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Exploring Circadian Blood Pressure Patterns

A thesis submitted to the National University of Ireland, Cork for the degree of Doctor of Philosophy in the Department of Epidemiology & Public Health

February 2017

Jamie Madden (B.Eng., MSc)

Head of Department

Prof. Ivan J. Perry

Supervisors

Dr. Anthony P. Fitzgerald

Prof. Patricia M. Kearney

Dr. Xia Li

CONTENTS

LIST OF TABLES 4

LIST OF FIGURES 6

LIST OF ABBREVIATIONS 10

DECLARATION 12

ACKNOWLEDGMENTS 13

THESIS ABSTRACT 14

1. INTRODUCTION 16

1.1 Introduction 17

1.2 Study Design – Motivating Dataset 26

1.3 Aims and Objectives 29

1.4 Thesis Outline 30

2. LONGITUDINAL DATA ANALYSIS & APPLICATION TO ABPM 32

2.1 Chapter Overview 33

2.2 Overview of Longitudinal Data 33

2.3 Statistical Approaches for Modelling Longitudinal Data 35

2.4 Statistical Approaches for Modelling Individual ABPM Profiles 45

3. CORRELATION BETWEEN SHORT-TERM BLOOD PRESSURE VARIABILITY AND LEFT VENTRICULAR MASS INDEX: A META-ANALYSIS (PAPER 1) 67

3.1 Abstract 68

3.2 Introduction 69

3.3 Methods 70

3.4 Results 72

3.5 Discussion 74

4. SHORT-TERM BLOOD PRESSURE VARIABILITY OVER 24 HOURS AND TARGET ORGAN DAMAGE IN MIDDLE-AGED MEN AND WOMEN (PAPER 2) 85

4.1 Abstract 86
4.2 Introduction 87
4.3 Methods 88
4.4 Results 91
4.5 Discussion 95
4.6 Conclusion 98
5. EXPLORING DIURNAL VARIATION USING PIECEWISE LINEAR SPLINES: AN EXAMPLE USING BLOOD PRESSURE (PAPER 3) 105
5.1 Abstract 106
5.2 Introduction 107
5.3 Methods 109
5.4 Results 115
5.5 Discussion 118
5.6 Conclusion 121
6. MORNING SURGE IN BLOOD PRESSURE USING A RANDOM-EFFECTS MULTIPLE-COMPONENT COSINOR MODEL (PAPER 4) 129
6.1 Abstract 130
6.2 Introduction 131
6.3 Methods 133
6.4 Statistical Analysis 134
6.5 Results 138
6.6 Discussion 142
7. SHORT REPORT: REPRODUCIBILITY 155
7.1 Introduction 156
7.2 Methods 157
7.3 Statistical Analysis 158

7.4 Results 159
7.5 Discussion 161
8. DISCUSSION 174
8.1 Summary of Main Findings 175
8.2 Implications of Findings 178
8.3 Strengths and Limitations 182
8.4 Future Recommendations for Research 187
8.5 Conclusion 190
9. REFERENCES: 191
Appendix A Supplementary material (Chapter 2) 209
Appendix B Supplementary material (Chapter 3) - search terms and search strategy for systematic review 214
Appendix C Supplementary material (Chapter 4) 220
Appendix D Supplementary material (Chapter 5) 227
Appendix E Supplementary material (Missing Data) 229
Appendix F Supplementary material (Chapter 6) 233
Appendix G Supplementary Material (Chapter 7) 242
Appendix H Sample R Code 250
Appendix I Research output, dissemination and training 257
Appendix J Published Papers (PDFs) 260

LIST OF TABLES

Table 1-1 Short-term BPV Summary Indices 22
Table 2-1 Comparison of different modeling approaches for the analysis of ABPM data. 66
Table 3-1 Study characteristics 80
Table 3-2 BPV definitions 81
Table 3-3 Extracted Results 82
Table 3-4 Quality Assessment 84
Table 4-1 Baseline Characteristics by LVH and Microalbuminuria 101
Table 4-2 Variability Indices and Nocturnal Dip by LVH 102
Table 4-3 Variability Indices and Nocturnal Dip by Microalbuminuria 102
Table 4-4 Association between SBP BPV and LVH 103
Table 4-5 Association between SBP BPV and Microalbuminuria 104
Table 5-1 Baseline Characteristics 124
Table 5-2 Various models with parameter estimates for slopes at each segment along with corresponding correlations and variances 126
Table 6-1 Baseline Characteristics 147
Table 6-2 Model parameter estimates (SBP) along with corresponding correlations and variances 149
Table 6-3 Maximum Morning Surge (mmHg/30mins) 151
Table 7-1 BP levels 2010 v 2014 165
Table 7-2 Dipping status 2010 v 2014 166
Table 7-3 BP categories 2010 v 2014 166
Table 7-4 Variability Indices 2010 v 2014 167
Table 7-5 Comparison of SBP parameters from two separate two-component random-effects cosinor models (2010 vs 2014) 169
Table 7-6 Comparison of SBP parameters from two separate two-component random-effects cosinor models (2010 vs 2014) 170
Table 9-1 Association between parameters extracted from polynomial regression model and microalbuminuria (logistic regression). 213
Table 9-2 Baseline Characteristics by anti-hypertensive treatment 220
Table 9-3 Variability Indices and Dip by White-Coat Hypertension 221

Table 9-4 Table of demographics comparing full sample to ABPM sample 231
Table 9-5 Peer reviewed publication from PhD 257
Table 9-6 Other research output during PhD 257
Table 9-7 Conference Presentations during PhD 258
Table 9-8 Training & workshops attend during PhD 259
Table 9-9 Placements Completed 259
Table 9-10 Awards 259

LIST OF FIGURES

Figure 1-1 Overview of thesis including aims and objectives. 31
Figure 2-1 Example of four individual ABPM readings 34
Figure 2-2 Illustration of simple linear regression model and simple linear mixed effects model where the data are clustered.....38
Figure 2-3 Raw data for four individuals along with predicted 6^{th} order polynomial model 47
Figure 2-4 Illustration of cubic and restricted splines 50
Figure 2-5 Illustration of piecewise linear splines 52
Figure 2-6 A simple cosinor fit to observed SBP readings of an individual (period=24h).....54
Figure 2-7 Fourier series – a linear combination of sines and cosines can produce a complex curve [127].....56
Figure 2-8 Illustration of the SITAR model for SBP over 24h. The black line indicates the mean BP curve. 63
Figure 3-1 Flow diagram of study selection 78
Figure 3-2 Pooled meta-analysis of z-scores by BPV index.....79
Figure 4-1 Four study participants and their 24h BP profiles. Panels (a) & (b) illustrate different patterns yet have similar 24h SD but different values based on the other indices. Both (c) & (d) demonstrate a similar issue (values rounded).....100
Figure 5-1 Plot of average SBP over 24h which helped identify 6pm and 4am as common knot points for all participants where there was a notable change in trajectory of BP. Also highlighted are the periods where individuals woke and went to sleep. In addition to the two common points, we were able to obtain additional (two) subject-specific knot points at wake and sleep times.....123
Figure 5-2 Individual SBP readings along with predicted subject-specific trajectories from a linear mixed effects model as a function of time only using two different approaches; polynomials (red line) and piecewise linear splines (blue lines).....125
Figure 5-3 Predicted average (95% CI) piecewise linear trajectory of those with/without presence of microalbuminuria adjusted for age, sex and BMI using a linear mixed-effects model (Model 2). Each linear spline represents the rate of SBP increase or decrease (slope) for that segment and has been given a corresponding

number which is referred to in Table 5-2. For the purposes of this plot we have set the sleep and wake time knots at 23.00 and 08.00 respectively. 128
Figure 6-1 ABPM readings (circles, thin black line) of four individuals along with predicted subject-specific trajectories from a random-effects model as a function of time using (i) single cosinor (thick black line) (ii) two-component cosinor (thick red line) and (iii) three-component cosinor (thick blue line) models. 148
Figure 6-2 ABPM readings of three individuals with fitted subject-specific trajectories from a two-component cosinor random-effects model (left panels). Their corresponding rate of change curves (first derivatives) are also plotted on right panels (red line indicating reference zero mark). 150
Figure 6-3 Histograms of maximum morning slope by simulations 151
Figure 6-4 FPCA: Each of the first three FPCs as variations about the mean along with the percentage of total variation explained by the component. The solid black line represents the mean SBP over the day and the functions obtained by adding and subtracting ± SD of the eigenfunctions to the mean. Plus signs indicate addition and minus signs indicate subtraction. 152
Figure 6-5 Scatter plots and the corresponding correlations between the two-component random-effects cosinor model parameters and the first three FPC scores from FPCA. 153
Figure 6-6 A two-component cosinor random-effects model implemented separately on those with and without presence of microalbuminuria. Subject-specific curves for those with (black lines) and without (light grey lines) evidence of microalbuminuria are also displayed. Red (microalbuminuria) and blue (no microalbuminuria) lines represent average curves for both groups. The corresponding first derivative curves indicating the rate of change over the day for both groups are also presented. 154
Figure 7-1 Data from four individuals 2010 vs 2014.....164
Figure 7-2 Bland-Altman scatter-plots of agreement of measurements of 24h SBP and 24h DBP four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values. 165

Figure 7-3 Bland-Altman scatter-plots of agreement of measurements of 24h SBP SD and 24h SBP ARV four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values. 168
Figure 7-4 Bland-Altman scatter-plots of agreement of measurements of the first and second amplitudes four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values. 171
Figure 7-5 Bland-Altman scatter-plots of agreement of measurements of the first and second phase shifts four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values. 172
Figure 7-6 Boxplots of differences between each of the cosinor parameters between the two time-points (2010 minus 2014). 172
Figure 7-7 Comparison of fits, 2010 vs 2014, for six individuals using two separate two-component random-effects cosinor models. 173
Figure 9-1 Population Level Effects for polynomial regression (Chapter 2) 210
Figure 9-2 Population Level Group Average (95 % CI) Linear Mixed Effects Model (Age, Sex, BMI Adjusted) (Chapter 2).....211
Figure 9-3 Subject-Specific Variation (function of Time only) 212
Figure 9-4 VPC (visual predictive check) plot. Black lines represent median of observed data with 90% interquantile range of observations. Red line is predicted mean along with 90% prediction interval. 227
Figure 9-5 Predicted average (95% CI) piecewise linear trajectory of those with/without presence of microalbuminuria adjusted for age, sex, BMI and including an interaction of the spline terms with microalbuminuria using a linear mixed-effects model (Model 3). Each linear spline represents the rate of BP increase or decrease (slope) for that time period which is referred to in Table 5-2. 228

Figure 9-6 ABPM readings of three individuals with fitted subject-specific trajectories from a two-component cosinor mixed-effects model (five parameters) and spline model (six parameters - cubic spline with four knots at 18:00, 24:00, 04:00 and 08:00) (left panels). Their corresponding rate of change curves (first derivatives) are also plotted (right panels). The plots indicated an extremely similar pattern thus giving further justification of the two-component cosinor model. 233
Figure 9-7 Histograms of maximum morning slope by different methods. 234
Figure 9-8 Scree plot with eigenvalues and the eight principle components which make up 99% of the variation. 235
Figure 9-9 FPCA: Each of the six three FPC as variations about the mean along with the percentage of total variation explained by the component. The solid black line represents the mean SBP over the day. The "+" curves illustrate what happens when a small amount of the component is added to the mean and the "-" curves illustrate the effect of subtracting the component. 236
Figure 9-10 Scatter plots and the corresponding correlations between all the two-component cosinor random-effects model parameters and the principle component scores from FPCA. Eight FPCs contribute to 99% of the total variance in the data.237
Figure 9-11 Simulated ARV values taken every 10mins and every 30mins. 239
Figure 9-12 Simulated data for one individual with readings taken 1) every 30min and 2) every 10mins. 240

LIST OF ABBREVIATIONS

Abbreviations	Term
ABP	Ambulatory Blood Pressure
ABPM	Ambulatory Blood Pressure Monitoring
ACEI	Angiotensin-Converting Enzyme Inhibitors
ACF	Autocorrelation Function
ACR	Albumin:Creatinine Ratio
AR	Autoregressive
ARB	Angiotensin Receptor Blockers
ARV	Average Real Variability
BMI	Body Mass Index
BP	Blood Pressure
BPV	Blood Pressure Variability
CCB	Calcium Channel Blockers
CI	Confidence Intervals
CV	Coefficient of Variation
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
EBLUP	Empirical Best Linear Unbiased Predictors
ECG	Electrocardiogram
FDA	Functional Data Analysis
FPC	Functional Principle Component
FPCA	Functional Principle Components Analysis
GEE	Generalised Estimating Equation
GP	General Practitioner
HR	Hazard Ratio
HSE	Health Services Executive
LHC	Livinghealth Clinic
LRT	Likelihood Ratio Test
LVH	Left Ventricular Hypertrophy
LVMI	Left Ventricular Mass Index
MESOR	Midline Estimating Statistic Of Rhythm
ML	Maximum Likelihood
MLE	Maximum Likelihood Estimates
NICE	National Institute For Health And Care Excellence
OR	Odds Ratio
PACE	Principal Component Analysis through Conditional Expectation
PCA	Principle Component Analysis
PRISMA	Preferred Reporting Items for Systematic reviews And Meta-Analyses
REML	Restricted (or Residual) Maximum Likelihood Estimation
ROC	Receiver Operating Characteristic
SBP	Systolic Blood Pressure
SD	Standard Deviation
SITAR	SuperImposition by Translation And Rotation
TILDA	The Irish Longitudinal Study On Ageing

TOD	Target Organ Damage
VPC	Visual Predictive Check
wSD	Weighted 24-h SD

DECLARATION

I declare that this thesis has not been submitted for another degree at this or at any other University. The work, upon which this thesis is based, was carried out in collaboration with a team of researchers and supervisors who are duly acknowledged in the text of the thesis.

Signed: _____ **Date:** _____

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THESIS ABSTRACT

Blood pressure (BP) is one of the most extensively researched topics. It is well established that elevated BP is the most prevalent treatable risk factor for cardiovascular disease. Despite our comprehensive knowledge of the importance of reducing mean levels of BP, we are less informed about the benefits of reducing other parameters of BP, specifically BP variability (BPV), which refers to the amount of variation over a period of time. Recent evidence has suggested that BPV may be an additional clinical target to mean level. However, its full prognostic significance and definition remains in doubt. BP does not remain stationary throughout the day but is constantly fluctuating and follows a circadian rhythm. Short-term BPV refers to fluctuations over this circadian rhythm. Ambulatory blood pressure monitoring (ABPM), which can be used to obtain estimates of BP usually every 30mins over a 24h period, offers a powerful tool in the analysis of circadian patterns and short-term BPV. Longitudinal circadian data with such a cyclical structure consisting of multiple repeated readings provides an opportunity to analysse BP in many different ways and an overview of possible approaches is outlined in this thesis. The main aims of this thesis were to explore and identify circadian BP patterns between individuals and groups, and extract meaningful measures that describe these patterns while appropriately accounting for the inherent cyclical structure of ABPM data. Specifically, the thesis includes a systematic review which identifies summary measures of BPV, such as standard deviation, that can easily be obtained from the observed data without the need for more advanced modelling. A meta-analysis exploring the correlation between short-term BPV and subclinical target organ damage (TOD), specifically left ventricular mass index, is included. The association between the identified summary measures and subclinical TOD is then explored in a group of middle aged adults. In an attempt to maximise the power of the repeated cyclical readings in ABPM and incorporate the data together in one model, different random-effects models were explored which allowed us to obtain estimates of both

within and between-individual variation of model parameters. A piece-wise linear mixed-effects model was considered as a simple but suitable approach to capture BP trajectory throughout the day. We attempt to relate factors such as the morning slope and within person variability (allowing this to be group-specific) to TOD (microalbuminuria). Finally, a two-component cosinor random-effects model is outlined where derivatives of the model fit presents a novel alternative method to locate and quantify the magnitude of slopes at critical points along the trajectory. This is used to obtain a measure of morning BP surge. We compare the random-effects from this model to principle component scores obtained through functional principle component analysis. Our motivating data comes from the Mitchelstown Study, a population based study of Irish adults where a subsample underwent 24h ABPM.

1. INTRODUCTION

1.1 Introduction

Hypertension

Cardiovascular disease (CVD) is now endemic worldwide. It is the leading cause of death, not only in Ireland but globally, accounting for 17.3 million deaths per year (30% of global deaths), a number that is expected to grow to greater than 23.6 million by 2030 [1]. Despite our comprehensive knowledge of prevention and treatment for hypertension, it is still the most prevalent treatable risk factor for CVD, affecting one billion people globally [2-4]. It has been estimated that 7.6 million deaths worldwide, 54% of strokes, and 47% of cases of ischaemic heart disease can be attributed to high blood pressure (BP) alone [1]. The most recent estimates for the Republic of Ireland (ROI) conducted by the Institute of Public Health using data from the SLÁN 2007 study estimated that more than 850,000 (25.1%) adults in ROI have hypertension with the figure set to rise to almost 1,220,000 adults (28.3%) by 2020 [5].

In order to accurately ascertain a "normal BP" level it is not simply enough to arbitrarily take one BP measurement and based on this single reading conclude whether an individual is above or below the generally accepted value of 140/90 mmHg. It is well known that BP does not remain stationary and is a haemodynamic variable that fluctuates from second to second making the diagnosis of hypertension challenging. In addition it has long been known that each individual has their own diurnal BP trajectory that varies over a 24h period, known as a circadian rhythm. Early evidence illustrated that BP tended to be higher during the day and much lower during the night [6]. This led to the term "dippers" being coined during the late 1980's referring to individuals whose BP fell or dipped at night [7]. Modelling and extracting features of longitudinal data, specifically BP data that is circadian in nature offers a unique challenge for researchers.

Awareness, treatment and control levels are problematic worldwide [4]. In Ireland, The Irish Longitudinal Study on Ageing (TILDA) reported that among those over 50 with hypertension, only 55% were aware of their hypertension status [8]. In addition, only 59% of those were on treatment, of whom only 52% were controlled

to suitable levels. Furthermore, there is on-going controversy that what we have always considered a normal hypertension cut-off (above 140/90mmHg) may still be too high. Results from the internationally acclaimed Systolic Blood Pressure Intervention Trial (SPRINT) suggest target levels of 120/70 mmHg are desirable [9]. SPRINT randomly assigned 9361 people without diabetes with systolic BP (SBP) above 130 mmHg and an increased cardiovascular risk to a target of less than 120 mmHg (intensive treatment) or a target of less than 140 mmHg (standard treatment). After 3 years the trial was stopped early due to a significant difference between treatment arms. Those assigned to the intensive group had a 25% lower relative risk of major cardiovascular events and 27% lower relative risk of all-cause mortality. Potential weaknesses of the study have been highlighted [10, 11]. Cushman *et al.* pointed out that BPs were measured with patients seated in a quiet room with an automated device without any observer present and argue this method can present BP values up to 20 mmHg lower than clinic readings [10]. The SPRINT trial is not alone in suggesting the threshold should be lower however and a recent meta-analysis examining the effect of intensive BP reduction found a 14% reduction in major cardiovascular events and 13% reduction in myocardial infraction when BP was reduced to 133/76 mmHg compared to standard treatment (140/90 mmHg) [12].

Measurement

Accurately measuring something that is fluctuating so frequently can be problematic. However, advancements in technology and wearable devices make the task of obtaining accurate BP readings throughout a 24h period much more achievable [13]. Ambulatory blood pressure monitoring (ABPM) is recognised as the gold standard of BP measurement [13] and involves wearing a small digital BP device that is attached to a belt around your body and which is connected to a cuff strapped around your upper arm. The devices are usually set to record BP values over a 24h period every 30mins, but can be set to 15mins or 20min [13]. After the device has been worn it can be removed and connected to a computer where the data are extracted, giving a print out of the average BP values and a visual representation of the circadian pattern. ABPM offers a unique insight into an

individual's underlying circadian rhythm and has many advantages over a single clinic reading; measurements can be obtained as individuals go about their daily lives giving a more accurate measure of their real BP values, it allows the detection of white-coat and masked hypertension, but also offers estimates of the night BP and dip parameters. Detecting white-coat is important as it may prevent the unnecessary commencement of antihypertensive medication. We now know that the dip at night is associated with more favourable outcomes, and mean night BP is established as a stronger predictor of outcomes than mean day values [14-16]. For example, in the Dublin Outcome Study, for each 10-mmHg increase in mean night SBP, the mortality risk increased 21% while adjusting for clinic BP [14].

In primary care according to NICE guidelines [17], ABPM is offered if the clinic BP (average of two single readings) is above 140/90 mmHg. Hypertension diagnosis is then based on thresholds that vary slightly based on average day/night values [13, 18]. Recently there have been strong calls advocating for the mandatory use of ABPM and even suggesting that failure to provide it amounts to medical ineptitude [13, 19, 20]. In Ireland the recent national cardiovascular health policy recommended general practises (GP) be encouraged to invest in BP technology (ABPM) that will improve BP management [21]. As a result the Health Service Executive (HSE) has acknowledged the importance of ABPM having recently approved the reimbursement of ABPM in primary care for those with a medical card [22]. Kario argues that in order to obtain perfect 24h BP control it is not enough to focus on the reduction of mean 24h BP, we must also reduce exaggerated blood pressure variability (BPV) and obtain an undisrupted or smooth circadian pattern [23]. Thus, one of the potential benefits of ABPM is that it offers us an opportunity to obtain measures beyond average day/night/24h BP values. More specifically, it allows us to quantify and explore the additional prognostic significance of variability in BP.

Blood Pressure Variability (BPV)

It is important to note there are primarily two types of BPV- long-term variability which refers to variation in readings often taken over months or years and short-term variability which refers to variation in readings taken over minutes or hours. There has been a recent surge in interest in the prognostic value of long-term BPV since the publication of work by Rothwell *et al.* [24, 25]. In treated hypertensive patients enrolled in ASCOT-BPLA (Anglo-Scandinavian Cardiac Outcomes Trial–Blood Pressure Lowering Arm), higher visit-to-visit variability in SBP was associated with stroke and coronary events. Rothwell also found that SBP variation between visits was a strong predictor of stroke and coronary events, independent of mean clinic BP. To synthesize findings from subsequent studies, Tai *et al.* recently conducted a meta-analysis of 13 studies to evaluate the prognostic value of visit-to-visit SBP variability by different parameters in 77,299 patients [26]. They found a pooled age and mean SBP-adjusted hazard ratio (HR) for all-cause mortality of 1.03 (95% CI, 1.02-1.04; p<0.001) per 1-mmHg increase in SBP standard deviation (SD) and 1.04 (1.02-1.06, p<.001) per one precent increase in SBP coefficient of variation (CV), with the corresponding values for cardiovascular mortality being 1.10 (1.02-1.17, p<.001) and 1.01 (0.99-1.03, p=.32), respectively. In addition, a 1-mm Hg increase in SD was significantly associated with an increased stroke risk, with an HR of 1.02 (1.01-1.03, P<.001).

The prognostic value of short-term BPV obtained from ABPM has also been examined, but with inconsistent results. Parati *et al.* were the first to demonstrate high BPV was associated with an increased risk of target organ damage (TOD) specifically left ventricular hypertrophy (LVH) [27]. There have been a number of other studies also reporting associations between short-term BPV and both TOD [28-31] and cardiovascular events [25, 32]. However, other studies have shown no or only weak associations after adjustment for the mean BP [32-35]. The majority of these studies however, have used SD as a measure of BPV. The appropriateness of such an index has been disputed because it only reflects the dispersion of measurements around a single value (mean) and does not account for the order in which BP measurements were obtained and the longitudinal variation in the

circadian data [36, 37]. Alternatively, studies have explored other indices of variability including:

- coefficient of variation which attempts to adjust for the tendency of those with a higher average BP to also have a higher SD [25, 38, 39];
- average real variability (ARV) which is the average absolute difference between successive readings, and is thought to give a true reflection of real variability [28, 33, 36] and;
- a weighted 24h SD (wSD) which attempts to remove the influence of the day-night BP difference from the estimate of BPV [28, 30, 33, 40].

Mule *et al.* demonstrated an association between BPV using ARV, SD and microalbuminuria among a group of 328 hypertensives after adjustment including mean BP [41]. In a separate study, Mule *et al.* found that ARV SBP was associated with microalbuminuria in 315 untreated essential hypertensives after adjustment for covariates including mean SBP [42]. However wSD, and SD of day and night periods were not found to be independently associated with microalbuminuria.

Moreover, there has also been extensive work examining variability during the morning period alone where BP rises rapidly to its peak, known as morning surge, before falling again throughout the day [23]. There is substantial evidence indicating that the morning is the most important period and is when cardiovascular events most frequently occur [23, 43, 44]. It has been shown that the morning surge (calculated as mean SBP during the 2 hours after awakening minus mean SBP during the hour that included the lowest sleep BP) is independently related to organ damage and risk of cardiovascular events [45-47]. Different indices of short-term BPV and various morning surge parameters can be seen in Table 1-1. The short-term summary measures are outlined in more detail in Chapter 3.

Table 1-1 Short-term BPV Summary Indices

Measure of BPV	Description
24h SD	Standard deviation over 24 hour period
Day SD	Standard deviation over day period usually 9am-9pm
Night SD	Standard deviation over night period usually 1am-6am
wSD	Weighted standard deviation (of day and night standard deviation)
CV	Coefficient of variation
ARV	Average real variability
Morning Surge	
Sleep-trough surge	2h morning BP minus 1h lowest night BP
Prewaking surge	2h morning BP minus 2h prewaking BP
Rising surge	BP on rising minus last ABPM in a supine position <30m before rising

*Kario [45]

Despite increased interest and research conducted on BPV we are still unclear as to what the best method or index is to quantify variability, and its clinical relevance. In addition, all the above studies have used direct summary measures of BPV. That is, measures that can be directly obtained from the raw data without the need for advanced statistical methods. This thesis aims to explore other possible novel measures that describe BPV. Considering the clinical importance of BP it is surprising to learn of so few studies that fully utilise the benefits of the longitudinal nature of ABPM. Lambert *et al.* [48] who explored the use of cubic splines to model the trajectory of ABPM emphasised the lack of research on the longitudinal analysis of 24h ABPM while Edwards *et al.* [49] who utilised orthonormal polynomials stressed there is no current "standard" method for analysing ABPM. These and other longitudinal models of ABPM variation are described in more detail in Chapter 2 (Longitudinal Data Analysis and Application to ABPM).

Biological Plausibility of BPV

Although the precise mechanisms responsible for BPV are not entirely understood [50], there are a number of acknowledged factors that influence variations in BP. Parati *et al.* [51, 52] argue it is primarily modulated by neural (increased central sympathetic drive and reduced arterial and cardiopulmonary reflexes), humoral (angiotensin II, insulin, bradykinin, nitric oxide) and vascular effects (elastic properties of arteries). The night-time dipping phenomenon that occurs during sleep is associated with a marked drop in sympathetic drive, while the morning

surge is associated with a remarkable sympatho-activation [53, 54]. In addition, behavioural influences such as sleep, physical activity and postural changes can induce significant changes to BP [52, 55]. Emotional or psychological stress can also sporadically increase BP values [50]. An emotional condition that is often seen in clinical practice is that of the "white-coat effect" [50]. This well-known phenomenon is characterised by a sharp increase in BP values during the course of a doctor's visit which then disappears once the doctor leaves. Even high altitude [56] and environmental (seasonal effects) [57] factors are known to have an effect on BP values.

BPV and Antihypertensive Medication

The interest in BPV and the importance of how it is quantified comes to the fore when we begin to examine therapeutic approaches to treat hypertension. In 2005, the ASCOT trial reported that antihypertensive medications (amlodipine and perindopril combination) significantly improved outcome compared to older dated alternatives [58]. However, of particular interest was that the difference in mean BP between treatments was only 1.6 mmHg at the end of the trial. This small difference suggested that the improvement in outcome between arms was unlikely to be as a result of reducing mean BP alone. In the subsequent years more analyses published from the study showed that the newer medication had significantly reduced central BP (pressure exerted on the heart and brain), night-time BP and BPV [25, 59, 60], suggesting we should not be solely focusing on mean BP. It highlighted the potential benefit of obtaining new indices of BP and the advantage of exploring ABPM data. There is growing evidence to suggest that although different antihypertensive classes have the same effect in terms of reducing mean BP, they have significantly different effects on BPV, particularly calcium channel blockers (CCB) or calcium antagonists [61, 62]. The X-CELLENT trial examined the efficacy of calcium antagonists in reducing BPV (measured as wSD and ARV) among 577 patients before and after anti-hypertensive treatment [63]. Larger reductions were obtained with amlodipine (CCB) compared to indapamide (diuretic), candesartan (angiotensin receptor blocker (ARB)) and placebo where the differences even remained significant after adjustment for mean BP. Further

evidence was found in a large cohort of 2780 hypertensive patients, where those receiving either CCBs or diuretics, alone or in addition to other drugs had significantly lower 24h SBP SD compared with angiotensin-converting enzyme inhibitors (ACEIs), ARBs or β-blockers alone or in combination [64]. CCB-based combinations again performed better than other combinations in post hoc analysis of the COPE (Combination Therapy of Hypertension to Prevent Cardiovascular Events) trial in reducing visit-to-visit BPV (SD & CV) [65]. The cope trial was the first clinical trial to examine the treatment of hypertension with combination therapies. This raises the question of whether BPV could be an additional therapeutic target of antihypertensive treatment to improve cardiovascular protection. And highlights potential benefits of obtaining new BP measures which can provide new information and bring about greater insights into the mode of action of new antihypertensive medication [52, 66].

These variability indices may be particularly useful for the analysis of chronotherapy effects which refers to the treatment of an illness or disease by administering a drug at a time of day believed to be in harmony with the body's natural rhythms. There are no current recommendations for when BP-lowering medications should be ingested but most people are instructed by their physicians and pharmacists to take the medication during the morning period. As explained previously, the morning period is a crucial time coinciding with a surge in BP and perhaps the administration of medication before this period may be more beneficial in reducing BP rather than during it. The MAPEC study was the first to examine the benefits of bedtime chronotherapy with one or more conventional hypertension medications on BP control and CVD risk reduction versus conventional morning therapy [67, 68]. They found subjects ingesting antihypertensive medication at bedtime showed significantly lower night BP, reduced prevalence of non-dipping and lower relative risk of both total CVD and major CVD events after a median follow-up of 5.6 years. In a similar study conducted among 448 hypertensive patients with type 2 diabetes, comparable results were found [69]. After a median follow up of 5.4 years patients with diabetes ingesting at least one hypertension medication at bedtime showed significantly lower night BP, higher prevalence of controlled ambulatory BP (ABP)

and a significantly lower cardiovascular risk than those taking medication upon awaking. Improved BP control was also shown in a separate study of 250 patients with resistant hypertension [70]. Indeed a systematic review [71] and a Cochrane review [72] have suggested that patients with evening dosing of antihypertensive drugs had better 24h BP control than those with a morning dosing regimen. Although the clinical significance of night-time administration needs to be explored further it does emphasize the need to be able to quantify the immediate effect of ingesting an antihypertensive drug so that we have the ability to compare different drug classes.

Summary

The primary focus of the management of hypertension to date has centred on lowering mean BP with little consideration for other factors, such as BPV, maximum BP reached, episodic hypertension or its circadian pattern. Hypertension guidelines have only focused on mean BP, which is clearly important, but fail to mention BPV [17]. As ABPM is more widely available and we begin to collect more and more data, it begs the question of whether we are maximising the rich data available. Recently published ABPM guidelines have highlighted that most ABPM studies have reported results derived from investigating mainly one unique ABPM characteristic, e.g., either a BP mean value, or a variability measure, or morning surge, without comparison or appropriate adjustment for the prognostic value of additional ABPM-derived characteristics [73]. It is clear that the full potential available from ABPM measurements is not currently being explored. This thesis explores current approaches and suggests novel alternatives to capture circadian BP patterns.

1.2 Study Design – Motivating Dataset

The motivating ABPM data for this thesis comes from a study examining the prevalence of major CVD risk factors in a middle-aged population in Ireland. The analysis utilises existing data obtained from the Mitchelstown Cohort Study, a population based study of middle-aged men and women, recruited in Ireland in 2010-2011. A detailed description of the study design is available elsewhere [74] but a summary is provided here.

The primary aim of the study was to provide a profile of cardiovascular health and their related factors in an Irish adult general population sample. The study comprised of 2047 adults aged 47-73 years (response rate: 67%) recruited from patients attending a single large primary care centre, the Livinghealth Clinic (LHC) in Mitchelstown, County Cork. The clinic is a GP developed independent advanced Primary Care Centre. The clinic serves a catchment area of 20,000 people, with a mix of urban and rural residents. At baseline, participants completed a detailed health and lifestyle questionnaire and were invited to attend the LHC for a physical examination to be carried out by a nurse trained in the study research protocols. Clinic measurements included height, weight, BP and in addition, fasting blood samples (minimum of 8-h fast) and urine samples were collected. Participants also underwent standard 12-lead electrocardiogram (ECG). ABPM was offered to all 2047 participants, and it was completed by 1207 (response rate: 58%). All participants provided written informed consent and ethical approval was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals.

BP measurement

With the participant in a seated position, study BP was measured using an OMRON M7. Three readings of BP were obtained and the average of the second and third measurements was used. ABP was measured using dabl ABPM system (dabl ltd., Ireland) with the Meditech ABOM-05 Monitor (Meditech LTD., Hungary). The monitors were programmed to obtain readings every 30mins and remained in place

for 24h. Participants kept diaries of wake and sleep periods, which were used to calculate day and night BP respectively. If no diary was kept, the period from 1am to 6am was used as the night period and from 9am to 9pm as the day period.

Subclinical Target Organ Damage (TOD)

To explore the prognostic value of BPV and for purposes of illustration of some of the methods, subclinical TOD is used as a surrogate marker of cardiovascular events where the association between BPV and TOD is investigated. Subclinical TOD, which is the development of asymptomatic functional and structural abnormalities in the human body, often precedes the occurrence of major cardiovascular events [75]. It usually refers to damage that occurs in major organs fed by the circulatory system e.g. heart, kidneys, brain and eyes. As they are subclinical, they are nearly or completely asymptomatic i.e. showing no signs or symptoms. Two examples of TOD are microalbuminuria (kidney) and LVH (heart).

Microalbuminuria is the persistent elevation of albumin in the urine [76]. It occurs when the kidney leaks small amounts of albumin into the urine. Specifically, it signals increased permeability (capacity of a blood vessel wall to allow for the flow of small molecules or whole cells in and out of the vessel) of the endothelial cells and signifies that some level of injury is present and vascular responsiveness is comprised [76]. It is a marker of increased risk for cardiovascular morbidity and mortality especially, but not exclusively, in high risk populations such as diabetes and hypertensives [77]. Microalbuminuria is measured in spot morning urine obtained from the patient and sent for measurement of both albumin and creatinine. A meta-analysis in 2010 demonstrated increased risk of mortality with urine - albumin:creatinine ratio (ACR) ≥ 1.1 mg/mmol and as a result microalbuminuria was defined using this cut-point [78].

LVH is enlargement and thickening (hypertrophy) of the walls of the heart's main pumping chamber (left ventricle). LVH can develop in response to factors such as increased BP or a heart condition that causes the left ventricle to work harder. Complications of LVH include atrial fibrillation, diastolic heart failure, systolic heart failure, and sudden death [79]. Both earlier recognition and improved

understanding of cardiac hypertrophy may lead to more effective therapeutic strategies for this cardiovascular risk factor [79]. An electrocardiogram (ECG) is usually used to detect the presence of LVH. In this study, a 12-lead ECG was obtained in the standard manner using a Siemens – Eclipse 850i machine. ECGs were reviewed and coded for LVH voltage criteria by a cardiologist. The presence of LVH was defined by Cornell Product ECG voltage criteria i.e. SV3 + RaVL (+6 in women) X QRS duration ≥ 2440 mm x ms [80].

Reproducibility Sub-study (Follow-up Data)

Based on the initial ABPM results the sample was divided into 4 groups: normotension, isolated nocturnal hypertension, isolated daytime hypertension and day-night hypertension [81]. Twenty participants were randomly selected from each group and invited to attend for follow-up ABPM measurements in 2014 using the Spacelabs 90217 monitor. Data was stored using the Spacelabs 92506 Ambulatory BP Report Management System software. At four years follow-up a subsample of fifty (response rate: 63%) of these participants consented to participation in this study. The mean period of follow up was 3.9 years.

1.3 Aims and Objectives

The aim of this thesis was to explore and identify circadian BP patterns between individuals and groups, and extract meaningful measures that describe these patterns.

Specifically the objectives were:

1. To conduct an extensive literature review to determine current summary measures that is used to quantify BPV and explore their association with the presence of TOD, specifically LVH.
2. To determine the association between short-term BPV extracted from 24h ABPM and subclinical TOD in middle aged adults based on the measures found in (1).
3. To implement models that adequately describe patterns of BP and which can provide novel measures of short-term BPV taking into account the full temporal and circadian nature ABPM.
4. To outline an approach that can be used to comprehensively interrogate the reproducibility of ABPM readings taken at two different occasions incorporating some of the approaches outlined in (3).

1.4 Thesis Outline

This thesis is comprised of four papers, Figure 1-1 illustrates each aim and objective and the corresponding chapter.

This thesis focuses on extracting features of longitudinal data, specifically data that is circadian. Although the focus is BP, the methods covered could be easily applied to other physiological processes that follow this circadian cycle. Chapter 2 first outlines approaches suitable for modelling longitudinal data in general but then focuses on specific techniques that may be appropriate for BP.

Chapter 3 is a systematic review that examines the prognostic significance of short-term BPV on the presence of TOD, specifically LVH. The review identifies variability summary measures that are currently used to quantify BPV. A meta-analysis exploring the correlation between short-term BPV and left ventricular mass index (LVMI) is included.

Chapter 4 explores the association between short-term BPV over 24h and subclinical TOD in middle aged adults using data from the Mitchelstown study.

Chapter 5 illustrates a method of describing and quantifying circadian BP patterns using piecewise linear mixed-effects models while ensuring periodicity.

Chapter 6 examines the traditional ABPM model (cosinor) using random-effects and illustrates how it can be extended and used as a method to determine morning BP surge. The model is compared to a functional principle component analysis.

Chapter 7 explores approaches to determine the reproducibility of ABPM trajectories taken at two different occasions.

Chapter 8 summarises the findings from the analysis, discussed the strengths and limitations of the thesis, and makes some suggestions for future research.

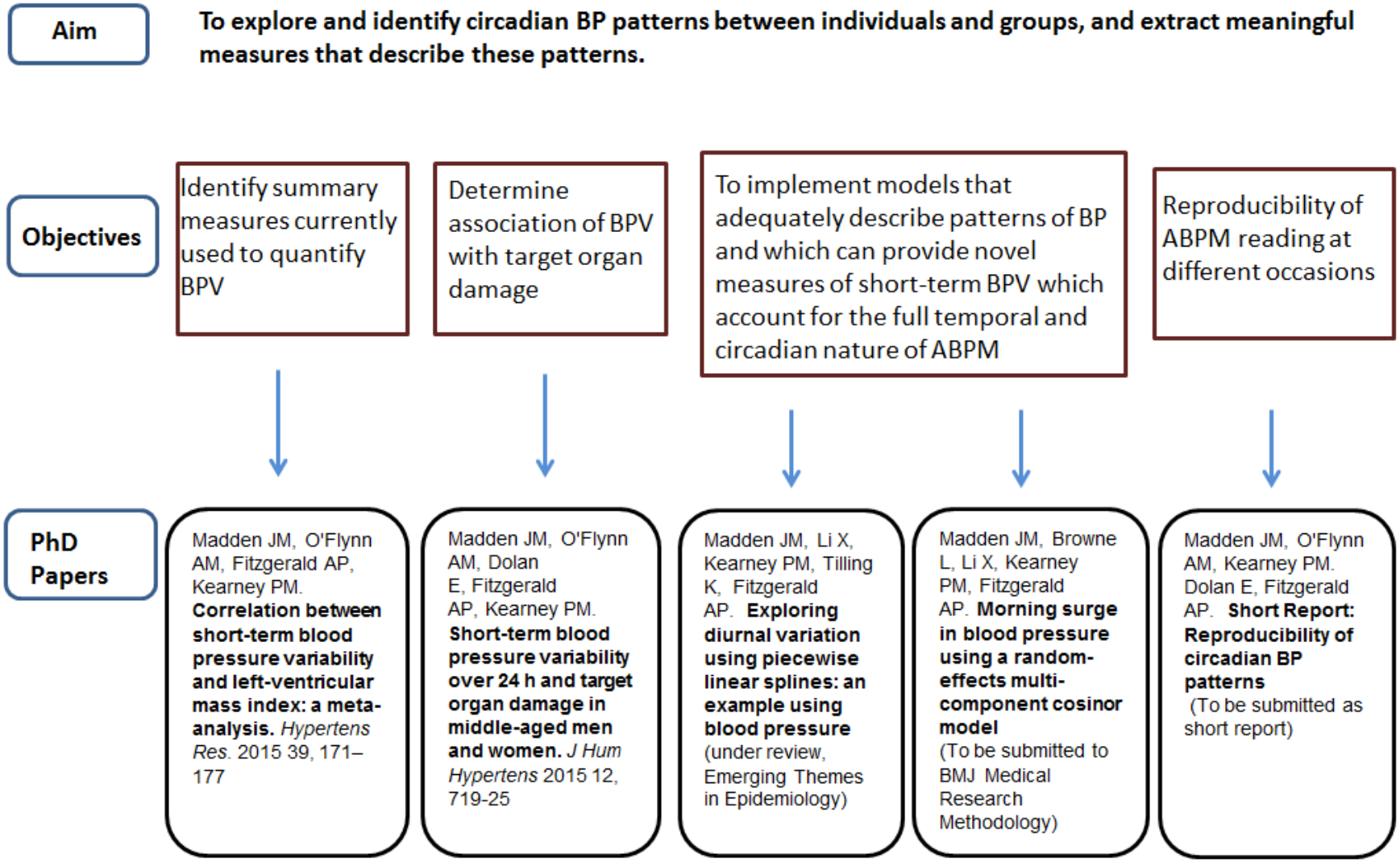


Figure 1-1 Overview of thesis including aims and objectives.

2. LONGITUDINAL DATA ANALYSIS & APPLICATION TO ABPM

2.1 Chapter Overview

This chapter introduces hierarchical and specifically longitudinal data structures. This is followed by a detailed description of statistical techniques available that are suitable for the analysis of ABPM both in terms of accounting for the longitudinal nature of the data but also methods that are capable of capturing non-linear circadian BP curves. A graphical illustration of each technique is presented using the motivating dataset as an example. This is followed by a summary of its application in the literature, paying particular attention to the use of the technique to analyse BP data. Particular attention is drawn to the techniques used in this thesis; however alternative methods are described briefly. A comparison of the different approaches is given at the end the chapter.

2.2 Overview of Longitudinal Data

Many epidemiological studies have a natural hierarchical data structure associated with them where there are different levels of information. For example, data from a number of different hospitals would comprise of hospital level information but within each hospital (or cluster) we could have patient level information. The term hierarchical (or nested/multilevel) data is given to data with this structure. Longitudinal data (panel data or repeated measures) can be considered a special case of hierarchical data where a sample is followed up over time with information collected at several occasions or time points. This type of data can be considered as a two level cluster where there are repeated measurements (level 1 units) with a natural ordering nested within individuals (level 2 units). It is important to highlight the natural ordering of the measurements which are not exchangeable, unlike for example patients nested within hospitals. Recognising both the hierarchical structure of the data points and the natural ordering in time of the data points is critical. Measurements within clusters or subjects will tend to be more similar than measurements from different subjects. In addition, the ordering of time points means that within-subject readings close together may be more correlated than points further away. Both issues of correlation must be accounted for in the analysis and renders traditional regression analysis techniques, which assume

independence of observations for non-hierarchical data to be inappropriate. ABPM is a perfect example of such hierarchical data where there are many repeated BP readings on an individual taken over a 24h period with a natural order (in time) to the observations. An example of four individual ABPM readings from the Mitchelstown ABPM dataset is provided in Figure 2-1.

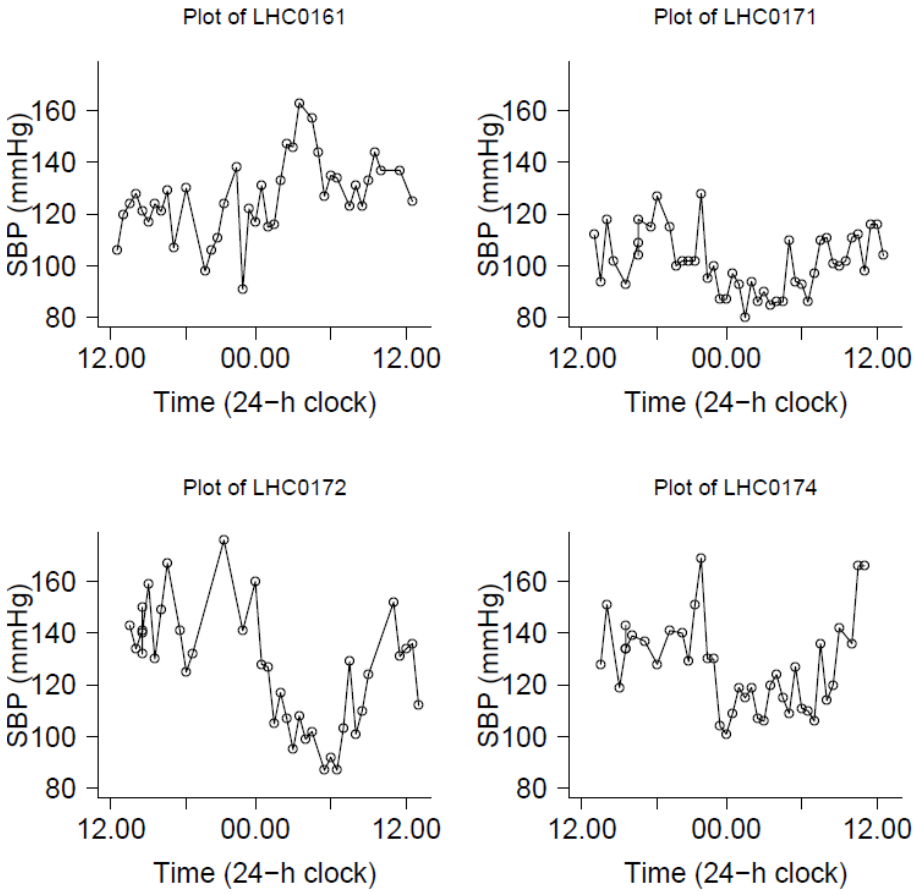


Figure 2-1 Example of four individual ABPM readings

2.3 Statistical Approaches for Modelling Longitudinal Data

Before the specific analysis of ABPM is introduced, a general description of statistical approaches for the modelling of longitudinal data is outlined.

There have been substantial developments in statistical methodology for the analysis of longitudinal data over the last 40 years. One of the key drivers has been improvements in technology which means computationally complex problems can now be solved in a fraction of the time taken previously. This, coupled with increased widespread availability of software programs and packages which are becoming more and more accessible to users, means researchers have access to a variety of more rigorous approaches for the analysis of longitudinal data [82]. Two of the most widely used approaches are generalised estimating equations (GEE) models [83-85] and mixed-effects models [86, 87]. As both methods make an adjustment for the dependency of the observations within an individual, both are suitable for the analysis of longitudinal data. However, there are differences in how both methods adjust for this correlated data leading to different model assumptions. In addition, the two models can be implemented to answer different questions relating to longitudinal data.

Generalised Estimating Equations

GEE models (or marginal models) are often referred to as population-average models where the target of inference is the population [88]. The term marginal is used as the focus is on the mean response, which depends only on the covariates of interest and does not specify the joint distribution of the individual's observations. An important point in the analysis of marginal models is that the mean response and the within-subject association are modelled separately. The latter is referred to as a nuisance characteristic that must be accounted for in the analysis so that correct inferences about the changes in the population mean response (primary goal) can be made [88, 89]. Separately modelling the mean response and the within-subject association has the important implication that the regression coefficients derived from GEE models have population-average interpretations, where they describe features of the mean response and how these relate to

covariates. GEE uses a partial-likelihood approach to estimate the parameter of interest.

A GEE model can be defined as the following three-part specification [88]:

The marginal expectation of the response, $E(Y_{ij}|X_{ij}) = \mu_{ij}$, depends on the covariates, X_{ij}, through a known link function

$$g\left(\mu_{ij}\right) = \eta_{ij} = X'_{ij}\beta \quad \textbf{(1)}$$

The variance of each Y_{ij}, given the covariates, depends on the mean

$$\mathrm{Var}(Y_{ij}|X_{ij}) = \phi v\left(\mu_{ij}\right) \quad \textbf{(2)}$$

where $v(\mu_{ij})$ is a known "variance function" (known function of the mean, μ_{ij}) and ϕ is a scale parameter that needs to be estimated when the response is continuous [88].

The pairwise (or two-way) within-subject association among the vector of repeated responses, given the covariates, is assumed to be a function of the means, μ_{ij}, and an additional set of within-subject association parameters, α. Given a model for the pairwise correlations, the corresponding covariance matrix can be constructed as the product of standard deviations and correlations

$$V_i = A_i^{1/2}\mathrm{Corr}(Y_i)A_i^{1/2}, \quad \textbf{(3)}$$

where A_i, is a diagonal matrix with $\mathrm{Var}(Y_{ij}|X_{ij}) = \phi v\left(\mu_{ij}\right)$ along the diagonal (and $A_i^{1/2}$ is a diagonal matrix with standard deviations, $\sqrt{\phi v\left(\mu_{ij}\right)}$, along the diagonal), and $\mathrm{Corr}(Y_i)$ is the correlation matrix (here a function of α). To distinguish it from the true underlying covariance among the Y_i, V_i is known as a working matrix which acknowledges the uncertainty about the assumed model for the variances and within-subject associations. Unless they have been modelled correctly, our model for the covariance matrix may not be correct [88].

The GEE estimator of β for marginal models (or generalised linear models for longitudinal data) can be obtained by minimizing the following function (where N is the sample size):

$$\sum_{i=1}^{N} \{y_i - \mu_i(\beta)\}' V_i^{-1} \{y_i - \mu_i(\beta)\} \quad \textbf{(4)}$$

It can be shown that if a minimum of the function given by equation (4) exists then, it must solve the following GEE:

$$\sum_{i=1}^{N} D_i' V_i^{-1} (y_i - \mu_i) = 0 \quad \textbf{(5)}$$

where $D_i = \partial \mu_{ij} / \partial \beta$ is the derivative matrix containing the derivative of μ_{ij} with respect to the components of β [88].

Mixed-Effects Regression Models

While GEE models are focused on inference for the population, mixed-effects (subject-specific) models target inference at the individual level. The full-likelihood approach associated with mixed-effects models provides estimates of subject-specific effects (e.g. subject-specific trajectories) that are useful for understanding between individual variability in the longitudinal response [82]. These are known as random-effects which are coefficients that are allowed to vary between individuals (clusters). The estimation of the random-effects determines the subject-specific curves and explains the correlation structure of the longitudinal data [90]. Mixed-effects models allow us to estimate both the degree of variation within a person (within-subject variation) and in the population of individuals (between-subject variation) [82].

The simple linear mixed-effects model [86-88], which is an extension of the simple linear regression model, can be written as:

$$y_{ij} = \beta_0 + b_{0i} + (\beta_1 + b_{1i}) t_{ij} + e_{ij} \quad \textbf{(6)}$$

where y_{ij} is the response value for the j^{th} measurement on the i^{th} subject, at time t_{ij}, the β*'s* are the fixed effects coefficients associated with the population (average) intercept at β_0 (equivalent to linear regression model) and the population slope (β_1), *b*'s are the random-effects where b_{0i} and b_{1i} are the intercept and slope deviations

respectively for the i^{th} subject, and e_{ij} represent the individual-level residuals from the model. A comparison between the traditional linear regression and the linear mixed-effects model is presented in Figure 2-2 where we have a response (y) plotted against time (t). In the fixed-effects linear regression plot all observations are considered independent. However, in reality the data may be clustered with repeat readings on different subjects, represented as colour coded dots in the mixed-effects plot. The black line represents the population average which is described by intercept β_0 and slope β_1 in the model. In addition, each individual obtains their own random-effects (b_{0i} and b_{1i}) which can be added to the population coefficients to obtain subject-specific curves (Figure 2-2).

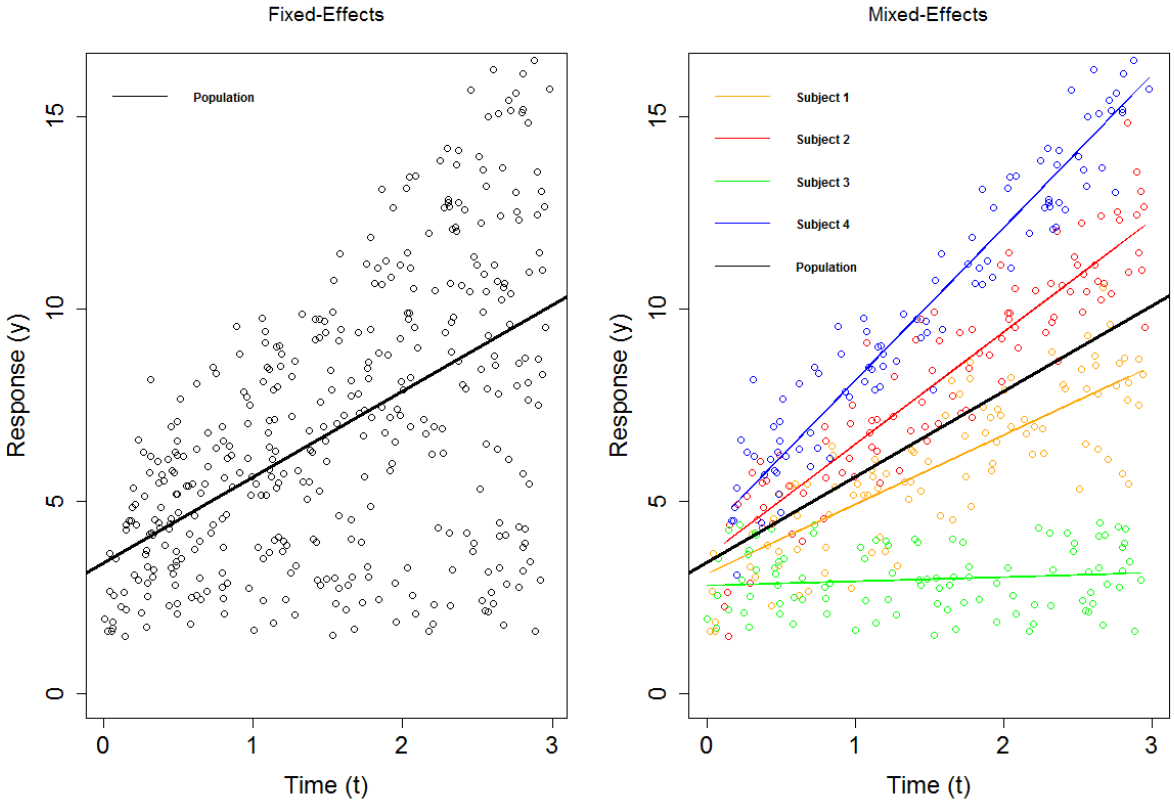


Figure 2-2 Illustration of simple linear regression model and simple linear mixed effects model where the data are clustered

The individual level residuals are assumed to be independent and have mean zero and variance σ^2. It is assumed the random-effects have zero mean and follow a bivariate normal distribution with a variance-covariance matrix Σ_b:

$$\sum_b = \begin{bmatrix} \sigma^2_{b0} & \sigma_{b0b1} \\ \sigma_{b0b1} & \sigma^2_{b1} \end{bmatrix} \quad \textbf{(7)}$$

where σ^2_{b0} and σ^2_{b1} represent the between-subject variation of the intercept and slope random-effects respectively. The magnitude and direction of the covariance term (σ_{b0b1}) gives information about the interaction between the random slope and intercept. In Figure 2-2 subjects with a high intercept have a steeper slope, giving a positive correlation between b_{0i} and $b_{1i,}$ resulting in a positive covariance. Accounting for this is an important aspect of longitudinal modelling.

Although the model presented above is quite flexible some of the assumptions may be too restrictive when examining certain data structures. It can however, easily be extended to accommodate many situations. Like linear regression, the model can adequately make adjustment for confounding variables. The model assumes the response changes linearly over time but for many biological processes, the mean profile is a non-linear function of time [91]. One approach is to model the non-linearity by including non-linear functions of time such as quadratic or cubic terms. It is important to note the model will remain linear in the random-effects. There are however, many other alternative approaches for modelling the mean (see section 2.4).

One of the defining properties of linear mixed-effects models is that it allows for the explicit analysis of between-subject and within-subject sources of variation i.e. the random-effects covariance structure can be explicitly defined [88]. This essentially involves defining two separate variance-covariance structures, one for the random-effects across subjects and one for the within-subject random errors [92]. This can be illustrated by rewriting the variance of *b'*s (random-effects) and e_{ij} (random errors) in matrix form:

$$var \begin{pmatrix} b_i \\ e_i \end{pmatrix} = \begin{pmatrix} G & 0 \\ 0 & R_i \end{pmatrix} \quad \textbf{(8)}$$

where *G* is the variance-covariance matrix for the random-effects across subjects. This is equivalent to the variance-covariance matrix defined in equation (6). The matrix R_i is the variance-covariance matrix for the within-subject random errors. The b_i (random-effects) and e_i (random errors) are assumed to be independent. As

assumed in the mixed-efffects model above, the R_i matrix is often simplified as $\sigma^2\mathbf{I}$, where **I** is the identity matrix, assuming within-subject random errors to be conditionally independent with homogeneous variance, that is, they are independent conditional on the b_i random-effects which accounts for the intraindividual correlation [90, 92]. In order to get the total variance of y_i, we first rewrite the simple linear mixed-effects model (equation (6)) in matrix form where the response is given as:

$$Y_i = X_i\beta + Z_i b_i + e_i \quad \textbf{(9)}$$

where X_i and Z_i are design matrix of covariates. From this, the total variance (V_i) becomes:

$$V_i = Z_i G Z_i' + R_i \quad \textbf{(10)}$$

By constructing the design matrix Z_i and specifying structures for G and R_i matrices, the variance of repeated measurements can be adequately specified in linear mixed models [92]. The subscript i on the V_i matrix indicates that the total variance-covariance matrix depends on the subjects covariates. Under matrix notion the repeated measurements of the response Y_i follows the following multivariate normal distribution:

$$Y_i \sim N(X_i'\beta, V_i) \quad \textbf{(11)}$$

where β is a vector of the population parameters, with the first element representing the intercept.

The structure of G is often left unspecified when more than one between-subjects random term is specified in a linear mixed-effects model. When there is no explicit structure assumed, it is referred to as an unstructured pattern. This has the advantage that no assumptions have to be made about the variances and covariances of the random-effects [88, 92].

Regarding the R_i matrix, Fitzmaurice *et al.* argue that in their experience of longitudinal data, variances are rarely constant over time and emphasize the benefits of the absence of restrictions on the variances [88]. Ideally we would like to use a unstructured pattern for all models but the number of parameters that

have to be estimated grows rapidly with the number of measurement occasions. This can affect the computational stability; where a large number of covariance parameters need to be estimated, relative to the sample size (both subjects and repeat readings), estimation is likely to be unstable [88]. There are alternatives that try to simplify the problem such as the compound symmetry or exchangeable structure that specifies that observations on the same subject have homogeneous covariance and variance. Other structures can be specified for the R_i matrix such as Toeplitz or autoregressive residual structure where the correlation between observations on the same subject are not equal, but decrease towards zero with increasing lag. We are not confined to these structures however. For example, the between-subject or within-subject variation could increase or decrease over time in which case the variance could be modelled as a function of time [93]. A similar situation could arise where we allow variation to vary depending on a grouping variable [94]. Selecting the appropriate structure involves finding a compromise between over fitting and under-fitting the model which results in a parsimonious model. Liu suggests the structure can be selected according to theory or empirically [92] while Zuur *et al.* emphasizes that it is "important to model the correlation structure in a reasonable and meaningful way rather than to model the correlation structure perfectly" [93].

The parameters from a mixed-effects model (fixed coefficients, variances, covariances) can be estimated by a number of methods. Maximum likelihood (ML) is the best known technique of obtaining estimates of unknown parameters. The maximum likelihood estimates (MLEs) of the parameters are the values of the parameters that maximise the likelihood function (i.e. the values of the parameters that make the observed values of the dependent variable most likely, given the distributional assumptions) [95]. For the case of the linear mixed-effect model, it can be shown by obtaining the likelihood of the multivariate normal probability density functions of the model that the log-likelihood function is defined as:

$$l = -\frac{n}{2} ln(2\pi) - \frac{1}{2} \sum_{i=1}^{n} ln|V_i| - \frac{1}{2} \left(\sum_{i=1}^{n} (Y_i - X_i\beta)' V_i' (Y_i - X_i\beta) \right) \qquad \textbf{(12)}$$

The natural log is introduced to simplify the mathematics as it converts the product of the individual density functions to a sum of log-density functions. In the case of linear regression (independent observations), maximising the likelihood function results in estimates that can be solved relatively easily as the equations have closed form solutions. In the case of linear mixed models, ML estimation can involve non-linear equations for which there is no closed form solution for the parameters [93]. Instead, the ML estimates can be obtained using an iterative technique. There are a number of different algorithms that can be applied to obtain MLEs including iterative generalised least squares and iteratively re-weighted least squares which are discussed in detail elsewhere [94]. One drawback of the ML approach is that the estimates for the parameters of the covariance matrix are biased by a factor of (n-2)/n. The reason for this is because it ignores the fact that the intercept and slope are estimated [93]. Restricted (or residual) maximum likelihood (REML) estimation was developed to address this problem based on a slight modification on the likelihood above where it is defined only in terms of the covariance matrix V_i, thus separating it from β (estimated parameter). It is recommended to use REML over ML when possible as it will provide less biased estimates, although it is less of an issue with significantly large sample sizes [88].

The likelihood ratio tests (LRT) can be used for hypothesis testing. The LRT compares the maximised log-likelihood of two models, a full model with a nested model. A formal test is obtained by taking twice the difference in the maximised log-likelihood and comparing the statistic to a chi-squared distribution [88]. Despite the recommendation to use REML, in order to be able to compare models using LRT we first estimated the models using MLE to obtain ML solution.

Comparison of GEE and Mixed-Effects Analysis

GEE models use partial-likelihood methods to estimate parameters of interest whereas mixed-effects models are based on full-likelihood methods. The advantages of partial-likelihood is that they are computationally easier than full-likelihood methods and they do not require distributional assumptions for the observations as there is no specification of the joint distribution, only the mean response [82, 88]. One of the appealing properties of the GEE approach is that when the data is balanced and under the condition that the mean response is modelled correctly, it produces consistent and unbiased estimates of regression parameters and corresponding standard errors even when the covariance structure is misspecified [88, 96]. This robustness can be attributed to the use of the empirical or sandwich estimator [97, 98] which produces valid standard errors for the parameters of the model even when the assumed covariance among the repeated readings is not correct. However, Fitzmaurice *et al.* argue that correctly specifying the covariance structure makes optimal use of the available data for estimation of the parameters of interest [88]. One of the main drawbacks of the sandwich estimator is that it is best suited to balanced longitudinal designs where there are a large number of individuals with a small number of observations. In situations where the design is severely unbalanced, with each individual having a unique sequence of measurement occasions in addition to having few subjects, sandwich based standard errors can be biased downward and result in underestimation of the variance of the regression coefficient [88]. For linear models with balanced data, GEE coefficients and the fixed-effects coefficients from a mixed-effects model will yield the same values. However, they are not equivalent for non-linear models [88]. By comparing the GEE function in equation (4) and the mixed-effects function in equation (12) similarities between the two can be seen. The larger mixed-effects function is essentially the GEE function with an additional variance component.

One of the key advantages of random-effects models over other techniques (e.g. GEE models, traditional analysis-of-variance approaches) is that they make full use of all available data from each individual and do not require balanced datasets. GEE

assumes that missing data are missing completely at random (MCAR). Full-likelihood estimation random-effects rely on a weaker assumption that missing data are missing at random (MAR). Missing data are known to be completely at random (MCAR) when their absence is not related to both the observed and unobserved data [99]. MAR however refers to missing data that depends on observed data but not on unobserved data [99]. In the case of random-effects, missing data are ignorable if the missing responses can be explained either by covariates in the model or by the available responses from a given subject [82, 88]. A mixed-effect analysis does not require a balanced dataset because the covariance can be expressed as an explicit function of times of measurement (when times of measurement, or functions of time are included in Z_i) [88]. In theory each individual can have their own unique sequence of time points. Additionally as highlighted previously, random-effects can allow the variance and covariance to be modelled as a function of time unlike other techniques which force the variance to be constant over time. Unbalanced data can however, have implications on specifying the autocorrelation residual structure when there are irregular gaps making it necessary to parameterise the autocorrelation function (ACF) explicitly [91]. For example, applying a continuous autoregressive error (CAR) structure is similar to applying a autoregressive structure but uses the actual value of measurement time which helps account for the issue of unbalanced data [94, 100].

It is the ability to estimate both between and within-subject variation that makes the use of mixed-effects models so appealing for the analysis of ABPM in general, but also specifically to help answer the questions posed in this thesis where the focus is on obtaining population and subject-specific measures of BPV and quantifying this variation both between and within individuals. For this reason the use of mixed-effects models is preferred over the GEE approach in this thesis.

2.4 Statistical Approaches for Modelling Individual ABPM Profiles

This section explores different approaches for modelling ABPM using random-effects models from which variability measures can be obtained. Previous longitudinal modelling approaches to ABPM from the literature are discussed as well as suggesting other novel techniques. A comparison of the suggested techniques is also included at the end.

Summary Measures of BPV

The simplest form of random-effects is in essence subject-specific summary parameters, where the parameter is random. The idea is that multiple measurements on an individual are collapsed into one (sometimes more than one) summary measurement which is an estimate of a subject-specific measure. This can then be related to an outcome measure. The advantage of this method is that data with a longitudinal structure are converted to a cross-sectional problem where traditional regression techniques are valid without worrying about correlation between readings. This can lead to straightforward analysis and does not require statistical knowledge of multilevel modelling.

With the focus of the thesis on patterns of BP, summary measures in this context relate to short-term summary measures of variability, as opposed to say mean BP values. As highlighted in Chapter 1, summary measures have been the focus of much of the literature to date in relation to short-term BPV. This simple approach can also be considered a simple two-stage model where the summary measures of variability such as SD over 24h, are obtained first (first-stage) and then these values are entered into a subsequent second model (second-stage), such as a logistic regression model, exploring its effect on an outcome such as TOD. The main disadvantage of this method is the loss of information as the statistical power associated with longitudinal data is removed by collapsing the data into one value. The other issue is that quantifying variability is difficult as highlighted in Chapter 1 and attempting to do this with one measure makes the task significantly more challenging. Nevertheless, this does not mean that summary measures should be discarded. In fact many ABPM software programs routinely include estimates of

variability measures such as SD (dabl ABPM system, dabl LTD, Ireland) in their reports and are an important reference point. Because of the ease with which these can be obtained and included in an ABPM report, the exploration of suitable summary measures that capture variability deserves substantial attention prior to exploring more complex methods. For this reason Chapter 3 is a systematic review identifying summary variability measures while Chapter 4 applies these measures to our dataset and explores their association with TOD.

Polynomials

As is evident from Figure 2-1, like many biological processes, BP is not linear over time. Polynomial regression is a simple, but useful tool in the analysis of longitudinal medical data to cope with non-linear or curvilinear relationships [101]. The general principle of polynomial regression is to use increasing powers of time (t) as separate predictor variables to model the mean of the response variable of BP. For an individual, the model can be expressed as:

$$BP_{ij} = \beta_{0i} + \beta_{1i}t_{ij} + \beta_{2i}t_{ij}^2 + \cdots + \beta_{pi}t_{ij}^p + e_{ij} \quad \textbf{(13)}$$

where BP_{ij} is the BP value for the j^{th} measurement on the i^{th} person, at time t_{ij}, the β*'s* are the polynomial regression coefficients that comprise of the average fixed-effect and the subject-specific random effect. The e_j represents the individual-level residuals from the model.

This model can be problematic however as the powers of *t* will be correlated, often quite highly so, which may not only lead to multicollinearity issues but also software convergence difficulties. Some of these issues can be overcome by centring the predictor variables, time in our case [102]. However, a better approach is to transform the powers into orthogonal polynomials, an equivalent set of predictor variables which are uncorrelated. These contain the same information, address the issue of collinearity while also helping to reduce the problem of

convergence. To illustrate the flexibility that can be obtained from polynomial analysis, raw data were plotted for four individuals along with the fitted values from a 6th order polynomial model, Figure 2-3.

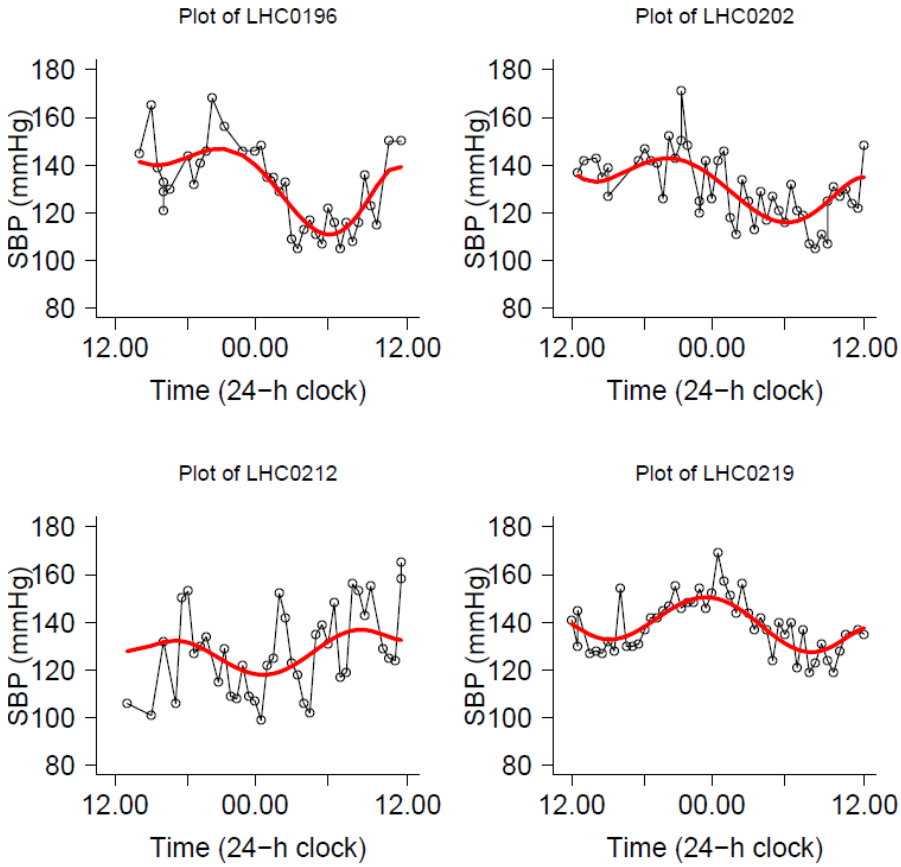


Figure 2-3 Raw data for four individuals along with predicted 6th order polynomial model

Corrao *et al.* implemented a 3rd degree polynomial on ABPM and demonstrated that the prevalence of TOD (LVH or ischemia) was higher in hypertensives with absence or inversion in BP circadian rhythm compared with hypertensives with preserved BP circadian rhythm [103]. Zwinderman *et al.* found that a 4th order polynomial fitted ABPM data quite well and could obtain trough-peak values from the predicted curves which could be used to compare antihypertensive medication but the sample size was a major limitation (n=10) [104]. More recently, Edwards *et al.* utilised a linear mixed model with orthonormal polynomials across time in both the fixed and random-effects in a group of hypertensive subjects [49]. Edwards demonstrated that a 9th order polynomial was the best fit to the data. Although a 9th order is unrealistic, Edwards correctly argues that using high order polynomials

is a valid method to smooth individual trajectories eliminating excess "noise" and providing researchers the ability to construct additional measures of 24h ABPM.

Splines – Cubic and restricted cubic splines

Another approach commonly used for modelling non-linear data involves the use of regression splines. Polynomials and regression splines are intrinsically related. Regression splines involve splitting a continuous covariate (usually time) into separate sections. Within each section a polynomial is fitted, and the fitted values from each section are then connected to form a smooth curve [93]. The points where these meet are known as knots. To ensure that there are smooth connections at these knots, certain conditions are imposed. In the instance when there are no knots, the function is a special case of regression splines and the function reverts to being a simple polynomial model. When a cubic polynomial is fitted within each interval it is known as a cubic regression spline with the constraint that it is has continuous first and second derivatives at each knot point. This is the most common type of spline but there is an array of splines now available in different software packages including B-splines, penalised splines, natural splines, thin-splines and smoothing splines, all of which have the capacity to produce well-fitting curves to non-linear data [105].

In the context of ABPM, Selwyn *et al.* used a hierarchical model incorporating a cubic spline model with eight knots [106] to model mean BP profiles. Lambert *et al.* expanded on this by incorporating restricted cubic splines to model the mean BP profiles [48]. Restricted cubic splines are an extension of regression splines with a constraint that the tails are linear. An unrestricted regression spline will have four more parameters to estimate than that of a restricted spline with the same number of knots. Figure 2-4 illustrates a cubic and restricted cubic curve. The assumption of linear tails is suggested as often there are sparse data at tails and suggesting that a polynomial fits this data best can be dangerous or unrealistic.

Both polynomials and cubic splines, by their nature, have the ability to produce well-fitting curves to the data. Increasing the degrees of freedom/knots that are added to these models inevitably increases the fit but can lead to over fitting of the

data. The main disadvantage of polynomials and splines is that the corresponding coefficients are challenging to interpret directly. Moreover, interpretation becomes more difficult with increasing complexity in the model when the number of parameters increases. Also, when the current sample data is over fitted it can also lead to difficulty in extrapolating to other data. However, using these methods to obtain smoothed curves from which new ABPM measures can be extracted is one practical way of using them. Although this approach was not considered as a separate chapter in the thesis, this method was applied to the dataset using a 6^{th} order polynomial (see Appendix A for details and results). Briefly, the maximum, minimum, number of minutes spent above certain hypertensive guidelines and variation about the curve (residual sums of squares) were calculated as measures of variability from the subject-specific fits from the polynomial random-effects model. The association between the extracted BPV measures and the presence of microalbuminuria was assessed using logistic regression with adjustment for age, sex, smoking status, BMI, diabetes and antihypertensive treatment. Additional models adjusted for mean BP. We found all measures were significantly related to microalbuminuria but the association did not persist after additional adjustment for mean BP.

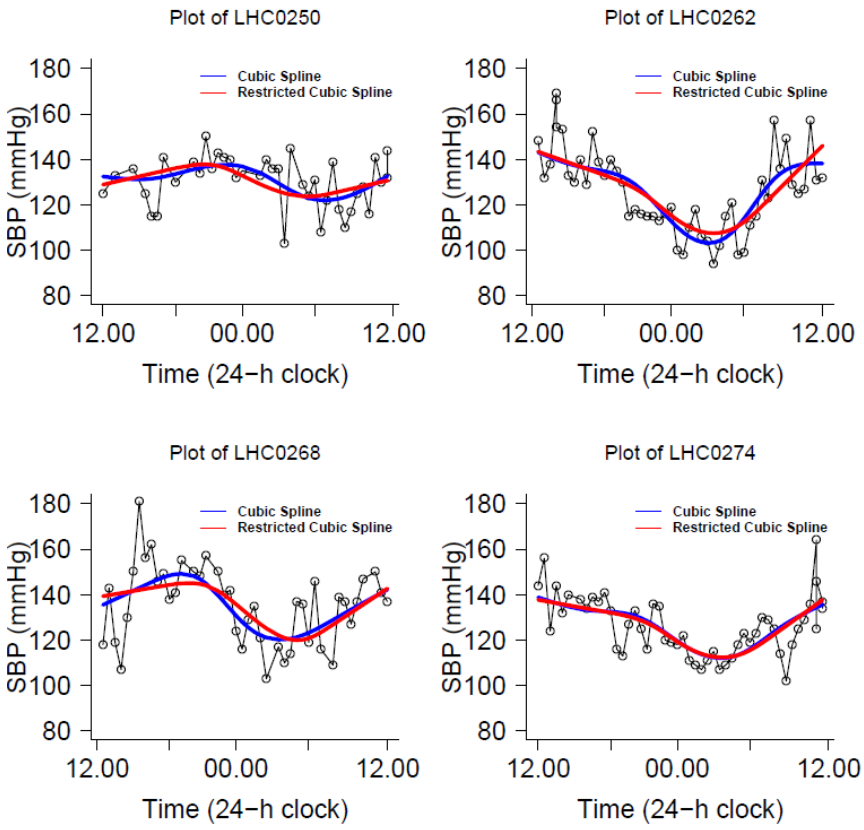


Figure 2-4 Illustration of cubic and restricted splines

Splines – Piecewise Linear Splines

In addition to the previous approaches a method that allowed direct meaningful interpretation of regression coefficients was a requisite of this thesis. Piecewise linear splines in a mixed-effects model were explored as an alternative approach for modelling ABPM. This approach can be seen as a simplification of the principle idea of smooth regression splines where a BP pattern is broken into different segments of time over the 24h period. Rather than fitting polynomials between knot points however, linear splines (straight lines) are used to connect knots. These simple spline models are known as piecewise linear or "broken-stick" models. The benefit of this simple modification is that coefficients represent something meaningful, in this case the slope of BP at different periods of the day. Applying piecewise linear splines in a mixed-effects model allows us to obtain new measures of short-term BPV; the variation about an individual's trajectory and also the variation in slopes

between individuals. This method also allows slopes at a group level to be easily compared. Although linear splines have been used extensively in growth models [107, 108] and even specifically to model BP change over years and gestational age [109], using them to explore daily patterns of BP represents a novel application for analysing ABPM data.

A full analysis using piecewise linear splines on ABPM curves is presented in Chapter 5 but a brief illustration is given here. For each individual *m* linear splines can be created, where the k^{th} spline:

$$\begin{aligned} &s_k(t) = 0 \text{ if } t \leq t_{ki} \\ &s_k(t) = t_i - t_{ki} \text{ if } t_k < t \leq t_{ki+1} \quad \text{for} \\ &k=1,\ldots,m \\ &s_k(t) = t_{ki+1} - t_{ki} \text{ if } t > t_{ki+1} \end{aligned} \tag{14}$$

These can easily be incorporated into the linear mixed effects model outlined previously:

$$BP_{ij} = (\beta_0 + b_{0i}) + \sum_{k=1}^{m} (\beta_k + b_{ki}) s_k + e_{ij} \tag{15}$$

Figure 2-5 illustrates an example of piecewise linear splines on individual ABPM curves. As Howe *et al.* explain there are a number of methods to determine the number and position of knot points [91]. One option is to place knot points at centiles of the distribution of the x-axis (time in this case), or implement a stepwise regression to select knots where there is statistical evidence of a difference between slopes either side of the knot point [110]. Obtaining a smooth curve for the data and extracting derivatives of this curve represents another approach to help inform the number and positions of knot points [111].

Crucially Howe *et al.* argue that subject knowledge of the underlying physiological process being examined can help the choice of knot point positioning [91]. Considering the circadian nature of BP, this is especially relevant for the analysis of

ABPM where we try to incorporate prior known characteristics of BP, see Chapter 5. Alternatively, the data itself can help decide knot positions [91]. There is no perfect way to determine the number and position of knots but in order to keep the number of parameters that have to be estimated to a minimum it is important to try and have as few knots as possible. Again, similar to picking the correct covariance structure, it is about achieving a compromise between over fitting and under-fitting the model which results in a parsimonious model.

One issue with these methods is there is no constraint to force the model to be periodic. In Chapter 5, a constraint is introduced and presented for a piecewise linear model that ensures periodicity, so that on the average subject-specific BP is the same 24h later.

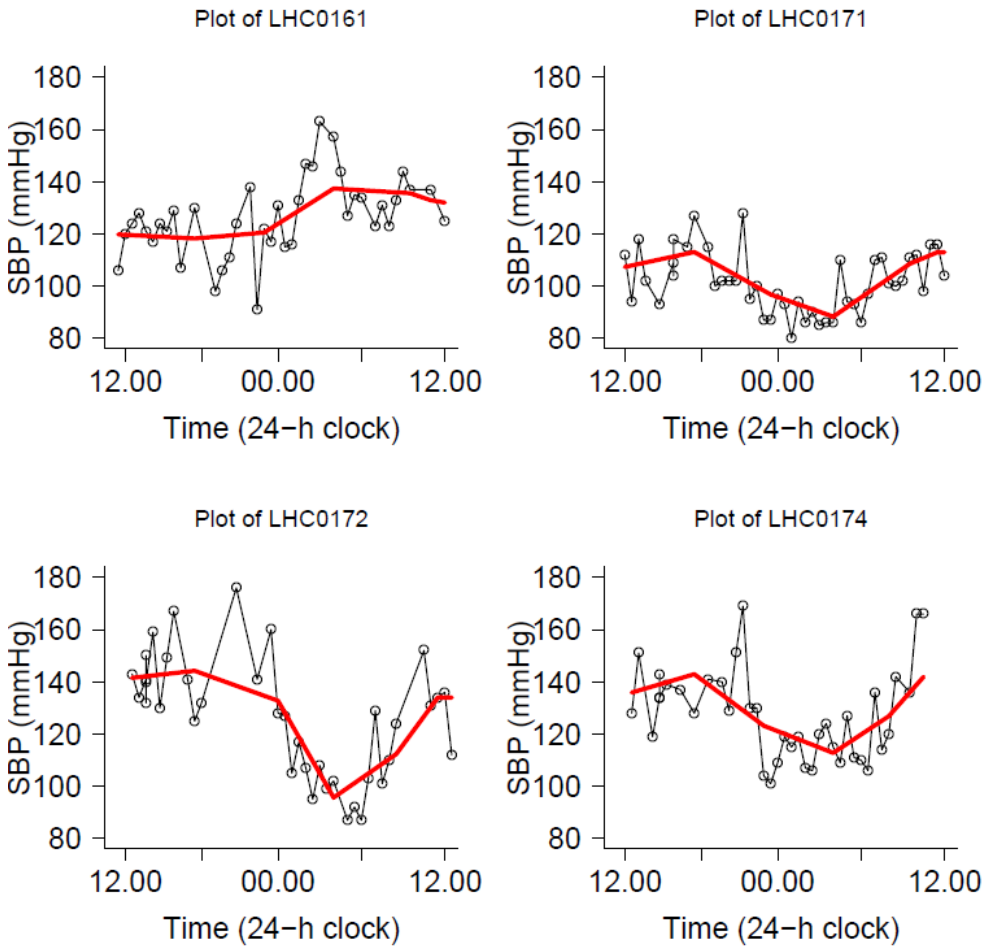


Figure 2-5 Illustration of piecewise linear splines

Cosinor Analysis

Cosinor analysis, which was first developed by Halberg [112, 113], has been the most common approach to modelling 24h BP [114-118]. The single-component cosinor uses a cosine function as a model for physiological processes that have a circadian rhythm:

$$f(t) = M + A\cos\left(\frac{2\pi t}{\tau} + \emptyset\right) + e_t \quad \textbf{(16)}$$

where *M* is the MESOR (Midline Estimating Statistic Of Rhythm, the average value over the period), *A* is the amplitude (half the difference between the highest and lowest values, or the distance between the MESOR and the highest (lowest) value), τ is the period or duration of one cycle, ϕ is the acrophase (a measure of the time of the overall high values recurring in each cycle) and *e* is the error term. Incorporating a 24h period and rewriting the equation in a linear form gives:

$$f(t) = M + \beta_1\cos\left(\frac{2\pi t}{24}\right) + \gamma_1\sin\left(\frac{2\pi t}{24}\right) + e_t \quad \textbf{(17)}$$

where the amplitude and acrophase can be obtained:

$$A = \sqrt{\beta_1^2 + \gamma_1^2} \quad \textbf{(18)}$$

$$\emptyset = arctan\left(\frac{-\gamma}{\beta_1}\right) \quad \textbf{(19)}$$

A graphical representation of this model fitted to raw data for an individual can be seen in Figure 2-6. Cosinor can be incorporated into a mixed-effects model. However, the majority of the studies to date exploring the use of sinusoidal functions have used fixed-effects models where the inference is on population

effects [114-118]. For example, typical inferences are based on estimated differences in model parameters between particular groups of patients, such as comparing the estimated amplitude or MESOR between-groups (fixed-effects) of individuals on different antihypertensive agents [116]. The single component cosinor has a number of limitations. It has been suggested that this method imposes too many restrictions on the shape of the profile and has been shown to fit real profiles poorly [119]. Wang *et al.* [120] suggest problems with fitting a sinusoidal function to a circadian pattern include (i) that the pattern over 24h may not be symmetric; that is, the peak and nadir may not be separated by 12 hours and/or the amplitude and width of the peak may differ from those of the nadir, (ii) sometimes there are local minimum and maximum points.

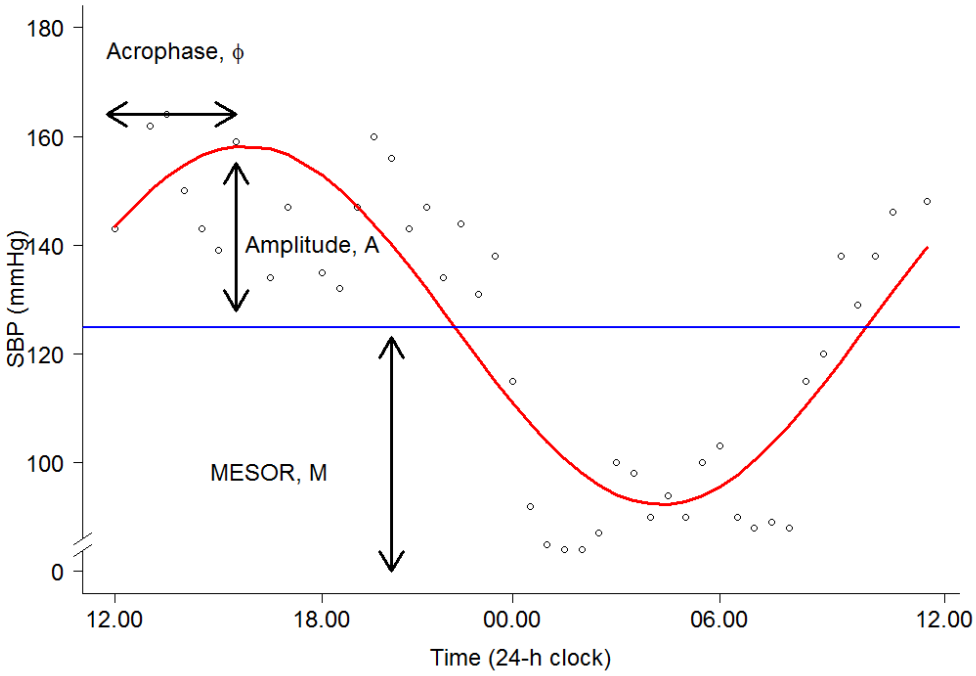


Figure 2-6 A simple cosinor fit to observed SBP readings of an individual (period=24h)

Additionally Wang *et al.* [121] suggests that the sinusoidal function is too restrictive and "rhythms with a shape closely approximating a cosine curve are uncommon" [122]. The method has some advantages however; a BP curve can be described with

the use of only three parameters that have been shown to be related to clinical markers. Also, inherent in the model is the assumption of periodicity – that BP on the average is the same 24h later. Extension of the simple cosinor model to include multiple sin and cosine terms result in more flexible models and overcome many of the disadvantages stated above. Although not as common as the single-component cosinor model, attempts to extend the model by including multiple cosine terms (Fourier analysis) allow more flexible curves to be obtained which has previously been implemented on BP data [123-125]. This is illustrated in Figure 2-7 where a complex curve (red curve) can be broken up into a linear combination of sines and cosines (blue curves). The main advantage of this method is that many complex curves can be obtained by including more terms. However, similar to the single-component model, the majority of studies to date exploring the use of multiple terms have only focused on fixed-effect models [123-125]. A key feature of ABPM analysis however is the exploration of subject-specific effects (random-effects) where its use in cosinor models has been limited [126]. The fitting of a multiple-component cosinor random-effects model is outlined in detail in Chapter 6. We determine how the rate of change or "morning surge" changes over time by using first-order derivatives of the multiple-component cosinor model. To the best of our knowledge this is the first use of the cosinor model to determine a measure of morning surge.

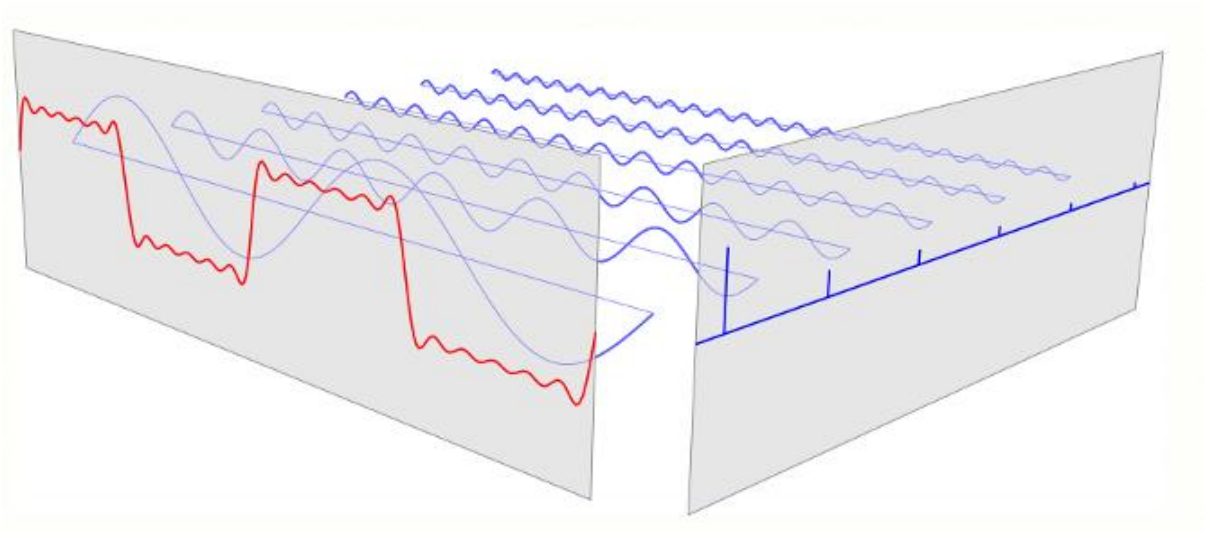


Figure 2-7 Fourier series – a linear combination of sines and cosines can produce a complex curve [127].

Functional Principle Component Analysis (FPCA)

Another possible approach to help identify patterns in BP that builds on the idea of combining multiple curves outlined in the previous section is a method known as functional data analysis (FDA), specifically FPCA [128, 129]. FDA refers to an advanced methodology that consists of a set of techniques designed for the analysis and smoothing of curves or functions which makes it appealing for analysing repeated measures data [129, 130]. In addition one of the major advantages of FDA is it does not make any a priori assumption about the curves. Often FDA is applied to data with high sampling frequency such as accelerometers, which takes readings as regularly as every second of a day. The initial aim is to replace the original observations with curves or functions which are then used for further analysis [131]. This is achieved by smoothing the data using a set of building blocks ϕ_k, $k=1,\ldots,K$ called basis functions, which are combined linearly [129]. A function $x(t)$ can be expressed as a base function expansion:

$$x(t) = \sum_{k=1}^{K} c_k \emptyset_k(t) = \boldsymbol{c}' \emptyset\ (t) \quad \textbf{(20)}$$

where $c_1, c_2, \ldots, c_k$ are the coefficients of the expansion, the matrix expression in the last term uses **c** to stand for the vector *K* coefficients and ϕ to denote a vector of length K containing the basis functions. There are two main types of basis systems; Fourier series and splines. Spline bases are more flexible and complex than Fourier series [129]. There are many types of splines but B-splines are typically used. Although Fourier series are generally used for periodic data, splines are flexible enough to capture a periodic cycle as long as the order of the spline is large enough [129].

To simplify, FPCA can be considered an extension of traditional principle component analysis (PCA) to the case of time series data. For example, rather than having separate variables measured on individuals and performing a PCA on these, as in the case of standard analysis, we instead have one variable (BP) measured multiple times for each individual which creates a time series or "function" over time. Each time-point can be essentially considered a variable similar to multivariate PCA. As in multivariate statistics, eigenvalues of the bivariate variance-covariance function are indicators of the importance of the principle components and plotting them, known as a scree plot, can help illustrate how many are necessary to produce a reasonable summary of the data [129]. Rather than an eigenvector, in FPCA there is an eigenfunction associated with each eigenvalue. The resulting output is also similar to that of regular PCA with the only difference being the focus is on functional data where we can identify the main forms of variability in curves [130]. The eigenfunctions describe the major variational components. Similarly, FPCA allows for each feature to be expressed in terms of its percentage of explained variance. FPCA is the most flexible approach outlined as it tries to estimate the "optimal" functions that explain the variability in the data without pre-specifying a particular pattern. As opposed to saying the pattern follows a certain function e.g. cosine function, it is a data driven process where we do not pick the form of the functions. It can be shown that the random function *x(t)* is $x(t) = \mu(t) + \sum_{k=1}^{\infty} \xi_k \emptyset_k(t)$, where $\mu(t) = E\{x(t)\}$ and $\xi_k = \int_0^1 \{x(t) -$

$\mu(t)\}\emptyset_k(t)dt$ are uncorrelated random variables with mean zero and variance λ_k (eigenvalues) [132]. These random variables are the principle component scores or loadings.

As mentioned, FDA has traditionally been implemented on high sampling frequency data (e.g. every second) but when there are fewer data points, several FDA methods become inefficient [133, 134]. In the case of sparse data, such as ABPM, Yao *et al.* developed a version of FPCA in which functional principle component (FPC) scores are framed as conditional expectations which is referred to as principal component analysis through conditional expectation (PACE) for longitudinal data [134]. In essence this approach is similar to a random-effects model where a mixed model framework is used to estimate curve-specific scores and variances. Unlike traditional FDA where curves are analysed and smoothed separately first, this method first ignores the hierarchical structure of the data and a smooth curve is fitted to the pooled data [133]. This estimate of the mean curve is then used to obtain the covariance matrix of the deviations from the mean for each pair of time-points. This covariance matrix is smoothed using a bivariate smoother and the main diagonal is removed. This smoothed covariance matrix is then decomposed (summarized) into a linear combination of orthogonal (uncorrelated) eigenfunctions and eigenvalues i.e. into its principal components and scores [133]. In the context of random-effects models these FPCs can be seen as patterns of within-subject variation remaining after the mean fit. The first FPC summarizes the main pattern of deviations from the mean trajectory. The second FPC which is uncorrelated to the first explains the next main pattern of variation from the mean and so on. Therefore, a linear combination of a few efficient functions can account for high proportion of the variance. The weights that define the optimal fit to each function are the principle component scores. These can be used to obtain individual BP curves by multiplying the weights by the functions. Zero scores for an individual would result in their trajectory following the mean pattern. The scores would usually be estimated through numerical integration but with sparse data, the approximation is sometimes deemed inadequate and in this case the scores are estimated by the PACE method [133, 134]. Using this method the scores are

estimated for each individual using their repeated measures while borrowing strength from the cohort with sample estimates of the mean function, covariance, eigenvalues and eigenfunctions [133, 134].

Although similar, this is not the same as a traditional random-effects model. FPCA will be inherently more flexible. For example, if we consider a two-component cosinor random-effects model as outlined in the previous section – it would assume a mean and two cosine terms with two different periods, FPCA however will try to estimate the "optimal" functions that explain the variability in the dataset. However, if the mean, a one-period cosine and two-period cosine are close to optimal then the rest of FPCA and the model fitting will be similar: random-effects will be used to estimate the subject-specific coefficients for each of these functions. To account for missing data, smoothing (denoising) and interpolation of the ABPM data is incorporated in the FPCA instead of a pre-processing step. Missing data can be problematic for pre-defined smoothing. To elaborate the process, we first estimate the mean and principle component basis functions (common to all ABPM profiles) by using all the available data across subjects. Secondly, we estimate subject-specific curve loadings by using the data available for that curve, and we combine them with the mean and basis functions to estimate the curve over the full domain. The issue of missing and sparse data is accounted for in this approach.

The implementation of FPCA on ABPM as described above is outlined in Chapter 6 and is compared to the multiple-component cosinor model. The rationale is that by using B-spline basis functions it allowed the FPCA to detect the primary patterns in the data without exactly specifying a model. In comparing the resulting individual FPC scores with the cosinor parameters using the individual random-effects estimates it allowed us to determine the adequacy of our proposed simpler multiple-component cosinor model. If the FPC scores from the more flexible approach correlated well with the cosinor model it would suggest that the model was close to the "optimal" model determined by the FPCA and would provide support for the use of our model. In addition, the use of FPCA enabled us to determine the main patterns of variation in the data and the percentage of the total variation that each principle component contributed. This offers a novel

method to describe variation within BP that to our knowledge has not been used before on ABPM data.

Other Possible Approaches

On occasion, there have been other attempts to model ABPM using different approaches than those outlined in the previous sections. Degaute *et al.* attempted to quantify the overall 24h variation by building a best-fit curve based on periodogram calculations [135, 136]. The method is quite similar to a Fourier analysis approach but is optimized for unevenly time-sampled data where trough-to-peak values can be obtained along with an acrophase parameter. Another method that has been purposed is the cumulative sums (cumsums) method [137]. This method draws a reference line across an individual's pattern at their mean value, and successive deviations of the data from that line are then summed and plotted. It is argued changes in the cusums reveal changes in the trend of data from the baseline much more sensitively than do the data themselves [137]. It is argued that the values obtained are not restricted to follow a symmetrical pattern or fixed period of time e.g. sleep or awake times. The resulting parameters include the crest and trough of BP which are similar to the values that can be obtained using the amplitude in cosinor analysis. However, multiple-component cosinor is more flexible and offers more parameters that describe the curve.

Another approach proposed to model ABPM trajectories was a method used in growth curve analysis literature. Beath introduced a method of data reduction to simplify comparisons between individual growth curves by describing a shape invariant model of infant weight [138]. Cole *et al.* extended this to create the SITAR model (SuperImposition by Translation and Rotation) [139, 140]. The idea is that there is an underlying mean curve which can be applied to all subjects and by applying just three subject-specific translations and rotations of the curve, individual trajectories can be obtained [140]. In the context of BP and mixed-effects, the model is:

$$BP_{it} = \alpha_i + h\left(\frac{t - \beta_i}{\exp(-\gamma_i)}\right) \quad \textbf{(21)}$$

where BP_{it} is BP for subject *i* at time *t*, *h(t)* is a natural cubic spline curve of BP vs time, and α_i, β_i and γ_i are subject-specific random-effects. Essentially the three parameters shift the curve up or down (α), left or right (β), and stretch or squash the time-axis (γ) [91]. Although the model has primarily been developed for examining data over years (growth curves), it does not mean it is not suitable for examining non-linear circadian data. Cole *et al.* recently applied the model to growth curves of insulin-like growth factor 1 (IGF-1) [141]. Unlike height for example, IGF-1 follows a curvilinear shape that does not stop when it reaches a maximum and is similar in shape to a polynomial (during adolescent years). Despite the non-linear pattern of the data Cole *et al.* found that the model explained 65% of the variance in IGF-1 [141].

A SITAR model was applied to our ABPM dataset. The model summarizes the set of BP curves with a mean BP curve as a cubic regression spline, plus the three fixed and random-effects (α_i, β_i and γ_i) defining how individual BP curves differ from the mean curve. Only these three parameters were random, with the spline terms fixed. The fitted models used regression splines with seven degrees of freedom. Figure 2-8 shows the raw ABPM data plotted against time along with the data re-plotted after adjustment was made for the subject-specific parameters (α_i, β_i and γ_i).

The plot indicates that although it is difficult to see a pattern in the raw data, after adjustment a somewhat clearer image can be seen. It suggests that adjusting for these three parameters can partially explain some of the variation in BP. These random-effects could then potentially be used as pseudo variability measures and applied in further models to explore their prognostic significance. One disadvantage of the method is that it is not periodic and additional work would need to be done to make it so. Furthermore the interpretation and clinical meaning of the three parameters that are obtained from the model are not instantly clear. However, if we compare the SITAR to the single-component cosinor model and their parameters, similarities can be seen between both models. They both essentially have parameters that represent a mean and phase shift (left to right). The SITAR also has a stretching parameter which is not directly equivalent but is similar to the

period in the cosinor which is fixed. Although not immediately obvious, with a little manipulation, the $exp(-\gamma_i)$ term could be considered similar to that of the amplitude parameter. The main difference between the models is that one uses a cubic spline (SITAR) and the other uses a cosine (cosinor) as a function of time. Although the parameters from the SITAR model may not seem that interpretable on first glance, they do have similarities to the cosinor parameters. However, implementing a cosinor analysis over a SITAR model seemed more appropriate for the analysis of BP data as the parameters are directly relevant and easier to understand with the advantage that the model is naturally periodic.

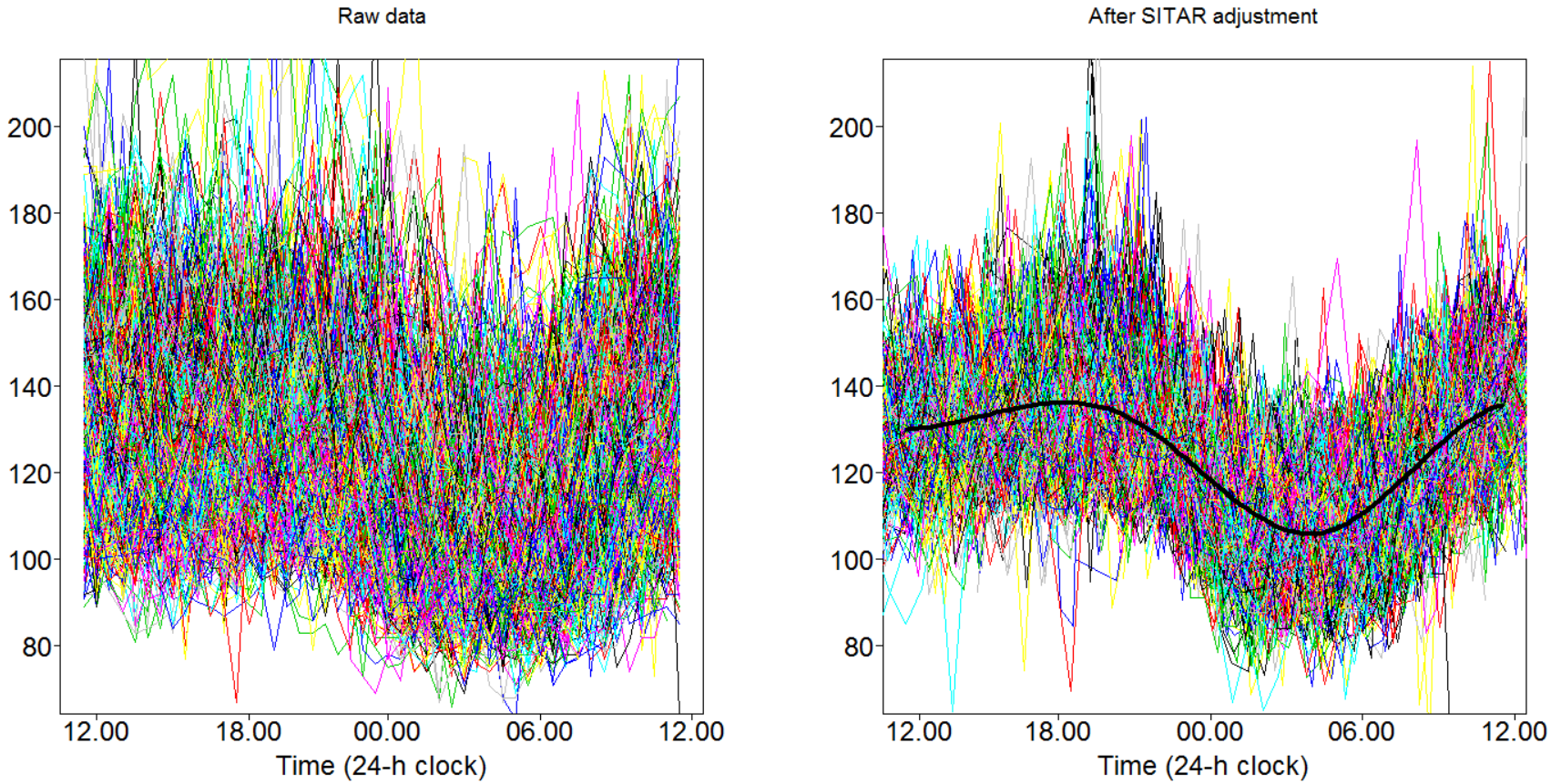


Figure 2-8 Illustration of the SITAR model for SBP over 24h. The black line indicates the mean BP curve.

Comparison of Potential ABPM Models

A number of different approaches to capture circadian BP patterns have been outlined and choosing a model amongst them is challenging. The ultimate goal remains the development of a physiological model that captures known features of the pattern while not over-fitting the data so that we can obtain clinically relevant parameters [142]. It is widely acknowledged that achieving this is a difficult task and there is no perfect model that captures all the features of the circadian rhythm simultaneously [49, 142-144]. To help compare models, Table 2-1 considers the main features that are deemed important in deciding on a final ABPM model. It reiterates the point that no model is optimal on all of the suggested features and choosing one involves making a compromise based on what the researcher deems most important for their particular research question.

We believed the two most important features that should influence our decision on model choice were flexibility and ease of parameter interpretability (which included clinical relevance). Flexibility referred to the ability of the model to capture the circadian pattern in the data. Although, as highlighted previously, summary measures such as SD are the crudest form of analysis, their ease of interpretation and widespread use (often included in standard ABPM reports) made their inclusion in the thesis mandatory (Chapter 3 and 4). By their nature polynomials and splines are flexible and can provide good fits to the data that can be particularly useful when exploring data. However, they have the problem that their resulting coefficients are not directly interpretable (excluding piecewise splines). For this reason they were not considered as a separate chapter in the thesis. Piecewise linear splines on the other hand are more interpretable and depending on the number of knots included can offer a flexible fit to the data. In addition, to the best of our knowledge the method has not been applied to ABPM data before thus offering novel measures of BPV (Chapter 5).

Considering the traditional single-component cosinor model has been the most commonly used approach for the longitudinal analysis of ABPM, further research in an attempt to extend the model seemed warranted for inclusion in this thesis. The

single-component model has the advantage of parameters being easy to understand but the disadvantage that the shapes available are very restricted. Although a multiple-component model is not as interpretable as a single-component model, it is still quite intuitive compared to spline models and their coefficients. The model also had the benefit of being periodic. For these reasons the exploration of a multiple-component cosinor model seemed reasonable (Chapter 6).

As outlined, FPCA is the most flexible approach and tries to estimate the "optimal" functions that explain the variability in the data without pre-specifying a particular pattern. Given a fixed number of functions, it will find the optimal fit. This was used in conjunction with the cosinor method to determine how well the model identified patterns in the data (Chapter 6).

Periodogram which has rarely been used is almost identical to Fourier analysis and was not considered due to the similarity with the resulting output from a cosinor analysis. Similarly, the method of cumulative sums has rarely been used and was not considered in the thesis as the resulting output is too similar to that of cosinor amplitude values. The SITAR model has never been used on BP data but does have similarities to a single-component cosinor model. We felt that the cosinor method gave similar output but with easier interpretation and for this reason the SITAR was not considered in a full analysis.

Table 2-1 Comparison of different modeling approaches for the analysis of ABPM data.

Modeling Technique	Flexibility	Implemented on ABPM	Naturally Periodic	Ease of Parameter Interpretability
Summary Measures	1	Often	No	Excellent
Polynomials	2	Sometimes	No†	Poor
Splines				
Cubic	3	Rarely	No†	Poor
Piecewise	2	Never*	No†	Excellent
Cosinor				
Single-component	1	Sometimes	Yes	Excellent
Multiple-component	3	Rarely	Yes	Good
FPCA	3	Never*	No	Not Implicitly known‡
SITAR	2	Never*	No	Poor
Periodogram	2	Rarely	Yes	Good
Cumulative Sums	2	Rarely	No	Good

Flexibility rating: 1,2, 3, where 1 is the least flexible and 3 represents the most flexible; Implemented on ABPM scale: Never, rarely, sometimes, often; Interpretability scale: Poor, good, excellent

*not to the best of our knowledge, †not directly but with manipulation they can be forced to be periodic, ‡Depending on the ease of interpretation of the FPCs – this won't be known until the results are explored

3. CORRELATION BETWEEN SHORT-TERM BLOOD PRESSURE VARIABILITY AND LEFT VENTRICULAR MASS INDEX: A META-ANALYSIS (PAPER 1)

J. M. MADDEN[a]

A. M. O'FLYNN[a]

A. P. FITZGERALD[a,b]

P. M. KEARNEY[a]

[a] Department of Epidemiology & Public Health, University College Cork, Ireland

[a,b] Department of Statistics, University College Cork, Ireland

This paper was published in Hypertension Research in 2015 (See Appendix J)

Another search was completed with no new papers identified (15th Sept 2016)

3.1 Abstract

Long term BPV has been associated with cardiovascular events but the prognostic significance of short-term BPV remains uncertain, including its influence on the presence of TOD, specifically LVH. A meta-analysis exploring the correlation between short-term BPV and LVMI was performed. Studies were identified by systematic searches in Pubmed and EMBASE. Any summary measure of short-term BPV obtained from ABPM was included. Twelve studies were included. SD, ARV, wSD and CV across 24h/day/night periods were identified as measures of variability. Meta-analysis showed the pooled subgroup correlation coefficients of LVMI with 24h SBP SD, day SBP SD, wSD SBP and 24h ARV SBP were 0.22 (95% CI: 0.12-0.31), 0.19 (95% CI: 0.15-0.25), 0.23 (95% CI: 0.13-0.33), 0.37 (95% CI: 0.01-0.65) respectively. This meta-analysis suggests there is a weak positive correlation, between BPV and LVMI.

3.2 Introduction

Hypertension is a well-established risk factor for CVD [3, 145]. To date guidelines on the management of hypertension have focused on reducing mean BP, which is clearly important, but don't mention BPV [17], for which there is increasing evidence of prognostic value. Evidence from meta-analyses suggest that although different antihypertensive-drug classes have similar effects in terms of reducing BP levels, pronounced differences in their ability to reduce BPV are observed [61, 62]. These differences additionally accounted for effects on stroke risk independent of mean BP. Studies have also shown that SBP variation from one visit to the next may be associated with a poor cardiovascular prognosis. In treated hypertensive patients enrolled in ASCOT-BPLA (Anglo-Scandinavian Cardiac Outcomes Trial–Blood Pressure Lowering Arm), higher visit-to-visit variability in SBP was associated with stroke and coronary events independent of mean BP [25]. In a population based observational study higher visit-to-visit variability in SBP was associated with increased mortality risk over a 14-year follow-up [38]. Importantly visit-to-visit BPV predicted all-cause mortality among those with normal BP, suggesting it may be a prognostic marker before hypertension develops.

Short-term BPV refers to fluctuations of BP across minutes or hours usually taken over a 24h period and can be obtained through the use of ABPM [146]. The predictive value of short-term BPV is less well established than that of visit-to-visit variability. Hansen *et al.* [33] using a large population cohort (8938 subjects) explored the relationship between BPV recorded at base line with cardiovascular events over a median period of 11.3 years and determined that although short-term reading-to-reading BPV was an independent predictor, it did not contribute significantly to risk stratification over and beyond 24h BP. Evidence from the ASCOT-BPLA trial which included both long and short-term variability suggests that although not as strong a predictor as visit-to-visit BPV, short-term BPV measured by the CV still predicted risk of vascular events independently of average daytime mean SBP [25].

The occurrence of major cardiovascular events is usually the result of long-term exposure to hypertension and other risk factors and is often preceded by the development of asymptomatic functional and structural abnormalities known as TOD [75]. Little is known about the influence of short-term BPV on the presence of TOD, specifically LVH. LVH can be determined by ECG or quantified more accurately by measuring left ventricular mass by echocardiography and indexing this to body surface area to give the LVMI [147]. In their seminal paper Parati *et al.* [27] demonstrated that higher diurnal BPV measured as 24h SD was associated with an increased risk of LVH (determined by ECG) in 108 mild-to-severe essentially hypertensive patients. They also showed that for nearly any level of 24h mean BP, subjects in whom the 24h BPV was low had a lower prevalence and severity of TOD those in whom BPV was high, indicating an independent association. However as highlighted evidence since suggests the predictive value of short-term BPV remains unclear and may not contribute much more than mean levels alone [33]. To advance our knowledge of short-term variability, this review attempts to assess and quantify the correlation between BPV and LVMI. A meta-analysis on the various correlation coefficients will be performed.

3.3 Methods

Types of studies

Cohort, cross-sectional or case-control studies that explored the relationship between 24h BPV and LVMI.

Study populations

Participants recruited to observational studies that underwent 24h non-invasive ABPM and an assessment of LVMI. Studies of pregnant women and children were excluded.

Predictor variables

Any summary measure of short-term BPV, where short-term refers to variations across minutes or hours taken over a 24h period obtained by non-invasive ABPM. Summary measures refer to those that can be obtained without the need for advanced statistical methods.

Outcomes

LVMI determined with echocardiography.

Search methods for identification of studies

Studies were identified by systematic searches in Pubmed and EMBASE (up to June 2015). The following search terms were used as keywords and/or MESH terms: ((ambulatory blood pressure) OR (blood pressure) OR (ambulatory blood pressure monitoring) OR (short-term blood pressure) OR (24 hour blood pressure)) AND (variability) AND ((left ventricular hypertrophy) OR (left ventricular mass index) OR ((end OR target) organ (damage OR disease))). The full search strategy can be seen in the Appendix B which includes different spellings and combinations of words.

Potentially relevant articles were identified and duplicates were removed. Only original research articles were included. We supplemented our electronic search by crosschecking the reference lists of all identified studies. There were no date or language restrictions. Non-English papers were translated with an online translation programme. The full texts of relevant articles were obtained and an independent reviewer reviewed selected papers against the inclusion criteria and assessed their quality using the guidelines recommended by Hayden *et al.* [148] for quality appraisal in systematic reviews of prognostic studies. Our systematic review and meta-analysis was conducted according to the checklist of Meta-analysis of Observational Studies in Epidemiology (MOOSE), and the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (PRISMA), see Appendix B.

Data Extraction

The study characteristics extracted included sampling approach, study design, sample size, mean age, BPV index and value, mean LVMI, correlation coefficients and relative information, such as p-values and if it was indicated that they were statistically significant or not. The data was extracted independently by two researchers (JMM and AMOF).

Statistical Analysis

For the meta-analysis, correlation coefficients were converted into Fisher's z-scores and standard errors which in turn were used to calculate 95% confidence intervals. The overall effect size was the weighted inverse variance of the adjusted individual effect sizes (z-scores). The overall effect sizes from the meta-analyses were then back transformed which corresponded to the overall correlation coefficients. Data from the various studies were pooled using the random-effects model. Heterogeneity between studies was assessed using the I^2 statistic. The Begg's test and Egger's test were used to assess the extent of publication bias. All analysis was performed using Stata software [149].

3.4 Results

Basic characteristics of studies

After removal of 218 duplicates a total of 440 articles were identified during the search, of which 416 were excluded based on their titles and abstracts alone, Figure 3-1. After reviewing the remaining 24 full-text articles, 12 were eligible for inclusion in the review, Table 3-1. Reasons for exclusion included articles didn't calculate LVMI as an outcome, summary measures of variability weren't calculated and no effect size was reported. Of the 12 studies, 11 were cross-sectional and one had a case-control design. The population sample sizes ranged from 33 to 1822. The

various indexes used in the studies along with their definitions are presented in Table 3-2. The SD of either 24h/day/night BP readings were used as indexes of BPV in all studies with the exception of two: one which only reported CV [150], and another study [151] only reported average real variability (ARV). In addition to SD, two studies also included CV [39, 152] and a further two included wSD [28, 40]. Leoncini *et al.* [28] also explored ARV. The average value of 24h SBP SD, day SD, night SD had range 13.0-19.7, 10.9-19 and 11.5-13.6 mmHg respectively. As there were so few studies exploring the other indices we have not reported their range here but can be found in Table 3-3. The correlation between 24h SBP SD, day SD, night SD and LVMI had range 0.05-0.52, 0.13-0.21 and 0.04-0.21 respectively. These correlations were all statistically significant with the exception of day SD (r=0.19) [153], 24h SBP SD (r=0.05) and night SD (r=0.04) [40]. In the three studies in which it was explored, wSD had a statistically significant correlation of r= 0.15, 0.26 and 0.31 [28, 30, 40]. Similarly in the two studies which examined 24h SBP ARV, a statistically significant correlation of 0.53 and 0.19 with LVMI was observed [28, 151].

Of the studies that adjusted for covariates (including mean BP), findings were mixed. Schillaci *et al.* [34] who considered 1822 untreated subjects with essential hypertension, reported a weak univarite statistically significant correlation between daytime and night-time SD and LVMI but the association didn't persist after adjustment for various confounders. Similar findings were found by Roman *et al.* [39] who found daytime and night-time SD were univariately associated with LVMI but the association did not persist after adjustment for confounders including average BP. Pascual *et al.* [154] also found similar results after adjustment for age, sex and mean BP.

In contrast, Tatasciore *et al.* [30] in a study examining 180 untreated hypertensive patients, found daytime SD and wSD to be statistically significantly associated with LVMI even after adjustment for other covariates, including mean BP. Similarly Bilo *et al.* [40] found wSD was statistically significantly related to LVMI in a study which investigated 339 hypertensive patients. Zhang *et al.* [151] also found 24h ARV to be significantly related to LVMI after adjustment.

Using the guidelines recommended by Hayden *et al.* [148], the quality appraisal of each paper was assessed and is presented in Table 3-4.

Meta-analysis

Figure 3-2 presents converted correlation coefficients (z-scores) with subgroup meta-analysis reported for each BPV index. An overall z-score for all studies was omitted as combining different indexes would not be appropriate. After conversion from z-scores the pooled subgroup correlation coefficients of LVMI with 24h SBP SD, day SBP SD, wSD SBP and 24h ARV SBP were 0.22 (95% CI: 0.12-0.31), 0.19 (95% CI: 0.15-0.25), 0.23 (95% CI: 0.13-0.33), 0.37 (95% CI: 0.01-0.65) respectively. All but one index (wSD) showed heterogeneity ($p<0.05$) across the studies and as a result random-effects models were used to combine coefficients. Begg's and Egger's tests indicated no evidence of publication bias within each variability index.

3.5 Discussion

Overall our review suggests that there is a weak positive correlation, between BPV and LVMI. We carried out a separate analysis for each measure of variability, resulting in reduced power in the meta-analysis. Our review highlights the lack of good epidemiological studies exploring the relationship between BPV and LVMI. As eleven of the twelve studies were cross-sectional, we cannot assess cause-effect relationships. Although all studies reported univariate coefficients we found just over half of the studies did any further analysis or appropriate adjustment for covariates. Despite these limitations, the results are still worth exploring and the review raised some important issues in relation to BPV in general and also specifically to LVMI.

Veerman *et al.* [153] reported a non-statistically significant correlation with day SD. We cited the small sample size (n=33) as a potential reason for the discrepancy between day SD compared to the other studies. Bilo *et al.* [40] also reported a non-statistically significant correlation of LVMI with 24h SD but interestingly in the same study found both day SD and wSD were significantly correlated with LVMI even

after adjustment. This finding highlights that results are sensitive to the index chosen and leads to the issue of variability measurement.

Most studies have used SD as a measure of BPV and the appropriateness of such an index has been disputed because it only reflects the dispersion of measurements around a single value (mean) not accounting for the order in which BP measurements were obtained [36, 37]. The discrepancies between day and 24h SD in the study by Bilo *et al.* [40] may be explained by the fall of BP at night (dip). A large dip which is known to be associated with healthier individuals will lead to a larger 24h SD. The wSD attempts to remove the effect of the dip and was found to be significantly correlated unlike the 24h SD. This suggests that perhaps SD, at least over 24h may not be a good measure of BPV. Mena *et al.* [36] first explored, and later Pierdomenico *et al.* [37], ARV in relation to BP which is the average absolute difference between successive readings, and is thought to give a true reflection of real variability. In both studies high ARV was found to be an independent predictor of cardiovascular risk in hypertension patients while high SD was not. The two studies that included ARV in this review both found a statistically significant correlation with LVMI [28, 151]. In one study association remained significant after adjustment [151] while the other study found it to be an independent predictor of multiple TOD where the majority of these had LVH [28]. As ARV is thought to give a true reflection of real variability it may be the most appropriate marker of short term BPV over other indexes and could potentially be used to predict outcome in patients even before BP becomes elevated and ultimately provide a means of identifying at risk patients before they develop hypertension.

Other studies exploring the relationship between BPV and TOD have found varied results. As mentioned Parati *et al.* [27] found an association between 24h BPV and severity of TOD (a score based on presence of LVH, chest x-ray abnormalities, abnormalities of the fundus plus a clinical event and/or a renal abnormality). The same group conducted another follow-up study with a follow-up period of 7 years to assess the prognostic relevance of short-term BPV on 73 hypertensive patients [155]. They found an independent association between 24h BPV at baseline and TOD at follow-up. Similarly, in another study of over 700 hypertensive and

normotensive patients, daytime systolic SD was found to be associated with degree of TOD. However in the same study, after adjustment for mean BP no strong association was found between BPV and LVH [156]. Hansen *et al.* explored the relationship between BPV and cardiovascular events [33]. ARV predicted all fatal and nonfatal outcomes even after adjustment for mean BP but found that it added only 0.1% to the explained risk of an event occurring. They concluded that the main risk factor remained mean BP.

Another point that must not be overlooked when considering the importance of the results from the individual studies is the issue of statistical and clinical significance. All the studies have decided on the importance of the correlation coefficient based solely on a statistical significance threshold level of 0.05. It is critical to remember that the smaller the p-level the more significant the relationship but the larger the correlation, the stronger the relationship. It is for this reason that we must be careful how we interpret findings from such studies. For example Bilo *et al*. found a statistically significant correlation between wSD and LVMI but the value of the coefficient was only 0.15 which would be considered a weak association [40]. In this case, with a large sample size (n=339), researchers should not solely focus on statistical significance. Here, we should not over emphasise the statistical significance finding as the correction coefficient is in fact quite low. Sterne and Smith argue that a p-value<0.001 provides much stronger evidence against the null hypothesis, in comparison to a p-value <0.05 and suggest that results of medical research should generally not be reported as “significant” or “non-significant” where appropriate [157]. It is important to take a practical but sensible approach where statistical and clinical significance are both given equal thought when deciding on the importance of a relationship.

As the studies are cross-sectional in nature we are not able to determine whether higher BPV initiates increases in LVMI or do increases in LVMI represent a risk factor for increased BPV rather than being a consequence of it. It is however argued that vascular hypertrophy induced by exaggerated and large BPV may lead to an impaired arterial distensibility of the large arteries, resulting in increased cardiac afterload and as a result increases LVMI [158]. Clinical trials have recently shown

that some classes of anti-hypertensive drugs significantly outperform others in terms of lowering BPV, and that this reduction in short-term and long-term BPV contributes to the prevention of cardiovascular events in hypertensive patients [25, 159]. Results indicate that CCB and to a lesser extent thiazide diuretics are superior to other drugs in reducing BPV and preventing stroke and other vascular events compared to the older β-blocker atenolol which increases BPV [61, 160]. Similar findings were reported in a more recent observational study assessing the efficacy of mono and combination therapy on short-term BPV of 2780 hypertensive patients [64]. Again CCB's, followed by diuretics were correlated with lower short-term BPV compared with ARB's, ACEI's and β-blockers. In addition combination of CCB's and diuretics resulted in the lowest BPV compared to others. In those with marked BPV, the prescribing of these drugs may offer a better alternative and could help reduce the risk of LVH especially in individuals where hypertension has not yet developed.

The major limitation of this review is that we have pooled together studies in a meta-analysis in regard to their correlation coefficients which are a very weak marker of association. As a result of using correlation coefficients there is an implicit assumption that the association between BPV and LVMI is linear which in reality may not be the case. The strength of this review is its focus on short-term BPV which has recently been receiving growing attention. It is also the first review to our knowledge that quantifies the correlation between BPV and LVMI. The review identifies a research gap where stronger epidemiological studies are needed to explore the relationship further and understand the prognostic value, if any, of short-term BPV.

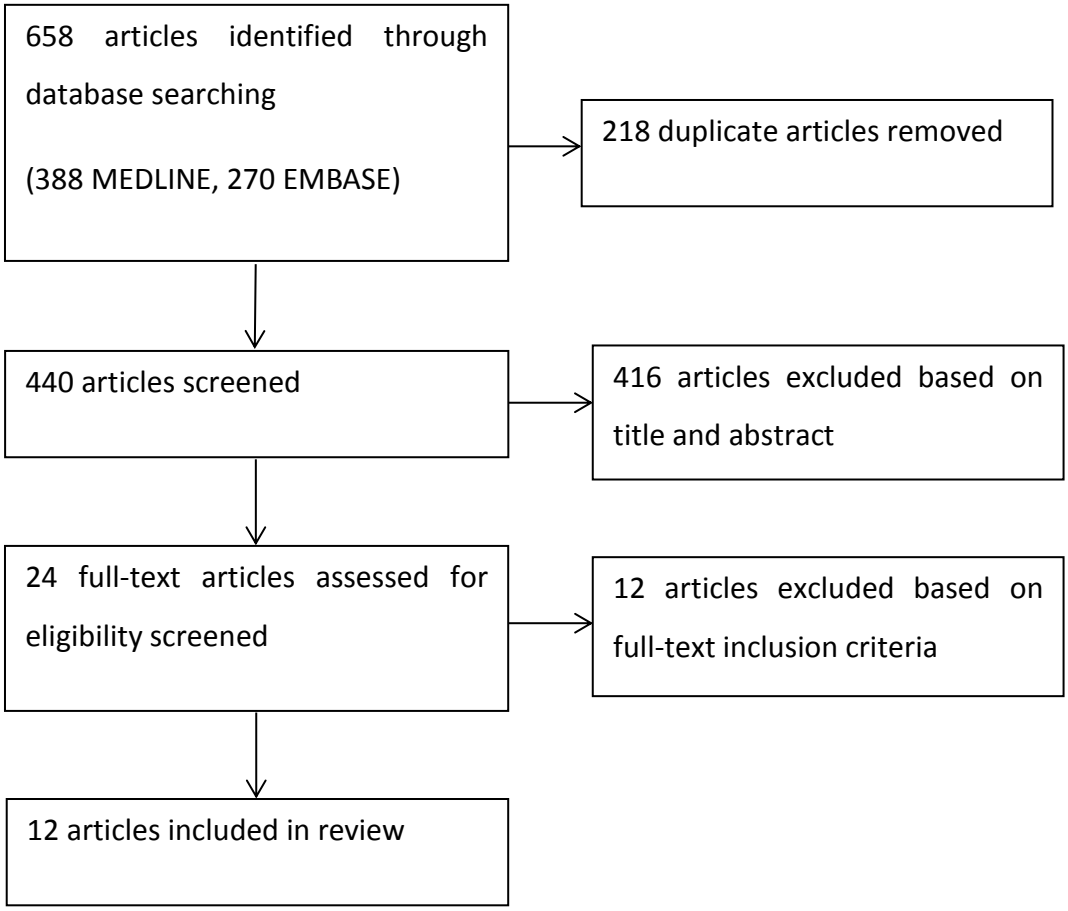


Figure 3-1 Flow diagram of study selection

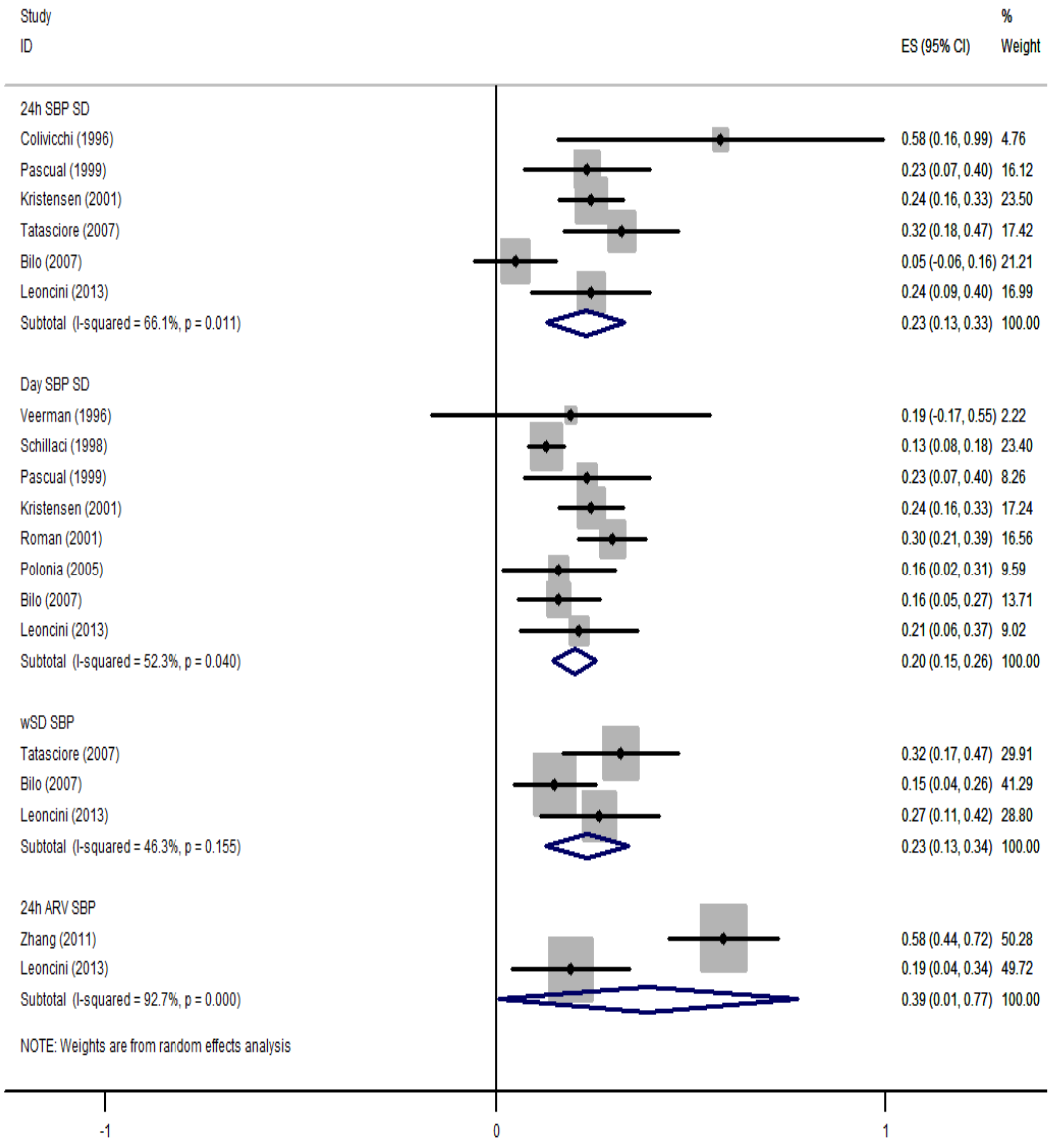


Figure 3-2 Pooled meta-analysis of z-scores by BPV index.

Table 3-1 Study characteristics

Study	Population	Sampling Approach	Design	n (women, %)	Mean age (SD or range), yr	BPV index	Outcome measure
Colivicchi (1996)	Elderly untreated HTN males with matched normotensives	Convenience	Case-control	50 (0%)	74 (4)	24h, day, night - SD	LVMI
Veerman (1996)	Referred to hypertension clinic due to suspected hypertension	Convenience	Cross-sectional	33 (48%)	41 (26-59)	Day- SD	LVMI
Schillaci (1998)	Hospital based untreated HTN	Convenience	Cross-sectional	1822 (47%)	50 (12)	24h, day, night- SD	LVMI
Pascual (1999)	Untreated HTN	Convenience	Cross-sectional	149 (33%)	38 (7)	24h, day, night – SD, CV	LVMI
Kristensen (2001)	Untreated HTN from general practice and subjects drawn at random from Danish national register	Convenience & random	Cross-sectional	566 (52%)	48 (20-79)	24h, day, night – SD,CV	LVMI
Roman (2001)	Subjects from a worksite-based study and who were evaluated at a hospital	NR	Cross-sectional	511 (44%)	50 (12)	Day, night – SD,CV	LVMI
Polonia (2005)	Population sample	NR	Cross-sectional	743 (56%) 185 (LVMI measured)	52 (14)	Day SD	LVMI
Tatasciore (2007)	Outpatients referred to clinic by GP	Convenience	Cross-sectional	180 (40%)	53 (8)	24h, day, night – SD wSD	LVMI
Bilo (2007)	Two hypertension centres	NR	Cross-sectional	3863 (54%) 339 (50%) (Echo taken)	54 (12)	24h, Day, night – SD, wSD	LVMI
Zhang (2011)	Elderly hospitalised HTN and normotensive controls	Convenience	Cross-sectional	197 (35%)	76.5 (7.8)	24h, day, night- ARV	LVMI
Ajayi (2011)	Nigerian HTN	Convenience	Cross-sectional	130 (26%)	54 (12) (31-85)	24h – CV	LVMI

Study	Population	Sampling Approach	Design	n (women, %)	Mean age (SD or range), yr	BPV index	Outcome measure
Continued on following page							
Leoncini (2013)	Untreated HTN attending outpatient clinic	Convenience	Cross-sectional	169 (33%)	47 (10)	24h, day, night – SD ARV, wSD	LVMI

Abbreviations: SD: Standard Deviation, CV: coefficient of variation, wSD: weighted standard deviation, LVMI: Left ventricular hypertrophy index, TOD: target organ damage, LVH: left ventricular hypertrophy, ARV: average real variability, HTN: Hypertensives, GP: general practitioner , NR: not reported

Table 3-2 BPV definitions

Measure of BPV	Description
24h SD	Standard deviation over 24h period
Day SD	Standard deviation over day period usually 9am-9pm
Night SD	Standard deviation over night period usually 1am-6am
wSD	Weighted standard deviation which is the mean of day and night standard deviation values corrected for the number of hours included in each of the two sub-periods which attempts to eliminate the effect of nocturnal fall. wSD=((day standard deviation x day hours) + ((night standard deviation x night hours)))/total number of hours
CV	Coefficient of variation = (Standard deviation over 24h/mean 24h) x 100
ARV	Average real variability which averages the absolute differences between successive readings which is the average absolute difference between successive readings. $ARV = \frac{1}{N-1}\sum_{k=1}^{N-1}\lvert BP_{k+1} - BP_k\rvert$, where *k* ranges from 1 to *N*-1 and *N* is the number of BP measurements

Table 3-3 Extracted Results

Study	SBP Variability SD	Mean LVMI (SD) g/m^2	Multivariate analysis adjusted for:	Method of adjustment for mean BP	Correlation Coefficient (r)	Beta coefficients (multivariate analysis) β (se)
Colivicchi (1996)	16.2 (3.5) 24h SD 19 (5.4) day SD 12 (2.9) night SD	134.9 (27.5) (LVH=16%)	-	-	r=0.52† 24h SD (HTN only) r=NS day & night SD	-
Veerman (1996)	12.7 (7.5-22) day SD	115 (67-153) (LVH=13%)	Day BP	Regression	r=0.19 day SBP SD r=0.35* day DBP SD	3.16 (1.18)* Day DBP SD
Schillaci (1998)	NR	NR	Age, body height, 24h BP, DBP, BMI, duration of hypertension, alcohol, smoking	Subjects divided into similar levels of BP, then subdivided if below or above median BPV & separately a regression analysis	(r=0.13, p<0.01) day SBP SD (r=0.1, p<0.01) night SBP SD r=NS day & night DBP SD	NS
Pascual (1999)	14.0 (3.4) 24h SD 10.3 (2.4) 24h CV 10.9 (2.8) day SD 13.8 (3.3) day CV 11.7 (3.5) night SD 11.7 (3.7) night CV	126 (34.6)	Age, sex, BP	Regression	r=0.23† (24h & day SBP SD) r=NS (24h, day, night DBP SD & CV; 24h, day, night SBP CV & night SBP SD)	0.87 (.83) 24h SBP SD
Kristensen (2001)	NR	102.7 (28.3)	-	-	r=0.24 (p<0.01)	NR
Roman (2001)	NR	NR	Age, age^2, sex, basal SBP or DBP, serum cholesterol, smoking, antihypertensive medication	Regression	r=0.29‡both day & night SBP SD r=0.19‡ DBP SD day r=0.26‡ DBP SD night r=0.08 SBP CV day r=0.022 SBP CV night r=0.00 DBP CV day r=0.06 DBP CV night	NS
Polonia (2005)	13.2 (3.4) day SBP SD 14.3 (3.4) 24h SBP SD 11.5 (3.8) night SBP SD	100 (40)	-	-	r=0.162* day SBP SD	-

Study	SBP Variability SD	Mean LVMI (SD) g/m^2	Multivariate analysis adjusted for:	Method of adjustment for mean BP	Correlation Coefficient (r)	Beta coefficients (multivariate analysis) β (se)
Tatasciore (2007)	13.0 (4.1) 24h SBP 10.9 (4.0) 24h DBP	96.7 (17.8)	Age, sex, alcohol, triglycerides, SBP, DBP, SBP load, DBP load	Regression	r=0.312‡ 24h SBP SD r=NS – 24h DBP r=0.310‡ wSD SBP r=NS wSD DBP	0.633 (p=0.028) 24h SBP
Bilo (2007)	NR for echocardiographic group	109.5 (33.8)	24h BP, sex	Regression	r=0.05 24h SBP SD r=0.15† 24h wSD r=0.16† day SBP SD r=0.04 night SBP SD	0.15 (p<0.01) 24h wSD SBP 0.16 (p<0.01) day SBP SD 0.04 (p=NS) 24h SD
Zhang (2011)	11.9 (2.6) 24 ARV SBP 8.6 (2.6) 24 ARV DBP 9.3 (2.3) day ARV SBP 8.2 (2.1) day ARV DBP 9.2 (3.3) night ARV SBP 7.8 (2.1) night ARV DBP	145.1 (43.2)	Age, duration of HTN, total cholesterol, low-density lipoprotein cholesterol, 24h BP	Regression	r=0.525† 24h ARV SBP r=NS NR 24h ARV DBP	0.593† 24h ARV SBP
Ajayi (2011)	NR	109.65 (38.1)	-	-	r=0.379* 24h CV SBP r=0.124 24h CV DBP	-
Leoncini (2013)	19.7 (5.9) 24h SD 18.5 (6.3) day SD 13.6 (5.5) night 16.8 (5.3) 24h wSD 15.1 (4.8) 24h ARV 15.9 (5.7) day ARV 13.0 (5.3) night ARV 15.1 (5.0) wARV (All SBP, DBP not displayed)	46 (11) (LVM g $m^{-2.7}$)	Office BP, age, BMI, sex, smoking, triglycerides, cholesterol, glucose, duration of HTN, AASI	Logistic regression	r=0.24† 24h SD r=0.19* 24h ARV r=0.26‡ 24h wSD r=0.21† day SD r=0.21† night SD r=0.19* day ARV r=0.14 night ARV all for LVMI indexed for BSA	Odds ratio reported but for overall TOD presence (2 or more) 1.103 (1.003-1.212)† 24h SD 1.114 (1.012-1.227)† day SD 1.140 (1.004-1.295)† 24h ARV

Abbreviations: SD: Standard Deviation, CV: coefficient of variation, wSD: weighted standard deviation, LVMI: Left ventricular hypertrophy index, TOD: target organ damage, LVH: left ventricular hypertrophy, ARV: average real variability, HTN: Hypertensives, NR: not reported, BP: blood pressure, SBP: systolic blood pressure, AASI: Arterial stiffness index based, NS: non-significant and no value was reported, *p<0.05; † p<0.01; ‡p<0.001

Table 3-4 Quality Assessment

Study	Study Participation	Study Attrition	Prognostic Factor Measurement /Outcome Measurement	Confounding Measurement and Account	Analysis
Colivicchi (1996)	No	No	Yes	No	No
Veerman (1996)	Partly	No	Partly	No	No
Schillaci (1998)	Yes	Partly	Yes	Yes	Yes
Pascual (1999)	Partly	Partly	Partly	Partly	Partly
Kristensen (2001)	Partly	No	Yes	No	No
Roman (2001)	Partly	No	Partly	Partly	Yes
Polonia (2005)	Partly	No	Partly	No	No
Tatasciore (2007)	Yes	Yes	Yes	Yes	Yes
Bilo (2007)	Partly	Partly	Partly	Partly	Partly
Zhang (2011)	Partly	No	Yes	Yes	Partly
Ajayi (2011)	Partly	No	Yes	No	Yes
Leoncini (2013)	Partly	Yes	Yes	Partly	Partly

4. SHORT-TERM BLOOD PRESSURE VARIABILITY OVER 24 HOURS AND TARGET ORGAN DAMAGE IN MIDDLE-AGED MEN AND WOMEN (PAPER 2)

J. M. MADDEN[a]

A. M. O'FLYNN[a]

E. DOLAN[c]

A. P. FITZGERALD[a,b]

P. M. KEARNEY[a]

[a] Department of Epidemiology & Public Health, University College Cork, Ireland

[a,b] Department of Statistics, University College Cork, Ireland

[c] Stroke and Hypertension unit, Connolly Hospital, Blanchardstown, Dublin, Ireland

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4.1 Abstract

BPV has been associated with cardiovascular events but the prognostic significance of short-term BPV remains uncertain. As uncertainty also remains as to which measure of variability most accurately describes short-term BPV, this study explores different indices and investigates their relationship with subclinical TOD.

We used data from the Mitchelstown Study, a population based study of Irish adults aged 47-73 years (n=2,047). A subsample (1,207) underwent 24h ABPM. As measures of short-term BPV we estimated the SD, wSD, CV and ARV. TOD was documented by microalbuminuria and ECG LVH.

There was no association found between any measure of BPV and LVH in both unadjusted and fully adjusted logistic regression models. Similar analysis found ARV (24h, day and night), SD (day & night) and wSD were all univariately associated with microalbuminuria and remained associated after adjustment for age, gender, smoking, BMI, diabetes and anti-hypertensive treatment. However, when the models were further adjusted for mean BP the association did not persist for all indices.

Our findings illustrate choosing the appropriate summary measure which accurately captures short-term BPV is difficult. Despite discrepancies in values between the different measures there was no association between any indexes of variability with TOD measures after adjustment for mean BP.

4.2 Introduction

The prognostic value of BPV in addition to mean BP has been receiving growing attention [161, 162]. In addition to showing a strong association with increased mortality risk, visit-to-visit variability in BP has been shown to predict stroke and coronary events independent of mean clinic BP [25, 38]. It has also been linked with TOD [38, 163, 164]. However, the predictive value of short-term BPV, i.e. fluctuations of BP across minutes or hours usually taken over a 24h period, and obtained through the use of ABPM remains contentious. While a number of studies have reported associations between short-term BPV and TOD [27-31] and cardiovascular events [25, 32], others have shown none or only weak associations after adjustment for mean BP [33, 34, 39].

There have been a number of different indices of variability proposed to describe short-term BPV with most studies considering SD [25, 27, 28, 30-34, 38, 39]. The appropriateness however, of such an index as an indicator of BPV has been disputed because it only reflects the dispersion of measurements around a single value (mean) not accounting for the order in which BP measurements were obtained [36, 37]. As a consequence, other indices of variability that have been used include: CV which attempts to adjust for the tendency of those with a higher average BP to also have a higher SD [25, 38, 39], ARV which is the average absolute difference between successive readings, and is thought to give a true reflection of real variability [28, 33, 36] and wSD which attempts to remove the influence of the day-night BP difference from the estimate of BPV [28, 30, 33, 40].

As the prognostic significance of short-term BPV remains uncertain with no consensus as to which measure of variability most accurately describes BPV, this study explores different indices of BPV and investigates their relationship with two parameters of subclinical TOD; LVH and microalbuminuria.

4.3 Methods

Study Population

The analysis utilises data from the Mitchelstown Study, a population based study of middle-aged men and women, recruited in Ireland 2010-2011. A detailed description of the study design is available from a previous publication [74]. In brief, the primary aim of the study was to provide a profile of cardiovascular health and their related factors in an Irish adult general population sample. The study recruited patients attending a single large primary care centre, the LHC, in Mitchelstown. Participants completed a detailed health and lifestyle questionnaire including a question on use of anti-hypertensive medication, and were invited to attend their primary care provider's surgery for a physical examination to be carried out by a nurse trained in the study research protocols. Study measurements included height, weight, BP and in addition, fasting blood samples (minimum of 8-h fast) and urine samples. Participants also underwent standard 12-lead electrocardiogram (ECG) and ABPM was offered to all participants. All participants provided written informed consent and ethical approval was obtained from the Clinical Research Ethics Committee Cork.

BP Measurements

Study BP was measured three times after 5 minutes of rest in a seated position by experienced research nurses using an OMRON M7 BP monitor (OMRON Healthcare, The Netherlands). The average of the second and third measurements was used. ABPM was measured using dabl ABPM system (dabl ltd., Ireland) with the Meditech ABOM-05 Monitor (Meditech LTD., Hungary). The monitors were programmed to obtain readings every 30mins and remained in place for 24h. Participants kept diaries of wake and sleep periods, which were used to calculate day and night BP respectively. If no diary was kept, the period from 1am to 6am was used as the night period and from 9am to 9pm as the day period. Only participants with a

minimum of 14 measurements during the day and a minimum of 7 measurements during the night period were included in the analysis [165].

As indices of short-term reading-to-reading BPV, we estimated the SD over 24h, wSD, CV ((SD 24h BP/mean 24h BP)*100) and ARV. The wSD is the mean of day and night SD values corrected for the number of hours included in each of these two sub-periods [40]. The ARV averages the absolute differences between consecutive measurements. To illustrate the subtle differences between each measure we have included 24h SBP profiles of four participants from the study along with their corresponding 24h variability measures (Figure 4-1.a-d). The two individuals illustrated in Figure 4-1.a and Figure 4-1.b have different overall levels of BP with the individual in Figure 4-1.b having substantially lower BP. In addition to this, the two BP patterns can be seen to fluctuate quite differently throughout the 24h but despite this, they have the same 24h SD, emphasizing the disadvantage of using SD as it only reflects dispersion around the average. Visually it is hard to justify that the two profiles have the same variability when it seems the first participant (Figure 4-1.a) has larger variation between each consecutive measurements compared to the second participant (Figure 4-1.b). In comparison, their 24h ARV differ considerably and highlights the benefit of using this measure as it accounts for the order of the BP readings. wSD and 24h CV values are also presented in Figure 4-1. The bottom two profiles are provided as another illustration where fluctuations and overall BP levels are different yet 24h SD values are similar but 24h ARV values vary (Figure 4-1.c & Figure 4-1.d).

Additional indices SD, CV, and ARV were calculated for both day and night periods. To investigate if BPV was influenced by nocturnal BP fall, a night to day BP fall (nocturnal dip) parameter was calculated as the ratio of mean BP between night-day periods. Additionally, the time rate of variability was calculated which is similar to ARV but is independent of the time intervals between measurements. All indices were calculated for both SBP and DBP.

Target organ damage

Each participant had a blood sample taken after a minimum of 8-h fast and in addition provided an early morning spot urine sample on the day of their appointment. Laboratory analyses included analysis for glycosylated haemoglobin (HbA1C) and ACR. A meta-analysis in 2010 demonstrated increased risk of mortality with urine ACR ≥ 1.1 mg/mmol and as a result microalbuminuria is defined using this cut-point [78]. A 12-lead ECG was obtained in the standard manner using a Siemens – Eclipse 850i machine. ECGs were reviewed and coded for LVH voltage criteria by a cardiologist. The presence of LVH was defined by Cornell Product ECG voltage criteria i.e. SV3 + RaVL (+6 in women) X QRS duration ≥ 2440 mm x ms [80].

Other Measurements

The questionaires provided data on participants smoking habits and were categorized as never, former and current smoker. Diabetes mellitus was defined as HbA1c level greater than or equal to 6.5% [166] or self-reported doctor diagnosis of diabetes. The classification of hypertension was based on SBP≥140 mmHg and/or DBP≥90 mmHg and/or on anti-hypertensive treatment. Weight was measured using a Tanita weighing scales and height measured with the use of a portable Seca length measure. Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Participants with a BMI ≥30, 25-30 and <25 kg/m^2 were classified as obese, overweight and normal/underweight respectively.

Statistical Analysis

All of the data are expressed as arithmetic mean (SD) or as percentages where appropriate. Normally distributed continuous variables were compared using student's t-test. Correlations among variables were assessed by Pearson correlation coefficient (r). The association between BPV and the presence of TOD was assessed using logistic regression with adjustment for age, sex, smoking status, BMI, diabetes and anti-hypertensive treatment. Additional models adjusted for mean 24h BP. Multicollinearity issues between mean BP and variability measures were assessed by checking for inflated standard errors, correlation coefficients and variance

inflation factors. Results are presented as odds ratios for a one-SD change with 95% confidence intervals (CI). The association between BPV and microalbuminuria was further explored by excluding those with diabetes from the analysis. Models were also separately run on those classified as hypertensive by ABPM and/or on anti-hypertensive treatment. In addition we examined just those on anti-hypertensive treatment alone. Receiver operating characteristic (ROC) curves were plotted to evaluate the predictive value of BPV. Additionally ACR was included in a linear regression model as a continuous outcome. To investigate the impact that the white-coat effect may have on variability, the analysis was repeated after the first hour of readings were excluded. Often, it is recommended to allow for multiple testing when examining independent measures and protect against data dredging where we might find a significant finding by chance alone when there is no real association. Typically an adjustment such as the Bonferonni correction is made but when measures are highly correlated as in our case, this method is inappropriate, as it will be highly conservative and may miss real differences [167]. Importantly in our study however, each variability index is in fact capturing different characteristics of variability and as we are interested in each measure separately or in isolation as opposed to seeing them as a collective, multiple testing is not required. If we were testing an overall hypothesis that BPV was related to our outcome then adjusting for multiple testing may have been considered. All of the statistical analyses were performed with the use of Stata version 12 (StataCorp., College Station, TX, USA). A value of $P<0.05$ was considered statistically significant. Figure 4-1 was plotted with the use of the R programming language.

4.4 Results

Of 3051 individuals invited to participate, 2047 (response rate: 67%) completed the questionnaire and physical examination component. ABPM was offered to all 2047 participants and it was completed by 1207 (response rate: 58%) of which 1134 had satisfactory amount of measurements recorded. The average numbers of day and night readings were 27 and 16 respectively. The main clinical characteristics of the

1134 participants are presented in Table 4-1 separately for those with and without LVH and microalbuminuria. Overall, participants had a mean (SD) age of 60.2 (5.5) and the majority were female (54%). Over half were classified as hypertensive (59%) and almost a tenth had diabetes (9.3%). The overall prevalence of LVH and microalbuminuria was 6.6% and 11.4% respectively.

Table 4-2 and Table 4-3 provides mean (SD) of different indices used to characterise BPV including the nocturnal dip, by 24h/day/night for both LVH and microalbuminuria respectively. With the exception of the nocturnal dip ratio there were no significant differences in BPV indexes between those with and without LVH (Table 4-2). However, night ARV, SD and wSD (both SBP & DBP) were significantly higher in those with microalbuminuria compared to those without. Additionally 24h ARV, day ARV, day SD (all SBP) and 24h ARV (DBP) were also higher in those with microalbuminuria (Table 4-3). Although the variability was higher in those with microalbuminuria, the nocturnal dip was significantly higher in those without microalbuminuria for both SBP and DBP. All four indices taken over the 24h period were significantly correlated with one another (correlation coefficient ranged 0.40-0.91 ($p<0.001$)).

Presented in Table 4-4 and Table 4-5 are results from regression analysis for both LVH and microalbuminuria respectively. Univariate logistic regression showed that none of the variability indices during any period were associated with LVH. In contrast, mean 24h ABPM was significantly associated with LVH in both univariate and adjusted models (OR=1.37(1.07-1.75)) (Table 4-4). The nocturnal dip was associated with a protective effect on LVH but the association did not persist when additionally adjusted for mean SBP (Table 4-4). In comparison ARV (24h, day and night), SD (day & night) and wSD were all univariately associated with microalbuminuria and the association persisted after adjustment for age, gender, smoking, BMI, diabetes and antihypertensive treatment (Table 4-5). However, when mean SBP was added to the model the association did not persist for all indices (Model 2, Table 4-5). Nocturnal dip was significantly associated with a protective effect on microalbuminuria in both the unadjusted and the fully adjusted models including mean BP (Table 4-5).

When analysis focused on only those classified as hypertensive by ABPM and/or taking antihypertensive treatment no differences to the findings above were observed. When those on antihypertensive treatment were analysed separately, models gave similar results to what we had seen already in relation to LVH and broadly similar in relation to microalbuminuria with the exception of 24h ARV. Despite adjustment for mean 24h BP in addition to age, gender, smoking, BMI and diabetes; 24h ARV was significantly associated with microalbuminuria (OR=1.43 (1.04-1.99). The prevalence of microalbuminuria in this sub-group was 14.7%.

The time rate of variability measure gave similar results to ARV (results not shown). No significant results were observed when mean BP was included in the models including when those on anti-hypertensive treatment were explored separately.

The stability of the models after the adjustment of mean BP was assessed to identify any multicollinearity issues. After adjustment standard errors were not inflated and were similar to the values before the inclusion of mean BP indicating stable models. Additionally; correlation coefficients were obtained between mean BP and all BPV indexes. The values ranged from -0.02-0.47. Finally, variance inflation factors were calculated in each model and all values were under 1.5 indicating that multicollinearity issues did not arise.

To evaluate the additional predictive value of BPV, ROC curves were plotted. The plots compared a model which included mean BP and BPV to a model which just included mean BP while adjusting for age, gender, smoking, BMI, diabetes and anti-hypertensive treatment. There was no significant difference between the areas under the curves regardless of which BPV measure or period of time was used (24h/day/night) (results not shown, plots in Appendix C).

All analyses were repeated for DBP. ARV (24h/day/night), night SD and wSD were found to be significantly related to microalbuminuria but the association did not persist once mean BP was added to the model. No other associations were observed with either LVH or microalbuminuria. Omitting the first hour of readings to explore the white-coat effect had no impact on our findings (data not shown). Results presented include the first hour of readings. The exclusion of participants

with diabetes in the analysis of microalbuminuria did not alter our findings. Similar findings to that of the logistic regression model were obtained when ACR was included in a linear regression model as a continuous outcome.

4.5 Discussion

In this large population based study we have explored the association between short-term BPV and subclinical TOD. Much debate remains in the literature as to the best measure of BPV and as a result four different indices were calculated and each assessed for their prognostic significance. Our work highlights the difficulties in accurately describing short-term variability with one summary measure. To illustrate this we provided cases of individuals who have distinctly different BP profiles and overall mean BP levels throughout the day yet have similar values in terms of SD but quite different values based on the other indices calculated. Despite the discrepancies between different measures our results indicated that there was no association between any index of variability we calculated and LVH even in unadjusted models. The associations between ARV (24h, day and night), SD (day & night), wSD, with microalbuminuria persisted following adjustment for a range of important potential confounders but did not persist after the models were additionally adjusted for mean BP. However, in a sub-analysis of those only taking anti-hypertensive treatment the association between 24h ARV with microalbuminuria persisted following adjustment for all confounders including mean BP.

The relationship between mean BP and TOD is well established and the literature does not question the importance of lowering mean levels of BP as is recommended in guidelines [18]. As a consequence, to identify BPV as an additional independent predictor we adjusted our analysis for mean 24h BP. The intrinsic relationship that can exist between mean BP and BPV can lead to invalid measures of variability and Hansen *et al.* [168] also claims the strong association between the two may cause problems in building stable regression models (although our analysis did not find such problems). Furthermore, SD is widely used as a measure of BPV but the appropriateness of such an index has been disputed because it only reflects the dispersion of measurements around a single value (mean) not accounting for the order in which BP measurements were obtained [36, 37]. This issue was highlighted in the different BP profiles presented in Figure 4-1. The CV has also been used and adjusts for the tendency of those with a higher average BP to also

have a higher SD as was found in this study. However these two measures (SD, CV) are still inherently linked to mean BP and this helps to advocate the use of ARV and wSD as more suitable measures of BPV.

Many studies have proposed ARV as an appropriate measure of variation which averages the absolute difference between successive readings and accounts for the order in which the BP readings are obtained [28, 33, 36], while wSD has also been utilised and is a weighted average of both day and night SDs which specifically attempts to remove the day-night difference in mean BP from the estimate of BPV [28, 30, 33, 40]. As a result 24h ARV and wSD are not strongly influenced by the difference between day-night mean BP levels. This is in contrast to SD and CV in which a large nocturnal dip will increase both values. The protective effect on the presence of microalbuminuria that was observed with nocturnal dip after adjustment for mean BP is not surprising. An absence in BP fall at night has been cited in many studies as a prognostic marker of cardiovascular events both in hypertensive [15, 169, 170] participants and the general population [171, 172].

Although the associations between ARV (24h, day and night), SD (day & night), wSD, with microalbuminuria did not persist after additionally adjusting for mean BP we plotted ROC curves to check if the combination of mean BP and BPV had more predictive power over that of just mean BP. Despite discrepancies between the different measures and the problems outlined above the plots revealed no additional benefit in including BPV over and beyond 24h BP irrespective of the BPV index used.

While there was no association overall between 24h ARV and microalbuminuria once adjusted for mean BP, among those on anti-hypertensive treatment the association persisted. We do not have data on specific treatment classes so we were unable to assess if this effect was mediated by a particular anti-hypertensive class. Some individual drug data was collected but sufficient numbers were not obtained to explore classes separately. Our finding does suggest however that anti-hypertensive treatment is having an effect on BPV. Evidence from meta-analyses suggest that although different anti-hypertensive-drug classes have similar effects

in terms of reducing BP levels, pronounced differences in their ability to reduce BPV [61, 62].

Considering the strong relationship that exists between mean BP and the presence of LVH, which was also evident in this study, it is interesting that no associations were identified with any measure of BPV even under unadjusted conditions alone. One contributing factor may be that LVH was determined based on the evaluation of a 12-lead ECG which is known to have low sensitivity in the detection of LVH [18]. Although it has its own technical issues, use of echocardiography would have been preferred as it is more sensitive than ECG in diagnosing LVH. Despite this however, guidelines promote the use of ECG as part of routine assessment of all hypertensive patients [18]. In addition to low sensitivity, we also had a low prevalence of LVH (6.6%) in the study. When these two factors are considered in combination, they may have had a large confounding effect on the relationship between BPV and TOD. When prevalence is low, there are few true positives in the sample, and false positives can be large compared to the number of true positives. It is clear that these factors may have masked the true underlying association in our data.

It is important to highlight that other studies have found an association between BPV and TOD even after adjustment for mean BP. Among hypertensive patients, Tatasciore *et al.* [30] found an association between awake SBP SD and LVMI in multivariate analysis even with the inclusion of awake SBP. Similarly, in multivariate analysis including daytime SBP Zakopoulos *et al.* [31] found an association between the daytime rate of SBP variation and left ventricular mass. Perhaps the use of echocardiography in the measurement of the outcome in these studies can, in part, explain discrepancies between these and our findings.

Another argument may be that visit-to-visit variability is a better prognostic predictor. Many large studies have demonstrated a strong relationship between visit-to-visit variability and all-cause mortality [38], cardiovascular events [25, 173] and TOD [163, 174]. However there is still no consensus on the matter and as highlighted in a recent review there is a need to design studies to prospectively determine the causes of visit-to-visit variability and determine if treatments that

reduce variability lead to improved clinical outcome [175]. Correctly determining the prognostic significance of both short-term and visit-to-visit BPV could have important implications for the prescribing of anti-hypertensive medication.

Limitations

The study is cross-sectional in nature and as mentioned above we are not able to determine whether increases in BPV promotes the development of TOD or if TOD represents a risk factor for increased BPV rather than being a consequence of it. Hansen *et al.* [168] argues that as TOD is a forerunner of cardiovascular complications, BPV will inevitably increase. This leads to the issue of reverse causality with increased BPV being a marker of underlying disease rather than being an independent predictor. The low sensitivity of ECG in the detection of LVH was a concern as previously highlighted. Although there are few guidelines focusing on BPV, O'Brien *et al.* suggests small intervals (15min) between measurements is best when measuring BPV [13]. Our monitors were set to intervals of length 30min and this is recognized as a limitation although there is still no strong consensus on the optimal interval size to measure BPV. Despite a response rate of only 58% the ABPM subsample was found to be representative of the full sample based on sex, age and education. In addition the prevalence of TOD in both the full sample and ABPM subsample were similar (LVH 6.0% vs 6.6%, ACR 10.6% vs 11.4%). The main strengths of the study lie in the robust community based design and large sample of ABPM data recorded.

4.6 Conclusion

The findings of this study have highlighted that accurately measuring short-term BPV over 24h is not straight forward and different indices can be heavily influenced by factors such as mean BP or nocturnal BP fall. Without consideration for these confounding factors inferences made on the true prognostic value of BPV could be misleading. Based on our findings variability indices such as ARV and wSD that are not influenced by the difference in mean BP between day-night periods best describe short-term BPV but were not associated with LVH or microalbuminuria after adjustment for mean BP. Future research should focus on long-term studies

where short-term BPV is measured at baseline and participants followed to view the development of TOD.

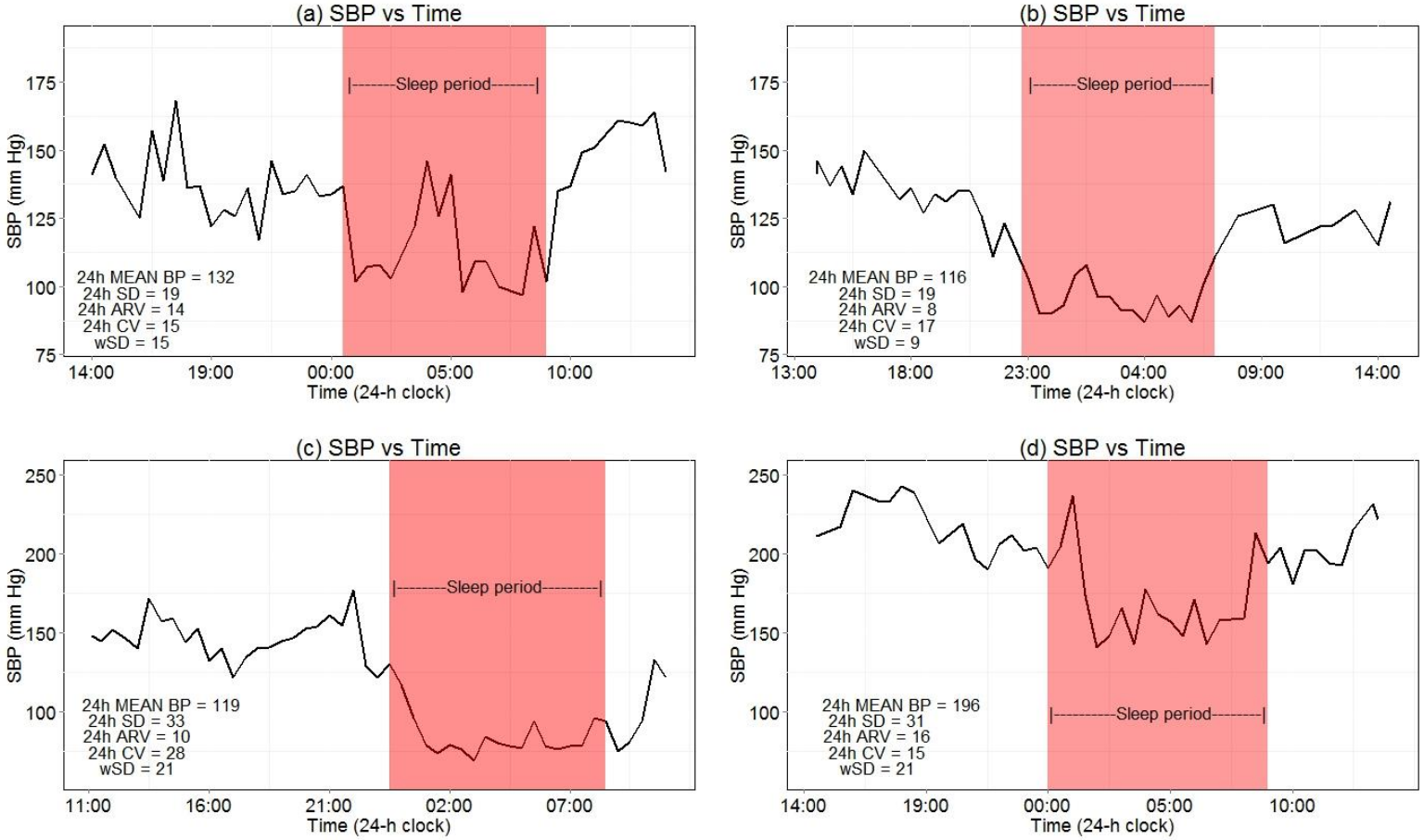


Figure 4-1 Four study participants and their 24h BP profiles. Panels (a) & (b) illustrate different patterns yet have similar 24h SD but different values based on the other indices. Both (c) & (d) demonstrate a similar issue (values rounded).

Table 4-1 Baseline Characteristics by LVH and Microalbuminuria

Characteristic	Total	LVH		Microalbuminuria	
		No	Yes	No	Yes
	(n=1134)	(n=1058)	(n=75)	(n=996)	(n=128)
Age, y	60.2 (5.5)	60.5 (5.5)	62.8 (4.7)**	60.1 (5.5)	61.1 (5.8)
Gender, Male n(%)	527 (46.5)	486 (45.9)	40 (53.3)	457 (45.8)	65 (50.8)
BMI, n (%)					
Underweight/normal	230 (20.3)	216 (20.4)	13 (17.3)	207 (20.8)	22 (17.3)**
Overweight	498 (44.0)	468 (44.3)	30 (40.0)	451 (45.3)	41 (32.3)
Obese	405 (35.7)	373 (35.3)	32 (42.7)	338 (33.9)	64 (50.4)
Smoking, n (%)					
Never	564 (51.9)	529 (52.2)	35 (48.6)	495 (52.1)	67 (52.8)
Former	355 (32.7)	325 (32.1)	30 (41.8)	307 (32.3)	42 (33.1)
Current	168 (15.4)	160 (15.8)	7 (9.7)	149 (15.7)	18 (14.2)
HbA1C	5.9 (0.8)	5.9 (0.7)	6.1 (1.2)	5.8 (0.7)	6.2 (1.2)**
Diabetes, n (%)	103 (9.3)	94 (9.1)	9 (12.2)	82 (8.4)	21 (16.4)**
Office SBP, mm Hg	134.2 (17.5)	133.8 (17.3)	140.4 (19.4)**	133.4 (17.1)	140.4 (20.4)**
Office DBP, mm Hg	82.7 (10.1)	82.5 (10.1)	84.7 (11.8)	82.4 (9.9)	84.8 (12.3)*
Hypertension, n (%)	665 (58.7)	607 (57.4)	58 (77.3)**	563 (56.6)	96 (75.0)**
Sleep time, hours	8.6 (1.3)	8.7 (1.3)	8.3 (1.3)	8.6 (1.3)	8.8 (1.3)
ABPM measurements					
24h SBP, mm Hg	124.1 (13.3)	123.8 (13.1)	128.9 (15.4)**	123.1 (12.7)	131.5 (15.4)**
24h DBP, mm Hg	71.8 (8.3)	71.8 (8.2)	72.0 (9.5)	71.4 (8.0)	74.6 (10.0)**
Awake SBP, mm Hg	131.4 (14.1)	131.1 (14.0)	135.1 (16.0)*	130.5 (13.6)	138.1 (16.1)**
Awake DBP, mm Hg	77.4 (9.0)	77.4 (9.0)	77.0 (9.9)	77.0 (8.7)	80.0 (11.0)**
Sleep SBP, mm Hg	112.3 (14.0)	111.9 (13.6)	118.6 (17.1)**	111.2 (13.3)	121.2 (16.2)**
Sleep DBP, mm Hg	62.8 (8.3)	62.7 (8.2)	63.6 (10.2)	62.3 (8.0)	66.4 (9.4)**

Data are mean (SD). SBP: Systolic Blood pressure, DBP: Diastolic blood pressure, BMI:Body mass index, LVH: Left ventricular hypertrophy, ABPM: Ambulatory blood pressure monitor. Microalbuminuria: albumin:creatinine ratio ≥ 1.1 mg/mmol. * p<0.05, **p-value<0.01, p-values comparing those with/without target organ damage

Table 4-2 Variability Indices and Nocturnal Dip by LVH

Variability	24-hour (mm Hg)		Day (mm Hg)		Night (mm Hg)	
Measure	No	Yes	No	Yes	No	Yes
ARV SBP	11.3 (2.5)	11.7 (2.4)	11.9 (3.1)	12.0 (3.1)	10.6 (3.3)	11.2 (2.9)
ARV DBP	7.8 (1.7)	7.5 (2.0)	8.1 (2.4)	7.8 (2.8)	7.3 (2.1)	7.1 (2.2)
SD SBP	15.8 (4.0)	16.0 (4.1)	12.8 (3.5)	13.4 (3.5)	11.2 (3.6)	11.5 (3.2)
SD DBP	11.5 (2.8)	11.1 (2.7)	8.9 (2.8)	8.7 (2.7)	8.0 (2.4)	7.7 (1.8)
CV SBP	12.8 (2.9)	12.4 (2.9)	9.8 (2.4)	10.0 (2.4)	10.0 (3.0)	9.7 (2.7)
CV DBP	16.1 (3.7)	15.6 (3.9)	11.7 (3.6)	11.5 (3.8)	12.9 (3.8)	12.4 (3.2)
wSD SBP	12.3 (2.9)	12.8 (2.7)	-	-	-	-
wSD DBP	8.7 (2.1)	8.4 (2.0)	-	-	-	-
Dip SBP	14.5 (6.7)	12.0 (9.0) *	-	-	-	-
Dip DBP	18.7 (7.5)	17.2 (9.8)	-	-	-	-

Data are mean (SD). ARV: Average real variability, SD: Standard deviation, wSD: Weighted standard deviation, CV: coefficient of variation, SBP: Systolic Blood pressure, DBP: Diastolic blood pressure, *p<0.05; represents significance between those with and without LVH during each period of the day

Table 4-3 Variability Indices and Nocturnal Dip by Microalbuminuria

Variability	24-hour (mm Hg)		Day (mm Hg)		Night (mm Hg)	
Measure	No	Yes	No	Yes	No	Yes
ARV SBP	11.2 (2.5)	12.4 (2.9) **	11.8 (3.1)	12.7 (3.5) **	10.5 (3.2)	11.9 (3.5) **
ARV DBP	7.7 (1.7)	8.2 (2.1) **	8.1 (2.3)	8.5 (3.0)	7.2 (2.1)	7.9 (2.3) **
SD SBP	15.8 (4.0)	16.2 (4.2)	12.8 (3.4)	13.8 (4.1) **	11.1 (3.5)	12.3 (3.7) **
SD DBP	11.4 (2.8)	11.5 (2.9)	8.9 (2.7)	9.2 (3.1)	7.9 (2.3)	8.7 (2.5) **
CV SBP	12.8 (2.9)	12.4 (2.9)	9.8 (2.3)	10.0 (2.8)	10.0 (3.0)	10.2 (2.9)
CV DBP	16.1 (3.7)	15.6 (3.9)	11.7 (3.6)	11.7 (4.2)	12.8 (3.8)	13.3 (4.0)
wSD SBP	12.2 (2.8)	13.3 (3.3) **	-	-	-	-
wSD DBP	8.6 (2.0)	9.0 (2.3) *	-	-	-	-
Dip SBP	14.6 (6.9)	12.1 (7.3) **	-	-	-	-
Dip DBP	18.8 (7.7)	16.7 (7.4) **	-	-	-	-

Data are mean (SD). ARV: Average real variability, SD: Standard deviation, wSD: Weighted standard deviation, CV: coefficient of variation, SBP: Systolic Blood pressure, DBP: Diastolic blood pressure, *p<0.05; ** p<0.01 represents significance between those with and without microalbuminuria during each period of the day

Table 4-4 Association between SBP BPV and LVH

Variability (per SD change in mm Hg)	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
ARV: 24h ARV	1.13 (0.90-1.41)	1.01 (0.79-1.30)	0.87 (0.66-1.14)
Day ARV	1.04 (0.83-1.31)	0.97 (0.76-1.25)	0.87 (0.67-1.13)
Night ARV	1.19 (0.96-1.49)	1.08 (0.84-1.39)	0.97 (0.74-1.26)
SD: 24h SD	1.03 (0.82-1.30)	1.01 (0.79-1.30)	0.91 (0.70-1.18)
Day SD	1.17 (0.94-1.46)	1.09 (0.86-1.39)	0.98 (0.76-1.28)
Night SD	1.09 (0.87-1.36)	1.02 (0.79-1.32)	0.90 (0.69-1.19)
CV: 24h CV	0.88 (0.68-1.12)	0.89 (0.69-1.14)	0.91 (0.71-1.17)
Day CV	1.08 (0.86-1.36)	1.01 (0.79-1.29)	1.02 (0.80-1.30)
Night CV	0.91 (0.71-1.16)	0.87 (0.67-1.13)	0.87 (0.67-1.14)
wSD	1.18 (0.94-1.46)	1.08 (0.85-1.40)	0.94 (0.71-1.24)
SBP Nocturnal Dip	0.71 (0.57-0.89)**	0.78 (0.62-0.98)*	0.81 (0.64-1.02)
24h SBP	1.42 (1.14-1.76)**	1.37 (1.07-1.75)*	-

ARV: Average real variability, SD: Standard deviation, wSD: Weighted standard deviation, CV: coefficient of variation, LVH: Left ventricular hypertrophy, SBP: Systolic blood pressure, BP: Blood pressure, BPV: Blood pressure variability, OR: Odds ratio, * p<0.05, **p-value<0.01

Each variability measure entered separately and adjusted as follows: Model 1: Age, gender, smoking, BMI, diabetes, anti-hypertensive treatment; Model 2: Age, gender, smoking, BMI, diabetes, anti-hypertensive treatment, 24h SBP

Table 4-5 Association between SBP BPV and Microalbuminuria

Variability (per SD change in mm Hg)	Unadjusted OR (95% CI)	Model 1 OR (95% CI)	Model 2 OR (95% CI)
ARV: 24h ARV	1.51 (1.27-1.79)**	1.44 (1.19-1.73)**	1.20 (0.97-1.47)
Day ARV	1.31 (1.11-1.56)**	1.31 (1.10-1.57)**	1.12 (0.93-1.37)
Night ARV	1.46 (1.24-1.74)**	1.35 (1.12-1.63)**	1.16 (0.95-1.42)
SD: 24h SD	1.10 (0.92-1.31)	1.09 (0.90-1.31)	0.89 (0.72-1.09)
Day SD	1.29 (1.09-1.53)**	1.29 (1.07-1.54)**	1.09 (0.89-1.33)
Night SD	1.35 (1.15-1.60)**	1.27 (1.06-1.52)**	1.06 (0.87-1.30)
CV: 24h CV	0.84 (0.70-1.02)	0.87 (0.72-1.06)	0.89 (0.73-1.08)
Day CV	1.09 (0.92-1.30)	1.11 (0.92-1.34)	1.12 (0.92-1.35)
Night CV	1.07 (0.89-1.27)	1.02 (0.84-1.23)	1.01 (0.84-1.23)
wSD	1.39 (1.18-1.64)**	1.35 (1.12-1.62)**	1.10 (0.89-1.35)
SBP Nocturnal Dip	0.70 (0.59-0.84)**	0.74 (0.62-0.90)**	0.78 (0.65-0.94)*
24h SBP	1.78 (1.49-2.13)**	1.67 (1.38-2.02)**	-

ARV: Average real variability, SD: Standard deviation, wSD: Weighted standard deviation, CV: coefficient of variation, SBP: Systolic blood pressure, BP: Blood pressure, BPV: Blood pressure variability, Microalbuminuria: albumin:creatinine ratio ≥ 1.1 mg/mmol, OR: Odds ratio *p<0.05; ** p<0.01

Each variability measure entered separately and adjusted as follows: Model 1: Age, gender, smoking, BMI, diabetes, anti-hypertensive treatment; Model 2: Age, gender, smoking, BMI, diabetes, anti-hypertensive treatment, 24h SBP

5. EXPLORING DIURNAL VARIATION USING PIECEWISE LINEAR SPLINES: AN EXAMPLE USING BLOOD PRESSURE (PAPER 3)

J. M. MADDEN[a]

X. Li[b]

P. M. KEARNEY[a]

K. TILLING[c]

A. P. FITZGERALD[a,d]

[a] Department of Epidemiology & Public Health, University College Cork, Ireland

[b] Graduate Entry Medical School, University of Limerick, Ireland

[c] School of Social and Community Medicine, University of Bristol, Bristol, UK

[d] Department of Statistics, University College Cork, Ireland

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5.1 Abstract

There are many examples of physiological processes that follow a circadian cycle and researchers are interested in alternative methods to illustrate and quantify this diurnal variation. Circadian BP deserves additional attention given uncertainty relating to the prognostic significance of BPV in relation to CVD. However, the majority of studies exploring variability in ABPM collapse the data into single readings ignoring the temporal nature of the data. Advanced statistical techniques are required to explore complete variation over 24h.

We use piecewise linear splines in a mixed-effects model with a constraint to ensure periodicity as a novel application for modelling daily BP. Data from the Mitchelstown Study, a population based study of Irish adults aged 47-73 years (n=2,047) was utilized. A subsample (1,207) underwent 24h ABPM. We compared patterns between those with and without evidence of subclinical TOD (microalbuminuria).

We were able to quantify the steepest rise and fall in SBP, which occurred just after waking (2.23 mmHg/30min) and immediately after falling asleep (-1.93 mmHg/30min) respectively. The variation about an individual's trajectory over 24h was 12.3mmHg (SD). On average those with microalbuminuria were found to have significantly higher SBP (7.6 mm Hg, 95%CI: 5.0-10.1) after adjustment for age, sex and BMI. Including an interaction term between each linear spline and microalbuminuria did not improve model fit.

We have introduced a practical method for the analysis of ABPM where we can determine the rate of increase or decrease for different periods of the day. This may be particularly useful in examining chronotherapy effects of antihypertensive medication. It offers new measures of short-term BPV as we can quantify the variation about an individual's trajectory but also allows examination of the variation in slopes between individuals (random-effects).

5.2 Introduction

There are many examples of physiological processes that follow a circadian cycle such as cortisol, intraocular pressure and body temperature where abnormalities in these patterns have been shown to be related to depression [176], glaucoma [177] and delayed sleep-phase disorder [178]. The ability to analyse and capture features of these cycles remains a challenge but is necessary to get a deeper understanding of the mechanisms behind them. For example, the cardiovascular system shows clear circadian rhythmicity where researchers are interested in alternative methods to illustrate and quantify this diurnal variation [179]. Circadian BP represents a situation where diurnal variation deserves additional attention given the uncertainty relating to the prognostic significance of BPV [25, 32-34]. The benefits of using ABPM in addition to clinic measurements in the diagnosis and management of hypertension are well established [13, 18]. As well as mean day, night and dip values, ABPM provides measures of short-term BPV and individual profile patterns. The majority of studies examining short-term BPV have focused on summary measures such as the SD of ABPM readings over the day. These summary measures are easily obtained without the need for advanced statistical techniques [25, 32-34, 37, 39] but ignore the temporal nature of the data. To date relatively little work has modelled 24h ABPM profiles to exploit the full potential of ABPM data to capture short-term BPV [48]. Moreover, there are a lack of studies exploring circadian patterns and specifically, studies examining differences in patterns among different groups of individuals.

Cosinor analysis which incorporates a sinusoidal function has been the most common approach to modelling 24h BP [114-117], while a similar method, Fourier analysis [123, 124], has also been implemented. These approaches have focused on between-group effects (fixed-effects) where typically inferences are based on estimated differences in model parameters between particular groups of patients, such as comparing the estimated amplitude or MESOR between groups of individuals on different antihypertensive agents obtained in cosinor analysis [116]. The focus of fixed-effects is on population trajectories. However one of the main

advantages of ABPM is that we obtain individual BP profiles and modelling subject-specific trajectories involves incorporating subject-specific effects (random-effects). To model mean profiles Selwyn *et al.* [106] used a hierarchical model incorporating a 4^{th} degree polynomial. Lambert *et al.* extended on this by incorporating restricted cubic splines to model the mean BP profiles [48]. More recently, Edwards *et al.* [49] utilised orthonormal polynomials in a linear mixed model in a group of hypertensive subjects. Both polynomials and cubic splines, by their nature, have the ability to produce well-fitting curves to the data but have the disadvantage that the corresponding coefficients are challenging to interpret directly.

As an alternative we propose using piecewise linear splines in a mixed-effects model as a different approach for modelling ABPM data. Although linear splines have been used to model BP change over years and gestational age [109], using them to explore daily patterns of BP represents a novel method for analysing ABPM. This approach has the advantage that coefficients represent something meaningful, in this case the slope of BP at different periods of the day. To date it is unclear if different underlying circadian BP patterns exist across various groups of the population. This method allows slopes at a group level (and individual level) to be easily compared. Furthermore, using random-effects we want to predict and plot curves at an individual level and to explore BPV within each period of the day. Thus the aim of this study is twofold 1) to introduce and describe a mixed-effects piecewise linear model in relation to BP; 2) to apply our method to a middle-aged population sample and explore their circadian BP patterns. We also introduce and present a constraint for our model that ensures periodicity, so that on the average BP is the same 24h later. We are particularly interested in identifying distinct differences in the shape of mean curves at a group level. For purposes of illustration of the models at a group level, we will compare those with and without evidence of subclinical TOD, specifically microalbuminuria.

5.3 Methods

Study Population

The analysis utilises data from the Mitchelstown Study, a population based study of middle-aged men and women, recruited in Ireland 2010-2011. A description of the study design is available from previous publications [74, 180]. The study recruited patients attending a single large primary care centre, the LHC, in Mitchelstown. Participants completed a detailed health and lifestyle questionnaire, and attended for a physical examination including height, weight, BP, fasting blood samples and urine samples. ABPM was offered to all participants. All participants provided written informed consent and ethical approval was obtained from the Clinical Research Ethics Committee Cork.

BP Measurements

Study BP was measured three times after 5 minutes of rest in a seated position by experienced research nurses using an OMRON M7 BP monitor (OMRON Healthcare, The Netherlands). The average of the second and third measurements was used for analyses. ABPM was measured using dabl ABPM system (dabl ltd., Ireland) with the Meditech ABOM-05 Monitor (Meditech LTD., Hungary). The monitors were programmed to obtain readings every 30mins and remained in place for 24h. Participants kept diaries of wake and sleep periods, which were used to calculate sleep and waking times. Only participants with a minimum of 20 measurements during the day and a minimum of 7 measurements during the night period were included in the analysis (see Appendix E) [13, 181]. Additionally, any participants with data lacking for more than two consecutive hourly intervals were excluded [13, 73, 181].

Target Organ Damage

Each participant provided an early-morning spot urine sample on the day of their appointment. Laboratory analyses included analysis for ACR. Microalbuminuria is defined as ACR$\geqslant$1.1 mg $mmol^{-1}$ [78].

Statistical Analysis

Linear Mixed Model - Linear Splines

The linear mixed model [87, 88] is a well-recognised tool in the analysis of longitudinal data and its ability to obtain both population (fixed-effects) and subject-specific (random-effects) trajectories makes it particularly appealing for the analysis of ABPM data. However, its use to-date has focused on BP following a smooth curvature trajectory which results in spline and polynomial coefficients that are of no direct clinical relevance. Piecewise linear functions or linear splines offer an alternative. These involve segregating the data into different segments across time initially assuming the segments are the same for everyone. Within each partition, a linear spline is fitted and where these are connected are known as knot points. The corresponding coefficient of each spline represents the rate of increase or decrease of BP during each time period.

Knot selection

The position of the knot points were determined based on a number of factors. Firstly, to get a general sense of the shape of the data and determine regions of interest (how many knots were required), we plotted an average curve of BP including all participants to determine common knot points. In addition we incorporated prior known characteristics of BP. The period of awakening corresponds with an abrupt and steep acceleration of BP and for many the maximum value obtained during this morning period corresponds to their maximum BP reached throughout the day [182]. We also know that BP gradually falls throughout the day and usually dips to its lowest value during the sleeping period [18]. Since waking and sleeping times are clearly important in terms of changes in BP we decided that the use of these times as two additional subject-specific knot points was appropriate. We were able to create these subject-specific knots using the wake and sleep times reported by the participant. We had 49 readings for each individual, where the first reading was t_1 (12pm) and the final reading was t_{49} (12pm the following day). Individual waking and sleeping times

were included within this range (t_1- t_{49}). For each individual we created *m* linear splines, where the k^{th} spline:

$$s_k(t) = 0 \text{ if } t \le t_{ki}$$
$$s_k(t) = t_i - t_{ki} \text{ if } t_k < t \le t_{ki+1} \quad \text{for } k=1,\ldots,m \qquad \textbf{(22)}$$
$$s_k(t) = t_{ki+1} - t_{ki} \text{ if } t > t_{ki+1}$$

Incorporating these linear splines into a linear mixed effects model for BP we get:

$$BP_{ij} = (\beta_0 + b_{0i}) + \sum_{k=1}^{m} (\beta_k + b_{ki}) s_k + \varepsilon_{ij}$$
$$b_i \sim MVN(0, \textstyle\sum_b), \qquad \varepsilon_{ij} \sim N(0, \textstyle\sum_\varepsilon) \qquad \textbf{(23)}$$

where BP_{ij} is the BP value for the j^{th} measurement on the i^{th} person, at time t_{ij}, the β*'s* are the fixed effects coefficients associated with the average intercept at β_0 (BP at 12pm) and the average slopes (β_k*'s*) between knot points, *b*'s are the random-effects associated with the average intercept (b_{0i}) and average slopes between knot points, and ε_{ij} representing the individual-level residuals from the model. The model is extended by incorporating the subject-specific knots in the s_k term. It is assumed the random-effects (b_i) have zero mean and an unstructured variance-covariance matrix Σ_b. The individual level residuals have mean zero and variance-covariance matrix Σ_ε.

To expand equation (23) to include the restriction that on the average BP is the same 24h later we define an equation which states average, subject-specific change in BP over 24h is zero:

$$\sum_{k=1}^{m} w_{ki} \beta_{ki} = 0 \qquad \textbf{(24)}$$

where w_{ki} is the width of the k^{th} interval (and where those involving wake and sleep times are subject-specific width intervals). Rewriting this in terms of β_1 gives:

$$\beta_{1i} = -\frac{\sum_{k=2}^{m} w_{ki}\beta_{ki}}{w_{1i}} \quad \textbf{(25)}$$

which implies:

$$\sum_{k=1}^{m} \beta_{ki} s_k = \beta_{1i} s_1 + \sum_{k=2}^{m} \beta_{ki} s_{ki} = \sum_{k=2}^{m} \beta_{ki} s_{ki}^* \quad \textbf{(26)}$$

where $s_{ki}^* = s_{ki} - \frac{w_{ki}}{w_{1i}} s_{1i}$, which allows us rewrite (23) as

$$BP_{ij} = (\beta_0 + b_{0i}) + \sum_{k=2}^{m} (\beta_k + b_{ki}) s_{ki}^* + \varepsilon_{ij} \quad \textbf{(27)}$$

To explore a group effect the model can easily incorporate a variable of interest, in this case TOD (microalbuminuria), as a dichotomous covariate. We further extended the model allowing the shape of the trajectory to depend on TOD by including interactions between TOD and each linear spline slope. Comparing this model with one without any interactions allowed us to test if the overall trajectory of BP was different between the two groups across the day. Additionally we were able to test if slopes between the groups differed at specific locations throughout the day. We adjusted for confounders by adding them into the model as fixed effects. In additional models we tested the effect of allowing the residual variance to differ between those with and without microalbuminuria. Similarly we tested the impact of allowing the interaction terms of microalbuminuria with each linear spline to be random to determine if there was heterogeneity of variance between the groups at any period of the day. Although we used an unstructured covariance structure for our models, we assumed these interaction terms to be independent of the other random-effects parameters. These interactions represented the difference in variation between the microalbuminuria groups within each segment. For all models explored we allowed all the linear spline terms to be random.

As individual ABPM readings taken close in time are likely to be correlated, a model with an independent residual correlation structure may not be appropriate. We

compared this to a model with a first-order autoregressive AR(1) structure and examined a plot of the ACF to detect violations of the assumption of independence. Allowing for temporal correlation can potentially result in a large improvement in the precision of parameter estimates [93].

Models were compared formally by a LRT [88, 183]. The appropriate variance and residual function structures were also identified using a LRT in addition with an ACF plot. R-squared (R^2) statistic is often presented as a summary measure for linear models but due to theoretical or practical problems is rarely presented for mixed-models. Nakagawa and Schielzeth discuss these issues and present a general but simple method for calculating an appropriate R^2 for random intercept mixed-models [184]. Johnson extended this to include random slope models which we implement in our analysis [185].

The parameters for our final models were estimated using REML as this method produces unbiased estimates unlike ML estimation [93]. Subject-specific trajectories were estimated using Empirical Best Linear Unbiased Predictors (EBLUPs) of the random-effects [88]. Residual diagnostic plots were examined to verify model distribution assumptions. In addition a visual predictive check (VPC) was performed in which the estimated mean and the 90% prediction interval from our model were plotted together with the observed BP values and the 90% interquantile range of the observations. The purpose of the VPC is to assess graphically if predictions from the fitted model reproduce the central trend and variability of BP in the observed data, when plotted against time. It is an internal validation method that assesses the goodness-of-fit. [186] All analysis was completed for both SBP and DBP. All analysis were implemented in R [187] and parameter estimation for the mixed-effect model was carried out by means of the lme command in nlme package [188].

Validation

Although difficult to interpret the coefficients, polynomial regression can still be a useful tool in the analysis of medical data to plot the trajectory of non-linear or curvilinear relationships [101]. As a method of validation for our approach we

additionally implemented a linear mixed model with orthogonal polynomials across time in both the fixed and random-effects, similar to that of Edwards *et al.* [49]. We wanted to determine if linear splines were capable of capturing the circadian rhythm of BP. This was investigated by comparing the trajectories obtained from both methods to determine if they followed similar patterns. A similar process to that of the piecewise model was followed when fitting the polynomial model. As we were only concerned to know if the general shape could be captured by our piecewise approach we were not worried about over-fitting the polynomial model. We implemented a model up to a 6^{th} order polynomial allowing all the terms to be random.

5.4 Results

Of 3051 individuals invited to participate, 2047 (response rate: 67%) completed the questionnaire and physical examination component. ABPM was offered to all 2047 participants and it was completed by 1207 (response rate: 58%) people, of whom 1008 had a minimum of 20 day and 7 night measurements respectively. Of these 886 had no data missing for more than two consecutive hourly intervals, and the main clinical characteristics of these participants are presented in Table 5-1. Overall, participants had a mean age of 59.9 (5.5) and the majority were female (55%). Sixty percent were classified as hypertensive. Also presented in Table 5-1 are the characteristics of the full sample which shows the ABPM sub-sample follows a similar distribution in terms of age, sex, BMI and the presence of microalbuminuria. However, the proportion of those with hypertension was higher amongst those in ABPM sub group than in overall study population (60% vs 47%).

The plot of average SBP for the 886 subjects is presented in Figure 5-1. Based on this plot we identified two common knot points where the trajectory of SBP changed notably at 6pm and 4am. In addition to these two points we were able to include two subject-specific knot points for each participant based on the time an individual woke and went to sleep (Figure 5-1). This meant each participant was assigned 4 knot points which in turn resulted in their SBP pattern being broken into 5 linear segments.

Figure 5-2 represents subject-specific trajectories as a function of time only from a linear mixed-effects model using both orthogonal polynomials (6^{th} order) (red line) and piecewise linear splines (blue lines). The plots suggest that the individual curves can be adequately captured by using piecewise linear splines.

We initially included the 5 linear splines as fixed-effects. A significant improvement in fit was observed when additionally including each term individually as a random-effect, based on a LRT (all $p<0.001$). As a consequence we included all the linear spline terms as random-effects. To allow for temporal correlation we incorporated an AR1 structure which resulted in a significant improvement in fit ($p<0.001$) ($rho=0.27$). Examining the ACF plot indicated that the inclusion of an AR1 residual

structure adequately accounted for the auto-correlation in the data. This unadjusted model, which only incorporates linear splines as a function of time was our base model, Table 5-2 (Model 1). Presented are the parameter estimates (fixed-effects, random-effects correlation matrix, the autocorrelation decay ρ along with fit criteria values). With the exception of the slope for the period from 12.00 to 18.00 (0.02(0.04) mmHg/30min), all slopes differed significantly from zero (all $p<0.001$). This suggests that on average this is the period during the day where average SBP remains constant. The largest rise and fall in SBP occurred between wake and 12.00 (2.23 mmHg/30min) and, between sleep and 04.00 (-1.93 mmHg/30min) respectively. These segments correspond to the period when an individual wakes up and the period immediately after they fall asleep. The variation in slopes was lowest from 12.00 to 18.00 where the variance was 0.51. The largest variation in slopes was observed between waking and 12.00 where the variance was 2.05 which is substantially larger in comparison to the rest of the day. The model R^2 value which illustrates the proportion of variance explained by both the fixed and random factors was quite high (0.67).

In subsequent models we adjusted for age, sex and BMI. We also included our variable of interest, microalbuminuria, to determine if it could help explain the larger variation in the period, wake to 12.00 (Model 2, Table 5-2). The residual variance, which represents the variation about an individual's trajectory, was 12.3mmHg. We additionally allowed the residual variance to vary between microalbuminuria groups (ratio of SD of those with to without microalbuminuria was 1.09). On average, over the day, those with microalbuminuria were found to have significantly higher SBP (7.6 mm Hg, 95% CI: 5.0-10.1, $p<0.001$). However, adjusting for age, sex, BMI and microalbuminuria had almost no effect on the model parameter estimates (except the intercept). To determine if slopes were different between groups at different times of the day we included an interaction between each linear spline and microalbuminuria (Model 3, Table 5-2). Although two of the interaction terms were marginally significant, a LRT suggested that including interaction terms did not improve the overall fit to the data ($p=0.12$). Based on additional models (results not shown) we found no evidence that the

variance of the random-effects varied with microalbuminuria. With the inclusion of age, sex, BMI and microalbuminuria we concluded that Model 2 offered the best fit to the data (Model 2 vs Model 1, $p<0.01$). Residual diagnostic plots of the models showed no violation of assumptions (results not shown). The VPC plot showed the model was adequately predicting central trend and variability of SBP in the observed data, when plotted against time (see Appendix C).

Figure 5-3 represents the average piecewise linear curve along with a 95% confidence interval for those with and without the presence of microalbuminuria using Model 2. The numbers on the plot correspond to the time periods presented in Table 5-2. It is clear that those with microalbuminuria have a higher average SBP throughout the day. For the purposes of this plot we have set the sleep and wake time knots at 23.00 and 08.00 respectively. A similar plot using model 3 can be found in Appendix C. Similar findings were found for all analysis when repeated using DBP (results not shown).

5.5 Discussion

In this large population based study we present an alternative method of modelling 24h BP that can easily be applied to any physiological process that follows a circadian cycle. Our novel but simple approach utilising a piecewise linear random-effects model, with an adjustment to ensure that the average level is the same at the beginning and end of each 24h period, offers a practical alternative to other methodological modelling techniques for researchers exploring circadian patterns. The flexible model has the ability to capture overall average, group and individual trajectories (in addition to being capable of examining slopes at different periods of the day).

Despite the large amounts of literature relating to BP, those specifically modelling 24h ABPM remain sparse. Our method offers new measures of short-term BPV as we can quantify the variation about an individual's trajectory but it also allows examination of the variation in slopes between individuals (random-effects). Our results indicated that after adjustment for age, sex and BMI the sharpest fall in BP occurred just after an individual went to sleep and the steepest rise occurred just after waking. Although there was a significant difference on average between those with and without microalbuminuria we found there was no overall improvement in fit after including interaction effects with the spline terms. However interestingly we found that the variation after awaking, representing what is known as the morning surge was considerably larger than the other periods of the day.

It has been acknowledged there is not a generally accepted "standard" method of analysing 24h ABPM [49]. Cosinor analysis has been highlighted as the most common approach [114-117] while Fourier analysis [123], has also been implemented which are both based on the idea that any time series can be described by a series of cosine (and sine) waves of various frequencies [189]. It has been suggested that these methods impose too many restrictions on the shape of the profile and have been shown to fit real profiles poorly [119]. Wang *et al.* [120] suggest problems with fitting a sinusoidal function to a circadian pattern include (i) that the pattern over time may not be symmetric; that is, the peak and nadir may

not be separated by 12 hours and/or the amplitude and width of the peak may differ from those of the nadir, (ii) sometimes there are local minimum and maximum points. Additionally Wang *et al.* [121] suggests that the sinusoidal function is too restrictive and "rhythms with a shape closely approximating a cosine curve are uncommon" [122]. Alternative methods have examined restricted cubic splines and more recently orthonormal polynomials [48, 49]. As we highlighted previously these approaches may model the data quite well and their curvature nature may look graphically appealing but it is difficult to understand and compare their resulting coefficients.

Piecewise regression which allows separate slopes to be fitted to observations before and after a certain period or event (knot points) has been cited as a useful tool that should be implemented more often in the context of epidemiological studies [190] but has not, to the best of our knowledge been used with ABPM data or other physiological processes that are circadian. The benefit of this method as opposed to polynomials is that the regression coefficients represent something meaningful directly without the need for further manipulation of the results - in our context, the rate of increase or decrease of BP for a certain time of day. The position of the knot points can easily be altered depending on the requirements of a specific study. For example if we were examining the effect of dialysis on BP in haemodialysis patients we could fix knot points at the time their dialysis began and at period(s) a number of hours later.

The morning is recognised as the most important period in relation to CVDs [23] and cardiovascular events occur more frequently in this period [23, 45, 46]. In our study we found that the steepest rise (slope) occurred during the period just after waking which is in line with the literature, thus verifying that our method is capturing known features of the data. It is suggested that the abrupt steep rise in BP may explain the link between cardiovascular events and the morning period [182]. In a review of morning surge with cardiovascular risk, 3 different definitions of morning surge were identified, all of which simply use BP differences where they subtracted some average night value minus an average of morning BP readings [45]. We argue that our method offers a more accurate estimate, as by definition of

a slope we can specifically quantify the rate of "surge". In fact, Parati *et al.* argue that a method that would be capable of capturing a slope similar to one purposed by our method would provide an accurate method of estimating the morning surge [142]. Considering that morning surge has been cited as a predictor of stroke and advanced TOD independent of ambulatory BP and nocturnal BP [23, 46], accurately quantifying it remains an important issue, particularly when we are assessing the benefits of antihypertensive medication in their ability to reduce this steep rise. This may not only have health implications but also financial benefits. A similar argument could be put forward for the dipping effect at night which is usually quantified just as a ratio of the mean BP between night-day periods. The slope at night obtained by our approach may represent a more accurate measure but further work would be needed to explore this.

Kario argues that the perfect 24h BP control is not limited to reducing mean BP but includes restoring disrupted circadian BP rhythms and reducing exaggerated BPV [23]. As highlighted previously most studies examining BPV have concentrated on summary measures of variability such as SD over 24h or separated into day and night values [25, 32-34, 37, 39, 180]. With the use of our mixed-effects model we were are able to obtain superior measures of BPV that take into account the temporal nature of the data. We were able to quantify the variation about an individual's trajectory but also the variation in slopes between individuals. Our work highlighted that the largest variation between individuals occurred during the morning surge period. Adjusting for age, sex and BMI did not help explain this variation. Similarly the presence of microalbuminuria had little impact on the variation. Ideally we would have preferred to explore if the variation could in part predict cardiovascular events but as data is currently only available for wave one, we have been restricted to explore a surrogate marker in microalbuminuria and have acknowledged this as a limitation. Further work is warranted to include CVD endpoints but perhaps an underlining physiological phenomenon of BP is that it is most variable in the morning possibly because this period of the day has an abrupt rise. Although some of the knots are subject-specific, others are at common fixed locations which may not represent the best position for a specific individual and this

assumption is recognised as a limitation. In addition to the average plot, we have attempted to incorporate our knowledge of the underlying pattern of BP to help inform our knot positions as suggested by Howe *et al.* [91].

As debate remains in relation to how to correctly quantify short-term BPV [180, 191] our approach offers new alternatives that utilise the full power of ABPM that is often lost when using summary measures such as SD because it only reflects the dispersion of measurements around a single value (mean) not accounting for the order in which BP measurements were obtained [37, 40]. The ability to determine variation over specific periods of the day offers a novel measure of variability in the analysis of BP which may have benefits when attempting to determine the optimal timing of antihypertensive medication administration in future studies. Finally, the approach and discussion outlined is not restricted to the use of BP and can easily be implemented on any physiological process that demonstrates a circadian cycle. BP is not the only biological process where disruptions to circadian rhythms are clinical relevant. Wang *et al.* found that those with Cushing syndrome exhibited no circadian rhythm of cortisol, while those with depression showed a dampened rhythm compared to the normal group [121]. Liu *et al.* found that larger short-term fluctuations in intraocular pressure are more common in glaucoma [192]. Similar to the morning BP surge, it was found that intraocular pressure was higher in the morning and more prevalent in those with glaucoma. This suggests that our approach may be beneficial to the exploration of other biological rhythms that have similar features to that of BP.

5.6 Conclusion

This study has introduced a novel but practical method for the analysis of ABPM data. Based on our work circadian BP patterns can be modelled using a mixed-effects model with piecewise linear splines. The main advantage of our method compared to other approaches is that the resulting regression coefficients have direct interpretation. We can determine the rate of increase or decrease at different periods of the day. In addition we can determine alternative measures of

variability compared to classical BPV indices. Future research in this area should focus on the association between the measures obtained from this method to stronger clinical outcomes.

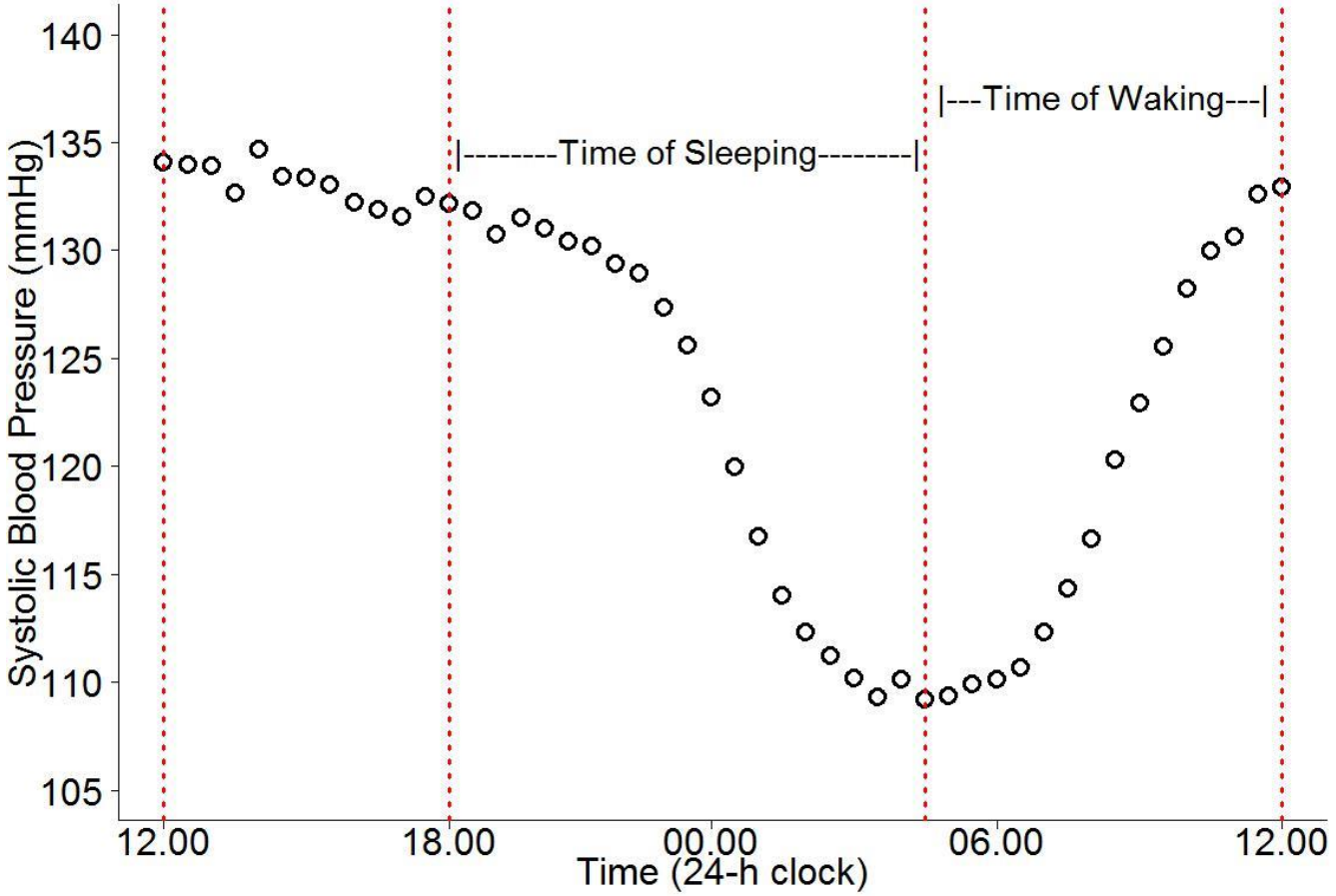


Figure 5-1 Plot of average SBP over 24h which helped identify 6pm and 4am as common knot points for all participants where there was a notable change in trajectory of BP. Also highlighted are the periods where individuals woke and went to sleep. In addition to the two common points, we were able to obtain additional (two) subject-specific knot points at wake and sleep times.

Table 5-1 Baseline Characteristics

Characteristic	Total (n=2047)	ABPM (sub-sample) Total (n=886)
Age, y	59.8 (5.5)	59.9 (5.5)
Gender, Male n(%)	1008 (49.2)	401 (45.3)
BMI, n (%)		
Underweight/normal (<25kg/m^2)	447 (21.9)	195 (22.0)
Overweight (25-30 kg/m^2)	925 (45.3)	380 (42.9)
Obese (≥30 kg/m^2)	668 (32.8)	310 (35.0)
Office SBP, mm Hg	129.6 (16.9)	134.7 (17.7)
Office DBP, mm Hg	80.1 (9.8)	83.1 (10.2)
Hypertension, n (%)	951 (46.5)	528 (59.7)
Microalbuminuria	215 (10.6)	62 (7.0)

Data are mean (SD). BMI:Body mass index, ABPM: Ambulatory blood pressure monitor. Hypertension: ≥140/90 mmHg and/or on antihypertensive treatment.

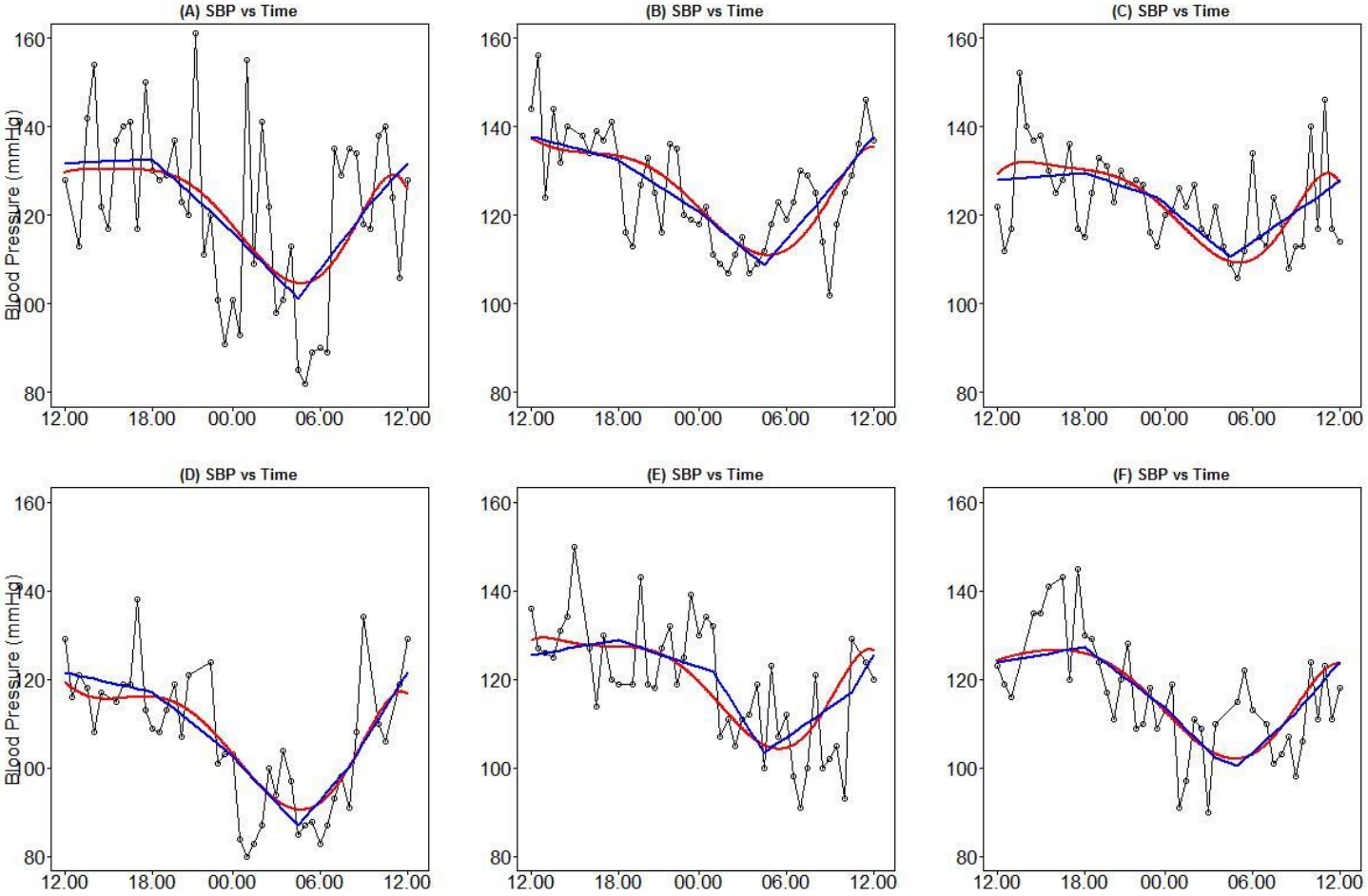


Figure 5-2 Individual SBP readings along with predicted subject-specific trajectories from a linear mixed effects model as a function of time only using two different approaches; polynomials (red line) and piecewise linear splines (blue lines).

Table 5-2 Various models with parameter estimates for slopes at each segment along with corresponding correlations and variances

Parameter	**Model 1**	**Model 2**	**Model 3**
Fixed effects (SBP mmHg/30mins)	**Estimate (SE)**	**Estimate (SE)**	**Estimate (SE)**
SBP at 12.00	134 (0.54)	119.2 (4.6)	119.3 (4.6)
Microalbuminuria	-	7.57 (1.30)*	5.79 (1.67)*
Slope for spline time period:			
1. 12.00 – 18.00	0.02 (0.04)	0.03 (0.04)	0.03 (0.04)
2. 18.00 - Sleep	-1.00 (0.04)*	-1.00 (0.04)*	-1.01 (0.04)*
3. Sleep – 04.00	-1.93 (0.05)*	-1.95 (0.06)*	-1.99 (0.06)*
4. 04.00 - Wake	1.69 (0.05)*	1.70 (0.05)*	1.71 (0.05)*
5. Wake – 12.00	2.23 (0.07)*	2.21 (0.07)*	2.26 (0.07)*
Microalbuminuria x Spline interaction			
1. 12.00 – 18.00	-	-	-0.06 (0.14)
2. 18.00 - Sleep	-	-	0.05 (0.13)
3. Sleep – 04.00	-	-	0.37 (0.18)**
4. 04.00 - Wake	-	-	-0.06 (0.16)
5. Wake – 12.00	-	-	-0.48 (0.22)**

Continued on following page

Parameter	Model 1	Model 2	Model 3
Fixed effects (SBP mmHg/30mins)	**Estimate (SE)**	**Estimate (SE)**	**Estimate (SE)**
Random effects			
Σ	223.6	199.5	200.5
	-0.23 0.51	-0.23 0.50	-0.24 0.51
	-0.23 -0.10 0.55	-0.25 -0.10 0.54	-0.25 -0.11 0.55
	-0.23 -0.45 0.03 1.39	-0.28 -0.46 0.02 1.41	-0.28 -0.44 0.02 1.40
	0.46 - 0.28 -0.74 -0.05 0.66	0.47 -0.31 -0.74 -0.05 0.65	0.49 -0.33 -0.73 -0.04 0.65
	0.34 -0.06 -0.21 -0.78 0.19 2.05	0.42 -0.03 -0.22 -0.80 0.23 2.00	0.42 -0.04 -0.20 -0.81 0.24 1.97
σ	12.3	12.3	12.2
ρ	0.27	0.27	0.27
R^2	0.67	0.68	0.68
Log-likelihood	-149608	-149505	-149502
		Model 2 vs Model 1 (p<0.001)	Model 3 vs Model 2 (p=0.12)

Microalbuminuria: albumin:creatinine ratio ≥ 1.1 mg/mmol

*P<0.001,**P<0.05

Model 1: Fixed effects (5 linear splines), random effects (5 linear splines).

Model 2: Fixed effects (5 linear splines, microalbuminuria, age, sex, BMI), random effects (5 linear splines).

Model 3: Fixed effects (5 linear splines and interaction with microalbuminuria, age, sex, BMI), random effects (5 linear splines).

Random Effects matrix shown has variances on the diagonal and correlation coefficients on off-diagonals.

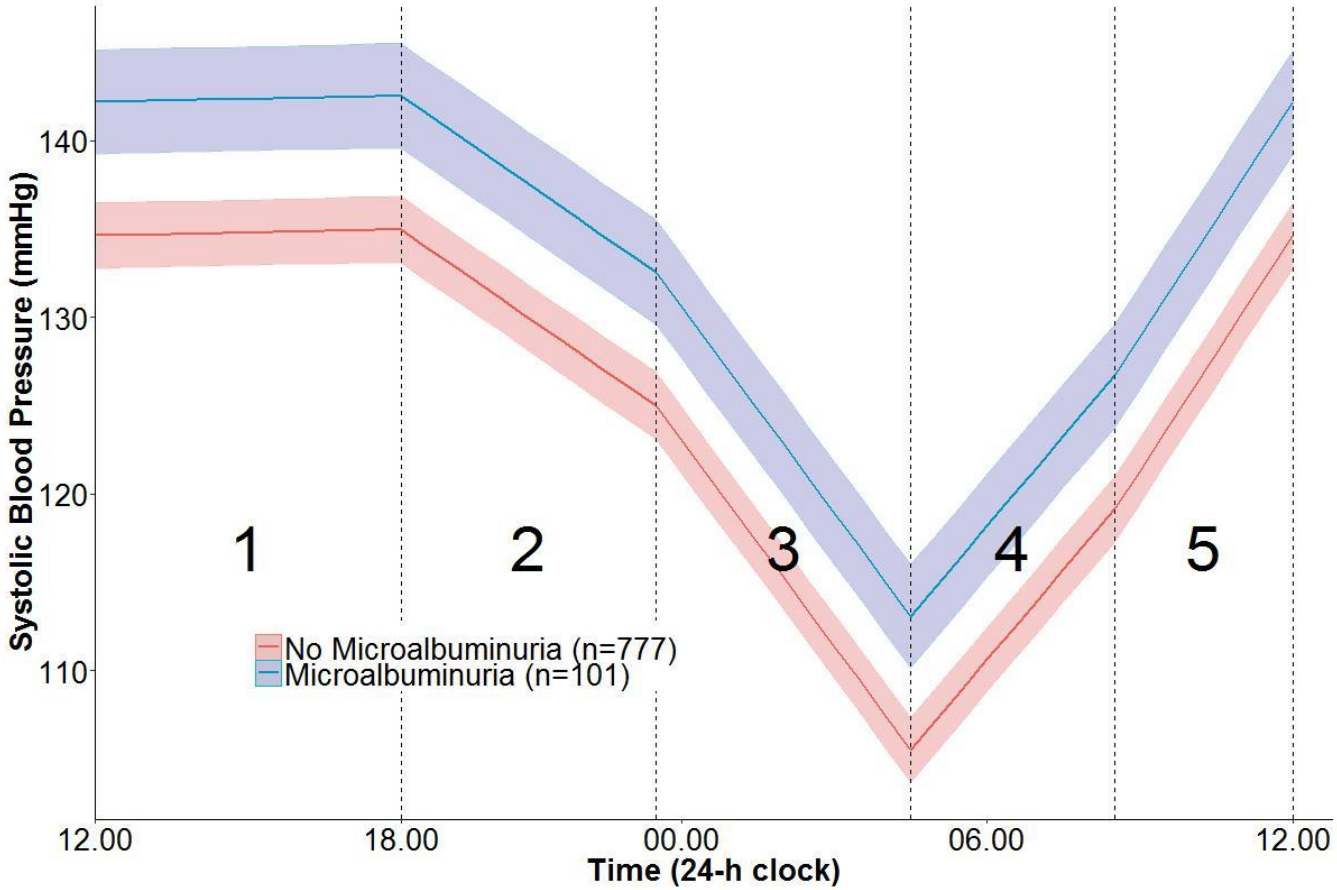


Figure 5-3 Predicted average (95% CI) piecewise linear trajectory of those with/without presence of microalbuminuria adjusted for age, sex and BMI using a linear mixed-effects model (Model 2). Each linear spline represents the rate of SBP increase or decrease (slope) for that segment and has been given a corresponding number which is referred to in Table 5-2. For the purposes of this plot we have set the sleep and wake time knots at 23.00 and 08.00 respectively.

6. MORNING SURGE IN BLOOD PRESSURE USING A RANDOM-EFFECTS MULTIPLE-COMPONENT COSINOR MODEL (PAPER 4)

J. M. MADDEN[a]

L. D. BROWNE[a]

P. M. KEARNEY[a]

X. LI[b]

K. TILLING[c]

A. P. FITZGERALD[a,d]

[a] Department of Epidemiology & Public Health, University College Cork, Ireland

[b] Graduate Entry Medical School, University of Limerick, Ireland

[c] School of Social and Community Medicine, University of Bristol, Bristol, UK

[d] Department of Statistics, University College Cork, Ireland

This paper is currently under review at Statistics in Medicine.

6.1 Abstract

BP fluctuates throughout the day. The pattern it follows represents one of the most important circadian rhythms in the human body. For example it is well known that the absence of a dip in night-time BP is associated with poor cardiovascular outcomes. Similarly the morning BP surge has been suggested as a potential risk factor for cardiovascular events occurring in the morning but the accurate quantification of this phenomenon remains a challenge. Here, we outline a novel method to quantify morning surge that can also be used to obtain other measures of BP variability throughout the day.

We demonstrate how the most commonly used method to model 24h BP, the single-cosinor approach, can be extended to a multiple-component cosinor random-effects model. We outline how this model can be used to obtain a measure of morning BP surge by obtaining derivatives of the model fit. The model is compared to a FPCA which determines the main components of variability in the data. Data from the Mitchelstown Study, a population based study of Irish adults (n=2,047) was utilized where a subsample (1,207) underwent 24h ABPM.

Our findings demonstrate that the most common approach to model ABPM, the single cosinor, does not provide the best fit to the data. Our two-component model developed with random-effects analysis provided a significant improvement in fit. In addition, it provided a similar fit to the more complex three-component model and a model captured by b-splines using functional principle component analysis. The estimate of the average maximum slope obtained from the derivatives from the model was 2.857 mmHg/30min (bootstrap estimates; 95% CI: 2.855-2.858 mmHg/30min). Simulation results allowed us to quantify the between-individual SD in maximum slopes which was 1.02 mmHg/30min.

By obtaining model derivatives we have demonstrated a novel approach to quantify morning BP surge and its variation between individuals. This is the first demonstration of a cosinor approach to obtain a measure of morning surge.

6.2 Introduction

Elevated BP is the most prevalent treatable risk factor for cardiovascular disease affecting one billion people globally [2, 3]. It is well known that BP does not remain stationary but fluctuates throughout the day and follows a circadian rhythm. Morning surge refers to the phenomenon that occurs in individuals during the first few hours after waking up in the morning when there is an exaggerated spike or surge in BP [23, 45]. It has frequently been suggested that this surge may be a risk factor for cardiovascular events occurring in the morning [23, 45, 46]. However, the accurate quantification of this phenomenon remains a challenge. A recent meta-analysis examining the prognostic significance of morning surge in 17 studies, identified seven different calculations for the term which highlights the problem [193]. All but one of these seven estimates involved simple subtraction of BP values where they subtracted some average night value minus an average of morning BP readings. While these measures can easily be calculated without the need for advanced methodology, they may not accurately quantify a surge or rate of change.

Rather than focusing on one value (mean BP) as obtained by traditional measurement techniques, ABPM which obtains multiple readings over a 24h period offers a unique insight into an individual's underlying circadian rhythm [13]. Parati *et al.* argue that rather than simplifying ABPM data into mean summary measures, incorporating all the data in more advanced models can lead to more robust estimates of clinically relevant parameters such as dipping status and morning BP surge [142]. In order to get a more advanced measure of surge, it is paramount that we can first accurately model ABPM.

To date, the main approaches proposed to model 24h BP that incorporate all the data and fully utilise the benefits of the longitudinal nature of ABPM include the cosinor method [114-118], cubic splines [48], polynomials [49] and recently a double logistic model [194, 195]. Moreover, there is no accepted "standard" method for analysing ABPM [49], and research on the longitudinal analysis of 24h ABPM is lacking [48]. Cosinor analysis has traditionally been the most common approach to modelling 24h BP. This method oversimplifies the data as it attempts to describe a 24h circadian pattern with the use of a single sinusoidal function. The

assumption of a simple symmetrical pattern for diurnal BP is unable to account for large variation in BP over a 24h period [142]. Although not as common as the single-component cosinor model, attempts to extend the model by including multiple cos terms (Fourier analysis) allow more flexible curves to be obtained while remaining periodic [123-125]. The majority of the studies to date exploring the use of sinusoidal functions have focused on fixed-effects where the inference is on population effects [114-118]. A key feature of ABPM analysis however is the exploration of subject-specific effects (random-effects) where its use in cosinor models has been limited [126].

Thus the purpose of this study is twofold. To first demonstrate that extending the traditional single cosinor to a multiple-component cosinor in a random-effects model can be achieved while offering a substantial improvement in fit to ABPM data compared to the single-component model. Moreover the model is compared to a FPCA which determines the main components that account for the majority of the variation in the data. The parameters from the cosinor model are compared to the functional principle component scores.

Secondly, by calculating first-order derivatives of the model fit, we present a novel alternative method to locate and quantify the magnitude of slopes at critical points on the trajectory. This simple application of derivatives allows us to quantify a measure of morning BP that specifically represents a surge parameter. This may be beneficial in future studies exploring the prognostic significance of morning BP and chronotherapy effects of antihypertensive medication. For purposes of illustration of the models at a group level, we compare first derivative curves in those with and without evidence of subclinical TOD, specifically microalbuminuria.

6.3 Methods

Study Design and ABPM

The analysis utilises data from the Mitchelstown Study, a population based study of middle-aged men and women, recruited in Ireland 2010-2011. A description of the study design is available from previous publications [74, 180]. The study recruited patients attending a single large primary care centre, the LHC, in Mitchelstown. Participants completed a detailed health and lifestyle questionnaire including a question on use of anti-hypertensive medication, and attended for a physical examination including height, weight, blood pressure and fasting blood samples. Each participant provided an early-morning spot urine sample on the day of their appointment. Laboratory analyses included analysis for ACR. Microalbuminuria is defined as ACR$\geqslant$1.1 mg $mmol^{-1}$ [78]. Study BP was measured three times after 5 minutes of rest in a seated position by experienced research nurses using an OMRON M7 BP monitor (OMRON Healthcare, The Netherlands). The average of the second and third measurements was used. The classification of hypertension was based on SBP≥140 mmHg and/or DBP≥90 mmHg and/or on anti-hypertensive treatment. ABPM was offered to all participants and was measured using dabl ABPM system (dabl ltd., Ireland) with the Meditech ABOM-05 Monitor (Meditech LTD., Hungary). The monitors were programmed to obtain readings every 30mins and remained in place for 24-h. Participants kept diaries of wake and sleep periods, which were used to calculate sleep and waking times. Only participants with a minimum of 20 measurements during the day and a minimum of 7 measurements during the night period were included in the analysis [13, 181]. Additionally, any participants with data lacking for more than two consecutive hourly intervals were excluded (see Appendix E) [13, 73, 181]. All participants provided written informed consent and ethical approval was obtained from the Clinical Research Ethics Committee Cork.

6.4 Statistical Analysis

Cosinor Analysis

The single-component cosinor model, which was first developed by Halberg [112, 113], uses a single cosine function as a model for physiological processes that have a circadian rhythm. This can be extended to a multiple-component model in the context of BP:

$$BP(t) = M + \sum_{i=1}^{n} A_i \cos\left(\frac{2\pi t}{\tau_i} + \emptyset_i\right) + e_t \text{ , i = 1,2…,n} \quad \textbf{(28)}$$

where *BP(t)* is BP as a function of time (*t*), *M* is the MESOR, the average value over the period, *A* is the amplitude for each cosine term (half the difference between the highest and lowest values, or the distance between the MESOR and the highest (lowest) value), τ is the period or duration of one cycle corresponding to each cosine term, ϕ is the acrophase (a measure of the time of the overall high values recurring in each cycle for each cosine term), *e* is the error term and *n* represents the number of cosine curves (*n*=1 represents the case of the single-component cosinor model).

The fixed-effects multiple-component model in equation (28) can be incorporated into a random-effects model. The mixed-effects model [87, 88] is a well-recognised tool in the analysis of longitudinal data that allows both population (fixed-effects) and subject-specific (random-effects) trajectories to be obtained which makes it useful for the analysis of ABPM data. It is assumed the random-effects have mean zero and an unstructured variance-covariance matrix. The individual level residuals (*e*) have mean zero and variance-covariance matrix Σ_e. As individual ABPM readings taken close in time are likely to be correlated, a model with an independent residual correlation structure may not be appropriate. We compared this to a model with a first-order autoregressive AR(1) structure and examined a plot of the ACF to detect violations of the assumption of independence. We determined the appropriate number of cosine terms by graphically comparing subject-specific predicted BP fits to the data (while increasing the number of terms) and formally by comparing models with different number of cosines using a LRT [88, 183]. Based on a LRT we univariately tested the inclusion of each term as a random-effect. If a

significant improvement was obtained, the term was included as a random-effect in the final model. The appropriate variance and residual function structures were also identified using a LRT in addition with the ACF plot.

The parameters for our final model were estimated using REML as this method produces unbiased estimates unlike ML estimation [93]. Subject-specific trajectories were based on empirical Bayes estimates of the random-effects [88].

Cosinor Derivatives and Morning Surge

To obtain an estimate of the maximum morning surge we first estimate *BP(t)* for each individual from our random-effects model and then obtain its first derivative, *BP(t)*'. This will give the rate of change or slope at each time of the day. Next, by limiting our analysis between 02:00 to 12.00 we can obtain the maximum slope and corresponding time during this period by numeric estimation for each individual which corresponds to their maximum morning slope or surge. As participants kept diary entries, we know their waking times. The earliest waking time is 05:00 but we broadened our period of interest further back to 02:00 in the unlikely event that an individual's maximum surge occurred just before waking. As the shape of the data is not overly complex over this period, assuming only one local maximum for this time is a reasonable assumption.

Cosinor Bootstrap and Simulations

Bootstrap estimation was performed in order to get an unbiased estimate of the standard error for the morning slope. A total of 1000 bootstrap datasets were created by randomly resampling from the original dataset with replacement. The bootstrap estimates were examined and determined to follow a normal distribution from which a standard error could be obtained. Results from the analysis were used to obtain bias corrected 95% CI for the slopes.

Additionally, to obtain an estimate of the between-individual variance in maximum slopes we ran 1000 simulations based on our final model. From this we obtained an estimate of the distribution of the slopes. This is similar to exploring the distribution

of the individual slopes from our final model but should result in a more precise estimate of between-person variation.

Functional Principle Component Analysis

The aim of FPCA is to find a combination of a few functions which capture the largest proportions of variation in the data. As a method of validation we compared model fits from the final multiple-component cosinor model to a model arising from FPCA. The purpose of this was to determine if the cosinor model was capable of capturing fluctuations in BP as well as the FPCA which is less restricted and can provide a flexible fit.

We implemented a FPCA which can be seen as an analogous to multivariate PCA where we can identify the main types of variation in patterns as a function of time as opposed to discrete measures [129, 130]. Instead of eigenvectors we obtain eigenfunctions which are associated with each eigenvalue and represent the FPCs which describe the different variations in the data.

To begin with, the mean curve was obtained through a method developed by Yao *et al.* which first ignores the hierarchical structure of the data and then fits a smooth curve to the pooled data [134]. This estimate of the mean curve is then used to obtain the covariance matrix of the deviations from the mean for each pair of time-points. This covariance matrix is smoothed using a bivariate smoother and the main diagonal is removed to normalise data. This smoothed covariance matrix is then decomposed into a linear combination of orthogonal (uncorrelated) eigenfunctions and eigenvalues i.e. into its principal components and scores [133]. The first principal component captures the most variation, the 2nd captures the second-most variation and so forth. Therefore, a linear combination of a few efficient functions can account for a high proportion of the variance. In our analysis, 10 B-spline basis functions were used to estimate the mean function, and for the bivariate smoothing of the covariance function. The number of FPCs to include was then determined by visual inspection of a scree plot. In the context of random-effects models these FPCs can be seen as patterns of within-subject variation remaining after the mean fit.

The weights that define the optimal fit to each function are the principle component scores. These can be used to obtain individual curves by multiplying the weights by the functions. Zero scores for an individual would result in their trajectory following the mean pattern. The scores would usually be estimated through numerical integration but with sparse data, as in the case of ABPM (compared to high sampling frequency data e.g. every second, that is often associated with functional data [131]), the approximation is sometimes deemed inadequate and in this case the scores were estimated by the principal component analysis through conditional expectation (PACE) method [133, 134]. Using this method the scores are estimated for each individual using their repeated measures while borrowing strength from the cohort with sample estimates of the mean function, covariance, eigenvalues and eigenfunctions [133, 134].

As a final step the random-effects of the multiple-component cosinor model were correlated with the individual FPC scores from the FPCA. This allowed us to determine if the cosinor model and their parameters were capturing the main components of 24h BP obtained through FPCA which was a more elaborate, flexible and data driven approach. If they correlated well it would help advocate the use of our model.

In a separate analysis and final method of validation we compared model fits from the final multiple-component cosinor model to a spline model. The purpose of this was to determine if the cosinor model was capable of capturing fluctuations in BP as well as a cubic spline model which is less restricted and can provide a flexible fit. We implemented a random-effects model cubic spline model with four knots at 18:00, 24:00, 04:00 and 08:00 allowing all terms to be random similar to our cosinor model. It was agreed that allowing four knots gave sufficient flexibility in the curve to capture BP pattern. Model fits were visually compared to the cosinor model.

Target Organ Damage

Finally, after the model has been compared to FPCA and as a method to illustrate the approach outlined we run the final model separately on those with and without evidence of microalbuminuria. Subject-specific curves are obtained and plotted for

each group. In addition, mean curves for both groups, over the 24h period are overlaid on the same plot giving a graphical comparison. We also obtain and compare first derivative curves in both groups.

Software

All analysis including bootstrapping and simulations were implemented in R [187]. Although the model can be rewritten in a linear form [196], when *n* (number of cosine terms) is greater than 1 it is easier to obtain estimates of the parameters directly from the non-linear model as opposed to calculating them post-hoc using trigonometry with the linear model. For this reason the nonlinear mixed-effects model was solved using the nlme command in the nlme R-package [188]. Initial starting values were obtained from a model incorporating fixed effects only. FPCA was utilised using the refund R-package [197]. A shiny app was also built in R using the shiny R-package [198] to illustrate different model fits that can be obtained from our final random-effects model using simulated data based on the model.

6.5 Results

The study questionnaire and physical examination was completed by 2047 participants (response rate: 67%). ABPM was completed by 1207 participants (response rate: 58%), of whom 886 had a minimum of 20 day and 7 night measurements and no data missing for more than two consecutive hourly intervals. Their main clinical characteristics are presented in Table 6-1.

Figure 6-1 is a graphical representation of ABPM for four individuals. Included are subject-specific fits from a random-effects cosinor model with varying number of cosine terms; single, two and three-component models (n=1,2,3). It can be seen that the single component model offers a very simplistic curve that struggles to capture the shape of the data. There is a large improvement observed however with both the two and three-component models where large fluctuations are accounted for more than in the restricted single model. When visually comparing all the two and three-component model fits, there was little difference between them.

For this reason and to obtain the most parsimonious model we identified the two-component as a satisfactory model to describe ABPM.

The final two-component SBP cosinor model parameters estimates are presented in Table 6-2. Initially all the parameters were included as fixed-effects. A significant improvement in fit was observed when additionally including each term individually as a random-effect, based on a LRT (all $p<0.001$). As a consequence we included all parameters as random-effects. The MESOR or average BP over 24h was 124 mmHg. The values of the parameters for the first and second cosine curves are presented separately. As expected, the first cosine with period 24h is the dominant curve with amplitude of 13.2 mmHg while the second cosine curve with period 12h has amplitude 5.6 mmHg. Similarly, the phase shift of the first cosine is larger than the second one which are both measured from 12:00 in units of 30mins, 5.3 (2.6h) compared to 1.0 (0.5h), which corresponds to a time of approximately 14:18 and 12:30 respectively. Exploring the random-effects covariance matrix suggests that there is a moderate positive correlation between the two amplitudes ($r=0.51$) and the two phase shifts ($r=0.44$). However, there is a weak correlation between the amplitudes and their corresponding phase shifts (-0.01 and -0.16). As some correlations were quite low we considered reducing the size of the covariance matrix by removing weak correlation terms but as Harrell *et al.* suggest removing separate terms in this way provides very little gains in terms of precision and power [199]. As a result the covariance structure was not altered. The variation between-individuals in the first cosine amplitude was greater compared to the second (SD, 6.1 mmHg vs 2.8 mmHg). However in contrast, there was less variation in the phase shift of the first cosine compared to the second (9mins vs 18mins). The within-subject SD (σ) was 11.9 mmHg. Examining the ACF plot indicated that the inclusion of an AR1 residual structure adequately accounted for the auto-correlation in the data. Incorporating an AR1 structure resulted in a significant improvement in fit ($\rho=0.22$, $p<0.001$).

After applying the final two-component cosinor model to the data and obtaining the random-effects coefficients, derivatives of the function were calculated. Figure 6-2 presents ABPM readings for three individuals with their fitted subject-specific

trajectories and corresponding first derivative curves. This represents the rate of change or slope at each time point during the day. By focusing on the morning period we can obtain the magnitude and location (time) of the maximum surge. The estimate of the maximum slope obtained from the derivatives from our final model was 2.857 mmHg/30min, Table 6-3. Also presented in Table 6-3 is the bias corrected bootstrap distribution estimates from which a standard error for the estimate of average maximum slope could be obtained (SE=0.0012), resulting in 95% CI: 2.855-2.858 mmHg/30min. The simulation results allowed us to quantify the between-individual SD in maximum slopes which was 1.01. The distribution of the maximum slopes from the simulations is presented in Figure 6-3. Further histograms of the slopes from the original model and the bootstrap estimates were also obtained which provided evidence of a normal distribution (see Appendix F).

As a separate analysis we compared a two-component model to a spline model as outlined previously. Model fits and derivative plots from a random-effects cubic spline model indicated a similar pattern giving further justification of the use of two-component cosinor model (see Appendix F).

B-splines were explored in FPCA. From a visual inspection of the scree plot, three principle components were retained (see Appendix F). Results indicated that the first three FPCs which accounted for 76.3% (FPC1), 9.2% (FPC2) and 6.8% (FPC3) accounted for 92.3% of total variation in the data. To help visualise and interpret the individual FPCs, Figure 6-4 illustrates the mean curve along with the effects to the pattern when a small amount of the component is added and subtracted from the mean. It is evident that the first component which accounts for the majority of the variance in the data represents a relatively constant shift in the mean. Individuals with positive scores on the second component have a slightly higher BP during the day and a lower BP at night indicating a large peak-to-tough value, those who score negatively have a slightly lower value during the day and higher value at night indicating a small peak-to-trough. It can be argued that this component is capturing dippers and non-dippers. Individuals with positive scores on the third component are associated with an earlier dip at night and a large morning rise, those with a low value seem to be shifted to the right and have a slightly less

pronounced morning rise. A larger version of Figure 6-4 can be found in the appendix with the first six FPCs included (see Appendix F). The correlation matrix presented in Figure 6-5 shows strong correlations between the FPC scores and the individual random-effects from the two-component cosinor model. It demonstrates, as expected that the first principle component score summarizes the MESOR (mean curve). The second principle component score has a strong correlation with both amplitudes especially the first one (r=0.9). Similarly the third principle component score has a strong correlation with both phases especially the first one (r=0.9). A similar correlation matrix including all the FPC scores is included in Appendix F.

The model was applied separately to those with and without evidence of microalbuminuria. A graphical comparison of the mean curves and their associated first-derivatives are presented in Figure 6-6. It can be seen that, on the average over 24h, those with microalbuminuria had higher SBP but the patterns were similar. As a result, the overall pattern in the rate of change over time is broadly similar in both groups. Although significantly lower in those with microalbuminuria, the difference in the maximum surge reached in the morning period between both groups was small, (2.6 vs 2.3 mm Hg/30mins, $p<0.01$). The time of the maximum surge reached in the morning was 08:24 and 08:39 for those with and without microalbuminuria respectively. At the point of their maximum first derivative or surge, a difference of 9 mmHg in average SBP was observed between those with and without microalbuminuria (129 vs 120 mmHg, $p<0.01$).

In addition, we have created a simple shiny app to illustrate different fits our model can provide to the data. We have simulated data based on our model and fits and derivatives are shown to different simulated individuals. The link to the app is https://user632.shinyapps.io/App_Double_Cosinor/

6.6 Discussion

In this study we have demonstrated that extending the traditional single cosinor to a two-component cosinor in a random-effects model results in a substantial improvement in fit. From our findings, the evidence suggested that the two-component model offered similar fits to that of a three-component model and a spline model. In addition, using FPCA we have demonstrated that the main components of variation in the data correlate extremely well with the parameters from our model. By obtaining model derivatives we have demonstrated a novel approach to quantify rate of change of BP throughout the day. This is the first demonstration of the cosinor model to obtain a measure of morning surge. The use of FPCA on ABPM data also offers a novel method to quantify BPV.

Considering the traditional single cosinor model has been the most commonly used approach for the longitudinal analysis of ABPM, further research in an attempt to extend the model seemed warranted. There has been much criticism of the single cosinor method [121, 122, 142] but we have illustrated that the inclusion of just one additional cosine term gives the model substantial flexibility which helps alleviate many of the concerns raised. The main criticism has focused on the unrealistic assumption that diurnal BP follows a simple symmetrical pattern. Unlike the single model, multiple-component models, while retaining the periodic property, can capture local minimum and maximum points as evident in this study. It has been stated that shapes closely approximating a single cosine curve are uncommon which is not under question [122]. We realise that a two-component model does not offer a perfect fit but is much more flexible than a single model and in order to compromise for a parsimonious model and interpretable terms, it offers a good alternative to other methods with fewer parameters and a similar fit (e.g. three-component cosinor).

The use of FPCA enabled us to obtain the main patterns of variation in the data and determine how much each component contributed to the total variation in the data. This offers a novel method to describe variation within BP, that to our knowledge, has not been used before on ABPM data. The use of functional data analysis allows flexible fits to be obtained without the need to pre-specify a model.

Although similar, this is not the same as a traditional random-effects model. FPCA will be inherently more flexible. For example, if we consider a two-component cosinor random-effects model – it would assume a mean and two cosine terms with two different periods, FPCA however will try to estimate the "optimal" functions that explain the variability in the dataset. However, if the mean, a one-period cosine and two-period cosine are close to optimal then the rest of FPCA and the model fitting will be similar: random-effects will be used to estimate the subject-specific coefficients for each of these functions. The fact that the random-effects from our model correlated well with the FPC scores help justify the use of our model. To elaborate, the two-component model correlates well with the optimal fit obtained through FPCA. Our finding that the first FPC correlates exactly with the mean value is not surprising where other studies have found in practise, the first FPC is essentially a mean shift [132, 200]. Not only do our values correlate, visually examining the effects of the scores on the mean pattern illustrate that the main components refer to a mean shift, peak-to-tough and a shift left to right which is being captured by the parameters of the cosinor model. In fact using FPCA and the cosinor model together complement each other well. Sometimes interpreting FPCs can be difficult and subjective but when it is used in parallel with a cosinor model and results correlate so well it is easier to explain findings. Although not as directly interpretable as a single-cosinor model, the two-component model is still more intuitive than for example, a spline model. An individual's amplitude being made up of the weighted sum of two cosine amplitudes throughout the day is more intuitive than an arbitrary spline coefficient. In addition, the MESOR which is the average over 24h represents the most important parameter of BP.

Of the definitions identified in the recent meta-analysis examining the prognostic significance of morning surge, only one used a more advanced technique than simply obtaining differences between arbitrary night and day averages [193]. Head *et al.* developed a six parameter double logistic model which is characterised by a day and night plateau of variable length, an independent slope for the fall and rise over the day and a midpoint for each transition [194, 195]. The double logistic model can be used to obtain the rate of rising during the morning period and the

power of the morning surge which is the derivative of the curve multiplied by the amplitude [201]. However, it has been suggested that the parametric structure of the model is very simple and because of the day and night plateaus important BP fluctuations may be averaged out [142]. The approach does however offer alternative morning BP measures that attempt to incorporate more data in a mathematical model than traditional methods and Head *et al.* have tried to refute some of the criticism. They argue that their model is quite flexible and can follow a single-component cosinor model, a saw tooth shape in either direction as well as a square wave-like shape [143]. There are, however, some limitations. Importantly, they stress that the model cannot capture complex fluctuations associated with multiple-component cosinor models and can miss short-term peaks. Research suggests that more complex patterns such as the ones obtained through our approach are not necessarily an advantage as it may be difficult for an investigator to obtain a coherent picture from wavy curves [143]. This may be true if the sole purpose is to decipher individual model fits. We argue however the main purpose of our method is to obtain an estimate of morning slope through derivative estimation and this requires capturing the most accurate curve possible while simultaneously obtaining interpretable parameters that describe it. Obtaining the balance between obtaining complex curves and interpretable parameters is difficult. Complex wavy curves will not alter our ability to describe in simple terms the rate in change or surge. In addition their analysis has focused on analysing each individual ABPM curve one by one which will result in inflated standard errors for their estimates unlike our model that incorporates random-effects.

The use of estimated derivatives in medical research can often offer new, intuitive clinical markers [202, 203]. In the context of modelling ABPM, considering the emphasis is on exploring curves, the use of derivatives is surprisingly rare. As highlighted we have demonstrated that obtaining derivatives from the cosinor model offers a novel method of determining morning slope that has not to the best of our knowledge been implemented elsewhere. An advantage of this approach is it is not restricted to the analysis of morning slope but can be used to obtain critical points throughout the day e.g. dip. We have focused our analysis in this study to

morning BP due to the substantial literature surrounding its potential prognostic significance and the debate surrounding its quantification [23, 45, 46, 193]. Morning parameters to date have focused on summary measures, usually the difference between a pre-awaking value and post-waking value [193]. The primary issue with this approach is that by definition, a morning surge represents a spike or rate of increase in BP during this period which is not accurately captured by differences between two time points. We propose that our method which specifically obtains a rate of change parameter is a better estimate. In fact Parati *et al.* argue that a tangent with the steepest slope to a curve from a model that accurately captures morning BP could be the most appropriate estimate of the morning rise in BP [142]. Provided the model fits the data well, estimates derived from it should be more robust which in turn will lead to more precise inferences being made to outcomes. Despite our comprehensive knowledge of BP, obtaining new measures and methods to model BP remains a crucial research priority which may help advance our understanding of different aspects of BPV including morning surge [52, 66]. This may be particularly useful in clinical trials where we may be able to provide evidence that a new antihypertensive medication outperforms another in a way we would not traditionally be able to detect e.g. their mean values may indicate no difference. In this study, we illustrated how the model can be compared in two groups using those with and without evidence of microalbuminuria as an example. Although the microalbuminuria group had higher SBP, there was little difference in the pattern of the curves. This was similar to findings observed in Chapter 5 when we examined the same data using a piecewise linear model. Using that approach, we found no evidence to suggest the overall shape differed, although the microalbuminuria group had significantly higher SBP. In addition, the slope parameter that was obtained for the morning period using the piecewise approach (2.21 mmHg/30mins) is similar to the cosinor approach (2.86 mmHg/30mins). It must be noted that the cosinor method obtained a maximum value while the piecewise approach obtained an average value. A comparison of both methods using hard endpoints (cardiovascular events) as opposed to a surrogate marker of CVD is recommended in future work.

There are a number of limitations to the study. Primarily, we note the model does not give the optimal fit to the data (although the parameters correlated well with the more flexible FPCA). In fact, it is acknowledged that it is extremely difficult for any model to capture all the features of a 24h BP profile simultaneously. Perhaps the chosen model should be dependent on the research question posed. For instance as highlighted complex models may not be the most intuitive to understand directly but if the purpose is to obtain a BP measure (e.g. morning surge, dip) from the model post-hoc through additional analyses (derivatives), an initial simple model may not necessarily be the best choice. Another limitation of the study was that we were not able to include the effect of antihypertensive medication in the analysis. Although we knew if a participant was on treatment, we had insufficient data on the specific class of antihypertensive medication the individual was prescribed which meant drug-class comparisons were not possible.

In conclusion, we have demonstrated a simple method to obtain a measure of morning BP surge using a random-effects multiple-component cosinor where our focus was not only at a group level but also the individual level. In addition to the ability of the model to obtain estimates for the morning BP, we derived derivatives of the circadian curves which allow us to locate and quantify the magnitude of other slopes at critical points on the trajectory. The approach offers novel alternative methods of quantifying new BP indices that may be useful in the exploration of BPV where there remains debate over its prognostic significance. The use of FPCA also offers a new alternative approach to quantify BPV. Considering the single-component cosinor has been the most common method of analysis for ABPM, a recommendation for future studies from the evidence presented in this study is to incorporate a second cosine in the context of a random-effects model. The method offers a substantial improvement in fit compared to the traditional cosinor that is capable of capturing short-term peaks and can be implemented in standard statistical software. Future studies should also investigate the clinical prognostic significance of the morning surge parameter obtained through the analysis outlined in this study.

Table 6-1 Baseline Characteristics

Characteristic	Total (n=2047)	ABPM (sub-sample) Total (n=886)
Age, y	59.8 (5.5)	59.9 (5.5)
Gender, Male n(%)	1008 (49.2)	401 (45.3)
BMI, n (%)		
Underweight/normal (<25kg/m^2)	447 (21.9)	195 (22.0)
Overweight (25-30 kg/m^2)	925 (45.3)	380 (42.9)
Obese (≥30 kg/m^2)	668 (32.8)	310 (35.0)
Office SBP, mm Hg	129.6 (16.9)	134.7 (17.7)
Office DBP, mm Hg	80.1 (9.8)	83.1 (10.2)
Hypertension, n (%)	951 (46.5)	528 (59.7)

Data are mean (SD). BMI:Body mass index, ABPM: Ambulatory blood pressure monitor. Hypertension: ≥140/90 mmHg and/or on antihypertensive treatment.

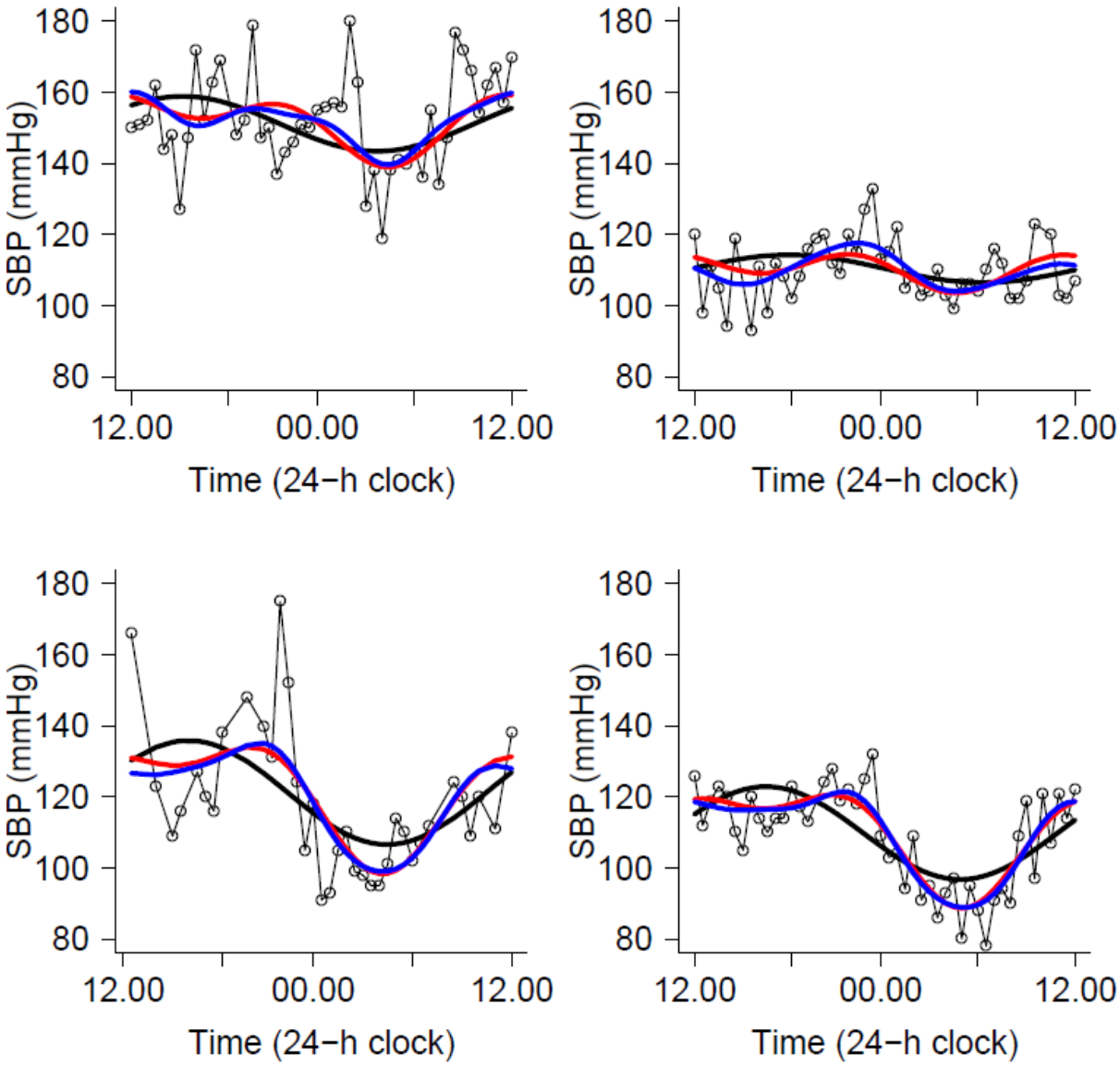


Figure 6-1 ABPM readings (circles, thin black line) of four individuals along with predicted subject-specific trajectories from a random-effects model as a function of time using (i) single cosinor (thick black line) (ii) two-component cosinor (thick red line) and (iii) three-component cosinor (thick blue line) models.

Table 6-2 Model parameter estimates (SBP) along with corresponding correlations and variances

Parameter	**Model**
Fixed effects	**Estimate (SE)**
24h MESOR (mmHg)	124 (0.44)*
First Cosine (24h period):	
Amplitude (mmHg)	13.2 (0.23)*
Phase shift (30mins)	5.3 (0.02)*
Time of phase shift	14:18
Second Cosine (12h period):	
Amplitude (mmHg)	5.6 (0.14)*
Phase shift (30mins)	1.0 (0.03)*
Time of phase shift	12:30
Random-effects	
	172.3
	0.18 37.0
Σ	-0.03 -0.01 0.1
	0.30 0.51 -0.14 7.8
	-0.03 0.01 0.44 -0.16 0.4
σ	11.9
ρ	0.22

*P<0.001, Random-effects matrix shown has variances on the diagonal and correlation coefficients on off-diagonals. Phase shift measured from 12:00 noon. Time presented in 24h clock.

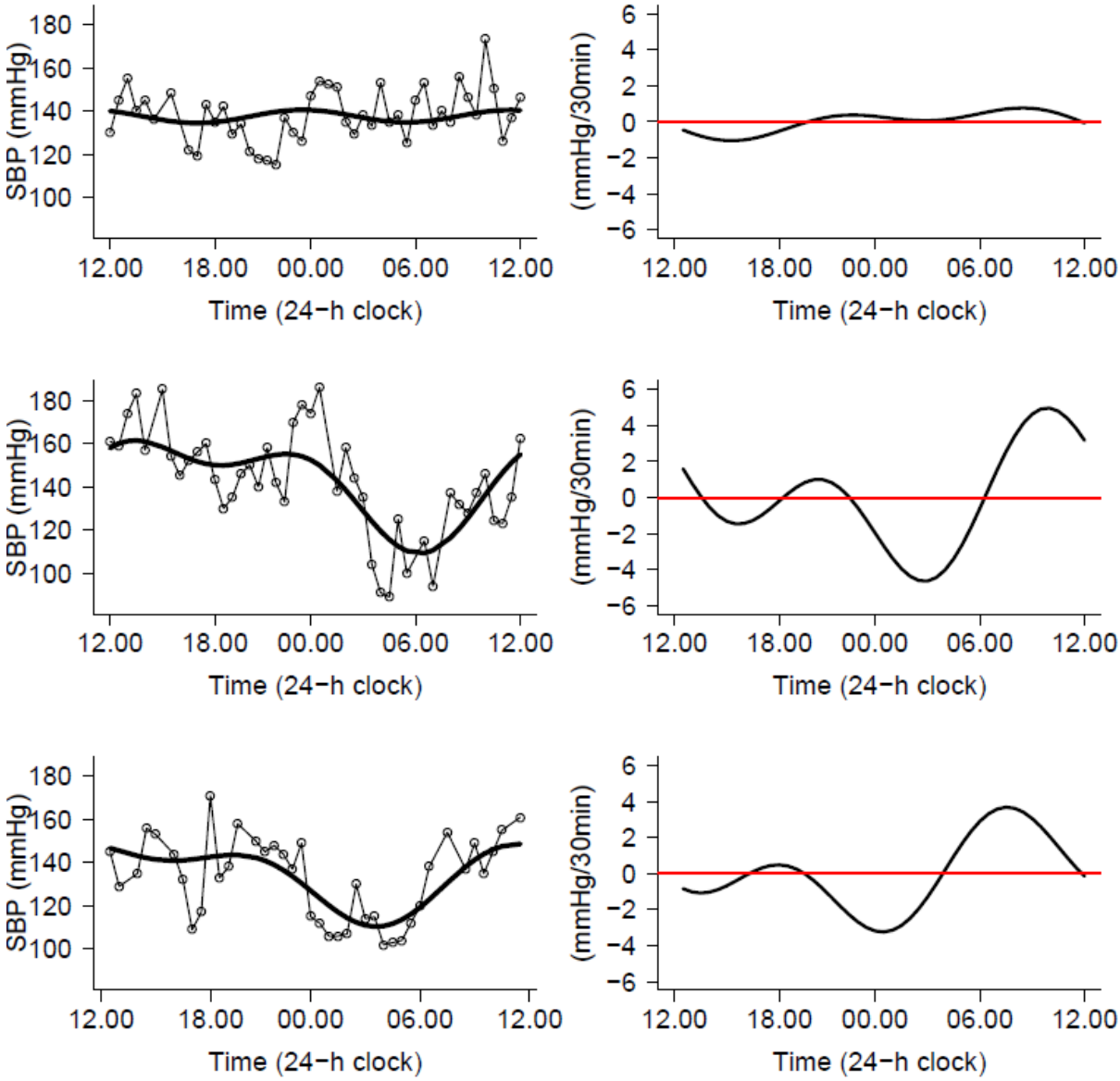


Figure 6-2 ABPM readings of three individuals with fitted subject-specific trajectories from a two-component cosinor random-effects model (left panels). Their corresponding rate of change curves (first derivatives) are also plotted on right panels (red line indicating reference zero mark).

Table 6-3 Maximum Morning Surge (mmHg/30mins)

	Median	Mean	Variance/CI
Original model	2.779	2.857	0.994
Simulations (1000)	2.840	2.840	1.040
Bias Corrected Bootstrap	2.857	2.857	CI (2.855 - 2.858)

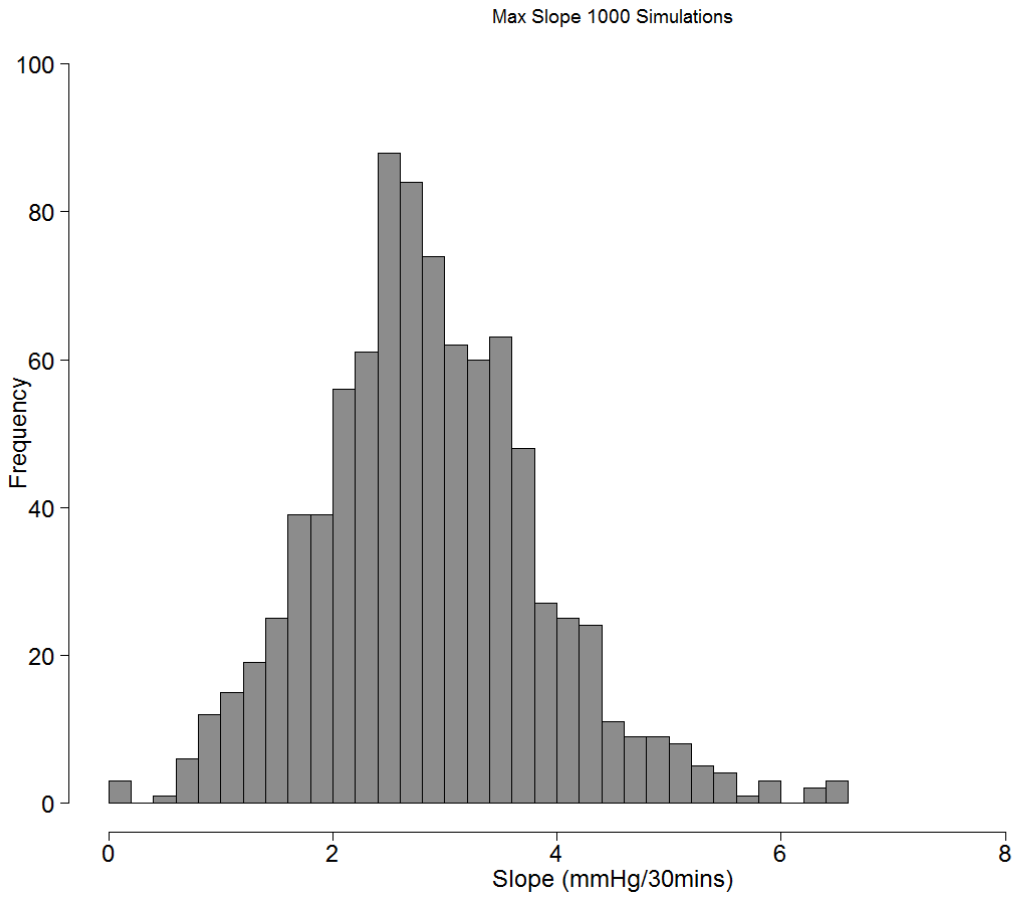


Figure 6-3 Histograms of maximum morning slope by simulations

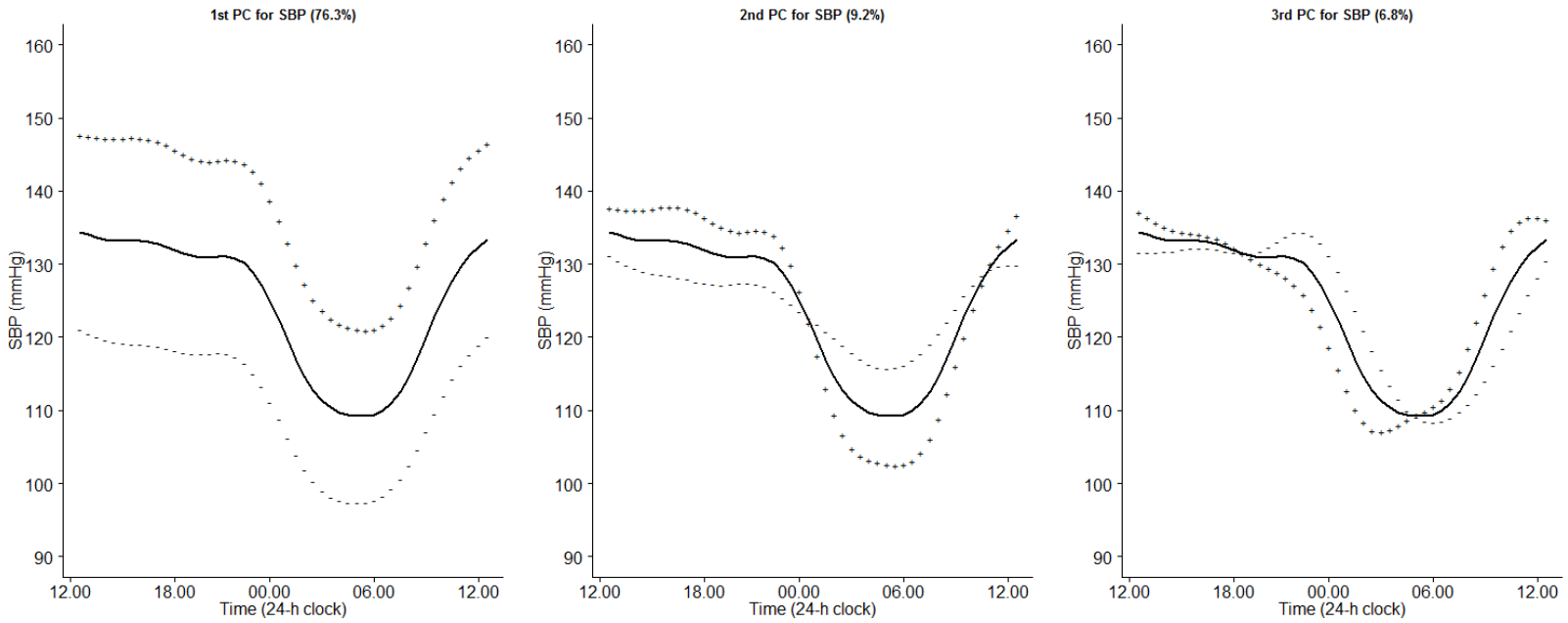


Figure 6-4 FPCA: Each of the first three FPCs as variations about the mean along with the percentage of total variation explained by the component. The solid black line represents the mean SBP over the day and the functions obtained by adding and subtracting ± SD of the eigenfunctions to the mean. Plus signs indicate addition and minus signs indicate subtraction.

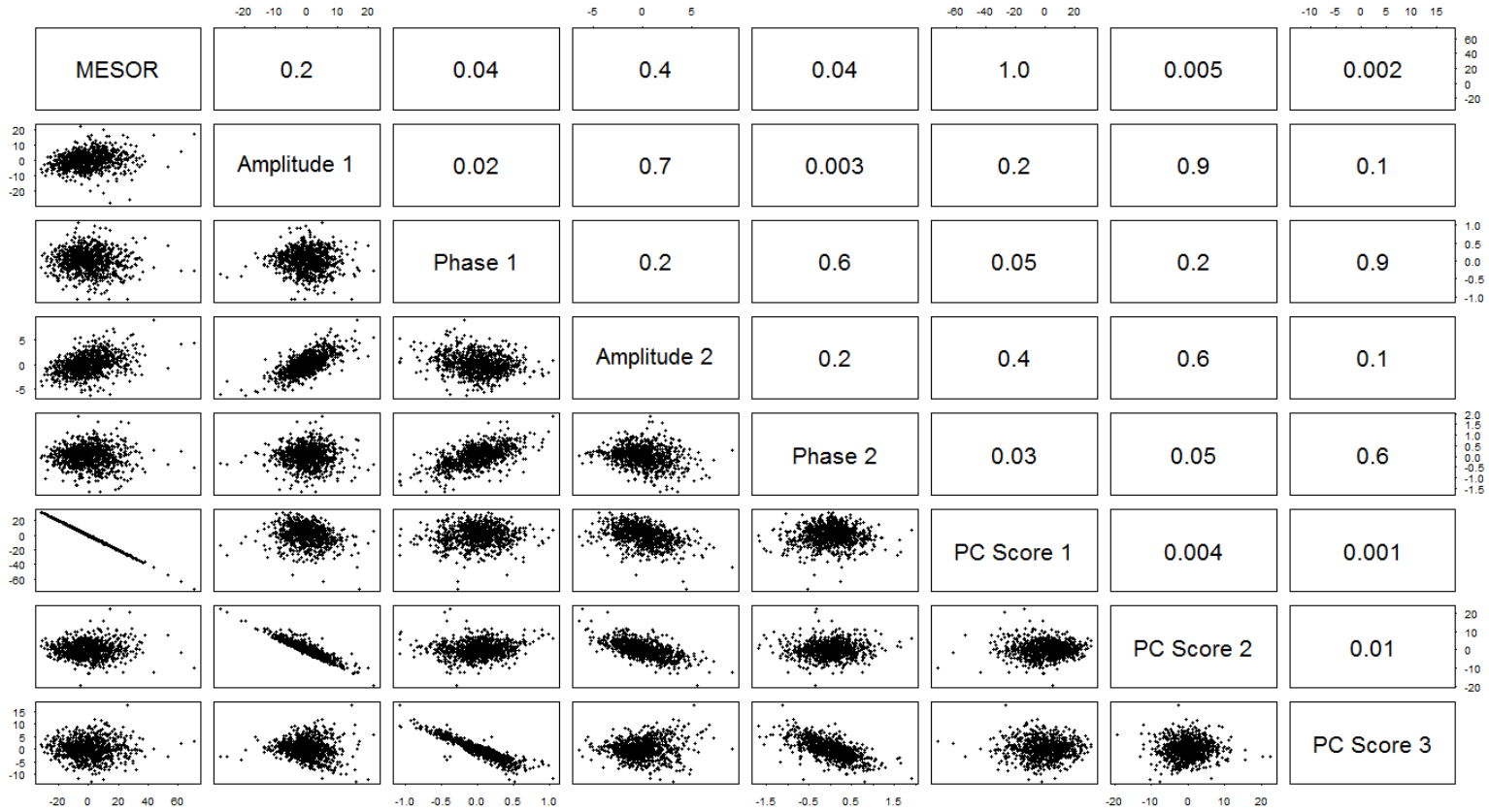


Figure 6-5 Scatter plots and the corresponding correlations between the two-component random-effects cosinor model parameters and the first three FPC scores from FPCA.

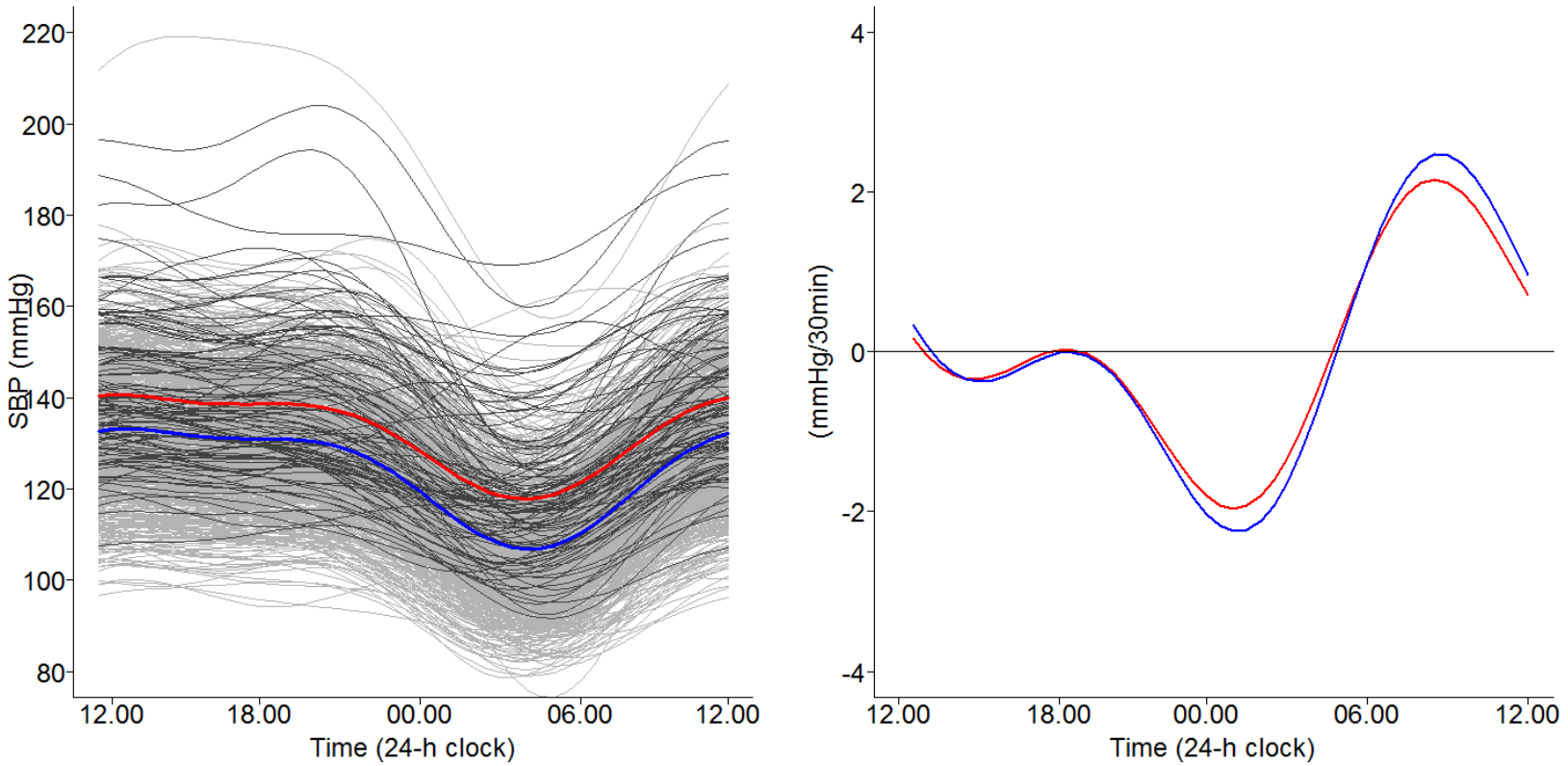


Figure 6-6 A two-component cosinor random-effects model implemented separately on those with and without presence of microalbuminuria. Subject-specific curves for those with (black lines) and without (light grey lines) evidence of microalbuminuria are also displayed. Red (microalbuminuria) and blue (no microalbuminuria) lines represent average curves for both groups. The corresponding first derivative curves indicating the rate of change over the day for both groups are also presented.

7. SHORT REPORT: REPRODUCIBILITY

J. M. MADDEN[a†]

A. M. O'FLYNN[a†]

P. M. KEARNEY[a]

E. DOLAN[b]

A. P. FITZGERALD[a,c]

[a] Department of Epidemiology & Public Health, University College Cork, Ireland

[b] Stroke and Hypertension unit, Connolly Hospital, Blanchardstown, Dublin, Ireland

[c] Department of Statistics, University College Cork, Ireland

† Both authors contributed equally to this work

7.1 Introduction

The use of ABPM provides the ability to classify individuals into different hypertension categories that cannot be achieved with a clinic BP reading. The reproducibility of these hypertension categories has been examined with inconsistent results [204-207]. Of particular interest is nocturnal hypertension which is a predictor of CVD and mortality [15, 16, 208]. However, the limited reproducibility of night-time BP patterns is recognised [209] and using absolute BP categories rather than dipping status may be more reproducible [210]. Yet, of those with isolated nocturnal hypertension (defined as nocturnal hypertension without daytime hypertension) at baseline only one third retained this pattern after 2 to 4 years in one small study [211]. This was again examined more recently in a community-based sample of adults (n=282) with similar findings where isolated nocturnal hypertension had poor reproducibility four weeks apart [212].

The majority of studies examining changes in ABPM readings between two time-points have focused on mean values and hypertension categories. Few longitudinal studies explore how variability changes between two ABPM readings. McDonald *et al.* found that while mean BP did not change, BPV, measured by day/night/24h SD and coefficient of variation, were significantly higher at 10 year follow-up compared to baseline in a community cohort of older people (n=83, median age 70 years) [213]. The percentage of patients taking antihypertensive medication increased from 46% at baseline to 69% at follow-up which may explain no change in mean BP. Conversely, in a community sample of 162 individuals aged 55 to 80 years, Goldstein *et al.* found that mean BP increased while day and night variability measured by SD decreased over two time-points 5 years apart [214]. Although there were no individuals diagnosed with hypertension at baseline, 14 subjects had BP with hypertensive levels. At baseline, no participant was on antihypertensive medication while there were 14 at follow-up.

In addition to exploring changes in hypertension categories and variability, we are interested in the reproducibility of the overall shape of the BP trajectories. Examining changes to variability measures only allows us to quantify changes in

fluctuation about the circadian rhythm but does not inform us if the pattern is changing. For example, an individual's BP trajectory may follow a similar pattern when measured at a second time-point but the variability about the pattern could increase or decrease. The purpose of this study is to outline an approach that can be used to comprehensively interrogate the reproducibility of ABPM readings over time. Changes in hypertension categories and variability measures are explored between two ABPM readings four years apart. In addition, parameters from a two-component cosinor model at each time-point are compared as a method of comparing changes in the shape of the circadian rhythm.

7.2 Methods

Baseline ABPM was obtained from the Mitchelstown Study, a population based study of middle-aged men and women, recruited in Ireland [74]. ABPM measurements were performed using the MEDITECH ABPM-05 in 2010 and data was stored using the dabl ABPM system (dabl ltd., Ireland). Based on the initial ABPM results the sample was divided into 4 groups: normotension, isolated nocturnal hypertension, isolated daytime hypertension and day-night hypertension [81]. Twenty participants were randomly selected from each group and invited to attend for follow-up ABPM measurements in 2014 using the Spacelabs 90217 monitor. Data was stored using the Spacelabs 92506 Ambulatory BP Report Management System software.

For both time-points, the monitors were programmed to record the BP every 30min throughout the 24h period. Participants kept a diary of the times they went to bed and got up. Diary times were used to calculate mean daytime and night-time BP. Mean 24h BP was calculated as the mean of all the readings throughout the 24h period. Use of antihypertensive medication was also recorded at both time-points.

Night-time BP was categorised by dipping status as follows:

(1) Dipping pattern: 10 to 20% fall in night-time SBP

(2) Non-dipping pattern: <10% fall in night-time SBP

(3) Extreme dipping pattern: >20% fall in night-time SBP

(4) Reverse dipping pattern: Rise in night-time SBP

BP was also categorised based on the absolute BP levels into four groups:
(1) Normotension: Daytime BP <135/85mmHg and night-time BP <120/70mmHg
(2) Isolated daytime hypertension: Daytime BP ≥135/85mmHg and night-time BP <120/70mmHg
(3) Isolated nocturnal hypertension: Daytime BP <135/85mmHg and night-time BP ≥120/70mmHg
(4) Sustained day-night hypertension: Daytime BP≥135/85mmHg and night-time BP ≥120/70mmHg

As indices of short-term BPV, we estimated the SD over 24h, wSD, coefficient of variation ((24h BP SD / 24h BP mean)*100) and ARV. The wSD is the mean of day and night standard deviation values corrected for the number of hours included in each of these two sub-periods. The ARV averages the absolute differences between consecutive measurements. Additional indices of SD, coefficient of variation, and ARV were calculated for both the day and night periods.

7.3 Statistical Analysis

Data are presented as the arithmetic mean (SD). The difference between the two ABPM results was assessed using a paired t-test. The reproducibility of night-time dipping status and absolute BP patterns were assessed using Cohen's kappa statistic. Agreement between the measurements was plotted using the Bland-Altman method. The per cent change in variability measures between the two time-points was calculated as the difference between the measures divided by the initial value. Changes of less than 5% were regarded as reproducible [205]. To quantify changes in the shape of BP as opposed to variability, a two-component random-effects cosinor model (see Chapter 6) was utilised on both time-points separately. Comparing model parameters between time-points identified differences in the shape of the curve. Empirical Bayes estimates were obtained for both time-points

for each individual and overlaid on the same plot giving a graphical comparison of the curves.

In addition, to determine how much of the variation in the difference between parameters over the two time-points can be attributed to measurement error, a simulation was performed:

(i) A random-effects cosinor model based on the data from the first time-point (2010) was used to estimate the mean parameter values, the variance covariance matrix of the random-effects, and the variance of the error term.

(ii) Subject-specific average curves were determined based on a random sample (n=47, same as the original sample size) from the estimated parameter coefficient distribution.

(iii) Observed values at 30min intervals were generated by adding random error to the values generated in (ii)

(iv) Step (iii) was repeated to generate a second dataset

The two simulated datasets were then analysed separately representing two different time-points. As before, the difference between model parameters was obtained. However, using the simulated datasets, any differences observed was due to measurement error. Thus, by comparing this variation to the variation observed between the actual readings taken at the two time-points, we can determine what percentage of the total variation is due to measurement error. This was repeated for n=1000 participants to examine if sample size effected the differences observed. Using the simulated data, a similar approach was used to explore how much of the variation in the differences in BPV summary measures over time could be attributed to measurement error. All analysis was implemented in R.

7.4 Results

At baseline, 1207 participants (response rate: 58%) underwent ABPM. At four years follow-up 80 participants were invited to attend for repeat ABPM and fifty

(response rate: 63%) of these participants consented to participation in the follow up study. We excluded three participants from the current analysis due to incomplete follow-up ABPM data giving a sample of 47 for this study. The mean period of follow up was 3.9 years. Raw SBP data for four individuals over 24h for the two time-points is presented in Figure 7-1, plots for all individuals are included in the Appendix G. Overall mean BPs were similar in 2010 and 2014, Table 7-1. The correlation between age and mean SBP was the same in 2010 and 2014 (r=0.17). Agreement between the two time-points for BP is given by the Bland-Altman plots in Figure 7-2. The reproducibility of BP profiles categorised by dipping status was low at 24% with a kappa statistic of -0.11 (p = 0. 89) while reproducibility based on categorisation by absolute BP was fair at 40% with a kappa statistic of 0.21 ($p < 0.005$), Table 7-2 and Table 7-3.

Overall, compared to baseline, BPV at follow-up had reduced, Table 7-4. However, only SBP variability, measured by wSD, SD and ARV, over 24h and the night period had fallen significantly. The Bland-Altman plots for both 24h SD and ARV are given in Figure 7-3, where a large spread is observed. Reproducibility of 24h SD (17%), ARV (10%) and wSD (23%) was poor. Parameters from a two-component cosinor model were obtained for both time-points, Table 7-5. With the exception of the second phase, all cosinor parameters were similar four years later. The variation in the difference in MESOR between time-points that could be attributed to measurement error based on simulations was low (3%), Table 7-6. Measurement error explained 19% and 32% of the variation in the difference in amplitudes, between the two-points, for the first and second cosines respectively. However, measurement error explained a larger amount of the total variation in the differences in the first (62%) and second phase shifts (88%). Repeating the simulations based on 1000 participants made little difference to our findings, Table 7-6. Bland-Altman agreement plots for the various parameters are presented Figure 7-4 and Figure 7-5. Reproducibility of MESOR (36%), first amplitude (14%), first phase shift (51%), second amplitude (21%) and second phase (4%) was poor. A separate boxplot of the differences between the parameters is given in Figure 7-6 showing a relatively even spread in the data. Using the simulated data,

measurement error between time-points accounted for 79%, 22% and 41% of the total variation in the differences of 24h ARV, SD and wSD respectively. Cosinor model fits for six individuals is provided in Figure 7-7 highlighting how the overall shape of the trajectory for two ABPM readings can easily be compared by overlaying the curves.

The use of antihypertensive medications increased between 2010 and 2014 from 42% (n=21) to 61% (n=30). In analysis limited to those not on medication (n=17) the kappa statistics were -0.2 ($p = 0.9$) for dipping status and 0.43 ($p <0.001$) for absolute BP categories. Mean BPs for those not on medication were similar in 2010 and 2014. Moreover, there was no change in variability measures for individuals not on medication. However, for those on medication, variability measured by wSD, SD and ARV, over 24h (all SBP) remained significantly reduced while the mean BP was similar.

7.5 Discussion

We have outlined methods to compare two ABPM readings taken at separate occasions. No one method can fully capture the reproducibility of a circadian BP trajectory but we have explored approaches that can account for the main features including the comparison of mean and variability values, hypertension categorization and a comparison of the shape of the curves. Our findings demonstrate the limited reproducibility of night-time BP profiles with poor reproducibility of dipping status and only fair reproducibility of absolute BP categories despite overall similar mean BPs. On average, variability measured by wSD, SD and ARV (all SBP only) significantly reduced over 4 years but reproducibility within the measures was fair. Finally, parameters from a two-component cosinor model which can be used to describe the shape of BP trajectories demonstrated that on the average patterns were similar but reproducibility of the parameters was poor.

Long-term changes in ABPM variability have rarely been explored. However, our findings are similar to Goldstein *et al.* who reported a reduction in variability over

two time-points 5 years apart in a similarly aged sample [214]. This is in contrast to McDonald *et al.* who reported BPV obtained by ABPM increased over a 10 year follow-up in an older sample [213]. Changes in mean SBP variability, despite no significant change in mean SBP is an interesting finding. There has been significant work over the last decade to control mean BP levels, coupled with advancements in antihypertensive therapy and perhaps an absence of an increase reflects these efforts. Much of the debate regarding BPV focuses on antihypertensive medications. While different classes of antihypertensives have similar BP lowering effects; significant differences between classes in their effects of BPV have been observed. Some studies suggest that calcium channel blockers and diuretics are superior to other drugs in reducing BPV and preventing stroke and other vascular events compared to other classes [61, 160]. In our study, the use of antihypertensive medications increased over the four years between the BP measurements. While the effect over time on mean BP was similar irrespective of medication use, BPV differed by medication use. Although numbers were small and hence, power was limited, we were still able to detect a significant reduction in BPV within the group on medication. In contrast, there was no difference in BPV over time for those not on medications. An analysis stratified by type of anti-hypertensive class was not possible and is recognised as a study limitation.

Overall reproducibility was poor on all features of the circadian pattern. BP is a continuous risk factor [215]. Thresholds define the levels where investigation and treatment do more good than harm [216], and for this reason are important but clinicians and public health professionals need to recognise the limitations of thresholds and that significant numbers of events occur in those below definitions of normal [217].

Using simulations, we were able to obtain the fraction of the total variation in the changes between time-points that was attributable to measurement error alone. If there was no change in the true readings between the two time-points, the only difference observed would be due to random error. Assuming a two-component cosinor model is an adequate fit for ABPM, which has been illustrated in Chapter 6,

we can estimate this random error. Comparing changes in summary BPV measures and model parameters from the two simulated datasets to the changes using the actual measured readings allow us to quantify the influence of measurement error between time-points. This highlights a benefit of using a random-effects model when examining reproducibility where the variance of differences between time-points can be separated into measurement error and true changes that have occurred during the intervening time-period.

The two-component cosinor model we have outlined provides a method that not only quantitatively assesses differences in the overall shape of two ABPM readings, but also offers a quick graphical comparison that may be useful in clinical practice when a clinician is trying to gauge changes in an individual's underlying circadian rhythm. The inclusion of the predicted curves in ABPM reports, in combination with the standard output may improve clinical decisions and ultimately, BP control.

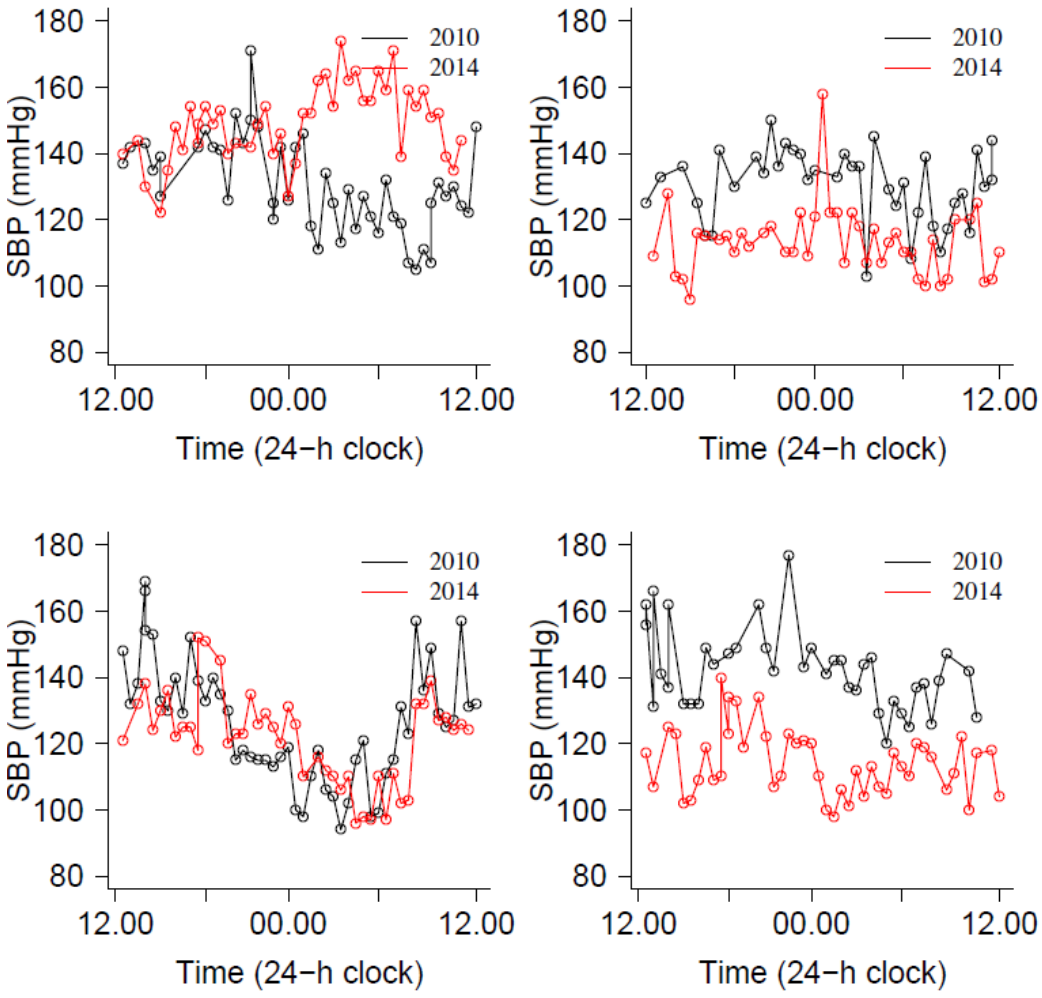


Figure 7-1 Data from four individuals 2010 vs 2014

Table 7-1 BP levels 2010 v 2014

N =47	2010	2014	p-value
Daytime systolic	133	129	0.05
Daytime diastolic	79	77	0.2
Night-time systolic	117	114	0.1
Night-time	66	66	0.9
Twenty four	127	123	0.05
Twenty four	74	73	0.3

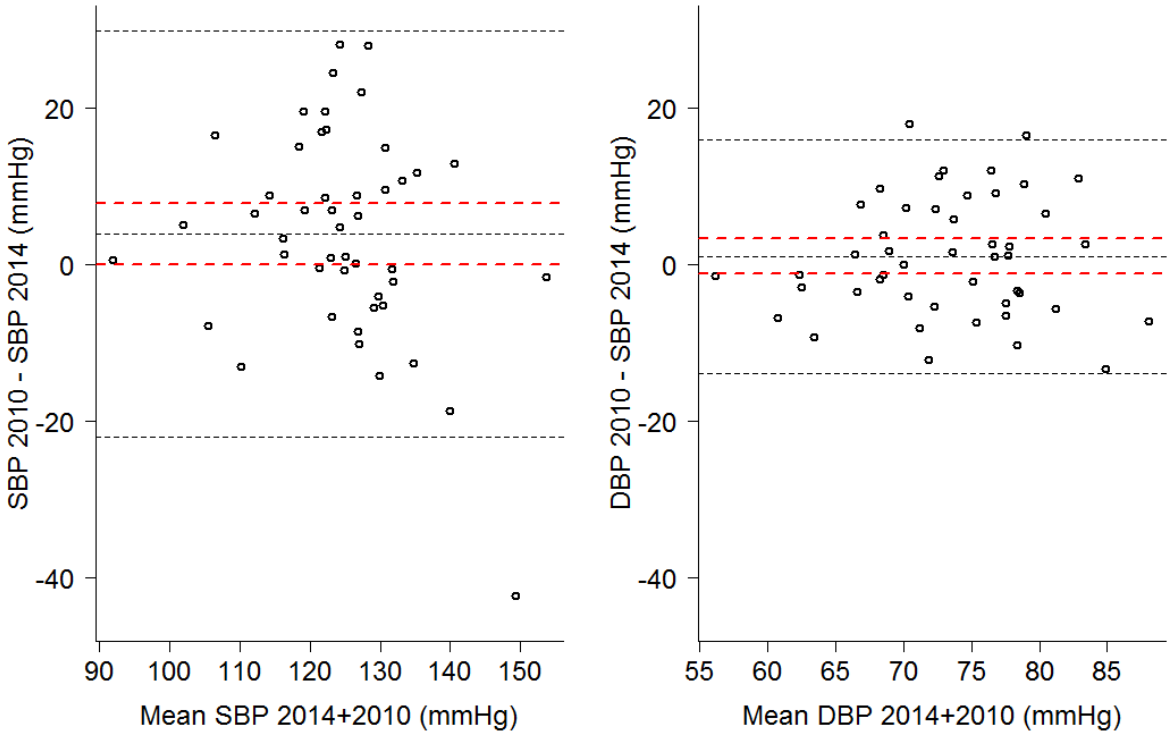


Figure 7-2 Bland-Altman scatter-plots of agreement of measurements of 24h SBP and 24h DBP four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values.

Table 7-2 Dipping status 2010 v 2014

	2014				
2010	Dippers	Non-dippers	Extreme dippers	Reverse dippers	Total
Dippers	5	11	5	1	22
Non-dippers	8	3	2	2	15
Extreme dippers	2	3	1	0	6
Reverse dippers	1	1	0	2	4
Total	15	18	8	5	47

kappa statistic = -0.11 (p = 0. 89)

Table 7-3 BP categories 2010 v 2014

	2014				
2010	Isolated nocturnal hypertension	Day/night hypertension	Isolated daytime hypertension	Normotension	Total
Isolated nocturnal hypertension	4	1	0	7	12
Day/night hypertension	2	3	2	4	11
Isolated daytime hypertension	1	6	3	4	14
Normotension	1	0	0	9	10
Total	8	10	5	24	47

kappa statistic = 0.21 (p < 0.005)

Table 7-4 Variability Indices 2010 v 2014

Variability Measure	24-hour (mm Hg)		Day (mm Hg)		Night (mm Hg)	
	2010	2014	2010	2014	2010	2014
ARV SBP	11.7 (2.5)	10.4 (2.2)**	12.2 (3.1)	11.2 (2.9)	11.1 (3.5)	9.2 (2.9)**
ARV DBP	7.8 (2.2)	7.5 (1.6)	8.2 (2.5)	8.0 (2.1)	7.1 (2.5)	6.7 (2.2)
SD SBP	15.9 (4.0)	14.6 (4.1)*	13.0 (3.0)	12.5 (3.7)	12.2 (4.1)	10.1 (3.7)**
SD DBP	11.3 (3.5)	10.1 (2.7)	8.9 (3.0)	8.8 (2.1)	8.4 (2.7)	7.2 (2.6)
CV SBP	12.5 (3.1)	12.0 (3.2)	9.8 (2.1)	9.8 (2.8)	10.4 (3.3)	8.8 (2.8)
CV DBP	15.3 (4.4)	14.1 (4.1)	11.3 (3.5)	11.6 (3.2)	12.8 (4.0)	11.1 (4.0)
wSD SBP	12.7 (2.7)	11.7 (3.2)*	-	-	-	-
wSD DBP	8.8 (2.4)	8.3 (1.8)	-	-	-	-

Abbreviations: ARV: Average real variability, SD: Standard deviation, wSD: Weighted standard deviation, CV: coefficient of variation, SBP: SBP, DBP: Diastolic BP. Data presented as mean(SD). *p<0.05; **p<0.01 represents significance between 2010 and 2014 for each period of the day

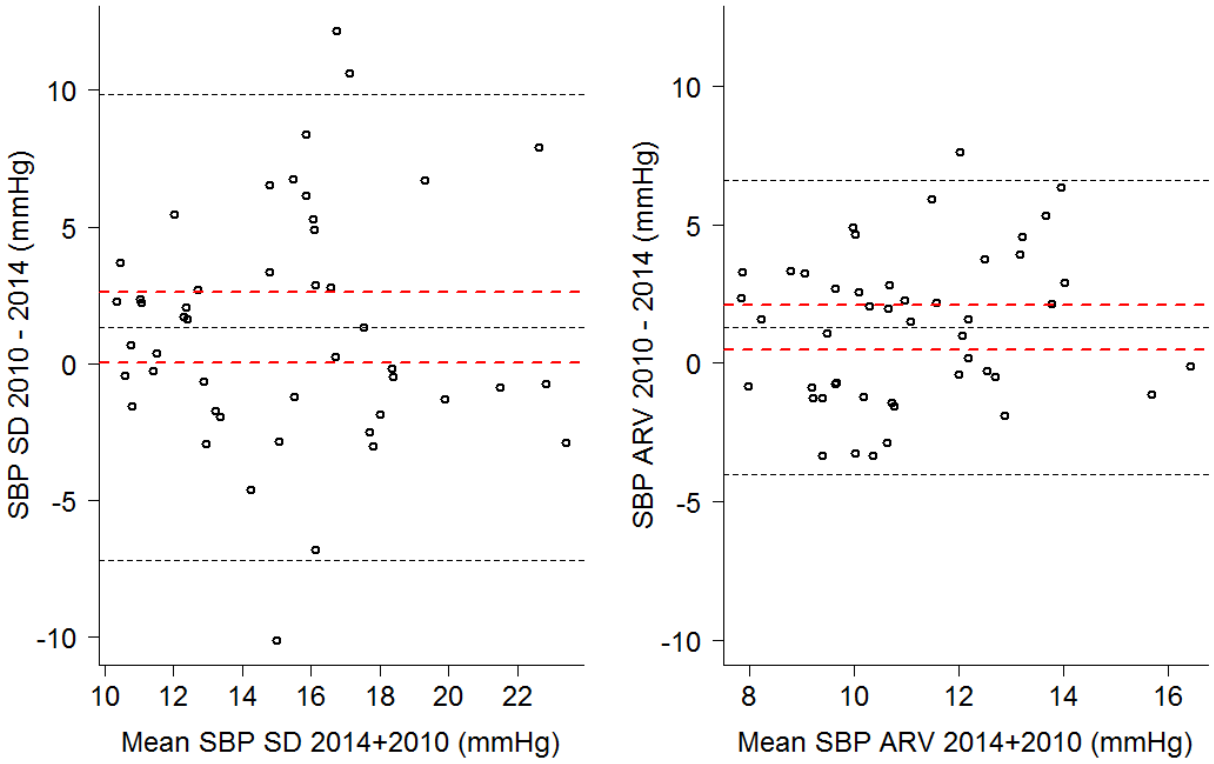


Figure 7-3 Bland-Altman scatter-plots of agreement of measurements of 24h SBP SD and 24h SBP ARV four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values.

Table 7-5 Comparison of SBP parameters from two separate two-component random-effects cosinor models (2010 vs 2014)

SBP **Parameter**	**Model Estimate (SE)** **Year = 2010**	**Model Estimate (SE)** **Year = 2014**
24h MESOR (mmHg)	127.1 (1.6)	123.2 (2.0)*
First Cosine (24h period):		
Amplitude (mmHg)	11.4 (1.3)	9.7 (1.1)
Phase shift (30mins)	5.0 (0.8)	5.1 (0.1)
Time of phase shift	14:30	14.33
Second Cosine (12h period):		
Amplitude (mmHg)	3.6 (0.6)	3.5 (0.7)
Phase shift (30mins)	0.6 (0.1)	1.6 (0.1)*
Time of phase shift	12:18	12.48
σ	12.7	11.5

*p<0.05 represents significance between 2010 and 2014, Phase shift measured from 12:00 noon. Time presented in 24h clock.

Table 7-6 Comparison of SBP parameters from two separate two-component random-effects cosinor models (2010 vs 2014)

SBP **Parameter**	**Total Variation in Differences**	**Variance due to Measurement Error (% of Total Variance)**	
		Based on 47 Simulations	**Based on 1000 Simulations**
24h MESOR (mmHg)	168.1	5.6 (3.3%)	6.1 (3.6%)
First Cosine (24h period):			
Amplitude (mmHg)	42.2	8.1 (19.2%)	8.8 (20.9%)
Phase shift (30mins)	2.1	1.3 (61.9%)	1.1 (52.4%)
Second Cosine (12h period):			
Amplitude (mmHg)	6.6	2.1 (31.8%)	1.9 (28.8%)
Phase shift (30mins)	1.7	1.5 (88%)	0.9 (52.9%)

*p<0.05 represents significance between 2010 and 2014, Phase shift measured from 12:00 noon. Time presented in 24h clock.

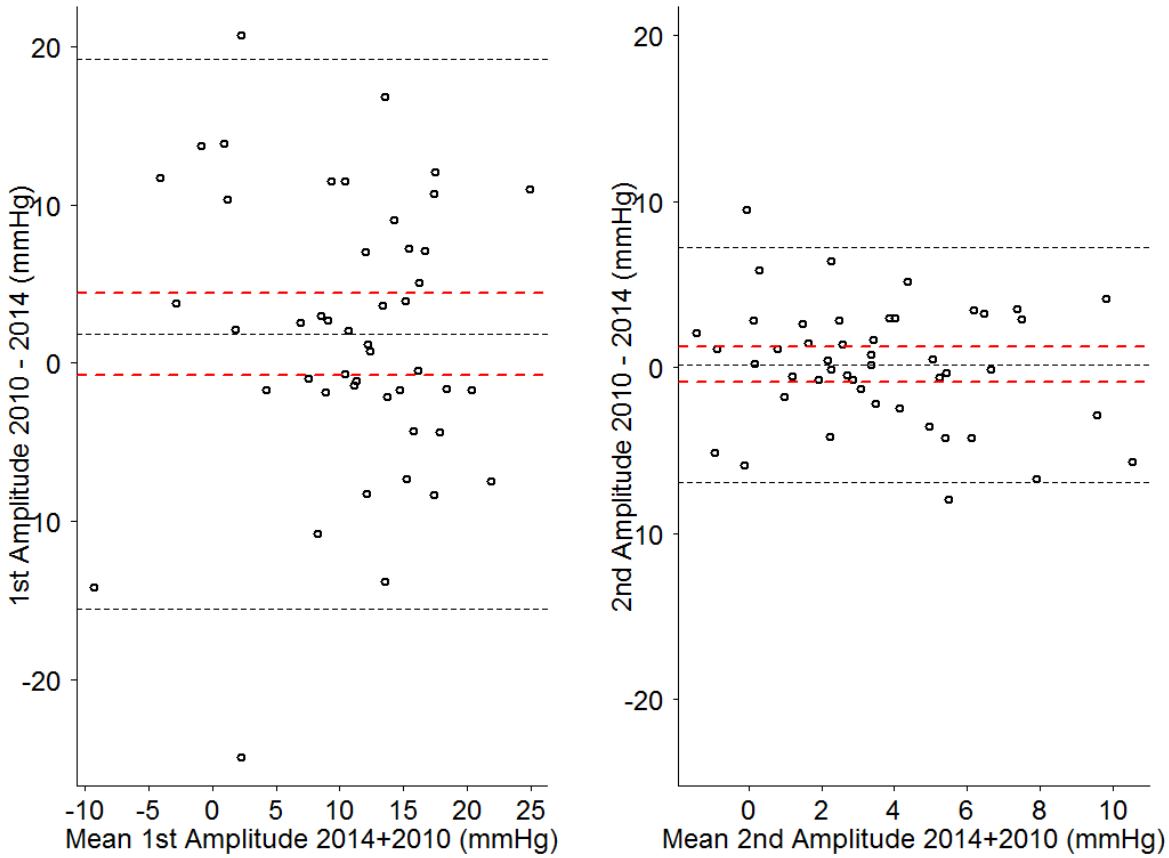


Figure 7-4 Bland-Altman scatter-plots of agreement of measurements of the first and second amplitudes four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values.

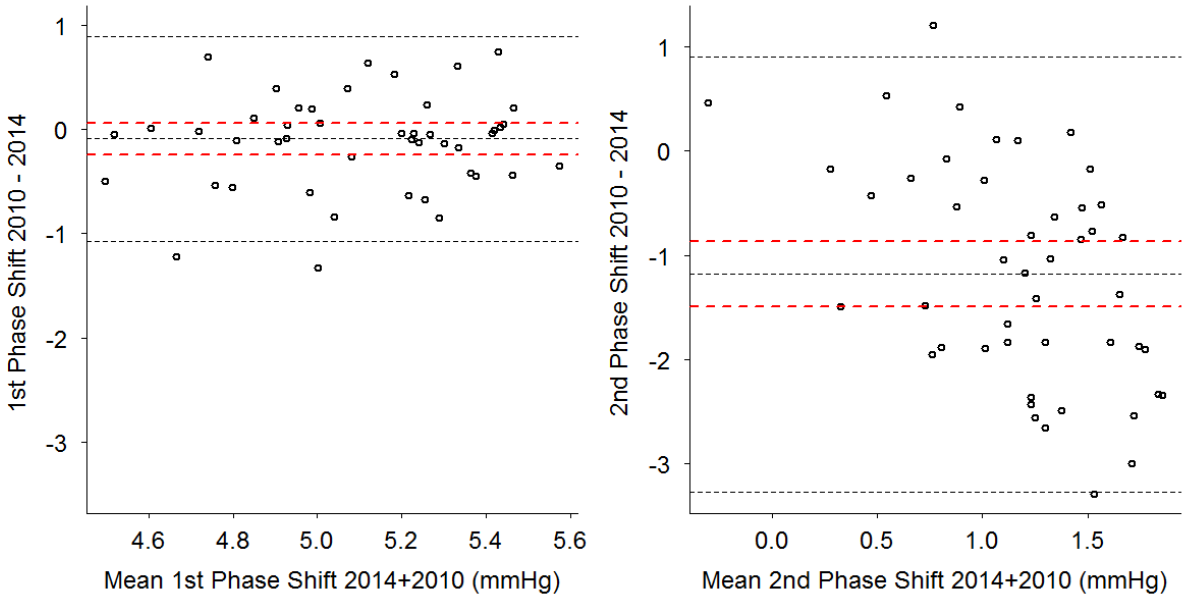


Figure 7-5 Bland-Altman scatter-plots of agreement of measurements of the first and second phase shifts four years apart. The x-axis represents the mean of the two measurements and the y-axis represents the difference between them. The black dashed lines represent the mean and limits of agreement. The dashed red lines represent the 95%CI for the differences in mean values.

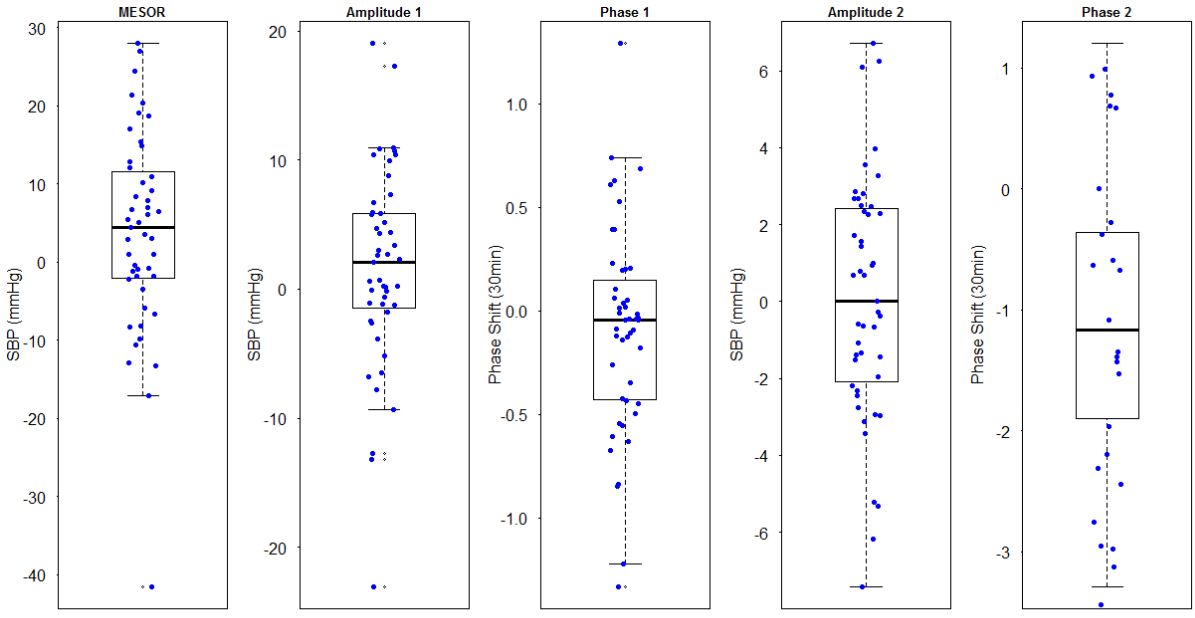


Figure 7-6 Boxplots of differences between each of the cosinor parameters between the two time-points (2010 minus 2014).

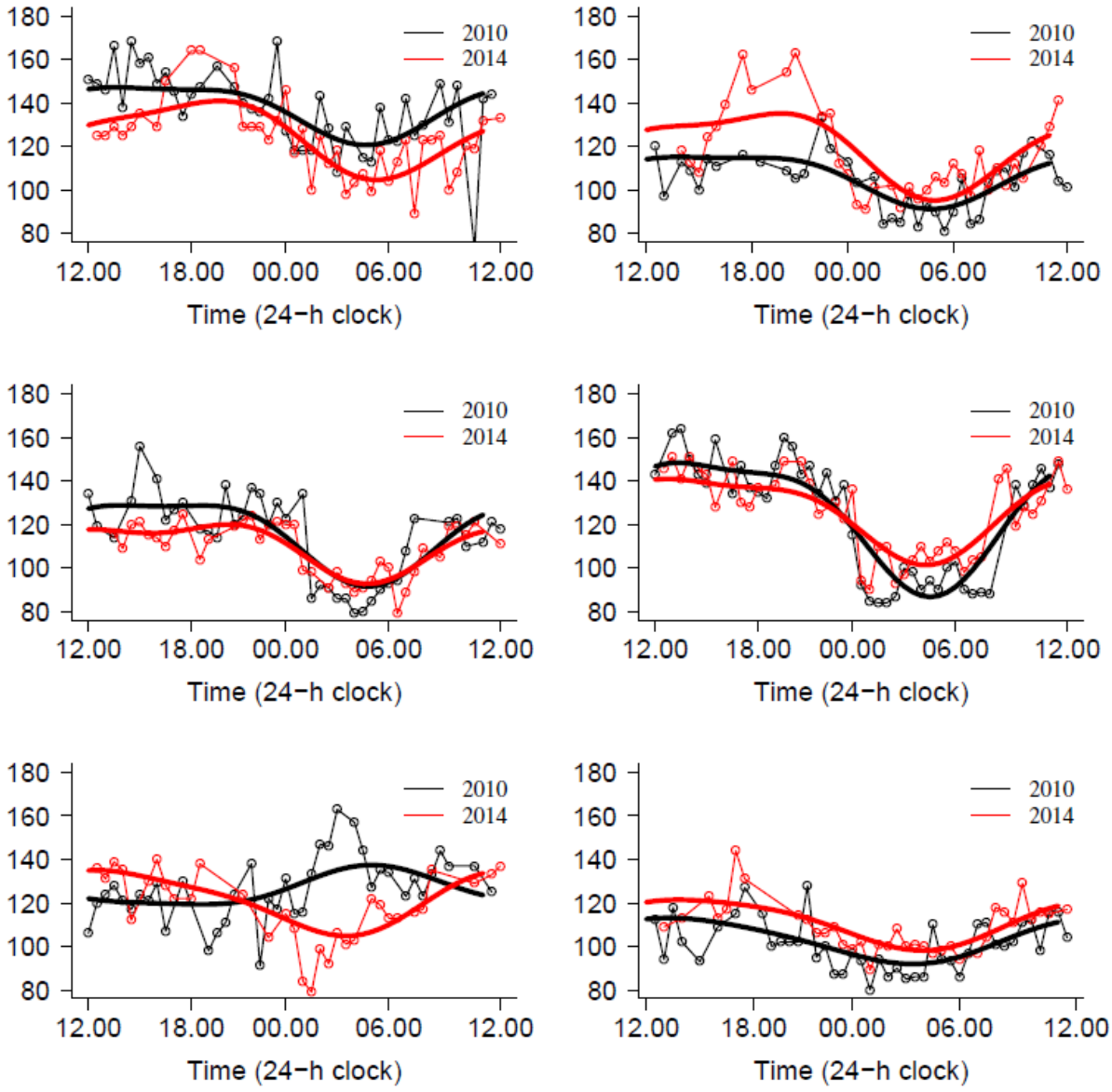


Figure 7-7 Comparison of fits, 2010 vs 2014, for six individuals using two separate two-component random-effects cosinor models.

8. DISCUSSION

This thesis aimed to explore circadian BP patterns by reviewing current and previous approaches for analysing ABPM data and describes novel methodologies that offer new measures of BP and may help us to obtain new insights into BP. The recent debate towards the prognostic significance of BPV and how it is quantified was the original motivating reason to undertake the analysis. The thesis first explores summary measures used to describe fluctuations in circadian patterns and then goes beyond this to obtain other indices through more advanced methods. This chapter outlines the main findings, and strengths and limitations of the thesis. Recommendations for areas of future research and a brief conclusion are also outlined.

8.1 Summary of Main Findings

In Chapter 3, the systematic review collated data to identify current summary measures that are used to quantify BPV and explored their association with the presence of TOD, specifically LVH. Four measures were identified; SD, ARV, wSD and CV. The meta-analysis suggested there is a weak positive correlation, between all measures of BPV and LVMI. Despite recent interest in the subject of BPV the systematic review highlighted the lack of good epidemiological studies exploring the relationship between BPV and LVH. The fact that there were so few studies (n=12) and only data available to perform a meta-analysis of such a weak marker (correlation coefficient) is evidence of this. In addition, a third of studies in the systematic review did not make adjustment for mean BP in any of their analysis which is a major methodological limitation. This is an important issue in the context of BPV: the adjustment for mean BP. The significance of mean BP has been well documented and is not under question but in order to determine if BPV is an additional clinic target it is paramount that any analysis adjusts for average BP.

In Chapter 4, following on from the systematic review, the measures identified were calculated for the Mitchelstown ABPM dataset and their association with subclinical TOD, documented by microalbuminuria and ECG LVH, were examined. The study found no association between any measure of BPV and LVH. Conversely, we found all but one measure (CV) associated with microalbuminuria and remained associated after adjustment for age, gender, smoking, BMI, diabetes and anti-

hypertensive treatment. However, when the models were further adjusted for mean BP the association did not persist for all indices.

Piecewise regression which is a simple but underutilised approach in epidemiological studies [190], allows separate slopes to be fitted to observations representing different periods throughout the day or more specifically periods before or after a "critical point". In Chapter 5, we implemented this technique to our data in the context of a mixed-effects model. This allowed us to obtain new measures of BP. In particular, the method allowed quantification of the steepest rise and fall in BP (along with measures of variation between-individuals for these parameters), which occurred just after waking (2.23 mmHg/30min) and immediately after falling asleep (-1.93 mmHg/30min) respectively. By definition of the model and in the context of the morning period this represents a novel measure of the rate of change of BP or morning surge. The within-subject variation about an individual's curve was also estimated (12.3 mmHg) and represents a measure similar to that of a SD summary measure. Despite a significant difference in mean BP values, we found no evidence that slopes were different within different periods of the day in those with and without TOD, suggesting a similar circadian pattern in both groups.

Cosinor analysis which uses a single cosine to model a circadian BP cycle has been the most common longitudinal method to analyse ABPM data. The method has rarely been extended to include more than one cosine term in the context of a random-effects model. In Chapter 6, we fitted a two-component cosinor random-effects model to our data and found that it provided a better fit than a single model and a similar fit to a more complex three-component model. Additionally, FPCA was performed on the data and it was determined that the first three principle components accounted for over 90% of the variation in the data. Not only did this offer an alternative method to quantify BPV, it also allowed us to determine if the two-component model was comparable to a more complex data driven approach

that obtained the optimal functions from the data. Our findings indicated that the parameters from the two-component cosinor model were highly correlated with the first three components from the FPCA. By obtaining first-order derivatives of the two-component cosinor model, we outlined a novel approach in the context of BP which can be used to visualise changes over time and, locate and quantify the magnitude of slopes at critical points along the circadian rhythm.

In Chapter 7, approaches to compare two ABPM readings obtained at different occasions were outlined. The purpose was to explore changes in variability over time and examine the reproducibility of patterns of circadian BP curves that go beyond looking at mean values and hypertension categories. To this end, we implemented the two-component model from Chapter 6 and compared parameters from the two time-points. Results indicated that mean levels of BP were similar four years later but SBP variability, measured by wSD, SD and ARV, over 24h and the night period had significantly fallen. When examining the variability measures for those on medication, wSD, SD and ARV, over 24h (all SBP) remained significantly reduced. Interestingly, there was no change in variability measures for individuals not on medication. Overall, the reproducibility of patterns was poor based on mean levels, variability measures, hypertension categories and cosinor parameters. Based on simulations we were also able to determine how much of the total variation in the difference between parameters and summary measures was attributable to measurement error alone.

8.2 Implications of Findings

What summary measure of BPV to use?

We have demonstrated that quantifying variability is difficult and attempting to do this with one summary measure makes the task significantly more challenging. The main disadvantage of using summary measures to quantify BPV is the loss of information as the statistical power associated with longitudinal data is lost by collapsing the data into one value. However, as previously stated, many ABPM software programs routinely include estimates of variability measures such as SD (dabl ABPM system, dabl LTD, Ireland) in their reports and are important reference points considering the ease with which they can be obtained.

We demonstrated that most but not all summary indices of BPV were associated (before adjustment for mean BP) with microalbuminuria (Chapter 4). This reiterates the problem of inconsistences among indices which makes understanding and collating of findings from studies particularly problematic. It is these inconsistences that illustrate how sensitive BPV measurement is and accentuates that each measure is in fact capturing different characteristics of variability. However, compared to other summary measures, we believe ARV is the most suitable index to quantify BPV. To reiterate, this measure is the mean of the absolute differences between successive readings which is independent of the mean value [36]. It has been argued that it is a more reliable representation of time series variability than 24h SD which only captures variability about a mean value [33, 36]. wSD has also been suggested as a better measure to use than 24h SD as it attempts to remove the influence of the day-night difference [33]. To elaborate, the primary reason that a number of studies have suggested SD is a poor measure is because it only reflects the dispersion of measurements around a single value (mean) and does not account for the order in which BP measurements were obtained and the longitudinal variation in the circadian data [36]. However, if we are only considering variability during a specific period i.e. awake or night period separately as opposed to the

whole 24h, the use of SD may not be as problematic as the day-night fall is not present.

What longitudinal model to use?

Parati *et al*. argue that rather than simplifying ABPM data into mean summary measures, incorporating all the data in more advanced models can lead to more robust estimates of clinically relevant parameters such as dipping status and morning BP surge [142]. In Chapter 2, a number of approaches to model ABPM data were outlined where we focused on the use of a piecewise linear and a cosinor model in subsequent chapters. Both a piecewise linear model and the use of derivative estimation is not new in medical research but in the context of BP they offer a simple but novel approach to analyse its circadian rhythm that help extract features from the pattern. As outlined in the thesis, there have been a number of modelling approaches applied to 24h BP but the ultimate goal remains the development of a physiological model that captures known features of the pattern while not over-fitting the data so that we can obtain clinically relevant parameters [142]. It is widely acknowledged that achieving this is a difficult task and there is no perfect model that captures all the features of the circadian rhythm simultaneously [49, 142-144]. Using a mathematical model over simple summary measures allows us to obtain a smooth predicted curve over the whole 24h period which removes some of the "noise" in the data. Removing noise in data is usually perceived as an advantage where we remove measurement error and the impact of any outliers and are left with the underlying pattern. In the case of BPV however where we are specifically trying to understand and capture fluctuations in BP, perhaps smoothing the data in a model that captures the whole 24h period may not be the optimal approach. The sudden jumps or spikes that are seen in ABPM data may be a response to emotional or psychological stress which can sporadically increase BP values and are therefore important to measure [50]. Although these spikes may be perceived as noise they may also represent an underlining feature of an individual's natural circadian rhythm. Some individuals may be more prone to abrupt changes due to stress or anxiety. Obtaining lower sampling frequency measurements such as beat-to-beat measurements may help in this regard but are not feasible in large

population based studies. We do however have to reach some compromise and perhaps modelling the data in separate segments similar to the piecewise model represents a suitable approach. However, graphically comparing circadian curves obtained at different time-points may be better achieved using the cosinor method which gives a smoother fit where differences in the pattern of the shape can easily be seen. We reiterate the point made in Chapter 2 that no model is optimal in terms of capturing all features of the curve and choosing one involves making a compromise based on what the researcher deems most important for their particular research question. Using mathematical models does allow us to obtain an almost infinite number of BP indices. Comparing these measures between new anti-hypertensive medications may allow us to determine if a drug outperforms another when there is no significant difference in means values.

Policy and Practice

With the HSE recently acknowledging the importance of ABPM by approving the reimbursement of ABPM in primary care for those with a medical card, obtaining values of BPV is now more accessible [22]. In practice, summary measures will continue to be the most common method of quantifying BPV due to the ease with which they can be calculated coupled with their automatic inclusion in some ABPM output reports. A recommendation to improve monitors and their output would be the inclusion of the ARV index which could be achieved with minimal effort. There have been calls in the most recent ABPM position paper to include all measures of BPV in ABPM research reports but many just include SD [13]. However, there are no current threshold levels for variability in BP guidelines which makes interpretation of BPV measures difficult for clinicians. A long-term goal would be to eventually have standardised cut-points for which levels of BPV would be controlled to, similar to that of mean BP. For this to happen however we will need stronger evidence of the importance of BPV. As highlighted in the position paper, the current threshold values for mean BP levels in the NICE guidelines [17], the JNC 7 guideline [218], and the ESH/ESC guidelines for 2003, 2007 and 2013 [13, 18, 219], and the results of

outcome studies, such as IDACO [220] and Ohasama [221] have contributed to the definition of ABPM consensus values currently being used in practice [13]. A similar level of research output is required to obtain conclusive evidence on the prognostic significance of BPV before we can begin to arrive at suitable variability thresholds.

More advanced methods to model BP and measure BPV have been suggested as particularly useful in research studies rather than directly used in practice [13, 142]. This is where we currently suggest the piecewise and cosinor approaches outlined in this thesis should be primarily implemented where they may be particularly beneficial in clinical trials. However, we suggest that the inclusion of the cosinor approach in an ABPM report could be quite useful when comparing two ABPM patterns from the same individual. As outlined in Chapter 7, it offers a quick graphical comparison that maybe helpful for a clinician when they are trying to gauge changes in an individual's underlying circadian rhythm. We argue the inclusion of the predicted curves in ABPM reports, in combination with the standard output may improve clinical decisions and ultimately, BP control.

8.3 Strengths and Limitations

The strengths and limitations of each paper have been discussed in previous chapters. In this section a summary is provided with a focus on the thesis as a whole.

The thesis inevitably has a number of limitations. Although the use of ABPM is considered the gold standard of BP measurement and is recommended by guidelines, the monitors will not always provide accurate readings. Undoubtedly, there will be some measurement error from the true BP value especially for example, during the night when an individual may be lying on the cuff. In the Mitchelstown cohort every effort was made by study nurses to emphasise the importance of wearing the monitor correctly. In addition, all monitors were recalibrated before the study began as recommended [17]. The physical inconvenience of wearing the monitor may introduce selection bias where certain groups of individuals do not feel comfortable wearing the device. For example, perhaps frailer individuals would be more concerned wearing a monitor than stronger individuals. Wood *et al*. explored reasons for ABPM uptake among 770 participants of different ethnicities in the United Kingdom [222]. In their qualitative analysis, reasons suggested for poor uptake included discomfort, sleep disruption, stress and embarrassment, and interruption of activities of daily living. In the Mitchelstown study, ABPM was offered to all participants but those with high BP were encouraged by the study nurses to wear a monitor. No qualitative data was recorded for reasons of not availing of ABPM. Despite a response rate of 58%, the ABPM subsample was found to be broadly representative of the full sample based on age, gender, BMI and diabetes prevalence (see Appendix E for details). However, the prevalence of hypertension (≥140/90 mmHg and/or on antihypertensive treatment) was higher in the baseline ABPM subsample (46% vs 59%). This introduces a selection bias based on hypertension only where there is a systematic difference between the subsample and the main sample. This may affect interpretation of the results where caution must be exercised when discussing the generalizability of the results to the population. It must be stressed that the only difference was that based on hypertension and this does not necessarily affect the

validity of the comparisons and inferences made within the study or the study's internal validity [223, 224].

Although a lot of the work in this thesis involved exploring the shape of circadian BP curves, Chapter 3 and 4 directly examined the association between BPV and TOD. A statistically significant association between an exposure (BPV measure) and an outcome (TOD) is not sufficient to imply causality. Hill's casual considerations offer guidance [225]. He listed nine considerations, none of which are essential, that are used in epidemiology to build up evidence for a causal relationship: strength, consistency, temporality, dose-response, plausibility, reversibility, coherence, analogy and specificity [223]. The strength of the association in this study between BPV and TOD was weak and did not persist after adjustment for mean BP. The systematic review examining the relationship between BPV and LVMI suggested a weak association and also provides evidence of poor consistency among studies. Temporality in this study is difficult to ascertain. BPV leading to the development of TOD and in turn CVD is the hypothesis made in most of the literature but perhaps as argued by Hansen *et al.*, as TOD is a forerunner of cardiovascular complications, BPV will inevitably increase [168]. This raises the question of reverse causality with increased BPV being a marker of underlying disease rather than being an independent predictor.

Although the precise mechanisms responsible for BPV are not entirely understood [50], there are a number of acknowledged factors that influence variations in BP which have been postulated as to why BPV may cause the development of TOD, and ultimately CVD. Parati *et al.* [51, 52] argue BPV is primarily modulated by neural (increased central sympathetic drive and reduced arterial and cardiopulmonary reflexes), humoral (angiotensin II, insulin, bradykinin, nitric oxide) and vascular effects (elastic properties of arteries). Variability has been shown to be correlated with arterial stiffness and peripheral vascular disease which is a blood circulation disorder that causes blood vessels outside of your heart and brain to narrow, block or spasm which can lead to CVD [25]. Rothwell *et al.* suggest that BPV could lead to cerebral ischaemia (insufficient blood flow to the brain) which could lead to both altered central autonomic control of BP and an increased risk of stroke [25].

Reversibility refers to whether an intervention to remove or reduce the exposure results in the elimination or reduction of the outcome [223]. We cannot assess this from our results. A clinical trial comparing the impact of different antihypertensive classes on BPV and ultimately TOD would help to address this. Coherence is also difficult to assess based on the evidence in this study. However, experimental data from animal models lend support to a causal link. It was previously shown that experimental sinoaortic denervation in rats, which increases variability in BP without changing mean BP, caused LVH and aortic vasoconstriction [24, 226]. Analogy refers to the similarity of the effect of interest to other established cause-effect relationships that would help support the argument for causality [223]. In our case, elevated mean BP has long been established as a causal factor for the development of TOD and CVD. As a result, it could be argued that small or large fluctuations in BP may also contribute to CVD but this is speculative. In conclusion, a causal relationship between BPV and TOD is difficult to prove based on the findings in this study and the literature available at present.

Another limitation of the thesis is the use of TOD as surrogate markers of CVD rather than the use of hard endpoints. The reason for this was simply the feasibility and time constraints of the thesis where only data from wave one is currently collected. Although hard endpoints were recorded in the study (MI, stroke), the number of events were small which precluded using them. Data collection for wave two is currently on-going but we may need to wait for subsequent waves to have adequate number of events to detect an association, if one exists.

Due to the nature of short-term BPV, we are examining a small time frame, only 24h. Ideally, we would like participants to continue to wear the monitors for a number of days or perhaps obtain separate ABPM recordings taken a few days apart to be sure of reliable observations. This would also allow the comprehensive analysis of the reproducibility of day-to-day circadian patterns. However, the practicalities and feasibility of wearing a monitor for a prolonged period of time in this cohort study meant we were unable to obtain more readings.

As highlighted in the thesis one of the key aspects of BPV has been the suggestion that different classes of antihypertensive medications have different effects in terms of increasing or reducing variability. Emerging evidence suggests that those receiving either CCBs or diuretics, alone or in addition to other drugs have significantly lower variability compared with ACEIs, ARBs or β-blockers alone or in combination [61-63, 65, 227]. A limitation of the study was that we were not able to include the effect of antihypertensive medication in the analysis. Although we knew if a participant was on treatment, we had insufficient data on the specific class of antihypertensive medication the individual was prescribed which meant drug-class comparisons were not possible. As one of the questions in the study questionnaire asked if the participant was taking antihypertensive drugs, it allowed analysis to be stratified by those taking and not taking medications. Findings were broadly similar across both groups (Chapter 4, 5). It could be the case that there are differences between classes but by analysing them all together the effect has been diluted. When examining changes to short-term variability over time (Chapter 7), evidence suggested that BPV in those on medication significantly reduced while there was no change in variability measures for individuals not on medication.

Despite the limitations outlined, the thesis has several strengths. Specifically, it addresses a relevant and highly topical area in BP research. To date, two of the chapters have been published in peer reviewed scientific journals while a third is under review. The work is both warranted and appropriate to increase awareness and to help develop a deeper understanding of the mechanisms responsible for BPV which are not entirely known. Methods to analyse circadian BP patterns that account for the longitudinal nature of the data are sparse and this thesis outlines new approaches. The thesis includes the first review to our knowledge that quantifies the correlation between BPV and LVMI which identifies a research gap where stronger epidemiological studies are needed in the area. To the best of our knowledge, the thesis is the first to apply a piecewise linear model and FPCA to

diurnal BP over 24h. We have demonstrated that incorporating more cosine terms into the cosinor model offers a substantial improvement in fit compared to the traditional method of ABPM analysis, the single-component model. One of the main strengths of the study lie in the robust community based design and large sample of ABPM data recorded. Although the inferences that are made may not be applicable across all age groups and populations there are no reasons why the methods outlined cannot be used on them to obtain valid estimates of BP. This argument can be extended where many of the chapters and methods are not limited to the exploration of BP but are flexible enough to be applied to other medical data that follows a circadian rhythm.

8.4 Future Recommendations for Research

As we have alluded to, it is evident that more evidence from large longitudinal studies examining the prognostic significance of short-term BPV is needed before suitable variability thresholds can be introduced. There is a need for standardisation of indices that we use to define BPV. This has been highlighted by a recent systematic review of BPV examining its prognostic value for all-cause mortality, cardiovascular mortality, all cardiovascular events, stroke and coronary heart disease [191]. Taylor et al. argue that the interpretation and use of 24h BPV in clinical practice, as a prognostic indicator of cardiovascular events is hampered by insufficient evidence and divergent methodologies [191]. Agreement and standardisation of indices may not be fully possible for a number of years until there is a plateauing effect in the number of methods used to quantify variability. We have however, made the recommendation to use the ARV index as the primary summary measure of BPV. Rather than using different indices, if all future studies included this measure it would make collating results easier. Indeed this thesis has developed yet further methods to explore circadian patters but as Dolan *et al.* argue each new measure helps to bring about new insights and is required to advance our understanding of BP [66]. We are still however, searching for the optimal measure to quantify variability over a short-period; this is as much a mathematical problem as it is clinical. This as the thesis has shown, is not easily achieved.

In the context of the single-component cosinor model which has been the most common method of analysis for ABPM, a recommendation for future studies from the evidence presented in this thesis is to incorporate a second cosine in the context of a random-effects model. The method offers a substantial improvement in fit compared to the traditional cosinor that is capable of capturing short-term peaks and can be implemented in standard statistical software.

In Chapter 7, we found that short-term BPV measured at two occasions, four years apart, decreased in a small sample. Few longitudinal studies explore how variability

changes between two ABPM readings. In those that have, inconsistent findings were reported [213, 214]. More research into how short-term variability changes over time is warranted and further studies exploring the reproducibility of ABPM patterns is needed. It must be remembered that the emphasis in this thesis has solely been on short-term patterns and not long-term variability which represents how much BP fluctuates between visits (months or years).

The prognostic significance of visit-to-visit BPV seems to be more transparent than in the case of short-term BPV. The most recent meta-analysis of 77,299 patients suggests that visit-to-visit SD was significantly predictive of all-cause mortality, cardiovascular mortality and stroke, after adjustment for mean BP [26]. As there is usually less data-points when analysing long-term compared to short-term data there is less opportunity to apply more advanced techniques. Studies have primarily used SD as a measure of variability. This has helped in collating data from studies, although it may not be the best measure of BPV. However, the issues associated with measuring short-term BPV may not be as evident for long-term BPV. The primary reason is that long-term variability does not have a cyclic component to it and there is no dipping effect like ABPM. Long-term variability usually refers to fluctuations in clinic BP values which have no night component. In this case, the use of SD as a measure of variation may be adequate. Another recommendation would be to investigate the association between short-term BPV and the development of long-term BPV which there is no data to the best of our knowledge. Perhaps long-term BPV is the cumulative aggregate of short-term BPV but we will not know the answer to this until large longitudinal studies are conducted. Perhaps obtaining multiple ABPM readings over the course of a short period e.g. one month, may yield the same information and benefits of running a long-term study over a couple of years which would save on time and reduce financial costs.

In context of the work presented in this thesis and following on from the limitation of examining surrogate markers, the most obvious recommendation is to explore the methods presented in the thesis with long-term follow up data that have hard endpoints e.g. cardiovascular events. The second wave of data collection is currently on-going for the Mitchelstown cohort. However, in order to have

adequate number of events to detect an association, if one exists; data from subsequent waves will probably have to be obtained.

The benefit of our findings may lie in the analysis of clinical trial data, particularly in the analysis of chronotherapy effects. The methods offer estimates of BP at important times of the day that define a rate of change. This could be applied after the ingestion of different antihypertensive drugs to identify which medication produced the quickest fall in BP levels. Debate is on-going into the benefits of night-time administration of antihypertensive medication compared to daytime administration but there has been substantial evidence in its favour [67-70]. The rationale behind the argument for this seems both plausible and reasonable. That is, administering medication at night will help pre-empt the magnitude of the morning surge by reducing BP during the night so that on waking, the surge is beginning at a lower value and thus not reaching as high a peak. When medication is ingested at night there should also be a larger reduction in night-time BP which has been highlighted as a stronger predictor of outcome than 24h mean. The latest on-going Treatment in Morning versus Evening (TIME) trial with 10,200 patients followed for 5 years is anticipated to provide definitive evidence of whether or not there is a benefit to administering antihypertensive medications in the evening to provide better protection against major adverse cardiovascular events [228, 229].

The methods outlined in this thesis are not restricted to the analysis of BP but can be applied to the many other physiological processes that follow a circadian rhythm such as cortisol, heart rate etc. In this regard it would be interesting to know if the methods, especially the more advanced approaches could be of use in analysing and answering questions in other medical fields. Indeed, it could be the case that the methods outlined could be more suited to important aspects of a particular circadian process other than BP. For purposes of dissemination of methods and particularly to act as a source for other researchers who are interested in extending on the work in the thesis, sample R code has been included in Appendix H. This may also be useful for researchers in other fields' aside from BP. Relevant R packages utilised are also included along with the version of R used. The run time for the non-linear model is also presented

8.5 Conclusion

This thesis has demonstrated different approaches to explore circadian BP patterns over a 24h period. Results indicated that most summary measures of BPV were associated with microalbuminuria but the association did not persist after adjustment for mean BP. There was no association between BPV and LVH. Where more advanced models were used incorporating all the data, the rate of increase or fall throughout different periods over 24h remained the same between those with and without TOD. A novel method to quantify morning surge was also presented which may be useful in future studies. FPCA may also be a novel method to determine major components of variations in 24h BP. BPV quantification, its predictive value and potential as a therapeutic target will remain controversial until more studies are conducted and an agreement on how best to accurately quantify BPV has been reached. The thesis, which had a large methods and statistical component, illustrated novel techniques that can be implemented with standard software and whose application may also be useful for analysing other medical datasets that follows a circadian process.

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Appendix A Supplementary material (Chapter 2)

Polynomial Regression

Briefly, a 6th order polynomial was applied to the dataset and measures of variability were obtained from subject-specific predictions. As one measure of variability the sum squared of the differences between the observed and subject-specific profiles was calculated which reflected a participant's individual BP variation across the 24h period. This is a similar measure to that calculated by Sega *et al.* except we used the variation about the individual curve rather than the mean [29]. Maximum, minimum and number of minutes spent above certain hypertensive guidelines were also calculated as measures of variability. The association between the extracted BPV measures and the presence of TOD was assessed using logistic regression with adjustment for age, sex, smoking status, BMI, diabetes and antihypertensive treatment. Additional models adjusted for mean clinic BP.

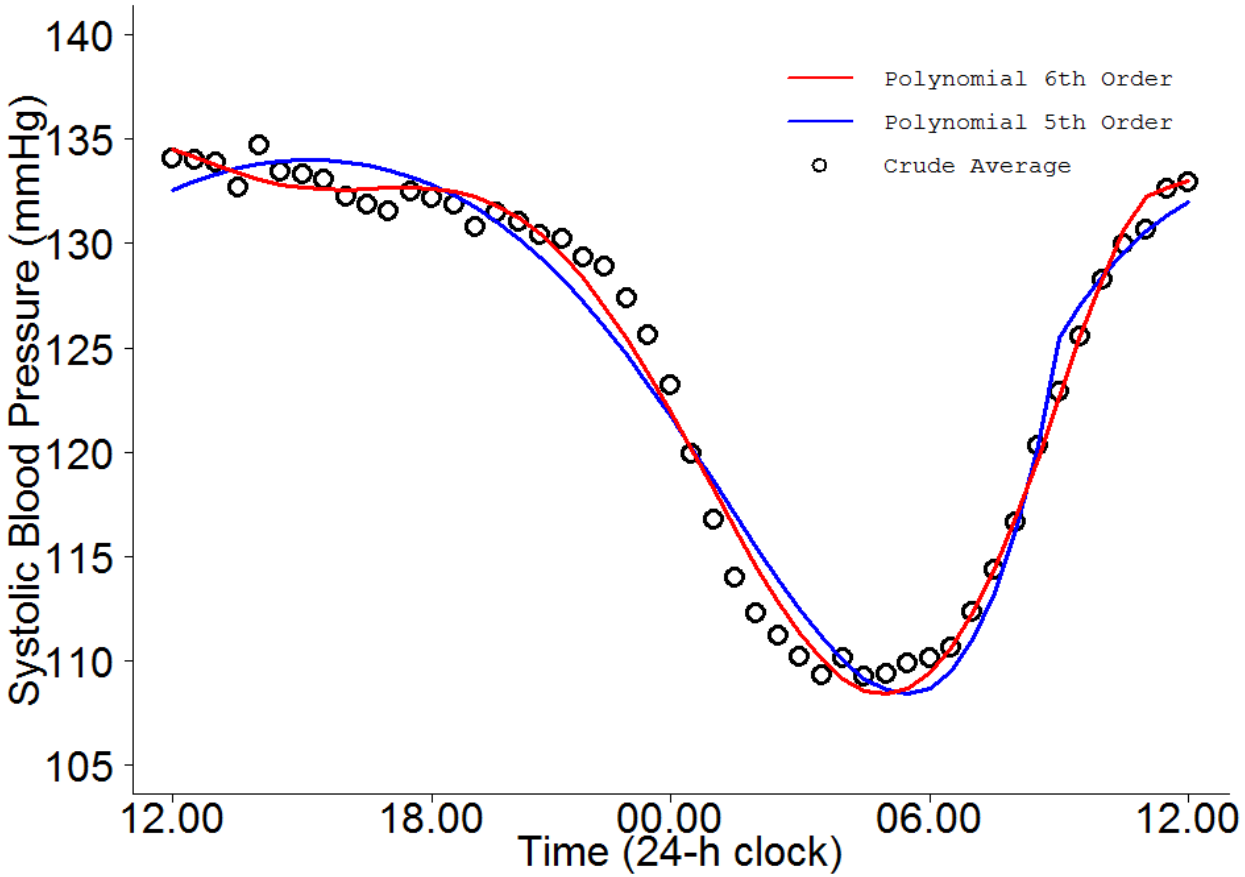


Figure 9-1 Population Level Effects for polynomial regression (Chapter 2)

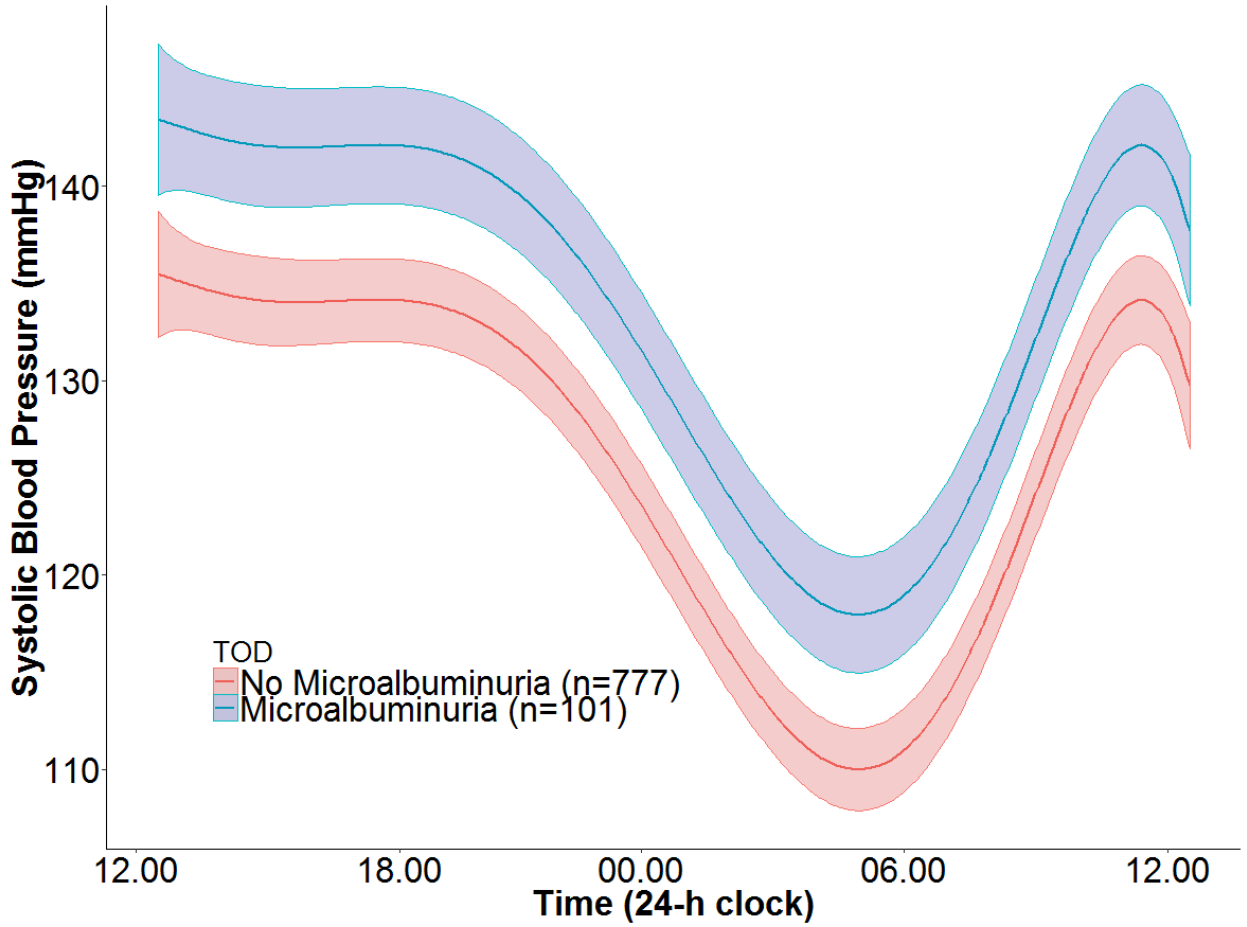


Figure 9-2 Population Level Group Average (95 % CI) Linear Mixed Effects Model (Age, Sex, BMI Adjusted) (Chapter 2)

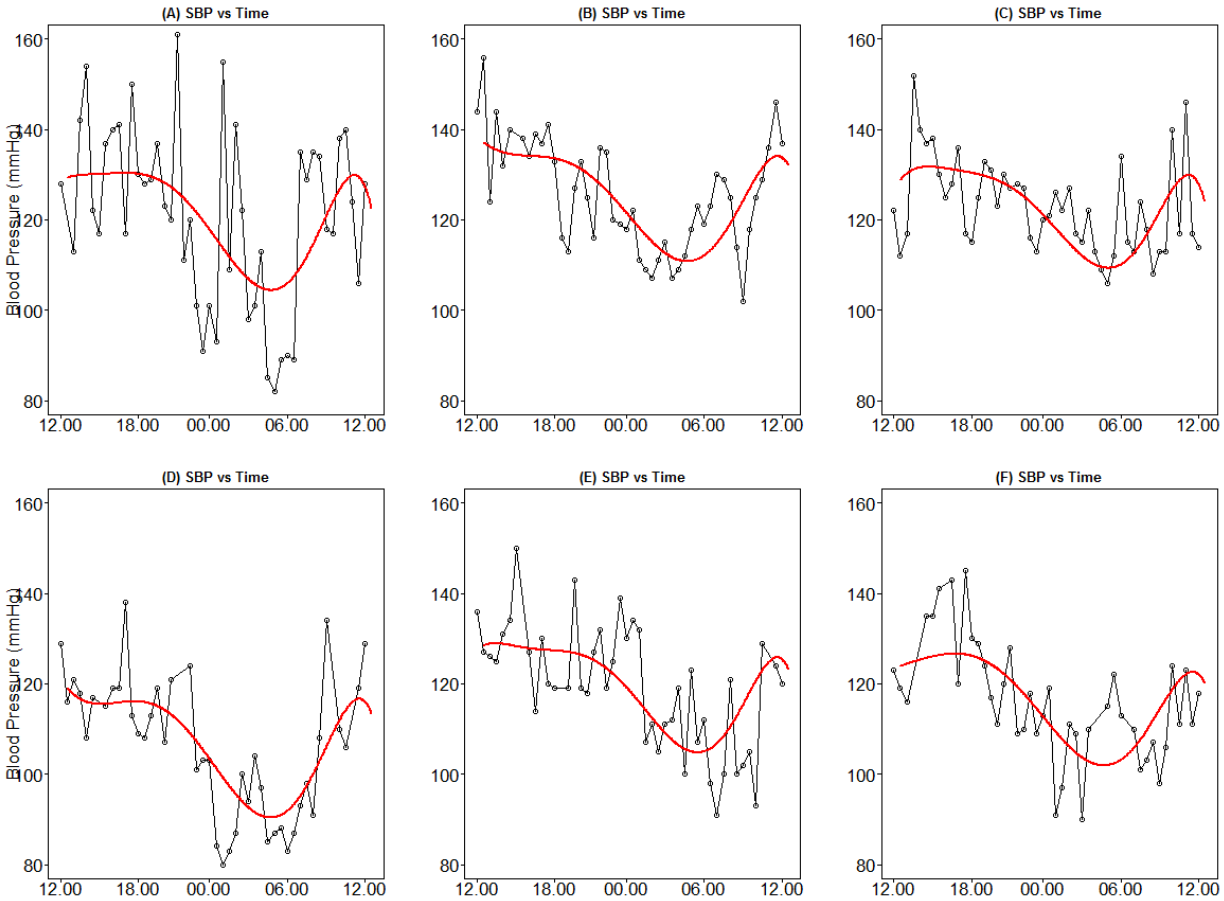


Figure 9-3 Subject-Specific Variation (function of Time only)

Table 9-1 Association between parameters extracted from polynomial regression model and microalbuminuria (logistic regression).

Characteristic (SBP)	Value (SD)	Microalbuminuria (10.6%)	
		Model 1	Model 2
		OR (CI%) per hour or per 10mmHg	OR (CI%) per hour or per 10mmHg
Max Morning, mm Hg	133 (13.0)	1.53 (1.30-1.81)*	0.24 (0.03-1.83)
Time	11.54pm (18min)	0.85 (0.59-1.24)	0.80 (0.55-1.15)
Minimum, mm Hg	108 (12.7)	1.60 (1.35-1.90)*	2.74 (0.90-8.33)
Time	4.51am (11min)	1.08 (0.59-0.97)	0.82 (0.45-1.49)
Hours ≥ 130 mm Hg (24 h)†	8.4 h (7.9h)	1.07 (1.04-1.10)*	0.98 (0.91-1.04)
Hours ≥ 135 mm Hg (Day)†	5.2 h (6.4h)	1.06 (1.02-1.10)*	0.95 (0.89-1.01)
Hours ≥ 120 mm Hg (Night)†	2.5 h (3.1h)	1.14 (1.07-1.21)*	0.96 (0.85-1.09)
Variability About Curve	123 (58.1)	1.05 (1.02-1.08)*	1.01 (0.97-1.05)

†European Society of Hypertension ABPM Guidelines: 24 h ≥130/80 mmHg; Day (awake) ≥135/85 mmHg; Night (sleep) ≥120/70 mmHg. *p-value <0.01. Model 1 adjusted for age, sex, smoking, BMI, diabetes, anti-hypertensive medication. Model 2 adjusted for age, sex, smoking, BMI, diabetes, anti-hypertensive medication, 24h SBP

Appendix B Supplementary material (Chapter 3) - search terms and search strategy for systematic review

EMBASE search strategy

#1. exp abpm variability/

#2. exp abp variability/

#3. exp ambulatory bp variability/

#4. exp ambulatory blood pressure variability/

#5. exp ambulatory blood pressure monitor variability/

#6. exp ambulatory blood pressure monitoring variability/

#7. exp 24hour BP variability/

#8. exp 24-hour blood pressure variability/

#9. exp 24h blood pressure variability/

#10. exp 24h bp variability/

#11. exp 24 hour ambulatory blood pressure variability/

#12. exp 24 hour ambulatory bp variability/

#13. exp 24 hour ambulatory blood pressure monitor variability/

#14. exp 24 hour ambulatory blood pressure monitoring variability/

#15. exp short term bp variability/

#16. exp short term abpm variability/

#17. exp short term blood pressure variability/

#18. exp short term abp variability/

#19. exp 24 hour abpm variability/

#20. exp 24 hour abp variability/

#21. exp 24h abp variability/

#22. exp 24-hour abp variability/

#23. exp 24-hour abpm variability/

#24. exp 24-hour ambulatory blood pressure monitoring variability/

#25. exp 24-hour ambulatory blood monitoring variability/

#26. exp bp variability/

#27. exp blood pressure variability/

#28. (#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27)

#29. left ventricular hypertrophy: ab,ti

#30. left ventricular hyperthrophy: ab,ti

#31. left ventricular hypertrophy: ab,ti

#32. left ventricular hypertrophic: ab,ti

#33. left ventricular mass: ab,ti

#34. left ventricular mass index: ab,ti

#35. target organ damage: ab,ti

#36. organ damage: ab,ti

#37. target-organ damage: ab,ti

#38. (#29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37)

#39. (#28 and #38)

#40.#39[Limit: humans & article]

PubMed search strategy

#1. abpm variability [all]

#2. abp variability [all]

#3. ambulatory bp variability [all]

#4. ambulatory blood pressure variability [all]

#5. ambulatory blood pressure monitor variability [all]

#6. ambulatory blood pressure monitoring variability [all]

#7. 24hour BP variability [all]

#8. 24-hour blood pressure variability [all]

#9. 24h blood pressure variability [all]

#10. 24h bp variability [all]

#11. 24 hour ambulatory blood pressure variability [all]

#12. 24 hour ambulatory bp variability [all]

#13. 24 hour ambulatory blood pressure monitor variability [all]

#14. 24 hour ambulatory blood pressure monitoring variability [all]

#15. short term bp variability [all]

#16. short term abpm variability [all]

#17. short term blood pressure variability [all]

#18. short term abp variability [all]

#19. 24 hour abpm variability [all]

#20. 24 hour abp variability [all]

#21. 24h abp variability [all]

#22. 24-hour abp variability [all]

#23. 24-hour abpm variability [all]

#24. 24-hour ambulatory blood pressure monitoring variability [all]

#25. 24-hour ambulatory blood monitoring variability [all]

#26. bp variability [all]

#27. blood pressure variability [all]

#28. (#1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #15 or #16 or #17 or #18 or #19 or #20 or #21 or #22 or #23 or #24 or #25 or #26 or #27)

#29. left ventricular hypertrophy [all]

#30. left ventricular hyperthrophy [all]

#31. left ventricular hypertrophy [all]

#32. left ventricular hypertrophic [all]

#33. left ventricular mass [all]

#34. left ventricular mass index [all]

#35. target organ damage [all]

#36. organ damage [all]

#37. target-organ damage [all]

#38. (#29 or #30 or #31 or #32 or #33 or #34 or #35 or #36 or #37)

#39. (#28 and #38)

#40. #39[Limit: humans & adults]

PRISMA checklist (Systematic review checklist for Chapter 3)

Section/topic	#	Checklist item	Reported on page # (see pdf version appendix)
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title page (p1)
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract (p2)
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction (p3,4)
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction (p4)
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods – Types of studies, Study populations, predictor variables, outcomes (p5)
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods – search methods for identification of studies (p5,6)
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Methods – search methods for identification of studies (p5,6)

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods – search methods for identification of studies, data extraction (p5,6)
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods –data extraction (p6)
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods –data extraction (p6)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Methods— search methods for identification of studies (p6), Quality assessment (Table 4)
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Methods—Statistical analysis (p6) Correlation Coefficient
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Methods—Statistical analysis (p6)

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Methods—Statistical analysis (p6)
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Methods—Statistical analysis (p6)
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Results (p7) & Flowchart (Figure 1)
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Results (p7) & Table 1,3
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Results (p8) & Quality assessment (Table 4)

Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Results (p7), Table 1,3 & Forest Plot (Figure 2)
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Results (p7) & Forest Plot (Figure 2)
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Results- Meta-analysis (p8,9)
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Results- Meta-analysis (p8,9) & Forest Plot (Figure 2)
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion (p9)
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Discussion (p9,10, 11, 12)
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Discussion (p9,10, 11, 12)
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Title page (p1) & Conflict of Interest (p12)

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Appendix C Supplementary material (Chapter 4)

Table 9-2 Baseline Characteristics by anti-hypertensive treatment

Characteristic	Total (n=1134)	Untreated (n=757)		Treated (n=377)	
		Men (n=362)	Female (n=395)	Men (n=165)	Female (n=212)
Age, y	60.2 (5.5)	59.5 (5.6)	59.5 (5.5)	61.6 (5.1)	61.9 (5.3)
BMI, n (%)					
Underweight/normal	230 (20.3)	56 (15.5)	130 (32.9)	8 (4.9)	36 (17.0)
Overweight	498 (44.0)	187 (51.8)	171 (43.3)	65 (39.4)	75 (35.4)
Obese	405 (35.7)	118 (32.7)	94 (23.8)	92 (55.7)	101 (47.6)
Smoking, n (%)					
Never	564 (51.9)	133 (38.8)	241 (63.3)	70 (43.2)	120 (59.7)
Former	355 (32.7)	142 (41.4)	86 (22.6)	71 (43.8)	56 (27.9)
Current	168 (15.4)	68 (19.8)	54 (14.2)	21 (13.0)	25 (12.4)
Diabetes, n (%)	103 (9.3)	34 (9.6)	14 (3.6)	31 (19.1)	24 (11.4)
Hypertension, n (%)	487 (43.0)	140 (38.7)	148 (37.6)	92 (55.8)	107 (50.5)
LVH, n (%)	75 (6.6)	34 (4.5)	15 (3.8)	21 (12.7)	20 (9.4)
Microalbuminuria, n (%)	128 (11.4)	73 (9.7)	32 (8.2)	24 (14.6)	31 (14.8)
ABPM measurements					
24-h SBP, mm Hg	124.1 (13.3)	126.0 (12.6)	119.9 (13.7)	128.0 (12.4)	125.8 (12.7)
24-h DBP, mm Hg	71.8 (8.3)	74.4 (8.4)	69.6 (7.6)	74.3 (8.6)	69.5 (7.4)
Awake SBP, mm Hg	131.4 (14.1)	133.5 (13.4)	127.2 (14.8)	134.8 (12.6)	132.9 (13.3)
Awake DBP, mm Hg	77.4 (9.0)	80.1 (9.0)	75.3 (8.5)	79.3 (9.3)	74.9 (8.3)
Sleep SBP, mm Hg	112.3 (14.0)	112.9 (13.4)	108.5 (13.5)	117.1 (14.3)	115.0 (13.9)
Sleep DBP, mm Hg	62.8 (8.3)	64.6 (8.7)	60.6 (13.5)	66.0 (9.2)	61.3 (7.2)

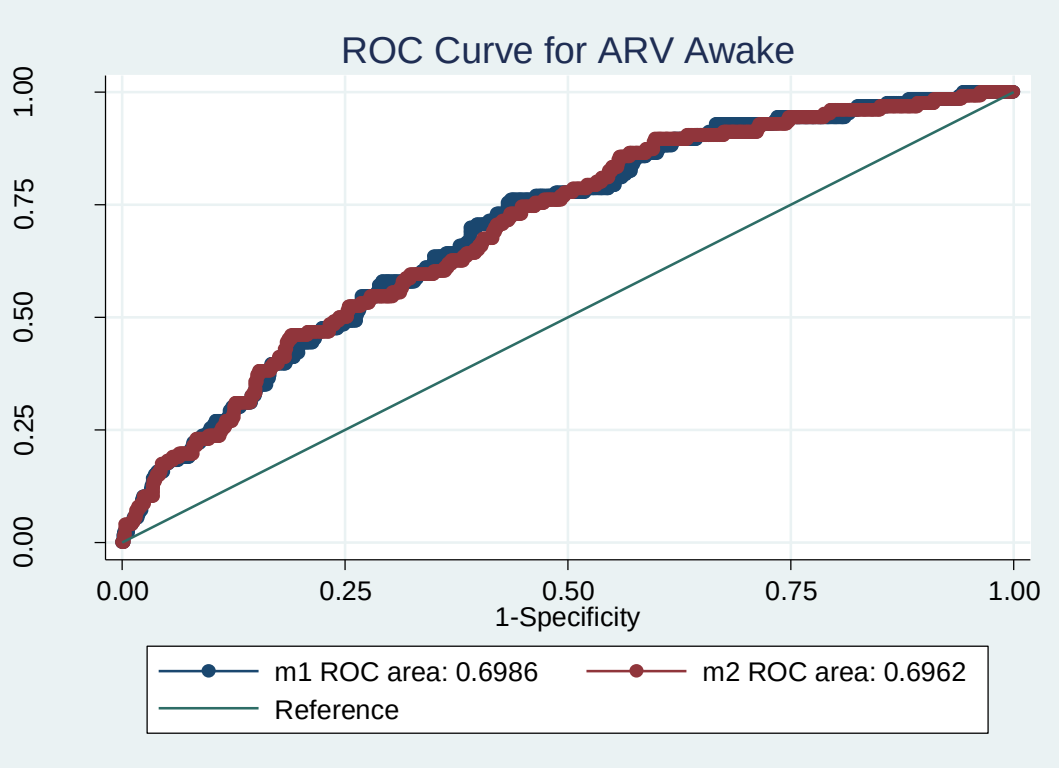
Data are mean (SD). ARV: Average real variability, SBP: Systolic Blood pressure, DBP: Diastolic blood pressure, BMI:Body mass index, LVH: Left ventricular hypertrophy, ABPM: Ambulatory blood pressure monitor. Microalbuminuria: albumin:creatinine ratio ≥ 1.1 mg/mmol

Table 9-3 Variability Indices and Dip by White-Coat Hypertension

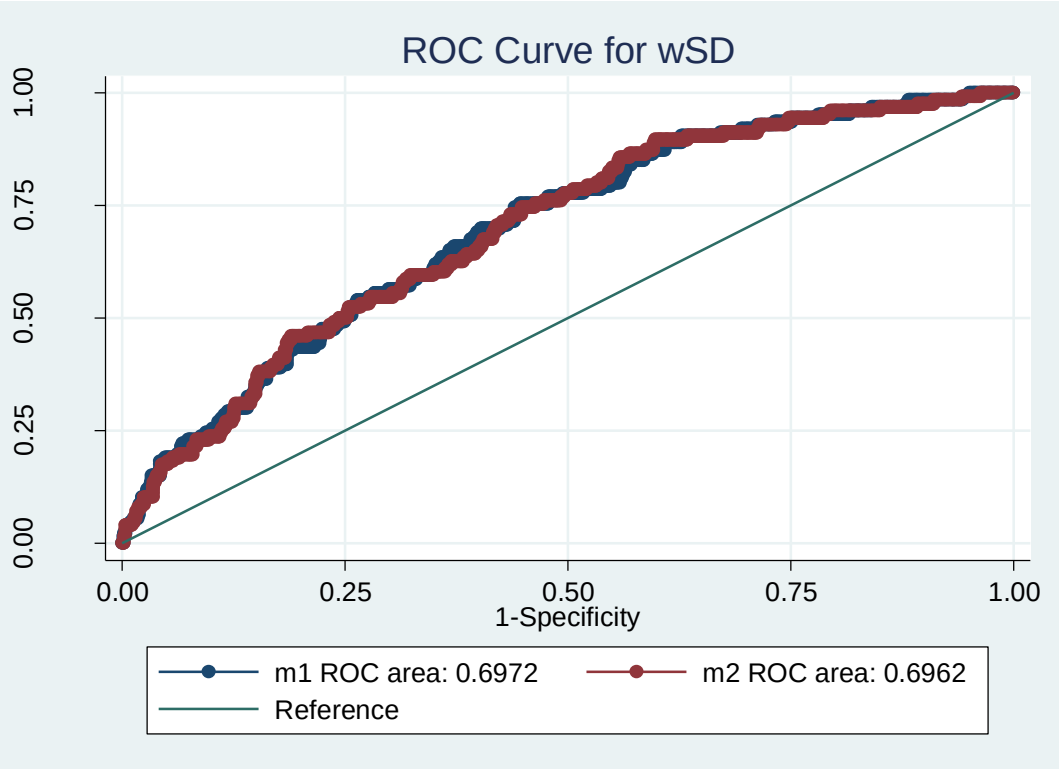
Variability	24-hour (mm Hg)		Day (mm Hg)		Night (mm Hg)	
Measure	No (n=986)	Yes (n=147)	No	Yes	No	Yes
ARV SBP	11.4 (2.6)	11.1 (2.0)	11.9 (3.2)	11.5 (2.7)	10.6 (3.3)	10.6 (2.6)
ARV DBP	7.8 (1.8)	7.3 (1.4) **	8.2 (2.4)	7.6 (2.2) **	7.3 (2.2)	6.9 (1.8) *
SD SBP	15.9 (4.0)	15.4 (3.0)	12.9 (3.6)	13.0 (3.0)	11.2 (3.6)	10.7 (2.7)
SD DBP	11.5 (2.8)	10.9 (2.4) *	9.0 (2.8)	8.6 (2.5)	8.1 (2.4)	7.6 (2.0) *
CV SBP	12.8 (2.9)	12.8 (2.8)	9.7 (2.4)	10.1 (2.4)	10.0 (3.0)	9.8 (2.5)
CV DBP	16.1 (3.8)	15.6 (3.4)	11.6 (3.7)	11.5 (3.5)	12.9 (3.8)	12.5 (3.5)
wSD SBP	12.3 (3.0)	12.2 (2.2)	-	-	-	-
wSD DBP	8.7 (2.1)	8.2 (1.7)	-	-	-	-
Dip SBP	14.4 (7.0)	14.2 (6.8) *	-	-	-	-
Dip DBP	18.7 (7.7)	18.2 (7.2)	-	-	-	-

Data are mean (SD). ARV: Average real variability, SD: Standard deviation, wSD: Weighted standard deviation, CV: coefficient of variation, SBP: Systolic Blood pressure, DBP: Diastolic blood pressure, *p<0.05; represents significance between those with and without white-coat hypertension during each period of the day

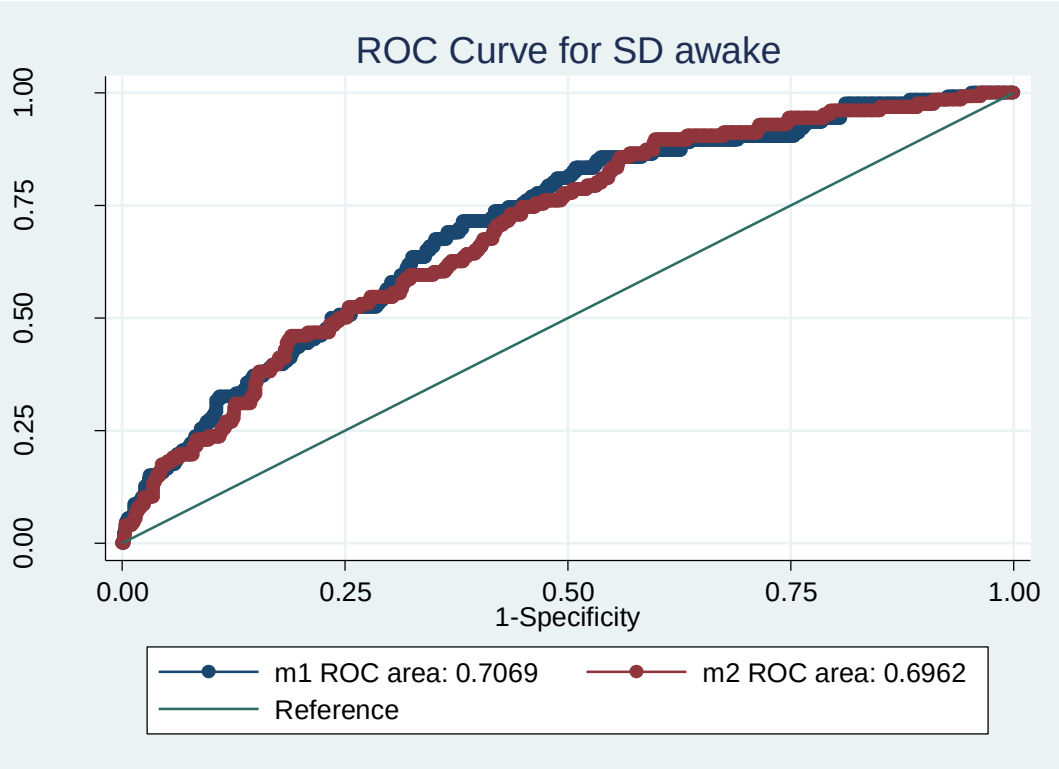
ROC curves for microalbuminuria (Chapter 4 supplementary material)



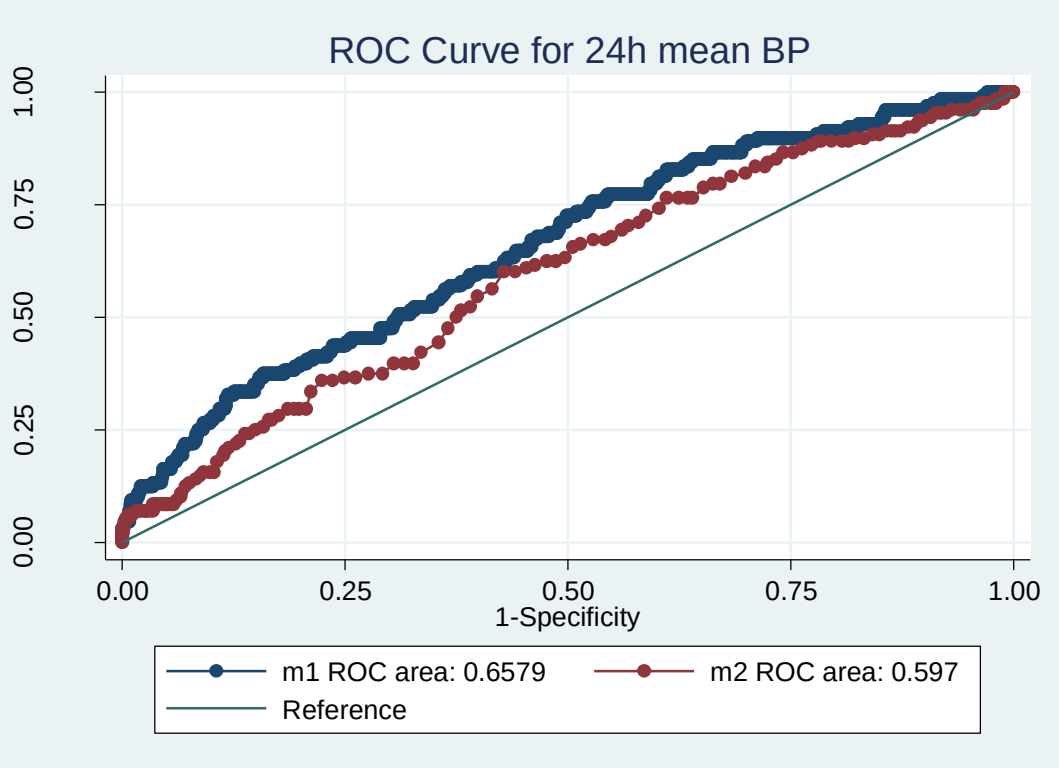
ARV awake ROC curve. M1: Age, Sex, 24-h mean BP, ARV awake, Smoking, Diabetic, bmi, anti-hypertensive; M2: Age, Sex, 24-h mean BP, Smoking, Diabetic, bmi, anti-hypertensive (p-value=0.56)



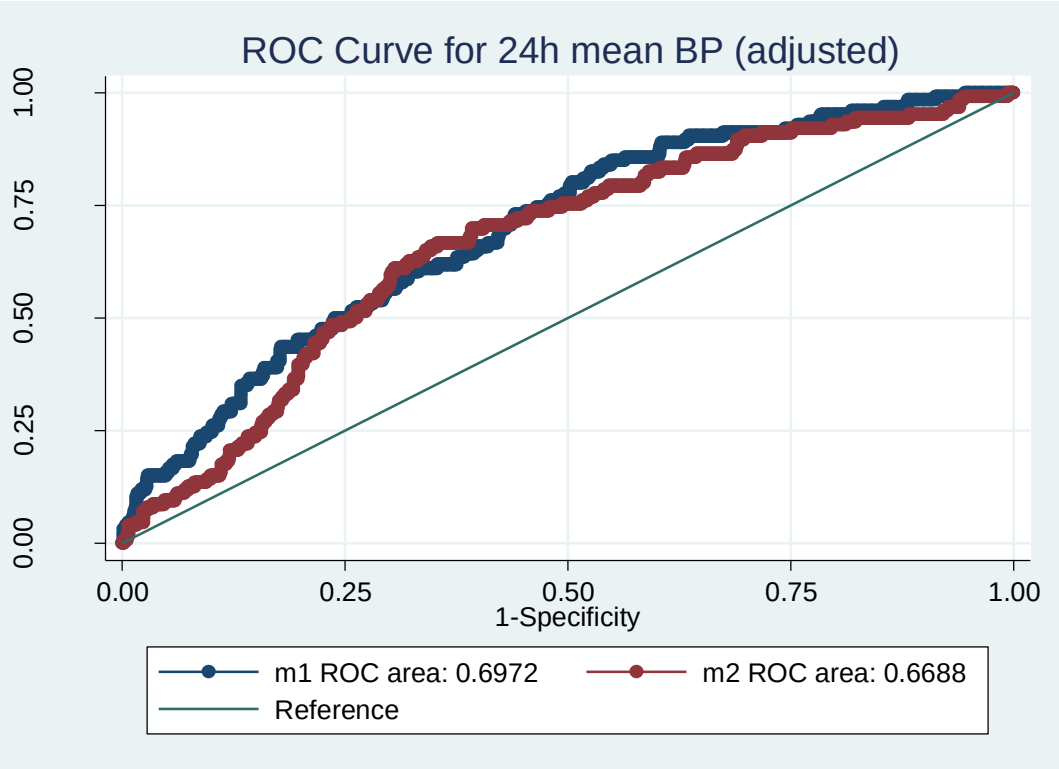
wSD ROC curve. M1: Age, Sex, 24-h mean BP, wSD, Smoking, Diabetic, bmi, anti-hypertensive; M2: Age, Sex, 24-h mean BP, Smoking, Diabetic, bmi, anti-hypertensive (p-value=0.73)



SD awake ROC curve. M1: Age, Sex, 24-h mean BP, SD awake, Smoking, Diabetic, bmi, anti-hypertensive; M2: Age, Sex, 24-h mean BP, Smoking, Diabetic, bmi, anti-hypertensive (p-value=0.25)



24-h mean BP ROC curve. M1: 24-h mean BP, clinic BP; M2: clinic BP (p-value=0.01)



24h mean BP ROC curve. M1: Age, Sex, 24-h mean BP, clinic BP, Smoking, Diabetic, bmi, anti-hypertensive; M2: Age, Sex, clinic BP, Smoking, Diabetic, bmi, anti-hypertensive (p-value=0.13)

Appendix D Supplementary material (Chapter 5)

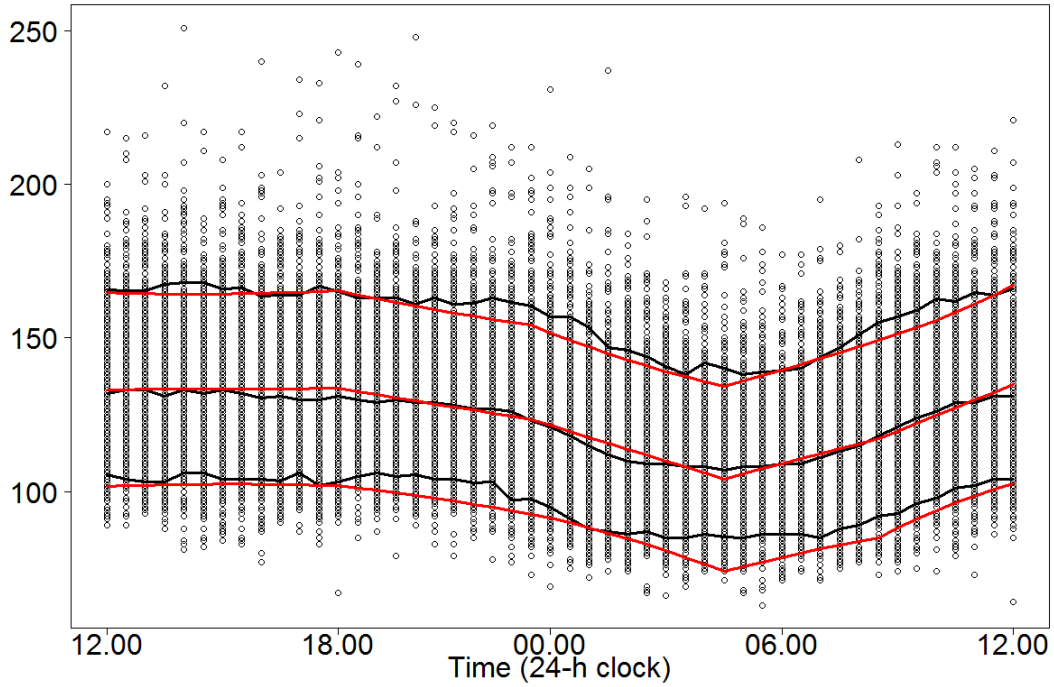


Figure 9-4 VPC (visual predictive check) plot. Black lines represent median of observed data with 90% interquantile range of observations. Red line is predicted mean along with 90% prediction interval.

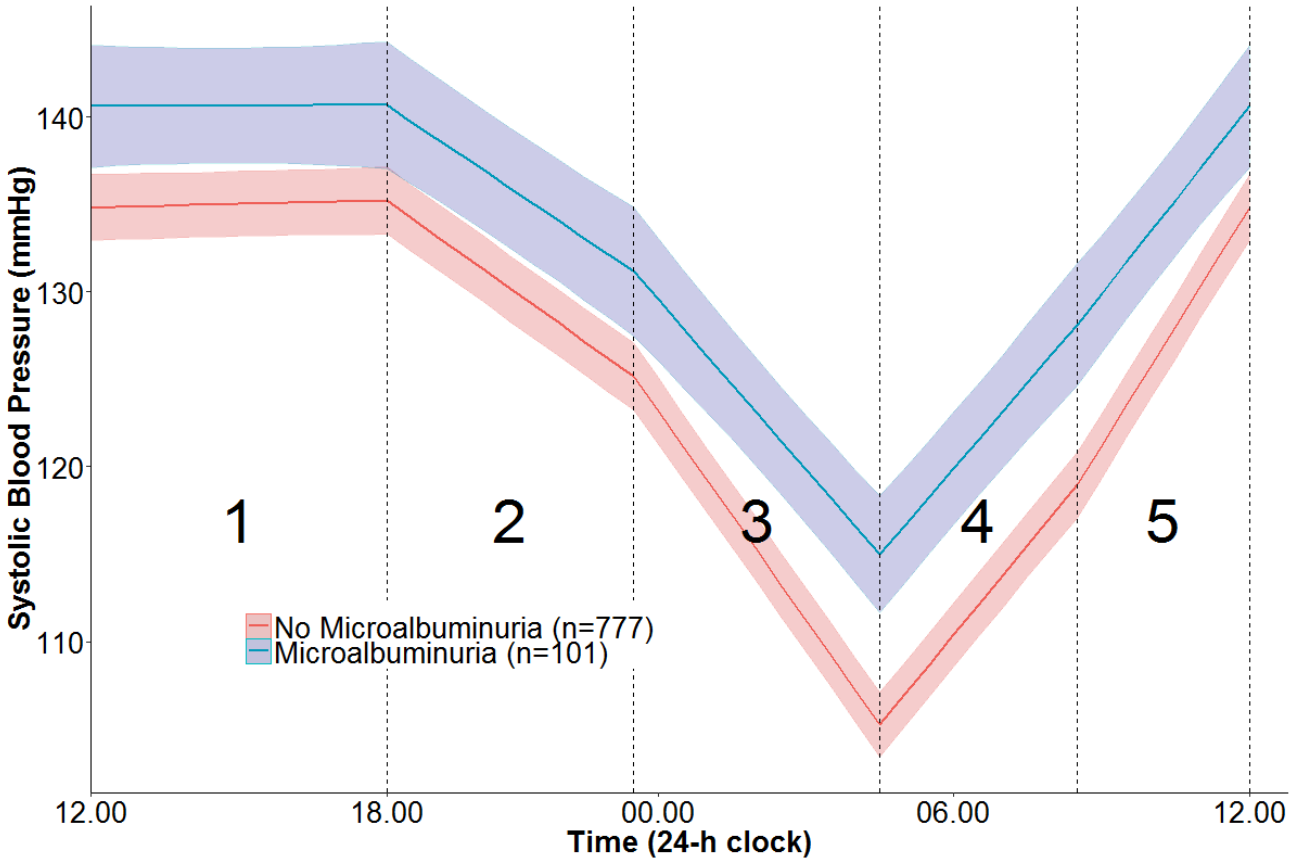


Figure 9-5 Predicted average (95% CI) piecewise linear trajectory of those with/without presence of microalbuminuria adjusted for age, sex, BMI and including an interaction of the spline terms with microalbuminuria using a linear mixed-effects model (Model 3). Each linear spline represents the rate of BP increase or decrease (slope) for that time period which is referred to in Table 5-2.

Appendix E Supplementary material (Missing Data)

(References for this appendix are at the end of the section.)

Although some studies have monitors set to collect data less frequently at night compared to daytime, sometimes every 30mins during the day and every hour at night, our study had the advantage that data was collected at the same sampling frequency, 30mins throughout 24h. Earlier ESH guidelines recommended a minimum of 14 measurements during the day and a minimum of 7 measurements during the night as the satisfactory number of data points for analysis [1-3]. However the more recent guidelines from the current position paper on ABPM say that stricter criteria should be used especially for research purposes [3, 4]. Specifically they recommend only participants with a minimum of 20 measurements during the day and a minimum of 7 measurements during the night period should be retained. Additionally, any participants with data lacking for more than two consecutive hourly intervals should be excluded [3, 4]. Additionally, when examining day and night values they recommended fixed time periods to identify these periods but we had the advantage of having diary entries of actual bed and rising times.

Unfortunately we had no available data that was collected on the reasons why there may be missing data in our study. However, common reasons for missing ABPM values include the patient disconnecting the device, suspension of a reading by use of the cancelation button, turning the monitor off, dead batteries, movement artefact, or kinks in the tubing [5]. Many of these reasons may lead to an occasional or sporadic missed reading but multiple missing data may suggest there is something else at fault such as a monitor malfunctioning and ignoring data from such a monitor seems appropriate. Another issue perhaps is that a certain group of individuals may have increased level of missing data. One study examining the influence of patient characteristics on the success of ABPM recording found that those with diabetes and elevated BMI were associated with less complete ABPM session results [6]. In relation to our study we explored differences between

those with and without the minimum measurements criteria, see Table 9-4. Similar to the Fravel *et al.* study we found that those with higher BMI were more likely to have missing data. However there was no significant difference in the prevalence of diabetes. BP was slightly lower in those with missing data but importantly there was no significant difference between groups based on hypertension classification (≥140/90 mmHg and/or on antihypertensive treatment). There was no difference in gender but the missing group were marginally older (59.9 vs 60.7 years, p=0.01). The difference in BMI may be explained by the practicality of wearing the monitor. Perhaps those with a higher BMI were given a standard size cuff resulting in the device either being too restricted and returning an error or it was too painful to wear and the participant had to remove it. Without any evidence however we are only speculating and the difference between groups was not that large (1.3 kg/m^2). Importantly from Table 9-4, the ABPM subsample was broadly representative of the full sample as prevalence rates were similar. However, those on ABPM had a higher prevalence of hypertension. ABPM was offered to all patients but those with high BP were encouraged to wear a monitor. This may affect the generalizability of the results but will not affect the estimates from our models for this study sample. It must be noted that even in the ABPM sample that met the criteria, there was still some missing data points. In terms of mixed-effects models, the approach is only valid when the incomplete dataset is MAR or MCAR [7-8]. In any real-life dataset missing data will never be completely MCAR, MAR or missing not at random (MNAR) but rather a combination of each. An assumption has to be made and in the case of our work we believe MAR is a reasonable assumption. As we have adhered to the most recent ABPM guidelines and feel our remaining ABPM sample is representative of the full sample it is reasonable to analysis those with the appropriate number of readings as set out by the guidelines. In addition there have been studies illustrating that methods that deal with missing data such as multiple imputation in mixed-effects models are not necessary and can result in unstable estimates and misleading inferences [7,9-10].

Table 9-4 Table of demographics comparing full sample to ABPM sample

		ABPM Subsample (n=1207)	
Characteristic	**Full Sample (n=2047)**	**ABPM ‡ (n=886)**	**ABPM Missing (n=321)**
Age, y	59.8 (5.5)	59.9 (5.5)	60.7 (5.6)*
Gender, Male n(%)	1008 (49.2)	401 (45.3)	163 (50.8)
BMI (kg/m^2)	28.6 (4.7)	28.6 (4.6)	29.9 (5.3)*
Office SBP, mm Hg	129.6 (16.9)	134.7 (17.7)	131.7 (17.1)*
Office DBP, mm Hg	80.1 (9.8)	83.1 (10.2)	80.7 (10.2)*
Hypertension, n (%)	951 (46.5)	528 (59.7)	182 (56.7)
Diabetes n (%)	174 (8.7)	81 (9.3)	34 (10.9)

Data are mean (SD) unless otherwise stated. BMI: Body mass index, ABPM: Ambulatory blood pressure monitor. Hypertension: ≥140/90 mmHg and/or on antihypertensive treatment. Diabetes: based on hbA1c greter than 6.5% and doctor diagnosed. ‡ABPM group based on minimum 20 valid awake and minimum 7 valid asleep and ≥2 valid daytime and 1 valid night-time measurement per h. *p-value<0.05, where p-value is comparing two ABPM groups.

References for Appendix E:

[1] O'Brien E, Coats A, Owens P, *et al.* Use and interpretation of ambulatory blood pressure monitoring: recommendations of the British Hypertension Society. BMJ 2000; 320: 1128.

[2] Fagard RH, Staessen JA, Thijs L. Optimal definition of daytime and night-time blood pressure. Blood Pressure Monitoring. 1997;2(6):315-21.

[3] O'Brien E, Parati G, Stergiou G. Ambulatory blood pressure measurements: What is the International Consensus? Hypertension. 2013;62:988–94.

[4] O'Brien E, Parati G, Stergiou G, Asmar R, Beilin L, Bilo G, *et al.* European Society of Hypertension position paper on ambulatory blood pressure monitoring. Journal of Hypertension. 2013;31(9):1731-68.

[5] Flynn JT, Daniels SR, Hayman LL, Maahs DM, McCrindle BW, Mitsnefes M, Zachariah JP, Urbina EM; American Heart Association Atherosclerosis, Hypertension and Obesity in Youth Committee of the Council on Cardiovascular Disease in the Young. Update: ambulatoryblood pressure monitoring in children and adolescents: a scientific statement from the American Heart Association. Hypertension. 2014; 63:1116–1135.

[6] Fravel MA, Ernst ME, Weber CA, Dawson JD, Carter BL, Bergus GR. Influence of patient characteristics on success of ambulatory blood pressure monitoring. Pharmacotherapy 2008; 28: 1341–47.

[7] Twisk J, de Boer M, de Vente W, Heymans M. Multiple imputation of missing values was not necessary before performing a longitudinal mixed-model analysis. J Clin Epidemiol 2013;66:1022

[8] Gibbons RD, Hedeker D, DuToit S. Advances in analysis of longitudinal data. Annual Review of Clinical Psychology. 2010;6:79-107.

[9] Little, RJA and Rubin, DB. Statistical analysis with missing data 2nd ed. Wiley, New York, NY, 2002

[10] Peters, SAE, Bots, ML, den Ruijter, HM, Palmer, MK, Grobbee, DE, Crouse, JR III, *et al.* Multiple imputation of missing repeated outcome measurements did not add to linear mixed-effects models. J Clin Epidemiol, 65 (2012), pp. 686–695

Appendix F Supplementary material (Chapter 6)

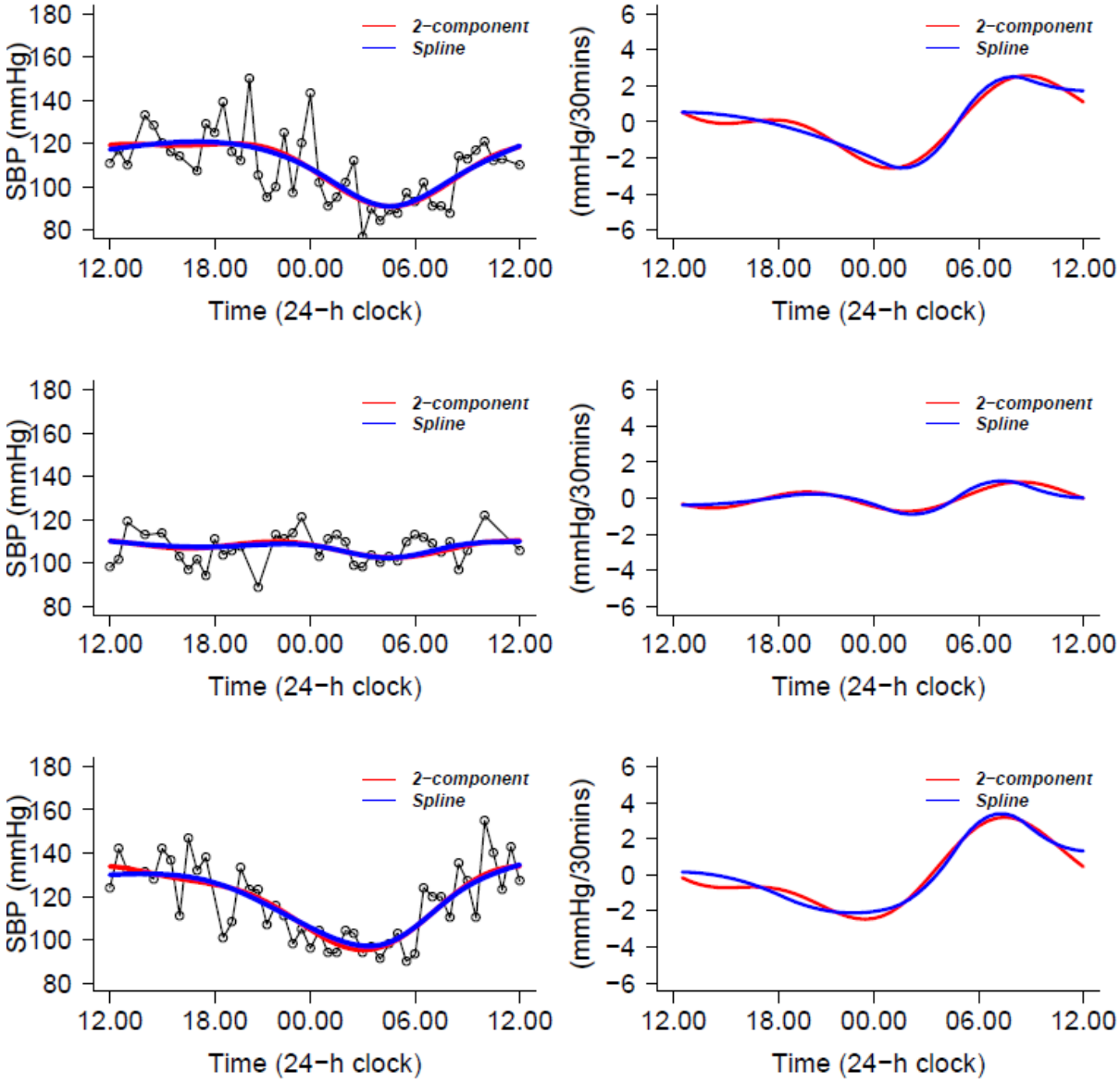


Figure 9-6 ABPM readings of three individuals with fitted subject-specific trajectories from a two-component cosinor mixed-effects model (five parameters) and spline model (six parameters - cubic spline with four knots at 18:00, 24:00, 04:00 and 08:00) (left panels). Their corresponding rate of change curves (first derivatives) are also plotted (right panels). The plots indicated an extremely similar pattern thus giving further justification of the two-component cosinor model.

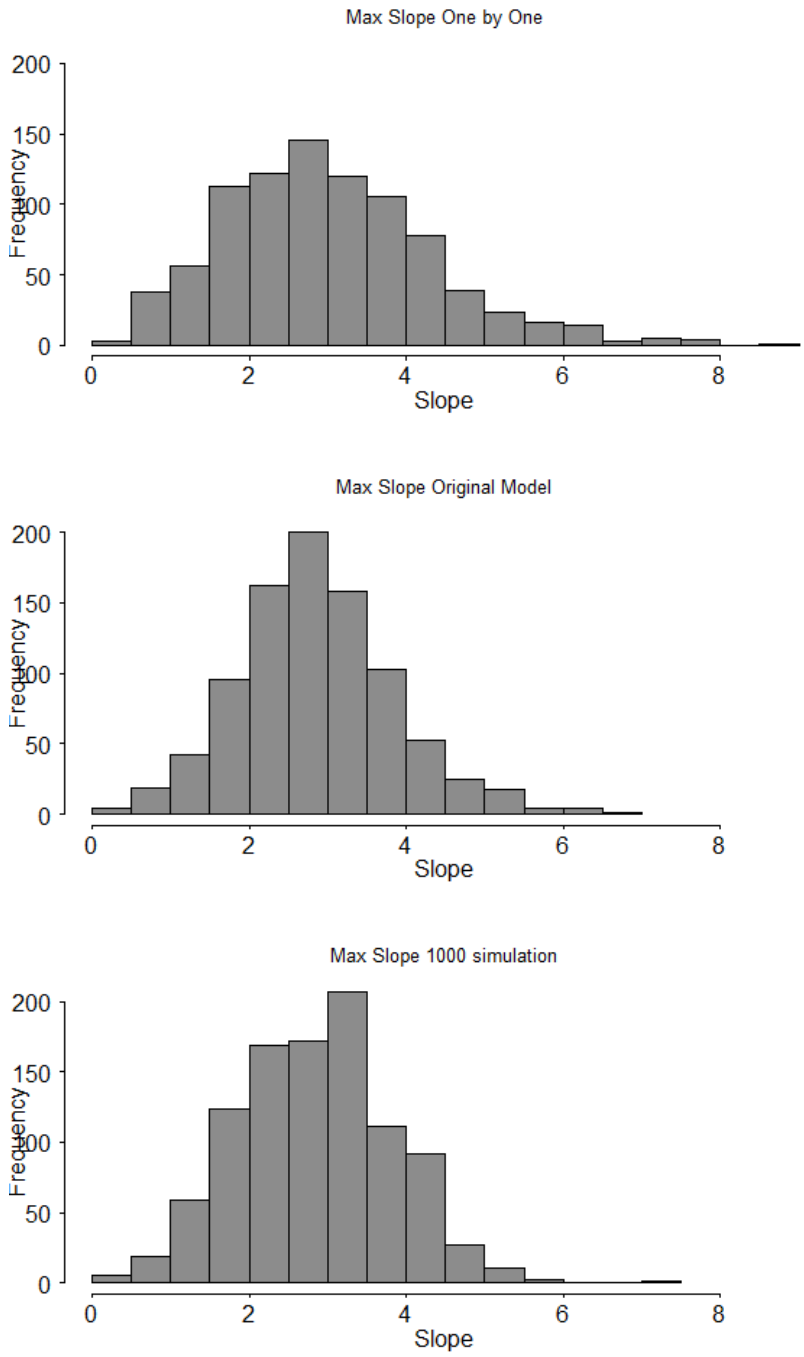


Figure 9-7 Histograms of maximum morning slope by different methods.

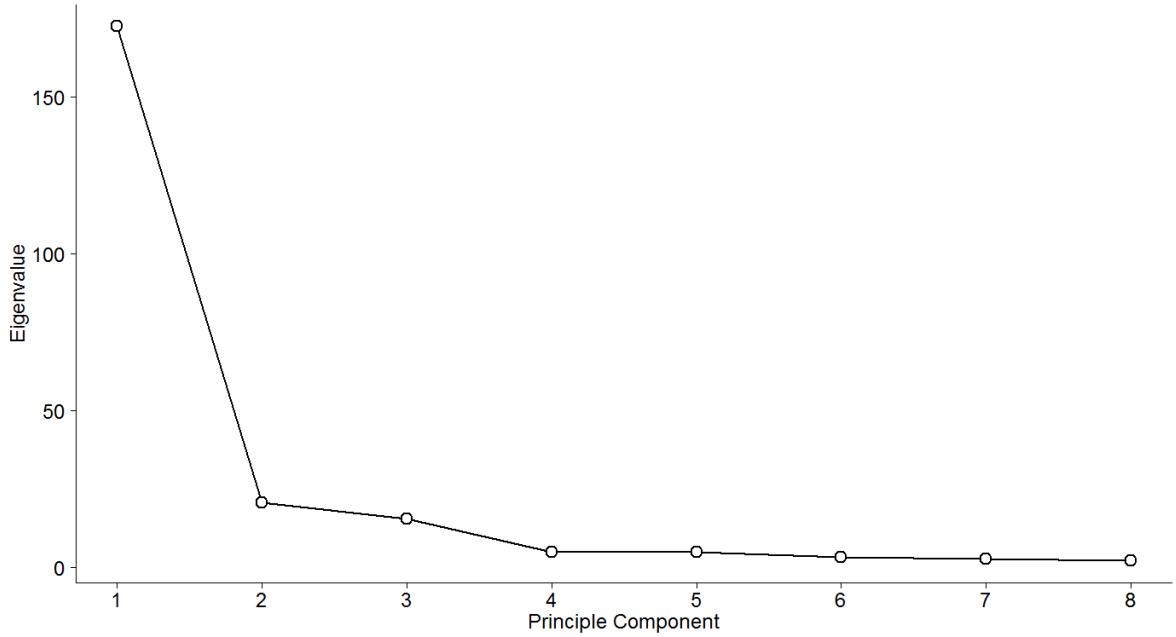


Figure 9-8 Scree plot with eigenvalues and the eight principle components which make up 99% of the variation.

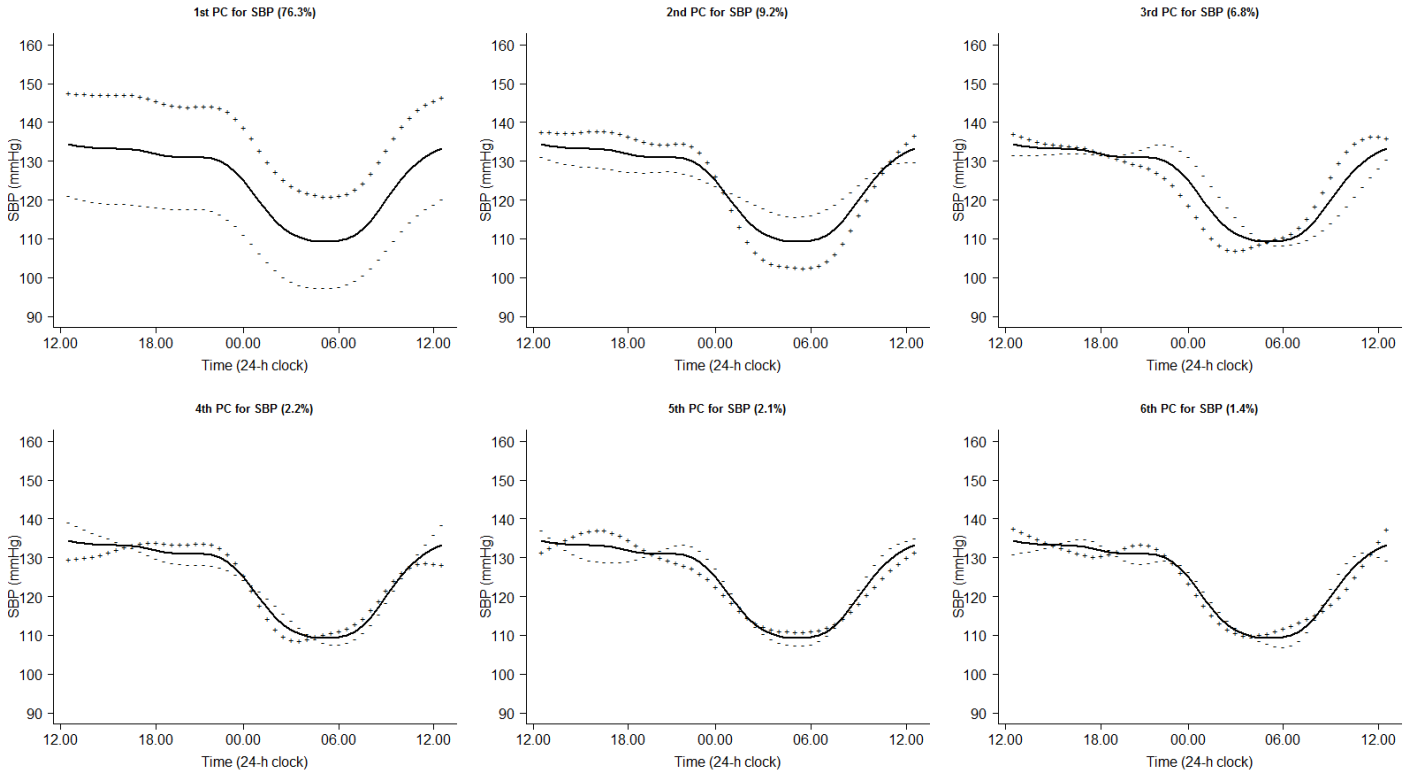


Figure 9-9 FPCA: Each of the six three FPC as variations about the mean along with the percentage of total variation explained by the component. The solid black line represents the mean SBP over the day. The "+" curves illustrate what happens when a small amount of the component is added to the mean and the "-" curves illustrate the effect of subtracting the component.

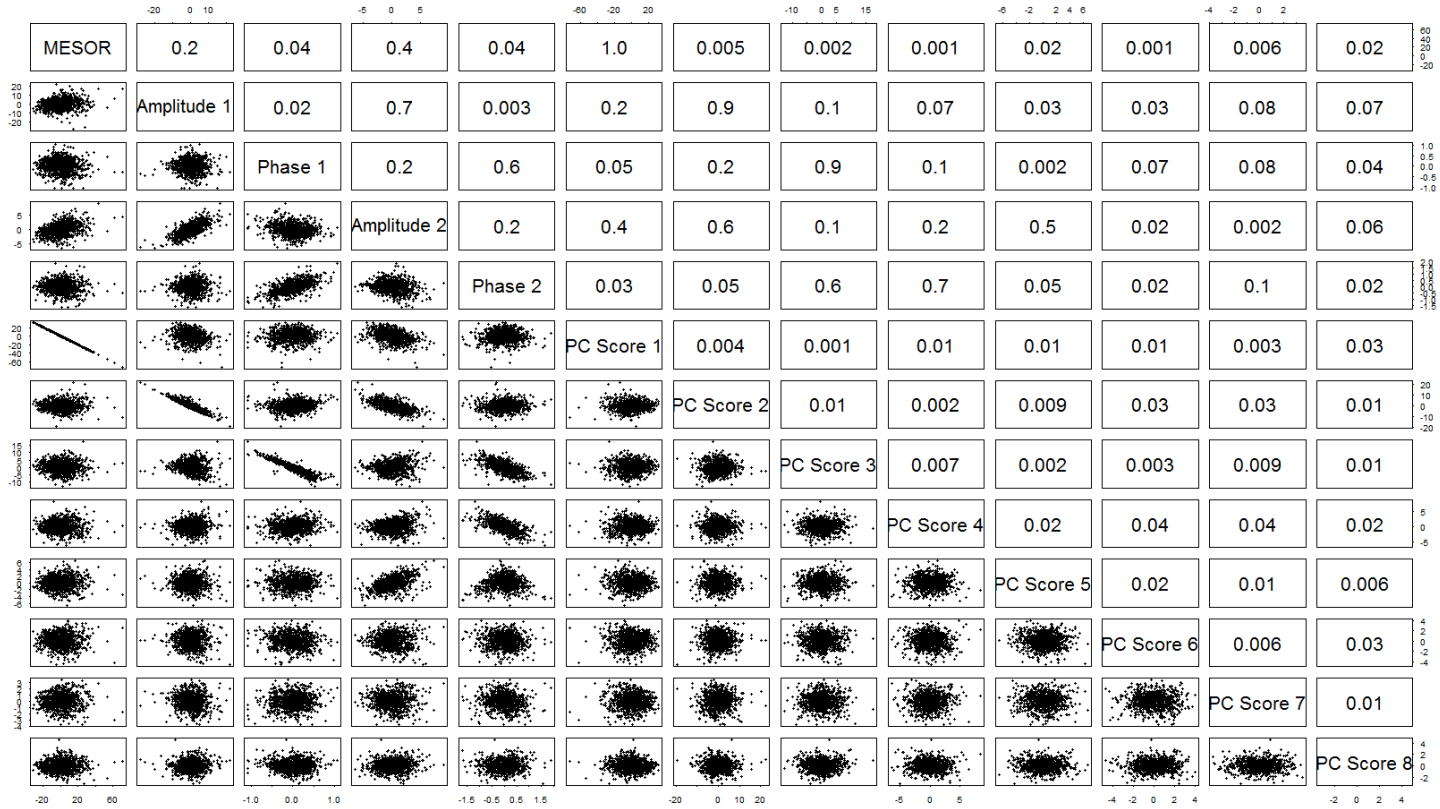


Figure 9-10 Scatter plots and the corresponding correlations between all the two-component cosinor random-effects model parameters and the principle component scores from FPCA. Eight FPCs contribute to 99% of the total variance in the data.

ARV Sampling Frequency

(References at the end of the section)

Using the simulated data based on the two-component randon-effects cosinor model in Chapter 6, ARV values were explored with respect to their sampling frequency. ARV was calculated from the simulated data (1) every 10mins and (2) every 30mins. Results indicated a substantial difference between the values taken at 10min and 30min intervals (p=0.02), see Figure 9-11 and Figure 9-12. The results are not surprising but the sampling frequency is not always referred to when ARV is mentioned in the literature, even those who developed ARV [1] failed to highlight it with only some attempting to adjust for the time interval [2,3]. We believe highlighting the findings is worthwhile. Zakopoulos *et al.* have however, suggested and advocated the use of the time rate of BP varation measure which is similar to ARV but is independent of the time intervals between measurements [4]. The measure was additionally obtained and included in Chapter 3 when summary measures were examined. We found the results were similar to the ARV value. Future studies using ARV should consider accounting for time between readings and determine the time rate of variation index. It must also be noted that although possible, in practise however, measurements taken every 10mins are unrealistic. The inconvience casued to the participant may result in them becoming iritated with the device and in turn lead to misleading values.

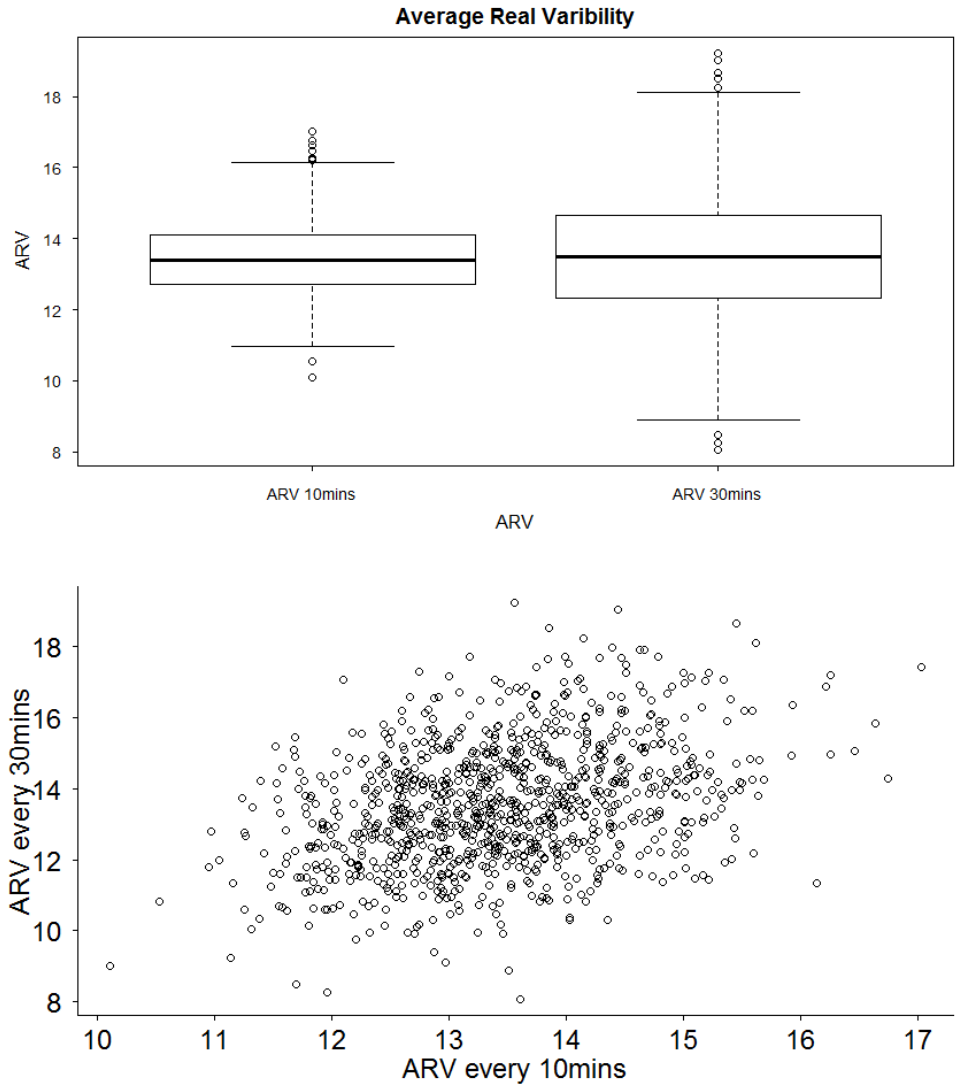


Figure 9-11 Simulated ARV values taken every 10mins and every 30mins.

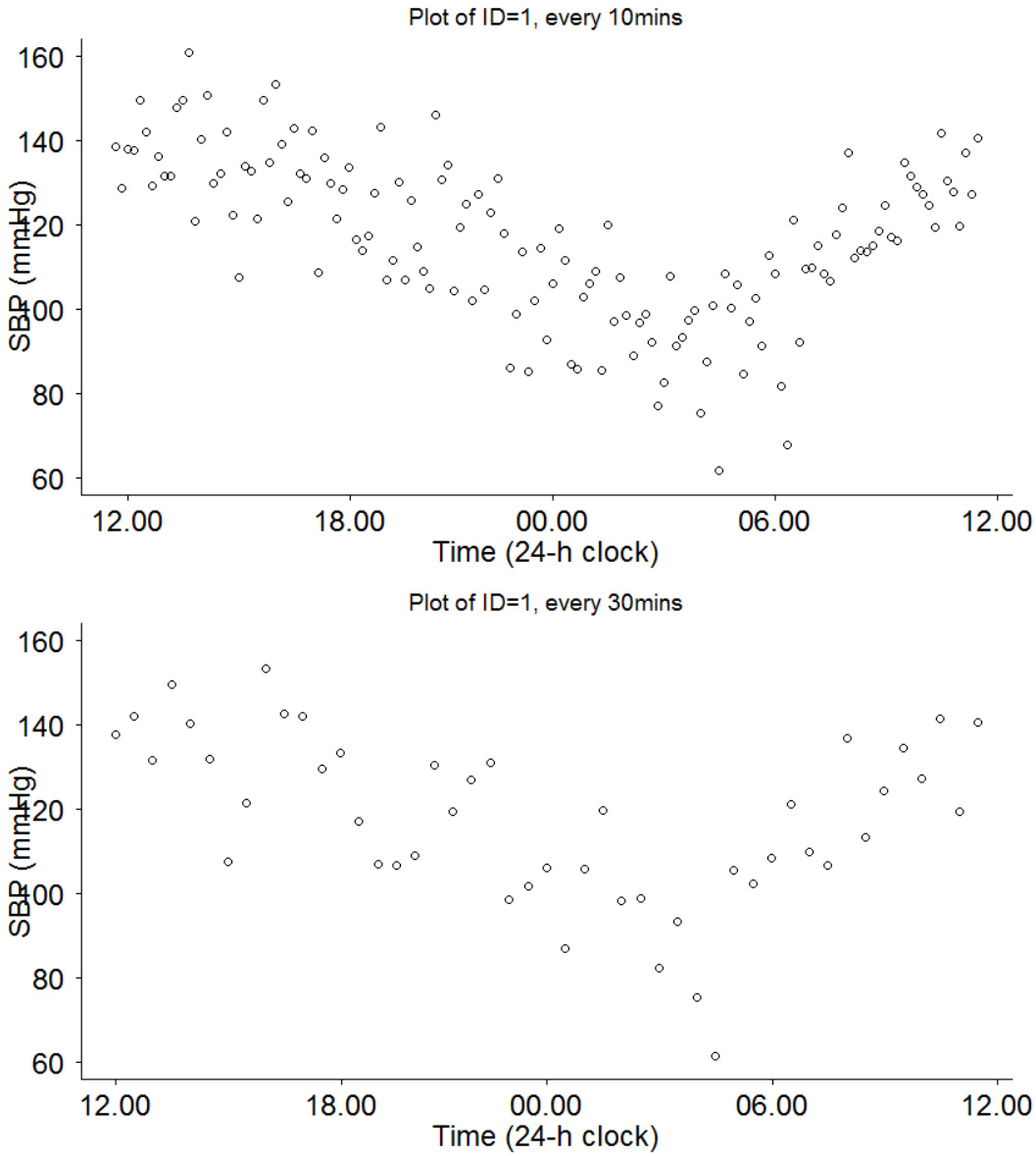


Figure 9-12 Simulated data for one individual with readings taken 1) every 30min and 2) every 10mins.

References

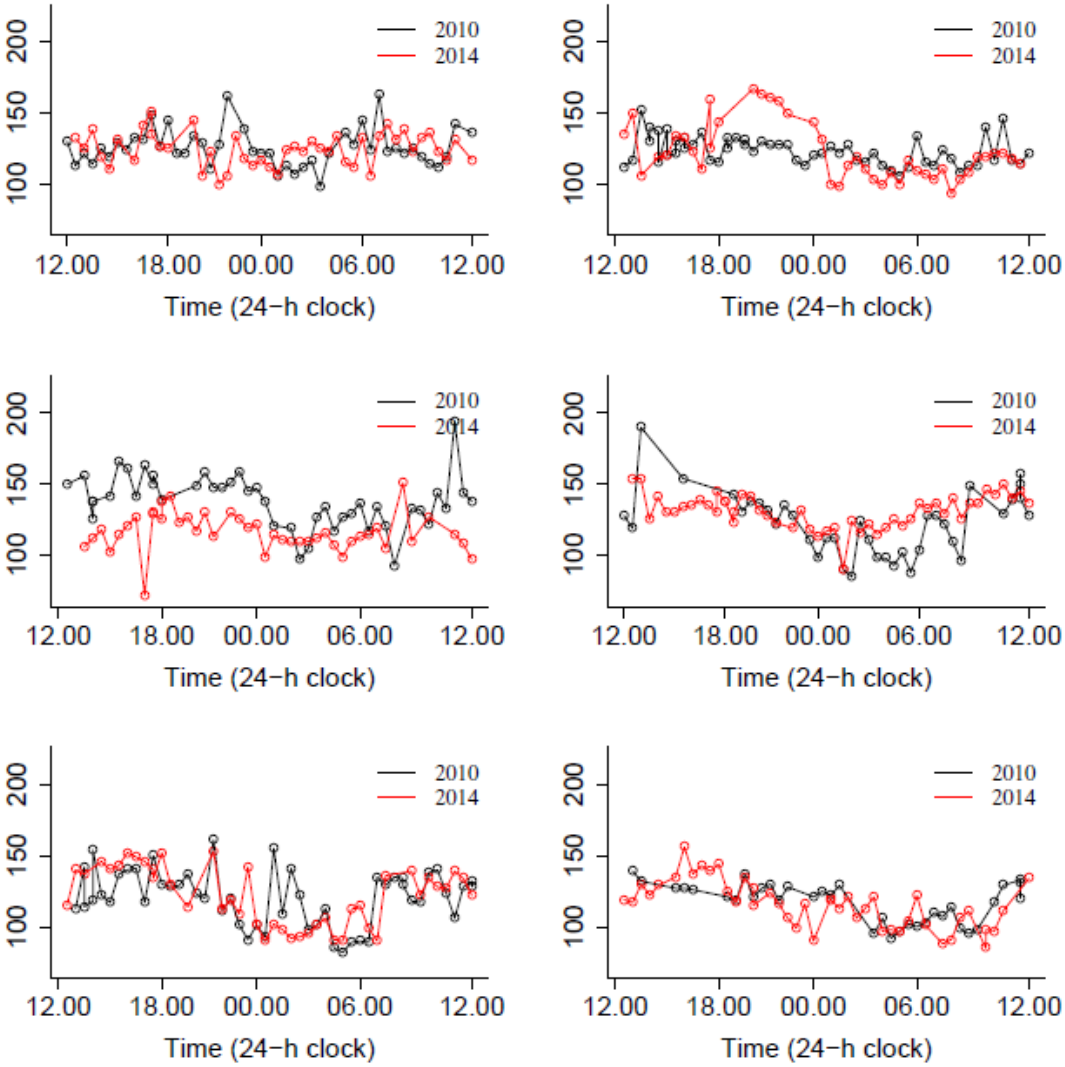
[1] Mena L, Pintos S, Queipo NV, Aizpurua JA, Maestre G, Sulbaran T. A reliable index for the prognostic significance of blood pressure variability. Journal of Hypertension. 2005;23(3):505-11.

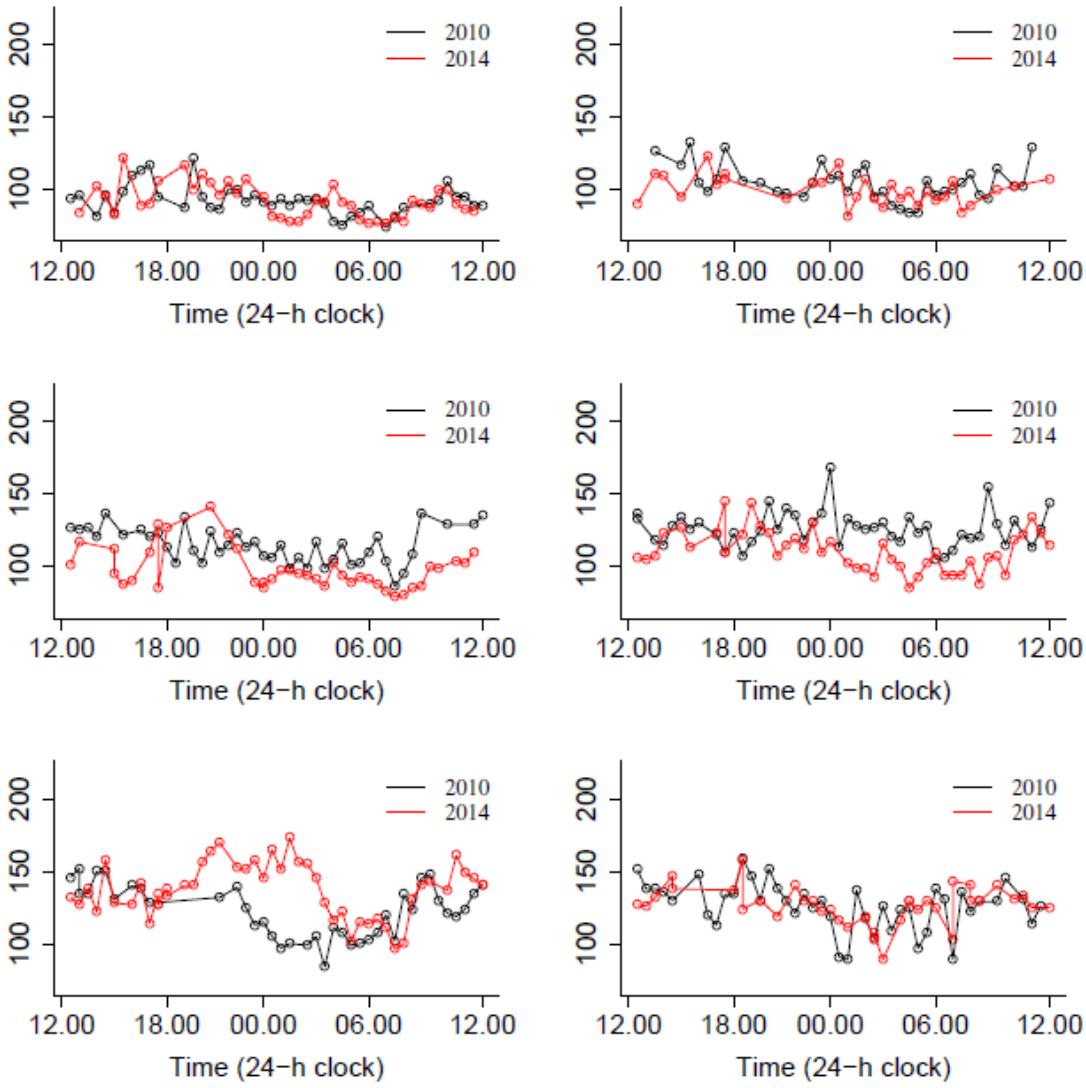
[2] Leoncini G, Viazzi F, Storace G, Deferrari G, Pontremoli R. Blood pressure variability and multiple organ damage in primary hypertension. Journal of Human Hypertension. 2013;27(11):663-70.

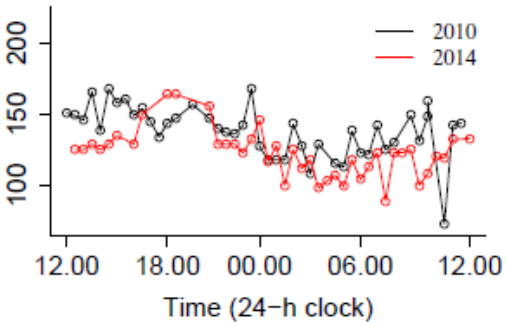
[3] Hansen TW, Thijs L, Li Y, Boggia J, Kikuya M, Bjorklund-Bodegard K, *et al.* Prognostic value of reading-to-reading blood pressure variability over 24 hours in 8938 subjects from 11 populations. Hypertension. 2010;55(4):1049-57.

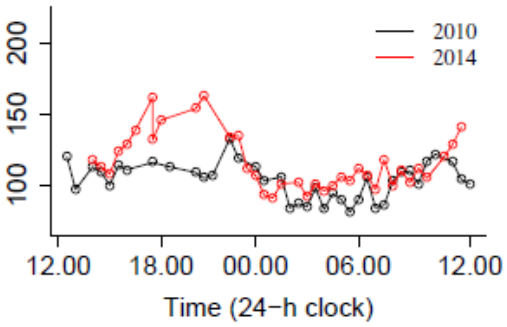
[4] Zakopoulos, NA, Tsivgoulis, G, Barlas, G, *et al.* (2005) Time Rate of Blood Pressure Variation Is Associated With Increased Common Carotid Artery Intima-Media Thickness. Hypertension. (4):505-512

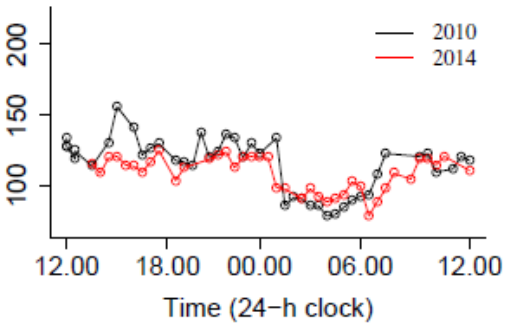
Appendix G Supplementary Material (Chapter 7)

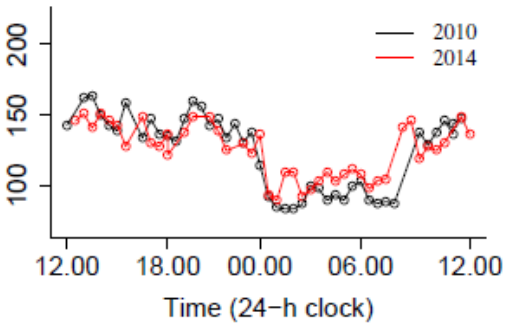


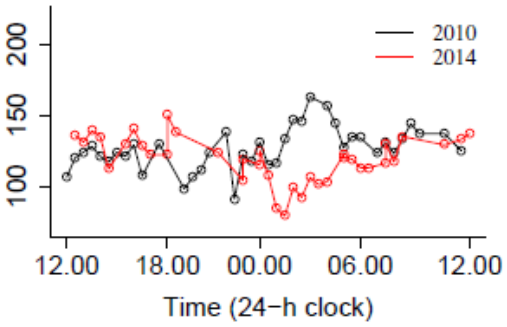

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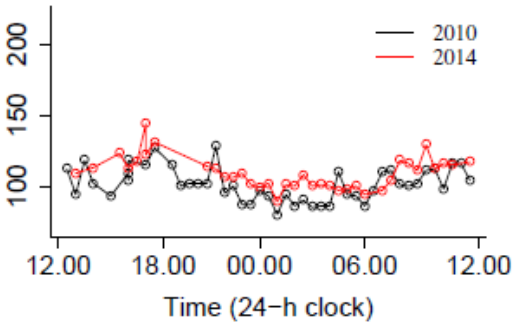

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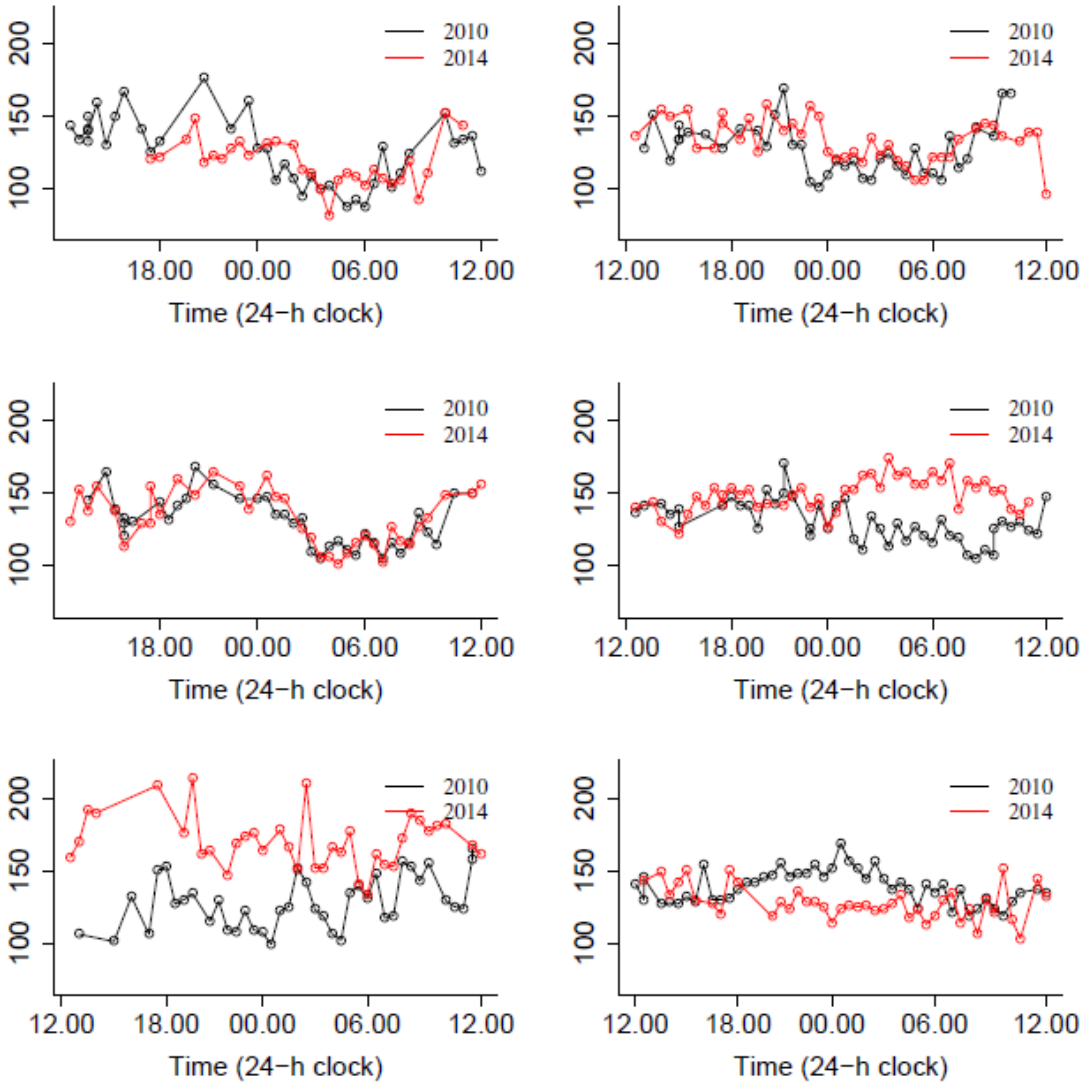

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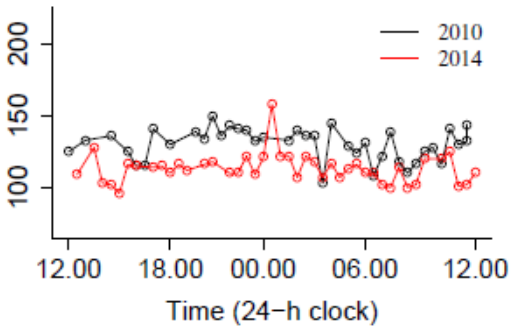

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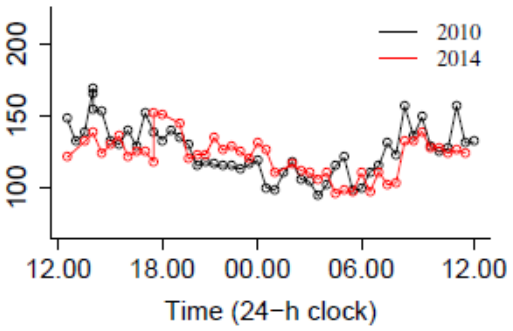

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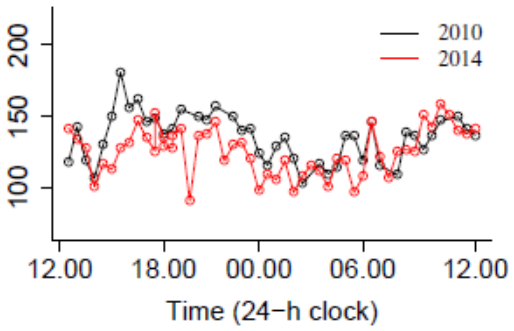

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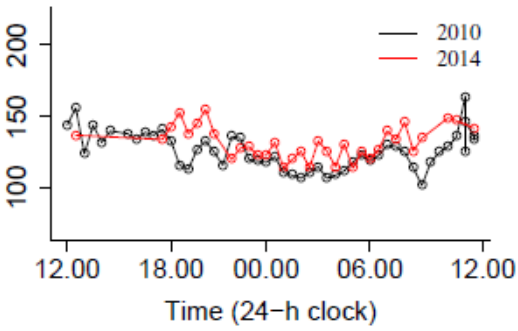

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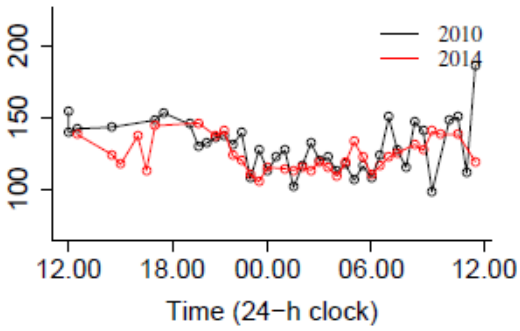

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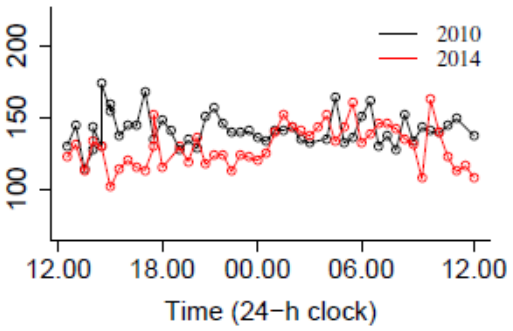

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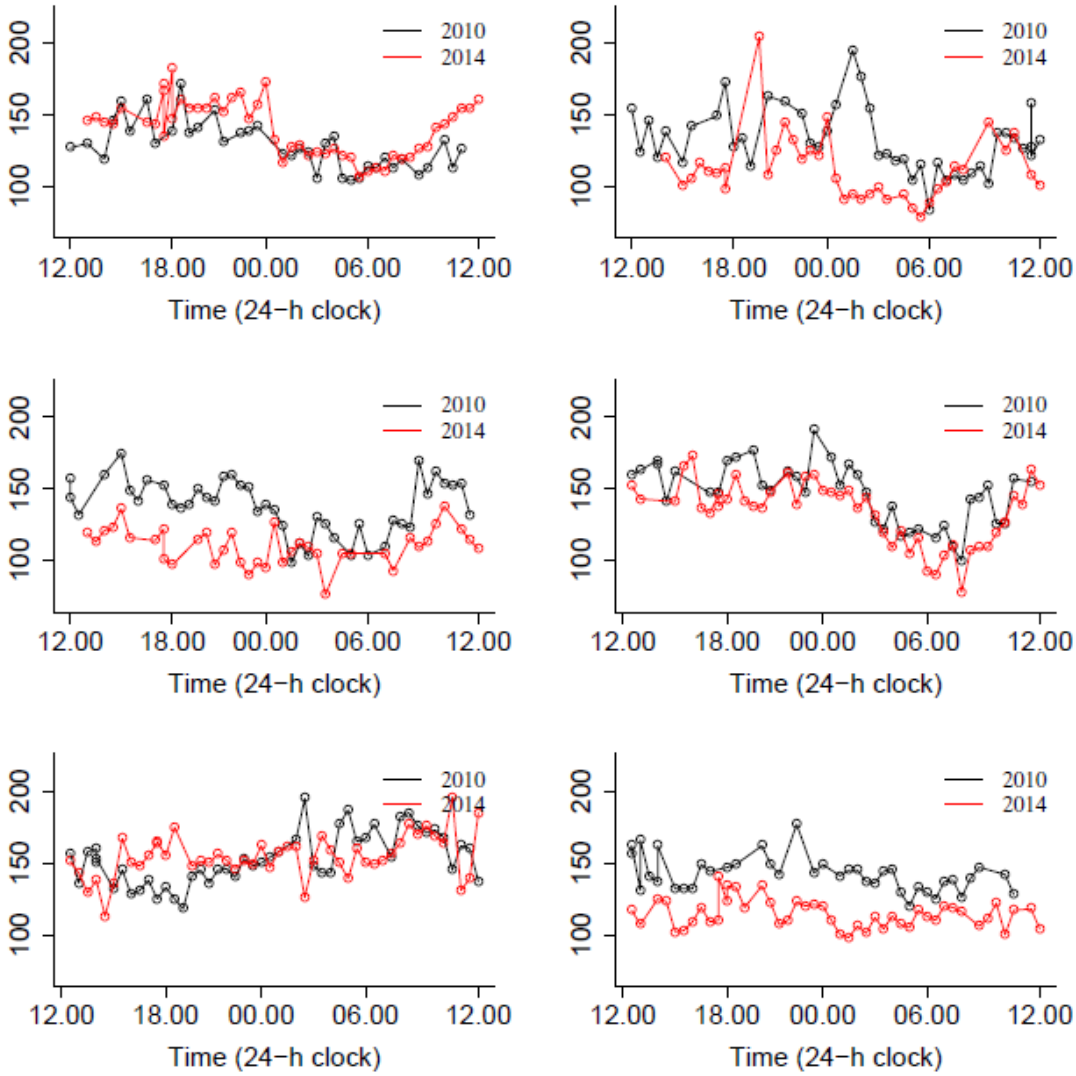

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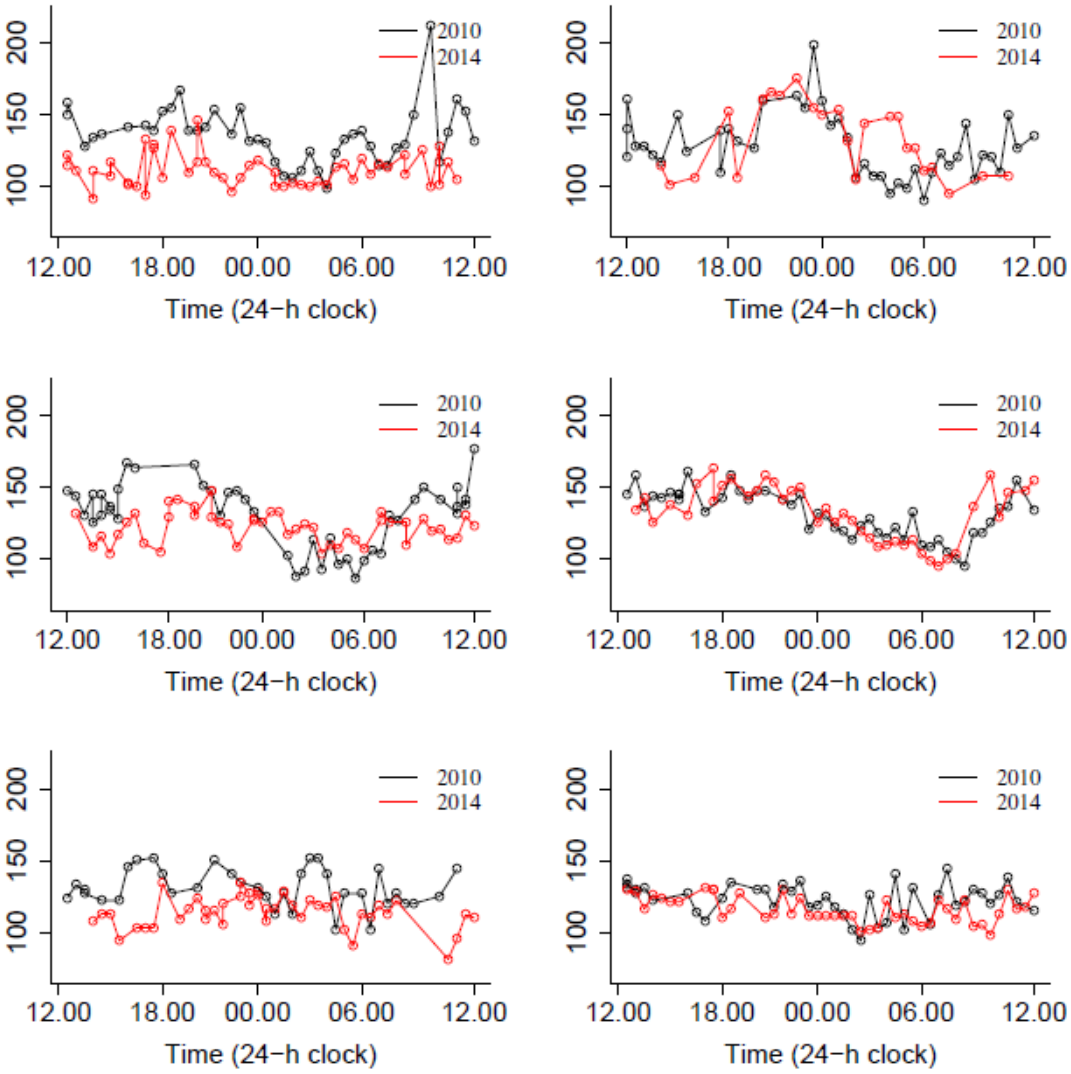

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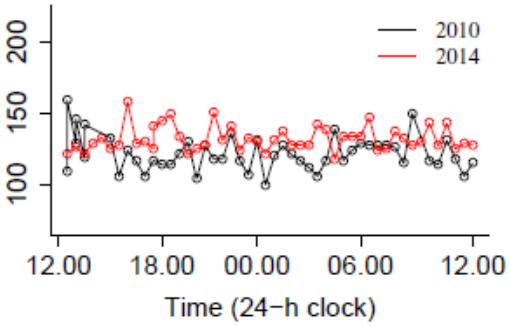

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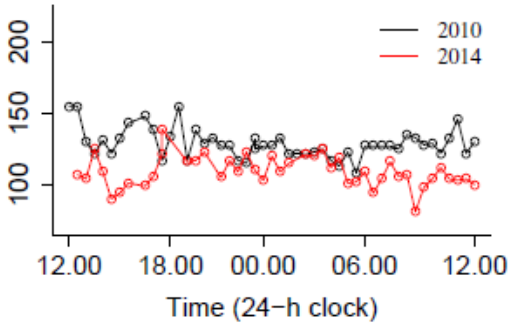

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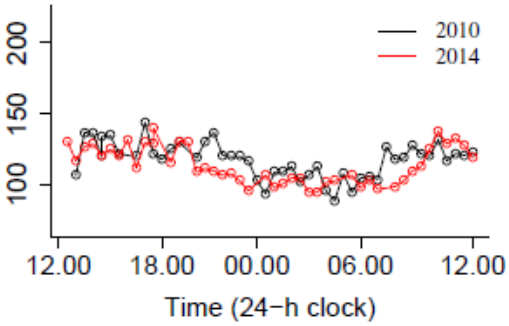

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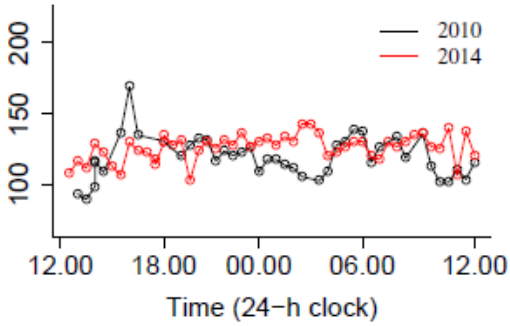

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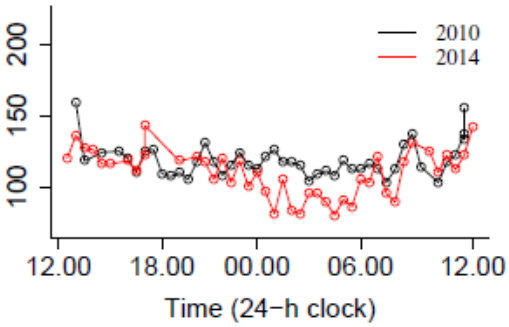

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Appendix H Sample R Code

```
#########################################################################
####Sample R code ################
#########################################################################
R>version
               _
platform       x86_64-w64-mingw32
arch           x86_64
os             mingw32
system         x86_64, mingw32
status
major          3
minor          3.1
year           2016
month          06
day            21
svn rev        70800
language       R
version.string R version 3.3.1 (2016-06-21)
nickname       Bug in Your Hair
#############################################################

#########################################################################
####Sample code: Chapter 5: Exploring diurnal variation using piecewise linear#################
####splines: an example using blood pressure#################################
#########################################################################

rm(list = ls()) #clear

library(foreign)   #imports stata file
library(nlme)
library(multcomp)

getwd()
setwd("C:/Users/JM")
w1 <- read.dta("w1.dta")
w1<-w1[order(w1$id, w1$newtime),]

####newtime 0-47 represents 24h clock
####newsleep represents subject-specific sleep time
####newwawke represents subject-specific wake time

####create individual splines with restriction BP on the average is cyclical ####
####create 5 splines - t12pm,t6pm,tsleep,t4am,twake
w1$t12pm<-ifelse(w1$newtime<=12, w1$newtime, 12)
w1$t6pm <- ifelse(w1$newtime<=12, 0, ifelse((w1$newtime>12) & (w1$newtime<=w1$newsleep),
(w1$newtime-12), (w1$newsleep-12)))
w1$tsleep<-ifelse(w1$newtime<=w1$newsleep, 0, ifelse((w1$newtime>w1$newsleep) & (w1$newtime<=32),
(w1$newtime-w1$newsleep), (32-(w1$newsleep))))
w1$t4am<- ifelse(w1$newtime<=32 ,0, ifelse((w1$newtime>32) & (w1$newtime<=w1$newwake),
(w1$newtime-32), (w1$newwake-32)))
w1$twake<-ifelse(w1$newtime<=w1$newwake, 0, ifelse((w1$newtime>w1$newwake) & (w1$newtime<=47),
(w1$newtime-w1$newwake), (47-(w1$newwake))))

####code introducing restriction ensuring pattern is periodic
w1$s2time<-w1$newsleep-12
w1$s3time<-32-w1$newsleep
```

```
w1$s4time<-w1$newwake-32
w1$s5time<-47-w1$newwake

#final splines - s2,s3,s4,s5
w1$s2<-w1$t6pm-((w1$s2time/12)*w1$t12pm)
w1$s3<-w1$tsleep-((w1$s3time/12)*w1$t12pm)
w1$s4<-w1$t4am-((w1$s4time/12)*w1$t12pm)
w1$s5<-w1$twake-((w1$s5time/12)*w1$t12pm)
############################################################


####Unadjusted Model 1 ####
m1r<- lme(sbp~ s2 + s3 + s4 + s5 ,
      random = ~ s2 + s3 + s4 + s5 | id ,method="REML",
      data=w1,control = lmeControl(msMaxIter=1000,opt = "optim",msVerbose=T),
      na.action="na.omit",correlation = corAR1(form=~1|id))
summary(mr1)
####variance-covariance matrix - random effects
getVarCov(m1r)
####need to go back to work out s1 estimate using linear combinations using glht command
####s1 refers to first spline rewritten
summary(glht(m1r, linfct = c("(s2*10+s3*10+s4*8+s5*7)/-12=0")))   #0.0158 se=0.037


##############################################################################
##############################################################################
```

```
##############################################################################
####Sample code: Chapter 6: Morning surge in blood pressure using a random-effects ######
####multiple-component cosinor model ##########################################
##############################################################################
rm(list = ls()) #clear

library(foreign)   #creates stata file
library(lme4)
library(nlme)
library(survey)
library(gmodels)
library(dplyr)
library(ggplot2)
library(multcomp)
library(mgcv)
library(lmerTest)
library(splines)
library(ares)   #only works with R2.13.2, lspline i.e. mkspline
library(car)
library(lme4)
library(RLRsim)
#library(MASS)     #ldahist, multiple histograms
library(Hmisc)
library(corrplot)
library(reshape)
library(reshape2)
library(zoo)
library(broom)
library(lmtest)
library(doBy)   #tabstat stata

getwd()
a1 <- read.dta("dataset.dta")


###2-component cosinor nonlinear method###
##system.time=2530 =42minutes run time
cos1qq<-nlme(sbp~m + am1*cos((2*pi*newtime/48)+ph1) + am2*cos((4*pi*newtime/48)+ph2),
         data=a1,
         fixed=m+am1+ph1+am2+ph2~1,
         random =m+am1+ph1+am2+ph2~1| Study_ID,
         start=c(m=124,am1=13.3,ph1=5.29,am2=4.2,ph2=0.87),
         control=nlmeControl(pnlsMaxIter=1000,pnlsTol=0.008,maxIter = 1000,msVerbose = TRUE),
         na.action="na.omit",correlation = corAR1(form=~1|Study_ID))
#saveRDS(cos1qq, "cos1qq.rds")
cos1qq <- readRDS("cos1qq.rds
summary(cos1qq)




####2 component cosinor####
#cos cos2 sin sin2
fm2 <- lme(sbp ~ cos(2*pi*newtime/48) + sin(2*pi*newtime/48) + cos(4*pi*newtime/48) +
sin(4*pi*newtime/48),
       random = ~ cos(2*pi*newtime/48) + sin(2*pi*newtime/48)+cos(4*pi*newtime/48) +
sin(4*pi*newtime/48) | Study_ID,method="ML",
       data=a1,control = lmeControl(msMaxIter=1000,opt = "optim",msVerbose=T),
       na.action="na.omit",correlation = corAR1(form=~1|Study_ID))
#saveRDS(fm2, "fm2.rds")
fm2 <- readRDS("fm2.rds")
summary(fm2)
anova(fm1,fm2)   #same models
```

```
####plot different fits- single,2 & 3 component - comparison####
somePDFPath = "some.pdf"
pdf(file=somePDFPath)
par(mar=c(5,4,1.5,1.5)+0.1)  #remove title space
par(las=1)    #y axis labels horizontal
par(mfrow = c(2,2))   #change depending how many per page
#par(mar = c(4,4.5,1.5,1.5) + 0.1)

for (i in unique(a1$Study_ID)) {

  plot(a1[a1$Study_ID==i, "newtime"], a1[a1$Study_ID==i, "sbp"],bty="l",
      #col=a1[a1$Study_ID==i, "visitno"],
      xaxt = "n",
      #xlim = range(a$bphour2), ylim = range(a$sbp), # base the axes on full data range
      ylim=c(80,180),
      # main = paste("Plot of", i),
      xlab="Time (24-h clock)", ylab="SBP (mmHg)",font.main = 1,cex.lab=1.5,cex.axis = 1.5)
  lines(a1[a1$Study_ID==i , "sbp"]~a1[a1$Study_ID==i , "newtime"], lwd=1)
  lines(a1[a1$Study_ID==i , "fitted_sub1"]~a1[a1$Study_ID==i , "newtime"], lwd=3)
  lines(a1[a1$Study_ID==i , "fitted_sub2"]~a1[a1$Study_ID==i , "newtime"], lwd=3,col="red")
  lines(a1[a1$Study_ID==i , "fitted_sub3"]~a1[a1$Study_ID==i , "newtime"], lwd=3,col="blue")
 axis(1, at=c(0,12,23,35,47), labels=c("12.00", "18.00", "00.00", "06.00", "12.00"),cex.axis = 1.5)
}
dev.off()

####derivatives, simulation & obtain max slope####
a1<-a1 %>% group_by(Study_ID) %>% mutate(derivative2 = c(NA,diff(fitted_sub2) / diff(newtime))) %>%
  ungroup()  %>%
  as.data.frame()

####prediction every minute####
z<-expand.grid(newtime=seq(0,47,by=1),Study_ID=unique(a1$Study_ID))    #prediction of lme for every minute
of day
z<-expand.grid(newtime=seq(0,47,by=1/30),Study_ID=unique(a1$Study_ID))      #prediction of lme for every
minute of day
z$pred <- predict(fm2, newdata=z,level=1)
z$pred <- predict(cos1qq, newdata=z,level=1)
z<-z %>% group_by(Study_ID) %>% mutate(derivative = c(NA,diff(pred) / diff(newtime))) %>%
  ungroup()  %>%
  as.data.frame()
detach(package:plyr)
z<-z %>% group_by(Study_ID) %>% mutate(derivative = c(NA,diff(pred) )) %>%
  ungroup()  %>%
  as.data.frame()

#manually getting derivative
library(mosaic)
D(124.32941 + 6.58314*cos(2*pi*t/P) + 10.35877*sin(2*pi*t/P) ~ t, P=48) # default values for parameters.


#########################################################################
####correct identification of max slope derivative ####
#coefficients
bc<-coef(fm2)
bc<-cbind(Study_ID = rownames(bc), bc)
colnames(bc)[2] <- "b0"
colnames(bc)[3] <- "b1"
colnames(bc)[4] <- "b2"
colnames(bc)[5] <- "b3"
colnames(bc)[6] <- "b4"

###one person
```

```
t<-rep(28:47)
n<-function(t,p=48){
 bc$b0[bc$Study_ID=="LHC0005"]                +          bc$b1[bc$Study_ID=="LHC0005"]*cos(2*pi*t/p)          +
bc$b2[bc$Study_ID=="LHC0005"]*sin(2*pi*t/p) +
   + bc$b3[bc$Study_ID=="LHC0005"]*cos(4*pi*t/p) + bc$b4[bc$Study_ID=="LHC0005"]*sin(4*pi*t/p)
}
max(grad(n, t))


##loop works
t<-seq(28,47,by=1/30)
for(row in 1:nrow(bc)){
 n<-function(t,p=48){
   bc$b0[row]     +   bc$b1[row]*cos(2*pi*t/p)   +   bc$b2[row]*sin(2*pi*t/p)   +   bc$b3[row]*cos(4*pi*t/p)   +
bc$b4[row]*sin(4*pi*t/p)
 }
 bc$newvar[row] <-max(grad(n, t))
}
summary(bc$newvar)

###################################
###################################



#############################################################################################
#########Chapter 6: FPCA Work#####################
#############################################################################

library(refund)
library(refund.shiny)

load("BPM.Rdata")

## Expand grid for all observations from 0 to 47 time points
res <- merge(
 expand.grid(newtime=unique(BPM$newtime), Study_ID=unique(BPM$Study_ID)),
 BPM, all=TRUE)
BPM=res[order(res$Study_ID),]
BPM$Study_ID<-as.character(BPM$Study_ID)

y=NULL
for(i in 1:length(unique(BPM$Study_ID))){
 y[[i]]=t(BPM$sbp[which(BPM$Study_ID==unique(BPM$Study_ID)[i])])}
names(y)<-sprintf(unique(BPM$Study_ID),1:length(y))
y=t(sapply(y, '[', 1:max(sapply(y, length))))


n = dim(y)[1]
s = seq(1, 48, length = dim(y)[2])

## do FPCA on the observed functions
fpca.sys = fpca.sc(y)
fpca.sys

par(las=1)    #y axis labels horizontal
par(mfrow = c(1,3))
par(mfrow = c(1,4))
par(mfrow = c(2,3))
par(mar=c(5,5,1.5,1.5)+0.1)  #remove title space


## plot FPCA effects for the first basis function
```

```
plot(s, fpca.sys$mu, type = 'l', ylim = c(90, 160), ylab = "", bty="l",
    pch = 19, lwd = 2, xaxt = "n",cex.lab=1.5,xlab="Time (24-h clock)",
    main = paste("1st PC for SBP (", 100*round(fpca.sys$evalues[1]/sum(fpca.sys$evalues),3), "%)", sep = "" ),
    cex.axis = 1.5)
axis(1, at=c(0,12,23,35,47), labels=c("12.00", "18.00", "00.00", "06.00", "12.00"),cex.axis = 1.5)
title(ylab = "SBP (mmHg)", cex.lab = 1.5, line = 3)
points(s, fpca.sys$mu - sqrt(fpca.sys$evalues[1]) * fpca.sys$efunctions[,1], pch = "+")
points(s, fpca.sys$mu + sqrt(fpca.sys$evalues[1]) * fpca.sys$efunctions[,1], pch = "-")

## plot FPCA effects for the second basis function
plot(s, fpca.sys$mu, type = 'l', ylim = c(90, 160), ylab = "",
    xaxt = "n",cex.lab=1.5,xlab="Time (24-h clock)", bty="l", pch = 19, lwd = 2, cex.axis = 1.5,
    main = paste("2nd PC for SBP (", 100*round(fpca.sys$evalues[2]/sum(fpca.sys$evalues),3), "%)", sep = "" ))
axis(1, at=c(0,12,23,35,47), labels=c("12.00", "18.00", "00.00", "06.00", "12.00"),cex.axis = 1.5)
title(ylab = "SBP (mmHg)", cex.lab = 1.5, line = 3)
points(s, fpca.sys$mu - sqrt(fpca.sys$evalues[2]) * fpca.sys$efunctions[,2], pch = "+")
points(s, fpca.sys$mu + sqrt(fpca.sys$evalues[2]) * fpca.sys$efunctions[,2], pch = "-")


## plot FPCA effects for the 3rd basis function
plot(s, fpca.sys$mu, type = 'l', ylim = c(90, 160), ylab = "",
    xaxt = "n",cex.lab=1.5,xlab="Time (24-h clock)", bty="l", pch = 19, lwd = 2, cex.axis = 1.5,
    main = paste("3rd PC for SBP (", 100*round(fpca.sys$evalues[3]/sum(fpca.sys$evalues),3), "%)", sep = "" ))
axis(1, at=c(0,12,23,35,47), labels=c("12.00", "18.00", "00.00", "06.00", "12.00"),cex.axis = 1.5)
title(ylab = "SBP (mmHg)", cex.lab = 1.5, line = 3)
points(s, fpca.sys$mu - sqrt(fpca.sys$evalues[3]) * fpca.sys$efunctions[,3], pch = "+")
points(s, fpca.sys$mu + sqrt(fpca.sys$evalues[3]) * fpca.sys$efunctions[,3], pch = "-")


## plot FPCA effects for the 4th basis function
plot(s, fpca.sys$mu, type = 'l', ylim = c(90, 160), ylab = "",
    xaxt = "n",cex.lab=1.5,xlab="Time (24-h clock)", bty="l", pch = 19, lwd = 2, cex.axis = 1.5,
    main = paste("4th PC for SBP (", 100*round(fpca.sys$evalues[4]/sum(fpca.sys$evalues),3), "%)", sep = "" ))
axis(1, at=c(0,12,23,35,47), labels=c("12.00", "18.00", "00.00", "06.00", "12.00"),cex.axis = 1.5)
title(ylab = "SBP (mmHg)", cex.lab = 1.5, line = 3)
points(s, fpca.sys$mu - sqrt(fpca.sys$evalues[4]) * fpca.sys$efunctions[,4], pch = "+")
points(s, fpca.sys$mu + sqrt(fpca.sys$evalues[4]) * fpca.sys$efunctions[,4], pch = "-")


## plot FPCA effects for the 5th basis function
plot(s, fpca.sys$mu, type = 'l', ylim = c(90, 160), ylab = "",
    xaxt = "n",cex.lab=1.5,xlab="Time (24-h clock)", bty="l", pch = 19, lwd = 2, cex.axis = 1.5,
    main = paste("5th PC for SBP (", 100*round(fpca.sys$evalues[5]/sum(fpca.sys$evalues),3), "%)", sep = "" ))
axis(1, at=c(0,12,23,35,47), labels=c("12.00", "18.00", "00.00", "06.00", "12.00"),cex.axis = 1.5)
title(ylab = "SBP (mmHg)", cex.lab = 1.5, line = 3)
points(s, fpca.sys$mu - sqrt(fpca.sys$evalues[5]) * fpca.sys$efunctions[,5], pch = "+")
points(s, fpca.sys$mu + sqrt(fpca.sys$evalues[5]) * fpca.sys$efunctions[,5], pch = "-")


## plot FPCA effects for the 6th basis function
plot(s, fpca.sys$mu, type = 'l', ylim = c(90, 160), ylab = "",
    xaxt = "n",cex.lab=1.5,xlab="Time (24-h clock)", bty="l", pch = 19, lwd = 2, cex.axis = 1.5,
    main = paste("6th PC for SBP (", 100*round(fpca.sys$evalues[6]/sum(fpca.sys$evalues),3), "%)", sep = "" ))
axis(1, at=c(0,12,23,35,47), labels=c("12.00", "18.00", "00.00", "06.00", "12.00"),cex.axis = 1.5)
title(ylab = "SBP (mmHg)", cex.lab = 1.5, line = 3)
points(s, fpca.sys$mu - sqrt(fpca.sys$evalues[6]) * fpca.sys$efunctions[,6], pch = "+")
points(s, fpca.sys$mu + sqrt(fpca.sys$evalues[6]) * fpca.sys$efunctions[,6], pch = "-")


#scree plot
par(mar=c(5,5,1.5,1.5)+0.1)  #remove title space
par(mfrow = c(1,1))
dd<-data.frame(y=fpca.sys$evalues,x=1:8)
```

```
plot(dd$x, dd$y, type = 'l',   ylab = "",
    cex.lab=1.5,xlab="Principle Component", bty="l", pch = 21,cex=2,lwd = 2, cex.axis = 1.5)
title(ylab = "Eigenvalue", cex.lab = 1.5, line = 3)
points(dd$x, dd$y, pch = 21,cex=2,bg="white",lwd = 2)


## Curve reconstruction for different values of k
plot(y[800,], pch=19, ylab = "mmHg", xlab="Timepoint", main= "Curve reconstruction for subject 800 with K
expansions")
lines(fpca.sys$mu+(fpca.sys$scores[800,1]*fpca.sys$efunctions[,1]+fpca.sys$scores[800,2]), col="blue")
lines(fpca.sys$mu+(fpca.sys$scores[800,1]*fpca.sys$efunctions[,1]+fpca.sys$scores[800,2]*fpca.sys$efunctions
[,2]
            +fpca.sys$scores[800,3]*fpca.sys$efunctions[,3]+fpca.sys$scores[800,4]),col="green")
lines(fpca.sys$mu+(fpca.sys$scores[800,1]*fpca.sys$efunctions[,1]+fpca.sys$scores[800,2]*fpca.sys$efunctions
[,2]
+fpca.sys$scores[800,3]*fpca.sys$efunctions[,3]+fpca.sys$scores[800,4]*fpca.sys$efunctions[,4]+fpca.sys$scor
es[800,5]*fpca.sys$efunctions[,5]+fpca.sys$scores[800,6]*fpca.sys$efunctions[,6]+fpca.sys$scores[800,7]*fpca.
sys$efunctions[,7]+fpca.sys$scores[800,8]*fpca.sys$efunctions[,8]), col="red")
legend("topright",legend=(c("k=2","K=4","k=8")),col=c("blue","green","red"),lty=1,bty="n",xpd=NA, seg.len = 2)

plot_shiny(fpca.sys)


####COMPARE FPCA to COSINOR####
uy<-data.frame(fpca.sys$scores)   #extract individuals scores
uy$Study_ID<-unique(BPM$Study_ID)
colnames(uy) = c("PC Score 1","PC Score 2","PC Score 3", "PC Score 4", "PC Score 5",
            "PC Score 6", "PC Score 7", "PC Score 8" ,"Study_ID")
uy

#bring in cosinor 2-component values
a<-ranef(cos1qq)
colnames(a) = c("MESOR","Amplitude 1","Phase 1", "Amplitude 2", "Phase 2")
a<-cbind(Study_ID = rownames(a), a)
a
names(a)
aw<-merge(a,uy,by= "Study_ID")
names(aw)
aw<-select(aw,-Study_ID)



#correlation plot
pairs(aw)
panel.cor <- function(x, y, digits=1, prefix="", cex.cor, ...)
{
 usr <- par("usr"); on.exit(par(usr))
 par(usr = c(0, 1, 0, 1))
 r <- abs(cor(x, y))
 txt <- format(c(r, 0.123456789), digits=digits)[1]
 txt <- paste(prefix, txt, sep="")
 if(missing(cex.cor)) cex.cor <- 0.8/strwidth(txt)
 text(0.5, 0.5, txt, cex = 2)
    #, cex = cex.cor * r)   #remove size proportional to correlation
}
pairs(aw, upper.panel=panel.cor, pch=20,cex.labels=2)
```

Appendix I Research output, dissemination and training

Table 9-5 Peer reviewed publication from PhD

Year	Peer reviewed journal
2016 (Under Review)	Madden JM, Li X, Tilling K, Kearney PM, Fitzgerald AP. Exploring Diurnal Variation Using Piecewise Linear Splines: An Example Using Blood Pressure. (Under review - Emerging Themes in Epidemiology)
2015	Madden JM, O'Flynn AM, Fitzgerald AP, Kearney PM. Correlation between short-term blood pressure variability and left-ventricular mass index: a meta-analysis. Hypertens Res. 2015 39, 171–177; doi:10.1038/hr.126
2015	Madden JM, O'Flynn AM, Dolan E, Fitzgerald AP, Kearney PM. Short-term blood pressure variability over 24h and target organ damage in middle-aged men and women. J Hum Hypertens 2015 doi: 10.1038/jhh.2015.18

Table 9-6 Other research output during PhD

Year	**Peer reviewed journal**
2015	O'Flynn AM, Madden JM, Russell AJ, Curtin RJ, Kearney PM. Isolated nocturnal hypertension and subclinical target organ damage: a systematic review of the literature. Hypertens Res. 2015 Apr 2. doi: 10.1038/hr.2015.43.
2015	O'Flynn AM, McHugh SM, Madden JM, Harrington JM, Perry IJ, Kearney PM. 'Applying the Ideal Cardiovascular Health Metrics to Couples: A Cross-Sectional Study in Primary Care'. Clinical Cardiology, 2015
2013	Harrington, J., Fitzgerald, A. P., Kearney, P. M., McCarthy, V. J., Madden, J., Browne, G., Dolan, E. & Perry, I. J. DASH Diet Score and Distribution of Blood Pressure in Middle-Aged Men and Women. Am J Hypertens 2013 Aug 6.
2013	Buckley, C. M., Madden, J., Balanda, K., Barron, S., Fahy, L., Harrington, J., Perry, I. J. & Kearney, P. M. Pre-diabetes in adults 45 years and over in Ireland: the Survey of Lifestyle, Attitudes and Nutrition in Ireland 2007. Diabet Med. 2013 May 10. doi: 10.1111/dme.12226
2013	Curtin, M., Madden, J., Staines, A. & Perry, I. J. Determinants of vulnerability in early childhood development in Ireland: a cross-sectional study BMJ Open 2013;3:e002387
2013	Balanda KP, Buckley CM, Barron SJ, Fahy LE, Madden JM, *et al.* (2013) Prevalence of Diabetes in the Republic of Ireland: Results from the National Health Survey (SLAN) 2007. PLoS ONE 8(10): e78406. doi:10.1371/journal.pone.0078406

2013	McKenna, G., Madden, J., Manton, S. & Cronin, M. Survey of Oral Health Behaviors of Patients Receiving Methadone Therapy at a Drug Rehabilitation Centre. Journal of Theory and Practice of Dental Public Health, 2013; Vol 1 No. 3. Pg 6-13
2013	Hayes, M., Burke, F. M., McKenna, G., Madden, J. & Cronin, M. An analysis of the attitudes of dental patients attending general dental practice in Galway. Journal of The Irish Dental Association, 2013; 59 (4): 179-182.

Table 9-7 Conference Presentations during PhD

Year	Title	Conference
2016	Blood Pressure Variability over 24 hours using Mixed-effects Models	SPHeRE Network 2nd Annual Conference, RCSI, Dublin, Ireland, February 29th, 2016
2015	Short-Term Blood Pressure Variability over 24-h Using Mixed-effects Models	International *Biometric* Society, *Eastern North* America *Region, Miami, USA March 15-18, 2015*
2015	Short-term Blood Pressure Variability over 24 hours and Target Organ Damage in Middle-Aged Men and Women	Conference on Applied Statistics in Ireland*, UCC, Cork, Ireland, May 11-13th, 2015*
2015	Short-term Blood Pressure Variability over 24 hours and Target Organ Damage in Middle-Aged Men and Women	SPHeRE Network 1st Annual Conference, RCSI, Dublin, Ireland, January 9th, 2015
2013	Intensity of Physical Activity and Obesity in an Irish Cohort Using GENEActiv Accelerometers.	3rd International Conference on Ambulatory Monitoring of Physical Activity and Movement Amherst, Massachusetts, USA June 17-19, 2013

Table 9-8 Training & workshops attend during PhD

Year	Course
2016	HRB-Trials Methodology Research Network: Statistical Considerations in Clinical Trial Design, UCC
2015	European Society of Hypertension Summer School, Vienna
2014	Newcastle R Course (1 week), Colin Gillespie
2014	Advanced programming in R: Royal Statistics Society RSS Colin Gillespie
2014	Multilevel Modeling using Stata, SPHeRE PhD programme, George Leckie
2014	An Introduction to Cochrane Systematic Reviews, Cochrane Collaboration, UCC
2013	Introduction to Propensity Score Methods with R, Predictive Modeling with R and the caret Package, User R Conference, Albacete, Spain
2012	Analysis of Repeated Measures, Centre for Multilevel Modeling, Bristol
2012	PG7016 Systematic reviews for the health sciences, UCC

Table 9-9 Placements Completed

Year	Supervisor	Award
Summer 2013	Dr. John Newell	Biostatistics Unit in the HRB Clinical Research Facility, NUI Galway
Summer 2015	Prof Kate Tilling	School of Social and Community Medicine, University of Bristol

Table 9-10 Awards

Year	Award
2014	Travel Bursary: College of Medicine and Health, UCC

Appendix J Published Papers (PDFs)