiffness and circulating biomarkers in isolated clinic hypertension. Cardiac Society of Australia and New Zealand Annual Scientific Meeting and the International Society for Heart Research, Australasian Section, Annual Scientific Meeting, 2009. Published Heart, Lung and Circulation. 2009; 18(S3):276

7. Martin CA, Chen SS , Camero JD' ,McGrath BP. Isolated clinic hypertension is associated with elevated two-hour glucose post oral glucose tolerance test. High Blood Pressure Research Council of Australia Annual Meeting, 2008.
8. Andrianopoulos N, Head G, Reid C, Lukoshkova E, McGrath B, Martin C, Carrington M, La Greca L, Chatzivlastou K. Predictors of mean arterial pressure morning rate of rise and power function in subjects undergoing ambulatory blood pressure recording. High Blood Pressure Research Council of Australia Annual Meeting, 2007.
9. Martin CA, Cameron JD, Zoungas S, Chen SS, Kotsopoulos, Head G, McGrath BP. Morning rate of blood pressure rise and aortic pulse wave velocity are increased in white-coat hypertension. High Blood Pressure Research Council of Australia Annual Meeting, 2006.

Letter

Martin CA, Cameron JD, Chen SS, McGrath BP. Hypertension. Measurement of blood pressure in the office. 2010. 56:e11.

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I want to thank all the participants who gave up their valuable time. The first visit alone took over three hours to complete. The barrage of testing included an oral glucose tolerance test, which required four blood samples; and pulse wave velocity, which required a pressure manometer pressed against the femoral artery, which I am sure for some was embarrassing, but they still came back each year.

Also thanks to Professor Rose Chapman for her friendship, support and encouragement.

Since I started this thesis life has continued. Two aunts, an uncle and two cousins have died. A friend who I have known for 30 years was diagnosed with cancer and has since died. My father's health has deteriorated over the last few years and he is now in high level aged care. My mother was diagnosed with cancer and developed a complication from the chemotherapy, which has caused a disability. Through all of this my good friend Sue Talbot has been my sounding board. She has given me common sense advice and encouragement to continue.

I want to acknowledge my family, in particular my mother, who being born in the 1930s did not have the educational opportunities I had but she worked to ensure all her nine children received a good education.

This work is dedicated to my brother Peter.

Glossary of abbreviations

24h	Twenty-four hour
2hPG	Two hour glucose post 75g oral dextrose
ABPM	Ambulatory blood pressure monitoring
ACR	Albumin creatinine ratio
ADMA	Asymmetric dimethyl arginine
AF	Autonomic function
ANOVA	Analysis of variance
BP	Blood pressure
BP_{Power}	Power of the morning blood pressure surge
BMI	Body mass index
CI	Confidence interval
CVE	Cardiovascular event
ESC	European Society of cardiology
ESH	European Society of Hypertension
FMD	Flow mediated dilation
HDL	High density lipoprotein
HF	High frequency
HOMA	Homeostatic model assessment
HR	Heart rate
HRV	Heart rate variability
hsCRP	high sensitive c-reactive protein

Hz	Hertz
IGT	Impaired glucose tolerance
IQR	Inter-quartile range
Kg	Kilogram
LDL	Low density lipoprotein
LF	Low frequency
LVMI	Left ventricular mass index
MAP	Mean arterial pressure
MBPS	Morning blood pressure surge
NO	Nitric oxide
OGTT	Oral glucose tolerance test
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor 1
PAMELA	Pressione Arteriose Monitorate E Loro Associazioni
PWVc	Central pulse wave velocity
QUICKI	Quantitative insulin sensitivity check index
ROR	Rate of rise
ROS	Reactive oxygen species
TOD	Target organ damage
UABP	Unobserved automated blood pressure
vWF	von Willebrand factor
WCE	White-coat effect
WCHT	White-coat hypertension

Chapter 1

Introduction

1.1 White-Coat Hypertension

The introduction of ambulatory blood pressure monitoring (ABPM) in the 1960s has provided new insights into the nature of high blood pressure (BP) disorders and BP can now be categorised into four quadrants: normotension, masked hypertension, hypertension and white-coat hypertension (WCHT) as shown in Figure 1.1. There is no universal definition for WCHT. WCHT has been loosely described as a condition where BP is elevated in a medical environment but is normal outside the medical environment. This raises important questions about standardisation of BP measurement, and the place of unobserved automated blood pressure (UABP) measurement in doctors' rooms.

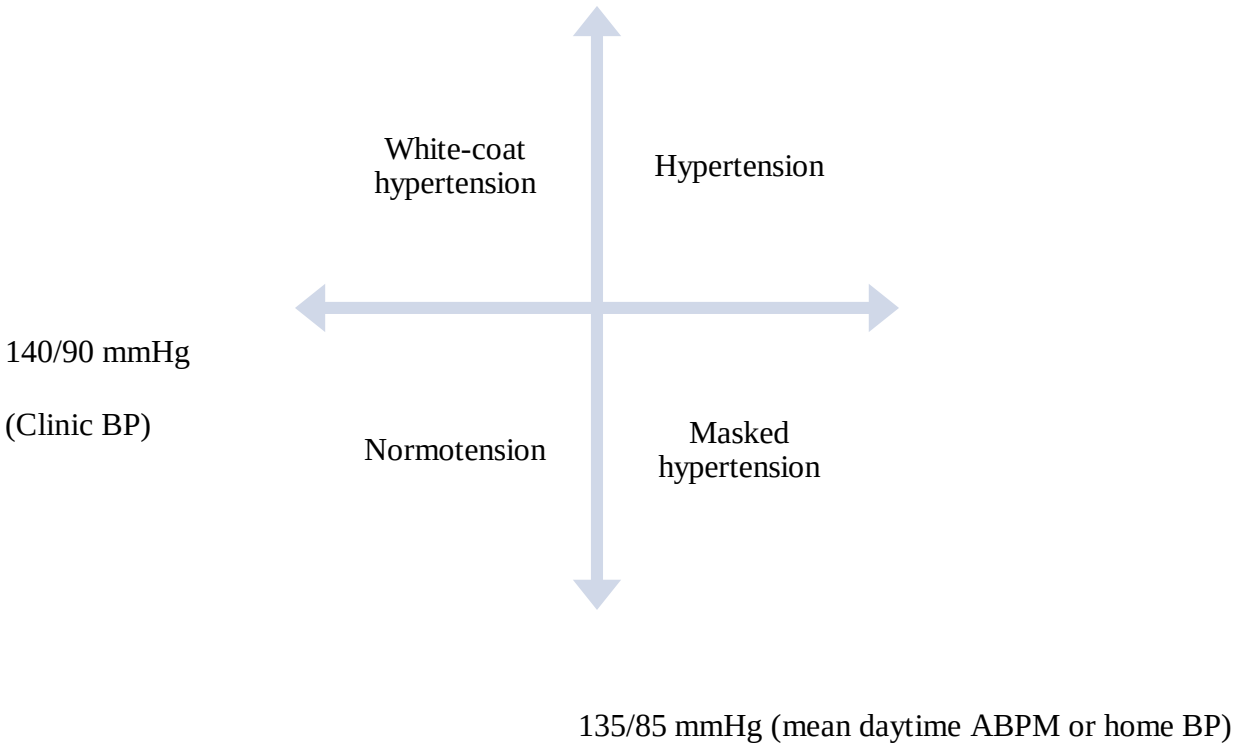

Figure 1.1. The four quadrants of BP defined by clinic and ambulatory blood pressure.

There are several controversies associated with WCHT including its terminology, definition and whether or not it is associated with increased cardiovascular risk. These will now be discussed.

1.1.1 Terminology

WCHT needs to be distinguished from the white-coat effect (WCE), which is a quantifiable numerical value that describes the actual rise in BP in the presence of a medical practitioner,[1] a response that is observed variably across all categories of BP from normotensive subjects to subjects with sustained hypertension. Other names have been put forth for this phenomenon, such as isolated office/clinic hypertension or out-of-office normotension in an effort to distinguish WCHT from the WCE but WCHT continues to be the name most commonly used.

1.1.2 Definition

There is no universal agreement on what is defined as normal BP outside of a physician's office or how that BP is measured. The 2013 European Society of Hypertension (ESH) and European Society of Cardiology (ESC) guidelines for the management of arterial hypertension recommends WCHT should be diagnosed when office BP is 140/90 mmHg or above on repeated occasions, with an average twenty-four hour (24h) BP less than 130/80 mmHg and average day ABPM less than 135/85 mmHg or the average of several home BP readings less than 135/85 mmHg.[2] The cut-off value 135/85 mmHg is the most accepted out-of-office definition for both mean day ABPM and home BP.[2]

Other important elements defining WCHT are that subjects must not be taking antihypertensive therapy,[2,3] the personnel and methods of recording office BP need to be clearly defined, and the

diagnosis requires more than one set of day ABPM or home BP recordings.[2] It is recommended that at least two 24h ABPM monitorings are recorded to confirm WCHT.[2,4]

1.1.3 Prevalence and characteristics associated with WCHT

Two population studies have examined WCHT in untreated participants.[5, 6] The Pressione Arteriose Monitorate E Loro Associazioni (PAMELA) study randomly selected participants representative of the city of Monza in Lombardy, Italy. The definition of WCHT was based on clinic BP ≥ 140/90 and a 24h ABPM < 125/79 mmHg or home BP < 135/83 mmHg, values obtained from population regression analysis of BPs to corresponding clinic BP of 140/90 mmHg. The PAMELA study estimated a population prevalence of 15% by ABPM, with only 70% of these participants having WCHT defined by home BP definition.[5]

The Finn-home study randomly selected participants from the general population of Finland and using home BP less than 135/85 mmHg as the out-of-office BP to define WCHT determined a general population prevalence of 15.6%. Participants with WCHT were less likely to smoke compared to normotensives and sustained hypertensive subjects, had similar body mass index (BMI) to normotensives, and mildly elevated clinic BP.[6]

The prevalence of WCHT amongst untreated hypertensive patients is estimated at 15 to 45% and is associated with non-smoking, female sex and increasing age.[7]

1.3.4 Is WCHT associated with increased cardiovascular risk?

Cross sectional studies have examined the prevalence of target organ damage (TOD) in WCHT with mixed results. Glucose dysregulation has been found in a cross sectional study of Japanese subjects.[8]

Prospective studies in WCHT have found that WCHT is associated with increased progression to diabetes[9] and hypertension[10-12]. Prospective studies examining the risk of cardiovascular events have found the risk in WCHT is low. A meta-analysis that examined the risk of stroke found WCHT appeared to have an increased risk of stroke in the long term,[13] suggesting missed progression to hypertension. A recent meta-analysis did not find the same result.[14]

The different definitions used to define WCHT, and the large proportion of subjects with WCHT who are commenced on antihypertensive treatment in prospective studies, likely largely based on repeated high BP readings in the doctor's office, have made it difficult to determine if WCHT is associated with increased cardiovascular risk compared to normotensives.

1.2 Biomarkers of essential hypertension

Essential hypertension is associated with a number of pathophysiological conditions, particularly endothelial dysfunction, vascular oxidative stress and inflammation, artery stiffness, insulin resistance and autonomic dysfunction. Figure 1.2 is a schematic representation of the interrelationships between BP and these vascular pathophysiological markers. Other pathophysiological markers associated with hypertension that are not examined in this thesis, such as renal, endocrine and salt status are not shown.

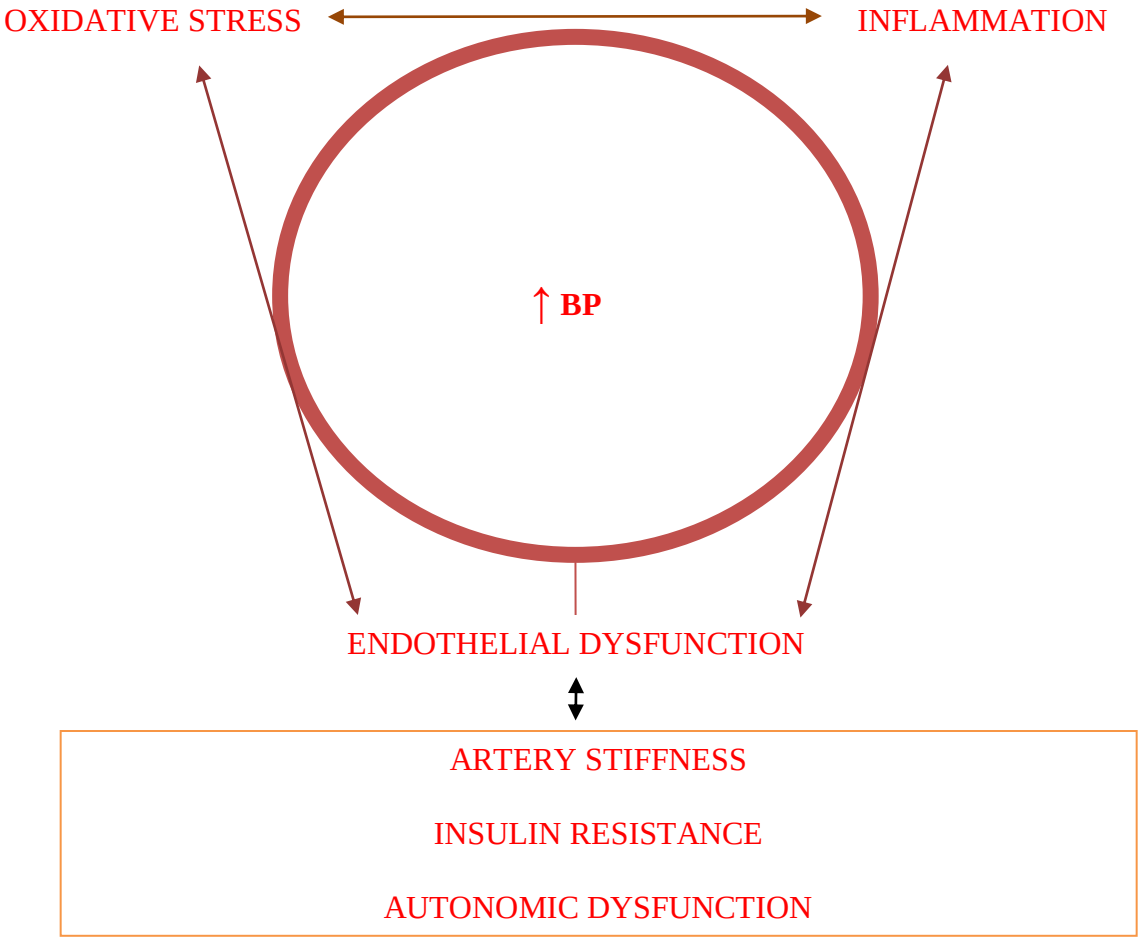

Figure 1.2. Schematic representation of interrelationships between BP and vascular pathophysiological markers: oxidative stress, inflammation, endothelial dysfunction and arterial stiffness.

As WCHT is a condition that is prone to the development of sustained hypertension then WCHT should have similar biomarkers seen in hypertension, particularly biomarkers associated with early vascular damage (endothelial dysfunction).

1.2.1 Endothelia dysfunction

The vascular endothelium generates nitric oxide (NO), which maintains the integrity of the vascular wall by inhibiting inflammation, cellular proliferation, and thrombosis. Endothelial dysfunction results in lower production of NO and the loss of its protective effects, resulting in atherosclerosis.[15] Numerous factors have been found to be associated with endothelial dysfunction, including ageing,[16] smoking,[17] diabetes,[18] oxidative stress[19] and inflammation.[20] Endothelial dysfunction is present in hypertension[21] and disruption to nitric oxide synthase, which generates NO, has been shown to affect BP in hypertensive subjects.[22, 23]

1.2.2 Oxidative stress

Reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet -}$), are highly reactive molecules that are important for vascular function but in excess cause damage to the vascular endothelium, resulting in a reduction of NO.[24] Oxidative stress has been implicated in the pathophysiology of hypertension.[25-28]

Nitric oxide is an important regulator of baroreceptor function[29] and ROS has been found to modulate baroreceptor function in animal models.[30, 31] Hypertension is associated with baroreflex dysfunction.[32]

1.2.3 Inflammation

Elevated markers of inflammation have been found in hypertension,[33, 34] normotensive offspring of parents with hypertension,[35] prehypertension,[36, 37] and have predicted new onset

hypertension.[38] This has led to the hypothesis that vascular inflammation contributes to the pathophysiology of essential hypertension.

1.2.4 Artery stiffness

Artery stiffness is associated with ageing and with pathological conditions such as diabetes[39] and hypertension.[40] It is present in borderline hypertension[41] and is implicated in the progression to sustained hypertension.[42] Increased artery stiffness predicts cardiovascular events.[43] The 2013 ESH/ESC guidelines for the management of arterial hypertension includes artery stiffness as a measure of TOD in hypertension.[2]

1.2.5 Insulin resistance

Insulin resistance is associated with endothelial dysfunction.[44] It is prevalent in hypertensives independent of body fat.[45] Its deleterious actions include reduced NO by impairment of NO synthase,[46, 47] increased sympathetic nervous system activity[48] and retention of sodium.[49] Insulin resistance has been found in offspring of hypertensive parents.[50]

1.2.6 Metabolic syndrome

The 2009 joint statement from the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity defined the metabolic syndrome as the presence of three or more of increased waist circumference, elevated blood glucose, low high density lipoprotein,

elevated triglycerides and high BP.[51] The metabolic syndrome is prevalent in hypertension[52, 53] and has been associated with incident hypertension.[54]

1.2.7 Autonomic dysfunction.

Hypertension is associated with increased sympathetic nervous activity.[55] It is hypothesized that sympathetic neural mechanisms are involved in the development and progression of hypertension.[56] Hypertension is also associated with baroreflex dysfunction.[32] Autonomic dysfunction in hypertension is associated with blunting of heart rate and BP variability.[57] A loss of nocturnal BP dipping is an end result. Autonomic dysfunction has been implicated in the morning BP surge.[58]

1.3 Thesis Questions

Questions addressed in this thesis are:

1) In strictly defined WCHT, using a consensus definition and two confirmatory ABPM recordings, is there evidence of endothelial dysfunction or its associated conditions?
2) In strictly defined WCHT, using a consensus definition and two confirmatory ABPM recordings, is there increased risk for the progression to sustained hypertension?
3) What biomarkers are associated with the progression to sustained hypertension in WCHT?
4) Is WCHT associated with increased morning BP surge?

1.4 References

1. Mancia G, Bertinieri G, Grassi G, Parati G, Pomidossi G, Ferrari A, et al. Effects of blood-pressure measurement by the doctor on patient's blood pressure and heart rate. *Lancet*. 1983; **2**: 695-8.

2. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur. Heart J. 2013; **34**: 2159-219.

3. Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, et al. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation*. 2005; **111**: 697-716.

4. Cuspidi C, Meani S, Sala C, Valerio C, Fusi V, Zanchett, A, et al. How reliable is isolated clinical hypertension defined by a single 24-h ambulatory blood pressure monitoring? *J. Hypertens*. 2007; **25**: 315-20.

5. Bombelli M, Sega R, Facchetti R, Corrao G, Friz HP, Vertemati AM, et al. Prevalence and clinical significance of a greater ambulatory versus office blood pressure ('reversed white coat' condition) in a general population. *J. Hypertens*. 2005; **23**: 513-20.

6. Niiranen TJ, Jula AM, Kantola IM, Reunanen A. Prevalence and determinants of isolated clinic hypertension in the Finnish population: the Finn-HOME study. *J. Hypertens*. 2006; **24**: 463-70.

7. Cuspidi C, Parati G. Isolated clinic hypertension in a general population. *J. Hypertens*. 2006; **24**: 437-40.

8. Hosaka M, Mimura A, Asayama K, Ohkubo T, Hayashi K, Kikuya M, et al. Relationship of dysregulation of glucose metabolism with white-coat hypertension: the Ohasama study. *Hypertens. Res*. 2010; **33**: 937-43.

9. Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Grassi G, et al. Increased long-term risk of new-onset diabetes mellitus in white-coat and masked hypertension. *J. Hypertens*. 2009; **27**: 1672-8.

10. Grandi AM, Solbiati F, Laurita E, Cassinerio E, Marchesi C, Piperno F, et al. Isolated office hypertension: a 3-year follow-up study. *Blood Press*. 2005; 14: 298-305.

11. Ugajin T, Hozawa A, Ohkubo T, Asayama K, Kikuya M, Obara T, et al. White-coat hypertension as a risk factor for the development of home hypertension: the Ohasama study. *Arch. Intern Med*. 2005; **165**: 1541-6.

12. Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Polo Friz H, et al. Long-term risk of sustained hypertension in white-coat or masked hypertension. *Hypertension*. 2009; **54**: 226-32.

13. Verdecchia P, Reboldi GP, Angeli F, Schillaci G, Schwartz JE, Pickering TG, et al. Short- and long-term incidence of stroke in white-coat hypertension. *Hypertension*. 2005; **45**: 203-8.

14. Pierdomenico SD, Cuccurullo F. Prognostic value of white-coat and masked hypertension diagnosed by ambulatory monitoring in initially untreated subjects: an updated meta analysis. *Am. J. Hypertens*. 2011; **24**: 52-8.

15. Vanhoutte,PM, Shimokawa H, Tang EH, Feletou M. Endothelial dysfunction and vascular disease. *Acta. Physiol. (Oxf)*. 2009; **196**: 193-222.

16. Taddei S, Virdis A, Mattei P, Ghiadoni L, Gennari A, Fasolo CB, et al. Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation*. 1995; **91**: 1981-7.

17. Puranik R, Celermajer DS. Smoking and endothelial function. *Prog. Cardiovasc. Dis*. 2003; **45**: 443-58.

18. Hadi HA, Suwaidi JA. Endothelial dysfunction in diabetes mellitus. *Vasc. Health Risk Manag*. 2007; **3**: 853-76.

19. Silva BR, Pernomian L, Bendhack LM. Contribution of oxidative stress to endothelial dysfunction in hypertension. *Front. Physiol*. 2012; **3**: 441.

20. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. *Basic Res Cardiol*. 2008; 103: 398-406.

21. Panza JA, Quyyumi AA, Brush JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N. Engl. J. Med*. 1990; **323**: 22-7.

22. Hong HJ, Loh SH, Yen MH. Suppression of the development of hypertension by the inhibitor of inducible nitric oxide synthase. *Br. J. Pharmacol*. 2000; **131**: 631-7.

23. Smith CJ, Santhanam L, Bruning RS, Stanhewicz A, Berkowitz DE, Holowatz LA. Upregulation of inducible nitric oxide synthase contributes to attenuated cutaneous vasodilation in essential hypertensive humans. *Hypertension*. 2011; **58**: 935-42.

24. Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol*. 1986; **250**: H822-7.

25. Zhao W, Swanson SA, Ye J, Li X, Shelton JM, Zhang W, et al. Reactive oxygen species impair sympathetic vasoregulation in skeletal muscle in angiotensin II-dependent hypertension. *Hypertension*. 2006; **48**: 637-43.

26. Li M, Dai X, Watts S, Kreulen D, Fink G. Increased superoxide levels in ganglia and sympathoexcitation are involved in sarafotoxin 6c-induced hypertension. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2008; **295**: R1546-54.

27. Datla SR, Griendling KK. Reactive oxygen species, NADPH oxidases, and hypertension. *Hypertension*. 2010; **56**: 325-30.

28. Touyz RM, Briones AM. Reactive oxygen species and vascular biology: implications in human hypertension. *Hyperten.s Res*. 2011; **34**: 5-14.

29. Chowdhary S, Vaile JC, Fletcher J, Ross HF, Coote JH, Townend JN. Nitric oxide and cardiac autonomic control in humans. *Hypertension*. 2000; **36**: 264-9.

30. Li Z, Mao HZ, Abboud FM, Chapleau MW. Oxygen-derived free radicals contribute to baroreceptor dysfunction in atherosclerotic rabbits. *Circ. Res*. 1996; **79**: 802-11.

31. Dang N, Pan YX. Central oxidative stress depresses baroreflex function in spontaneously hypertensive rats. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2012; **28**: 445-8.

32. Gribbin B, Pickering TG, Sleight P, Peto R. Effect of age and high blood pressure on baroreflex sensitivity in man. *Circ. Res*. 1971; **29**: 424-31.

33. Sesso HD, Buring JE., Rifai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. *JAMA*. 2003; **290**: 2945-51.

34. Liao TD, Yang XP, Liu YH, Shesely EG, Cavasin MA, Kuzie, WA, et al. Role of inflammation in the development of renal damage and dysfunction in angiotensin II-induced hypertension. *Hypertension*. 2008; **52**: 256-63.

35. Lieb W, Pencina MJ, Wang TJ, Larson MG, Lanier KJ, Benjamin EJ, et al. Association of parental hypertension with concentrations of select biomarkers in nonhypertensive offspring. *Hypertension*. 2008; **52**: 381-6.

36. Chrysohoou C, Pitsavos C, Panagiotakos DB, Skoumas J, Stefanadis C. Association between prehypertension status and inflammatory markers related to atherosclerotic disease: The ATTICA Study. *Am. J. Hypertens*. 2004; **17**: 568-73.

37. King DE, Egan BM, Mainous AG , Geesey ME. Elevation of C-reactive protein in people with prehypertension. *J. Clin. Hypertens (Greenwich)*. 2004; **6**: 562-8.

38. Dauphinot V, Roche F, Kossovsky MP, Schott AM, Pichot V, Gaspoz JM, et al. C-reactive protein implications in new-onset hypertension in a healthy population initially aged 65 years: the Proof study. *J. Hypertens*. 2009; **27**: 736-43.

39. Schram MT, Henry RM, van Dijk RA, Kostense PJ, Dekker JM, Nijpels G, et al. Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. *Hypertension*. 2004; **43**: 176-81.

40. Cameron JD, Asmar R, Struijker-Boudier H, Shirai K, Sirenko Y, Kotovskaya Y, et al. Current and future initiatives for vascular health management in clinical practice. *Vasc. Health Risk Manag*. 2013; **9**: 255-64.

41. Messerli FH, Frohlich ED, Ventura HO. Arterial compliance in essential hypertension. *J. Cardiovasc. Pharmacol*. 1985; **7** Suppl 2: S33-5.

42. Tomiyama H, Yamashina A. Arterial stiffness in prehypertension: a possible vicious cycle. *J. Cardiovasc. Transl. Res*. 2012; **5**: 280-6.

43. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. *J. Am. Coll. Cardiol*. 2010; **55**: 1318-27.

44. Muniyappa R, Sowers JR. Role of insulin resistance in endothelial dysfunction. *Rev. Endocr. Metab. Disord*. 2013; **14**: 5-12.

45. Modan M, Halkin H, Almog S, Lusky A, Eshkol A, Shefi M, et al. Hyperinsulinemia. A link between hypertension obesity and glucose intolerance. *J. Clin. Invest*. 1985; **75**: 809-17.

46. Calver A, Collier J, Vallance P. Inhibition and stimulation of nitric oxide synthesis in the human forearm arterial bed of patients with insulin-dependent diabetes. *J. Clin. Invest*. 1992; **90**: 2548-54.

47. Kashyap SR, Roman,LJ, Lamont J, Masters BS, Bajaj M, Suraamornkul S, et al. Insulin resistance is associated with impaired nitric oxide synthase activity in skeletal muscle of type 2 diabetic subjects. *J. Clin. Endocrinol Metab.* 2005; **90**: 1100-5.

48. Rowe JW, Young JB, Minaker KL, Stevens AL, Pallotta J., Landsberg L. Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes*. 1981; **30**: 219-25.

49. Ter Maaten JC, Voorburg A, Heine RJ, Ter Wee PM, Donker AJ, Gans RO. Renal handling of urate and sodium during acute physiological hyperinsulinaemia in healthy subjects. *Clin. Sci. (Lond)*. 1997; **92**: 51-8.

50. Beatty OL, Harper R, Sheridan B, Atkinson AB, Bell PM. Insulin resistance in offspring of hypertensive parents. *BMJ*. 1993; **307**: 92-6.

51. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International

Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009; **120**: 1640-5.

52. Bulhoes K, Araujo L. Metabolic syndrome in hypertensive patients: correlation between anthropometric data and laboratory findings. *Diabetes Care*. 2007; **30**: 1624-6.

53. Kabakci G, Koylan N, Kozan O, Buyukozturk K, Ilerigelen B and Investigators. Evaluation of the metabolic syndrome in hypertensive patients: results from the ICEBERG Study. *J. Cardiometab. Syndr*. 2007; **2**: 168-73.

54. Cheung BM, Wat NM, Man YB, Tam S, Cheng CH, Leung GM, et al. Relationship between the metabolic syndrome and the development of hypertension in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2). *Am. J. Hypertens*. 2008; **21**: 17-22.

55. DiBona GF. Sympathetic nervous system and hypertension. *Hypertension*. 2013; **61**: 556-60.

56. Grassi G, Seravalle G, Quarti-Trevano F. The 'neuroadrenergic hypothesis' in hypertension: current evidence. *Exp. Physiol*. 2010; **95**: 581-6.

57. Mann S, Altman DG, Raftery EB, Bannister R. Circadian variation of blood pressure in autonomic failure. *Circulation*. 1983; **68**: 477-83.

58. Yano Y, Kario K. Possible difference in the sympathetic activation on extreme dippers with or without exaggerated morning surge. *Hypertension*. 2009; **53**: e1; author reply e2.

Chapter 2

Circulating and mechanical biomarkers in isolated clinic hypertension.

2.1 Introduction

The published paper in this Chapter reviewed the evidence for altered mechanical and circulating biomarkers in WCHT. Studies were included in the review if they examined biomarkers of artery stiffness, endothelial dysfunction, inflammation and oxidative stress in both WCHT and normotensives. Studies that examined urinary albumin excretion in WCHT were also reviewed as microalbuminuria is considered a biomarker of renal vascular endothelial dysfunction.

Questions addressed

- Is WCHT associated with endothelial dysfunction?
- Is WCHT associated with oxidative stress?
- Is WCHT associated with vascular inflammation?
- Is WCHT associated with artery stiffness?

2.2 Declaration

In the case of Chapter Two, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Review of evidence, interpretation of evidence and writing of paper.	70

The following co-authors contributed to the work.

Name	Nature of contribution
Professor Barry McGrath	Conception, writing and review of paper
Professor Jams Cameron	Conception, writing and review of paper

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	18.9.13
Main Supervisor's Signature	[redacted]	18.9.13

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MECHANICAL AND CIRCULATING BIOMARKERS IN ISOLATED CLINIC HYPERTENSION

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SUMMARY

1. This review examines the current evidence for altered mechanical and circulating biomarkers in isolated clinic hypertension and their potential significance.

2. Arterial stiffness, as assessed by central pulse wave velocity, is influenced by multiple cardiovascular risk factors; however, an independent association with isolated clinic hypertension (ICHT) has not been convincingly shown in four small studies.

3. Endothelial dysfunction, as assessed by brachial artery flow-mediated vasodilation, circulating levels of endothelial markers (e.g. nitrite/nitrate, von Willebrand factor, endothelin-1) and/or circulating levels of inhibitors of vascular nitric oxide (plasma asymmetric dimethylarginine, homocysteine), has been shown to be present in established hypertension and to a variable and inconsistent extent in subjects with ICHT.

4. Evidence of increased oxidative stress in ICHT versus normotensive subjects was found in two of three studies.

5. Circulating inflammatory markers C-reactive protein and plasminogen activator inhibitor-1 were significantly increased in two of three and two of two studies, respectively, in ICHT compared with normotensive subjects.

6. Urinary albumin excretion is a marker of both arterial and renal disease. The consensus from seven studies in patients with ICHT is that albuminuria is not an independent marker for ICHT.

7. Studies to date assessing biomarkers in ICHT have been small and cross-sectional. Larger, long-term longitudinal studies of arterial functional and circulating biomarkers are required to assess the potential vascular impact of ICHT.

Key words: arterial stiffness, endothelial dysfunction, inflammation, isolated clinic hypertension, oxidative stress.

INTRODUCTION

Blood pressure measured in the doctor's office or clinic has been the most established biomarker of future cardiovascular disease, but it is no longer sufficient if used as the sole method of assessing the usual blood pressure of an individual. Twenty-four hour ambulatory blood pressure and home blood pressure monitoring have identified the discrepancy that exists between clinic and out-of-clinic blood pressures in a significant number of patients and in these patients, with either isolated clinic or masked hypertension, risk calculations based on clinic readings may lead to erroneous risk prediction of cardiovascular risk and inappropriate treatment.

Isolated clinic hypertension (ICHT), also known as 'white-coat' hypertension, is a common clinical condition, where clinic measurements are elevated (systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg) but home and 24 h ambulatory blood pressure measurements are within the normotensive range (day average SBP < 135 mmHg and day average DBP < 85 mmHg). The prevalence depends on blood pressure cut-off levels and accurate classification requires more than one 24 h blood pressure recording.[1] There has been considerable debate about the significance of the condition in predicting future cardiovascular disease.[2] In an 8-year follow-up period in the Ohasama study, ICHT was identified as a significant predictor for the development of hypertension as assessed by home measurement[3] confirming the results of an earlier study.[4] Recently, Verdecchia *et al*.[5] described a trend towards increasing incidence of stroke, with the hazard curve for isolated clinic hypertension crossing that of the established hypertensive group at 9 years of follow-up.

Over the past 10 years there has been a concerted effort to explore the clinical usefulness of surrogate markers of arterial function. There have been many indices proposed for this purpose, including those indicative of arterial stiffness in central or peripheral arterial segments (pulse wave velocity, stiffness index), indices of arterial compliance (systemic arterial compliance, distensibility) and a composite measure of wave reflection and systemic arterial stiffness (aortic augmentation index). Dynamic or functional influences such as endothelial-mediated changes can influence these indices, but the scope of these dynamic components has generally not been well characterized. Central (aorto-femoral) pulse wave velocity (PWV) is the index of arterial stiffness with most promise as a functional biomarker.[6] Other surrogate non-mechanical biomarkers of cardiovascular disease can be broadly classified into three categories: measures of endothelial function, circulating inflammatory markers and markers of oxidative stress (Fig. 1). In established hypertension there is good evidence for disturbed vascular

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function and structure as shown by changes in each of these biomarkers.

This review examines the evidence for alteration in biomarkers of vascular disease in ICHT.

ARTERIAL STIFFNESS

Central PWV in a variety of patient groups and in normal individuals has consistently been shown to be an independent predictor of cardiovascular outcome;[7] it tends to track appropriately with disease severity and there is a strong case for its inclusion in risk assessment algorithms.[8] An age-adjusted reference curve for PWV has recently been reported with demonstrable construct validity in regards to identification of groups at medium and high risk of cardiovascular disease.[9]

Four studies of PWV measurement in patients with ICHT are reported[10–13] (Fig. 2). Only two of the studies used central (carotid/aortic to femoral) PWV, which is now generally accepted as the best mechanical predictor of cardiovascular disease. Tillin *et al*.[14] reported that central (carotid-femoral) PWV was significantly associated with coronary artery calcification score and with carotid or femoral artery intima-media thickness, whereas carotid-radial PWV and femoral-posterior tibial PWV were not. Pannier *et al*.[15] showed that only central PWV, not brachial artery or femoro-tibial PWV, was able to predict cardiovascular mortality in patients with end-stage renal failure.

Silva *et al*.[10] showed that, unadjusted for concomitant risk factors, there was a progressive increase in mean carotid-femoral PWV from a normotensive to an ICHT group and then to an established hypertensive group. Ribeiro *et al*.[11] found that patients with ICHT had significantly higher aorto-femoral PWV compared with normotensive subjects, but that this was influenced by the number of coexisting risk factors. In patients at low overall risk, with one or no cardiovascular risk factors, this difference was not significant. A recently reported study in Japanese subjects used radial-femoral PWV measurements and showed that after adjustment for age, gender, body mass index (BMI), habitual alcohol drinking, lifetime smoking and SBP during PWV measurement, there was no significant difference between normotensive and ICHT subjects.[12] Longo *et al*.[13] who used carotid to radial PWV, found a significant difference between the ICHT group and the normotensive group after adjusting for age, sex, heart rate, weight, height, blood pressure, smoking, alcohol use, physical activity, fasting glucose, total cholesterol and triglycerides. However this should be interpreted with caution given the lack of outcome predictability of this measure as discussed above. There have been no long-term studies of ICHT that have looked at baseline central PWV as an outcome predictor.

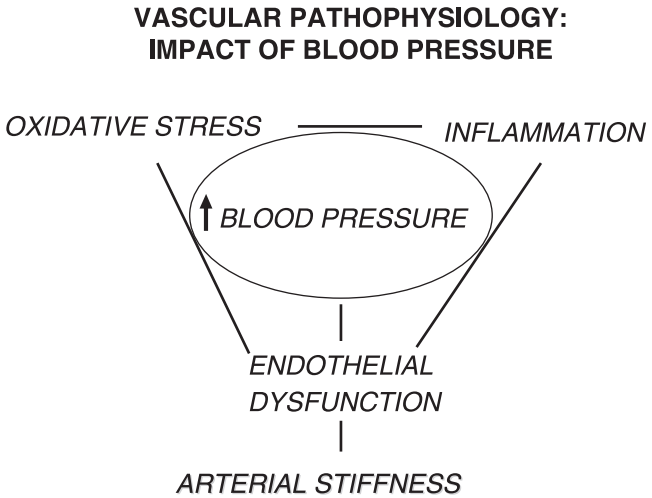


Fig. 1 Schematic representation of interrelationships between blood pressure and vascular pathophysiological markers: oxidative stress, inflammation, endothelial dysfunction and arterial stiffness.

BIOMARKERS OF ENDOTHELIAL FUNCTION

Vascular endothelial cells have multiple, often counter-regulatory activities relating to vasodilatation and vasoconstriction, thrombosis and fibrinolysis, platelet aggregation and adhesion, leucocyte adhesion and activation, smooth muscle cell proliferation and migration, immunological and inflammatory processes. Nitric oxide (NO) is produced by endothelial cells in response to various stimuli, with shear-stress being the most important. It has been estimated that NO tonically restrains BP in humans by approximately 30 mmHg.[16]

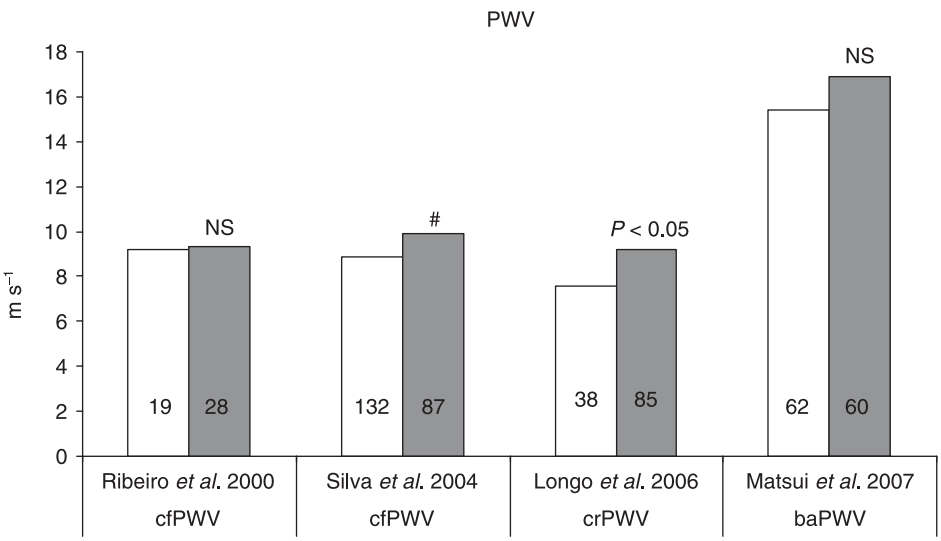


Fig. 2 Pulse wave velocity (PWV) in four published studies of isolated clinic hypertension (ICHT) (shaded bars) versus normotensive controls (open bars). baPWV, brachial-ankle PWV; cfPWV, carotid-femoral PWV; crPWV, carotid-radial PWV; NS, not significant at the 5% level. #*P*-value ICHT versus normotension not given.

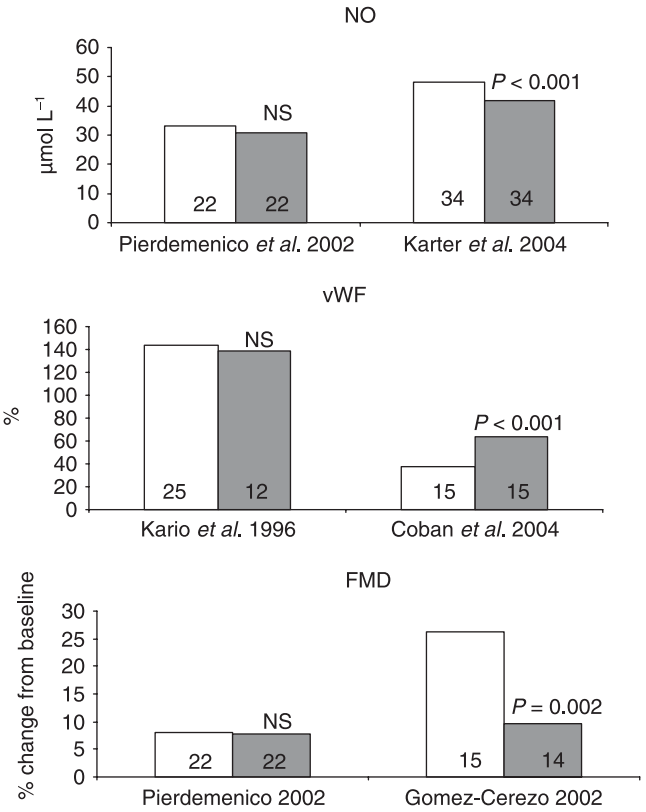


Fig. 3 Summary of published studies of biomarkers of endothelial function in isolated clinic hypertension (ICHT) (shaded bars) versus normotensive controls (open bars). FMD, brachial artery flow-mediated dilation; NO, plasma nitrite and nitrate; NS, not significant at the 5% level; vWF: Von Willebrand Factor.

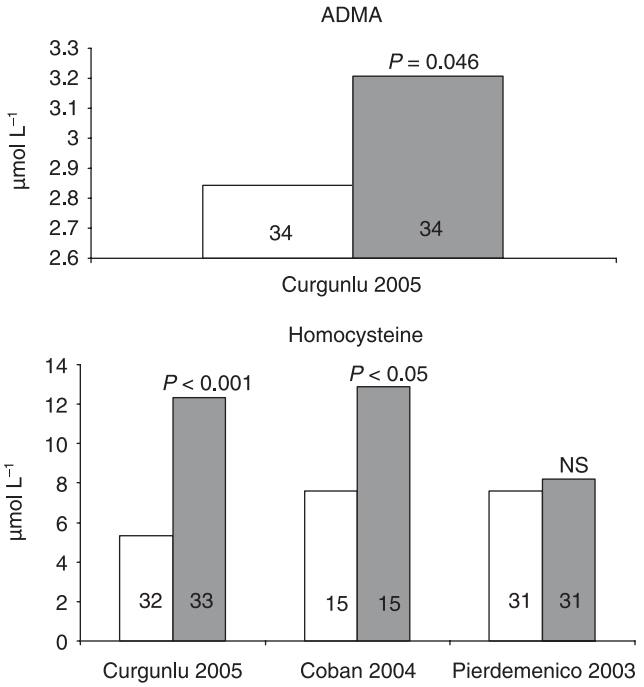


Fig. 4 Summary of published studies of plasma levels of inhibitors of nitric oxide (NO) synthesis, asymmetric dimethylarginine (ADMA, top panel) and homocysteine (bottom panel) in isolated clinic hypertension (ICHT) (shaded bars) versus normotensive controls (open bars). NS, not significant at the 5% level.

Shear-stress induced NO can be assessed by the technique of brachial arterial flow-mediated dilation (FMD) in response to postischaemic reactive hyperaemia, to determine a measure of endothelium-dependent dilation (EDD). This is impaired in established hypertension.[17] Gomez-Cereza *et al*.[18] reported a linear relationship between 24-h SBP and EDD ($r = -0.48$, $P = 0.0001$), with significant differences between ICHT and normotensive groups. Although not statistically significant, there were higher proportions of females (57% versus 33%) and overweight subjects (57% versus 36%) in the ICHT group. Pierdemenico *et al*.[19] found no significant difference in EDD between ICHT and normotensive subjects (Fig. 3).

Plasma levels of NO can be estimated by levels of its metabolites, NO_2 and NO_3. Karter *et al*.[20] showed that mean plasma nitrite/nitrate was reduced in ICHT compared with normotensive controls (42 ± 2 versus 48 ± 6 µmol/L, Fig. 3) and further reduced in established hypertension (32 ± 3 µmol/L, $P < 0.001$). Curgunlu *et al*.[21] reported very similar results, with the same numbers of subjects and likely from the same population. Pierdomenico *et al*.[19] found no significant difference between normotensive and ICHT groups (Fig. 3), but mean plasma nitrite/nitrate level on a standardized low-nitrate diet was lower in established hypertension compared with ICHT (22 ± 8 versus 31 ± 12 µmol/L, $P < 0.05$).

Von Willebrand factor (vWF) is a glycoprotein, required for platelet aggregation and adhesion and for factor VIII survival. It is stored in endothelial cells and secreted into the plasma. Increased vWF levels are associated with hypertension and progression of cardiovascular disease.[22] Two studies have examined vWF levels in groups of normotensive, ICHT and established hypertensive subjects.[23,24] Both showed increased vWF levels in groups of subjects with established hypertension, but only the study by Coban *et al*.[24] showed a difference between ICHT and normotensive groups (Fig. 3). In that study groups were matched for age, gender and body mass index. Kario *et al*.[23] studied an older population, in which groups were age-matched, but not matched for other factors.

Endothelin-1 (ET-1) is a potent vasoconstrictor expressed by endothelial cells in response to a number of stimuli and has been found to be elevated in hyperlipoproteinaemia, insulin resistance, diabetes, smokers and obese subjects with metabolic syndrome. High concentrations have also been found in atherosclerotic plaques. Plasma levels of ET-1 are normal in most subjects with essential hypertension but are elevated in other types of hypertension such as renal hypertension. Plasma ET-1 and vascular endothelial growth factor levels were increased in subjects with ICHT compared with normotensive controls in the study of Karter *et al*.[25] Urinary ET-1 excretion was reported to be increased in male adolescent ICHT subjects[26] compared with male adolescent normotensives. Although the male ICHT group had a higher mean BMI, the difference persisted after adjustment for BMI. No difference was found in females. Potential confounders other than BMI were not reported.

INHIBITORS OF VASCULAR NO

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO synthesis. ADMA levels are elevated in chronic renal disease, hypercholesterolaemia, hyperhomocysteinaemia and hypertension. ADMA synthesis is triggered by shear stress and this is the likely

Table 1 Oxidative markers and anti-oxidant activity

			NT	ICHT	EHT	
LDL oxidation						
Pierdomenico *et al*. 1998			$n = 21$	$n = 15$	$n = 27$	
	FPL	URF/mg LDL protein	10.1 ± 1.9	10.4 ± 3.6	14.2 ± 3.8	NS ICHT versus NT $P < 0.05$ ICHT versus EHT
	Lag phase	min	89 ± 9	86.5 ± 10	62 ± 19	NS ICHT versus NT $P < 0.05$ ICHT versus EHT
	PR	Nmol diene/min per mg LDL-C	4.8 ± 1.3	5.1 ± 1.8	8.1 ± 2.3	NS ICHT versus NT $P < 0.05$ ICHT versus EHT
	LDL Vitamin E	Nmol/mg LDL-C	10.5 ± 1.5	10.5 ± 1.9	8.8 ± 1.3	NS ICHT versus NT $P < 0.05$ ICHT versus EHT
	Plasma Vitamin C	μmol/L	58 ± 13	56 ± 7.5	45 ± 14	NS ICHT versus NT $P < 0.05$ ICHT versus EHT
Uzun *et al*. 2004			$n = 26$	$n = 30$	$n = 30$	
	MDA	μmol/L	4.54 ± 0.67	5.22 ± 0.62	5.9 ± 0.9	$P = 0.026$ ICHT versus NT NS ICHT versus EHT
	oxLDL	U/L	51.21 ± 16.35	60.15 ± 20.33	70.54 ± 25.75	$P = 0.023$ EHT versus NT NS ICHT versus NT NS ICHT versus EHT
	PON1	U/L	94.05 ± 39.17	48.49 ± 16.54	46.81 ± 21.43	$P < 0.001$ ICHT versus NT NS ICHT versus EHT
Protein oxidation						
Caner *et al*. 2006			$n = 37$	$n = 37$	$n = 37$	
	PCO	nmol/L/mg pr	0.65 ± 0.04	0.70 ± 0.07	0.82 ± 0.12	$P < 0.01$ ICHT versus NT NS ICHT versus EHT
	P-SH	μM	562.56 ± 44.45	516.35 ± 52.09	475.97 ± 53.84	$P < 0.01$ ICHT versus NT NS ICHT versus EHT
	GSH	μmol/g Hb	4.32 ± 0.39	3.68 ± 0.28	3.44 ± 0.28	$P < 0.001$ ICHT versus NT NS ICHT versus EHT
	SOD	U/mL	24.43 ± 2.36	22.76 ± 2.91	22.60 ± 2.4	$P < 0.01$ ICHT versus NT NS ICHT versus EHT

EHT, essential hypertension; FPL, fluorescent products of lipid peroxidation in native LDL; GSH, glutathione; ICHT, isolated clinic hypertension; lag phase, a measure of LDL resistance to oxidation *in vitro*; LDL, low-density lipoprotein; MDA, malondialdehyde; NS, not significant at the 5% level; NT, normotension; oxLDL, oxidized low-density lipoprotein; PCO, protein carbonyls; PON1, paraoxonase; P-SH, protein thiol; PR, peroxidation rate which is an indication of the autocatalytic chain reaction of lipid peroxidation after depletion of anti-oxidant content; SOD, superoxide dismutase.

cause for increased levels in hypertension. It is inversely related to endothelial function in hypertension as measured by peak haemodynamic response to acetylcholine.[27] Oxidative stress may also increase ADMA levels by inhibiting dimethylaminohydrolase (DDAH), which metabolises ADMA to citrulline.[28] As shown in Fig. 4 only one study was found that measured ADMA levels in ICHT subjects.[21] Mean ADMA level in ICHT subjects was statistically increased when compared with the normotensive group, but less than that of the established hypertensive group (3.21 ± 0.49 versus 2.84 ± 0.58 versus 4.24 ± 0.38).

Increased plasma total homocysteine is associated with increased risk of cardiovascular disease, particularly in hypertensive subjects. Three studies have compared mean plasma homocysteine levels in ICHT and normotensive subjects;[21,29,30] in two of these levels were significantly higher in ICHT subjects[21,30] (Fig. 4). However these studies did not adjust for potential confounding factors, such as renal function.

BIOMARKERS OF OXIDATIVE STRESS

Oxidative stress occurs when reactive oxygen species (ROS) react with and damage tissue and organs. It is hypothesized that oxidative stress plays a role in the pathogenesis of hypertension.[31] but this has been challenged.[32] Results of three studies that have examined various markers of oxidative stress in ICHT are summarized in Table 1.[33–35] Two of the three studies reported evidence of increased oxidative stress in ICHT compared with normotensive controls.[34,35]

CIRCULATING INFLAMMATORY MARKERS

Inflammation is well established as a major contributing factor in atherogenesis. Cardiovascular risk factors, including smoking, obesity, hypertension, hyperglycaemia and atherogenic lipoproteins all cause injury to the vascular endothelium, which initiates a local inflammatory response. The inflammatory biomarker that is most extensively studied is C-reactive protein (CRP), an acute phase protein considered the most sensitive circulating marker of inflammation. CRP has been associated with cardiovascular events[36,37] and has been suggested as an independent risk marker for cardiovascular disease.[38] Recent evidence suggests that CRP itself is deleterious to the vascular endothelium: CRP induces coronary and aortic endothelial release of inflammatory cytokines[39] increases expression of angiotensin II type 1 receptors in vascular smooth muscle cells,[40] reduces production of NO and increases the uptake of low-density

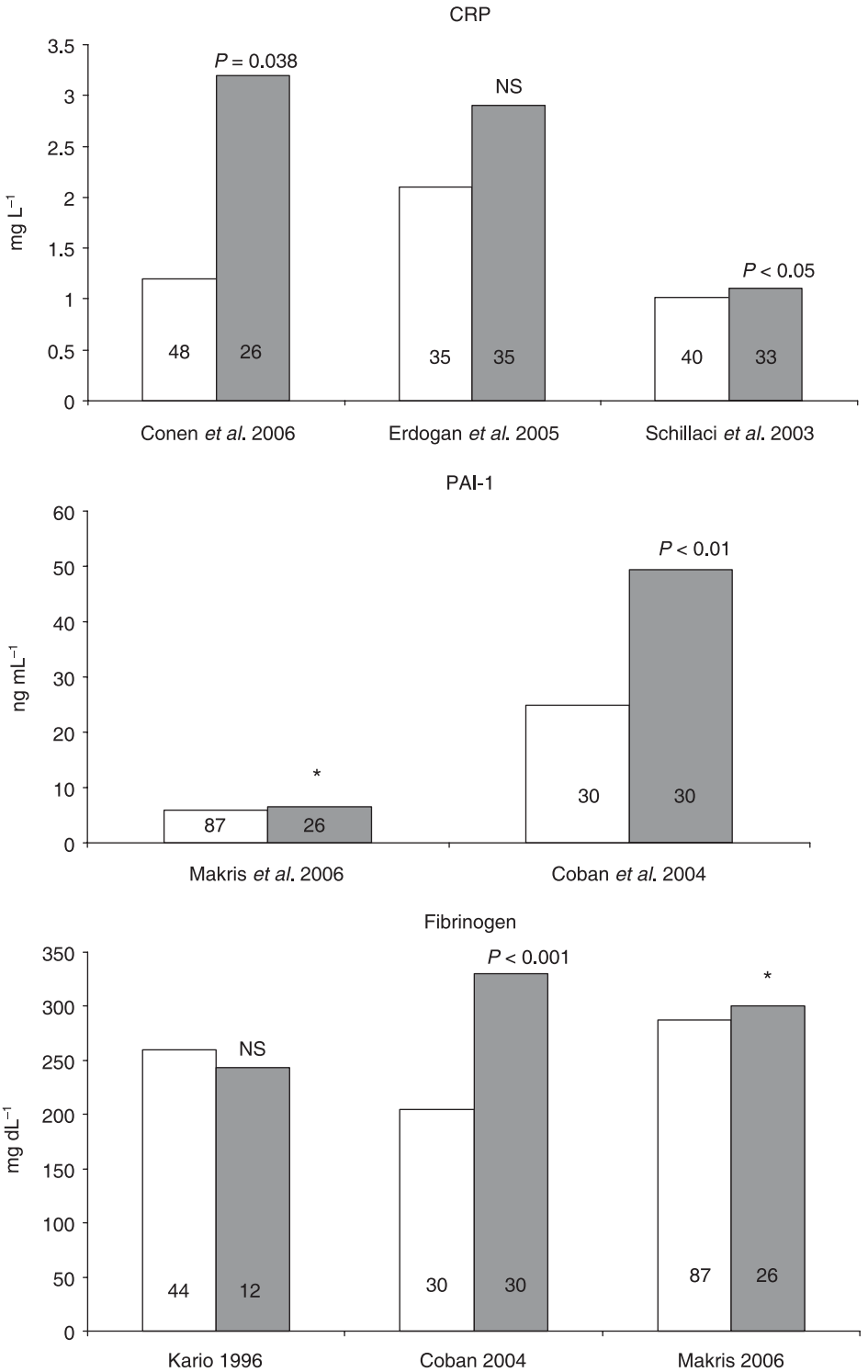


Fig. 5 Summary of published studies of plasma levels of inflammatory biomarkers, C-reactive protein (CRP, top panel), plasminogen activator inhibitor-1 (PAI-1, middle panel) and fibrinogen (bottom panel), in isolated clinic hypertension (KHT) (shaded bars) versus normotensive controls (open bars). *P*-value KHT versus normotension not given.

lipoprotein (LDL) by macrophages.[41] CRP has been found in other prospective studies to be an independent predictor for the development of hypertension[42,43] suggesting that inflammation may precede hypertension and endothelial dysfunction, but causal evidence is yet to be established.

Results of studies comparing inflammatory markers in ICHT and normotensive subjects are shown in Fig. 5. Three studies were identified that compared CRP in ICH subjects in comparison to normotensives and sustained hypertensives.[44–46] These studies used similar definitions of ICH and were matched for age and gender. Two of the studies showed significant difference in CRP (between ICH and normotensive groups).[44,45]

Plasminogen activator inhibitor-1 (PAI-1) is a marker of impaired fibrinolysis and atherothrombosis. PAI-1 is induced by CRP.[47] Both PAI-1 and CRP are increased in obesity, diabetes and the metabolic syndrome. PAI-1 is also found in platelets and can be released during blood taking.[48] PAI-1 levels are elevated in established hypertension[49] and PAI-1 levels were found to be associated with

blood pressure levels in children with hypertensive parents.[50] In a study of the Framingham population both CRP and PAI-1 were found to be independent markers of incident hypertension.[51]

Two studies were identified that compared PAI-1 in ICH subjects compared to normotensives and sustained hypertensives.[52,53] Both studies report a significant difference between PAI-1 levels in the ICHT group and the normotensives group.

URINARY ALBUMIN EXCRETION

Seven studies have reported urinary albumin excretion in ICHT.[21,54–59] Although increased urinary albumin excretion was reported in established hypertension in four of the seven studies, in only one study was there a significant difference between ICHT and normotensive groups.

CONCLUSIONS

Established hypertension is associated with arterial stiffness, endothelial dysfunction, increased vascular oxidative stress and an increase in circulating inflammatory markers. It is likely that ICHT is a prehypertensive state that requires close monitoring. This review of many cross-sectional studies provides suggestive evidence for altered vascular function in ICHT, as shown by arterial endothelial dysfunction and circulating markers of oxidative stress and inflammation, but arterial stiffness is not increased. There is a need for large, long-term longitudinal studies of arterial functional and circulating biomarkers to assess the potential vascular effect of ICHT. These studies need to take into account the multiple variables that can influence these biomarkers.

REFERENCES

1. Cuspidi C, Meani S, Sala C *et al*. How reliable is isolated clinic hypertension defined by a single 24-hr ambulatory blood pressure monitoring? *J. Hypertens*. 2007; **25**: 315–20.
2. McGrath BP. Is white-coat hypertension innocent? *Lancet* 1996; **348**: 630–1.
3. Ugajin T, Hozawa A, Ohkubo T *et al*. White-coat hypertension as a risk factor for the development of home hypertension: The Ohasama study. *Arch. Intern. Med*. 2005; **165**: 1541–6.
4. Bidlingmeyer I, Burnier M, Bidlingmeyer M, Waeber B, Brunner HR. Isolated office hypertension: A prehypertensive state? *J. Hypertens* 1996; **14**: 327–32.
5. Verdecchia P, Reboldi GP, Agneli F *et al*. Short- and long-term incidence of stroke in white-coat hypertension. *Hypertension* 2005; **45**: 203–8.
6. McGrath BP, Laurent S. The current status of arterial functional biomarkers in health and disease. *Clin. Exper. Pharmacol. Physiol*. 2007; **34**: 645–6.
7. Zoungas S, Asmar R. Arterial stiffness and cardiovascular outcome. *Clin. Exp. Pharmacol. Physiol*. 2007; **34**: 647–51.
8. Mancia G, De Backer G, Dominiczak A *et al*. The Taskforce for the Management of Arterial Hypertension of the European Society of Hypertension and the European Society of Cardiology. 2007 guidelines for the management of arterial hypertension. *J. Hypertens*. 2007; **25**: 1105–87.
9. Khoshdel AR, Thakkinstian A, Carney SL, Attia J. Estimation of an age-specific reference interval for pulse wave velocity: A meta-analysis. *J. Hypertens*. 2006; **24**: 1231–7.
10. Silva JA, Barbosa L, Bertoquini S, Maldonado J, Polonia J. Relationship between aortic stiffness and cardiovascular risk factors in a population of normotensives, white-coat normotensives, white-coat hypertensives, sustained hypertensives and diabetic patients. *Rev. Port. Cardiol*. 2004; **23**: 1533–47.
11. Ribeiro L, Gama G, Santos A, Asmar R, Martins L, Polonia J. Arterial distensibility in subjects with white-coat hypertension with and without diabetes or dyslipidaemia: comparison with normotensives and sustained hypertensives. *Blood Press. Monit*. 2000; **5**: 11–17.
12. Matsui Y, Eguchi K, Ishikawa J, Hoshide S, Shimada K, Kario K. Subclinical arterial damage in untreated masked hypertensive subjects detected by home blood pressure measurement. *Am. J. Hypertens*. 2007; **20**: 385–91.
13. Longo D, Zaetta V, Perkovic D *et al*. Impaired arterial elasticity in young patients with white-coat hypertension. *Blood Press. Monit*. 2006; **11**: 243–9.
14. Tillin T, Chambers J, Malik I *et al*. Measurement of pulse wave velocity: Site matters. *J. Hypertens*. 2007; **25**: 383–9.
15. Pannier B, Guérin AP, Marchais SJ, Safar ME, London GM. Stiffness of capacitive and conduit arteries: Prognostic significance for end-stage renal disease patients. *Hypertension* 2005; **45**: 592–6.
16. Gamboa A, Shibao C, Diedrich A *et al*. Contribution of endothelial nitric oxide to blood pressure in humans. *Hypertension*, 2007; **49**: 170–7.
17. Panza JA, Quyyumi AA, Brush Jr JE, Epstein SE. Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N. Engl. J. Med*. 1990; **323**: 22–7.
18. Gomez-Cerezo J, Rios Blanco JJ, Suarez GI *et al*. Noninvasive study of endothelial function in white coat hypertension. *Hypertension* 2002; **40**: 304–9.
19. Pierdomenico SD, Cipollone F, Lapenna D, Bucci A, Cuccurullo F, Mezzetti A. Endothelial function in sustained and white coat hypertension. *Am. J. Hypertens*. 2002; **15**: 946–52.
20. Karter Y, Erturk NT, Aydin S *et al*. Endothelial dysfunction in sustained and white coat hypertension. *Am. J. Hypertens*. 2003; **16**: 892.
21. Curgunlu A, Karter Y, Uzun H *et al*. Hyperhomocysteinemia: An additional risk factor in white coat hypertension. *Int. Heart J*. 2005; **46**: 245–54.
22. Blann AD, W M. Von Willebrand factor and soluble E-selectin in hypertension: Influence of treatment and value in predicting the progression of atherosclerosis. *Coron Artery Dis*. 1996; **7**: 143–7.
23. Kario K, Matsuo T, Kobayashi H *et al*. Factor VII hyperactivity and endothelial cell damage are found in elderly hypertensives only when concomitant with microalbuminuria. *Arterioscler. Thromb. Vasc. Biol*. 1996; **16**: 455–61.
24. Coban E, Timuragaoglu A, Ozdogan M. Endothelial dysfunction in patients with white coat hypertension. *J. Hum. Hypertens*. 2004; **18**: 71–2.
25. Karter Y, Aydin S, Curgunlu A *et al*. Endothelium and angiogenesis in white coat hypertension. *J. Hum. Hypertens*. 2004; **18**: 809–14.
26. Vaindirlis I, Peppa-Patrikiou M, Dracopoulou M, Manoli I, Voutetakis A, Dacou-Voutetakis C. 'White coat hypertension' in adolescents: Increased values of urinary cortisol and endothelin. *J. Pediatr*. 2000; **136**: 359–64.
27. Perticone F, Sciacqua A, Maio R *et al*. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. *J. Am. Coll. Cardiol*. 2005; **46**: 518–23.
28. Stühlinger MC, Oka RK, Graf EE *et al*. Endothelial dysfunction induced by hyperhomocysteinemia. Role of asymmetric dimethylarginine. *Circulation* 2003; **108**: 933–8.
29. Coban E, Ozdogan M, Ermis C. Plasma levels of homocysteine in patients with white-coat hypertension. *Int. J. Clin. Pract*. 2004; **58**: 997–9.
30. Pierdomenico SD, Bucci A, Lapenna D *et al*. Circulating homocysteine levels in sustained and white coat hypertension. *J. Hum. Hypertens*. 2003; **17**: 165–70.
31. Kumar KV, DU. Are free radicals involved in the pathobiology of human essential hypertension? *Free Radic Res. Commun*. 1993; **19**: 59–66.
32. Cracowski JL, Baguet JP, Ormezzano O *et al*. Lipid peroxidation is not increased in patients with untreated mild-to-moderate hypertension. *Hypertension* 2003; **41**: 286–8.

33. Pierdomenico SD, Costantini F, Bucci A, De Cesare D, Cuccurullo F, Mezzetti A. Low-density lipoprotein oxidation and vitamins E and C in sustained and white-coat hypertension. *Hypertension* 1998; **31**: 621–6.
34. Caner M, Karter Y, Uzun H *et al*. Oxidative stress in humans in sustained and white coat hypertension. *Int. J. Clin. Pract*. 2006; **60**: 1565–71.
35. Uzun H, Karter Y, Aydin S *et al*. Oxidative stress in white coat hypertension; role of paraoxonase. *J. Hum. Hypertens*. 2004; **18**: 523–8.
36. Morrow DA, Rifai N, Antman EM *et al*. C-reactive protein is a potent predictor of mortality independently of and in combination with Troponin T in acute coronary syndromes: A TIMI 11A substudy. Thrombolysis in myocardial infarction. *J. Am. Coll. Cardiol*. 1998; **31**: 1460–5.
37. Koenig W, Meisinger C, Baumert J *et al*. Systemic low-grade inflammation and risk of coronary heart disease: Results from the MONICA/KORA Augsburg cohort studies. *Gesundheitswesen* 2005; **67** (Suppl. 1): S62–7.
38. Albert CM, Ma J, Rifai N *et al*. Prospective study of C-reactive protein, homocysteine, and plasma lipid levels as predictors of sudden cardiac death. *Circulation* 2002; **105**: 2595–9.
39. Devaraj S, Venugopal S, Jialal I. Native pentameric C-reactive protein displays more potent pro-atherogenic activities in human aortic endothelial cells than modified C-reactive protein. *Atherosclerosis* 2006; **184**: 48–52.
40. Wang CH, Li SH, Weisel RD *et al*. C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation* 2003; **107**: 1783–90.
41. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jiala I. Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation* 2002; **106**: 1439–41.
42. Sesso HD, Buring JE, Rifai N, Blake GJ, Gaziano JM, Ridker PM. C-reactive protein and the risk of developing hypertension. *JAMA* 2003; **290**: 2945–51.
43. Lakoski SG, Herrington DM, Siscovick DM, Hulley SB. C-reactive protein concentration and incident hypertension in young adults: The CARDIA study. *Arch. Intern. Med*. 2006; **166**: 345–9.
44. Conen D, Dieterle T, Utech K, Rimner M, Martina B. C-reactive protein and B-type natriuretic peptides in never-treated white coat hypertensives. *Hypertens. Res*. 2006; **29**: 411–15.
45. Schillaci G, Pirro M, Gemelli F *et al*. Increased C-reactive protein concentrations in never-treated hypertension: The role of systolic and pulse pressures. *J. Hypertens*. 2003; **21**: 1841–6.
46. Erdogan D, Caliskan M, Gullu H *et al*. Aortic elastic properties and left ventricular diastolic function in white-coat hypertensive individuals. *Blood Press. Monit*. 2006; **11**: 191–8.
47. Devaraj S, Yan Xu D, Jialal I. C-reactive protein increases plasminogen activator inhibitor-1 expression and activity in human aortic endothelial cells. Implications for the metabolic syndrome and atherothrombosis. *Circulation* 2003; **107**: 398–404.
48. Blann AD, Taberner DA. A reliable marker of endothelial cell dysfunction: Does it exist? *Br. J. Haematol*. 1995; **90**: 244–8.
49. Srikumar N, Brown NJ, Hopkins PN *et al*. PAI-1 in human hypertension: Relation to hypertensive groups. *Am. J. Hypertens*. 2002; **15**: 683–90.
50. Poli KA, Tofler GH, Larson MG *et al*. Association of blood pressure with fibrinolytic potential in the Framingham offspring population. *Circulation* 2000; **101**: 264–9.
51. Wang TJ, Gona P, Larson MG *et al*. Multiple biomarkers and the risk of incident hypertension. *Hypertension* 2007; **49**: 432–8.
52. Makris T, Stavroulakis G, Papadopoulos D *et al*. White coat hypertension and haemostatic/fibrinolytic balance disorders. *Eur. Cytokine Net*. 2006; **17**: 137–41.
53. Coban E, Ozdogan M. The plasma levels of plasminogen activator inhibitor-1 in subjects with white coat hypertension. *Int. J. Clin. Pract*. 2004; **58**: 541–4.
54. Martinez MA, Garcia-Puig J, Martin JC *et al*. Frequency and determinants of white coat hypertension in mild to moderate hypertension. A primary care-based study. *Am. J. Hypertens*. 1999; **12**: 251–9.
55. Pierdemenico SD, Lapenna D, Guglielmi MD *et al*. Target organ status and serum lipids in patients with white coat hypertension. *Hypertension* 1999; **26**: 801.
56. Palatini P, Mormino P, Santonastaso M *et al*. Target-organ damage in stage 1 hypertensive subjects with white coat and sustained hypertension: Results from the HARVEST study. *Hypertension* 1998; **31**: 57–63.
57. Høegholm A, Bang LE, Kristensen KS, Nielsen JW, Holm J. Microalbuminuria in 411 untreated individuals with established hypertension, white coat hypertension and normotension. *Hypertension* 1994; **24**: 101–5.
58. Bang LE, Holm J, Svendsen TL. Retinol-binding protein and transferrin in urine. New markers of renal function in essential hypertension and white coat hypertension? *Am. J. Hypertens*. 1996; **9**: 1024–8.
59. Björklund K, Lind L, Vessby B, Andren B, Lithell H. Different metabolic predictors of white-coat and sustained hypertension over a 20-year follow-up period. A population-based study of elderly men. *Circulation* 2002; **106**: 63–8.

Chapter 3

Methodology

Objective

This purpose of this chapter is to comprehensively describe the methods that form the basis of the following chapters which include published and unpublished work.

3.1 Sample size

A total sample size of 64, assuming the proportion of normotensives who progress to sustained hypertension in three years is 10% , was 80% powered to pick up a 30% difference in the WCHT group progressing to sustained hypertension, one sided, α=0.05. To take into account 20% dropout a total sample of 77 was required.

This sample size was powered to pick up a minimum difference of one metre per second in the cross-sectional analysis of central pulse wave velocity (PWVc), with standard deviation of one metre per second, between the normotensive and WCHT groups. This sample size was also powered to pick up a ratio of 1.25 in the two-hour plasma glucose post glucose load (2hPG) between the normotensive and WCHT groups. Significance level α=0.05, two-sided comparisons.

3.2 Participants

Ethics approval for the study was obtained from Monash Health and Monash University human research ethics committees.

Participants were included if they were aged 18 to 80 years. Participants were excluded if they were smokers, had known diabetes, were taking anti-hypertensive medication, or had existing liver, renal, autonomic or cardiovascular disease or any malignancy. Hypertensive participants were recruited from the Department of Vascular Sciences at the time of referred ambulatory blood pressure (BP) measurement (ABPM), before antihypertensive treatment

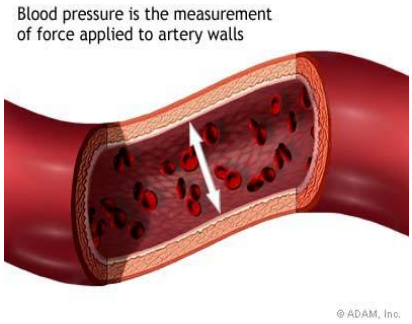
was commenced. WCHT participants were recruited by 1) screening all new patients presenting to the Department of Vascular Sciences for ABPM. 2) screening the ABPM records of the Department of Vascular Sciences, Monash Health and from a private cardiology group, both services located in Dandenong, Victoria, for patients who had an ABPM reported as WCHT and 3) by advertisements.

Figure 3.1 shows a pamphlet that was mailed out to patients who had had a previous ABPM reported as WCHT at the Department of Vascular Sciences, Monash Health or the private cardiology group.

An advertisement was placed in local newspapers, Dandenong hospital hallways and the Monash University staff newsletter asking for participants who have been told by general practitioner that they have WCHT and for people who had normal BPs taken at their general practitioner's room. All normotensive participants were recruited by advertisement. A Participants Information sheet was sent out and the person was rung a week later and asked if they were happy to participate. The participant was then given a visit date and asked to fast (except water) from 9pm the night before their appointment. Patients presented at 9am to the Department of Vascular Sciences.

The order of testing after arrival was sitting observed and unobserved blood pressure, autonomic function testing, artery stiffness testing, fasting blood sampling, oral glucose tolerance test, after which the ABPM was applied.

WHITE COAT HYPERTENSION STUDY
(Blood Pressure Study)



White Coat Hypertension, has been considered for many years by doctors to be not important. This has meant that if you are labeled as having White Coat Hypertension then you are considered at low risk for developing problems that are associated with hypertension, such as stroke or heart attack. Some researchers think that if you have White Coat Hypertension you will eventually develop hypertension.

We want to see if we can predict which people with White Coat Hypertension develop hypertension. To be able to answer this question we need your participation over a few years.

BENEFITS OF BEING INVOLVED IN THIS STUDY.

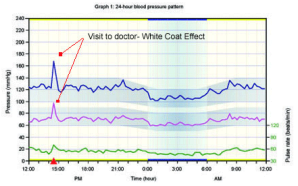
- Yearly assessment of blood pressure status. (24-hour blood pressure monitoring)

- Yearly assessment of `stiffness` of arteries. A good indicator if blood pressure is causing any organ damage.

At the first year visit only you will have:

- Cholesterol testing-good and bad cholesterol checked. Triglyceride testing-another blood fat.
- Oral Glucose Tolerance Test-tests diabetic status. This means whether you have diabetes or are have pre-diabetes.
- Autonomic Nervous System function testing

All results can be given to your doctor.



Twenty four hour recording of blood pressure showing a normal blood pressure pattern, except for one raised blood pressure recording during a visit to the doctors. To be eligible for this study you must -not be on any blood pressure medication -not have diabetes
-not have any cardiovascular illness
-not have any other major illnesses such as cancer, kidney or liver disease.

I hope you will really consider being involved in this study so that we can help to determine whether White Coat Hypertension should be considered a minor condition or not.

If you want further information or want to book to participate in the study please contact Cathy Martin

[redacted]

Or Email

[redacted].

Figure 3.1. Pamphlet sent out to potential WCHT participants

3.3 Blood pressure measurement

The 2013 ESH/ESC guidelines for the management of arterial hypertension recommend "To take at least two BP measurements, in the sitting position, spaced 1–2 min apart, and additional measurements if the first two are quite different". The guidelines recommend that the BP should be taken after resting in an isolated room for 3-5 minutes.[1] ABPM is not currently subsidised by Medicare in Australia and in the United States of America ABPM is subsidised only if WCHT is suspected. Hence the diagnosis of hypertension is generally based on clinic BP.

ABPM has been shown to better predict cardiovascular events[2, 3] or target organ damage[4] than clinic BP. Home BP also better predicts cardiovascular events than clinic BP[5] but as with clinic BP, night BP and the morning rate of BP rise cannot be assessed.

Methods for the diagnosis of hypertension by clinic BP have been proposed using automatic unobserved BP, particularly in an attempt to eliminate the white-coat response.[6] Myers et al (2010) proposed an algorithm assessing clinic BP using automated BP measuring instead of using manual clinic BP.[7] The algorithm proposed three pathways to follow depending on the unobserved automated BP (UABP). For an UABP ≥ 140/90 mmHg a diagnosis of hypertension is made. For an UABP 130-139/80-89 mmHg ABPM or home BP monitoring is recommended and for an UABP < 130/80 mmHg the recommendation is the patient goes down the "continue to follow" pathway.

This algorithm does not propose clinic observed BP to be taken, which means that a patient with WCHT cannot be identified. It is important to identify patients with WCHT as several recent studies have suggested that patients with WCHT are at increased risk of developing

diabetes and sustained hypertension. These patients require close monitoring and should be discriminated from normotensives.

Observed clinic BP tends to overestimate a patient's true BP whereas UABP potentially can underestimate the true BP as it reflects resting conditions and not the BP of active daily life. UABP provides a better baseline BP in the clinic setting than observed clinic BP. How UABP relates to WCHT will be dealt with in this thesis. ABPM although inconvenient is more likely to reflect a patient's typical daily activity.

Algorithms for the detection of hypertension should incorporate both observed clinic BP and UABP, which will enable patients to be categorized into one of the four quadrants of BP. A letter was published in the journal "Hypertension" regarding our concerns about the algorithm and the statistical method used in the paper (See appendix 1).[8]

3.3.1 Blood pressure devices

Observed and unobserved BPs were taken using an automated oscillometric blood pressure device (Task Force monitor®, CNSystems**,** Graz, Austria). ABPM was performed using a portable device (MeditechCardiotens, Meditech Ltd, Hungary).

3.3.2 Recruited participants-definition of BP category

In this study the definition of BP category was determined as per the 2007 Guideline**s** for the Management of Arterial Hypertension of the European Society of Hypertension, which were current at the time of study commencement and data analysis.[9] The definition of BP category remains unchanged in the new 2013 ESH/ESC guidelines for the management of arterial hypertension.[1] Normotension was defined as both clinic systolic BP < 140 mmHg and

diastolic < 90 mmHg, and both day ABPM systolic < 135 mmHg and diastolic < 85 mmHg. WCHT was defined as either general practitioner clinic systolic BP ≥ 140 mmHg or diastolic ≥ 90 mmHg, and both day ABPM systolic < 135 mmHg and diastolic < 85 mmHg. Sustained hypertension was defined as either clinic systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg and either day ABPM systolic ≥ 135 mmHg or diastolic ≥ 85 mmHg. In this thesis sustained hypertensive participants refer to untreated hypertensive subjects.

WCHT was also determined by two confirmatory ABPM recordings as previous studies have shown that approximately 50% of WCHT on a second ABPM were found to be hypertensive.[10, 11]

Four WCHT subjects did not have a second confirmatory ABPM and were not included in published results. One subject that met the criteria for WCHT based on 24h day ABPM but had a night BP of 140/87 mmHg was classified as hypertensive. One WCHT subject was also removed from analysis as it was found out after recruitment that the participant had undisclosed IgA nephropathy. One normotensive participant was also removed from further analysis after recruitment as it was later revealed the participant had known elevated glucose and was undergoing investigation for diabetes.

3.4 Morning blood pressure surge

Four measures were used to assess the morning BP surge. Figure 3.2 displays the schematic example of BP definitions used to define two measures of the morning BP surge.
"Morning BP" was defined as the average of the first four BPs post waking and the "BP before waking" was the average of the last four BPs before waking.

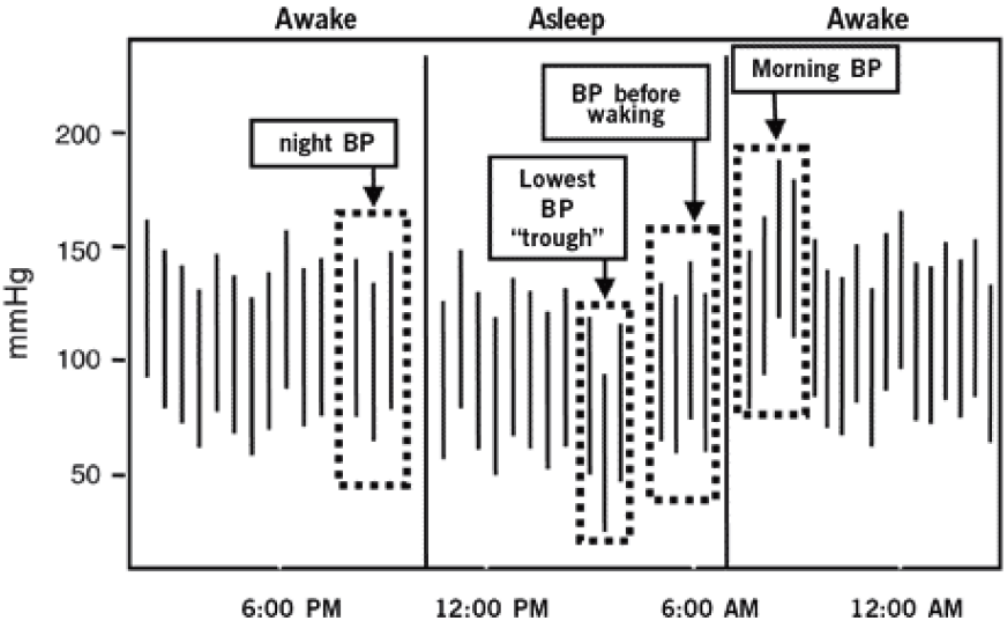


Figure 3.2. Definition of components of the morning blood pressure surge (Kario et al. 2003).

Sleep-trough surge = Morning BP – BP before waking

Pre-awake surge= BP before waking - lowest sleep BP, where the lowest sleep BP is the average of three BP reading centred by lowest night BP (”trough”) reading.

Figure 3.3, along with equations I and 2 describes the methods as per Head et al. (2005).[12] Briefly six parameters are determined from fitting ABPM data using double logistic regression analysis.

$$\hat{y} = P1 + \frac{P2}{1+e^{P3(P4-x)}} + \frac{P2}{1+e^{P5(P6-x)}} \qquad \text{(Equation 1)}$$

The non-symmetrical double logistic equation (Equation 1) is used to model the 24h blood pressure profile. Where P1 is the nighttime plateau, P2 is the difference between the day and night plateau, P3 is the rate of transition from day to night, P5 is the rate of transition

between night and day, P4 and P6 are the mid times for these transitions and x represents time. The morning BP surge, termed the 'rate of rise' by Head et al (2005) is calculated as (P2 x P5)/4.

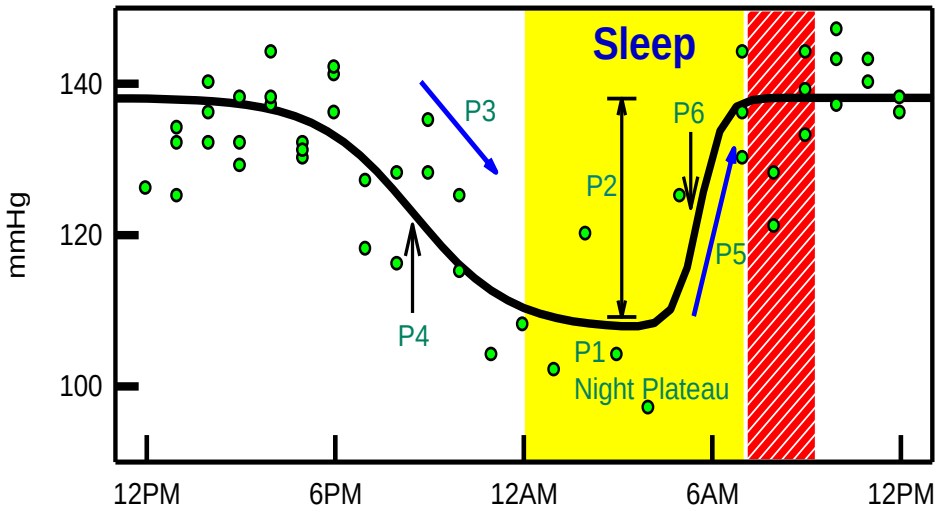


Figure 3.3. Non-symmetrical double logistic analysis of ABPM recordings. (Figure courtesy of Professor Geoffrey Head)

A Power function was derived by Head (2010)[13], to take into account the rate of rise as well as the magnitude of the rise. The Power function (Equation 2) is equal to the first derivative of Equation 1, with respect to time (x), multiplied by the amplitude (the day-night BP difference).

$$Power\ function = \frac{P2 \times P2 \times P5 \times e^{P5(P6-x)}}{(1+e^{P5(P6-x)})^2} \qquad \text{(Equation 2)}$$

ABPM 2008 software was used to analyse twenty four hour ABPM. A copy of the software was given for use by Professor Geoffrey Head from the Baker IDI Heart and Diabetes Institute.

The published paper in Chapter 5 describes the results of the analysis of the morning BP surge in WCHT. The submitted paper in Chapter 6 expands on the findings from Chapter 5.

3.5 The Metabolic Syndrome and glucose status definitions.

The metabolic syndrome was defined according to the joint statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.[14] Glucose status was defined as per World Health Organisation "Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications" (1999).[15]

3.6 Hypertension risk calculation.

The hypertension risk score developed from the Framingham Heart study was used to determine the risk of near-term hypertension in each subject.[16]

3.7 Biomarkers

Figure 3.4 is a schematic representation of interrelationships between BP and vascular pathophysiological markers: oxidative stress, inflammation, endothelial dysfunction and arterial stiffness, which were first shown in the introduction (figure 1.2). The chosen methodologies for each marker have been added to the figure in blue. Each marker and its chosen methodology are discussed.

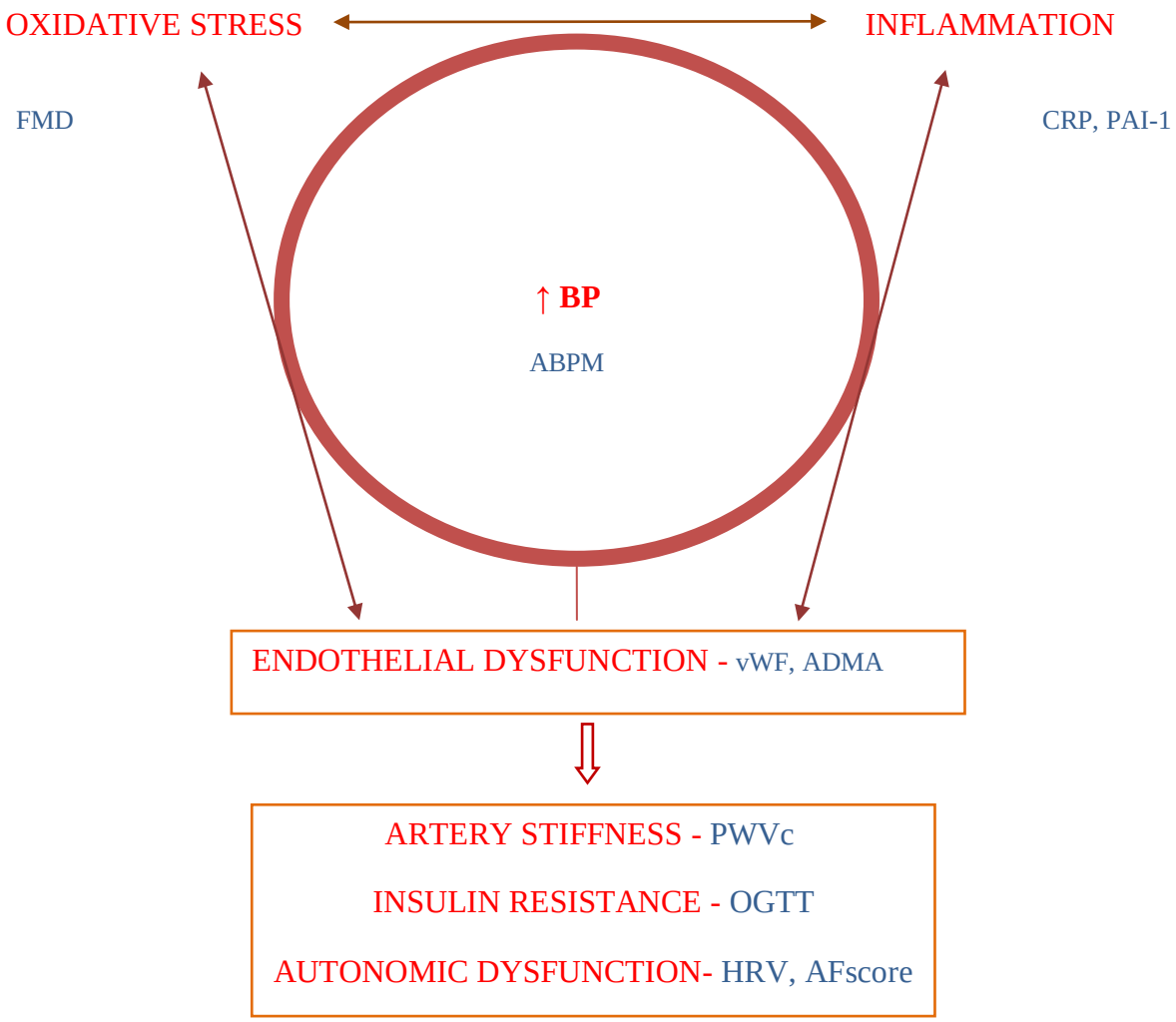


Figure 3.4. Schematic representation of interrelationships between BP and vascular pathophysiological markers: oxidative stress, inflammation, endothelial dysfunction and arterial stiffness (shown in red) and chosen methods of assessment (shown in blue).

3.7.1 Artery stiffness

The gold standard for measuring artery stiffness is central pulse wave velocity (PWVc).[17] PWVc has good repeatability[18] and predicts incident cardiovascular events in a range of disease populations.[19-21] PWVc has several important confounders that must be adjusted for

in any analysis. Arterial stiffness increases with age and analysis of PWVc has shown that the association is curvilinear.[22] Although healthy artery walls are elastic an increased pressure load on the artery wall can cause the artery to stiffen, hence analysis of PWVc must adjust for mean arterial pressure (MAP).[23]

3.7.1.1 Assessment of PWVc

PWVc measurements were performed by the same two operators (CM and SC) in all participants, in a quiet air-conditioned room after 10 minutes of rest and with the subject lying supine. Continuous pulse pressure wave signals were recorded with hand-held tonometers (Millar Mikro-tip, SPT-301; Millar Instruments, Houston, Texas, USA) positioned at the base of the right common carotid artery and over the right common femoral artery. Transit distance was defined as the measured distance from the sternal notch to femoral artery minus distance from sternal notch to carotid. At the time of commencement of this study the method used for estimating the transit distance was one of three acceptable methods and the same method has been applied for consistency.[24] More recently magnetic resonant imaging has shown that the most accurate determination of transit distance is 80% of the direct measure between the carotid and femoral sites, which is the current recommended method.[25] The start of systole was defined by the local maximum of the first derivative of the pressure signal. Mean transit time (Δt) between the feet of simultaneously recorded waves was determined from 10 consecutive cardiac cycles**.** MAP was estimated from an oscillometric BP measurement taken immediately post recording of the arterial waveforms.

3.7.1.2 PWVc repeatability

Twenty-four participants recruited for this study underwent repeat PWVc at three months.

Analysis of variance (ANOVA) was used to calculate the within-subject standard deviation. The mean difference between the two measures of PWVc was -0.33 m/sec, with the 95% coefficient of precision estimated at ± 1.19 m/sec. Figure 3.5 shows the Bland-Altman plot of PWVc difference versus PWVc average. There was no apparent drift with increasing PWVc.

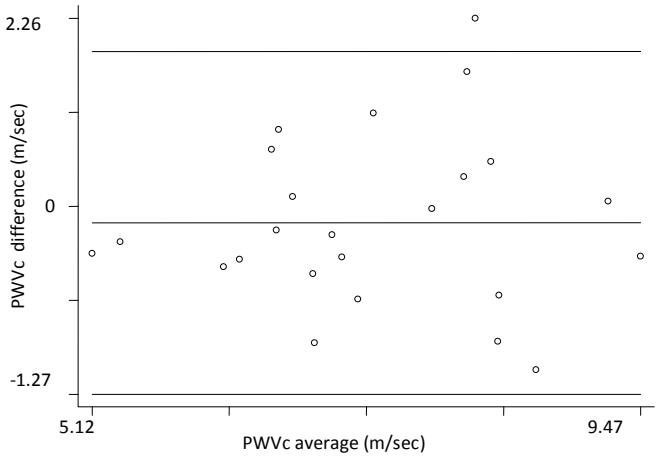


Figure 3.5. Bland-Altman plot of PWVc repeatability.

3.7.2 Autonomic function

TaskForce monitor

Autonomic function testing was done using the TaskForce monitor (CN Systems, Graz, Austria), which allowed continuous real time beat-to-beat heart rate and BP recording. The patient wore electrocardiographic monitoring leads, an arm BP cuff and a finger BP monitoring device.

Ewing's bedside autonomic function tests

For almost 40 years doctors and researchers have used different combinations of Ewing's autonomic function tests[26] to assess autonomic dysfunction. Ewing's autonomic function

tests are comprised of five tests: heart rate response to standing, heart rate response to deep breathing, heart rate response to the Valsalva manouevre, BP response to standing, and BP response to a sustained hand grip.

Heart rate response to standing

Ewing described the methodology for this test as "The subject lies quietly on a couch and then stands up unaided". The maximum heart rate response occurs at around, the 15th beat, followed by a bradycardia around the 30th beat. Ewing termed this the 30:15 ratio, which is the ratio of the longest R-R interval around the 30th beat divided by the ratio of the shortest R-R interval around the 15th beat. The ratio of the maximum and minimum heart rate in the first 30 seconds from standing has also been proposed to take into account individual differences.[27]

Figure 3.6 shows the heart rate response to standing in a normal subject with the 15th, 30th, maximum and minimum beats identified.

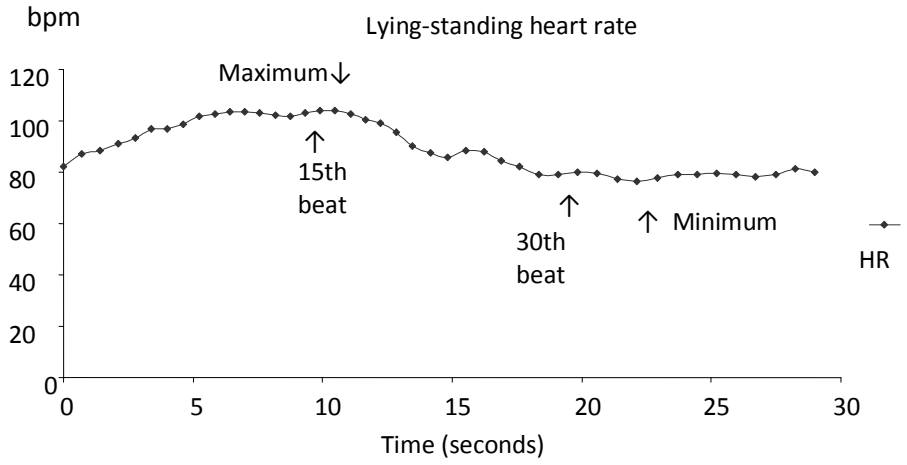


Figure 3.6. Heart rate response to standing in a normal control subject.

For the purpose of this study both ratios were measured. To ensure consistency the 30:15 ratio was defined as

$$LS_{ratio} = \frac{MAXR \quad [30^{th} beat \pm 2beats]}{MINRR \quad [15^{th} beat \pm 2beats]}$$

Where MAXRR=maximum RR interval and MINRR=minimum RR interval.

Repeatability of heart rate response to standing

The mean difference in repeated lying-standing heart rate ratio obtained in 13 participants, at three months, was -0.01. The 95% confidence interval (CI) of the mean difference was -0.1 to 0.08. The 95% limits of agreement were -0.30 to 0.29 and the Bland-Altman plot is shown (See figure 3.7).

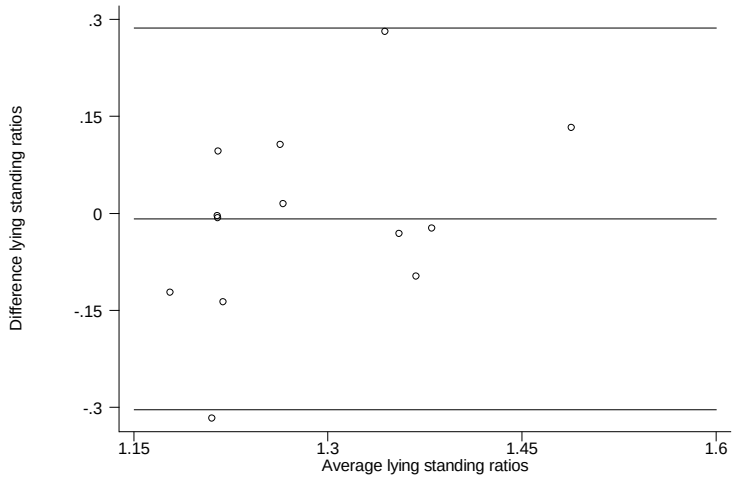


Figure 3.7. Bland-Altman plot of lying-standing heart rate ratio repeatability.

Heart rate response to deep breathing

Ewing described the methodology for this test as "The patient sits quietly and breathes deeply at six breaths a minute (five seconds in and five seconds out) for one minute". The mean of the difference between the maximum and minimum heart rates for the six measured cycles is calculated. The result can also be expressed as the average of the maximum/minimum ratio, which is known as the E:I ratio. Alternative methods to assess the

heart rate response to deep breathing include the heart rate response to a single deep breath,[28] which has poorer repeatability than the six breaths.[29] The addition of a single deep breath, along with the average of six deep breaths was added to a battery of autonomic function tests, which predicted increased mortality in type 2 diabetes .[30]

In this study the heart rate response to deep breathing was assessed by a single deep breath and for one minute at six breaths per minute. Only the response to six breaths in participants with normal fasting plasma glucose was published (See Chapter 4). Table displays results of a single deep breath and for six breaths for the entire study population.

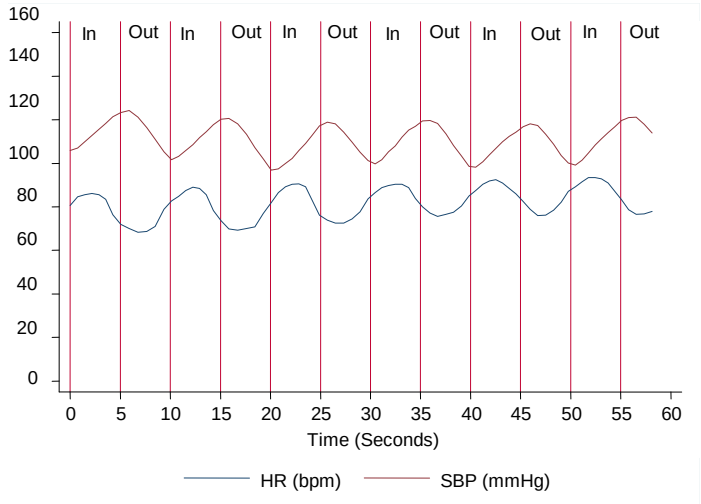


Figure 3.8. Heart rate and blood pressure response to controlled respiration in a normal control subject (In: participant breathed in over 5 seconds, Out: participant breathed out over 5 seconds).

To assess heart rate response to six breaths a metronome was used, with the instructor (CM) counting out aloud "in, 2, 3, 4, 5, 6, out, 2, 3, 4, 5, 6 …" for one minute. Figure 3.8 shows the heart rate and BP response to one minute of deep breathing in a normal subject.

Repeatability of controlled breathing

The mean difference in heart rate (HR) during repeated controlled breathing obtained in 14 participants, at three months, was -0.09 beats/min. The 95% CI of the mean difference was -2.8 to 2.6 beats/min. The 95% limits of agreement were -9.4 to 9.2 beats/min and the Bland-Altman plot is shown (See Figure 3.9).

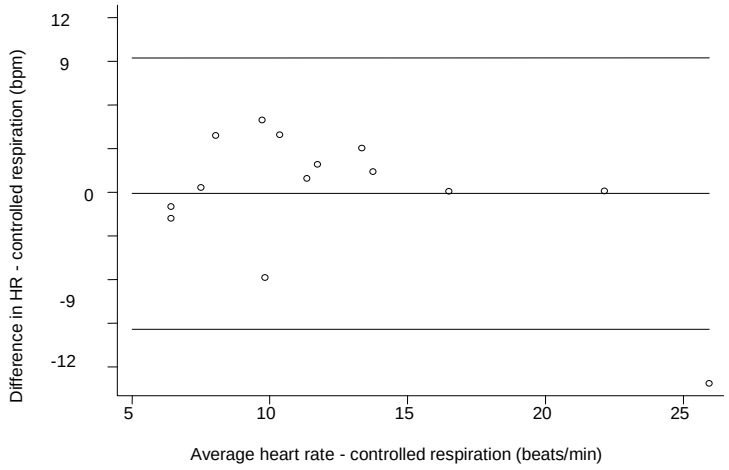


Figure 3.9. Bland-Altman plot of controlled respiration repeatability.

Heart rate response to Valsalva Manoeuvre

The Valsalva test was performed as per Ewing[31] "The test is performed by the patient blowing into a mouthpiece connected to an aneroid manometer or a modified sphygmomanometer and holding it at a pressure of 40 mm Hg for 15 seconds while a continuous electrocardiogram is recorded. The manoeuvre is performed three times with one minute intervals between". A small air leak was induced to prevent the subject generating pressure from the buccal muscles, ensuring that the pressure generated was from contraction of the expiratory muscles.

The Valsalva manoeuvre is divided into four phases. Phase I: The increase in intrathoracic pressure compresses the aorta causing a rise in BP, which induces a reflex bradycardia. Phase II: The prolonged increase in intrathoracic pressure causes a reduction in venous return, lowering BP. The baroreceptor reflex is activated, causing vasoconstriction and tachycardia, raising BP towards normal. Phase III: The strain is abruptly ceased; intrathoracic pressure suddenly drops causing blood to pool in the pulmonary vessels, which results in a further BP drop and associated further rise in heart. Phase IV: Venous return is restored and due to the raised systemic vascular resistance induced in phase II, BP overshoots inducing a baroreceptor mediated bradycardia. Ewing termed the result the Valsalva Ratio, which is defined as the ratio of longest R-R interval after the manoeuvre to the shortest R-R interval during the manoeuvre.

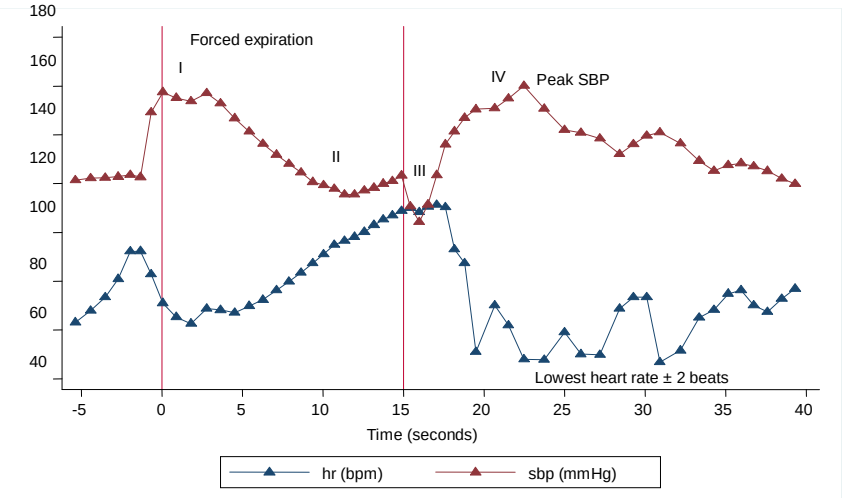


Figure 3.10. Heart rate and BP response to the Valsalva manouevre (normal control subject).

O'Brien et al. (1986) proposed only one Valsalva is required.[28] In this study three Valsalvas were obtained and the average taken.

To ensure consistency of measurement the minimum heart rate post Valsalva was determined at ± 2 beats from the peak systolic BP during the BP overshoot (phase IV) (See figure 3.10).

$$Valsalva_{ratio} = \frac{MAXRR \ \ [phaseIV_{valsalva}]}{MINRR \ \ [phaseII_{valsalva}]}$$

Where MAXRR=maximum RR interval and MINRR=minimum RR interval.

Repeatability of Valsalva manoeuvre

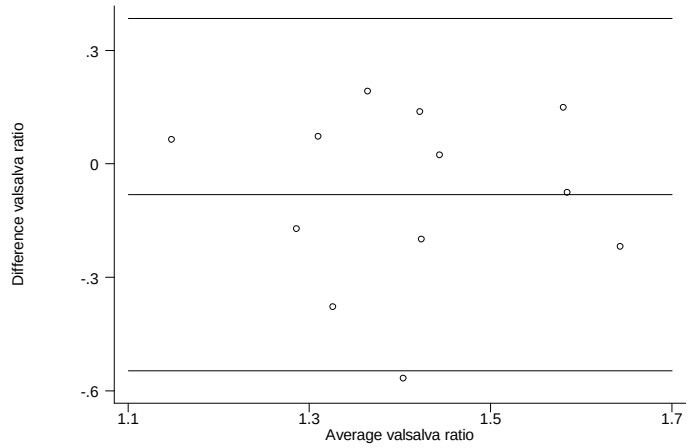


Figure 3.11. Bland-Altman plot of valsalva repeatability.

The mean difference in Valsalva ratio obtained in 12 participants, at three months, was -0.08. The 95% CI of the mean difference was -0.23 to 0.07. The 95% limits of agreement were -0.55 to 0.39 and the Bland-Altman plot is shown (Fig 3.11).

Normal values of autonomic function

Ewing reported normal values for the three heart rate response tests for the total population as determined the responses were independent of age[26] whereas others found an age relationship.[28, 32]

The following table displays age values obtained from this study and the repeatability. The normal age values were obtained after exclusion of participants that had a 2hPG ≥ 7.8 mmol/l or who said they regularly drank ≥ three standard drinks of alcohol per day.

Table 3.1: Values of heart rate response to autonomic function tests by age categorisation

	30:15 ratio			Deep breathing (bpm)			Valsalva ratio		
Age (years)	40-49	50-59	60-69	40-49	50-59	60-69	40-49	50-59	60-69
Number	10	33	19	7	21	11	10	32	21
Median	1.37	1.26	1.14	15.1	14.4	11.7	1.73	1.43	1.30
Range	1.06-1.85	1.05-1.70	0.95-1.32	7.0-33.7	4.8-27.9	4.9-20.8	1.21-2.14	1.12-2.22	1.07-1.79

bpm: beats per minute, N: Number, IQR: Inter-quartile range.

BP response to standing

Oscillatory BP was taken whilst the participant was supine, after two minutes of rest. The participant was asked to stand, in a timely fashion, in less than 5 seconds, without assistance. The participant was instructed to stand still, no talking or movement until the BP was finished. BP was taken one minute post standing.

Repeatability of BP response to standing

The mean difference in systolic BP to standing in 12 participants was 3 mmHg. The 95% CI of the mean difference was 0 to 6 mmHg. The 95% limits of agreement were -13 to 7 mmHg and the Bland-Altman plot is shown (See Figure 3.12).

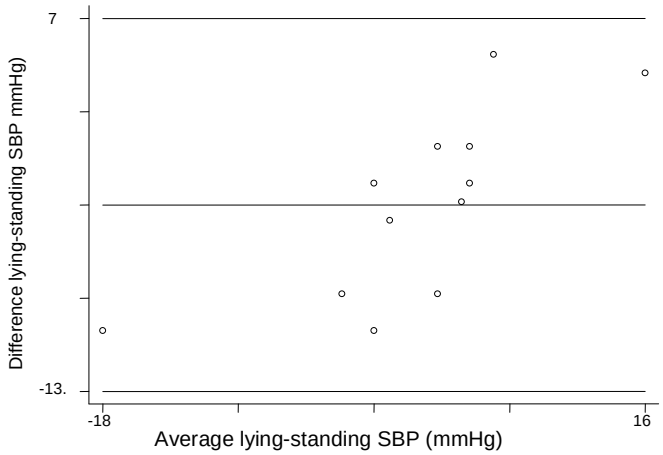


Figure 3.12. Bland-Altman plot of lying-standing SBP repeatability.

The mean difference in diastolic BP to standing in 12 participants, at three months, was 0 mmHg. The 95% CI of the mean difference was -3 to 2 mmHg. The 95% limits of agreement were -8 to 8 mmHg and the Bland-Altman plot is shown (See Figure 3.13).

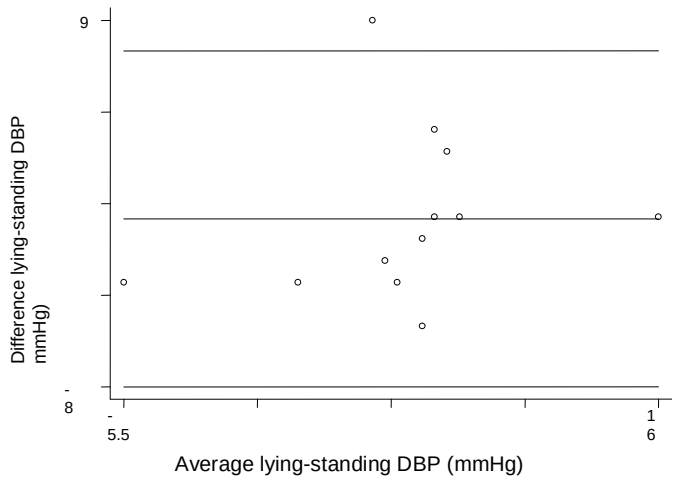


Figure 3.13. Bland-Altman plot lying-standing DBP repeatability

BP response to sustained hand grip

The participant was asked to use their dominant hand to grip a slightly inflated BP cuff. They were asked to squeeze the BP cuff as hard as possible. The maximum rise in pressure mmHg was noted. The participant was rested until heart rate and BP returned to baseline values. The participant was then asked to grip the inflated BP and apply pressure so that the mercury in the manometer reached one-third of the noted grip pressure. They were asked to hold the grip at this pressure for five minutes. Minutely oscillatory BPs were recorded. BP was taken on the non-dominant arm. The BP response was recorded as the difference between the maximum diastolic BP during the grip and the diastolic BP pre grip.

Repeatability of BP response to sustained hand grip test

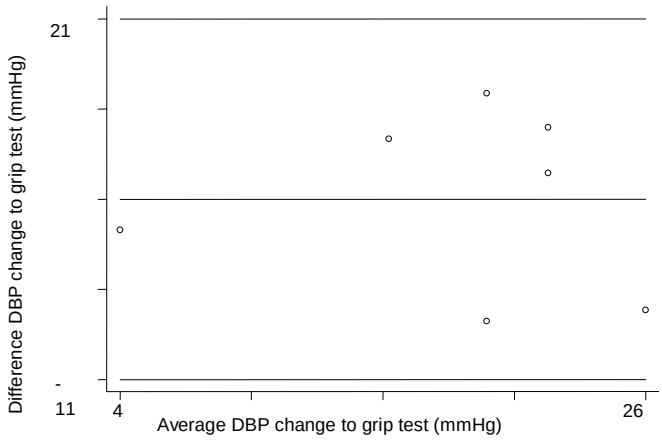


Figure 3.14. Bland-Altman plot of sustained hand-grip repeatability.

The mean difference in sustained grip diastolic BP in eight participants, at three months, was 0 mmHg. The 95% CI of the mean difference was -3 to 2 mmHg. The 95% limits of agreement were -8 to 8 mmHg and the Bland-Altman plot is shown (See Figure 3.14).

Ewing autonomic function score

Ewing developed a scoring system to grade the results of the five tests.[31] A score of zero is given for a normal result, half a score for a borderline result and a score of one for an abnormal result for each test. The individual scores are added, with a potential range for the total score of zero to five. The total score results were published for subjects with normal fasting glucose (chapter 4).

Mental arithmetic stress test

After resting for two minutes an oscillometric BP was taken. Subjects were then instructed to subtract out loud the value 13 from the value 4,300. Subjects were instructed to either start again or continue from the current value if they felt they had made a mistake and that no assistance would be given by the examiner. If a subject verbalised an inability to do this subtraction then they were offered the subtraction of 13 from 430. An oscillometric BP measurement was taken at one minute. The difference in diastolic BP at one minute, from the resting diastolic BP was calculated.

Repeatability of the mental stress test

The mean difference in diastolic BP to the mental stress test in 7 participants, at three months, was -1 mmHg. The 95% CI of the mean difference was -9 to 8 mmHg. The 95% limits of agreement were -19 to 18 mmHg and the Bland-Altman plot is shown (See Figure 3.15).

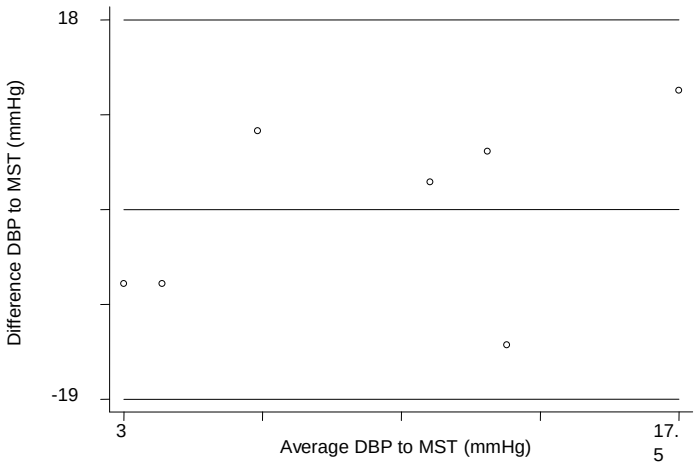


Figure 3.15. **Figure 3.15**. Bland-Altman plot of mental stress test repeatability.

Other methods assessing heart rate variability

Other methods to assess heart rate variability include Time Domain analysis and Frequency Domain analysis. Time domain analysis generally requires 24h recording whereas Frequency Domain analysis can be assessed using two to five minutes of heart rate recordings.

Frequency Domain analysis

Heart rate can be decomposed into different cycles. The heart rate cycle related to respiration (sinus arrhythmia), usually > 9 cycles per minute (0.15-0.4 Hz) is considered a high frequency (HF) cycle. Heart rate from 2.4 to 9 cycles per minute (0.04-0.15 Hz) is low frequency (LF). Ultra low (<0.03 Hz) and very low (0.03-04 Hz) frequency cycles can only be measured when heart rate is monitored for 24 hours. To assess autonomic function the ratio of LF/HF is used as a measure of autonomic function (Task Force of The European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996). The HF cycle is considered a measure of parasympathetic function. Many researchers have used the LF cycle as a measure of sympathetic function, hence the LF/HF ratio is used as a measure of sympathetic /parasympathetic balance but this use is controversial.[33-35]

Respiratory rate confounds the LF/HF ratio. If a person breaths at < 9 breaths per minute the result is respiratory cycling in the low frequency range. As the testing is done with the patient resting it is possible the respiratory rate could fall below this level.

The TaskForce® monitor (CN Systems, Graz, Austria), assesses real time heart rate variability using an autoregressive algorithm. Recordings were taken for ten minutes in the supine position and for two minutes with the participant breathing at a rate of 15 breaths per minute with the aid of a metronome. Figure 3.12 shows the effect on LF and HF when the heart rate is cycled at 15 breaths per minute Controlled respiration (15 breaths per minute) caused the LF to drop from 27.6% during uncontrolled respiration (the participant controlled own respiratory rate) to 16.6% , whereas HF rose from 72.4% to 83.4%.

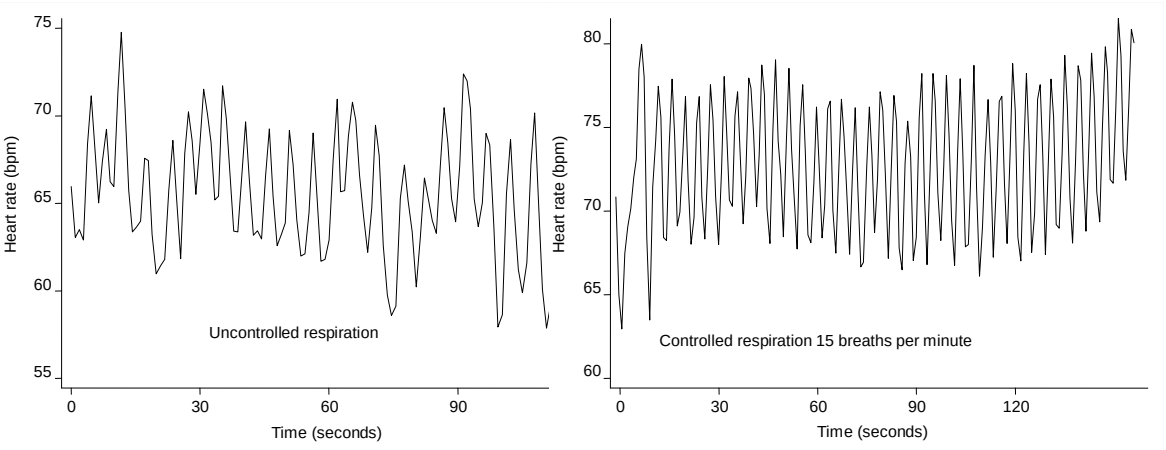


Figure 3.16. The effects of controlled respiration on heart rate cycling (normal control subject).

Autonomic function testing protocol

Autonomic function testing was done under standardised conditions. All participants were tested, in a quiet room, at a comfortable temperature around 21°C. Participants were all tested

in the morning after fasting (except water) from 9pm the night before. Participants on medication that are known to affect autonomic function were excluded from analysis. Subjects lay supine for five minutes before testing began.

The order of autonomic function testing (TaskForce® monitor recording) was 1) five minutes lying supine in a quiet room, alone, before 10 minutes electrocardiography (HRV) recording, 2) two minutes electrocardiography recording (HRV), with the participant breathing at 15 breaths per minute with the aid of a metronome, 3) lying-standing heart rate and BP, 4) sitting-deep breathing for one minute with one minute practice prior to recording, 5) sitting-three Valsalva manouevres, 6) mental stress test and 7) sustained hand grip test.

3.7.3 Insulin resistance/glucose status

The gold standard for measurement of insulin resistance is the hyperinsulinaemic euglycemic clamp test,[36] which is invasive and labour intensive. The oral glucose tolerance test (OGTT) is considered an acceptable alternative for research studies.[37] The reproducibility of the OGTT is a limitation[38] but the OGTT reflects physiological conditions rather than those under laboratory conditions.

Derived indices from OGTT include measures based on fasting plasma insulin and glucose. The most commonly used are the Homeostasis model assessment (HOMA)[39] and quantitative insulin sensitivity check index (QUICKI)[40] tests, which have been validated against the gold standard.

$$\text{HOMA} = \frac{\text{Fasting plasma glucose} * \text{Fasting plasma insulin}}{22.5}$$

The QUICKI formula attempts to normalize the right skewed nature of plasma insulin and glucose levels by taking their logarithms. Lower levels reflect insulin resistance.

$$\text{QUICKI} = \frac{1}{\log(\text{Fasting insulin } \mu\text{U/mL}) + \log(\text{Fasting glucose mg/dL})}$$

Both formulas are functions of fasting plasma glucose and insulin hence can be considered surrogate markers of hepatic insulin resistance.

The classification of insulin resistance with tests based on the oral glucose administration may allow earlier identification of insulin resistance than indices based on the fasting measures.[42] Measures that utilize glucose and insulin plasma levels obtained immediately prior to and two to three hours post OGTT have been used to derive estimates of both hepatic and peripheral insulin resistance, these include area-under-the-curve insulin and glucose and mathematical models that include both of these measures.[37] The 2hPG can be considered a measure that reflects peripheral insulin resistance,[41] which is known to be present in hypertension.[44]

Many of these derived tests have not been validated in longitudinal studies, whereas the 2hPG has been shown to be associated with endothelial dysfunction in hypertensives[45] and has been shown to predict future hypertension.[46, 47]

Participants had an OGTT with insulin and glucose measured at 0, 30, 60 and 120 minutes. The 2hPG was used as a measure of peripheral insulin resistance and the area-under-the-curve glucose and insulin (using trapezoid method to calculate the area) was used as a

measure of hepatic and peripheral insulin resistance. Chapter 4 displays results of these measures in subjects with normal fasting glucose, comparing the different groups.

3.7.4 Endothelial Dysfunction

Flow mediated-dilatation

Flow-mediated dilatation (FMD) is a procedure that measures brachial artery response to shear stress. Shear stress induces nitric oxide vasodilation. FMD can be considered a surrogate measure of oxidative stress and endothelial dysfunction. Studies have found reduced FMD in hypertensives[48] and FMD was associated with cardiovascular events in hypertensives.[49]

Two prospective studies found no association between FMD and risk of development of hypertension.[50,51]

Participants rested for ten minutes prior to FMD measurement. A BP cuff was placed above the antecubital fossa and a baseline rest image was acquired. Arterial occlusion was created by cuff inflation to 50 mmHg above systolic pressure (measured just prior to FMD) for five minutes. The longitudinal image of the artery was recorded continuously for two minutes after cuff deflation. The diameter of the brachial artery (the media-adventitia interfaces) was identified manually with electronic calipers. The change in artery diameter was assessed at 60 seconds post deflation. Analysis of the change in artery diameter was undertaken using linear regression adjusting for baseline artery diameter. FMD was measured by the same person (SC).

Repeatability of FMD

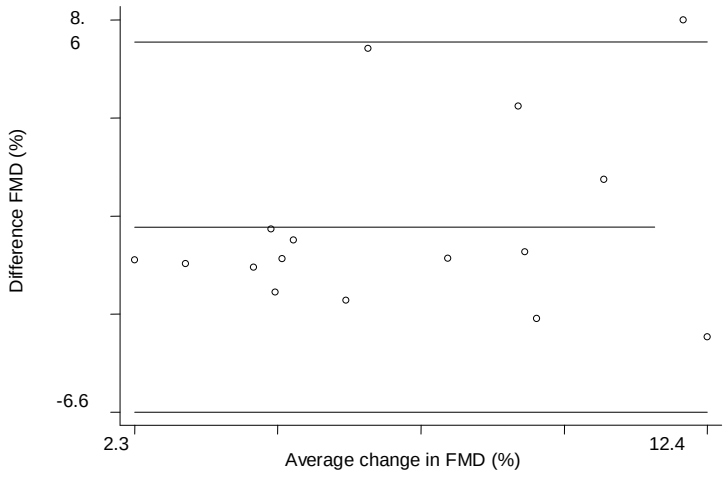


Figure 3.17. Bland-Altman plot of flow-mediated dilation repeatability.

The mean difference in diastolic BP to the mental stress test in 16 participants, at three months, was 0.6% mmHg. The 95% CI of the mean difference was -1.3 to 2.5%. The 95% limits of agreement were 6 to 8% and the Bland-Altman plot is shown (See Figure 3.17).

3.7.5 Circulating biomarkers

Chapter two reviewed studies that measured circulating biomarkers in WCHT. Circulating measures of vascular endothelial dysfunction, inflammation and fibrinolytic measured were

- Von Willebrand factor (vWF). vWF is released by endothelial cells and has been found to negatively correlate with FMD[52] and is associated with target organ damage in hypertensives.[53]

- Asymmetric dimethylarginine (ADMA) is an inhibitor of NO synthase[54] and elevated levels are associated with conditions associated with increased cardiovascular risk including hypertension,[55] and insulin resistance[56] and predicts cardiovascular events.[57]

- High sensitivity c-reactive protein (hsCRP) is commonly used to assess vascular inflammation in research.

- Plasminogen Activator 1 (PAI-1) assesses fibrinolytic vascular function and inflammation as it is induced by CRP.[58]

Two of these circulating biomarkers (CRP and PAI-1) along with urinary albumin-creatinine ratio were associated with incident hypertension in a multivariate analysis of biomarkers from the Framingham Offspring Study.[59]

3.7.6. Plasma biochemistry methods

Bloods for lipid profiling, insulin and glucose were sent immediately to Southern Cross pathology, Monash Health for analysis. Blood samples for cRP, vWF, plasminogen activator I (PAI-1), ADMA were spun and the plasma frozen at -70^{oC} until analysis.

Table 3.2 shows the repeatability of the bloods. Information was obtained from available information sheet from the kit.

Table 3.2: Repeatability of circulating biomarkers.

Test		Coefficient of Variation (Intra-assay)	Kit Information sheet
Triglycerides	Beckman Coulter commercial enzymatic assay	7.4% at 1.0 mmol/l 4.7% at 2.0 mmol/l	389951AA 1A TG
Cholesterol	Beckman Coulter commercial enzymatic assay	1.9% at 3.4 mmol/l 1.3% at 7.0 mmol/l	389895AA 5A CHOL
High density lipoprotein	Beckman Coulter commercial enzymatic assay	4.4% at 0.65 mmol/l 4.5% at 1.4 mmol/l	389918AA 8A HDLD
Low density lipoprotein	CHOL – HDLC – (TRIG/2.25)		389918AA 8A HDLD
Glucose	Beckman Coulter commercial rate assay	2.6% at 4.7 mmol/l 1.6%at 15.7 mmol/Ll	389747 AA GLU3
Insulin	Beckman Coulter Access/DXI Ultrasensitive Insulin assay	6.9% at 14.2 mIu/l 6.1% at 50.4 mIU/l 4.6% at 127.7 mIU/l	N/A
ADMA	ImmunDiagnostik AG Bensheim ELISA assay	9.3% at 0.43 µmol/l 13.5% at 0.74 µmol/l	N/A
Plasminogen activator 1	Behring commercial assay kit	7.7% at 2.6 Inhibitory units/ml 7.3% at 4.1 Inhibitory units/ml	N/A
Von Willebrand factor	DakoCytomation ELISA assay	7.1% at 1.29 U/ml 5.1% at 0.44 U/ml	N/A
Leptin	N/A	N/A	N/A
Urinary protein	Beckman Diagnostics Nepholemetric technology on a Beckman Immage Analyser	4.2% at 12.1 mg/l 5.3% at 45.0 mg/l	Beckman Immage Chemistry information manual. 988643, Sept 2003

N/A: Not available.

Leptin

Leptin analysis was performed by Dr Nina Eikelis at the Baker Institute. Leptin is an adipokine that is associated with insulin resistance,[60] induces oxidative stress[61] and the sympathetic nervous system[62] and is implicated in the development of hypertension.[63]

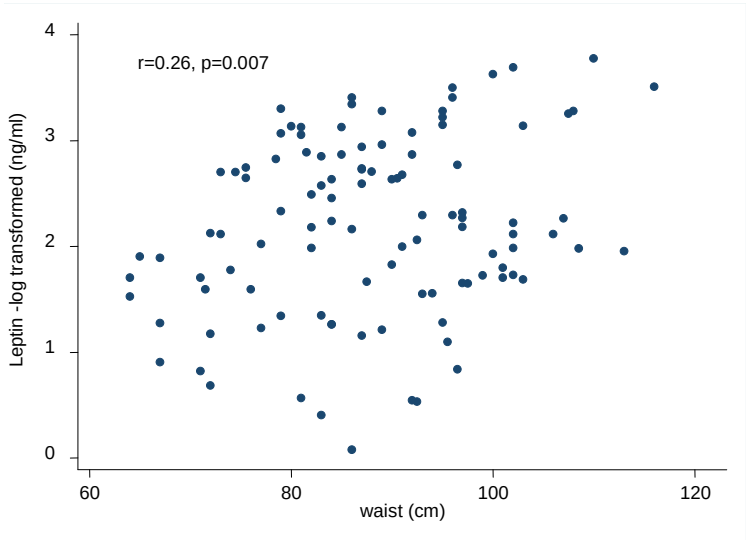


Figure 3.18. Scatter plot of leptin and waist circumference.

Leptin is known to be right skewed and is associated with waist circumference, which was confirmed in this study (see figure 3.13) hence leptin measurements were log-transformed and adjusted for waist circumference in all analyses.

3.7.7 Proteinuria

Microalbuminuria can be considered a marker of renal vascular endothelial dysfunction.[64] The gold standard for assessing microalbuminuria is a 24h urine collection but is logistically inconvenient for studies, particularly the cost and storage. An early morning spot urine test is considered an acceptable screening test for microalbuminuria.[65] European Hypertension

guidelines suggest a normal albumin-creatinine ratio: < 22 mg/g for males and < 31 mg/g for females.

Participants were asked to provide an early morning urine specimen. Urine samples were frozen at at -70^{oC} until analysis.

As the results were given in mg/mmol, the albumin-creatinine ratio was converted to mg/g by being multiplied by 8.85.

(http://www.sydpath.stvincents.com.au/other/Conversions/ConversionMasterF3.htm)

3.7 References

1. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Bohm M, Christiaens T, et al. 2013 ESH/ESC Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur. Heart J.*. 2013; **34**: 2159-219.

2. Dolan E, Stanton A, Thijs L, Hined, K, Atkins N, McClory S, et al. Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin outcome study. *Hypertension*. 2005; **46**: 156-61.

3. Eguchi K, Pickering TG, Hoshide S, Ishikawa J, Ishikawa S, Schwartz JE, et al. Ambulatory blood pressure is a better marker than clinic blood pressure in predicting cardiovascular events in patients with/without type 2 diabetes. *Am. J. Hypertens*. 2008; **21**: 443-50.

4. Bliziotis IA, Destounis A, Stergiou GS. Home versus ambulatory and office blood pressure in predicting target organ damage in hypertension: a systematic review and meta-analysis. *J. Hypertens*. 2012; **30**: 1289-99.

5. Niiranen TJ, Hanninen MR, Johansson J, Reunanen A, Jula AM. Home-measured blood pressure is a stronger predictor of cardiovascular risk than office blood pressure: the Finn-Home study. *Hypertension*. 2010; **55**: 1346-51.

6. Scherpbier-de Haan N, van der Wel M, Schoenmakers G, Boudewijns S, Peer P, van Weel C, et al. Thirty-minute compared to standardised office blood pressure measurement in general practice. *Br. J. Gen. Pract*. 2011; **61**: e590-7.

7. Myers MG. A proposed algorithm for diagnosing hypertension using automated office blood pressure measurement. *J. Hypertens*. 2010; **28**: 703-8.

8. Martin CA, Cameron JD, Chen SS, McGrath BP. Measurement of blood pressure in the office. *Hypertension*. 2010; **56**: e11; author reply e2.

9. Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R., Germano, G., et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens*. 2007; 25: 1105-87.

10. Palatini P, Dorigatti F, Roman E, Giovinazzo P, Piccolo D, De Venuto G, et al. White-coat hypertension: a selection bias? Harvest Study Investigators. Hypertension and Ambulatory Recording Venetia Study. *J. Hypertens*. 1998; **16**: 977-84.

11. Cuspidi C, Meani S, Sala C, Valerio C, Fusi V, Zanchetti A, et al. How reliable is isolated clinical hypertension defined by a single 24-h ambulatory blood pressure monitoring? *J. Hypertens*. 2007; **25**: 315-20.

12. Head GA, Reid CM, Lukoshkova EV. Nonsymmetrical double logistic analysis of ambulatory blood pressure recordings. *J. Appl. Physiol*. 2005; **98**: 1511-8.

13. Head GA, Chatzivlastou K, Lukoshkova EV, Jennings GL, Reid CM. A novel measure of the power of the morning blood pressure surge from ambulatory blood pressure recordings. *Am. J. Hypertens*. 2010; **23**:1074-81.

14. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et. al; International Diabetes Federation Task Force on Epidemiology and Prevention;

Hational Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation.* 2009; **120**:1640-5.

15. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009; **120**: 1640-5.

16. Parikh NI, Pencina MJ, Wang TJ, Benjamin EJ, Lanier KJ, Levy D, et al. A risk score for predicting near-term incidence of hypertension: the Framingham Heart Study. *Ann. Intern. Med*. 2008; **148**: 102-10.

17. Laurent S, Boutouyrie P. Arterial stiffness: a new surrogate end point for cardiovascular disease? *J. Nephrol*. 2007; **20** Suppl 12: S45-50.

18. Liang YL, Teede H, Kotsopoulos D, Shiel L, Cameron JD, Dart AM, et al. Non-invasive measurements of arterial structure and function: repeatability, interrelationships and trial sample size. *Clin. Sci. (Lond)*. 1998; **95**: 669-79.

19. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkitachalam L, Kupelian V, Simonsick EM, et al. Elevated aortic pulse wave velocity, a marker of arterial

stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation*. 2005; **111**: 3384-90.

20. Mitchell GF, Hwang SJ, Vasan RS, Larson MG, Pencina MJ, Hamburg NM, et al. Arterial stiffness and cardiovascular events: the Framingham Heart Study. *Circulation*. 2010; **121**: 505-11.

21. Karras A, Haymann JP, Bozec E, Metzger M, Jacquot C, Maruani G, et al. Large artery stiffening and remodeling are independently associated with all-cause mortality and cardiovascular events in chronic kidney disease. *Hypertension*. 2012; **60**: 1451-7.

22. Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, et al. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. *Hypertension*. 2004; **43**: 1239-45.

23. McEniery CM, Wilkinson IB, Avolio AP. Age, hypertension and arterial function. *Clin. Exp. Pharmacol. Physiol*. 2007; **34**: 665-71.

24. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur. Heart J*. 2006; **27**: 2588-605.

25. Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank JK, De Backer T, et al. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J. Hypertens*. 2012; **30**: 445-8.

26. Ewing DJ, Clarke BF. Diagnosis and management of diabetic autonomic neuropathy. *Br. Med. J. (Clin Res Ed)*. 1982; **285**: 916-8.

27. Wieling W, van Brederode JF, de Rijk LG, Borst C, Dunning AJ. Reflex control of heart rate in normal subjects in relation to age: a data base for cardiac vagal neuropathy. *Diabetologia*. 1982; **22**: 163-6.

28. O'Brien IA, O'Hare P, Corrall RJ. Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. *Br. Heart J.* 1986; **55**: 348-54.

29. Espi F, Ewing DJ, Clarke BF. Testing for heart rate variation in diabetes: single or repeated deep breaths? *Acta Diabetol. Lat*. 1982; **19**: 177-81.

30. Chen HS, Hwu CM, Kuo BI, Chiang SC, Kwok CF, Lee SH, et al. Abnormal cardiovascular reflex tests are predictors of mortality in Type 2 diabetes mellitus. *Diabet. Med*. 2001; **18**: 268-73.

31. Ewing DJ, Martyn CN, Young RJ., Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care*. 1985; **8**: 491-8.

32. Gerritsen J, TenVoorde BJ, Dekker JM, Kingma R, Kostense PJ, Bouter LM, et al. Measures of cardiovascular autonomic nervous function: agreement, reproducibility, and reference values in middle age and elderly subjects. *Diabetologia*. 2003; **46**: 330-8.

33. Parati G, Mancia G, Di Rienzo M Castiglioni P. Point: cardiovascular variability is/is not an index of autonomic control of circulation. *J. Appl. Physiol*. 2006; **101**: 676-8; discussion 81-2.

34. Pagani M, Lucini D, Porta A. Sympathovagal balance from heart rate variability: time for a second round? *Exp. Physiol*. 2012; **97**: 1141-2.

35. Prakash ES. 'Sympathovagal balance from heart rate variability: an obituary', but what is sympathovagal balance? *Exp. Physiol*. 2012; **97**: 1140.

36. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol*. 1979; **237**: E214-23.

37. Borai A, Livingstone C, Kaddam I, Ferns G. Selection of the appropriate method for the assessment of insulin resistance. *BMC Med. Res. Methodol*. 2011; **11**: 158.

38. Balion CM, Raina PS, Gerstein HC, Santaguida PL, Morrison KM, Booker L, et al. Reproducibility of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) classification: a systematic review. *Clin. Chem. Lab. Med.* 2007; **45**: 1180-5.

39. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985; 28: 412-9.

40. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*. 2000; 85: 2402-10.

41. Bonora E, Kiechl S, Willeit J, Oberhollenzer F, Egger G, Meigs JB, et al. Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in caucasian subjects from the general population: the Bruneck study. *Diabetes Care*. 2007; 30: 318-24.

42. Borai A, Livingstone C, Ferns GA. The biochemical assessment of insulin resistance. *Ann Clin Biochem*. 2007; 44: 324-42.

43. Meyer C, Pimenta W, Woerle HJ, Van Haeften T, Szoke E, Mitrakou A, et al. Different mechanisms for impaired fasting glucose and impaired postprandial glucose tolerance in humans. *Diabetes Care*. 2006; 29: 1909-14.

44. Natali A, Santoro D, Palombo C, Cerri M, Ghione S, Ferrannini E. Impaired insulin action on skeletal muscle metabolism in essential hypertension. *Hypertension*. 1991; 17: 170-8.

45. Tomiyama H, Kimura Y, Okazaki R, Kushiro T, Abe M, Kuwabara Y, et al. Close relationship of abnormal glucose tolerance with endothelial dysfunction in hypertension. *Hypertension*. 2000; 36: 245-9.

46. Boyko EJ, Barr EL, Zimmet PZ, Shaw JE. Two-hour glucose predicts the development of hypertension over 5 years: the AusDiab study. *J. Hum. Hypertens*. 2008; **22**: 168-76.

47. Boyko EJ, Shaw JE, Zimmet PZ, Chitson P, Tuomilehto J, Alberti KG. A prospective study of glycemia, body size, insulin resistance and the risk of hypertension in Mauritius. *J. Hypertens*. 2008; **26**: 1742-9.

48. Muiesan ML, Salvetti M, Monteduro C, Corbellini C, Guelfi D, Rizzoni D, et al. Flow-mediated dilatation of the brachial artery and left ventricular geometry in hypertensive patients. *J. Hypertens*. 2001; **19**: 641-7.

49. Muiesan ML, Salvetti M, Paini A, Monteduro C, Galbassini G, Poisa P, et al. Prognostic role of flow-mediated dilatation of the brachial artery in hypertensive patients. *J. Hypertens*. 2008; **26**: 1612-8.

50. Shimbo D, Muntner P, Mann D, Viera AJ, Homma S, Polak JF, et al. Endothelial dysfunction and the risk of hypertension: the multi-ethnic study of atherosclerosis. *Hypertension*. 2010; **55**: 1210-6.

51. Lytsy P, Lind L, Sundstrom J. Endothelial function and risk of hypertension and blood pressure progression: the prospective investigation of the vasculature in Uppsala seniors. *J. Hypertens*. 2013; **31**: 936-9.

52. Felmeden DC, Spencer CG, Chung NA, Belgore FM, Blann AD, Beevers DG, et al. Relation of thrombogenesis in systemic hypertension to angiogenesis and endothelial damage/dysfunction (a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial [ASCOT]). *Am. J. Cardiol*. 2003; **92**: 400-5.

53. Spencer CG, Gurney D, Blann AD, Beevers DG, Lip GYand Ascot Steering Committee, ASCOT. Von Willebrand factor, soluble P-selectin, and target organ damage in hypertension: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). *Hypertension*. 2002; **40**: 61-6.

54. Li J, Zhou Z, Jiang DJ, Li D, Tan B, Liu H, et al. Reduction of NO- and EDHF-mediated vasodilatation in hypertension: role of asymmetric dimethylarginine. *Clin. Exp. Hypertens*. 2007; **29**: 489-501.

55. Perticone F, Sciacqua,A, Maio R, Perticone M, Maas R, Boger RH, et al. Asymmetric dimethylarginine, L-arginine, and endothelial dysfunction in essential hypertension. *J. Am. Coll. Cardiol*. 2005; **46**: 518-23.

56. Nakhjavani M, Karimi-Jafari H, Esteghamati A, Khalilzadeh O, Asgarani F, Ghadiri-Anari A. ADMA is a correlate of insulin resistance in early-stage diabetes independent of hs-CRP and body adiposity. *Ann. Endocrinol. (Paris)*. 2010; **71**: 303-8.

57. Boger RH, Sullivan LM, Schwedhelm E, Wang TJ, Maas R, Benjamin EJ, et al. Plasma asymmetric dimethylarginine and incidence of cardiovascular disease and death in the community. *Circulation*. 2009; **119:** 1592-600.

58. Chen C, Nan B, Lin P, Yao Q. C-reactive protein increases plasminogen activator inhibitor-1 expression in human endothelial cells. *Thromb Res*. 2008; 122: 125-33.

59. Wang TJ, Gona P, Larson MG, Levy D, Benjamin EJ, Tofler GH, et al. Multiple biomarkers and the risk of incident hypertension. *Hypertension*. 2007; **49**: 432-8.

60. Haffner SM, Miettinen H, Mykkanen L, Karhapaa P, Rainwater DL, Laakso M. Leptin concentrations and insulin sensitivity in normoglycemic men. *Int. J. Obes. Relat. Metab. Disord*. 1997; **21**: 393-9.

61. Bouloumie A, Marumo T, Lafontan M, Busse R. Leptin induces oxidative stress in human endothelial cells. *FASEB J*. 1999; **13**: 1231-8.

62. Satoh N, Ogawa Y, Katsuura G, Numata Y, Tsuji T, Hayase M, et al. Sympathetic activation of leptin via the ventromedial hypothalamus: leptin-induced increase in catecholamine secretion. *Diabetes*. 1999; **48**: 1787-93.

63. Shek EW, Brands MW, Hall JE. Chronic leptin infusion increases arterial pressure. *Hypertension*. 1998; **31**: 409-14.

64. Perticone F, Maio R, Tripepi G, Sciacqua A, Mallamaci F, Zoccali C. Microalbuminuria, endothelial dysfunction and inflammation in primary hypertension. *J. Nephrol*. 2007; **20** Suppl 12: S56-62.

65. Wu HY, Huang JW, Peng YS, Hung KY, Wu KD, Lai MS, et al. Microalbuminuria screening for detecting chronic kidney disease in the general population: a systematic review. *Ren. Fail*. 2013; **35**: 607-14.

Chapter 4

Two hour glucose post loading: a biomarker of cardiovascular risk in isolated clinic hypertension

4.1 Introduction

The published paper in Chapter 2 showed that studies looking at biomarkers in WCHT have reported mixed results. The studies used different definitions for WCHT and many of the studies did not adjust for potential confounders making it difficult to determine if WCHT is associated with any biomarkers of cardiovascular disease.

The published paper in this chapter reported the results of a carefully controlled study in a WCHT group (non-smoking, normal fasting plasma glucose and no known diabetes), defined by strict criteria (two ABPM recordings) and using a consensus definition for WCHT. This study examined markers of specific pathophysiological mechanisms that may potentially be involved in WCHT.

Questions addressed:

- Is WCHT associated with glucose dysregulation or insulin resistance?
- Is WCHT associated with artery stiffness?
- Is WCHT associated with autonomic dysfunction?
- Is WCHT associated with circulating biomarkers of endothelial dysfunction and inflammation?

The addendum to Chapter Four reports the results of an analysis looking at specific sub-groups (across blood pressure and glucose dysregulation spectra) and individual test components.

4.2 Declaration

In the case of Chapter four, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Conception, data collection, analysis of data, writing paper.	80

The following co-authors contributed to the work.

Name	Nature of contribution
Professor Barry McGrath	Conception, writing and review of paper
Professor James Cameron	Conception, writing and review of paper
Dr Suzie Chen	Assistance with data collection, analysis of arterial function, review of paper.

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	18/9/13
Main Supervisor's Signature	[redacted]	18 9 13

Two hour glucose post loading: a biomarker of cardiovascular risk in isolated clinic hypertension

Catherine A. Martin[a], James D. Cameron[b], Suzi S. Chen[a] and Barry P. McGrath[a]

Background **Isolated clinic hypertension (ICHT) may be an indicator of both future hypertension and diabetes. This study examines the 2-h plasma glucose level post load (2hPG), and measures of arterial stiffness, autonomic function and circulating biomarkers in ICHT, normotension and hypertension.**

Methods **Participants aged 39–75 years, who were untreated for hypertension, nonsmokers and not known diabetic ($n = 105$) were categorized as normotension, ICHT and hypertension, based on clinic and mean daytime ambulatory blood pressures. Participants had measurements of autonomic function, aorto-femoral pulse wave velocity (PWVc), as well as blood sampling for lipids and potential circulating biomarkers [high sensitivity C-reactive protein (hsCRP), plasminogen activator inhibitor 1 (PAI-1), asymmetric dimethylarginine (ADMA), and von Willebrand factor (vWF)], followed by a glucose tolerance test.**

Results **A total of 8.3% normotension, 37.9% ICHT and 15% hypertension patients had impaired glucose tolerance. Mean 2hPG adjusted for age and waist circumference was 5.7 mmol/l [interquartile range (IQR) 5.2–6.4] for normotension, 7.4 mmol/l (IQR 6.5–8.3) for ICHT ($P = 0.002$ vs. normotension) and 6.2 mmol/l (IQR 5.6–6.9) for hypertension group. Other measures of insulin resistance were similar in the three groups. Mental stress testing induced a greater blood pressure response in the ICHT group ($P = 0.01$ vs. normotension); other autonomic function measures were similar in the three groups. Mean PWVc, adjusted for age and blood pressure, was similar in ICHT and normotension but increased in the hypertension group. Circulating biomarker levels were not different in the three groups.**

Conclusion **Assessment of total cardiovascular risk in patients with ICHT should include measurement of postprandial glucose.** *J Hypertens* **29:749–757 © 2011 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: autonomic function, circulating biomarkers, glucose, insulin resistance, isolated clinic hypertension, pulse wave velocity, white-coat hypertension

Abbreviations: 2hPG, two-hour glucose post glucose load; AusDiab, The Australian Diabetes, Obesity and Lifestyle Study; dABP, daytime ambulatory blood pressure; HRV, heart rate variability; ICHT, isolated clinic hypertension; PAMELA, Pressioni Arteriose Monitorate E Loro Associazioni Study; PWV_c, central pulse wave velocity

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Introduction

Isolated clinic hypertension (ICHT), also known as 'white coat' hypertension, is estimated to have a population prevalence of about 15% [1,2]. In the Pressioni Arteriose Monitorate E. Loro Associazioni (PAMELA) study patients with ICHT were not only more likely to progress to sustained hypertension compared to normotensive participants [3], but were also more likely to develop blood glucose abnormalities [4]. It was considered that the progression towards blood glucose abnormalities was predominantly attributable to metabolic abnormalities associated with presence of ICHT.

Two large prospective studies have found an association between 2-h plasma glucose post oral glucose load (2hPG) and incident hypertension. The Australian Diabetes, Obesity and Lifestyle (AusDiab) study, which followed 4413 participants, found a baseline 2hPG was associated with incident hypertension over a 5-year period with the results remaining statistically significant after adjusting for confounding factors [5]. A Mauritian study which prospectively followed 3581 participants found that at 5 years the baseline 2hPG was also associated with incident hypertension after adjusting for similar confounders [6].

Previous studies have shown that the 2hPG appears to be an important biomarker of cardiovascular risk. In the AusDiab study cardiovascular and all-cause mortality rates were linearly associated with the 2hPG in nondiabetic individuals after adjustment for age and sex [7]. A large meta-analysis, involving 95 783 participants, showed a progressive relationship between the 2hPG and cardiovascular events [8]. In addition therapy targeted at exaggerated postprandial glucose rise in diabetic patients has cardiovascular benefit [9].

In this cross-sectional study we compare the 2hPG, adjusted for confounding variables (age and waist

circumference), in groups of participants with normal blood pressure (normotension), isolated clinic hypertension (ICHT) and hypertension. Only individuals with normal fasting plasma glucose were included in the analysis. To investigate potential mediators that might cluster with, or be potential mediators of ICHT, we also examined central aorto-femoral pulse wave velocity (PWVc), autonomic function, and circulating markers of endothelial dysfunction and inflammation.

Methods

The study was approved by the institutional human research ethics committee. Adult individuals (18–79 years) were recruited through referrals to an ambulatory blood pressure monitoring service and by advertisement. Patients with ICHT were recruited by review of all ABPM reports, for the previous 2 years. This service receives referrals from 120 general practitioners from a service population of 150 000 people. Any patient reported as having ICHT or hypertension and not receiving antihypertensive drug therapy were contacted by mail inviting them to participate in this study. Hypertensive and normotensive individuals were also recruited by advertisements in local newspapers in the same geographic areas as the referring general practitioners.

Individuals were excluded if they were smokers, had known diabetes, were taking antihypertensive medication, or had existing liver, renal or cardiovascular disease or any malignancy. Written informed consent was obtained from all participants.

Participants presented in the morning, after a minimum fast of 10 h. Two sitting blood pressure measurements were taken on the left arm at 1-min intervals using an automated oscillometric blood pressure device (Task Force monitor; CNSystems, Graz, Austria). One of the authors (C.M.) remained in the room for these measurements in order to mimic usual clinical practice. The study participant was then left alone in the room and after a further 5 min of rest three sitting blood pressure measurements were automatically recorded at 3-min intervals. All anthropomorphic measurements were then taken by the same person (S.C.). Waist circumference was measured at the halfway point between the inferior margin of the ribs and the superior border of the iliac crests.

Each participant completed a questionnaire about previous and present illnesses, family history, medication, diet, physical activity and alcohol habits. Alcohol consumption was categorized into none, low health risk (regular $\leq$ two standard drinks on any day or occasional $\leq$ four standard drinks) or high health risk (regular $>$ two standard drinks on any day or occasional $>$ four standard drinks). Presence of the metabolic syndrome was defined on the basis of waist circumference, blood pressure and lipid profile [10].

Measurement of autonomic function

Heart rate variability

Testing was performed and analysed by the same operator (C.M.) using the Task Force monitor, which uses an adaptive autoregressive parametric algorithm allowing time-varying spectral estimation of heart rate variability (HRV). Testing occurred in a quiet, air-conditioned room with the participant resting supine for 10 min prior to testing. Two recordings for analysis of HRV were taken: one of 10 min with no constraints on the participant's breathing rate and the second for 2 min with the participant breathing at a rate of 15 breaths per minute (0.25 Hz) with the aid of a metronome. Analysis was performed on the middle 5 min of the 10-min unconstrained measurements and on the entire 2-min recording during constrained breathing. Any participant's data displaying more than three ectopics per minute were not included in the analysis. Low frequency (LF) was defined as 0.05–0.15 Hz and high frequency (HF) was defined as 0.15–0.4 Hz. Normalized LF (LF_nu) is defined as LF/(LF+HF) and normalized HF (HF_nu) as HF/(LF+HF).

Postural blood pressure and heart rate responses

After lying supine for 2 min an oscillometric blood pressure measurement was taken. The participant was then asked to promptly stand, without assistance, and to stand still with no movement or talking for 1 min. Standing oscillometric blood pressure was taken 1 min post standing. From the continuous recording, the maximum heart rate at 15 ± 2 s and the minimum heart rate at 30 ± 2 s were identified.

Heart rate responses to respiration

Participants were asked to take a deep breath in and out. The minimum heart rate during expiration was subtracted from the maximum heart rate during inspiration. Each participant then had controlled breathing, at a rate of six breaths per minute for 1 min, with the operator, using a metronome, pacing the participant's breathing rate. The maximum heart rate during inspiration and the minimum heart rate during expiration were identified in each cycle and the difference calculated. The average of the difference for the six cycles was calculated.

Heart rate response to the Valsalva manoeuvre

Each participant performed three Valsalva manoeuvres in the sitting position with continuous beat-to-beat finger blood pressure recording. Participants rested for 2 min between each manoeuvre. The maximum heart rate, during the forced exhalation strain against a closed glottis (phase 2), and the minimum heart associated with the maximum systolic blood pressure immediately after exhalation ceased (phase 4), were measured.

Mental arithmetic stress test

After resting for 2 min an oscillometric blood pressure was taken. Participants were then instructed to subtract out loud the value 13 from the value 4300. Participants were instructed to either start again or continue from the current value if they felt they had made a mistake and that no assistance would be given by the examiner. If a participant verbalized an inability to do this subtraction then they were offered the subtraction of 13 from 430. An oscillometric blood pressure measurement was taken at 1 min. The difference in systolic and diastolic blood pressures at 1 min, from the resting systolic and diastolic blood pressures was calculated.

Sustained handgrip test

After resting for 2 min an oscillometric blood pressure was taken. Participants were asked to squeeze a cuff connected to a sphygmomanometer and the maximal pressure was noted. Participants were then asked to squeeze the cuff to one-third of the noted maximum pressure for 5 min, and oscillometric blood pressure measurements were taken on the other arm at 1-min intervals. The diastolic blood pressure difference from resting to the peak response during the grip was calculated.

Central pulse wave velocity

Central aorto-femoral PWV (PWVc) measurements were performed by the same two operators (C.M., S.C.), in a quiet air-conditioned room after 10 min of rest and with the participant lying supine. Continuous pulse pressure wave signals were recorded with hand-held tonometers (Millar Mikro-tip, SPT-301; Millar Instruments, Houston, Texas, USA) positioned at the base of the right common carotid artery and over the right common femoral artery. Transit distance was defined as the measured distance from the sternal notch to femoral artery minus distance from sternal notch to carotid. The start of systole was defined by the local maximum of the first derivative of the pressure signal. Mean transit time (Δt) between the feet of simultaneously recorded waves was determined from 10 consecutive cardiac cycles. Mean arterial pressure (MAP) was estimated from an oscillometric blood pressure measurement taken immediately post recording of the arterial waveforms.

Biochemical measures

Blood samples were taken under fasting conditions, following which participants consumed a 75 g glucose solution over a period of 5 min. Plasma glucose was measured at 30, 60 and 120 min post glucose load. Blood samples were processed immediately after collection. Plasma glucose was analysed by the glucose oxidase method and insulin determined by the Access/DXI Ultrasensitive one-step immunoenzymatic assay. WHO criteria were used to define newly diagnosed diabetes as a 2hPG level at least 11.1 mmol/l and impaired glucose tolerance (IGT) was defined as a 2hPG level at least 7.8 mmol/l and less than 11.1 mmol/l [11].

Plasma samples for fasting plasma cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were analysed immediately, whereas plasma for asymmetric dimethylarginine (ADMA), von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), and C-reactive protein (hsCRP) was stored at −70°C until analysis. Commercial assay kits were used for these analyses.

Ambulatory blood pressure monitoring

Ambulatory blood pressure monitoring was performed using a portable device (Meditech Cardiotens; Meditech Ltd, Hungary) and was commenced after the glucose tolerance test between 1200 and 1400 h. The blood pressure cuff was placed on the nondominant arm. Participants wore the device for 24 h with blood pressure measurements recorded every 30 min day and night. Day and night-time periods were determined from diary information provided by the participant noting the time they fell asleep and the time they woke and stayed awake. Participants were told to continue normal activities during the 24-h blood pressure monitoring period. Normotensive individuals were defined as reported clinic blood pressure below 140/90 mmHg and daytime ABP (dABP) less than 135/85 mmHg. ICHT was defined as reported clinic blood pressure at least 140/90 mmHg and dABP less than 135/85 mmHg. Sustained hypertension was defined as reported clinic at least 140/90 mmHg and dABP at least 135/85 mmHg. Repeat 24-h ABPM within a 12-month period was required in all patients with ICHT to confirm this diagnosis.

Statistical analysis

For each participant the observed blood pressure recorded was the second reading. The average sitting blood pressure (no observer present) was calculated as the average of the three recordings. Participants were classified as having the metabolic syndrome based on measurements of waist circumference, blood pressure and lipid profile [10]. As ICHT is a condition in which ambulatory and home BP readings are within the normal range, two blood pressure measurements were used in determining if a participant had the metabolic syndrome. In the first of these, the observed blood pressure was used consistent with usual clinical practice. The second determination was based on the average daytime ambulatory blood pressure. An autonomic function score was obtained [12]. Normal fasting blood glucose was defined as less than 5.6 mmol/l [10].

Statistics

STATA, version 10, (StataCorp LP, College Station, Texas, USA) was used to analyse the data. Normally

Table 1 Baseline characteristics of study participants

	NT	ICHT	HT	*P* value ICHT vs. NT	*P* value HT vs. NT
Number	36	29	40		
Age (years)	54.9 ± 9.3	55.8 ± 8.3	57.9 ± 9.4	0.70	0.15
Female	26 (72.2)	25 (86.2)	23 (57.5)	0.17	0.18
Female menopause	24 (92.3)	22 (88.0)	22 (95.5)	0.85	0.66
Alcohol consumption					
None	11 (32.3)	15 (45.4)	13 (33.3)	0.22	0.55
Low health risk	19 (55.9)	10 (33.5)	18 (46.2)		
High health risk	4 (11.8)	4 (13.8)	8 (20.5)		
Waist (cm)	86.0 ± 13.1	84.1 ± 11.1	93.2 ± 10.7	0.50	0.009
BMI (kg/m^2)	26.3 (22.8–28.5)	25.4 (22.2–26.4)	26.4 (24.2–28.7)	0.76	0.21
Metabolic syndrome defined on basis of:					
observed BP	7 (19.4)	6 (20.7)	16 (40.0)	0.44	0.05
ambulatory daytime BP	7 (19.4)	2 (6.9)	21 (52.5)	0.15	0.003
Cholesterol (mmol/l)	5.6 ± 1.1	5.4 ± 0.9	5.5 ± 1.0	0.47	0.67
Triglycerides (mmol/l)	0.95 (0.60–1.40)	1.01 (0.60–1.30)	1.25 (0.75–1.55)	0.57	0.16
HDL (mmol/l)	1.41 ± 0.39	1.43 ± 0.36	1.21 ± 0.40	0.91	0.03
LDL (mmol/l)	3.60 ± 1.10	3.52 ± 0.84	3.70 ± 0.97	0.75	0.65

Data presented as mean ± standard deviation, number (%) or median (25th–75th quartile). HDL, high-density lipoprotein; HT, hypertension; ICHT, Isolated clinic hypertension; LDL, low-density lipoprotein; n, number; NT, normotension.

distributed data are expressed as mean ± standard deviation, whereas skewed data are expressed as median [interquartile range (IQR)]. Categorical data are expressed as percentages. Positive skewed data was log-transformed prior to analysis.

The 2hPG is positive and right skewed, hence it required log-transformation for analysis. Sample size was calculated using the method of sample size calculation for log-transformed data [13]. Estimated sample size was 33 participants per group, based on the coefficient of variation of 2hPG in a normotensive population in the AusDiab study [5] and allowing a ratio of 1.25 in the 2hPG of the ICHT group compared to the normotensive group. To allow the comparisons of the two hypertensive groups against the normotensive group the following conditions were applied: power = 0.8 and $\alpha = 0.025$. All comparisons were two-sided. Logistic regression was used to analyse binary outcomes, whereas linear regression was used to analyse continuous outcomes. Fisher's exact test was used to analyse count data. Statistical significance was considered to be met at $\alpha = 0.05$.

Results

One hundred and twenty-eight participants were recruited. Eight individuals were excluded as their fasting plasma glucose was elevated (> 5.6 mmol). Two individuals with ICHT were commenced on drug therapy by their general practitioners before repeat ABPM. Two individuals with ICHT had elevated repeat ABPM showing borderline or elevated dABP (≥135/85). One met the definition of ICHT (based on average daytime blood pressure on two occasions) but had an elevated night-time blood pressure and the decision was made to classify this patient in the hypertensive group. To control for the disparity in age ranges of the groups, the analysis was confined to the age range 39–75 years. Thirty-six normotension, 29 ICHT and 40 hypertension patients remained in the analysis. Nineteen of the 29 ICHT patients were recruited via our ABPM service with the remainder by advertisement. Twelve of the 40 untreated hypertensive patients were recruited via our ABPM service and 28 by advertisement. All of the normotensive patients were recruited by advertisement.

As shown in Table 1, baseline characteristics in the ICHT group were similar to the normotension group. The hypertension group is statistically different from the normotension group with regard to greater waist circumference, lower HDL and presence of the metabolic syndrome. This is the case whether the metabolic syndrome was defined using observed or ambulatory daytime blood pressure measurements.

The groups were similar in ethnicity with the majority of patients being of British or European descent (97% for normotension, 90% for both ICHT and hypertension groups) with the remainder being of Asian descent.

Comparison of blood pressure measurements in the three groups

These results are shown in Table 2. The observed sitting blood pressure, average unobserved sitting blood pressure and first ABPM blood pressures for the ICHT group were intermediate between those of the normotension and hypertension groups. In all three groups unobserved readings were lowest. The average 24-h, day and night ABPM blood pressures in the ICHT group were similar to those of the normotension group. The observed sitting blood pressure for the normotension and ICHT groups were similar to their first ABPM blood pressure; their unobserved sitting average blood pressures were similar to their 24-h day average ABPM blood pressure.

2hPG measurements and indices of insulin resistance

Two normotension (5.5%), two hypertension (5.0%) and one ICHT patients (3.0%) were found to be diabetic on

Table 2 Blood pressure measurements by blood pressure category

	NT	ICHT	HT
Observed[a] sitting BP	121/80 ± 9/8	133/87 ± 9/10	143/92 ± 19/9
Average sitting BP, no observer present	117/77 ± 9/6	124/81 ± 9/8	137/89 ± 14/9
First ABPM BP	123/75 ± 13/9	134/83 ± 14/9	150/89 ± 21/9
Average day ABPM BP	121/72 ± 9/7	126/76 ± 6/5	143/85 ± 9/5
Average night ABPM BP	104/59 ± 8/6	106/60 ± 7/6	121/69 ± 13/8

Data is displayed as mean ± SD. ABPM, ambulatory blood pressure monitoring; BP, blood pressure; HT, hypertension; ICHT, isolated clinic hypertension; NT, normotension. Measures, mmHg. [a] Observer: registered nurse.

the 2hPG. Three (8.3%) normotension, 11 (37.9%) ICHT and 6 (15.0%) hypertension patients were diagnosed with IGT (Table 3). No statistical difference was found, after adjusting for age and waist circumference, between measures of insulin resistance and sex. After adjusting for age and waist circumference, there was a statistical difference in the 2hPG in the ICHT group compared to the normotension group. The estimated geometric mean values for the average study age of 56 years and average study waist circumference of 88.6 cm was 7.4 mmol/l (95% CI 6.5–8.3) compared with 5.7 mmol/l (95% CI 5.2–6.4) for a normotension patient of same age and waist circumference ($P = 0.002$). The 2hPG for the hypertension group was elevated at 6.2 mmol/l (95% CI 5.6–6.9) but was not statistically different from the normotensive group ($P = 0.28$). Although not statistically significant and considering the constraint on fasting plasma glucose levels in this study, there was a trend towards higher mean fasting plasma insulin and homeostasis model of insulin resistance (HOMA_IR) levels in the hypertension group. The ICHT group had a significantly higher glucose area-under-the-curve (AUC) ($P < 0.001$) compared to the normotensive group. As women predominated in the normotensive and ICHT groups, a separate analysis of glucose and insulin measures was performed for women (Table 4). The ICHT group had higher estimated geometric mean values for 2hPG and glucose AUC, adjusted for age, menopause status and waist circumference.

The ICHT group displayed a similar median glucose profile over time to that of the hypertension group (see Fig. 1). Log-transformed glucose AUC and log-transformed insulin AUC were significantly correlated with unobserved systolic blood pressures ($r = 0.32$, $P < 0.001$; $r = 0.32$, $P = 0.001$) and dABPs ($r = 0.23$, $P = 0.02$; $r = 0.24$, $P = 0.01$) across the study population.

Pulse wave velocity

Central pulse wave velocity was positive and right skewed so was log-transformed for analysis. Age and mean arterial pressure were linearly associated with log PWVc ($r = 0.50$, $P < 0.001$ and $r = 0.49$, $P < 0.001$, respectively). There was no statistical difference in PWVc between the sexes, or for different levels of alcohol use. At the average age of 56 years and average MAP of 95mmHg, mean PWVc was 7.8m/s in NT, 8.0m/s in ICHT and 8.6 m/s in HT group ($P = 0.006$ for HT vs. NT) (Table 5). PWVc correlated with fasting insulin ($r = 0.29$, $P = 0.003$), fasting glucose ($r = 0.24$, $P = 0.01$) and HOMA_IR ($r = 0.30$, $P = 0.002$) but not with the 2hPG.

Autonomic function, endothelial function and circulating biomarkers

Normalized low frequency and high frequency, during constrained (15 breaths per minute) and unconstrained breathing, did not differ statistically between the blood pressure groups (Table 6). Values given are estimates for women, nonalcohol drinkers aged 56 years. Normalized low frequency coefficients were 44.8% (IQR 35.8–53.8) in normotensive, 46.5% (IQR 38.1–54.9) in ICHT and 48.9% (IQR 40.4–57.5) in the hypertensive group. The greatest fall in normalized low frequency and the greatest

Table 3 Measures of insulin resistance

	NT	ICHT	HT	*P* value ICHT vs. NT	*P* value HT vs. NT
Female/male	26/10	25/4	23/17		
Diabetes	2 (5.6)	1 (3.4)	2 (5.0)	0.69	0.91
Impaired glucose tolerance	3 (8.3)	11 (37.9)	6 (15.0)	0.004	0.38
Unadjusted values					
Fasting glucose (mmol/l)	4.8 ± 0.3	4.9 ± 0.3	4.9 ± 0.4	0.18	0.64
Fasting insulin (mmol/l)	4.4 (2.8–6.3)	4.2 (3.2–5.1)	5.1 (3.6–6.3)	0.89	0.05
2hPG (mmol/l)	5.8 (4.5–7.0)	7.1 (5.9–9.0)	6.3 (5.5–7.4)	0.002	0.12
Adjusted values [β (95% CI)][a]					
HOMA_IR	0.89 (0.76–1.03)	0.96 (0.81–1.14)	0.97 (0.84–1.13)	0.49	0.39
2hPG (mmol/l)	5.7 (5.2–6.4)	7.4 (6.5–8.3)	6.2 (5.6–6.9)	0.002	0.28
Glucose AUC (mmol/l. 120 min)	822 (766–882)	1015 (938–1098)	906 (846–970)	< 0.001	0.06
Insulin AUC (mmol/l. 120 min)	5391 (4483–6483)	6218 (5054–7649)	5919 (4942–7089)	0.30	0.48

Data presented as mean ± standard deviation or number (%). 2hPG, 2-h plasma glucose post glucose load; AUC, area under curve; HOMA_IR, homeostasis model of insulin resistance; HT, hypertension; ICHT, isolated clinic hypertension; NT, normotension. [a] Estimated geometric means [β (95% CI)] for study average age of 56 years and average waist 88.6 cm.

Table 4 Measures of insulin resistance (women)

	NT	ICHT	HT	*P* value ICHT vs. NT	*P* value HT vs. NT
Number (postmenopause)	26 (24)	25 (22)	23 (22)		
Waist (cm)	81.8 ± 11.2	82.5 ± 10.6	90.3 ± 10.8	0.65	0.008
Diabetes	2 (7.7)	1 (4.0)	1 (4.3)	0.52	0.55
Impaired glucose tolerance	2 (7.7)	9 (36.0)	3 (12.0)	0.018	0.56
Unadjusted values					
Fasting glucose (mmol/l)	4.8 ± 0.3	4.9 ± 0.2	4.9 ± 0.4	0.14	0.37
Fasting insulin (mmol/l)	3.9 (2.7–6.2)	4.2 (3.2–5.1)	4.9 (3.6–5.7)	0.68	0.07
2 hPG (mmol/l)	5.7 (4.5–6.5)	6.8 (5.9–8.6)	6.6 (5.6–7.5)	0.008	0.10
Adjusted values [β (95% CI)][a]					
HOMA_IR	0.85 (0.71–1.01)	0.91 (0.76–1.09)	0.94 (0.77–1.15)	0.56	0.44
2 hPG (mmol/l)	5.8 (5.2–6.5)	7.0 (6.3–7.9)	6.3 (5.5–7.1)	0.016	0.39
Glucose AUC (mmol/l, 120 min)	817 (751–888)	978 (898–1066)	880 (800–968)	0.003	0.26
Insulin AUC (mmol/l, 120 min)	5366 (4314–6676)	5898 (4727–7359)	6084 (4766–7765)	0.54	0.46

Data presented as mean ± standard deviation or number (%). 2hPG, 2-h plasma glucose post-glucose load; AUC, area under curve; HOMA_IR, homeostasis model of insulin resistance; HT, hypertension; ICHT, isolated clinic hypertension; NT, normotension. [a]Estimated geometric means β and (95% CI) for postmenopausal female study average age of 58 years and average waist 84.9 cm.

rise in normalized high frequency, from unconstrained to constrained breathing occurred in the ICHT group, but these were not significantly different from the changes seen in the normotensive group.

The mean increases in DBP with the mental stress test were 12 mmHg (IQR 6–18) in normotensive, 21 mmHg (IQR 15–27) in ICHT ($P = 0.01$ vs. normotensive) and 17 mmHg (IQR 10–23) in the hypertensive group ($P =$ NS vs. normotensive). There were no significant differences in handgrip blood pressure responses among the three groups (Table 6).

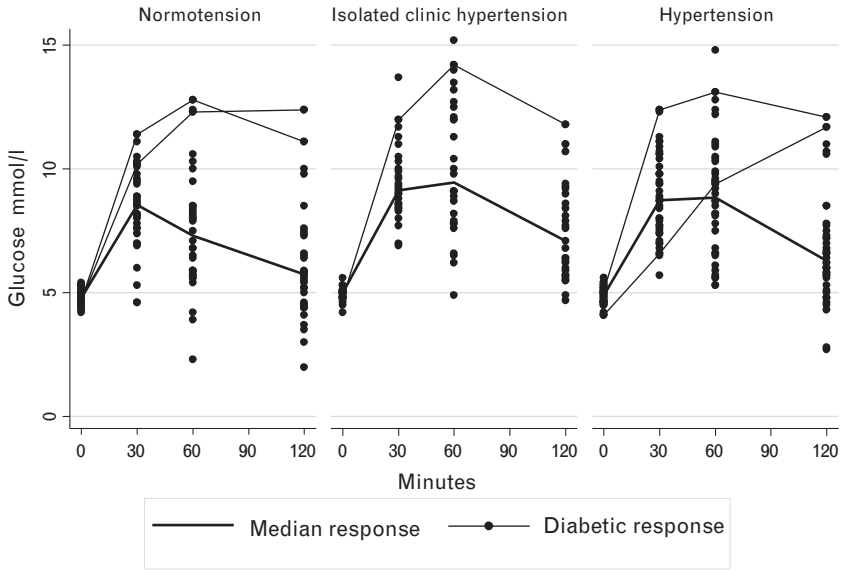
There were no significant differences in numbers of patients with autonomic dysfunction: six (17%) in normotensive, seven (24%) in ICHT and five (13%) in the hypertensive group (Table 6).

Mean values for circulating inflammation markers (hsCRP, PAI-1) and endothelial function (vWF, ADMA) were not significantly different in the three groups (Table 7).

Discussion

The study found that in an ICHT group, with normal fasting plasma glucose, the 2hPG glucose was significantly elevated compared to a normotensive group, similar for age and mean waist circumference. Women were predominant in this study, in particular in the ICHT group, and a separate analysis of women, with the additional adjustment of menopausal status again showed that the 2hPG was significantly elevated in the ICHT group compared to normotension.

Fig. 1



Graph of glucose profiles over time.

Table 5 Pulse wave velocity

PWVc (m/s)	NT	ICHT	HT		
>12	0	0	3 (7.5)		
9–12	5 (13.9)	6 (20.7)	15 (37.5)		
				P value ICHT vs. NT	*P* value HT vs. NT
Adjusted values [β (95% CI)][a]	7.8 (7.4–8.2)	8.0 (7.6–8.4)	8.6 (8.2–9.0)	0.38	0.006

CI, confidence interval; HT, hypertension; ICHT, isolated clinic hypertension; NT, normotension; PWV_c, central pulse wave velocity; PWVc, number (%). [a] Estimated mean values (95% CI) for PWVc adjusted for average study population arterial pressure (95 mmHg) and age (56 years).

The prevalence of IGT in the ICHT group (38%) was much higher than expected for age and sex [14], and could not be readily attributed to the metabolic syndrome, whether defined on observed or ambulatory blood pressure measurements. The ICHT group displayed a similar median glucose profile across time as the hypertensive group. Two previous studies have examined the 2hPG in the ICHT population. A Turkish study, which enrolled consecutive clinic patients aged 15–75 years, including smokers, diabetic patients not on insulin and patients with known coronary artery disease, reported a prevalence of IGT or diabetes of 17.2% in the ICHT group ($n = 324$) compared with 7.2% in the normotensive group ($n = 510$) and 32.2% in the hypertensive group ($n = 121$) [15]. However, in that study ABPM was only performed on a single occasion in patients with high home or office blood pressure and no information was given regarding antihypertensive medication use. In the 20-year follow-up of participants in the Uppsala longitudinal study of adult Swedish men, aged 70 years, patients with ICHT had an average 2hPG of 7.9 mmol/l compared to 6.9 mmol/l in the normotensive group and 8.2 mmol/l in the hypertensive group [15]. The analysis excluded individuals taking antihypertensive medication but included smokers. The results of the present study in nonsmoking, nondiabetic individuals who were not taking antihypertensive therapy, are in substantial agreement with the results of these two studies.

The question arises, as to whether this apparent link between glucose metabolism and blood pressure is mediated through insulin resistance or some other mechanism. The AusDiab and Mauritian studies showed that the 2hPG predicted incident hypertension at 5 years, independent of baseline anthropometric measures, fasting plasma glucose and insulin sensitivity at baseline as assessed by the homeostasis model [5,6]. However, euglycaemic hyperinsulinaemia clamp studies have shown that the 2hPG is associated with peripheral insulin resistance [17,18], which is known to be present in patients with untreated hypertension [19]. Measures of liver and pancreatic insulin resistance – fasting plasma glucose, insulin HOMA and insulin AUC – were not significantly different between the hypertensive and the

Table 6 Measures of autonomic function

	NT	ICHT β (95% CI)	HT	*P* value ICHT vs. NT	*P* value HT vs. NT
LF_nu (%)	44.8 (35.8–53.8)	46.5 (38.1–54.9)	48.9 (40.4–57.5)	0.72	0.36
HF_nu (%)	55.2 (46.2–64.2)	53.5 (45.1–61.9)	51.1 (42.5–59.6)		
Controlled respiration 0.25 Hz					
LF_nu Δ	−3.4 (−13.4–6.5)	−10.5 (−20.5–0.5)	−4.5 (−14.4–5.3)	0.19	0.84
HF_nu Δ	3.3 (−6.2–12.8)	11.2 (1.6–20.8)	4.4 (−5.1–13.9)		
MST, MAP difference	12 (6–18)	21 (15–27)	17 (10–23)	0.01	0.20
Grip test, DBP difference	18 (13–24)	14 (10–19)	19 (13–24)	0.24	0.91
Autonomic score					
Number abnormal (%)	6 (17)	7 (24)	5 (13)	0.45	0.64

DBP, diastolic blood pressure; HT, hypertension; ICHT, isolated clinic hypertension; MAP, mean arterial pressure; MST, Mental Stress Test; NT, normotension. Estimated coefficients are for a mean age of 56 years, female sex and nonalcohol use. LF_nu, normalized low frequency, HF_nu, normalized high frequency. LF_nu Δ and HF_nu Δ estimated coefficients for mean age of 56 years, female sex and nonalcohol use, with adjustment for baseline LF_nu or HF_nu.

Table 7 Measures of circulating biomarkers

	NT	ICHT β (95% CI)	HT	*P* value ICHT vs. NT	*P* value HT vs. NT
hsCRP (mg/l)	1.7 (1.1–2.7)	1.3 (0.9–2.1)	1.1 (0.8–1.7)	0.41	0.16
PAI-1 (U/ml)	3.17 (2.84–3.81)	3.16 (2.77–3.61)	3.11 (2.77–3.48)	0.99	0.81
ADMA (μmol/l)	0.75 (0.62–0.88)	0.78 (0.65–0.90)	0.80 (0.67–0.94)	0.75	0.54
vWF (U/ml)	1.10 (0.93–1.27)	1.08 (0.90–1.26)	1.10 (0.95–1.25)	0.88	0.97

ADMA, asymmetric dimethylarginine; CI, confidence interval; hsCRP, high-sensitivity C-reactive protein; HT, hypertension; ICHT, isolated clinic hypertension; NT, normotension; PAI-1, plasminogen activator inhibitor 1; vWF, von Willebrand factor. hsCRP and PAI-1 log-transformed for analysis. hsCRP adjusted for mean waist, PAI-1 adjusted for mean waist, ADMA adjusted for alcohol use.

normotensive groups in our study. This may be due to the constraint on fasting plasma glucose of less than 5.6 mmol/l. However, these measures were generally associated with the study population systolic blood pressures (unobserved or ABP), indicating the importance of insulin resistance in relation to blood pressure. Longitudinal studies have shown that the 2hPG better predicted cardiovascular events and mortality than fasting plasma glucose [20–23], HbA_{1c} [22,23] or the presence of the metabolic syndrome [24]. The Stop Non-Insulin Dependent Diabetes Mellitus study which randomized patients with IGT to acarbose or placebo therapy, showed a beneficial effect for acarbose therapy, with a reduction in cardiovascular events and hypertension, but the number of events were small [9]. Further studies are required to test the hypothesis that targeting the 2hPG is beneficial for reducing risk of future hypertension and cardiovascular events.

The prevalence of the metabolic syndrome in hypertension is elevated compared to normotenive individuals [25] and in this study we found that the proportion of hypertensive patients with the metabolic syndrome was high whether defined by clinic (40%) or mean day 24-h ABPM (52.5%). Population studies have shown that ICHT patients are more likely to have metabolic abnormalities compared to normotensive individuals [1,2,16,26]. To test the hypothesis that ICHT is related to the metabolic syndrome Helvaci *et al.* [27] examined the effects on blood pressure of treating hyperglycaemia in overweight patients with ICHT. Patients were given the choice of metformin 850 mg t.d.s. or a low-calorie diet to reduce their weight. Compared to the diet-alone group, the metfomin group had significantly higher rates of resolved ICHT, resolved excess weight and obesity as well as lowered lipid parameters over the 6-month period of study [28]. The results of this study need to be interpreted with caution given the lack of randomization of patients to the two groups. Moreover the present study challenges this hypothesis as the numbers of patients with the metabolic syndrome, whether defined on the basis of observed or ambulatory daytime average blood pressure, were similar in the normotensive and ICHT groups.

Previous studies that have looked at PWVc in ICHT have described increased mean values compared to normotensive groups [29,30], but in these studies PWVc values were not adjusted for the critical variables of age and mean arterial pressure, which are well established important determinants of PWVc [31]. In the present study PWVc, when adjusted for age and MAP, was increased in the hypertensive group but there was no significant difference in the ICHT group compared to the normotensive group. A plausible explanation for these results is that the apparent 24-h blood pressure load, to which the central arterial system is exposed, is clearly different between hypertensive and normotensive but not between ICHT and normotensive groups and it is the sustained blood pressure load that contributes to increased arterial stiffness.

A number of studies have examined autonomic function in the ICHT group compared to normotension [32–40]. Increased efferent sympathetic activity as detected by peroneal nerve recordings has been reported in patients with ICHT but to a lesser degree than in hypertensive patients [32,33]. Heart rate variability studies in ICHT patients have given ambiguous results [34–37]. Two mental stress studies have found the ICHT group had a greater blood pressure rise than normotension [38] and hypertension [39], whereas Garcia-Vera *et al.* [40] found no significant difference between ICHT and normotension. In the present study a range of autonomic function tests were evaluated; only the mental stress test induced a significantly different response in the ICHT group compared to normotension. Mental stress test response predicts incident hypertension and is considered an adrenergic response [41].

In a previous review of studies that have examined circulating biomarkers in ICHT, inconsistent results were evident for vWF, CRP and ADMA between ICHT and normotensive groups; two studies have reported increased mean PAI-1 levels in ICHT [42]. In the present study levels of circulating biomarkers were similar in the three groups. Studies have shown that hsCRP is associated with levels of hypertension [43] and incident hypertension [44]. We did not find a difference in hsCRP between the hypertensive groups and the normotensive group but this may be due to the small sample size and the low cardiovascular risk population examined.

The limitations to this study include its cross-sectional design and potential selection bias as all normotensive individuals were recruited by advertisement, whereas the majority of the ICHT patients were recruited from a blood pressure monitoring service. Data on family history of diabetes were not available for all participants.

In conclusion, isolated clinic hypertension patients, with normal fasting plasma glucose and similar metabolic profile to normotensive individuals, have a high prevalence of IGT and a greater blood pressure response to a mental stress test. This may help to explain why ICHT patients are more likely to develop future diabetes and hypertension than normotensive individuals. Assessment of total cardiovascular risk in patients with ICHT should include measurement of postprandial glucose.

Acknowledgement

There are no conflicts of interest.

References

1 Sega R, Trocino G, Lanzarotti A, Carugo S, Cesana G, Schiavina R, *et al.* Alterations of cardiac structure in patients with isolated office, ambulatory, or home hypertension: data from the general population (Pressione Arteriose Monitorate E Loro Associazioni [PAMELA] Study). *Circulation* 2001; **104**:1385–1392.

2 Niiranen TJ, Jula AM, Kantola IM, Reunanen A. Prevalence and determinants of isolated clinic hypertension in the Finnish population: the Finn-HOME study. *J Hypertens* 2006; **24**:463–470.
3 Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Polo Friz H, *et al.* Long-term risk of sustained hypertension in white-coat or masked hypertension. *Hypertension* 2009; **54**:226–232.
4 Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Grassi G, Sega R. Increased long-term risk of new-onset diabetes mellitus in white-coat and masked hypertension. *J Hypertens* 2009; **27**:1672–1678.
5 Boyko EJ, Barr EL, Zimmet PZ, Shaw JE. Two-hour glucose predicts the development of hypertension over 5 years: the AusDiab study. *J Hum Hypertens* 2008; **22**:168–176.
6 Boyko EJ, Shaw JE, Zimmet PZ, Chitson P, Tuomilehto J, Alberti KG. A prospective study of glycemia, body size, insulin resistance and the risk of hypertension in Mauritius. *J Hypertens* 2008; **26**:1742–1749.
7 Barr EL, Boyko EJ, Zimmet PZ, Wolfe R, Tonkin AM, Shaw JE. Continuous relationships between nondiabetic hyperglycaemia and both cardiovascular disease and all-cause mortality: the Australian Diabetes, Obesity, and Lifestyle (AusDiab) study. *Diabetologia* 2009; **52**:415–424.
8 Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events: a metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. *Diabetes Care* 1999; **22**:233–240.
9 Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M. STOP-NIDDM Trial Research Group. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. *JAMA 2003;* **290***:486–494.*
10 Alberti KG, Zimmet P, Shaw J. Metabolic syndrome: a new world-wide definition. A Consensus Statement from the International Diabetes Federation. *Diab Med* 2006; **23**:469–480.
11 WHO. *Definition, diagnosis and classification of diabetes mellitus and its complications; part 1: diagnosis and classification of diabetes mellitus.* Geneva, Switzerland: World Health Organisation, Department of Noncommunicable Disease Surveillance; 1999.
12 Ho LT, Chou TY, Chen RL, Deng PR, Lin WH, Jap TS, Tsai ST. The diagnostic criteria of diabetic autonomic neuropathy. *Proc Natl Sci Counc ROC* 1982; **6**:286–291.
13 Wolfe R, Carlin JB. Sample-size calculation for a log-transformed outcome measure. *Control Clin Trials* 1999; **20**:547–554.
14 DECODE Study Group. Age- and sex-specific prevalences of diabetes and impaired glucose regulation in 13 European Cohorts. *Diab Care* 2003; **26**:61–69.
15 Helvaci MR, Kaya H, Duru M, Yalcin A. What is the relationship between white coat hypertension and dyslipidemia? *Int Heart J* 2008; **49**:87–93.
16 Bjorklund K, Lind L, Vessby B, Andren B, Lithell H. Different metabolic predictors of white-coat and sustained hypertension over a 20-year follow-up period: a population-based study of elderly men. *Circulation* 2002; **106**:63–68.
17 DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique, a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**:E214–E223.
18 Meyer C, Pimenta W, Woerle HJ, Van Haeften T, Szoke E, Mitrakou A, Gerich J. Different mechanisms for impaired fasting glucose and impaired postprandial glucose tolerance in humans. *Diabetes Care* 2006; **29**:1909–1914.
19 Natali A, Santoro D, Palombo C, Cerri M, Ghione S, Ferrannini E. Impaired insulin action on skeletal muscle metabolism in essential hypertension. *Hypertension* 1991; **17**:170–178.
20 DECODE Study Group, the European Diabetes Epidemiology Group. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2 h diagnostic criteria. *Arch Intern Med* 2001; **161**:397–405.
21 Tominaga M, Igarashi K, Eguchi H, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease but not impaired fasting glucose. *Diab Care* 1999; **22**:920–924.
22 de Vegt F, Dekker JM, Ruhe HG, Stehouwer CD, Nijpels G, Bouter LM, Heine RJ. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999; **42**:926–931.
23 Meigs JB ND, D'Agostino RB, Wilson PW. Fasting and postchallenge glycaemia and cardiovascular disease risk: The Framingham Offspring Study. *Diab Care* 2002; **25**:1845–1850.
24 Lin HJ, Lee BC, Ho YL, Lin YH, Chen CY, Hsu HC, *et al.* Postprandial glucose improves the risk prediction of cardiovascular death beyond the metabolic syndrome in the nondiabetic population. *Diab Care* 2009; **32**:1721–1726.
25 Mancia G, Parati G, Borghi C, Ghironzi G, Andriani E, Marinelli L, *et al.* Hypertension prevalence, awareness, control and association with metabolic abnormalities in the San Marino population: the SMOOTH study. *J Hypertens* 2006; **24**:837–843.
26 Julius S, Mejia A, Jones K, Krause L, Schork N, van de Ven C, *et al.* 'White coat' versus 'sustained' borderline hypertension in Tecumseh, Michigan. *Hypertension* 1990; **16**:617–623.
27 Helvaci MR, Kaya H, Seyhanli M, Yalcin A. White coat hypertension in definition of metabolic syndrome. *Int Heart J* 2008; **49**:449–457.
28 Helvaci MR, Sevinc A, Camci C, Yalcin A. Treatment of white coat hypertension with metformin. *Int Heart J* 2008; **49**:671–679.
29 Silva JA, Barbosa L, Bertoquini S, Maldonado J, Polonia J. Relationship between aortic stiffness and cardiovascular risk factors in a population of normotensives, white-coat normotensives, white-coat hypertensives, sustained hypertensives and diabetic patients. *Rev Port Cardiol* 2004; **23**:1533–1547.
30 Ribeiro L, Gama G, Santos A, Asmar R, Martins L, Polonia J. Arterial distensibility in subjects with white-coat hypertension with and without diabetes or dyslipidaemia: comparison with normotensives and sustained hypertensives. *Blood Press Monit* 2000; **5**:11–17.
31 McEniery CM, Wilkinson IB, Avolio AP. Age, hypertension and arterial function. *Clin Exp Pharmacol Physiol* 2007; **34**:655–671.
32 Grassi G, Seravelle G, Quarti Trevano F, Dell'Oro R, Bolla G, Cuspidi C, *et al.* Neurogenic abnormalities in masked hypertension. *Hypertension* 2007; **50**:537–542.
33 Smith PA, Graham LN, Mackintosh AF, Stoker JB, Mary DA. Relationship between central sympathetic activity and stages of human hypertension. *Am J Hypertens* 2004; **17**:217–222.
34 Madsen LB, Rasmussen JK, Moller DS, Nyvad O, Pedersen EB. Heart rate variability in white-coat hypertension. *Blood Press Monit* 2008; **13**:65–71.
35 Fagard RH, Stolarz K, Kuznetsova T, Seidlerova J, Tikhonoff V, Grodzicki T, *et al.* Sympathetic activity, assessed by power spectral analysis of heart rate variability, in white-coat, masked and sustained hypertension versus true normotension. *J Hypertens* 2007; **25**:2280–2285.
36 Neumann SA, Jennings JR, Muldoon MF, Manuck SB. White-coat hypertension and autonomic nervous system dysregulation. *Am J Hypertens* 2005; **18**:584–588.
37 Pierdomenico SD, Bucci A, Costantini F, Lapenna D, Cuccurullo F, Mezzetti A. Twenty-four-hour autonomic nervous function in sustained and 'white coat' hypertension. *Am Heart J* 2000; **140**:672–677.
38 Pannarale G, Isea JE, Coats AJ, Conway J, Sleight P. Cardiac and blood pressure response to mental stress test in reactive hypertensives. *Clin Exp Hypertens* 1991; **A13**:1–12.
39 Nakao M, Shimosawa T, Nomura S, Kuboki T, Fujita T, Murata K. Mental arithmetic is a useful diagnostic evaluation in white coat hypertension. *Am J Hypertens* 1998; **11**:41–45.
40 Garcia-Vera MP, Sanz J, F.J. L. Orienting-defense responses and psychophysiological reactivity in isolated clinic versus sustained hypertension. *Clin Experiment Hypertens* 2007; **29**:175–188.
41 Flaa A, Eide IK, Kjeldsen SE, Rostrup M. Sympathoadrenal stress reactivity is a predictor of future blood pressure: an 18-year follow-up study. *Hypertension* 2008; **52**:336–341.
42 Martin C, Cameron J, McGrath B. Mechanical and circulating biomarkers in isolated clinic hypertension. *Clin Exp Pharmacol Physiol* 2008; **35**:402–408.
43 Zhang Y, Thompson AM, Tong W, Xu T, Chen J, Zhao L, *et al.* Biomarkers of inflammation and endothelial dysfunction and risk of hypertension among inner Mongolians in China. *J Hypertens* 2010; **28**:35–40.
44 Sung KC, Suh JY, Kim BS, Kang JH, Kim H, Lee MH, *et al.* High sensitivity C-reactive protein as an independent risk factor for essential hypertension. *Am J Hypertens* 2003; **16**:429–433.

Addendum

OBJECTIVE

The published results in Chapter 4 reported the results of an analysis in WCHT participants to determine if WCHT is associated with biomarkers of increased cardiovascular risk unconfounded by the potential cardiovascular risk of hyperglycaemia, using a conservative fasting plasma glucose cut-off of 5.6 mmol/l. This study encompassed a number of other elements which could not be included in the publication because of space and specificity constraints, the results of which are now presented. This addendum reports on:

- Results related to blood pressure (BP) measurement methods, questioning which BP in WCHT best approximates the patients 'true' BP, as defined by mean daytime ambulatory blood pressure measurement (ABPM).
- Results related to autonomic function measurements. The published paper in this chapter reported on the results of an autonomic score generated from Ewing's test.[1] Here we report the individual results of each of the Ewing's tests. This addendum also examines if autonomic function is associated with alcohol or glucose status.

This addendum also addresses these additional questions:

- Is WCHT associated with altered brachial artery flow-mediated dilation?
- Is WCHT with impaired glucose tolerance associated with increased artery stiffness or altered brachial artery flow-mediated dilatation?
- Is WCHT associated with plasma leptin?
- Is WCHT associated with proteinuria?

METHODS

The methodology is as per Chapter three.

RESULTS

4.1 Blood pressure measurements.

The normotensive group displayed normal mean BP for all categories of measurement (See table 4.1). The WCHT group displayed labile mean BP dependent on the BP measurement. The hypertensive group tended to remain hypertensive at all measures.

Table 4.1: Blood pressure measurements of study participants.

Blood pressure (mmHg)	Normotensive	WCHT	Hypertensive
Number of participants	44	30	53
BP on arrival (first reading)	124/80±10/8	135/88±15/8	147/93±19/10
Observed automated BP	120/80±9/8	132/86±9/8	143/92±18/9
Unobserved automated BP	116/77±9/6	123/81±9/8	136/88±14/9
BP immediately post PWV measurement	119/70±14/7	132/77±13/9	146/82±19/11
First BP ABPM application	123/76±12/9	134/84±13/10	150/89±19/8
24h day	122/73±8/6	125/76±6/5	143/85±9/6
24h night	104/59±8/6	105/60±6/5	122/69±13/9

Data are mean ± standard deviation. BP: blood pressure, ABPM: ambulatory blood pressure monitoring, PWV: pulse wave velocity

The sitting observed automated BP was elevated in WCHT and sustained hypertensives. The sitting unobserved automated BP (UABP) dropped in all three BP categories and was similar to mean 24 hour day ABPM in the normotensive and WCHT groups, whereas the observed BP was similar to mean 24 hour day ABPM in the hypertensive group. These results were reported in a letter published in Hypertension regarding our concerns about an algorithm designed to determine BP status based solely on UABP (See Appendix one).

4.2 Central pulse wave velocity (PWVc)

Table 4.2 shows the result of analysis of PWVc, adjusted for confounders. No significant difference was found between WCHT and normotensive subjects, however median PWVc for the WCHT group was intermediate between normotensive and hypertensive subjects.

Table 4.2: Pulse wave velocity analysis.

	Number	PWV$^{\Omega}$	PWV†	P-value$^{¥}$
Normotensive	44	7.2 [6.4-8.1]	7.6 {7.2-8.0}	
WCHT	30	8.1 [7.2-9.1]	8.0 {7.6-8.5}	0.19
Hypertensive	53	8.9 [7.9-10.7]	8.8 {8.4-9.2}	<0.001

Data are $^{\Omega}$median [inter-quartile range] and †geometric mean {95% confidence interval} adjusted for age and mean arterial pressure, ¥: versus normotensive group.

PWVc was not significantly different in the WCHT participants with impaired glucose tolerance compared to WCHT participants with normoglycaemia (See Table 4.3).

Table 4.3: Pulse wave velocity in WCHT by glucose status.

	Number	PWVc[Ω]	PWVc†	P-value[¥]
WCHT-normoglycaemia	17	7.8 [7.2-8.9]	7.9 {7.4-8.5}	
WCHT-IGT	9	8.5 [7.0-9.1]	8.0 {7.2-8.8}	0.85

Data are [Ω]median [inter-quartile range] and [†]geometric mean {95% confidence interval} adjusted for age and mean arterial pressure, IGT: impaired glucose tolerance, ¥: versus WCHT-normoglycaemia.

4.3 Brachial artery flow mediated dilation (FMD)

No difference was found between normotensives and WCHT (P=0.95) in FMD, adjusting for age and baseline diameter (See Table 4.4).

Table 4.4: Flow-mediated dilation with measures taken end diastole

	N	Baseline diameter[£] (mm)	60secs diameter (mm)	Δ diameter (mm)	Δ diameter (%)	Adjusted Δ diameter [Σ]	P value[¥]
Normotensive	33	0.386	0.417	0.030	8.10	0.028 (0.022-0.034)	
WCHT	19	0.380	0.409	0.032	7.75	0.028 (0.021-0.036)	0.95
Hypertensive	19	0.396	0.428	0.029	8.07	0.027 (0.021-0.036)	0.93

N: Number, £: measure taken end diastole, Δ: Difference, Σ: Adjusted for confounder's age and baseline diameter, ¥versus normotensive group.

No difference was found in FMD in the WCHT group by impaired glucose status (See Table 4.5).

Table 4.5: Flow-mediated dilation in WCHT by glucose status

	N	Baseline diameter (mm)	60secs diameter£ (mm)	Δ diameter (mm)	Δ diameter (%)	Adjusted Δ diameter Σ	P value¥
WCHT	9	0.36	0.39	0.03	8.3	0.03 {0.02-0.05}	
WCHT-IGT	7	0.36	0.38	0.02	5.6	0.02 {0.01-0.03}	0.14

N: Number, £: measure taken end diastole, Δ: Difference, Σ: Adjusted for confounder's age and baseline diameter, IGT: impaired glucose tolerance, ¥: versus normotensive group.

4.4 Autonomic function test results.

The heart rate response to controlled deep breathing, lying to standing and Valsalva ratio tended to be reduced in diabetics (See Tables 4.4 to 4.6). The number of diabetics was small so statistical difference was not tested. No discernible difference was found in the three autonomic measures by alcohol category. No difference in heart rate response to controlled deep breathing, lying to standing and Valsalva ratio was found between WCHT and normotensives, adjusting for age.

Figures 4.1 to 4.3 show the variation in heart rate and blood pressure response to standing, slow-controlled breathing and the Valsalva Manoeuvre, by age categorization, for all subjects. In Figure 4.3 the duration of the Valsalva Manoeuvre is marked by two vertical lines. The beginning of Valsalva is at time zero and the end of Valsalva is at 15 seconds. Median response lines are highlighted.

Table 4.6: Heart rate response to standing: measure 30:15 ratio and maximum/minimum ratio.

	N	30:15 ratio	P-value†Ω€	Maximum/Minimum	P-value†¥€
All	130	1.24 [1.13-1.34]		1.29 [1.19-1.40]	
Normoglycaemia	91	1.25 [1.16-1.35]		1.31 [1.22-1.40]	
IGT	21	1.21 [1.10-1.34]	0.89	1.31 [1.19-1.41]	0.88
IFG	3	1.26 [1.13-1.35]	NT	1.35 [1.14-1.47]	NT
Diabetes	8	1.16 [1.10-1.30]	NT	1.23 [1.19-1.38]	NT
No alcohol	44	1.24 [1.10-1.32]		1.28 [1.21-1.39]	
Moderate alcohol	62	1.23 [1.16-1.34]	0.22	1.29 [1.21-1.41]	0.61
Heavy alcohol	20	1.24 [1.12-1.34]	0.73	1.32 [1.19-1.37]	0.54
Normotensives	41	1.27 [1.14-1.39]		1.33 [1.19-1.48]	
WCHT	36	1.24 [1.18-1.34]	0.99	1.29 [1.22-1.34]	0.78
Hypertensives	57	1.22 [1.13-1.32]	0.51	1.29 [1.21-1.37]	0.56

Data are median [inter-quartile range]. †Diabetics removed from analysis of blood pressure category, data log-transformed, ¥: P value versus first (normoglycaemic, no alcohol or normotensive) group, €: adjusted for age, NT: not tested.

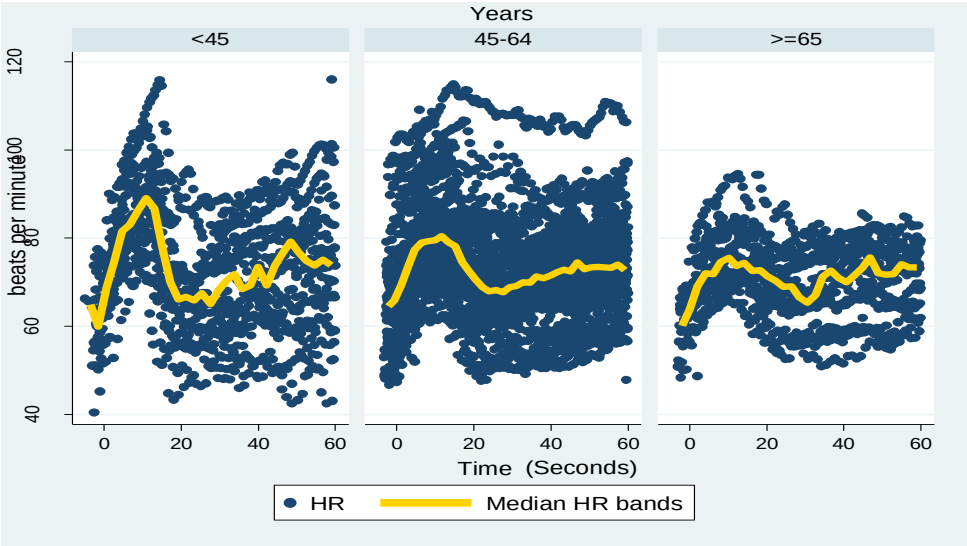


Figure 4.1. Scatter graph of heart rate response to standing by age categorisation (all subjects)

Table 4.7: Heart rate response to a single deep breath and for six controlled deep breaths.

	N	Single deep breath	P Value¥	Six breaths/minute	P Value¥
All	129	14.4 [9.7-19.7]		12.1 [7.9-16.7]	
Normoglycaemia	90	14.5 [10.6-19.3]		12.4 [7.8-17.1]	
IGT	24	13.9 [8.0-21.7]	0.75	11.8 [8.7-18.4]	0.79
IFG	3	15.8 [9.8-24.3]	NT	9.9 [8.3-13.1]	NT
Diabetes	8	13.0 [8.2-19.0]	NT	9.4 [7.7-11.7]	NT
No alcohol	44	15.1 [11.0-20.6]		12.7 [9.1-17.1]	
Moderate alcohol	62	13.7 [9.2-20.1]	0.78	11.5 [7.9-15.1]	0.99
Heavy alcohol	20	14.6 [8.4-19.0]	0.77	12.6 [6.3-15.3]	0.30
Normotensives	41	14.5 [11.1-18.1]		12.2 [7.8-14.7]	
WCHT	30	15.1 [10.1-21.3]	0.72	13.5 [6.8-17.6]	0.14
Hypertensives	45	14.4 [9.2-18.9]	0.50	12.4 [9.4-16.7]	0.20

Data are median [inter-quartile range]. †Diabetics removed from analysis of blood pressure category, data log-transformed. ¥: P value versus first (normoglycaemic, no alcohol or normotensive) group, €: adjusted for age, NT: not tested.

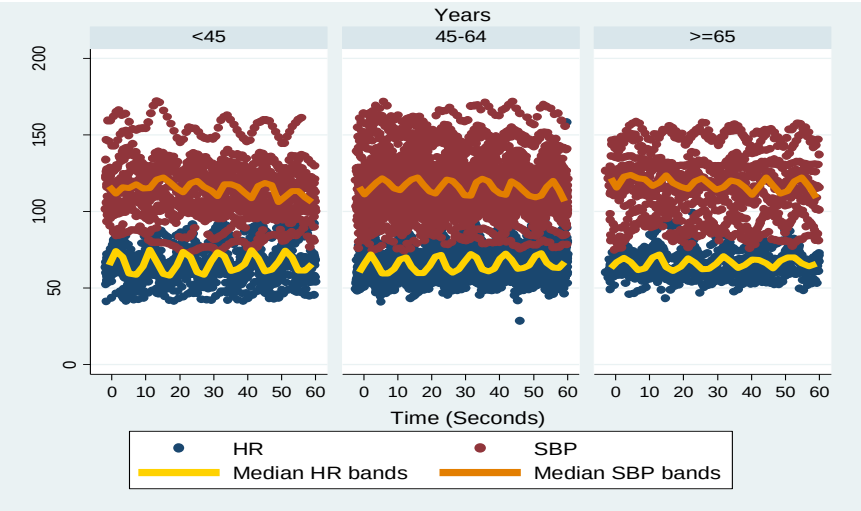


Figure 4.2. Scatter graph of heart rate and blood pressure response to slow deep breathing (all subjects)

Table 4.8: Heart rate response to the Valsalva Manoeuvre: average of three manoeuvres and the maximum of the three.

	Number	Maximum Valsalva	P value¥	Average of three Valsalvas	P value¥
All	130	1.45 [1.30-1.67]		1.38 [1.23-1.58]	
Normoglycaemia	91	1.45 [1.31-1.76]		1.38 [1.25-1.65]	
IGT	24	1.47 [1.22-1.63]	0.44	1.43 [1.20-1.57]	0.65
IFG	3	1.44 [1.41-153]	NT	1.33 [1.33-1.36]	NT
Diabetes	8	1.46 [1.26-1.53]	NT	1.36 [1.19-149]	NT
No alcohol	44	1.43 [1.29-1.76]		1.35 [1.23-1.69]	
Moderate alcohol	62	1.48 [1.35-1.60]	0.51	1.38 [1.25-1.53]	0.60
Heavy alcohol	20	1.43 [1.17-1.57]	0.39	1.38 [1.16-1.45]	0.24
Normotensives	40	1.45 [1.39-1.65]		1.38 [1.28-1.56]	
WCHT	30	1.56 [1.36-1.79]	0.12	1.47 [1.26-1.72]	0.21
Hypertensives	47	1.43 [1.26-1.55]	0.93	1.36 [1.20-1.56]	0.91

Data are median [inter-quartile range]. †Diabetics removed from analysis of blood pressure category, data log-transformed, ¥: P value versus first (normoglycaemic, no alcohol or normotensive) group, NT: not tested.

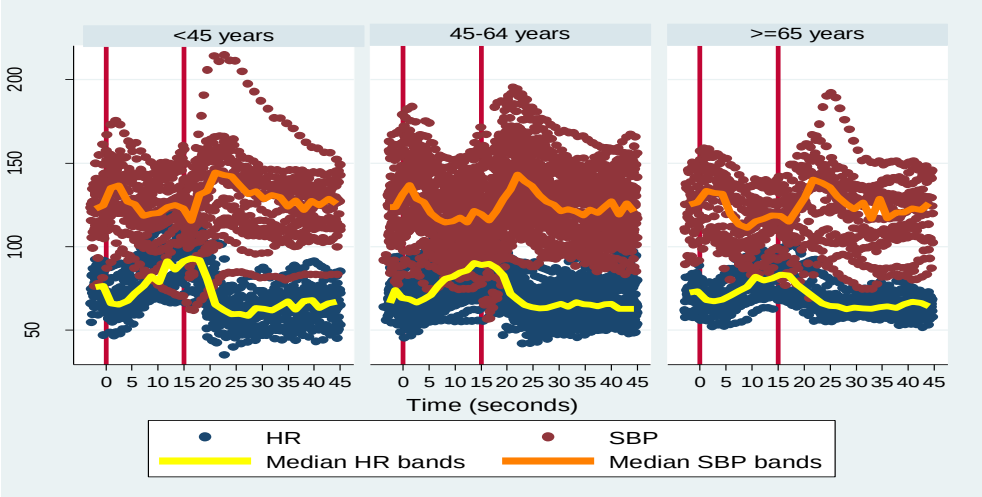


Figure 4.3. Scatter graph of heart rate and blood pressure response to Valsalva Manoeuvre (all subjects).

4.5 Proteinuria

Table 4.9 displays the results of an analysis of a sample of our participants, age and sex matched. No difference was found between normotensives and WCHT subjects.

Table 4.9: Microalbuminuria by blood pressure status.

	N	Urinary albumin mg/l	Number ≥ 20 mg/l	ACR (mg/g)	p^{Ω}
Normotensive	15	2.5 [2.0-3.2]	0	2.46 [0.003- 4.13]	
WCHT	14	4.5 [2.8-6.5]	0	0.005 [0.003-2.55]	0.48
Hypertensive	20	2.7 [2.1-5.2]	1	0.005 [0.003- 4.75]	0.18

Data are median [inter-quartile range]. ACR: Albumin-creatinine ratio. Σ: data log-transformed prior to analysis, Ω: versus normotensive group.

4.6 Leptin

No difference was found in log-transformed leptin between normotensives and WCHT (P=0.71), adjusting for waist circumference (See Table 4.10).

Table 4.10: Leptin levels.

	Number	$Leptin^{\Omega}$	$Leptin^{\dagger}$	P $Value^{¥}$
Normotensive	39	9.2 [4.9-21.2]	9.7 {7.5-12.6}	
WCHT	26	13.2 [5.5-15.5]	10.5 {7.6-14.5}	0.71
Hypertensive	41	8.3 [5.4-16.0]	7.9 {6.1-10.3}	0.29

Data are $^{\Omega}$median [inter-quartile range] and †geometric mean {95% confidence interval} adjustedfor waist circumference, ¥: versus normotensive group.

No difference in plasma leptin was found in the WCHT group by impaired glucose status.

Table 4.11: Leptin levels in WCHT by glucose status

	Number	Leptin$^{\Omega}$	Leptin†	P Value$^{¥}$
WCHT-normoglycaemia	15	13.4 [3.8-18.0]	10.4 {7.0-15.3}	
WCHT-IGT	7	8.3 [3.2-8.9]	7.8 {4.1-14.8}	0.40

Data are $^{\Omega}$median [inter-quartile range] and †geometric mean {95% confidence interval} adjusted for waist circumference. IGT: impaired glucose tolerance, ¥: versus WCHT-normoglycaemia.

4.9 Summary

The results from this addendum show the following: Clinic UABP best approximates mean day ABPM in subjects with confirmed WCHT. Autonomic function was not found to be different for any of the individual tests that comprised the Ewing battery of tests. No difference in autonomic function was found for alcohol or glucose status for any of the autonomic tests; however in this subgroup analysis the number of participants with diabetes or impaired fasting glucose was small hence statistical testing was not done.

Although not statistically different from normotensives, PWVc in WCHT is intermediated between normotensive and hypertensive groups, a result that is consistent with previous reports.[3, 4] PWVc was not elevated in WCHT with impaired glucose tolerance (IGT) compared to WCHT with normoglycaemia.

No difference was found for FMD, proteinuria or leptin levels between the BP groups.

4.10 References

1. Ewing DJ, Martyn CN, Young RJ and Clarke BF. The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care*. 1985; **8**: 491-8.
2. Alberti KG and Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet. Med*. 1998; **15**: 539-53.
3. Schillaci G, Pucci G, Pirro M, et al. Combined effects of office and 24-h blood pressure on aortic stiffness in human hypertension. *J. Hypertens*. 2011; **29**: 869-75.
4. Silva JA, Barbosa L, Bertoquini S, Maldonado J and Polonia J. Relationship between aortic stiffness and cardiovascular risk factors in a population of normotensives, white-coat normotensives, white-coat hypertensives, sustained hypertensives and diabetic patients. *Rev. Port. Cardiol*. 2004; **23**: 1533-47.

Chapter 5

The morning blood pressure surge is related to serum cholesterol

5.1 Introduction

The morning blood pressure surge is put forth as a possible reason why the morning period is associated with increased cardiovascular events.

The published paper in this chapter reported the results of an analysis to determine if WCHT is associated with an increased morning blood pressure surge, which could put subjects with WCHT at increased cardiovascular risk.

Four common measures of the morning blood pressure rise were used to assess the morning blood pressure surge.

Questions addressed were:

- Is WCHT associated with an increased morning blood pressure surge?
- Are there any factors which show consistent association across the four measures of the morning blood pressure surge?

5.2 Declaration

In the case of Chapter five, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Conception, data collection, analysis of data, writing paper.	80

The following co-authors contributed to the work.

Name	Nature of contribution
Professor Barry McGrath	Conception, writing and review of paper
Professor James Cameron	Conception, writing and review of paper
Professor Geoff Head	Conception, review of paper
Dr Nina Eikelis	Analysis of leptin levels, review of paper
Dr Suzie Chen	Assistance with data collection and review of paper.

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	8/9/13
Main Supervisor's Signature	[redacted]	18.9.13

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ORIGINAL ARTICLE

The morning blood pressure surge is related to serum cholesterol

CA Martin[1,2], JD Cameron[3], GA Head[4], SS Chen[1], N Eikelis[4] and BP McGrath[1,2,5]

A morning blood pressure surge (MBPS) may be either a mechanism for, or a marker of, increased cardiovascular events. This study has examined factors which may influence the morning surge: age, gender, metabolic factors, sympathetic function, blood pressure and arterial stiffness. Four measures of the MBPS were examined—sleep-trough surge, pre-awake surge, rate of blood pressure rise and a Power function. Subjects underwent ambulatory blood pressure monitoring, glucose tolerance test, central pulse wave velocity, sympathetic autonomic function tests (mental stress and sustained handgrip). MBPS was associated with age, hypertension, blood pressure variability and serum lipids. After adjustment for age and waist circumference, all four measures of MBPS remained positively associated with low-density lipoprotein (LDL) cholesterol. The novel finding of a significant relationship between measures of MBPS and LDL-cholesterol is an intriguing link between two major cardiovascular risk factors.

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Keywords: morning blood pressure surge (MBPS); ambulatory blood pressure monitoring; mild hypertension; white-coat hypertension; lipids

INTRODUCTION

Cardiovascular events and sudden cardiac death display a circadian pattern with the peak incidence occurring in the morning period.[1,2] It is postulated that an exaggerated morning blood pressure surge (MBPS) may explain this finding.[3]

The most widely used definitions of a MBPS are the sleep-trough surge and the pre-waking surge,[3,4] both of which are calculated from differences of blood pressure averages over specific time-intervals around waking. A MBPS, by either definition, has been found to be associated with incident cardiovascular events.[5,6,7,8] In a study of elderly hypertensive subjects who had undergone carotid endarterectomy for asymptomatic high-grade lesions, Marfella *et al.*,[9] found that patients with an exaggerated MBPS had unstable atherosclerotic lesion phenotypes compared with those that did not.

Head *et al.*[10] proposed a mathematical approach, using a double logistic curve fitting technique that models the rate of MBPS to define a rate of morning blood pressure rise (RoR). Head *et al.*[11] further determined the 'Power' of the MBPS as the product of the RoR with the day–night blood pressure difference. The RoR has not been validated against cardiovascular event outcome data, whereas the Power function was associated with reduced mortality in a post-hoc analysis of the Second Australian National Blood Pressure study—ANBP2.[12]

The mechanism(s) underlying the rate of MBPS have not been clearly defined. Potential contributors include sympathetic activity[13] and/or activation of the renin–angiotensin system.[14] In the present study, we examine a range of factors, including age, sex, metabolic factors, sympathetic function, blood pressure and central arterial stiffness, using four measures of the MBPS, to assess which factor/s are consistently associated across the measures of the MBPS. This study also examines the four measures of the MBPS in subjects with normotension, white-coat hypertension and newly diagnosed hypertension.

MATERIALS AND METHODS

The study was approved by the institutional human research ethics committee. Adult subjects (40–75 years) were recruited by advertisement in local newspapers and through an ambulatory blood pressure monitoring (ABPM) service, which receives referrals from 120 general practitioners.

Subjects were excluded if they were smokers, had known diabetes, were taking anti-hypertensive medication or had existing liver, renal or cardiovascular disease or any malignancy. Written informed consent was obtained from all the subjects.

Subjects presented in the morning, after a minimum 10 h fast. Each subject completed a questionnaire about previous and present illnesses, family history, medication, diet, physical activity and alcohol habits. Alcohol consumption was categorised into none, mild (one to two standard drinks on any day) or heavy (more than two standard drinks on any day). The metabolic syndrome was defined according to the criteria set out by the International Diabetic Federation Task Force.[15] Glucose status was defined as per World Health Organisation definitions.[16]

Ambulatory blood pressure monitoring

ABPM was performed using a portable device (Meditech Cardiotens, Meditech Ltd, Hungary). ABPM cuff was placed on the non-dominant arm and automatic blood pressure recordings were programmed to occur at 30 min intervals day and night. Normotension was defined as reported clinic blood pressure $<140/90$ mm Hg and day 24 h ABPM $<135/85$ mm Hg and night ABPM $<120/70$ mm Hg. White-coat hypertension was defined as reported clinic blood pressure $\geqslant 140/90$ mm Hg and day 24 h ABPM $<135/85$ mm Hg and night ABPM $<120/70$ mm Hg. Repeat 24 h ABPM within a 12-month period was required in all subjects with white-coat hypertension to confirm this diagnosis. Sustained hypertension

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was defined as reported clinic $\geqslant$140/90 mm Hg and day 24 h ABPM $\geqslant$135/85 mm Hg or night ABPM $\geqslant$120/70 mm Hg.

Recordings were not included in the analysis if reported sleep duration was $<$5 h, if more than one blood pressure recording was missing in the pre-awake or post-awake period, or if there was missing recordings contiguous to the lowest systolic blood pressure (SBP) recorded during sleep. Recordings were also excluded if $<$10 SBP readings were recorded during the sleep period.

Morning blood pressure surge

The sleep-trough MBPS was defined as the morning SBP minus the lowest night-time SBP, where the morning SBP was defined as the average of four 30 min SBP readings just after waking and the lowest night-time SBP was the 1 h average of three SBPs readings centred on the lowest night-time reading.

The pre-awake MBPS was defined as the morning SBP minus the pre-awake SBP, where the pre-awake SBP was the average of four 30 min SBP readings just before wakening.

The morning SBP RoR and the Power function were calculated according to the method of Head *et al.*[11]

SBP variability was assessed by the day s.d. ($s.d._{Day}$), the night s.d. ($s.d._{Night}$), the 24-h weighted s.d. ($s.d._{W}$)[17] and the 24-h absolute real variability ($s.d._{ARV}$).[18]

Assessment of sympathetic function

Autonomic function testing was performed using the Task Force monitor, CNSystems, Graz, Austria. After resting for 2 min an oscillometric blood pressure was taken. Subjects were then instructed to subtract out loud the value 13 from the value 4300. Subjects were instructed to either start again or continue from the current value if they felt they had made a mistake and that no assistance would be given by the examiner. If a subject verbalised an inability to do this subtraction then they were offered the subtraction of 13 from 430. An oscillometric blood pressure measurement was taken at 1 min. The difference in systolic and diastolic blood pressures at 1 min, from the resting systolic and diastolic blood pressures was calculated.

After resting for 2 min an oscillometric blood pressure was taken. Subjects were asked to squeeze a partially inflated cuff connected to a sphygmomanometer and the maximal pressure was noted. Subjects were then asked to squeeze the cuff to one-third of the noted maximum pressure for 5 min, and oscillometric blood pressure measurements were taken on the other arm at 1 min intervals. The difference between resting diastolic blood pressure and the maximum diastolic blood pressure during sustained handgrip was determined.

Central pulse wave velocity

Central aorto-femoral PWV (PWVc) measurements were performed by the same two operators (CAM, SSC), in a quiet air-conditioned room after 10 min of rest and with the subject lying supine. Continuous pulse pressure wave signals were recorded with hand-held tonometers (Millar Mikro-tip, SPT-301; Millar Instruments, Houston, TX, USA) positioned at the base of the right common carotid artery and over the right common femoral artery. Transit distance was defined as the measured distance from the sternal notch to femoral artery minus distance from sternal notch to carotid. The start of systole was defined by the local maximum of the first derivative of the pressure signal. Mean transit time (Δt) between the feet of simultaneously recorded waves was determined from 10 consecutive cardiac cycles. Mean arterial pressure was estimated from an oscillometric blood pressure measurement taken immediately post recording of the arterial waveforms.

Biochemical measures

Blood samples were taken under fasting conditions, following which subjects consumed a 75-g glucose solution over a period of 5 min. Plasma glucose was measured at 120 min post glucose load (2hPG). Blood samples were processed immediately after collection. Plasma glucose was analysed by the glucose oxidase method.

Plasma samples for fasting total plasma cholesterol, triglycerides, low-density lipoprotein (LDL-cholesterol) and high-density lipoprotein (cholesterol) were analysed immediately, whereas plasma for high-sensitivity C-reactive protein and Leptin were stored at -70^{0}C until analysis. Commercial assay kits were used for these analyses.

Statistical analysis

STATA, version 10, (StataCorp LP, College Station, TX, USA) was used to analyse the data. Normally distributed data is expressed as mean $\pm$ s.d., whereas skewed data is expressed as median (interquartile range). Categorical data is expressed as percentages. Non-normal data was transformed before analysis. All comparisons were two-sided. Univariate and multivariate linear regression was used to analyse continuous variables, whereas the chi-squared (χ^2) or Fischer's exact test was used to analyse categorical variables. Univariate and multivariate logistic regression was used to analyse the dichotimisation of each measure of the MBPS at the 90th percentile. Each outcome variable was examined for potential confounders. Analysis of outcome variable was adjusted for each confounder. To prevent co-linearity between related variables, such as the lipid variables, the strongest linearly associated related variable was included in the model. Assessment of MBPS measures, which involved measures of differences, (sleep-trough and pre-awake), were correlated against their baseline measure to assess for regression to the mean. The baseline measures were included in regression analysis if regression to the mean was present. For data, which required log transformation, that contained negative values, the minimum value of the data plus one was added to each value before transformation, to anchor the log-transformed data on zero. The minimum value, plus one, was removed from the back-transformed data to determine the geometric mean. Statistical significance was considered to be met at $\alpha = 0.05$.

RESULTS

One hundred and twenty-two participants met the study criteria. Fifteen participants (12%) were excluded from the analysis as four reported sleep times of $<$5 h and 11 participants had insufficient night or morning SBP readings. Thirty-eight normotensive subjects, 28 white-coat hypertensive subjects and 42 newly diagnosed, untreated hypertensive subjects remained in the analysis. Three subjects had a mean night SBP greater than their mean day SBP. These subjects were excluded from the analysis of the modelled rate of MBPS and its Power function.

As shown in Table 1, subject characteristics, PWVc, sympathetic function and plasma biochemistry were similar in the normotensive and white-coat hypertensive groups, except for an increase in the 2hPG in the white-coat hypertensive group.

The sustained hypertensive group was statistically different from the normotensive and white-coat hypertensive groups with regard to a higher male to female ratio, greater waist circumference, higher triglycerides, lower high-density lipoprotein-cholesterol and the presence of the metabolic syndrome. The sustained hypertensive group had a higher diastolic blood pressure response to the grip-test compared with white-coat hypertensive group ($P = 0.04$) but not compared with the normotensive group. The sustained hypertensive group had a borderline significantly different high-sensitivity C-reactive protein compared with the normotensive group. PWVc, adjusted for mean arterial pressure, was significantly higher in the sustained hypertensive group compared with the normotensive group.

Analysis of the 24-h ABP measurements in the three groups is reported in Table 2. The recorded sleep time was similar in the three groups. The sustained hypertensive group had significantly higher day, night and 24 h s.d. of SBP, as well as higher night minimum average SBP, pre-awake SBP and morning SBP compared with the normotensive and the white-coat hypertensive groups. No difference was found in these measures between the white-coat hypertensive and normotensive groups.

MBPS measurements

The sleep-trough MBPS was right skewed and ranged from -6 to 56 mm Hg, (median 17, interquartile range: 11–26, 90th percentile 33.2 mm Hg). The pre-awake MBPS was normally distributed, ranging from -13 to 43 mm Hg, (mean $\pm$ s.d.: 13 $\pm$ 10, 90th percentile 27.3 mm Hg). The RoR was extremely right skewed and ranged from 0.5 to 39.8 mm Hg per hour, (median 9.29,

Table 1. Subject characteristics, PWVc, sympathetic function and serum biochemistry

	NT (n = 38)	*WCHT (n = 28)*	*HT (n = 42)*	*NT vs WCHT (P-value)*	*NT vs HT (P-value)*	*WCHT vs HT (P-value)*
Age (years)	54 ± 11	55 ± 8	57 ± 10	0.59	0.15	0.44
Female (%)	28 (73.7)	24 (85.7)	23 (54.8)	0.24	0.08	0.01
Menopause (%)	15 (53.6)	12 (50)	16 (69.6)	0.78	0.89	0.69
Alcohol use (%)						
Mild	48.7	42.9	53.7	0.44	0.86	0.46
Heavy	18.9	10.7	14.6			
Lipid-lowering medication (%)	2 (5.3)	2 (7.1)	5 (11.9)	0.57	0.26	0.41
Metabolic syndrome (%)	7 (18.4)	4 (14.3)	19 (45.2)	0.66	0.002	0.001
Waist circumference (cm)	85.2 ± 12.1	83.8 ± 11.0	93.0 ± 9.5	0.61	0.002	< 0.001
BMI ($Kg\,m^{-2}$)[a]	25.3 ± 4.0	25.4 ± 4.1	26.5 ± 3.5	0.95	0.13	0.18
PWVc ($m\,s^{-1}$)[a,b]	7.2 (6.5–8.2)	7.8 (7.2–8.8)	8.9 (7.8–10.6)	0.37	0.03	0.13
Mental stress test − Δ DBP (mm Hg)	8 ± 6	9 ± 6	10 ± 7	0.42	0.21	0.68
Grip-test − Δ DBP[a] (mm Hg)	17 (12–25)	16 (10–21)	19 (13–27)	0.11	0.57	0.04
Fasting glucose ($mmol\,l^{-1}$)[a]	4.9 ± 0.4	5.0 ± 0.4	4.9 ± 0.5	0.37	0.61	0.65
2hPG ($mmol\,l^{-1}$)	5.8 (5.0–7.4)	7.1 (5.7–9.0)	6.3 (5.4–7.8)	0.04	0.58	0.12
Triglycerides ($mmol\,l^{-1}$)[a]	1.1 ± 0.6	1.0 ± 0.5	1.3 ± 0.6	0.56	0.05	0.02
HDL cholesterol ($mmol\,l^{-1}$)	1.4 ± 0.4	1.4 ± 0.4	1.2 ± 0.4	0.88	0.02	0.02
LDL-cholesterol ($mmol\,l^{-1}$)[a]	3.5 ± 1.1	3.4 ± 0.8	3.6 ± 1.0	0.79	0.32	0.52
hs-CRP ($mg\,l^{-1}$)[a]	1.3 (0.4–3.5)	0.7 (0.3–1.5)	1.2 (0.8–2.2)	0.19	0.64	0.08

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C reactive protein; 2hPG, 2 h plasma glucose post 75 g glucose load; LDL, low-density lipoprotein; NT, normotension; PWVc, central pulse wave velocity; WCHT, white-coat hypertension. Results presented as mean ± s.d. or median (interquartile range).
[a]Log-transformed for analysis.
[b]Adjusted for mean arterial pressure.

Table 2. Analysis of 24-h ABP measurements in the three blood pressure groups

	NT	*WCHT*	*HT*	*NT vs WCHT (P-value)*	*NT vs HT (P-value)*	*WCHT vs HT (P-value)*
Day SBP (mm Hg)	120 ± 8	125 ± 6	142 ± 9			
Night SBP (mm Hg)	103 ± 8	105 ± 6	121 ± 12			
Twenty-four hour SBP (mm Hg)	115 ± 7	119 ± 5	135 ± 7			
Sleep duration (h)	7.5 ± 1.3	7.4 ± 1.2	7.4 ± 1.5	0.64	0.64	0.97
Day–night SBP Δ (mm Hg)	17 ± 7	20 ± 7	20 ± 11	0.26	0.13	0.79
S.d. Day (mm Hg)	12 ± 2	12 ± 2	14 ± 3	0.53	< 0.001	0.005
S.d. Night (mm Hg)	8 ± 3	8 ± 2	11 ± 4	0.87	< 0.001	< 0.001
Twenty-four hour $s.d._w$ (mm Hg)	11 ± 2	11 ± 2	13 ± 3	0.72	< 0.001	< 0.001
Twenty-four hour $s.d._{ARV}$ (mm Hg)	10 ± 2	10 ± 2	12 ± 2	0.44	< 0.001	< 0.001

Abbreviations: ABP, ambulatory blood pressure; HT, hypertension; NT, normotension; SBP, systolic blood pressure; $s.d._w$, weighted 24 h s.d.; $s.d._{ARV}$, s.d. of the absolute real variability; WCHT, white-coat hypertension. Results presented as mean ± s.d.

interquartile range: 5.1–17.4 mm Hg per hour, 90th percentile 25 mm Hg per hour). The Power function was extremely right skewed with a range of 4.4–2068.9 $mm\,Hg^2$ per hour, (median 305.2, interquartile range: 111.51–561.90 $mm\,Hg^2$ per hour, 90th percentile 774 $mm\,Hg^2$ per hour).

Factors relating to MBPS

A scatterplot of the sleep-trough MBPS and the pre-awake MBPS against age suggested a quadratic relationship ($P < 0.05$). The RoR and the Power function were not associated with age.

The only variable to show a relationship across all four measures of the MBPS was LDL-cholesterol ($r = 0.28$, $P = 0.004$ for sleep-trough MBPS, $r = 0.29$, $P = 0.002$ for the pre-awake MBPS, $r = 0.20$, $P = 0.04$ for the RoR and $r = 0.26$, $P = 0.008$ for the Power function) (Figure 1).

After adjustment for age, waist circumference, menopause, ABPM day SBP, LDL-cholesterol remained significantly associated with the sleep-trough MBPS ($P = 0.01$), the pre-awake MBPS ($P = 0.003$), RoR ($P = 0.045$) and the Power function ($P = 0.007$).

Analysis of the dichotomised MBPS measures

The dichotomisation of MBPS measures at the 90th percentile created binary variables with 11 subjects in the top 10%. Subjects in the top 10% of the sleep-trough (odds ratio 2.6, 95% confidence interval (CI): 1.4–4.9) and the pre-awake (odds ratio 2.4, 95% CI: 1.3–4.4) MBPS were more likely to have an elevated LDL-cholesterol than subjects in the lower 90% (Table 3). This difference remained significant after adjusting for potential confounders including age, menopausal status, gender and ABPM day SBP. There was no significant difference between LDL levels for subjects in the top decile of the RoR, or the Power function and subjects in the lower 90%.

Both the sleep-trough MBPS and the pre-awake MBPS were associated with their baseline measure (lowest night-time SBP for

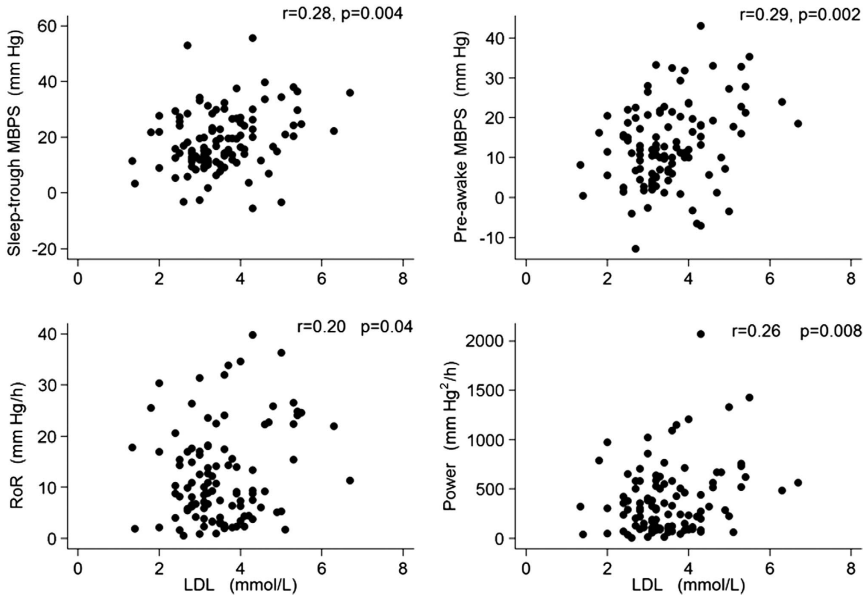

Figure 1. Scatterplot of low-density lipoprotein (LDL) and measures of morning blood pressure surge (MBPS). RoR, rate of rise.

Table 3. Analysis of dichotomised MBPS measures and LDL-cholesterol

MBPS measures	*Sleep-trough*	*Pre-awake*	*RoR*	*Power*
90th percentile	33 mm Hg	27 mm Hg	25 mm Hg per hour	774 mm Hg^2 per hour
Median (IQR)				
LDL non-MBPS group (mmol l^{-1})	3.3 (2.8–3.9)	3.3 (2.8–4.0)	3.3 (2.8–4.0)	3.3 (2.8–4.0)
LDL MBPS group (mmol l^{-1})	4.6 (3.0–5.3)	4.3 (3.6–5.3)	3.7 (2.8–4.8)	3.7 (3.0–4.3)
OR (95% CI)				
LDL	2.6 (1.4–4.9)**	2.4 (1.3–4.4)**	1.2 (0.6–2.2)	1.1 (0.6–2.1)
Adjusted LDL$^{\Sigma}$	2.8 (1.4–5.7)**	2.7 (1.3–5.4)**	1.2 (0.6–2.4)	1.2 (0.6–2.3)

Abbreviations: CI, confidence interval; IQR, interquartile range; LDL, low-density lipoprotein; MBPS, morning blood pressure surge; OR, odds ratio; RoR, rate of rise. $^{\Sigma}$Adjusted for age, waist circumference, menopause status and ambulatory blood pressure day systolic blood pressure. **$P<0.01$.

Table 4. Analysis of morning SBP surge across blood pressure categories

	NT	*WCHT*	*HT*	*NT vs WCHT (P-value)*	*NT vs HT (P-value)*	*WCHT vs HT (P-value)*
Sleep-trough MBPS[a,b] (mm Hg)	13 (11–15)	15 (12–18)	23 (19–28)	0.23	<0.001	0.003
Pre-awake MBPS[c] (mm Hg)	8 (5–11)	11 (8–15)	19 (16–22)	0.12	<0.001	0.004
RoR[a,d] (mm Hg per hour)	6.3 (4.4–8.9)	8.3 (5.8–12.1)	11.1 (7.8–15.9)	0.23	0.05	0.32
Power[a,b] (mm Hg^2 per hour)	141.2 (94.3–211.9)	191.8 (125.2–293.7)	425.9 (282.3–642.3)	0.26	0.001	0.02

Abbreviations: HT, hypertension; MBPS, morning blood pressure surge; NT, normotension; RoR, rate of rise; SBP, systolic blood pressure; WCHT, white-coat hypertension.
[a]Estimated geometric mean (95% confidence interval).
[b]Adjusted for age, age^2, LDL-cholesterol and night minimum SBP average.
[c]Adjusted for age, age^2, LDL-cholesterol and pre-awake SBP average.
[d]Adjusted for LDL-cholesterol and night SBP.

the sleep-trough MBPS and pre-awake SBP for the pre-awake MBPS) suggesting regression to the mean.

None of the four measures of MBPS were associated with sex, alcohol use, waist, body mass index, PWVc, fasting plasma glucose or the 2-h plasma glucose post oral glucose load, high-sensitivity C-reactive protein, leptin, sleep duration, grip or mental stress tests.

MBPS across blood pressure groups

These results are shown in Table 4. After adjusting the sleep-trough MBPS for age and age^2, LDL-cholesterol and the night minimum SBP no significant difference was found between the normotensive and white-coat hypertensive groups, $P=0.23$, whereas a significant difference was found between the sustained hypertensive and the normotensive groups ($P<0.001$) and between the sustained hypertensive and white-coat hypertensive groups ($P=0.003$). Estimated, adjusted sleep-trough MBPS was 13 mm Hg (95% CI: 11–15 mm Hg) for the normotensive group, 15 mm Hg (95% CI: 12–18 mm Hg) for the white-coat hypertensive group and 23 mm Hg (95% CI: 19–28 mm Hg) for the sustained hypertensive group.

Adjusting the pre-awake MBPS for age and age^2, LDL-cholesterol and the pre-awake SBP no significant difference was found between the normotensive and white-coat hypertensive groups ($P = 0.12$), whereas a significant difference was found between the sustained hypertensive and the normotensive groups ($P < 0.001$), and between the sustained hypertensive and white-coat hypertensive groups ($P = 0.004$). Estimated, adjusted pre-awake MBPS was 8 mm Hg (95% CI: 5–11 mm Hg) for the normotensive group, 11 mm Hg (95% CI: 8–15 mm Hg) for the white-coat hypertensive group and 19 mm Hg (95% CI: 16–22 mm Hg) for the sustained hypertensive group.

Adjusting the RoR by LDL-cholesterol and the night SBP, no difference was found for the adjusted RoR between the normotensive and white-coat hypertensive groups, or the white-coat hypertensive and sustained hypertensive groups, whereas a significant was found between the adjusted RoR between the normotensive and sustained hypertensive groups. Estimated, adjusted RoR was 6.3 mm Hg per hour (95% CI: 4.4–8.9 mm Hg per hour) for the normotensive group 8.3 mm Hg per hour (95% CI: 5.8–12.1 mm Hg per hour) for the white-coat hypertensive group and 11.1 mm Hg per hour (95% CI: 7.8–15.9 mm Hg per hour) for the sustained hypertensive group.

No significant difference was found between the normotensive and white-coat hypertensive groups for the Power function, whereas a significant difference was found between the sustained hypertensive and normotensive groups ($P = 0.001$) and between the sustained hypertensive and white-coat hypertensive groups ($P = 0.02$). Estimated, adjusted Power values were 141.2 mm Hg^2 per hour (95% CI: 94.3–211.9 mm Hg^2 per hour) for the normotensive group, 191.8 mm Hg^2 per hour (95% CI: 125.1–293.7 mm Hg^2 per hour) for the white-coat hypertensive group and 425.9 mm Hg^2 per hour (95% CI: 282.3–642.3 mm Hg^2 per hour) for the sustained hypertensive group.

DISCUSSION

In our study we found in an untreated population, significant linear associations across all four measures of the MBPS and LDL-cholesterol. This consistent finding could not be explained by waist circumference, age, blood pressure or menopause status, all of which are potential confounders.[3,19] A significant positive relationship was found for both the RoR and the Power function with fasting total cholesterol and LDL-cholesterol in a study, which was presented to the 23rd meeting of the International Society of Hypertension. (Head GA, Andrianopoulos N, Reid C, McGrath B, Martin C, La Greca C, Chatzivlastou K, unpublished work, 2010)

In a meta-analysis of eight studies, which included 5645 patients, Li *et al.*[6] reported that the top decile of the sleep-trough MBPS had higher mean total cholesterol compared with the rest of the study population.

Similarly, in the present study the top deciles of the sleep-trough and pre-awake MBPS had higher mean fasting LDL-cholesterol compared with the lower 90 per cent, but this did not hold for the RoR or the Power function. This could be due to the small sample size and the loss of information and power that occurs when continuous variables are categorised.[20] Two outcome studies of the MBPS in hypertensive subjects, reported similar lipid profiles in the MBPS and non-MBPS groups.[5,7]

Several studies have shown subjects with an elevated MBPS tend to be older than those with a lower MBPS.[6,21] Lee *et al.*[22] found age was linearly associated with the sleep-trough MBPS in untreated hypertensive subjects. Head *et al.*[11] found a relationship between age and the RoR and the Power function, although the relationship could be described as weak.

A number of studies have examined factors that may potentially contribute to MBPS. A positive relationship has been reported for fasting plasma glucose and MBPS.[23] Conflicting results have been found between measures of sympathetic activity and MBPS.[24–26] Polonia *et al.*[27] in a study of 711 subjects, which included treated hypertensives and diabetics, found a significant correlation between PWV and the MBPS. The present study found no significant relationships between metabolic factors, sympathetic function or PWV and any of the measures of the MBPS.

Two studies have assessed the MBPS in a white-coat hypertensive population.[11,28] Polonia *et al.*,[28] using the sleep-trough as a measure of MBPS, with subjects matched for age gender and weight, found a higher MBPS and day time variability in white-coat hypertensive compared with the normotensive. However, the definition of white-coat hypertensive in that study was based on a single 24-h ABP recording. Moreover the results did not take into account different baseline measurements. The sleep-trough surge and the pre-awake surge both require adjustment for their baseline measure to take into account the regression to the mean. The present study found no differences between white-coat hypertension and the normotensive group for any of the four measures of MBPS.

There are limitations to this study. The subjects were not randomly selected, with all the normotensives recruited by advertisement, whereas the majority of white-coat hypertensive and sustained hypertensive subjects were recruited from our ABPM service. There was also a predominance of female subjects. However, subjects were all drawn from the same geographic area. Information of morning activity was not obtained in study subjects.

The novel finding of a significant relationship between measures of MBPS and fasting LDL-cholesterol in a population of untreated subjects is an intriguing link between the two major cardiovascular risk factors. The factor(s) responsible for this linkage have yet to be defined.

What is known about this topic

- An increased morning blood pressure rise is associated with incident cardiovascular events.
- The morning blood pressure rise, by a variety of definitions, has been shown to be associated with cardiovascular events, particularly stroke.
- Factors associated with the morning blood pressure rise have previously been identified.
- Non-modifiable factors: age
- Modifiable risk factors: hypertension, blood pressure variability, sympathetic activity and fasting glucose.

What this study adds

- A comprehensive analysis of several measures of the MBPS and cardiovascular risk factors.
- The present study confirmed that age, blood pressure variability and sustained hypertension were related to the MBPS.
- The novel finding in the present study was the significant positive correlation between measures of the MBPS and serum lipids.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Willich SN, Lowel H, Lewis M, Arntz R, Baur R, Winther K *et al.* Association of wake time and the onset of myocardial infarction. Triggers and mechanisms of myocardial infarction (TRIMM) pilot study. TRIMM Study Group. *Circulation* 1991; **84**: VI62–V167.

2 Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. *Am J Cardiol* 1987; **60**: 801–806.
3 Kario K. Morning surge in blood pressure and cardiovascular risk: evidence and perspectives. *Hypertension* 2010; **56**: 765–773.
4 Head GA, Lukoshkova EV. Understanding the morning rise in blood pressure. *Clin Exp Pharmacol Physiol* 2008; **35**: 516–521.
5 Kario K, Pickering TG, Umeda Y, Hoshide S, Hoshide Y, Morinari M *et al.* Morning surge in blood pressure as a predictor of silent and clinical cerebrovascular disease in elderly hypertensives: a prospective study. *Circulation* 2003; **107**: 1401–1406.
6 Li Y, Thijs L, Hansen TW, Kikuya M, Boggia J, Richart T *et al.* Prognostic value of the morning blood pressure surge in 5645 subjects from 8 populations. *Hypertension* 2010; **55**: 1040–1048.
7 Amici A, Cicconetti P, Sagrafoli C, Baratta A, Passador P, Pecc T *et al.* Exaggerated morning blood pressure surge and cardiovascular events. A 5-year longitudinal study in normotensive and well-controlled hypertensive elderly. *Arch Gerontol Geriatr* 2009; **49**: e105–e109.
8 Metoki H, Ohkubo T, Kikuya M, Asayama K, Obara T, Hashimoto J *et al.* Prognostic significance for stroke of a morning pressor surge and a nocturnal blood pressure decline: the Ohasama study. *Hypertension* 2006; **47**: 149–154.
9 Marfella R, Siniscalchi M, Portoghese M, Di Filippo C, Ferraraccio F, Schiattarella C *et al.* Morning blood pressure surge as a destabilizing factor of atherosclerotic plaque: role of ubiquitin-proteasome activity. *Hypertension* 2007; **49**: 784–791.
10 Head GA, Reid CM, Lukoshkova EV. Nonsymmetrical double logistic analysis of ambulatory blood pressure recordings. *J Appl Physiol* 2005; **98**: 1511–1518.
11 Head GA, Chatzivlastou K, Lukoshkova EV, Jennings GL, Reid CMA. Novel measure of the power of the morning blood pressure surge from ambulatory blood pressure recordings. *Am J Hypertens* 2010; **23**: 1074–1081.
12 Reid C, Head G, Lukoshkova E, Shiel L, Owen A, Wing L. The Power of the morning blood pressure surge and its relation to long-term survival in the 2nd Australian National Blood Pressure Study (Anbp2) [abstract]. *J Hypertens* 2010; **28**: e15.
13 Panza JA, Epstein SE, Quyyumi AA. Circadian variation in vascular tone and its relation to alpha-sympathetic vasoconstrictor activity. *N Engl J Med* 1991; **325**: 986–990.
14 Brandenberger G, Follenius M, Goichot B, Saini J, Spiegel K, Ehrhart J *et al.* Twenty-four-hour profiles of plasma renin activity in relation to the sleep-wake cycle. *J Hypertens* 1994; **12**: 277–283.
15 Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA *et al.* Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640–1645.
16 WHO. *Definition, Diagnosis and Classification of Diabetes Mellitus and Its Complications; Part 1: Diagnosis and Classification of Diabetes Mellitus*. In World Health Organisation. Department of Noncommunicable Disease Surveillance: Geneva, Switzerland, 1999.
17 Bilo G, Giglio A, Styczkiewicz K, Caldara G, Maronati A, Kawecka-Jaszcz K *et al.* A new method for assessing 24 h blood pressure variability after excluding the contribution of nocturnal blood pressure fall. *J Hypertens* 2007; **25**: 2058–2066.
18 Mena L, Pintos S, Queipo NV, Aizpurua JA, Maestre G, Sulbaran T. A reliable index for the prognostic significance of blood pressure variability. *J Hypertens* 2005; **23**: 505–511.
19 Cicero AF, D'Addato S, Veronesi M, Rosticci M, Santi F, Dormi A *et al.* Brisighella Heart Study Group. Relationship between blood pressure, cholesterolemia and serum apolipoprotein B in a large population sample: the Brisighella Heart Study. *J Hypertens* 2012; **30**: 492–496.
20 Altman DG. Royston P. The cost of dichotomizing continuous variables. *BMJ* 2006; **332**: 1080.
21 Neutel JM, Schumacher H, Gosse P, Lacourciere Y, Williams B. Magnitude of the early morning blood pressure surge in untreated hypertensive patients: a pooled analysis. *Int J Clin Pract* 2008; **62**: 1654–1663.
22 Lee DH, Ihm SH, Youn HJ, Choi YS, Park CS, Park CS *et al.* Age is an independent risk factor for the early morning blood pressure surge in patients never-treated for hypertension. *Korean Circ J* 2009; **39**: 322–327.
23 Shimizu M, Ishikawa J, Eguchi K, Hoshide S, Shimada K, Kario K. Association of an abnormal blood glucose level and morning blood pressure surge in elderly subjects with hypertension. *Am J Hypertens* 2009; **22**: 611–616.
24 Hering D, Kucharska W, Kara T, Somers VK, Narkiewicz K. Resting sympathetic outflow does not predict the morning blood pressure surge in hypertension. *J Hypertens* 2011; **29**: 2381–2386.
25 Chatzivlastou K, Lukoshkova EV, Schlaich M, Lambert GW, Lambert EA, Head GA. Does the reactivity of the sympathetic nervous system contribute to the morning surge in blood pressure [abstract]. *Hypertension* 2008; **53**: 1104.
26 Yano Y, Kario K. Possible difference in the sympathetic activation on extreme dippers with or without exaggerated morning surge. *Hypertension* 2009; **53**: e1.
27 Polonia J, Amado P, Barbosa L, Nazare J, Silva JA, Bertoquini S *et al.* Morning rise, morning surge and daytime variability of blood pressure and cardiovascular target organ damage. A cross-sectional study in 743 subjects. *Rev Port Cardiol* 2005; **24**: 65–78.
28 Polonia J, Carvalho N, Barbosa L, Silva JA. Blood pressure on arising, morning blood pressure surge and blood pressure variability in white coat hypertensives and in matched normotensives and sustained hypertensives. *Rev Port Cardiol* 2006; **25**: 693–704.

Chapter 6

Predictors of mean arterial pressure morning rate of rise and power function in subjects undergoing ambulatory blood pressure recording

6.1 Introduction

The submitted paper in this chapter builds on the published work in Chapter 5, where it was reported that lipids, in particular low-density lipoprotein, are positively associated with the morning blood pressure surge. The analysis in this chapter was undertaken in a larger population and included treated hypertensive participants. The morning blood pressure surge was determined using a mathematical modeling technique.

The questions addressed in this paper are:

- What variables are associated with the morning blood pressure surge in subjects that includes normotensive, treated and untreated hypertensive participants?
- Does commencing lipid therapy have an effect on the morning blood pressure surge in the short term?

The paper also attempts to explain possible mechanisms for the relationship between lipids and the morning blood pressure surge.

6.2 Declaration

In the case of Chapter six, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Collection of data and review of paper	25

The following co-authors contributed to the work.

Name	Nature of contribution
Professor Geoff Head	Conception, writing, data analysis
Professor Barry McGrath	Conception, review of paper
Professor Gary Jennings	Review of paper
Professor Christopher Reid	Review of paper
Melinda Carrington	Review of paper
Dr Nick Andrianopoulos	Data analysis
Pamela J. Davern[1]	Review of paper
Elena Lukoshkova	Developed mathematical model

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature [redacted] 18/9/13

Main Supervisor's Signature [redacted] 18.9.13

PREDICTORS OF MEAN ARTERIAL PRESSURE MORNING RATE OF RISE AND POWER FUNCTION IN SUBJECTS UNDERGOING AMBULATORY BLOOD PRESSURE RECORDING

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Running Title: Cholesterol predicts morning blood pressure surge

Disclosure: none

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Abstract

We determined clinical predictors of the rate of rise (RoR) in blood pressure (BP) in the morning as well as a novel measure of the power of the BP surge (BP_{power}) derived from ambulatory BP (AMBP) recordings. BP_{power} and RoR were calculated from 409 ABP recordings from subjects attending a cardiovascular risk clinic. Anthropometric data, blood biochemistry, and history were recorded. A 1-2 year follow up visit of 213 subjects identified predictors of long term changes in BP surge. Subjects were 20-82 years old (average 57, SD 13), 46% male, 9% with hypertension but not on medication, 34% on antihypertensive medication. Average RoR was 11.1 mmHg/hour (SD=8) and BP_{power} was 273 $mmHg^2$/hour (SD=235). Only cholesterol, low density lipoprotein and body mass index (BMI) were associated with higher BP_{power} and RoR ($P<0.05$) from the 25 variables assessed. BP_{power} was lower in those taking beta-blockers or diuretics. Multivariate analysis identified that only BMI predicted RoR (4.2% increase/unit BMI, P=0.020) while cholesterol was the only remaining predictor for BP_{power} (17.5% increase/mmol/L cholesterol, p=0.047). Baseline cholesterol was the only predictor for an increasing RoR and BP_{power} ($p<0.05$) in 213 subjects with repeated ABP after 1.8 years on average. In 22 patients who commenced statin, they had subsequently lower day-night difference and BP_{power}. We conclude that cholesterol is an independent predictor of a greater and more rapid rise in morning BP as well as of further increases over several years. Reduction of cholesterol with statin therapy is very effective in reducing the morning BP surge.

Key Words : Antihypertensive drugs; Ambulatory blood pressure; Cholesterol; Double logistic equation; Hypertension; Morning blood pressure surge.

The circadian variation in blood pressure (BP) in humans has been established since the first chronic intra-arterial recordings were made in the late 1960's [1]. It is well known that many factors such as physical activity and periods of rest strongly influence the diurnal BP pattern as well as circadian variation in autonomic and hormonal systems. The importance of this circadian pattern has been brought to the fore by the extensive literature that has been developed over the last two decades to show that cardiovascular events, such as stroke, transient ischemic attacks, myocardial infarction and sudden cardiac death occur most frequently during the morning hours which coincides with the rapid rise in BP and heart rate (HR) [2, 3, 4, 5, 6]. Stroke is up to 3 times more likely in the morning[5, 7] while the Framingham study showed that the incidence of sudden death was nearly twice as likely than at any other time[8].

We have developed a novel mathematical approach to measure the morning surge in BP using a logistic equation that contains separate parameters for the rate of rise (RoR) and the rate of fall as a parameter for the night plateau and the difference between the day and night plateau[9]. The model is therefore able to provide a non-symmetrical fit of any circadian data and can estimate the RoR independent of the rate of fall. When applied to data such as human 24 hour BP and HR recordings, it provides a measure of the RoR of BP and HR in the morning period which is independent of what happens at any other time of the day.

In a prospective study of over 300 subjects, we found that there was a markedly greater RoR in BP and HR in the upper quartile of daytime ambulatory mean arterial pressure (MAP) [10]. This finding was not simply because the underlying BP is higher, as we found no correlation between the RoR in morning systolic BP and the night time systolic BP[10]. Our study showed that the duration of the morning surge is similar between the normotensive and hypertensive

participants.[10] We have extended this approach and developed a method of assessing the power (effective force) of the morning BP surge (BP_{power})[11]. We found that patients with hypertension and also those with "white coat" hypertension have markedly exaggerated morning surge BP_{power}[11]. The question is whether this is predicted by any of the standard clinical and biochemical measures collected as part of the monitoring of cardiovascular health. In a previous analysis with 54 normotensive subjects and 70 untreated hypertensive patients, we found an association between fasting LDL cholesterol and morning BP surge as assessed by 4 different methods including the RoR and BP_{power}. These associations were independent of age or waist circumference[12].

The aim of the current study was to perform a much larger study in order to be able to statistically confirm the independence of cholesterol from other confounders, to include subjects treated with hypertensive agents and to determine what predicted long term changes in the morning BP surge (rate and power) in a 1-2 year follow up. We also examined the effect of statin therapy and included other more established methods of calculating the morning BP surge[13].

METHODS

A total of 416 subjects were prospectively recruited from the Healthy Hearts Clinic of Baker IDI Heart and Diabetes Institute or from patients attending the Hypertension Diagnostic Service at the Alfred Hospital Heart Centre (funded and staffed by the Baker IDI Heart and Diabetes Institute) or Monash Health (Dandenong Hospital). Seven subjects were excluded as having nocturnal rising rather than dipping. Of the 409 remainder, 213 were re-examined in follow up after a median 1.8 years (range 1.1 - 2.2). The procedures were approved by The Alfred and Monash Health Human Research Ethics Committee. All subjects gave their

written informed consent. Participants fasted overnight and arrived in the morning for clinic assessment of BP, blood sampling for biochemistry, anthropometrics and questionnaires for medical history after which an ABP device was fitted to the non-dominant arm and the patients briefed on the correct use of the device. Hypertension was defined as being on antihypertensive treatment or having a 24 hour ambulatory systolic BP ≥ 130 mmHg and diastolic BP ≥ 80 mmHg [14]. Normotension was defined as having a 24 hour ambulatory systolic BP < 130 mmHg or diastolic BP < 80 mmHg.

Cardiovascular measurements

Ambulatory BP was recorded during a typical day using SpaceLabs 90207 or 90217 units (SpaceLabs Medical inc., Redmond, WA, USA) or Meditech CardioTens (Meditech Ltd, Budapest, Hungary), which were set to measure BP every 30 minutes from late morning for 26 hours. The first and last hour of the recordings were not included in the analysis as they involved fitting and removing the device in the clinic. Diaries were kept by some patients to record daily activities including awake and asleep times. Clinic BP was determined in the reclining position using a mercury sphygmomanometer after 5 minute rest and an average of three readings.

Analysis of ambulatory curves

Ambulatory BP recording data were fitted to a 6 parameter double logistic equation as described previously[15]. The principle involves the multiplication of the RoR by the amplitude of the rise which are calculated from our standard six parameter logistic equation[9]. The novel power function is the first derivative of the logistic curve multiplied by the amplitude which is the day night difference between plateaus [11]. In addition, we included previously used methods of calculating the morning BP surge (MBPS) which were the night minimum minus the post awake 2 hour period ($MBPS_{NightMin}$)and the pre awake minus the post awake 2 hour

period ($MBPS_{pre\text{-}awake}$) according to the method of Kario and colleagues [13]. Our modification was to use mean BP rather than Systolic BP in order to be comparable to the morning BP power.

Statistical analysis

Data are presented as mean ± standard deviation (SD) of the between-patient variation for continuous variables and frequency (%) for categorical variables. Using the natural log of RoR and BP_{power} as outcomes, baseline univariate predictors were determined using linear regression analysis. The significant univariate predictors were then used in the multivariate analysis. Similarly, for predictors of the change in RoR and BP_{power} as the outcome, the difference between follow up and baseline were used in both the uni- and multi-variate analysis. Slopes were considered significant when $P<0.05$. Analysis was performed with STATA version 10 Data Analysis and Statistical Software (StataCorp, College Station, Texas, USA.).

RESULTS

Subject characteristics

The study group of 409 subjects was made up of 54% female and 46% male subjects with an average age of 57 years (SD = 13, range 20-83, median 58, Table 1). Average body mass index (BMI) was 26.5 kg/m^2 with plasma total cholesterol of 5.2 mmol/L, low density lipoprotein (LDL) 3.4 mm/L, high density lipoprotein (HDL) 1.4 mmol/L and triglycerides 1.3 mmol/L (Table 1). While males and females were of similar ages and BMI plasma cholesterol including HDL and LDL were slightly higher in females and triglycerides were lower (Table 1, $p<0.05$ for all). Males also showed elevated daytime, night time MAP as well a lesser day night difference than females but there was no difference between the clinic

systolic BP or diastolic BP values (Table 2). Males and females had similar RoR and BP_{power} being on average 11 mmHg/h (SD=8) and 273 $mmHg^2$/h (SD=235).

Based on clinic BP measurements from the entire group, 14% would be considered hypertensive (SBP/DBP>140/90) and 33% were taking antihypertensive therapy with the most common therapy being angiotensin converting enzyme inhibitors (ACEI) at 14%. Based on ABP recordings, 226 (55%) were considered normotensive compared with 183 subjects (45%) with hypertension which included 137 on antihypertensive therapy and 46 not taking therapy (Table 1). As expected, clinic and ABP day and night BP values were elevated in those patients classified as hypertensive (Table 2, $p<0.001$). Surprisingly the hypertensive group had a reduced morning RoR compared with normotensives (10.2 vs 11.8 mm Hg/h, $p=0.04$) but we did not detect a statistical difference between the morning BP_{power} ($p=0.1$). Patients with hypertension were also older, heavier, had greater BMI, triglycerides (all $p<0.001$) and elevated fasting glucose (Table 1, $p=0.02$). However, plasma total, HDL and LDL cholesterol were not different between these groups (Table 1).

Univariate analysis of rate of morning rise in BP and power

An initial univariate analysis was performed using the entire 409 recordings. The morning RoR and BP_{power} were first normalised by natural log transformation. Total cholesterol and LDL and BMI but not HDL predicted higher BP_{power} ($p<0.05$) and RoR ($P<0.05$) (Table 3, Fig 1 and Fig 2). By contrast, a lower BP_{power} and RoR was predicted by age (Fig 2). BP_{power} was proportionally lower in those patients taking beta-blockers or diuretics (Table 3).
We also found there was an association of $MBPS_{NightMin}$ and $MBPS_{pre\text{-}awake}$ with cholesterol ($p<0.05$), $MBPS_{pre\text{-}awake}$ was associated with LDL cholesterol but neither were associated with HDL (Fig 3).

Multivariate analysis of rate of morning rise in BP and power

Multivariate analysis determined that only BMI predicted morning RoR (4.2% increase in per unit BMI, p=0.020), while total cholesterol was the only remaining predictor for BP_{power} (17.5% increase/mmol/L, p=0.047, Table 3).

Predicting change in analysis of rate of morning rise in BP and power

A total of 213 subjects from the main study underwent follow up ABP monitoring (median follow-up time of 1.8 years) from whom we calculated the predictors of a change in RoR and BP_{power} between baseline and analyzed this by univariate and multivariate covariance. The univariate analysis indicated that baseline total cholesterol, LDL, triglycerides, fasting glucose and diabetic medication were predictors of increases in RoR and BP_{power} (Table 4, $p<0.05$). However, multivariate analysis showed after adjustment for baseline level of RoR and BP_{power} as well as time between visits, that only baseline total cholesterol was a predictor for increasing RoR and BP_{power} ($p<0.05$, Table 4). No association was found with 24 hour MAP or statin use in any of the analyses.

Effect of starting statin therapy.

Statin therapy was commenced between the initial and subsequent ABP assessments in a limited number of subjects that contributed to a reduction in plasma cholesterol by 1.0 mmol/L (Table 5, $p<0.001$). Subjects were excluded if they had started antihypertensive therapy between visits. A within subject comparison revealed that after commencing a statin the BP_{power} was reduced by 44%, the day night difference was reduced by 32% (P=0.04, Table 5) on the subsequent visit, and there was a tendency to reduce the morning RoR (-11%, p=0.065).

DISCUSSION

The aim of the current study was to determine independent predictors of the morning surge in BP using a novel method of analysis of the RoR as well as an index of the "power" of the BP surge derived from the product of the rate and amplitude[9, 10]. We examined over 20 clinical anthropomorphic, biochemical and treatment indices from 409 subjects to find that the only independent predictor of the morning BP_{power} was plasma levels of total cholesterol and the best predictor of morning RoR was BMI. Importantly, we also found that plasma cholesterol at baseline was the only independent predictor of an increase in the RoR and an increase in the morning BP_{power} over the one to two year follow up examination within the same subject. The latter analysis was adjusted for baseline levels of BP_{power} which allows for the possibility that the cross-sectional analysis association between cholesterol and morning power might be influencing this result. We also found that commencing statin therapy markedly reduced the BP_{power} while no change was observed in those that did not commence a statin between visits. These findings suggest that plasma cholesterol may be intimately linked with the underlying pathological process that is leading to a greater morning BP surge rather than a simple association.

The mechanism involved in the increase in BP and HR in the morning period involves activation of a number of systems associated with arousal as well as circadian rhythms associated with transitioning from dark to light. The average duration of the rise is approximately 3 hours [9] and is not a simple step change at the point of waking. For these reasons we have used the double logistic method for calculating the RoR [9] and the BP_{power}[11] which has the advantage of providing a line of best fit rather than individual points to better estimate the overall trend in BP. The method is independent of the recorded waking time as we have previously shown these to be unreliable in predicting the peak in BP surge[16]. The

transition from sleep to awake is associated with activation of the sympathetic nervous system and increasing levels of plasma catecholamines leading to greater sympathetic vasoconstrictor tone [17]. Also during the morning period compared with evening, sympathetic baroreflex gain is reduced [18], plasma cortisol is elevated[19] and endothelium dependent forearm vasodilatation is reduced[19]. By contrast there is little circadian rhythm in plasma cholesterol[19] suggesting that the link between cholesterol and the morning BP_{power} in both the cross sectional and longitudinal study is not simply a co-incidence of an association of circadian rhythms. However, we do acknowledge that upright posture increases plasma cholesterol concentration slightly due to a reduction in blood volume[20]. Furthermore, we also observed significant correlations with previous methods of determining the morning BP surge and cholesterol and LDL cholesterol. These methods developed by Kario and colleagues encapsulate the change in BP pre and post waking or post awake minus night minimum. The latter essentially indicates the magnitude of the morning rise[13, 21, 22].

Activation of the sympathetic vasomotor drive associated with arousal in the morning period is a particularly important mechanism influencing the rise in BP. Schofl and colleagues showed using frequent sampling of central venous blood to measure the rhythm of plasma norepinephrine and epinephrine that there is not an intrinsic surge in plasma catecholamines that would likely contribute to an increase in BP and HR in the morning[23]. Dodt and colleagues found similarly a lack of association with norepinephrine but did show an increase in epinephrine that was related to waking[24]. One limitation of plasma concentrations is that there is no consideration of clearance. An alternative approach has been used to evaluate the effect of α-adrenergic blockade which produces the largest hypotension in the morning being approximately double that produced during the evening[17]. Furthermore, Kawano and colleagues showed that the α-adrenergic antagonist doxazosin abolished the morning surge in

BP[25]. Muscle sympathetic nerve activity measured using microneurography was markedly lower during slow wave sleep compared with the awake state[18, 26]. We have recently found that there was a close association between an acute increase in muscle sympathetic activation due to a cold pressor test and the morning BP_{power} [27]. Thus it would appear that there is a major contribution of the morning surge in BP and hence BP_{power} from the sympathetic nervous system.

The major finding of the current study that plasma cholesterol and in particular LDL is associated with the morning BP surge might be explained if there was a known link between these plasma lipids and the sympathetic nervous system. There has been considerable controversy in this area with diverse protocols of acute and long term duration combined with the difficulty of assessing sympathetic activity [28, 29]. For example acute infusion of lipid increases plasma noradrenaline[30, 31] and renal noradrenaline spillover[32]. By contrast a 2 day infusion of lipid has been reported to cause a reduction in plasma and urinary norepinephrine[33]. However, these studies focused on fatty acids rather than on cholesterol per se. We have recently published that dyslipidaemia (high cholesterol) in young females is associated with high levels of sympathetic activity compared to normal as measured by microneurography[34]. This association was independent of age, height, weight or BMI. Furthermore Ekstedt and colleagues found that total cholesterol, LDL and cortisol but not HDL, triglycerides or insulin were associated with the frequency of microarousals during sleep[35]. This pattern of association in metabolic markers is remarkably similar to the current study and together suggests that inappropriate activation of the sympathetic nervous system during sleep (microarousals) and at the end of sleep (morning power) can be predicted by elevated cholesterol.

A possible mechanism has only recently come to light with the discovery that high levels of cholesterol can induce cerebral oxidative stress[36-38]. Using an LDL receptor knockout mouse model fed a high cholesterol diet; it was found that mitochondrial oxidative capacity was reduced due to greater consumption of NADPH-linked substrates[37]. Furthermore, the LDL knockout mouse and a mouse fed a high cholesterol diet display an increased vascular sympathetic modulation[36] as determined by spectral analysis [39]. We suggest that a possible mechanism underlying the association between the morning BP_{power} and cholesterol involves increased oxidative stress in central presympathetic pathways leading to a greater sympathetic contribution to the morning arousal surge in BP. The key finding in our study was the dramatic effect of commencing statin therapy which reduced BP_{power} by approximately 50%. This did not occur in the control group that did not commence statin therapy. In support of our hypothesis, studies have previously shown an association between oxidative stress and sympathetic contributions to obesity induced hypertension [40], renovascular hypertension[41, 42], salt sensitive hypertension [43], a mouse model of neurogenic hypertension [44]. Conversely inhibiting oxidative stress leads to sympatho-inhibition [45].

The strength of our study is that the analysis has used a large data set with follow up assessments allowing for within subject analysis which complements the cross sectional analysis. Furthermore we have included all subjects including those on antihypertensive and other therapies which more closely reflect the general population except for a higher incidence of hypertension. Hypertension was present in 44% of our cohort compared with the AusDiab population study from 2005 (Age < 65 years) that showed that 31% of patients had hypertension[46]. The multivariate analysis model has allowed us to account for significant factors such as types of treatment. Interestingly, beta blockers and diuretics were negatively correlated to morning BP_{power} but none of these treatments were independent of cholesterol.

We had previously found that patients taking diuretics had reduced BP_{power}[11] and those taking beta blockers trended similarly but did not reach statistical significance[11]. At first glance the absence of an association with statin treatment might be considered to challenge the association between cholesterol and morning power. However, only those patients with high cholesterol levels would be given statins in order to try to normalize the hyperlipidemia. As such the statistical analysis would be expected to show little difference between those on treatment and untreated patients. When we compared the same subjects before and after commencing statin therapy, a clear reduction in morning BP_{power} was observed.

In conclusion, we used a new measure of the morning BP surge which incorporates the RoR in BP multiplied by the amplitude, namely BP_{power} to show that in a large well characterized group of subjects that plasma cholesterol was the sole independent correlate. Importantly cholesterol was the sole predictor of a long term increase in morning BP_{power} after adjustment for baseline BP_{power}. We suggest that the mechanism may relate to increased cerebral oxidative stress leading to an activation of the sympathetic nervous system. Importantly, we found from a limited number of subjects who had commenced statin therapy that reducing cholesterol had a marked effect in reducing BP_{power}. These studies suggest an important link between two major risk factors in cardiovascular disease, namely hypertension and dyslipidaemia and offer new insights into their interaction within the central nervous system. BP_{power} may therefore be a useful measure to highlight those subjects at greatest risk of cardiovascular events and for determining the most benefit of antihypertensive and cholesterol lowering therapy.

Novelty and Significance

- Plasma cholesterol was an independent predictor associated with a greater and more rapid rise in morning BP
- Plasma cholesterol at baseline independently predicting further increases in morning surge over several years.
- Statin therapy that reduced plasma cholesterol, reduced morning BP surge.
- The suggested mechanism involves cholesterol induced cerebral oxidative stress that activates pre-sympathetic pathways.

What is Relevant?

- An extreme morning BP surge is a risk factor for stroke.
- A calculated parameter that is a product of the rate of rise and the amplitude of the BP surge known as BP_{power} encapsulates the power of the morning BP surge.
- Morning BP surge is partly driven by activation of the sympathetic nervous system.
- High cholesterol levels activates the central nervous system pre-sympathetic pathways through increased oxidative stress.

Summary

- Plasma cholesterol is an independent predictor of a greater and more rapid rise in morning BP as well as a predictor for further increases in morning surge over several years. Reduction of cholesterol with statin therapy is very effective in reducing the morning BP surge.

Disclosures

We have no conflicts of interest to declare.

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References

1. Bevan AT, Honour AJ, Stott FH. Direct arterial pressure recording in unrestricted man. *Clin. Sci*. 1969; **36**: 329-44.

2. Sirgo MA, Mills RJ, DeQuattro V. Effects of antihypertensive agents on circadian blood pressure and heart rate patterns. Review. *Arch. Intern Med*. 1988; **148**: 2547-52.

3. Muller JE, Stone PH, Turi ZG, Rutherford JD, Czeisler CA, Parker C, Poole WK, Passamani E, Roberts R, Robertson T, et al. Circadian variation in the frequency of onset of acute myocardial infarction. *N. Engl. J. Med*. 1985; **313**: 1315-22.

4. Nademanee K, Intarachot V, Josephson MA, Singh BN. Circadian variation in occurrence of transient overt and silent myocardial ischemia in chronic stable angina and comparison with Prinzmetal angina in men. *Am. J. Cardiol*. 1987; **60**: 494-8.

5. Argentino C, Toni D, Rasura M, Violi F, Sacchetti ML, Allegretta A, Balsano F, Fieschi C. Circadian variation in the frequency of ischemic stroke. *Stroke*. 1990; **21**: 387-9.

6. Willich SN. Epidemiologic studies demonstrating increased morning incidence of sudden cardiac death. *Am. J. Cardiol*. 1990; **66**: 15G-17G.

7. Elliott WJ. Circadian variation in the timing of stroke onset: a meta-analysis. *Stroke*. 1998; **29**: 992-6.

8. Willich SN, Levy D, Rocco MB, Tofler GH, Stone PH, Muller JE. Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. *Am. J. Cardiol*. 1987; **60**: 801-6.

9. Head GA, Lukoshkova E, Reid CM. Nonsymmetrical double logistic analysis of ambulatory blood pressure recordings. *J. App.l Physiol*. 2005; **98**: 1511-8.

10. Head GA, Reid CM, Shiel LM, Jennings GL, Lukoshkova EV. Rate of morning rise in blood pressure is elevated in hypertensives. *Am. J. Hypertens*. 2006; **19**: 1010-17.

11. Head GA, Chatzivlastou K, Lukoshkova EV, Jennings GL, Reid CM. A novel measure of the power of the morning blood pressure surge from ambulatory blood pressure recordings. *Am. J. Hypertens*. 2010; **23**: 1074-81.

12. Martin CA, Cameron JD, Head GA, Chen SS, Eikelis N, McGrath BP. The morning blood pressure surge is related to serum cholesterol. *J. Hum. Hypertens*. 2012; **27**: 315-320.

13. Kario K, Pickering TG, Hoshide S, Eguchi K, Ishikawa J, Morinari M, Hoshide Y, Shimada K. Morning blood pressure surge and hypertensive cerebrovascular disease: role of the alpha adrenergic sympathetic nervous system. *Am. J. Hypertens*. 2004; **17**: 668-75

14. Head GA, McGrath BP, Mihailidou AS, Nelson MR, Schlaich MP, Stowasser M, Brown MA, Mangoni AA, Cowley D, Brown MA, Ruta L-A, Wilson A. Ambulatory blood pressure monitoring in Australia: 2011 consensus position statement. *J. Hypertens*. 2012; **30**: 253-66.

15. Head GA, Lukoshkova EV, Mayorov DN, Van den Buuse M. Non-symmetrical double logistic analysis of 24 hour blood pressure recordings in normotensive and hypertensive rats. *J. Hypertens*. 2004; **22**: 2075-85.

16. Head GA, Lukoshkova EV. Understanding the morning rise in blood pressure. *Clin. Exp. Pharmacol. Physiol*. 2008; **35**: 516-21.

17. Panza JA, Epstein SE, Quyyumi AA. Circadian variation in vascular tone and its relation to alpha- sympathetic vasoconstrictor activity. *N. Engl. J. Med*. 1991; **325**: 986-90.

18. Nakazato T, Shikama T, Toma S, Nakajima Y, Masuda Y. Nocturnal variation in human sympathetic baroreflex sensitivity. *J. Auton. Nerv. Syst*. 1998; **70**: 32-7.

19. Shaw JA, Chin-Dusting JP, Kingwell BA, Dart AM. Diurnal variation in endothelium-dependent vasodilatation is not apparent in coronary artery disease. *Circulation*. 2001; **103**: 806-12.

20. Muldoon MF, Bachen EA, Manuck SB, Waldstein SR, Bricker PL, Bennett JA. Acute cholesterol responses to mental stress and change in posture. *Arch. Intern. Med.* 1992; **152**: 775-80.

21. Kario K, Ishikawa J. Exaggerated morning surge in blood pressure in hypertension. *Am. J. Hypertens*. 2006; **19**: 1018.

22. Kario K, Pickering TG, Umeda Y, Hoshide S, Hoshide Y, Morinari M, Murata M, Kuroda T, Schwartz JE, Shimada K. Morning surge in blood pressure as a predictor of silent and clinical cerebrovascular disease in elderly hypertensives: a prospective study. *Circulation*. 2003; **107**: 1401-6.

23. Schofl C, Becker C, Prank K, von zur Muhlen A, Brabant G. Twenty-four-hour rhythms of plasma catecholamines and their relation to cardiovascular parameters in healthy young men. *Eur. J. Endocrinol*. 1997; **137**: 675-83.

24. Dodt C, Breckling U, Derad I, Fehm HL, Born J. Plasma epinephrine and norepinephrine concentrations of healthy humans associated with nighttime sleep and morning arousal. *Hypertension*. 1997; **30**: 71-6.

25. Kawano Y, Tochikubo O, Watanabe Y, Miyajima E, Ishii M. Doxazosin suppresses the morning increase in blood pressure and sympathetic nervous activity in patients with essential hypertension. *Hypertens. Res*. 1997; **20**: 149-56.

26. Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic-nerve activity during sleep in normal subjects. *N. Engl. J. Med*. 1993; **328**: 303-7.

27. Chatzivlastou K, Lukoshkova EV, Schlaich M, Lambert GW, Lambert EA, Head GA. Does the reactivity of the sympathetic nervous system contribute to the morning surge in blood pressure. *30th Annual Scientific Meeting of the High Blood Pressure Research Council of Australia*. 2008; **53**: 1104.

28. Guild SJ, Barrett CJ, McBryde FD, Van Vliet BN, Head GA, Burke SL, Malpas SC. Quantifying sympathetic nerve activity; problems and pitfalls, the need for standardization. *Exp. Physiol*. 2010; **95**: 41-50.

29. Burke SL, Lambert E, Head GA. New approaches to quantifying sympathetic nerve activity *Curr. Hypertens. Rep*. 2011; **13**: 249-57.

30. Grekin RJ, Vollmer AP, Sider RS. Pressor effects of portal venous oleate infusion. A proposed mechanism for obesity hypertension. *Hypertension*. 1995; **26**: 193-8.

31. Manzella D, Barbieri M, Rizzo MR, Ragno E, Passariello N, Gambardella A, Marfella R, Giugliano D, Paolisso G. Role of free fatty acids on cardiac autonomic nervous system in noninsulin-dependent diabetic patients: effects of metabolic control. *J. Clin. Endocrinol. Metab*. 2001; **86**: 2769-74.

32. Grekin RJ, Ngarmukos CO, Williams DM, Supiano MA. Renal norepinephrine spillover during infusion of nonesterified fatty acids. *Am. J. Hypertens*. 2005; **18**: 422-6.

33. Magnan C, Cruciani C, Clement L, Adnot P, Vincent M, Kergoat M, Girard A, Elghozi JL, Velho G, Beressi N, Bresson JL, Ktorza A. Glucose-induced insulin hypersecretion in lipid-infused healthy subjects is associated with a decrease in

plasma norepinephrine concentration and urinary excretion. *J. Clin. Endocrinol. Metab*. 2001; **86**: 4901-7.

34. Lambert E, Ika Sari C, Hering D, Head GA, Straznicky N, Dixon JB, Esler M, Schlaich MP, Lambert G. Dyslipidemia is associated with sympathetic nervous activation and impaired endothelial function in young females. *Am. J. Hypertens*. 2013; **26**: 250-6.

35. Ekstedt M, Akerstedt T, Soderstrom M. Microarousals during sleep are associated with increased levels of lipids, cortisol, and blood pressure. *Psychosom. Med*. 2004; **66**: 925-31.

36. Evangelho JS, Casali KR, Campos C, De Angelis K, Veiga AB, Rigatto K. Hypercholesterolemia magnitude increases sympathetic modulation and coagulation in LDLr knockout mice. *Auton. Neurosci*. 2011; **159**: 98-103.

37. Paim BA, Velho JA, Castilho RF, Oliveira HC, Vercesi AE. Oxidative stress in hypercholesterolemic LDL (low-density lipoprotein) receptor knockout mice is associated with low content of mitochondrial NADP-linked substrates and is partially reversed by citrate replacement. *Free Radic. Biol. Med*. 2008; **44**: 444-51.

38. de Oliveira J, Hort MA, Moreira EL, Glaser V, Ribeiro-do-Valle RM, Prediger RD, Farina M, Latini A, de Bem AF. Positive correlation between elevated plasma cholesterol levels and cognitive impairments in LDL receptor knockout mice: relevance of cortico-cerebral mitochondrial dysfunction and oxidative stress. *Neuroscience*. 2011; **197**: 99-106.

39. Guzzetti S, Borroni E, Garbelli PE, Ceriani E, Della Bella P, Montano N, Cogliati C, Somers VK, Malliani A, Porta A. Symbolic dynamics of heart rate variability: a probe to investigate cardiac autonomic modulation. *Circulation*. 2005; **112**: 465-70.

40. Nagae A, Fujita M, Kawarazaki H, Matsui H, Ando K, Fujita T. Sympathoexcitation by oxidative stress in the brain mediates arterial pressure elevation in obesity-induced hypertension. *Circulation*. 2009; **119**: 978-86.

41. Campos RR, Oliveira-Sales EB, Nishi EE, Boim MA, Dolnikoff MS, Bergamaschi CT. The role of oxidative stress in renovascular hypertension. *Clin. Exp. Pharmacol. Physiol*. 2011; **38**: 144-52.

42. Oliveira-Sales EB, Nishi EE, Carillo BA, Boim MA, Dolnikoff MS, Bergamaschi CT, Campos RR. Oxidative stress in the sympathetic premotor neurons contributes to sympathetic activation in renovascular hypertension. *Am. J. Hypertens*. 2009; **22**: 484-92.

43. Fujita M, Ando K, Nagae A, Fujita T. Sympathoexcitation by oxidative stress in the brain mediates arterial pressure elevation in salt-sensitive hypertension. *Hypertension*. 2007; **50**: 360-7.

44. Marques FZ, Campain AE, Davern PJ, Yang YH, Head GA, Morris BJ. Global identification of the genes and pathways differentially expressed in hypothalamus in early and established neurogenic hypertension. *Physiol. Genom*. 2011; **43**: 766-71.

45. Fujita M, Kuwaki T, Ando K, Fujita T. Sympatho-inhibitory action of endogenous adrenomedullin through inhibition of oxidative stress in the brain. *Hypertension*. 2005; **45**: 1165-72.

46. Barr ELM, Magliano DJ, Zimmet PZ, Polkinghorne KR, Atkins RC, Dunstan DW, Murray SG, Shaw JE. The Australian diabetes, obesity and lifestyle study. Tracking the accelerating epidemic: Its causes and outcomes. 2005

Table 1. Clinical characteristics of subject groups.

Subjects	**Total**	**Females**	**Males**	***P*1**	**NT**	**HT**	***P*2**
Number	409	222	187		226	183	
Age (years)	56.8 ± 12.8	56.6 ± 12.7	57.1 ± 12.9	NS	54.6 ± 12.6	59.5 ± 12.5	***
Weight	77.7 ± 14.6	72.9 ± 15	83 ± 12.1	***	75.5 ± 14.8	81 ± 13.8	***
BMI	26.5 ± 3.9	26.4 ± 4.4	26.7 ± 3.3	NS	25.7 ± 3.5	28 ± 4.2	NS
Cholesterol (mmol/L)	5.2 ± 0.9	5.4 ± 0.9	5.1 ± 0.9	***	5.2 ± 0.9	5.3 ± 0.9	***
LDL (mmol/L)	3.4 ± 0.9	3.5 ± 0.9	3.2 ± 0.9	*	3.4 ± 0.9	3.3 ± 0.8	*
HDL (mmol/L)	1.4 ± 0.4	1.5 ± 0.4	1.2 ± 0.3	***	1.3 ± 0.4	1.4 ± 0.4	***
Triglycerides (mmol/L)	1.3 ± 0.7	1.2 ± 0.6	1.5 ± 0.9	***	1.2 ± 0.6	1.4 ± 0.8	***
Fasting glucose (mmol/L)	5.4 ± 1.4	5.2 ± 1.3	5.6 ± 1.4	*	5.3 ± 1.1	5.6 ± 1.7	*
Clinic BP>140/90 mmHg (%)	14.4	13.5	15.5		9.7	20.2	
Antihypertensive medication (%)	33.5	33.3	33.7		0.0	74.9	
ACE Inhibitors (%)	13.9	13.1	15.0		0.0	31.1	
ARB (%)	11.7	10.4	13.4		0.4	25.7	
Beta blockers (%)	9.3	7.2	11.8		0.0	20.8	
Calcium channel blockers (%)	10.0	9.5	10.7		0.0	22.4	
Diuretics (%)	10.3	9.0	11.8		0.0	23.0	
Statins (%)	18.1	17.6	18.7		11.1	26.8	

Values are Mean +/- SD, *P*1 is the probability for the comparison between Females and males. *P*2 is between normotensive (NT) and patients with hypertension (HT), * $P<0.05$, ** $P<0.01$, NS $P>0.05$.

Table 2. Clinic blood pressures, day and night MAP, day–night difference, morning rate of rise in MAP, and peak morning MAP surge power in subject groups.

Subjects	**Total**	**Females**	**Males**	***P*1**	**NT**	**HT**	***P*2**
Recordings	**409**	**222**	**187**		**226**	**183**	
Clinic SBP (mmHg)	137 ± 19	138 ± 21	136 ± 17	NS	131 ± 16	145 ± 20	***
Clinic DBP (mm Hg)	83.8 ± 10.6	83 ± 11	84.8 ± 9.9	NS	81.5 ± 9.5	86.6 ± 11.1	***
Clinic BP>140/90 mmHg (%)	14.4	13.5	15.5		9.7	20.2	
Daytime MAP (mm Hg)	98.2 ± 8.7	97.2 ± 9.6	99.5 ± 7.4	***	95.6 ± 6.9	101.4 ± 9.6	***
Night MAP (mm Hg)	92.8 ± 8.3	91.4 ± 9.1	94.4 ± 6.9	***	90 ± 6	96.2 ± 9.3	***
Day-Night difference (mm Hg)	12.9 ± 6.7	13.5 ± 6.7	12.2 ± 6.5	*	13.4 ± 5.9	12.3 ± 7.5	NS
Morning rate of MAP increase (mm Hg/h)	11.1 ± 8.2	11.4 ± 8.2	10.7 ± 8.2	NS	11.8 ± 8.3	10.2 ± 7.9	*
Peak morning surge power (mm Hg^2/h)	273 ± 235	289 ± 237	254 ± 231	NS	290 ± 234	252 ± 235	NS

Values are Mean +/- SD, *P*1 is the probability for the comparison between Females and males. *P*2 is between normotensive (NT) and patients with hypertension (HT), * $P<0.05$, ** $P<0.01$, NS $P>0.05$.

Table 3. Linear regression coefficient (b), 95% CI and p-value of baseline predictors of the RoR and BPPower as natural log (Ln) from a univariate and multivariate analysis.

Univariate	Ln RoR			Ln BPPower		
Baseline Predictor	b	95%CI	*P*-value	b	95%CI	*P*-value
Age (yrs)	-0.01	-0.02,-0.001	0.018	-0.01	-0.02,-0.005	0.001
BMI (kg/m^2)	0.04	0.004,0.07	0.030	0.03	-0.005,0.07	0.087
Male (%)	-0.06	-0.25,0.12	0.488	-0.11	-0.32,0.09	0.282
Clinical systolic HPT (%)	-0.01	-0.21,0.19	0.921	0.06	-0.17,0.29	0.603
Clinical diastolic HPT (%)	-0.12	-0.35,0.11	0.307	-0.13	-0.40,0.13	0.319
BP >140/90 mmHg (%)	-0.10	-0.36,0.16	0.432	-0.10	-0.40,0.19	0.494
Baseline hypertension (%)	-0.10	-0.29,0.09	0.299	-0.09	-0.31,0.13	0.418
Cholesterol (mmol/L)	0.11	0.003,0.22	0.043	0.16	0.04,0.28	0.009
LDL (mmol/L)	0.15	0.03,0.27	0.018	0.20	0.07,0.34	0.004
HDL (mmol/L)	0.09	-0.20,0.37	0.552	0.14	-0.18,0.46	0.402
Triglycerides (mmol/L)	-0.04	-0.17,0.09	0.531	-0.03	-0.18,0.12	0.675
Fasting glucose (mmol/L)	-0.02	-0.09,0.05	0.599	-0.04	-0.12,0.05	0.389
Current Smokers (%)	0.09	-0.38,0.56	0.711	0.04	-0.49,0.58	0.882
Family history of CHD (%)	0.16	-0.07,0.40	0.177	0.25	-0.02,0.51	0.067
Diabetic (%)	-0.15	-0.59,0.30	0.515	-0.13	-0.63,0.37	0.610
Any alcohol (%)	0.05	-0.05,0.15	0.304	0.07	-0.04,0.18	0.219
Antihypertensive medication (%)	-0.14	-0.33,0.05	0.144	-0.13	-0.34,0.09	0.247
ACE Inhibitors (%)	-0.04	-0.29,0.22	0.761	-0.04	-0.33,0.25	0.772
ARB (%)	-0.12	-0.40,0.15	0.378	-0.01	-0.33.0.30	0.938
Beta blockers (%)	-0.30	-0.62,0.01	0.060	-0.49	-0.85,-0.14	0.007
Calcium channel blocker (%)	-0.18	-0.48,0.11	0.221	-0.23	-0.56,0.11	0.186
Diuretics (%)	-0.25	-0.54,0.04	0.094	-0.37	-0.70,-0.04	0.029
Statins (%)	-0.02	-0.25,0.22	0.888	-0.06	-0.33,0.20	0.635
Diabetic medication (%)	-0.03	-0.64,0.60	0.948	-0.10	-0.80,0.61	0.789

Multivariate	Ln RoR			Ln BPPower		
Baseline Predictor	b	95%CI	p-value	b	95%CI	p-value
Age (yrs)	-0.01	-0.2,0.006	0.405	-0.01	-0.02,0.002	0.113
BMI (kg/m^2)	0.04	0.01,0.08	0.020	-	-	-
Cholesterol (mmol/L)	0.07	-0.09,0.23	0.379	0.16	0.003,0.32	0.047
Beta Blocker	-	-	-	-0.23	-0.71,0.24	0.336
Diuretic	-	-	-	-0.23	-0.71,0.24	0.337

Table 4 . Linear regression coefficient (b), 95% CI and p-value of baseline predictors of the change in natural log (ln) RoR and BP_{power} between first follow-up and baseline visits from a univariate and multivariate ANCOVA analysis.

ANCOVA - Univariate	Δ ln RoR[#]			Δ ln BPPower[^]		
Baseline Predictor	b	95%CI	*P*-value	b	95%CI	*P*-value
Cholesterol (mmol/L)	0.21	0.05,0.37	0.009	0.29	0.11,0.48	0.002
LDL (mmol/L)	0.24	0.05,0.44	0.015	0.32	0.09,0.56	0.008
Triglycerides (mmol/L)	0.23	0.05,0.42	0.012	0.31	0.10,0.52	0.004
Fasting glucose (mmol/L)	-0.11	-0.20,-0.01	0.024	-0.12	-0.23,-0.02	0.025
Diabetic medication	-0.95	-1.71,-0.18	0.015	-1.49	-2.38,-0.60	0.001
ANCOVA -Multivariate	Δ ln RoR[#]			Δ ln BPPower[^]		
Baseline Predictor	b	95%CI	*P*-value	b	95%CI	*P*-value
Cholesterol (mmol/L)	0.21	0.05,0.37	0.012	0.29	0.11,0.48	0.002
Fasting glucose (mmol/L)	-0.08	-0.18,0.01	0.091	-0.09	-0.20,0.02	0.108

[#]Also adjusted for baseline ln ROR and time between visits, [^]Also adjusted for baseline ln BP_{power} and time between visits.

Table 5. Comparison of the day–night difference, morning rate of rise in MAP, and peak morning MAP surge power in subjects before (baseline) and after commencing chronic statin therapy.

n= 37	Baseline	Subsequent visit	P	Baseline	Subsequent visit	P
Statin	No	Yes		No	No	
n		37			116	
Total Cholesterol (mmol/l)	5.2 ± 0.7	4.2 ± 0.7	***	5.3 ± 0.8	5.4 ± 0.9	NS
24 hour MAP	93.8 ± 8	92.1 ± 7.9	NS	92 ± 7.5	91.5 ± 7.5	NS
Day-Night difference (mm Hg)	27.9 ± 8.2	23.4 ± 6.5	**	25.5 ± 9.3	24.5 ± 9.3	NS
Morning rate of MAP increase (mm Hg/h)	12.9 ± 11.6	9.7 ± 7.4	NS	11 ± 8	10.8 ± 8.4	NS
Peak morning surge power (mm Hg^2/h)	385 ± 417	229 ± 204	*	279 ± 231	268 ± 262	NS

Values are Mean ± SD. Subject who changed antihypertensive medication or who had less than 25% of the total variance explained by the 6 parameter double logistic fit were excluded.

P is the probability for the comparison between baseline and subsequent visit, * $P<0.05$, ** $P<0.01$, NS $P>0.05$.

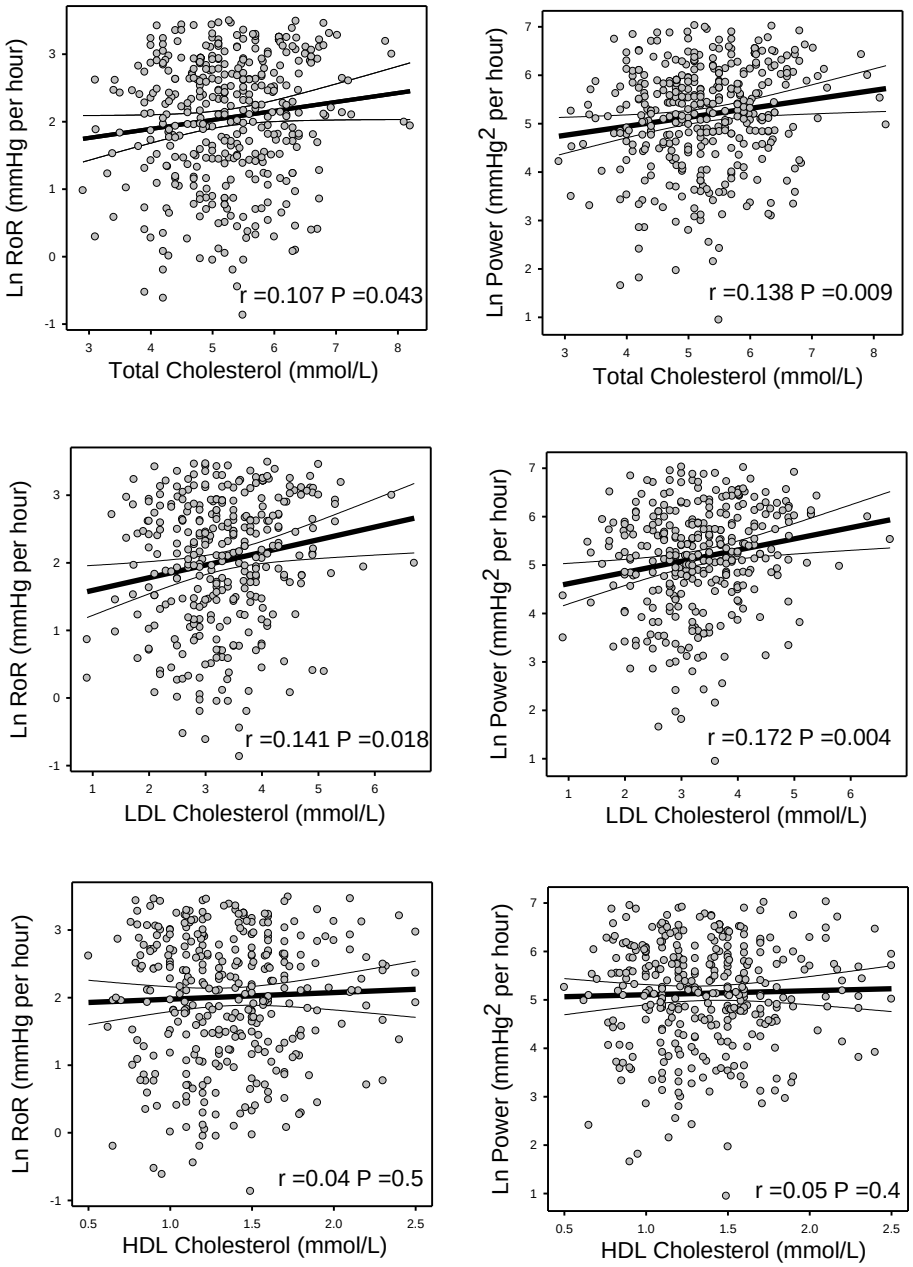

Figure 1: Correlations between natural log morning rate of rise (RoR) in mean arterial pressure (MAP) (left panels) and power (right panels) and plasma levels of cholesterol (top), low density lipoprotein (LDL, middle) and high density lipoprotein (HDL) from subjects (n=409). Thick line represents least squares regression lines and thin lines are 99% confidence limits. r is the correlation co-efficient and P is the probability.

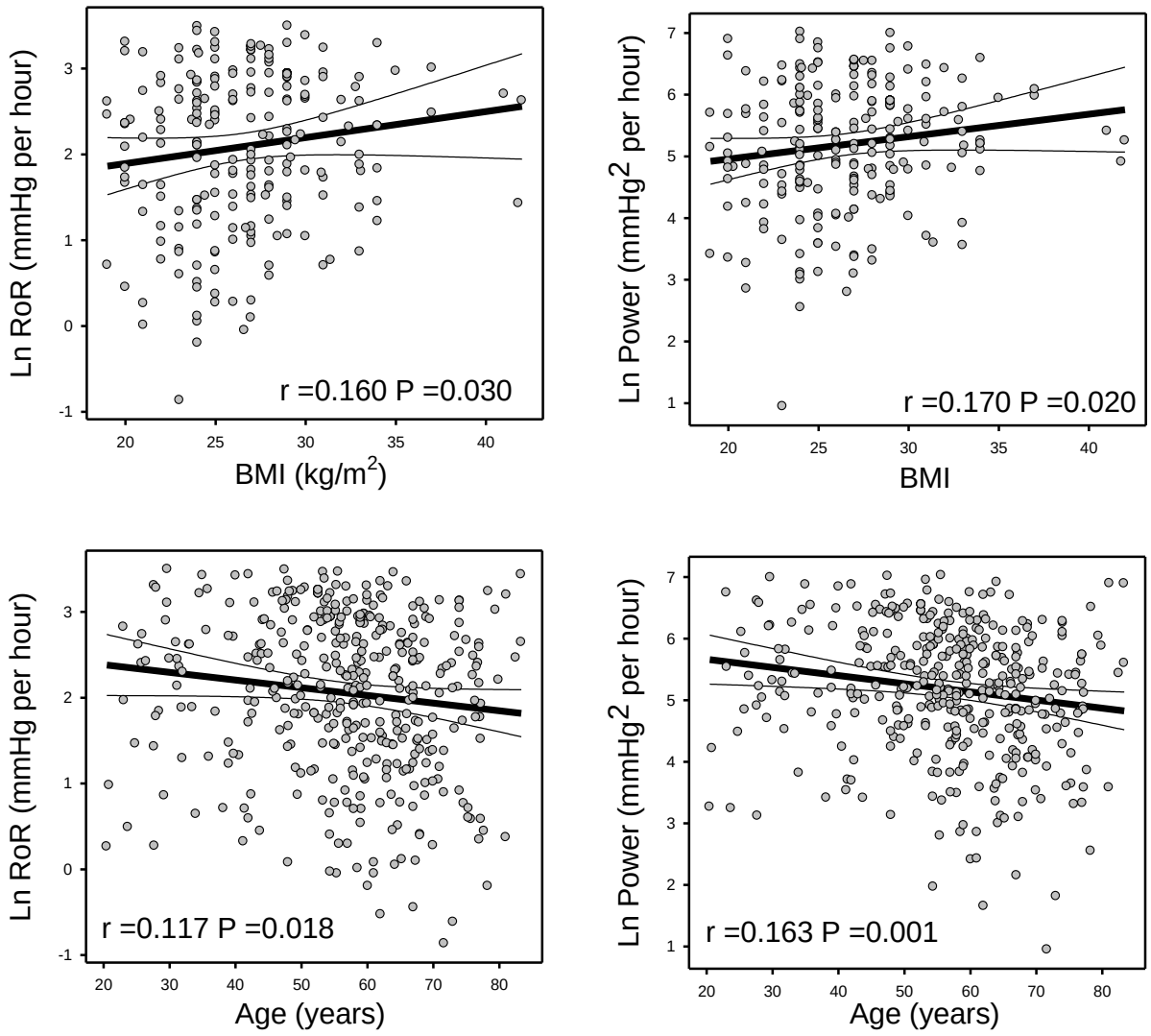

Figure 2: Correlations between natural log morning rate of rise (RoR) in mean arterial pressure (MAP) (left panels) and power (right panels) and body mass index (BMI) and age from subjects (n=409). Thick line represents least squares regression lines and thin lines are 99% confidence limits. r is the correlation co-efficient and P is the probability.

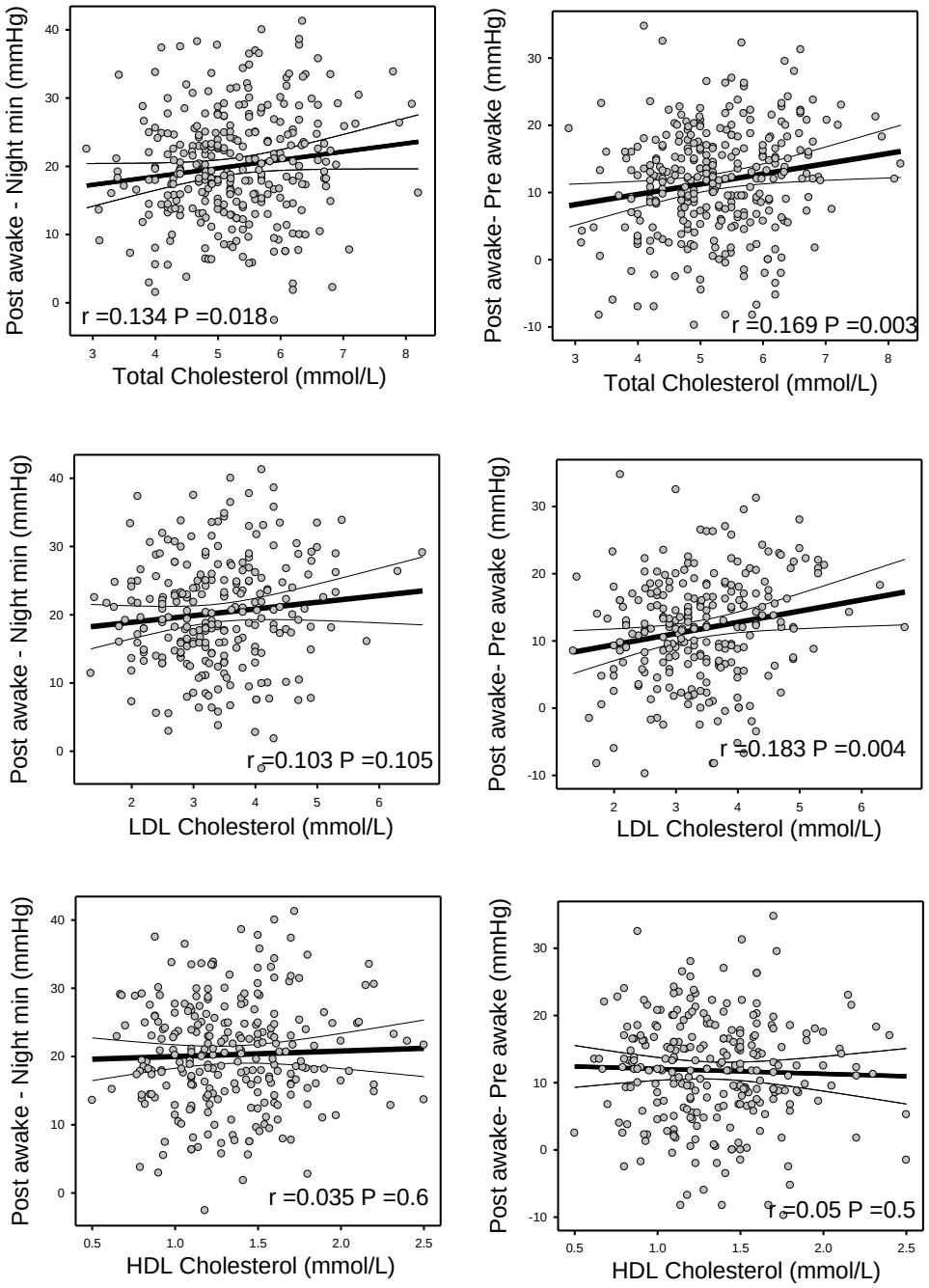

Figure 3: Correlations between post awake 2 hours minus night minimum 2 hours in mean arterial pressure (MAP) (left panels) and post awake 2 hours minus pre awake 2 hours (right panels) and plasma levels of cholesterol (top), low density lipoprotein (LDL, middle) and high density lipoprotein (HDL) from subjects (n=307). Thick line represents least squares regression lines and thin lines are 99% confidence limits. r is the correlation co-efficient and P is the probability.

Chapter 7

Biomarkers of future hypertension in white-coat hypertension

7.1 Introduction

Previous studies have suggested that subjects with WCHT are at increased risk of developing sustained hypertension, but due to the various definitions used to define WCHT it is not clear if WCHT is associated with increased risk of sustained hypertension.

This chapter reports the results of a prospective study, in which normotensive and WCHT participants were followed for a period of three years to observe for the development of sustained hypertension. The WCHT group was carefully defined with two ABPM's required to determine WCHT status.

The study examined and compared the rate of progression to sustained hypertension in the two groups. It builds on the results detailed in Chapter 4, which defined markers of specific pathophysiological mechanisms that may potentially be involved in WCHT. Subject anthropomorphic features, glucose dysregulation, autonomic function, arterial function and circulating biomarkers were examined to investigate which factor(s) best predicted future sustained hypertension in the WCHT group.

7.2 Declaration

In the case of Chapter seven, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Conception, data collection, data analysis, writing of paper	80

The following co-authors contributed to the work.

Name	Nature of contribution
Professor Barry McGrath	Conception, writing and review of paper
Professor James Cameron	Conception, writing and review of paper

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature	[redacted]	18/9/13
Main Supervisor's Signature	[redacted]	18 9 13

Baseline biomarkers of future hypertension in white-coat hypertension.

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None

Key words

Ambulatory blood pressure monitoring, incident hypertension, prospective, white-coat hypertension.

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Abstract

It is yet to be determined if white-coat hypertension, which is prevalent in the general population, can be considered a condition of increased cardiovascular risk. In this exploratory prospective study the risk of the development of hypertension in a WCHT cohort, defined by two ambulatory blood pressure monitorings, is examined along with the identification of baseline predictors of future hypertension in white-coat hypertension. Two out of 33 normotensives (6.1%, median follow-up 3.1 years [inter-quartile range: 2.3-3.3 years]) and 10 of the 28 white-coat hypertensives (35.7%, median follow-up 2.9 years [inter-quartile range: 1.1-3.6 years]) developed sustained hypertension. White-coat hypertension participants who developed sustained hypertension had higher baseline waist circumference (80.1±9.7 versus 91.9±13.1 centimetres, p=0.01), tended to be male (5.6 versus 40%, p=0.04), and had higher initial ambulatory blood pressure measurement (129/82±9/7 versus 143/87±14/14 mmHg, p=0.02) compared to white-coat hypertensives who remained white-coat. Although not statistically different the white-coat hypertensives who developed sustained hypertension had higher two-hour plasma glucose post 75 gram dextrose challenge and higher central pulse wave velocity compared to white-coat hypertensives who did not develop sustained hypertension. Waist circumference, which is a modifiable risk factor for hypertension, should be measured and closely monitored in white-coat hypertensive patients. Studies are required to determine if interventions to reduce waist circumference delay or prevent the conversion to sustained hypertension in white-coat hypertension. Further research is required to determine if the oral glucose tolerance test and pulse wave velocity are useful measures to define increased cardiovascular risk in white-coat hypertension

White-Coat hypertension (WCHT) is suggested to be associated with increased progression to sustained hypertension[1-3] Regular ABPM or home BP monitoring is required to monitor for conversion to sustained hypertension in patients with WCHT. The population prevalence of WCHT is estimated at 15%,[4] making it logistically and financially difficult to monitor all WCHT subjects for conversion to sustained hypertension. If predictors of future hypertension in WCHT can be identified then the focus of monitoring and intervention can be undertaken in WCHT patients at high risk.

In this exploratory prospective study, the progression to sustained hypertension, over a three year period, in a strictly defined WCHT group, was determined and compared to normotensives. Baseline predictors of sustained hypertension in WCHT were also examined, including anthropometric, metabolic and arterial mechanical measures.

Methodology

The study was approved by the institutional human research ethics committee. Adult subjects (18-79 years) were recruited by advertisement and through an ABPM service. Subjects were excluded if they were smokers, had known diabetes, were taking anti-hypertensive medication, or had existing liver, renal or cardiovascular disease or any malignancy. Written informed consent was obtained from all subjects.

Subjects presented to The Department of Vascular Sciences, Monash Health, Melbourne, Australia, at 9am. They were asked to fast from 2100hrs the evening before but were told they could still drink water. Each subject completed a questionnaire about previous and present

illnesses, family history, medication, diet, physical activity and alcohol habits. Alcohol consumption was categorised into none, mild (one to two standard drinks per day) or heavy (more than two standard drinks per day). The metabolic syndrome was defined according to the joint statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity.[5] Glucose status was defined as per World Health Organisation definition.[6] Risk score for the development hypertension was calculated using the Framingham hypertension risk calculator.[7]

Anthropomorphic measures

Measurements of weight occurred without shoes and with the subject in light clothing. Waist circumference was measured at the halfway point between the inferior margin of the ribs and the superior border of the iliac crests**.** All anthropomorphic measurements were taken by the same person (CM).

Blood pressure measurement

At five minutes post arrival three BPs were taken on the left arm at one minute intervals using an automated oscillometric blood pressure device. (Task Force monitor®, CNSystems**,** Graz, Austria). One of the authors (CM) remained in the room for these measurements in order to determine observed automated BP. The study subject was then left alone in the room and after a further five minutes of rest three sitting BP measurements were automatically recorded at three minutely intervals to determine unobserved automated blood pressure.

Ambulatory Blood Pressure Monitoring

ABPM was performed using a portable device (MeditechCardiotens, Meditech Ltd, Hungary). ABPM cuff was placed on the non-dominant arm and automated BP recordings were programmed to occur at thirty minute intervals day and night. Normotension was defined as clinic BP < 140/90 mmHg and day 24-hour ABPM < 135/85 mmHg and night ABPM < 120/70 mmHg. WCHT was defined as reported clinic systolic BP ≥ 140mmHg or clinic diastolic BP ≥ 90mmHg and day 24-hour ABPM < 135/85 mmHg and night ABPM < 120/70 mmHg. Two ABPM were required to confirm WCHT, either by prior ABPM with a reported diagnosis of WCHT or repeat ABPM within a 12 month period of study commencement. Sustained hypertension was defined as clinic systolic BP ≥ 140 mmHg or clinic diastolic BP ≥ 90 mmHg; and day systolic ABPM ≥ 135 mmHg or day ABPM diastolic ≥ 90 mmHg.

Measurement of artery stiffness

Central aorto-femoral PWV (PWVc) measurements were performed by the same two operators (CM, SC), in a quiet air-conditioned room after 10 minutes of rest and with the subject lying supine. Continuous pulse pressure wave signals were recorded with hand-held tonometers (Millar Mikro-tip, SPT-301; Millar Instruments, Houston, Texas, USA) positioned at the base of the right common carotid artery and over the right common femoral artery. Transit distance was defined as the measured distance from the sternal notch to femoral artery minus distance from sternal notch to carotid. The start of systole was defined by the local maximum of the first derivative of the pressure signal. Mean transit time (Δt) between the feet of simultaneously recorded waves was determined from 10 consecutive cardiac cycles**.** Mean arterial pressure

(MAP) was estimated from an oscillometric blood pressure measurement taken immediately post recording of the PWVc.

Flow-mediated dilation (FMD)

Participants rested for ten minutes prior to FMD measurement. A BP cuff was placed above the antecubital fossa and a baseline rest image was acquired. Arterial occlusion was created by cuff inflation to 50 mm Hg above systolic pressure (measured just prior to FMD) for five minutes. The longitudinal image of the artery was recorded continuously for two minutes after cuff deflation. The diameter of the brachial artery (the media-adventitia interfaces) was identified manually with electronic calipers. The change in artery diameter was assessed at 60 seconds post deflation. Analysis of the change in artery diameter was undertaken using linear regression adjusting for baseline artery diameter. FMD was measured by the same person (SC).

Mental arithmetic stress test

After resting for two minutes an oscillometric BP was taken. Subjects were then instructed to subtract out loud the value 13 from the value 4,300. Subjects were instructed to either start again or continue from the current value if they felt they had made a mistake and that no assistance would be given by the examiner. If a subject verbalised an inability to do this subtraction then they were offered the subtraction of 13 from 430. An oscillometric BP measurement was taken at one minute. The difference in diastolic BP at one minute, from the resting diastolic BP was calculated.

Glucose Tolerance

Each subject had an oral glucose tolerance test with measurements of blood glucose at baseline and at 30, 60 and 120 minutes post glucose loading of 75g dextrose.

Circulating biomarkers

Blood samples were taken in the morning after a 12 hour fast. Samples for lipid profiling, glucose and insulin were immediately sent to Southern Cross Pathology, Monash Health, for analysis. Samples for leptin, von Willebrand factor (vWF), plasminogen activator 1 (PAI-1), high sensitivity c-reactive protein (hsCRP) and asymmetric dimethylarginine (ADMA) were spun immediately and the plasma was frozen at -70^{c} for later batch measurement.

Return visits and definition of conversion to sustained hypertension

Subjects returned for yearly visits for three years. At each visits subjects were interviewed about their health since the previous visit and current medication. Measurements of weight and waist circumference were taken using the same scales and measuring tape used at baseline.

Conversion to sustained hypertension was defined as day ABPM $\geq$ 135/85 mmHg. Subjects commenced on anti-hypertensive therapy by the subject's general practitioner without prior confirmatory ABPM required day ABPM $\geq$ 135/85 mmHg, on anti-hypertensive therapy, to be considered as having developed sustained hypertension.

Participants were invited to continue yearly ABPM beyond three years whilst the study continued.

Statistical analysis

STATA, version 12, (StataCorp LP, College Station, Texas) was used to analyse the data. Normally distributed data is expressed as mean ± standard deviation, whereas skewed data is expressed as median [interquartile range (IQR)]. Categorical data is expressed as percentages. Non-normal data was transformed prior to analysis. Comparisons were two-sided. Univariate and multivariate linear regression was used to analyse continuous variables, whereas the chi-squared (χ^2) or Fischer's exact test was used to analyse categorical variables. Each outcome variable was examined for potential confounders. Analysis of outcome variable was adjusted for each confounder. Log-rank test was used to compare equality of new-onset hypertension curves. Statistical significance was considered met at α=0.05. A total sample size of 64, assuming the proportion of normotensives who progress to sustained hypertension in three years is 10% , will be powered to pick up an additional difference of 30% in the WCHT group, one sided, α=0.05. To take into account 20% dropout a total sample size of 77 was required.

Results

Forty two normotensives (NT)'s and 35 WCHT subjects were recruited. Four WCHT subjects were removed from the analysis as they did not return for a second confirmatory ABPM. The youngest WCHT subject recruited was 39 years, and the oldest NT recruited was 71 years ; in order to standardize the age of groups, the decision was taken to exclude subjects aged < 39 and > 71 years from both groups.

Data from 36 NT and 30 WCHT subjects was available for analysis at baseline. Baseline characteristics in the WCHT group were similar to the NT group, with regard to age, gender and

anthropomorphic measures (See Table 1). Sitting observed automated BP, unobserved automated blood pressure, ABPM (initial reading) and ABPM day average pressure were all higher in the WCHT group. Median PWVc was elevated in the WCHT group compared to the controls but the difference was no longer significant after adjustment for age and mean arterial pressure determined from BP taken immediately after PWV (See Table 2). Two hour plasma glucose post 75g dextrose challenge (2hPG) remained elevated in the WCHT group, after adjustment for waist circumference (See Table 3).

Three NT and two WCHT subjects had no follow-up data. Two WCHT were commenced on beta-blocker treatment for non-blood pressure reasons, and their data, from the study visit prior to beta-blocker commencement, was used in the analysis of WCHT who remained WCHT. Table 6.4 shows baseline characterisitics by the participant's final BP category. There were 31 NT who remained NT (NT→NT). Two NT subjects (5.6%) became hypertensive. Eighteen WCHT subjects (64.3%) remained WCHT (WCHT→WCHT) and ten WCHT subjects (35.7%) converted to sustained hypertension (WCHT→HT) (P=0.02). Six of the ten WCHT subjects had commenced antihypertensive therapy; of these subjects three subjects had a previous elevated day ABPM prior to commencing antihypertensive therapy and three subjects who did not have confirmatory ABPM prior to commencing antihypertensive therapy had elevated ABPM whilst on antihypertensive therapy.

Eighteen of the WCHT subjects (64.3%) had a previous ABPM identified within the two years prior to study commencement. Figure 1 shows hypertension onset (survival curve) analysis from the time of study commencement (p=0.005) and from time of the first identified ABPM

(p=0.06) in WCHT. Although not statistically different, the analysis from time of first identified ABPM shows diverging free from hypertension curves between normotension and WCHT.

WCHT subjects who developed sustained hypertension had significantly higher baseline waist circumference (91.9±13.1 cm) compared to both NT's who stayed NT (82.4±11.7 cm) and WCHT who did not develop sustained hypertension (80.1±9.7 cm) (See Table 4). They also had higher sitting unobserved automated BP and initial ambulatory blood pressure measurement compared to the two groups who did not develop hypertension (See Table 5 and Figure 2).

Compared to NT who stayed NT the WCHT participants who developed sustained hypertension had higher median PWVc, adjusted for age and MAP (See Table 6), and higher median 2hPG adjusted for waist circumference (See Table 7). There was a trend towards higher median adjusted PWVc and 2hPG in the WCHT participants who developed hypertension compared to WCHT who did not but the differences were not significant. Baseline flow-mediated dilation was not found to be a predictor of sustained hypertension in WCHT. No significant difference was found in leptin plasma levels or circulating biomarkers of inflammation or endothelial dysfunction (see Table 8).

The Framingham score of developing hypertension showed statistically different scores for WCHT who developed hypertension compared to WCHT who did not, when the sitting unobserved blood pressure was used rather than the sitting observed automated BP but the estimated score corresponds to a risk of approximately 23%. Figure 6.2 shows hypertension outcome by baseline BP measurement technique.

Discussion

This comprehensive prospective study of a carefully defined cohort of WCHT subjects has provided further evidence that WCHT is associated with increased risk of developing sustained hypertension.[1-3] Moreover, this study has shown that WCHT subjects who developed sustained hypertension had elevated baseline measures including waist circumference that may help to predict the onset of hypertension in the WCHT population. After adjustment for potential confounders the WCHT groups had higher median values for PWVc and 2hPG than the NT group, with a trend towards a higher value in the group that progressed to sustained hypertension. This suggests both are important in the development of hypertension in WCHT. The WCHT group who did not develop hypertension had intermediate PWVc and 2hPG between the NTs and those WCHT who developed hypertension but waist circumference was similar to NTs suggesting leanness may prevent or delay the onset of hypertension in WCHT.

In 1998 Saito et al. reporting on a study in adolescent males, showed that BP at five years follow-up was related to changes in body mass index in both WCHT and sustained hypertensives.[8] Subsequence studies that have examined baseline predictors of hypertension in the general population have found that body mass index is a predictor of incident hypertension[7, 9] and prospective studies that have used weight loss as an intervention have shown that the incidence of new-onset hypertension is reduced in the intervention group.[10, 11]

It is postulated that an increased body mass index can cause hypertension by the secretion of adipocytokines.[12] Leptin, which is released in proportion to the amount of body fat, is known to increase sympathetic nervous activity,[13] and has been shown to be a predictor for the

development of hypertension[14, 15] was not associated with new-onset hypertension in the WCHT group in the present study.

An elevated 2hPG[16, 17] has been shown to predict the onset of hypertension in the general population. Hyperglycaemia is associated with vasoconstriction[18] and impairs vasodilation[19] in animal models. In the only other study identified that examined baseline predictors of sustained hypertension in WCHT, the first hour of the 24h ABPM and BP variation during a mental stress test were found to be predictors of sustained hypertension in WCHT.[20] The present study found an elevated initial ABPM was associated with the development of hypertension in WCHT but the BP response to the mental stress test was not.

Myers et al. in an algorithm designed to diagnose hypertension in the doctor's office suggested unobserved automated blood pressure should be used instead of clinic observed blood pressure to eliminate the white-coat response.[21] This algorithm raises several concerns for patients with WCHT.[22] A particular concern is that patients with WCHT would not be identified and would go down the "continue to follow" pathway. Both the present study and the study by Colombo et al. (2000) suggest that the initial ABPM BP may be a better baseline BP to predict future hypertension risk in WCHT but larger studies are required to determine this.

As clinic observed and unobserved BP may not be the best BP to estimate the risk of future hypertension in WCHT the use of hypertension risk calculators may not be appropriate. The Framingham hypertension risk calculator also assigns a higher score for female sex, whereas this

study along with other studies found male sex to be associated with increased risk of hypertension.[18, 19]

Artery stiffness, measured by PWVc, has been shown to be elevated in hypertensives and the relationship is well-known to be reciprocal in that increased contained BP results in increased arterial stiffness and vice versa. Artery stiffness also predicts the onset of hypertension in the general population.[25,26] Artery stiffness is associated with endothelial dysfunction, and with increased inflammatory states[27] as well as associated with detrimental changes in media/lumen ratio in small resistance arteries which may contribute to an increase in central blood pressure by increasing the magnitude of wave reflections.[28] In spite of these established associations it remains unclear and a point of debate whether increased arterial stiffness is causal in the pathogenesis of hypertension or if it merely represents a biomarker of a prolonged increase in "operating" BP over time and an associated mechanical deterioration. It may be that primary arterial degeneration is causal in isolated systolic hypertension while being secondary phenomena in younger and/or combined hypertensives.

There are several limitations to this study. This study was not powered to pick up a difference between the WCHT groups n many variables tested. The study was not large enough to develop a new prediction model for hypertension in WCHT. Participants were only formally followed for three years and even though there was ABPM data on a cohort up to five years, a longer period of observation would almost certainly have identified more subjects who progressed to hypertension. Factors that may help to determine the pathway to hypertension in WCHT, such as inflammatory markers (e.g. Interleukins) were not examined.

Waist circumference, which is a modifiable risk factor for hypertension, should be measured and closely monitored in WCHT patients. Interventional studies are required to examine if the maintenance or reduction of waist measurement prevents or delays the onset of the development of sustained hypertension in WCHT. Larger studies are also required, with longer follow-up, to determine if the 2hPG and PWVc are associated with the development of sustained hypertension in WCHT. Studies are also required to develop hypertension risk in WCHT as current hypertension risk calculators are not suitable in WCHT.

References

1. Grandi AM, Solbiati F, Laurita E, Cassinerio E, Marchesi C, Piperno F, Maresca A, Guasti L, Venco A. Isolated office hypertension: a 3-year follow-up study. *Blood Press*. 2005;14:298-305.
2. Ugajin T, Hozawa A, Ohkubo T, Asayama K, Kikuya M, Obara T, Metoki H, Hoshi H, Hashimoto J, Totsune K, Satoh H, Tsuji I, Imai Y. White-coat hypertension as a risk factor for the development of home hypertension: the Ohasama study. *Arch Intern Med*. 2005;165:1541-1546.
3. Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Polo Friz H, Grassi G, Sega R. Long-term risk of sustained hypertension in white-coat or masked hypertension. *Hypertension*. 2009;54:226-232.
4. Niiranen TJ, Jula AM, Kantola IM, Reunanen A. Prevalence and determinants of isolated clinic hypertension in the Finnish population: the Finn-HOME study. *J Hypertens*. 2006;24:463-470.
5. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr., International Diabetes Federation Task Force on E, Prevention, Hational Heart L, Blood I, American Heart A, World Heart F, International Atherosclerosis S, International Association for the Study of O. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120:1640-1645.

6. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;15:539-553.

7. Parikh NI, Pencina MJ, Wang TJ, Benjamin EJ, Lanier KJ, Levy D, D'Agostino RB, Sr., Kannel WB, Vasan RS. A risk score for predicting near-term incidence of hypertension: the Framingham Heart Study. *Ann Intern Med*. 2008;148:102-110.

8. Saito II, Murata K, Tsujioka M, Kawabe H, Saruta T. Long-term changes in clinic blood pressure in patients with white-coat hypertension. *Blood Press Monit*. 1998;3:97-100.

9. Lee SK, Kim SH, Cho GY, Baik I, Lim HE, Park CG, Lee JB, Kim YH, Lim SY, Kim H, Shin C. Obesity phenotype and incident hypertension: a prospective community-based cohort study. *J Hypertens*. 2013;31:145-151.

10. He J, Whelton PK, Appel LJ, Charleston J, Klag MJ. Long-term effects of weight loss and dietary sodium reduction on incidence of hypertension. *Hypertension*. 2000;35:544-549.

11. Stevens VJ, Obarzanek E, Cook NR, Lee IM, Appel LJ, Smith West D, Milas NC, Mattfeldt-Beman M, Belden L, Bragg C, Millstone M, Raczynski J, Brewer A, Singh B, Cohen J, Trials for the Hypertension Prevention Research G. Long-term weight loss and changes in blood pressure: results of the Trials of Hypertension Prevention, phase II. *Ann Intern Med*. 2001;134:1-11.

12. Mendizabal Y, Llorens S, Nava E. Hypertension in metabolic syndrome: vascular pathophysiology. *Int J Hypertens*. 2013;2013:230868.

13. Satoh N, Ogawa Y, Katsuura G, Numata Y, Tsuji T, Hayase M, Ebihara K, Masuzaki H, Hosoda K, Yoshimasa Y, Nakao K. Sympathetic activation of leptin via the ventromedial

hypothalamus: leptin-induced increase in catecholamine secretion. *Diabetes*. 1999;48:1787-1793.

14. Asferg C, Mogelvang R, Flyvbjerg A, Frystyk J, Jensen JS, Marott JL, Appleyard M, Jensen GB, Jeppesen J. Leptin, not adiponectin, predicts hypertension in the Copenhagen City Heart Study. *Am J Hypertens*. 2010;23:327-333.
15. Thomopoulos C, Tsioufis C, Makris T, Stefanadis C. Free leptin predicts incident (clinic) hypertension in a Danish cohort. *Am J Hypertens*. 2010;23:814; author reply 815.
16. Boyko EJ, Barr EL, Zimmet PZ, Shaw JE. Two-hour glucose predicts the development of hypertension over 5 years: the AusDiab study. *J Hum Hypertens*. 2008;22:168-176.
17. Boyko EJ, Shaw JE, Zimmet PZ, Chitson P, Tuomilehto J, Alberti KG. A prospective study of glycemia, body size, insulin resistance and the risk of hypertension in Mauritius. *J Hypertens*. 2008;26:1742-1749.
18. Radi S, Lang T, Lauwers-Cances V, Chatellier G, Fauvel JP, Larabi L, De Gaudemaris R, Group I. One-year hypertension incidence and its predictors in a working population: the IHPAF study. *J Hum Hypertens*. 2004;18:487-494.
19. Zhang H, Thijs L, Kuznetsova T, Fagard RH, Li X, Staessen JA. Progression to hypertension in the non-hypertensive participants in the Flemish Study on Environment, Genes and Health Outcomes. *J Hypertens*. 2006;24:1719-1727.

Table 1: Baseline characteristics of normotensive and white-coat hypertensive groups.

	NT	WCHT	P-value
Number	36	30	
Time followed (years)	3.1 [2.3-3.3]	2.9 [1.1-3.6]	0.62
Age (years)	56.0±8.9	56.0±7.7	0.99
Female	26 (72.2)	25 (83.3)	0.28
Menopause	15 (57.7)	13 (52.0)	0.68
Waist (cm)	84.8±12.7	84.8±12.0	0.99
Weight (Kg)	69.8±14.7	69.8±13.1	0.99
Family history hypertension	8/28 (29)	8/22 (36)	0.56

Data is presented as mean ± standard deviation, median [inter-quartile range], geometric mean {95% confidence interval}, and number (%). NT: normotension, WCHT: white-coat hypertension, HT: sustained hypertension, cm: centimetres, Kg: kilogram.

Table 2: Baseline blood pressure and artery function characteristics of normotensive and white-coat hypertensive groups.

	NT	WCHT	P-value
Number	36	30	
Sitting arrival BP (mmHg)	124/80±11/8	138/89±16/9	<0.001[Π]
Sitting observed BP (mmHg)	121/80±9/8	133/86±10/9	<0.001[Π]
Sitting unobserved BP (mmHg)	116/77±9/6	124/81±10/8	0.003[Π]
ABPM initial (mmHg)	122/75±13/9	136/84±15/10	<0.001[Π]
Day ABPM (mmHg)	121/72±9/6	125/76±6/5	0.004[Π]
Night ABPM (mmHg)	104/59±8/6	107/61±9/7	0.16[Π]
PWV_c (m/s)	7.4 [6.7-8.3]	8.4 [7.3-9.1]	0.008
PWV_c adjusted for age and MAP[¥]	7.5 {7.1-7.9}	8.1 {7.6-8.7}	0.07

Π: Statistical comparison of MAP. ¥: MAP calculated from BP taken at time of PWVc. Data is presented as mean ± standard deviation, median [inter-quartile range] and geometric mean {95% confidence interval}. NT: normotension, WCHT: white-coat hypertension, HT: sustained hypertension, MAP: mean arterial pressure, BP: blood pressure, ABPM: ambulatory blood pressure, PWVc: central pulse wave velocity.

Table 3: Baseline lipid and glucose status of normotensive and white-coat hypertensive groups.

	NT	WCHT	P-value
Number	36	30	
Cholesterol treatment	2	2	
Cholesterol (mmol/l)	5.5±1.1	5.3±0.9	0.47
Triglycerides (mmol/l)	0.9 [0.6-1.4]	0.9 [0.5-1.3]	0.62
HDL (mmol/l)	1.4±0.4	1.4±0.4	0.82
LDL (mmol/l)	3.5±1.1	3.5±0.8	0.97
Insulin (mmol/l)	4.2 [2.8-6.2]	4.1 [3.2-5.0]	0.98
Fasting glucose (mmol/l)	4.9 [4.6-5.2]	5.0 [4.8-5.1]	0.13
2hPG (mmol/l)	5.8 [4.8-7.1]	7.6 [5.9-9.2]	0.003
2hPG adjusted for waist	5.9 {5.4-6.5}	7.5 {6.7-8.4}	0.002

Data is presented as mean ± standard deviation, median [inter-quartile range], geometric mean {95% confidence interval}, and number (%). NT: normotension, WCHT: white-coat hypertension, HT: sustained hypertension, HDL: high density lipoprotein, LDL: low density lipoprotein, 2hPG: two hour plasma glucose post 75g dextrose.

Table 4: Baseline characteristics of outcome blood pressure groups.

	NT → NT (1)	WCHT → WCHT (2)	WCHT → HT (3)	2 vs 1	3 vs 1	3 vs 2
Number	31	18	10			
Length of follow up (years)	3.1 [2.3-3.4]	3.6 [2.6-4.6]	2.3 [1.9-3.8]	0.07	0.14	0.10
Age (years)	56.6±8.4	54.3±7.1	58.1±9.0	0.35	0.62	0.25
Male	8 (25.8)	1 (5.6)	4 (40.0)	0.08	0.44	0.04
Menopause	13 (56.5)	7 (41.2)	4 (66.7)	0.63	0.99	0.37
Waist (cm)	82.4±11.7	80.1±9.7	91.9±13.1	0.49	0.03	0.01
Weight (Kg)	66.7± 13.4	66.3± 11.2	75.4± 15.3	0.92	0.08	0.09
Metabolic syndrome (sitting observed BP)	4 (12.9)	4 (22.2)	5 (50.0)			
Metabolic syndrome (sitting unobserved BP)	4 (12.9)	2 (11.1)	3 (30.0)			

Data is presented as mean ± standard deviation, median [inter-quartile range] and number (%). NT→NT: normotensive who remained normotensive, WCHT→WCHT: white-coat hypertensive who did not develop sustained hypertension, WCHT→HT: WCHT who developed sustained hypertension, WCHT: white-coat hypertension, cm: centimetres, Kg: kilogram, BP: blood pressure.

Table 5: Baseline blood pressures by blood pressure outcome.

	NT → NT (1)	WCHT → WCHT (2)	WCHT → HT (3)	2 vs 1	3 vs 1	3 vs 2
Number	31	18	10			
Sitting arrival BP (mmHg)	124/81±12/8	137/87±18/9	138/91±8/5	0.004	0.001	0.33
Sitting observed BP (mmHg)	120/80±10/8	132/84±9/7	135/87±7/10	0.002	0.001	0.47
Sitting unobserved BP (mmHg)	116/77±9/6	122/80±9/8	130/83±9/9	0.09	<0.001	0.04
ABPM initial BP (mmHg)	121/74±13/9	129/82±9/7	143/87±14/14	0.002	<0.001	0.02
Day ABPM (mmHg)	120/71±9/6	124/76±6/4	129/77±4/6	0.002	<0.001	0.30
Night ABPM (mmHg)	103/59±7/5	103/60±4/4	114/62±12/11	0.84	0.006	0.02
Mental stress test ΔSBP/DBP (mmHg)	12/6 [3-16]/[0-10]	15/12 [11-22]/ [2-14]	15/12 [11-27]/[6-17]	0.06	0.11	0.81

II: Statistical comparison of MAP. Data is presented as mean ± standard deviation and median [inter-quartile range]. NT→NT: normotensive who remained normotensive, WCHT→WCHT: white-coat hypertensive who did not develop sustained hypertension, WCHT→HT: WCHT who developed sustained hypertension, WCHT: white-coat hypertension, SBP: systolic blood pressure, DBP: diastolic blood pressure..

Table 6: Baseline artery function by blood pressure outcome.

	NT → NT (1)	WCHT → WCHT (2)	WCHT → HT (3)	2 vs 1	3 vs 1	3 vs 2
Number	31	18	10			
PWV_c (m/s)	7.2 [6.9-7.9]	7.8 [7.3-8.9]	8.5 [7.2-10.2]	0.07	0.003	0.15
$PWV_c \geq 10$ ms^{-2}	0	1	3			
PWV_c adjusted for MAP and age	7.4 {6.8-7.8}	7.9 {7.3-8.6}	8.7 {7.7-9.8}	0.11	0.03	0.22
FMD (% Δ) adjusted for baseline diameter	7.0 [4.3-10.6]	8.5 [4.7-10.5]	6.1 [4.0-8.0]	0.42	0.71	0.64

Data is presented as mean ± standard deviation, median [inter-quartile range] and geometric mean {95% confidence interval}. NT→NT: normotensive who remained normotensive, WCHT→WCHT: white-coat hypertensive who did not develop sustained hypertension, WCHT→HT: WCHT who developed sustained hypertension, WCHT: white-coat hypertension, MAP: mean arterial pressure, BP: blood pressure, ABPM: ambulatory blood pressure, PWVc: central pulse wave velocity, FMD: flow-mediated dilatation.

Table 7: Baseline participant characteristics by blood pressure outcome.

	NT → NT (1)	WCHT → WCHT (2)	WCHT → HT (3)	2 vs 1	3 vs 1	3 vs 2
Number	31	18	10			
Family history HT	7/26 (27)	6/15 (40)	2/6 (33)	0.26	0.07	0.16
Risk of HT at 4yrs (clinic observed BP)	13.5 [10-17]	18 [15-19]	17.5 [16-21]	0.01	0.003	0.36
Risk score of HT at 4 years (clinic unobserved BP)	12 [9-15]	12.5 [9.5-16]	16.5 [15-18]	0.61	0.007	0.03
Cholesterol med	2	0	2			
Cholesterol (mmol/l)	5.4±1.1	5.6±0.9	4.9±0.7	0.66	0.10	0.06
Triglycerides (mmol/l)	0.94±0.46	0.94±0.54	1.1±0.62	0.99	0.52	0.55
HDL (mmol/l)	1.5±0.4	1.5±0.4	1.4±0.3	0.85	0.39	0.52
LDL (mmol/l)	3.5±1.1	3.7±0.8	3.0±0.6	0.49	0.19	0.09
Insulin (mmol/l)	3.9 [2.6-5.6]	4.1 [3.2-4.7]	4.1 [2.7-5.0]	0.68	0.70	0.93
Fasting glucose (mmol/l)	4.7 [4.6-5.1]	5.0 [4.8-5.1]	5.0 [4.8-5.3]	0.63	0.09	0.22
2hPG (mmol/l)	5.7 [4.5-6.4]	6.4 [5.7-8.1]	8.4 [7.2-9.3]	0.04	0.001	0.09
2hPG adjusted for waist (mmol/l)	5.7 {5.1-6.3}	6.7 {5.9-7.7}	8.1 {6.7-9.9}	0.04	0.002	0.13

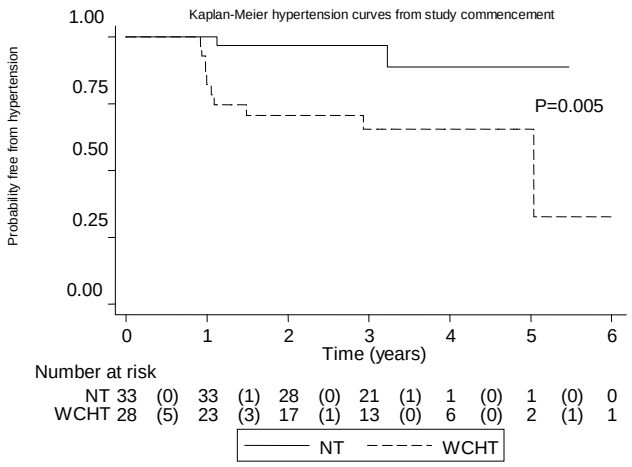
Data is presented as mean ± standard deviation, median [inter-quartile range] and geometric mean {95% confidence interval}. NT→NT: normotensive who remained normotensive, WCHT→WCHT: white-coat hypertensive who did not develop sustained hypertension, WCHT→HT: WCHT who developed sustained hypertension, WCHT: white-coat hypertension, HDL: high density lipoprotein, LDL: low density lipoprotein, 2hPG: two hour plasma glucose post 75g dextrose.

Table 8: Baseline circulating biomarkers by blood pressure outcome.

	NT → NT (1)	WCHT → WCHT (2)	WCHT → HT (3)	2 vs 1	3 vs 1	3 vs 2
Number	31	18	10			
Leptin (ng/ml)	7.6 [4.7-17.3]	13.1 [7.6-15.5]	11.3 [4.5-18.4]	0.50	0.60	0.94
ADMA (umol/L)	0.66 [0.40-0.73]	0.70 [0.54-0.80]	0.94 [0.68-1.01]	0.65	0.17	0.28
hsCRP (mg/L)	0.9 [0.3-2.1]	0.6 [0.6-1.9]	1.1 [0.5-1.5]	0.81	0.84	0.97
PAI-1 (U/ML)	2.8 [2.5-3.4]	2.8 [2.5-3.2]	3.4 [3.3-3.4]	0.73	0.26	0.18
VWF (IU/L)	1.1 [0.7-1.4]	0.9 [0.7-1.3]	1.1 [0.9-1.4]	0.70	0.93	0.71

Data is presented as median [inter-quartile range. NT→NT: normotensive who remained normotensive, WCHT→WCHT: white-coat hypertensive who did not develop sustained hypertension, WCHT→HT: WCHT who developed sustained hypertension, WCHT: white-coat hypertension, ADMA: asymmetric dimethyl arginine, hsCRP: high sensitivity c-reactive protein, PAI-1: plasminogen activator inhibitor 1, VWF: von willebrand factor.

a)



b)

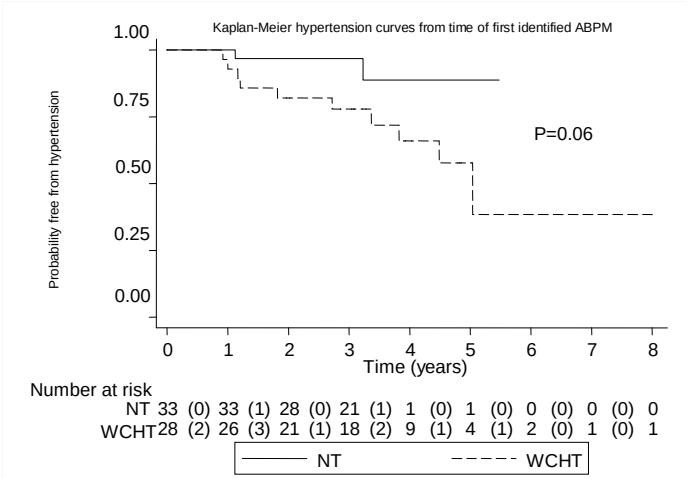


Figure 1: New onset hypertension curves-probablity of remaining free from sustained hypertension. NT: normotensive, WCHT: white-coat hypertension.

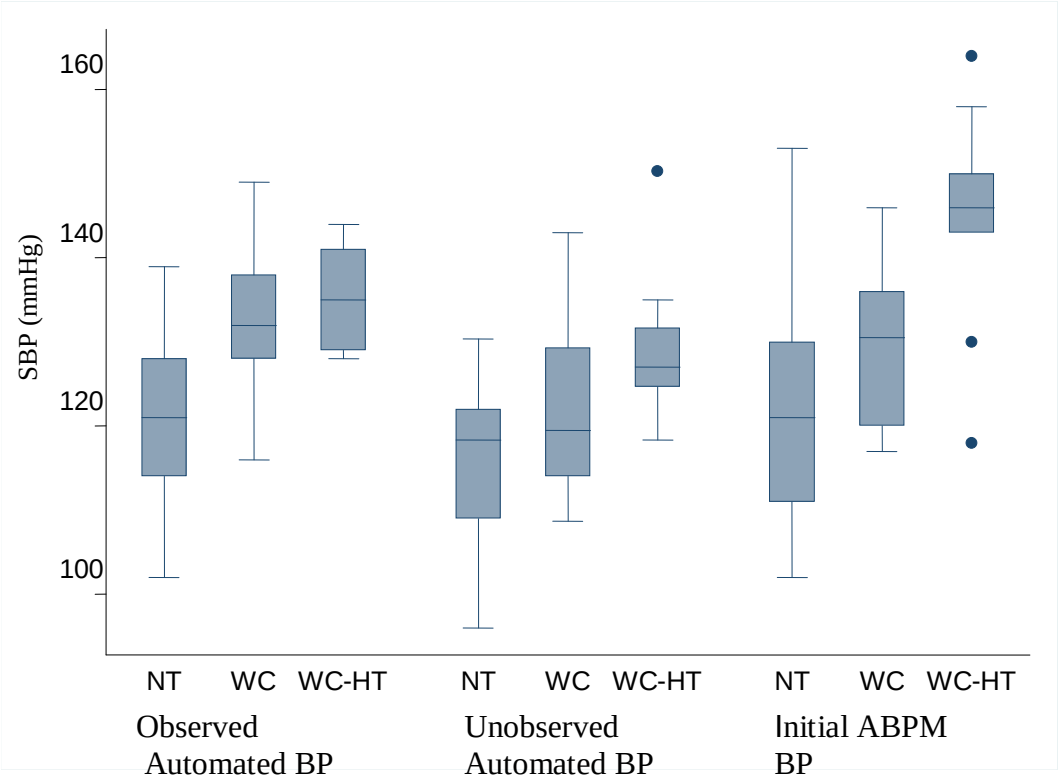


Figure 2: Boxplots of blood pressures measured at baseline by final blood pressure outcome ABPM: ambulatory blood pressure monitoring, BP: Blood pressure, NT: normotensive, WC: white-coat hypertensive, WC-HT: WCHT who developed sustained hypertension in the study period.

Chapter 8

White-coat hypertension

8.1 Introduction

The published paper in this chapter provides an up to date review examining the evidence for possible vascular and circulating biomarkers of WCHT, its association with cardiovascular target organ damage, and whether or not WCHT can predict future sustained hypertension and cardiovascular events. It is different from previous reviews in that it attempts to standardize the evidence about WCHT by only including studies with similar definitions of WCHT in untreated populations. Studies were included in the review if the definition of WCHT used met the 2007 European Society of Hypertension and European Society of Cardiology hypertension guidelines, which were current at the time of the review.

Usually reviews are placed at the front of a thesis but this review includes recent published work, including work from this thesis and recommendations for treating physicians, based on the conclusions from this thesis.

Questions addressed in this paper are

- Is WCHT associated with the Metabolic Syndrome?
- Is WCHT associated with vascular biomarkers of hypertension?
- Is WCHT associated with increased sympathetic activity?
- Is WCHT associated with target organ damage?
- Is WCHT associated with increased risk of future hypertension?
- Is WCHT associated with increased cardiovascular events?

8.2 Declaration

In the case of Chapter eight, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, writing paper	90

The following co-authors contributed to the work.

Name	Nature of contribution
Professor Barry McGrath	Conception, review of paper

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the candidate's and co-authors' contributions to this work.

Candidate's Signature [redacted] 18/9/13

Main Supervisor's Signature [redacted] 18 9 13

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CEPP

Frontiers in Research

Frontiers in Research Review:

Ambulatory and Home Blood Pressure Measurement in the Management of Hypertension

White-coat hypertension

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SUMMARY

1. Numerous studies have examined whether white-coat hypertension (WCHT) is associated with increased cardiovascular risk, but with definitions of WCHT that were not sufficiently robust, results have been inconsistent. The aim of the present review was to standardize the evidence by only including studies that used a definition of WCHT consistent with international guidelines.

2. Published studies were reviewed for data on vascular dysfunction, target organ damage, risk of future sustained hypertension and cardiovascular events.

3. White-coat hypertension has a population prevalence of approximately 15% and is associated with non-smoking and slightly elevated clinic blood pressure. Compared with normotensives, subjects with WCHT are at increased cardiovascular risk due to a higher prevalence of glucose dysregulation, increased left ventricular mass index and increased risk of future diabetes and hypertension.

4. In conclusion, management of a patient with WCHT should focus on cardiovascular risk factors, particularly glucose intolerance, not blood pressure alone.

Key words: cardiovascular risk, glucose intolerance, isolated clinic hypertension, white-coat hypertension.

INTRODUCTION

The 2005 American Heart Association guidelines for the measurement of blood pressure in humans defined white-coat hypertension (WCHT) as persistently elevated blood pressure (BP) 'in the presence of a health care worker, particularly a physician' (≥ 140/90 mmHg) in patients not taking medication, with an average awake ambulatory blood pressure monitoring (ABPM) < 135/85 mmHg.[1] In 2007, the European Society of Hypertension and European Society of Cardiology published guidelines for the management of hypertension and recommended WCHT should be diagnosed when office BP ≥ 140/90 on at least three occasions, with normal 24 h (< 125–130/80 mmHg) and day ABPM (< 130–135/85 mmHg) or home BP (average of several readings < 130–135/85 mmHg).[2]

Key elements of the definition of WCHT are that subjects should not be taking antihypertensive therapy, the personnel and methods of recording office blood pressure need to be clearly defined and that more than one set of day ABPM or home BP recordings are made. Office blood pressures recorded by medical practitioners give higher readings on average compared with readings obtained by nurses or non-medical trained health professionals.[3] The differences between office and average daytime ABPM or home BP measurements can be minimized by use of non-observed automated BP recordings in the clinic.[4] It is recommended that at least two 24 h ABPM readings are recorded to confirm WCHT.[5] Not having consistency in the definition of WCHT has made comparison of research studies difficult.

"Need at least two 24h ABPM recordings to confirm WCHT"

White-coat hypertension needs to be distinguished from the white-coat effect, which is a numerical value that describes the rise in BP in the presence of a medical practitioner, a response that is observed variably across all categories of blood pressure from normotensive subjects to subjects with sustained hypertension.

The present review focuses on studies in untreated adult populations in which WCHT was defined on the basis of 2007

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Frontiers in Research

List of abbreviations:

ABPM	ambulatory blood pressure monitoring	OR	odds ratio
BMI	body mass index	PAMELA	Pressione ArterioseMonitorate E Loro Associazioni
BP	blood pressure	TOD	target organ damage
CVE	cardiovascular event	WCHT	white-coat hypertension
CI	confidence interval	24h	twenty-four hour
LVMI	left ventricular mass index		

European Society of Hypertension and European Society of Cardiology hypertension guidelines. Studies were excluded if: (i) WCHT was defined by either systolic or diastolic BP alone; (ii) different definitions of out-of office BP were used for men and women; (iii) different definitions of out-of-office BP were used for WCHT and normotensive groups; (iv) not all participants had ABPM in ABPM studies; (v) it was unclear how normotensives were obtained; (vi) results were not given; or (vii) statistical comparisons were not made between WCHT and normotensive groups.

"General population (Europe) prevalence of WCHT ~15%"

In the present review we examine the prevalence of WCHT, its associations with other clinical conditions and vascular biomarkers and the risk of future hypertension and cardiovascular disease in subjects with WCHT compared with normotensives.

Information about WCHT has been determined from three sources, all of which have inherent biases: (i) population studies; (ii) medical clinics; and (iii) ABPM services. Population studies screen for WCHT on one clinic visit, whereas studies from ABPM services and medical clinics are biased towards patients who are referred and/or more likely to seek health care.

PREVALENCE AND CHARACTERISTICS OF WHITE-COAT HYPERTENSION

Two population studies have examined WCHT in untreated participants.[6,7] The Finn-HOME study randomly selected 1440 participants, aged 45–74 years, from the general population of Finland and, using home BP < 135/85 mmHg as the out-of-office BP to define WCHT, determined a general population prevalence of 15.6%; among untreated hypertensives, the prevalence was 37.5%.[6] Participants with WCHT were less likely to smoke compared with normotensives and sustained hypertensives, had a similar body mass index (BMI) to normotensives and mildly elevated clinic BP. Mean values of a number of other cardiovascular risk factors (age, lipid profile, fasting glucose, prevalence of diabetes) were intermediate between normotensive and sustained hypertensives.[6]

The Pressione Arteriose Monitorate E Loro Associazioni (PAMELA) study randomized a sample of 3200 people, aged 25–74 years, representative of the city of Monza in Lombardy, Italy.[7] The definition of WCHT was based on clinic BP ≥ 140/90 and a 24 h ABPM reading of < 125/79 mmHg or home BP < 135/83 mmHg, values obtained from population regression analysis of BP to corresponding clinic BP of 140/90 mmHg. The PAMELA study estimated a population prevalence of WCHT of 15% by ABPM, with only 70% of these participants also having WCHT based on home BP definition.[7]

The prevalence of WCHT among untreated hypertensive patients is estimated to be 15–45% and is associated with non-smoking, female sex and increasing age.[8]

IS WHITE-COAT HYPERTENSION ASSOCIATED WITH METABOLIC SYNDROME?

It has been hypothesized that WCHT is associated with metabolic syndrome.[9] The 2009 Joint Statement on the Metabolic Syndrome defines metabolic syndrome as the presence of three of five abnormal findings of waist circumference, triglycerides, high-density lipoprotein (HDL), BP or fasting glucose.[10]

Bjorklund *et al.*,[11] in a study that recruited men aged 50 years of age, found that participants who had WCHT (day ABPM < 135/85 mmHg) at the 20 year follow up had similar baseline BMI (23.9 kg/m^2) compared with normotensives (23.8 kg/m^2), but had slightly higher BMI at 70 years of age (25.3 vs 24.8 kg/m^2, respectively). Baseline mean triglycerides were higher in the WCHT group compared with normotensives (1.76 vs 1.59 mmol/L, respectively). Mean fasting insulin was higher in the WCHT compared with normotensive group (47.7 vs 39.0 mmol/L, respectively). Participants underwent intravenous glucose tolerance test at baseline and those in the WCHT group had higher plasma glucose at 60 min (11.0 vs 10.2 mmol/L) and a lower glucose disappearance rate (1.7 vs 1.96 *K* values).[11]

In a follow up of the PAMELA study, which excluded diabetics at study entry, participants with WCHT were more likely to develop future diabetes and impaired fasting glucose at 10 years.[12] The baseline BMI of the WCHT group was similar to that of sustained hypertensives and higher than that of normotensives

(26.4 vs 27.2 and 23.9 kg/m^2, respectively). At baseline, participants with WCHT had higher levels of fasting plasma glucose compared with normotensives (5.0 vs 4.7 mmol/L, respectively). Unadjusted odds ratios (ORs) for the development of diabetes in WCHT compared with normotensives based on 24 h ABPM and home BP were 2.9 and 3.0, respectively. After adjustment for baseline metabolic factors, the risk of developing impaired fasting glucose or diabetes was no longer significant.[12]

In a substudy of the OHASAMA study, a longitudinal study of residents in Ohasama, Japan, 466 participants not on antihypertensive or diabetic treatment volunteered to undergo an oral glucose tolerance test. The sample contained 71% of women, reflecting the bias of female gender towards seeking health care and participating in trials. The BMI in the WCHT group was similar to that in the normotensive group (23.5 vs 22.7 kg/m^2, respectively). The WCHT group had similar HDL but higher triglycerides compared with normotensives. The prevalence of impaired fasting glucose was 11.1% and 2.2% in the WCHT and normotensives, respectively. The prevalence of impaired glucose tolerance was 24.4% and 17.2% in the WCHT and normotensives, respectively, whereas the prevalence of diabetes was 8.9% and 3.7% normotensives, respectively.[13]

A link between impaired glucose tolerance and WCHT was also found in a study of 105 subjects who were aged 39–75 years, untreated, non-smokers and with normal fasting glucose levels.[14] Mean 2 h plasma glucose level post-load, adjusted for age and waist circumference, was 5.7 mmol/L in normotensives and 7.4 mmol/L in the WCHT group (P = 0.002). As a result of this finding, it was suggested that assessment of total cardiovascular risk in subjects with WCHT should include measurement of postprandial glucose.[14]

It appears that WCHT is associated with glucose dysregulation and that the presence of metabolic risk factors in WCHT increases the risk for future diabetes.

IS WHITE-COAT HYPERTENSION ASSOCIATED WITH VASCULAR BIOMARKERS OF HYPERTENSION?

In a review in 2008, we examined studies that assessed measures related to endothelial dysfunction, oxidative stress, inflammation and arterial function in WCHT.[15] The studies were all cross-sectional, tended to be small and many did not take into account potential confounding factors in their analyses. Overall, the studies provided equivocal evidence of altered vascular biomarkers in WCHT. Since that review, there have been a small number of additional studies that have examined circulating biomarkers of altered vascular function in WCHT. In the present review we examine studies that meet the inclusion criteria given above.

Six studies were identified that have examined C-reactive protein (CRP) in WCHT, with only one finding a significant difference between the WCHT and normotensive groups.[14,16–20] Only one study was identified that examined plasminogen activator I and von Willebrand factor levels in WCHT, with no significant difference found between the WCHT and normotensive groups.[14] Two studies examined homocysteine[21,22] and asymmetric dimethylarginine[14,23] in WCHT with conflicting results. Three studies examined measures of nitrite–nitrate (metabolites of nitric oxide); two found no significant difference between WCHT and normotensive groups.[18,24,25] One study was identified that examined endothelial microparticles, which are defined as small, membrane-bound vesicles derived from cells such as endothelial cells. Microparticles retain the signature membrane of the parent cell; hence, endothelial microparticles can be identified and used as a biomarker of endothelial dysfunction. The study by Chen *et al.*[26] found raised endothelial microparticles in sustained hypertensives, but the level of endothelial microparticles in WCHT was similar to that in normotensives.

"No consistent evidence of endothelial dysfunction in WCHT"

Assessment of endothelial function by brachial artery flow-mediated vasodilation has been examined in two studies, with conflicting results.[25,27] Gomez-Cerezo *et al.*[27] found that WCHT had a lower percentage decrease in brachial artery diameter compared with both normotensives and sustained hypertensives, but no adjustment for baseline diameter or overweight participants was taken into account. The WCHT group had the higher basal diameter of the three groups and the result may be due to regression to the mean.

Arterial stiffness, assessed by aortofemoral (central) pulse wave velocity has been measured in fours studies.[14,28–30] Adjustment for mean arterial pressure (MAP), an important confounder[31] occurred in only two of these studies.[14,29] Both studies, after adjustment for MAP, found no significant difference between normotensive and WCHT.

Based on the inclusion criteria give above, the number of studies assessing an association between WCHT and circulating vascular biomarkers is limited, with no consistent evidence to indicate vascular endothelial dysfunction is present in WCHT.

IS WHITE-COAT HYPERTENSION ASSOCIATED WITH INCREASED SYMPATHETIC ACTIVITY?

Essential hypertension is associated with increased sympathetic activity.[32] Only one study using direct measure of sympathetic nerve activity (microneurography) was found that met the inclusion criteria for this review. Grassi *et al.*[33] found WCHT had significantly higher resting sympathetic nerve activity than

normotensives. One of four studies assessing the ratio of low frequency to high frequency (LF/HF) as an indirect measure of sympathovagal balance found WCHT had a higher LF/HF ratio than normotensives.[14,34–36] One study was identified that assessed BP response to mental stress, with the WCHT group having a significantly greater increase in BP compared with the normotensive group.[14]

IS WHITE-COAT HYPERTENSION ASSOCIATED WITH TARGET ORGAN DAMAGE?

Only 10 studies meeting our inclusion criteria were found that assessed target organ damage in WCHT and normotensive groups.[17,19,33,37–43] Most studies measured left ventricular mass index (LVMI) as the measure of target organ damage. Six of eight studies, which used body surface area to normalize LVMI, found that the WCHT group had increased LVMI compared with normotensives (Fig. 1). Two of the studies that found no significant difference between the normotensive and WCHT groups had excessive LVMI as an exclusion criterion,[17,40] which may have influenced the results. Many of the studies were small, determined clinic BP and out-of-office BP on one occasion and many did not adjust for potential confounders. Palatini *et al.*[37] required WCHT to have two ABPM readings < 130/80 mmHg 3 months apart and found that the WCHT group, after adjustment for age, sex and BMI, had significantly higher LVMI compared with the normotensive group. However, the normotensive group had only one ABPM.[37] The only other study that had a large sample size normalized LVMI by height and found no significant difference after adjusting for the same confounders.[42]

"WCHT is associated with increased LVMI"

Three studies assessed carotid intima–media thickness with mixed results.[19,39,42] Two studies were identified that measured 24 h urinary albumin, with no significant difference found between the WCHT and normotensive groups.[19,43]

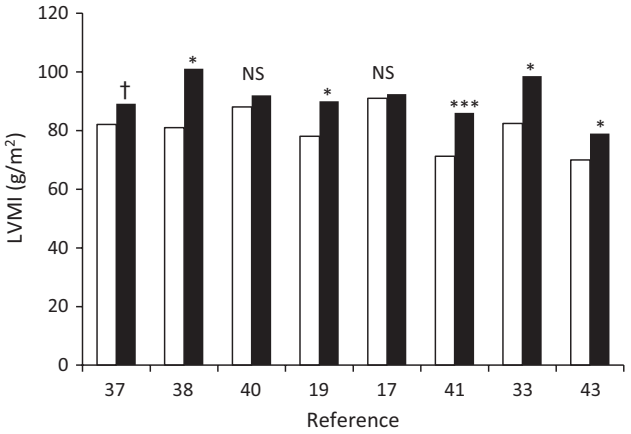

Fig. 1 Comparison of left ventricular mass index (LVMI) in subjects with white-coat hypertension (■) and normotensive subjects (□) in different studies. Data are reported mean values. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with normotensives. $^{\dagger}P$ value not given.

No studies were found that met our inclusion criteria examining fundoscopy in WCHT.

Based on this analysis WCHT appears to have an increased risk for target organ damage, based on increased LVMI compared with normotensives.

IS WHITE-COAT HYPERTENSION ASSOCIATED WITH AN INCREASED RISK OF FUTURE HYPERTENSION?

Three prospective studies were identified that met our inclusion criteria and assessed the development of sustained hypertension in WCHT and normotensive groups (Table 1).[44–46]

Table 1 Studies examining progression to sustained hypertension in white-coat hypertensives compared with normotensives

Study	BP methodology	Years followed	No. progressing to sustained HT/ participants (%)		OR (95% CI)
			WCHT	NT	
Grandi *et al.*[44]	ABPM	3	44/61 (72.1)	0/38 (0)	
Ugajin *et al.*[45]	Home BP	8.2	31/128 (24.2)	71/649 (10.9)	3.1 (1.8–5.2)
Mancia *et al.*[46]	Home BP	10	Not given	Not given	3.7 (2.2–6.3)
	ABPM				3.3 (2.1–5.1)

WCHT, white-coat hypertensives; NT, normotensives; HT, hypertension; OR, odds ratio; CI, confidence interval; ABPM, ambulatory blood pressure (BP) monitoring.

Grandi *et al.*,[44] using baseline day ABPM < 130/80 mmHg to define out-of-office normal BP, followed participants for 3 years; progression to sustained hypertension was defined as a day ABPM > 135/85 mmHg. In the WCHT group, 44 of 61 subjects (72%) developed sustained hypertension, whereas no normotensive developed sustained hypertension. In an analysis of participants who were untreated at follow up, LVMI was elevated at baseline in the WCHT group that developed sustained hypertension compared with both the WCHT group that WCHT and normotensives.[44]

Two population studies have examined the progression of WCHT to sustained hypertension, with both studies defining WCHT based on one clinic screening visit. The OHASAMA study[45] examined incident hypertension defined by home BP > 135/85 mmHg or commencement of antihypertensive medication over 8 years, with 22.2% of normotensives progressing to sustained hypertension and 46.9% of WCHT progressing to sustained hypertension (OR 2.9; 95% confidence interval (CI) 1.9–4.3). For the development of hypertension defined by home hypertension alone, 10.9% in the normotensive group and 22.7% in the WCHT group developed sustained hypertension (OR 3.1; 95% CI 1.9–5.3).[45]

The PAMELA study followed participants for 10 years and assessed new-onset hypertension by home BP and ABPM. The WCHT participants were more likely to develop sustained hypertension compared with normotensives. The OR for age- and sex-adjusted new-onset hypertension was 3.3 (95% CI 2.1–5.1) for 24 h ABPM and 3.7 (95% CI 2.2–6.3) for home BP in participants not taking antihypertensive therapy at baseline or follow up.[46]

It is evident that individuals with WCHT are at increased risk of developing sustained hypertension compared with normotensives.

IS WHITE-COAT HYPERTENSION ASSOCIATED WITH INCREASED CARDIOVASCULAR EVENTS?

Six prospective studies that met our inclusion criteria and examining the incidence of cardiovascular events (CVE) were identified (Table 2).[47–52] Four of these studies included treated participants in the WCHT group but had done separate analysis for participants who were not on antihypertensive therapy at baseline.[47–50]

"10 year risk of sustained hypertension is > 3 fold"

In the six studies identified, the WCHT groups did not differ significantly from the normotensive groups in terms of the risk of CVE, but because the WCHT groups tended to have much greater rates of antihypertensive treatment at follow up than the normotensive groups the results are not conclusive. Two studies documented the rate of antihypertensive treatment in WCHT (32% and 46%) compared with normotensives (2% and 7%).[51,52]

A meta-analysis was identified that analysed untreated participants at baseline using European hypertension guidelines.[53] Pierdomenico *et al.* identified six prospective studies in WCHT and found similar risks between normotensives and WCHT, with an adjusted OR of 0.96 (95% CI 0.65–1.42) compared with normotensives.[53] Antihypertensive therapy at follow up was more frequent in WCHT compared with normotensives, which may have confounded the result. One study from that meta-analysis was excluded from the present review due to different ABPM definitions for men and women.[54]

Because of the high rate of antihypertensive treatment in WCHT, it is difficult to determine whether WCHT are at greater risk of CVE than normotensives based on prospective studies.

The risk of CVE in WCHT was shown in a study by Verdecchia *et al.*[55] to be dependent on baseline ABPM BP. In that study, WCHT was categorized into day ABPM < 130/80 mmHg and day ABPM 130–135/80–85 mmHg. The WCHT with the higher day ABPM had event rates significantly higher than normotensives and similar to sustained hypertensives; however, no information regarding baseline antihypertensive therapy was provided.[55]

Table 2 Prospective studies comparing cardiovascular events in white-coat hypertensives and normotensives

		No. CVE/participants (%)		
	Years followed	WCHT	NT	WCHT vs NT
Kario *et al.*[51]	3.5	5/236 (2.1)	3/147 (2.0)	NS
Fagard *et al.*[47]	10.9	8/50 (16.0)	16/117 (13.7)	NS
Ohkubo *et al.*[48]	10.2	6/93 (6.5)	25/581 (4.3)	NS
Hansen *et al.*[49]	9.5	Data not shown	Data not shown	NS
Pierdomenico *et al.*[52]	6.4	12/399 (3.0)	7/305 (2.3)	NS
Hanninen *et al.*[50]	7.5	16/237 (6.8)	47/789 (6.0)	NS

CVE, cardiovascular events; WCHT, white-coat hypertensive; NT, normotensives.

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OVERALL MESSAGE FOR TREATING CLINICIANS

The purpose of the present review was to determine, using European hypertension guidelines, whether WCHT is associated with an increased cardiovascular risk. We have shown that, compared with normotensives, individuals with WCHT are more likely to have higher LVMI, glucose dysregulation and are at increased risk of future sustained hypertension. We are of the opinion that this shows that WCHT is associated with an increased cardiovascular risk and that WCHT itself may be regarded as a biomarker of an increased cardiovascular risk.

"Focus on CV risk, particularly glucose intolerance"

Patients with WCHT need to be identified. Diagnosis needs to be based on repeated high clinic readings; hence, patients presenting with a high reading need to have clinic BP measurements taken on subsequent occasions. Practitioners need to focus on a patient's overall cardiovascular risk. Assessment, monitoring and education of WCHT patients should include the following.

1. Assessment for the presence of target organ damage.
2. Assessment of cardiovascular risk, including an oral glucose tolerance test.
3. Education regarding increased cardiovascular and diabetes risk, with particular emphasis on maintaining and/or losing weight.
4. Education regarding limiting salt intake, in particular processed foods.[56]
5. Monitoring weight.
6. Monitoring for conversion to sustained hypertension (1–2 yearly ABPM) and/or regular home BP monitoring.
7. Monitoring for the development of diabetes, particularly in WCHT with weight gain.

FUTURE RESEARCH DIRECTIONS

One of the main problems in the area of WCHT research is that of poor definition of the condition, and it is recommended that treated hypertensives, subjects with insufficient definition of clinic BP and only a single set of 24 h ABPM recordings or poorly characterized home BP should not be included in research studies.

More extensive evaluation is required of automated non-observed clinic BP, its relationships with 24 h ABPM and home BP and long-term predictability of CVE.

White-coat hypertension can be considered a form of hypertension in evolution. The links between progression to sustained hypertension, glucose dysregulation and insulin resistance should be further evaluated. In a small study, baseline waist circumference, glucose dysregulation and central pulse wave velocity were found to be associated with new-onset hypertension in participants with WCHT.[57]

Newer biomarkers of cardiovascular risk, such as circulating cellular inflammatory biomarkers (tumour necrosis factor-α, interleukins) and microRNAs (miRNAs) have not been examined in WCHT. Two potential areas of research for miRNAs in WCHT are the analysis of miRNAs associated with endothelial dysfunction and left ventricular hypertrophy.[58]

CONCLUSION

White-coat hypertension is a high cardiovascular risk condition because patients with WCHT are more likely to develop sustained hypertension and diabetes compared with normotensives. The risk increases with additional metabolic risk factors, particularly obesity. Management of an individual with WCHT should focus on cardiovascular risk factors, particularly obesity and glucose intolerance, not BP alone.

REFERENCES

1. Pickering TG, Hall JE, Appel LJ *et al.* Recommendations for blood pressure measurement in humans: An AHA scientific statement from the Council on High Blood Pressure Research Professional and Public Education Subcommittee. *J. Clin. Hypertens. (Greenwich)* 2005; **7**: 102–9.
2. Mancia G, De Backer G, Dominiczak A *et al.* 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J. Hypertens.* 2007; **25**: 1105–87.
3. Mancia G, Bertinieri G, Grassi G *et al.* Effects of blood-pressure measurement by the doctor on patient's blood pressure and heart rate. *Lancet* 1983; **2**: 695–8.
4. Myers MG, Godwin M, Dawes M, Kiss A, Tobe SW, Kaczorowski J. Measurement of blood pressure in the office: Recognizing the problem and proposing the solution. *Hypertension* 2010; **55**: 195–200.
5. Palatini P, Dorigatti F, Roman E *et al.* White-coat hypertension: A selection bias? Harvest Study Investigators. Hypertension and Ambulatory Recording Venetia Study. *J. Hypertens.* 1998; **16**: 977–84.
6. Niiranen TJ, Jula AM, Kantola IM, Reunanen A. Prevalence and determinants of isolated clinic hypertension in the Finnish population: The Finn-HOME study. *J. Hypertens.* 2006; **24**: 463–70.
7. Sega R, Trocino G, Lanzarotti A *et al.* Alterations of cardiac structure in patients with isolated office, ambulatory, or home hypertension: Data from the general population (Pressione Arteriose Monitorate E Loro Associazioni [PAMELA] Study). *Circulation* 2001; **104**: 1385–92.
8. Cuspidi C, Parati G. Isolated clinic hypertension in a general population. *J. Hypertens.* 2006; **24**: 437–40.
9. Helvaci MR, Sevinc A, Camci C, Yalcin A. Treatment of white coat hypertension with metformin. *Int. Heart J.* 2008; **49**: 671–9.

10. Alberti KG, Eckel RH, Grundy SM *et al.* Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* 2009; **120**: 1640–5.
11. Bjorklund K, Lind L, Vessby B, Andren B, Lithell H. Different metabolic predictors of white-coat and sustained hypertension over a 20-year follow-up period: A population-based study of elderly men. *Circulation* 2002; **106**: 63–8.
12. Mancia G, Bombelli M, Facchetti R *et al.* Increased long-term risk of new-onset diabetes mellitus in white-coat and masked hypertension. *J. Hypertens.* 2009; **27**: 1672–8.
13. Hosaka M, Mimura A, Asayama K *et al.* Relationship of dysregulation of glucose metabolism with white-coat hypertension: The Ohasama study. *Hypertens. Res.* 2010; **33**: 937–43.
14. Martin CA, Cameron JD, Chen SS, McGrath BP. Two hour glucose post loading: A biomarker of cardiovascular risk in isolated clinic hypertension. *J. Hypertens.* 2011; **29**: 749–57.
15. Martin C, Cameron J, McGrath B. Mechanical and circulating biomarkers in isolated clinic hypertension. *Clin. Exp. Pharmacol. Physiol.* 2008; **35**: 402–8.
16. Conen D, Dieterle T, Utech K, Rimner M, Martina B. C-Reactive protein and B-type natriuretic peptides in never-treated white coat hypertensives. *Hypertens. Res.* 2006; **29**: 411–15.
17. Erdogan D, Caliskan M, Gullu H *et al.* Aortic elastic properties and left ventricular diastolic function in white-coat hypertensive individuals. *Blood Press. Monit.* 2006; **11**: 191–8.
18. Guven A, Tolun F, Caliskan M, Ciftci O, Muderrisoglu H. C-Reactive protein and nitric oxide level in patients with white coat hypertension. *Blood Press.* 2012; **21**: 281–5.
19. Kamel N, Gursoy A, Koseoglulari O, Dincer I, Gullu S. Isolated office hypertension: Association with target organ damage and cardiovascular risk indices. *J. Natl Med. Assoc.* 2006; **98**: 601–6.
20. Schillaci G, Pirro M, Gemelli F *et al.* Increased C-reactive protein concentrations in never-treated hypertension: The role of systolic and pulse pressures. *J. Hypertens.* 2003; **21**: 1841–6.
21. Curgunlu A, Karter Y, Uzun H *et al.* Hyperhomocysteinemia: An additional risk factor in white coat hypertension. *Int. Heart J.* 2005; **46**: 245–54.
22. Pierdomenico SD, Bucci A, Lapenna D *et al.* Circulating homocysteine levels in sustained and white coat hypertension. *J. Hum. Hypertens.* 2003; **17**: 165–70.
23. Curgunlu A, Uzun H, Bavunoglu I, Karter Y, Genc H, Vehid S. Increased circulating concentrations of asymmetric dimethylarginine (ADMA) in white coat hypertension. *J. Hum. Hypertens.* 2005; **19**: 629–33.
24. Karter Y, Aydin S, Curgunlu A *et al.* Endothelium and angiogenesis in white coat hypertension. *J. Hum. Hypertens.* 2004; **18**: 809–14.
25. Pierdomenico SD, Cipollone F, Lapenna D, Bucci A, Cuccurullo F, Mezzetti A. Endothelial function in sustained and white coat hypertension. *Am. J. Hypertens.* 2002; **15**: 946–52.
26. Chen LW, Huang PH, Huang SS, Chen JW, Lin SJ. Different levels of circulating endothelial microparticles among patients with normotension, hypertension or white coat hypertension. *Int. J. Cardiol.* 2009; **137** (Suppl. 1): S1–2 (Abstract).
27. Gomez-Cerezo J, Rios Blanco JJ, Suarez Garcia I *et al.* Noninvasive study of endothelial function in white coat hypertension. *Hypertension* 2002; **40**: 304–9.
28. Ribeiro L, Gama G, Santos A, Asmar R, Martins L, Polonia J. Arterial distensibility in subjects with white-coat hypertension with and without diabetes or dyslipidaemia. Comparison with normotensives and sustained hypertensives. *Blood Press. Monit.* 2000; **5**: 11–17.
29. Schillaci G, Pucci G, Pirro M *et al.* Combined effects of office and 24-h blood pressure on aortic stiffness in human hypertension. *J. Hypertens.* 2011; **29**: 869–75.
30. Silva JA, Barbosa L, Bertoquini S, Maldonado J, Polonia J. Relationship between aortic stiffness and cardiovascular risk factors in a population of normotensives, white-coat normotensives, white-coat hypertensives, sustained hypertensives and diabetic patients. *Rev. Port. Cardiol.* 2004; **23**: 1533–47.
31. McEniery CM, Wilkinson IB, Avolio AP. Age, hypertension and arterial function. *Clin. Exp. Pharmacol. Physiol.* 2007; **34**: 665–71.
32. Parati G, Esler M. The human sympathetic nervous system: Its relevance in hypertension and heart failure. *Eur. Heart J.* 2012; **33**: 1058–66.
33. Grassi G, Seravalle G, Trevano FQ *et al.* Neurogenic abnormalities in masked hypertension. *Hypertension* 2007; **50**: 537–42.
34. Pierdomenico SD, Bucci A, Costantini F, Lapenna D, Cuccurullo F, Mezzetti A. Twenty-four-hour autonomic nervous function in sustained and 'white coat' hypertension. *Am. Heart J.* 2000; **140**: 672–7.
35. Fagard RH, Stolarz K, Kuznetsova T *et al.* Sympathetic activity, assessed by power spectral analysis of heart rate variability, in white-coat, masked and sustained hypertension versus true normotension. *J. Hypertens.* 2007; **25**: 2280–5.
36. Madsen LB, Rasmussen JK, Moller DS, Nyvad O, Pedersen EB. Heart rate variability in white-coat hypertension. *Blood Press. Monit.* 2008; **13**: 65–71.
37. Palatini P, Mormino P, Santonastaso M *et al.* Target-organ damage in stage I hypertensive subjects with white coat and sustained hypertension: Results from the HARVEST study. *Hypertension* 1998; **31**: 57–63.
38. Owens PE, Lyons SP, Rodriguez SA, O'Brien ET. Is elevation of clinic blood pressure in patients with white coat hypertension who have normal ambulatory blood pressure associated with target organ changes? *J. Hum. Hypertens.* 1998; **12**: 743–8.
39. Nakashima T, Yamano S, Sasaki R *et al.* White-coat hypertension contributes to the presence of carotid arteriosclerosis. *Hypertens. Res.* 2004; **27**: 739–45.
40. Polonia JJ, Gama GM, Silva JA, Amaral C, Martins LR, Bertoquini SE. Sequential follow-up clinic and ambulatory blood pressure evaluation in a low risk population of white-coat hypertensive patients and in normotensives. *Blood Press. Monit.* 2005; **10**: 57–64.
41. Mule G, Nardi E, Cottone S *et al.* Metabolic syndrome in subjects with white-coat hypertension: Impact on left ventricular structure and function. *J. Hum. Hypertens.* 2007; **21**: 854–60.
42. Kotsis V, Stabouli S, Toumanidis S *et al.* Target organ damage in 'white coat hypertension' and 'masked hypertension'. *Am. J. Hypertens.* 2008; **21**: 393–9.

43. Ihm SH, Youn HJ, Park CS *et al.* Target organ status in white-coat hypertensives: usefulness of serum procollagen type I propeptide in the respect of left ventricular diastolic dysfunction. *Circ. J.* 2009; **73**: 100–5.
44. Grandi AM, Solbiati F, Laurita E *et al.* Isolated office hypertension: A 3-year follow-up study. *Blood Press.* 2005; **14**: 298–305.
45. Ugajin T, Hozawa A, Ohkubo T *et al.* White-coat hypertension as a risk factor for the development of home hypertension: The Ohasama study. *Arch. Intern. Med.* 2005; **165**: 1541–6.
46. Mancia G, Bombelli M, Facchetti R *et al.* Long-term risk of sustained hypertension in white-coat or masked hypertension. *Hypertension* 2009; **54**: 226–32.
47. Fagard RH, Van Den Broeke C, De Cort P. Prognostic significance of blood pressure measured in the office, at home and during ambulatory monitoring in older patients in general practice. *J. Hum. Hypertens.* 2005; **19**: 801–7.
48. Ohkubo T, Kikuya M, Metoki H *et al.* Prognosis of 'masked' hypertension and 'white-coat' hypertension detected by 24-h ambulatory blood pressure monitoring 10-year follow-up from the Ohasama study. *J. Am. Coll. Cardiol.* 2005; **46**: 508–15.
49. Hansen TW, Jeppesen J, Rasmussen S, Ibsen H, Torp-Pedersen C. Ambulatory blood pressure monitoring and risk of cardiovascular disease: A population based study. *Am. J. Hypertens.* 2006; **19**: 243–50.
50. Hanninen MR, Niiranen TJ, Puukka PJ, Johansson J, Jula AM. Prognostic significance of masked and white-coat hypertension in the general population: The Finn-Home Study. *J. Hypertens.* 2012; **30**: 705–12.
51. Kario K, Shimada K, Schwartz JE, Matsuo T, Hoshide S, Pickering TG. Silent and clinically overt stroke in older Japanese subjects with white-coat and sustained hypertension. *J. Am. Coll. Cardiol.* 2001; **38**: 238–45.
52. Pierdomenico SD, Lapenna D, Di Mascio R, Cuccurullo F. Short- and long-term risk of cardiovascular events in white-coat hypertension. *J. Hum. Hypertens.* 2008; **22**: 408–14.
53. Pierdomenico SD, Cuccurullo F. Prognostic value of white-coat and masked hypertension diagnosed by ambulatory monitoring in initially untreated subjects: An updated meta analysis. *Am. J. Hypertens.* 2011; **24**: 52–8.
54. Verdecchia P, Porcellati C, Schillaci G *et al.* Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. *Hypertension* 1994; **24**: 793–801.
55. Verdecchia P, Angeli F, Gattobigio R *et al.* The clinical significance of white-coat and masked hypertension. *Blood Press. Monit.* 2007; **12**: 387–9.
56. Strazzullo P, D'Elia L, Cairella G, Scalfi L, Schiano di Cola M. Recommending salt intake reduction to the hypertensive patient: More than just lip service. *High Blood Press. Cardiovasc. Prev.* 2012; **19**: 59–64.
57. Martin CA, Cameron JD, McGrath BP. Baseline predictors of future hypertension in white-coat hypertension. *J. Hypertens.* 2012; **30** (Suppl. 1): e65 (Abstract).
58. Batkai S, Thum T. MicroRNAs in hypertension: Mechanisms and therapeutic targets. *Curr. Hypertens. Rep.* 2012; **14**: 79–87.

Chapter 9

Conclusion and future research directions

This thesis has examined the evidence for the presence of biomarkers, associated with increased cardiovascular risk, in white-coat hypertension.

The first step undertaken in this thesis was a careful literature review. Given that by definition WCHT cannot be readily identified in the clinic setting, it was important to try to identify whether there were any significant alterations in biomarkers of artery stiffness, endothelial dysfunction, vascular inflammation and/or oxidative stress in WCHT. The review at that time, shown in Chapter Two, highlighted the lack of a consistent definition used to define WCHT, which made it difficult to compare studies in WCHT. Many of the studies reviewed were small, did not adjust for potential confounders and many did not use correct statistical techniques to analyse the data. There was no clear evidence that WCHT was associated with biomarkers of increased cardiovascular risk.

Chapter Three gives the details of the methodology used in this thesis. It was important to determine how the different blood pressure (BP) measurements in the clinic - casual by doctor or other health professional, observed automated, unobserved automated - related to the gold-standard daytime average ambulatory blood pressure measurement (ABPM). The best approximation of daytime mean ABPM was obtained by non-observed automated BP recording with some caveats (see Letter to Editor, Hypertension in the Appendix).

Bedside autonomic function tests methods were refined as a result of utilising beat-to-beat analysis obtained using a Task Force monitor (CN Systems, Graz, Austria). This has enabled the derivation of a global autonomic function score for use in the various studies reported herein, but

also confirmed the important need to adjust for age when interpreting autonomic function, particularly parasympathetic function.

The published paper in Chapter Four reported the results of a carefully controlled study in a WCHT group (non-smoking, normal fasting plasma glucose and no known diabetes), defined by strict criteria (two ABPM) and using a consensus definition for WCHT. This study examined markers of specific pathophysiological mechanisms that may potentially be involved in WCHT, in comparison with normotensive and established hypertension groups from the same community. This paper had a particular emphasis on exploring the potential subclinical links between glucose intolerance/insulin resistance and BP, as previous literature has shown a clear association between hyperglycaemia and increased risk of future diabetes and sustained hypertension. Impaired glucose tolerance or diabetes, as defined by the 2h plasma glucose post load, was present in 40% of the WCHT group, compared to 15% of normotensive and 16% of sustained hypertension groups, and this could not be readily attributed to the metabolic syndrome. Mental stress testing induced a greater blood pressure response in the WCHT group than the normotensive group; other autonomic function measures and circulation biomarkers were not different in the three groups. This novel finding of a strong association of glucose intolerance with WCHT indicates the potential need for careful monitoring of postprandial glucose in these subjects.

One of the potential mechanisms for an increase in cardiovascular risk in WCHT, as has been shown for established hypertension, is the morning BP surge. The published paper in Chapter Five reported the results of a cross-sectional analysis of four measures of the morning BP surge.

The morning BP surge was not found to be elevated in WCHT. Univariate analysis found that lipids, in particular low-density lipoprotein, were consistently correlated with all four measures of the of the morning BP surge. This novel finding of an intriguing link between two major cardiovascular risk factors needs further evaluation.

Chapter Six is a manuscript submitted for publication which combines the data published from our centre with a larger database from a second institution and shows again the clear association between morning BP surge and plasma cholesterol. Moreover this study also showed the association was also present in treated hypertensives, and showed in a subgroup that use of a Statin drug to lower plasma cholesterol was associated with a significant reduction in the morning BP surge.

The prospective study presented in Chapter Seven confirmed that WCHT is a condition of increased cardiovascular risk due to increased progression to sustained hypertension. By 5 years approximately 36% of subjects with WCHT had progressed to established hypertension. This study has also shown that subjects with WCHT who progressed to sustained hypertension tend to have increased waist circumference, higher 2hPG and PWVc. This is in line with population studies, which have shown WCHT is associated with increased progression to hypertension and also with the development of diabetes. This highlights the importance of monitoring and managing cardiovascular risk in WCHT, not just BP alone, which is an important message for medical practitioners.

The WCHT group who developed hypertension had higher initial ABPM blood pressure compared to WCHT who did not develop hypertension. In recent years algorithms have been put forth to identify patients who have hypertension that rely solely on unobserved clinic BP. As WCHT is a biomarker of increased hypertension risk patients then people with WCHT need to be identified. These people need to be examined for the presence of target organ damage, they need education, particularly in relation to their cardiovascular risk, and they need to be monitored for progression to sustained hypertension. This means both observed and unobserved automated clinic BP's are required in any BP algorithms to identify patients with WCHT.

Further studies are required to determine 1) the relationship between the 2hPG, PWVc and the development of sustained hypertension in WCHT, 2) the hypertension risk in WCHT as current hypertension risk calculators are not suitable in WCHT and 3) if an intervention of maintenance or reduction of waist measurement in WCHT can prevent or delay the onset of the development of sustained hypertension are required.

Chapter Eight is a Frontiers in Research Review in a series of papers from a satellite meeting of the International Society of Hypertension Meeting, Sydney 2012. It is usual to place reviews at the beginning of a thesis but this review incorporated studies that were published during the time of this thesis, since the first review, and more importantly for this thesis the review encompasses my studies. It shows that WCHT is associated with increased LVMI, increased progression to sustained hypertension and development of diabetes. The review concludes with a message to treating physicians that subjects with WCHT need to be identified. Diagnosis needs to be based on a solid definition of WCHT, based on two ABPM and repeated clinic recordings in untreated

subjects. Once defined, practitioners need to focus on the overall cardiovascular risk for the subject. Assessment, monitoring and education of WCHT patients should include the following.

1. Assessment for the presence of target organ damage.
2. Assessment of cardiovascular risk, including an oral glucose tolerance test.
3. Education regarding increased cardiovascular and diabetes risk, with particular emphasis on central body weight.
4. Education regarding limiting salt intake, in particular processed foods[55]
5. Monitoring waist circumference.
6. Monitoring for conversion to sustained hypertension (1–2 yearly ABPM) and/or regular home BP monitoring.
7. Monitoring for the development of diabetes, particularly in WCHT with weight gain.
8. Measurement of PWVc is of potential value as an additional risk marker.

APPENDIX

Letter to the Editor

Letters to the Editor will be published, if suitable, as space permits. They should not exceed 1000 words (typed double-spaced) in length and may be subject to editing or abridgment.

Measurement of Blood Pressure in the Office

To the Editor:

The algorithm for office blood pressure recently proposed by Myers et al[1] does not allow for the discernment of isolated clinic or masked hypertension. In a recent study we measured observed automated blood pressure (nurse present), unobserved automated blood pressure (3 readings at 3-minute intervals after 5 minutes of rest), and ambulatory blood pressure in groups of subjects with normal blood pressure, isolated clinic hypertension, and untreated essential hypertension. Mean daytime awake and automated unobserved blood pressures were similar in subjects with isolated clinic hypertension, whereas in normotensive and hypertensive groups, mean daytime awake blood pressures were similar to observed automated blood pressures (Table). Applying the proposed algorithm, 29 subjects (75%) with isolated clinic hypertension would go along the "continue-to-follow" pathway.

Isolated clinic hypertension is known to be associated with an increased risk of both diabetes mellitus[2] and hypertension.[3] Moreover, in our study, impaired glucose tolerance was a common finding in subjects with isolated clinic hypertension, even in subjects with normal fasting glucose.[4] There is currently no outcome data to suggest that isolated clinic hypertension subjects who go along the proposed "continue-to-follow" pathway have any less risk of future cardiovascular events than those who go along the proposed "home or 24-hour ambulatory blood pressure monitoring" pathway. Until outcome data are available for unobserved automated blood pressure readings, all subjects with isolated clinic hypertension need home and/or 24-hour ambulatory blood pressure monitoring over time to be able to discern if and when these patients develop sustained hypertension. This is particularly important given the frequency of impaired glucose tolerance in these subjects.

There is another important methodological issue in the article by Myers et al[1] that needs to be considered. The Bland-Altman plots suggest that the relationship between the average of the 2 blood pressures (in particular, the automated and ambulatory awake blood pressures) and the difference of the 2 blood pressures are linear, suggesting proportional bias. Also, the scatter of the 2 blood pressures appears to increase as the average of the 2 blood pressures increases, indicating likely heteroscedacity. Hence, using normal 95% CIs on untransformed data may not be the correct technique to use, as was highlighted in a recent review of the Bland-Altman method by Ludbrook.[5]

Table. Automated Office (Observed and Unobserved) Blood Pressure vs Ambulatory Blood Pressure

BP	Normotensive (n=45)	Isolated Clinic Hypertension (n=39)	Hypertension (n=52)
Automated observed BP	120/79±9/8	135/87±12/10	143/92±18/9
Automatic unobserved BP	116/77±9/6	126/82±13/9	136/88±14/9
Mean awake BP	122/73±8/6	126/76±6/5	143/85±9/6

BP indicates blood pressure in millimeters of mercury±SD.

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Disclosures

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1. Myers MG, Godwin M, Dawes M, Kiss A, Tobe SW, Kaczorowski J. Measurement of blood pressure in the office: recognizing the problem and proposing the solution. *Hypertension*. 2010;55:195–200.
2. Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Grassi G, Sega R. Increased long-term risk of new-onset diabetes mellitus in white-coat and masked hypertension. *J Hypertens*. 2009;27:1672–1678.
3. Mancia G, Bombelli M, Facchetti R, Madotto F, Quarti-Trevano F, Friz HP, Grassi G, Sega R. Long-term risk of sustained hypertension in white-coat or masked hypertension. *Hypertension*. 2009;54:226–232.
4. Martin C, Cameron J, McGrath BP. Insulin resistance, arterial stiffness and circulating biomarkers in isolated clinic hypertension. *Heart Lung Circ*. 2009;18:S276.
5. Ludbrook J. Confidence in Altman-Bland plots: a critical review of the method of differences. *Clin Exp Pharmacol Physiol*. 2010;37:143–149.

(***Hypertension*. 2010;56:e11.**)