An investigation of underlying biophysical mechanisms in the use of pulse wave velocity for cuffless measurement of blood pressure

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Declaration

I hereby declare that the work presented throughout this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree to any other university or institution other than Macquarie University. I also declare, to the best of my knowledge, that this thesis is an original piece of research and it has solely been written by me. Any assistance that I have received throughout this research and during the preparation of the thesis itself has been appropriately acknowledged. In addition to this, I declare that all information, sources, and literature used are indicated within the thesis.

The research presented in this thesis was approved by the Macquarie University Human Research Ethics Committee (Non-invasive measurement of blood pressure without a cuff, Reference number: 5201700226).

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October 12, 2019

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List of publications

The work presented in this thesis has been presented previously at the following conferences, with the abstracts being published in the conference proceedings of those conferences:

1. **Cox J**, Butlin M, Tan I, Avolio AP. Repeatability of individual calibration for cuffless estimation of blood pressure from arterial pulse wave velocity. (Pulse of Asia, Shanghai, 2019)

2. Butlin M, **Cox J**, Spronck B, Tan I, Avolio AP. Repeatability and predictors of a potentially blood pressure-independent parameter of arterial stiffness (Artery 19, Budapest, 2019)

3. **Cox J**, Avolio AP, Spronck B, Tan I, Butlin M. Predictors and repeatability of a subject-specific calibration term for cuffless estimation of blood pressure using arterial pulse wave velocity (15th Asian-Pacific Congress of Hypertension, Brisbane, 2019)

4. Butlin M, **Cox J**, Tan I, Avolio AP. Assessing carotid-femoral pulse wave velocity: How many measurements does it take to account for measurement variability? (15th Asian-Pacific Congress of Hypertension, Brisbane, 2019)

Abstract

Background: Blood pressure (BP) is a health-based risk factor predictive of cardiovascular complications. Conventional cuff-based techniques measuring BP are limited by being discontinuous and inconvenient. This thesis investigates the underlying components of an alternative cuffless approach, utilising a subject-specific calibration method. This approach aims to minimise the use of a cuff, therefore, addressing these limitations by estimating BP through its relationship with arterial pulse wave velocity (PWV), a measure of arterial stiffness.

Methods: Carotid-femoral PWV measurements were taken under baseline conditions and following two interventions; a postural change and a cold pressor test. The postural change generated a calibration factor that was investigated for repeatability. Consecutive PWV measurements assessed reliability and measurement variability. A cross-sectional multivariate statistical analysis was performed to determine possible predictors of the calibration factor. The cold pressor test investigated potential PWV differences in different arterial segments in response to a BP change.

Results: The mean calibration factor was 18.78±7.39 mmHg/m/s with good repeatability (difference: 1.40±9.76 mmHg/m/s, p=0.48). This factor correlated with weight (standardised β=0.247, p=0.003) and diastolic BP (β=0.244, p=0.004). The cross-sectional stepwise linear regression model predicted 15% of the calibration factor variability (p<0.05, R^2=0.146). Waveform quality and distance were a source of potential variability. There were significant differences in regional PWV following a change in BP (p<0.05).

Conclusions: These findings demonstrate the capacity of the subject-specific calibration factor to estimate BP. Variability present was primarily associated with the PWV measurement. Pulse transit time was significantly affected by changes in BP. As this thesis has only articulated the potential of the calibration approach and factors contributing towards its variability, future research is still required to validate BP estimation incorporating the calibration factor.

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List of Acronymns

BP	blood pressure
cfPWV	carotid-femoral pulse wave velocity
DBP	diastolic blood pressure
ECG	electrocardiogram
HR	heart rate
MAP	mean arterial pressure
PPG	photoplethysmography
PEP	pre-ejection period
PAT	pulse arrival time
PTT	pulse transit time
PWV	pulse wave velocity
SBP	systolic blood pressure

CHAPTER 1

Introduction

Philosophically the human heart is often envisioned as an organ of emotion, spirit, and soul, however, physiologically there is but one definitive role, being the pumping of blood throughout the body's extensive vascular system. The muscular contraction of this organ is what allows life to be sustained through the transportation of oxygen, nutrients, carbon dioxide, and waste products to and from the respective tissues. The rhythmic contraction within this enclosed system will alter the volume of blood that is present within blood vessels. The volume of blood will in turn alter the tension experienced against the vessel wall. This is due to vessel compliance, as determined by the wall's stiffness. This tension is more commonly known as blood pressure (BP) and will fluctuate with the ejection of blood during each cardiac cycle. During the initial period of ventricular contraction the blood will begin to be pushed through the vessels. Shortly after contraction the vessels will experience a peak pressure due to the increase in tension, known as systolic BP (SBP). Following ventricular relaxation the tension experienced will gradually decline to a period where the pressure is at its minimum, known as diastolic BP (DBP). Clinically these two values are universally accepted as a marker of an individual's cardiovascular health. Notably, these values will change with time due to physiological responses, conditions, and lifestyle factors such as smoking status, diet, and exercise (Appel et al., 1997; Cornelissen and Fagard, 2005; Primatesta et al., 2001). BP is of importance because depending on the measured values, or change in pressure following certain interventions, it may be utilised to signify cardiovascular abnormalities.

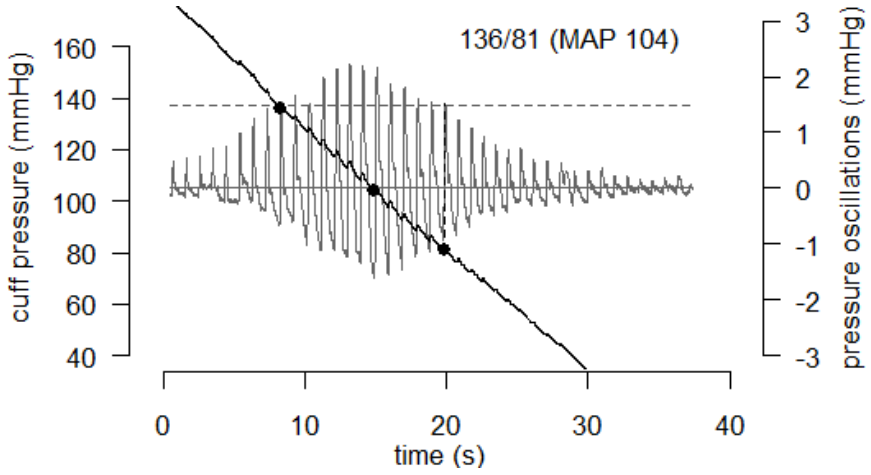

Figure 1.1: The estimation of blood pressure from an oscillometric waveform generated from the deflation of a pneumatic cuff. As the pressure in the cuff decreases (black line) the pressure oscillations increase in amplitude until they reach a peak point and then begin to decline back to zero.

BP may be measured through either invasive or non-invasive methods, each with their own strengths and weaknesses. However, conventionally this physiological parameter is acquired through non-invasive, brachial cuff-based techniques that typically follow an auscultatory or oscillometric method of measurement. This involves a pneumatic cuff being placed around the upper arm, in line with the heart, and inflated to a suprasystolic pressure to momentarily occlude the artery. The cuff is then deflated at a steady rate, approximately 2-3 mmHg/heartbeat (bpm) according to current guidelines (NHFA, 2016), with SBP and DBP ascertained by physiological markers associated with those pressures. For the auscultatory method the hallmark Korotkoff sounds act as the physiological marker of SBP and DBP whilst the amplitude of the pulsations transduced in the cuff act as the marker for the oscillometric method (Fig. 1.1). Despite cuff-based approaches being well-validated against intra-arterial pressure and other validated devices (O'Brien et al., 2001; Reshetnik et al., 2016), there has been extensive research undertaken on alternate methods of BP measurement.

Research into other methods of measurement have been sought to address the two primary disadvantages associated with cuff-based techniques: (1) their discontinuity (Gesche et al., 2012) and (2) their inconvenience (Ogedegbe and Pickering, 2010). These issues arise due to the

requirement of a pneumatic cuff on the arm and its nature of inflation and deflation to obtain a single measurement. In conjunction with this, studies have further highlighted the personal preference of cuffless devices (Islam et al., 2019). Currently, there are numerous approaches that are being investigated which aim to address these disadvantages. One approach estimates BP through its relationship with arterial pulse wave velocity (PWV), a measure of arterial stiffness attained by measuring the time delay of the arterial pulse (transit time) across an arterial distance. Although this approach is cuffless, non-invasive, and has the capacity to estimate BP continuously, it requires accurately characterising the relationship between BP and PWV (calibration). At present there is no consensus in the literature for the ideal calibration method to define this relationship. Thus, for PWV to be utilised as a method in BP estimation it is important to explore the underlying biophysical mechanisms of the relationship between BP and PWV in order to address this issue of calibration.

This thesis henceforth investigates the underlying biophysical mechanisms of an alternative cuffless approach, utilising a subject-specific calibration method to generate a calibration factor, with contents as described below:

Aim 1 (Chapter 3): To quantify the repeatability of the subject-specific calibration factor for cuffless estimation of blood pressure.

For the calibration factor to be useful it must be repeatable within the individual. This chapter investigates the repeatability of a subject-specific calibration factor that may be used for the cuffless estimation of BP. This calibration factor integrates the use of a postural change and quantifies within individual repeatability of measurements taken by the same operator on separate visits.

Aim 2 (Chapter 4): To examine potential sources of error in the measurement of pulse wave velocity.

Error observed in measurements is a contributing factor of variability. This chapter seeks to examine potential sources of error that may arise in the measurement of PWV. This requires multiple consecutive measurements of PWV to be undertaken by two operators, experienced and inexperienced. The effect of changing the waveform quality and distance between the waveforms was also examined.

Aim 3 (Chapter 5): To establish predictors of the subject-specific calibration factor.

This chapter goes on to perform a cross-sectional multivariate statistical analysis on the calibration factor to determine whether the it is generalisable across the population and may be estimated based on predictors.

Aim 4 (Chapter 6): To investigate regional differences of pulse wave velocity following a change in blood pressure.

This is the last experimental chapter and investigates the regional differences of PWV following a change in BP, as achieved by a cold pressor test.

This thesis is not proposing a new method of BP estimation. It is an exploration of the relationship between BP and PWV in order to further characterise a potential approach that may be incorporated in the cuffless estimation of BP.

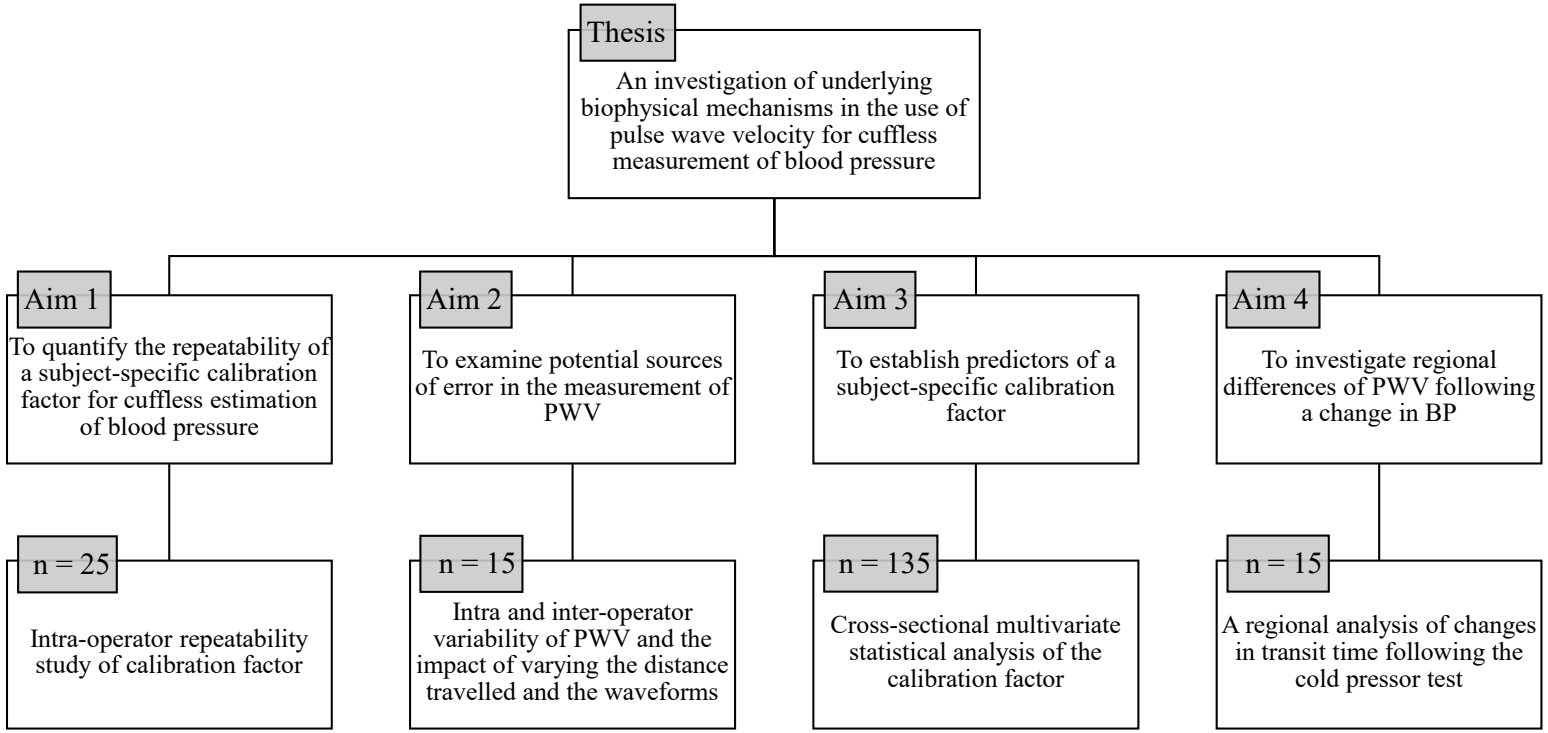

Figure 1.2: A general overview of the thesis and its respective chapters, including the aims and samples sizes (n).

CHAPTER 2

Literature Review

The measurement of BP is becoming ever more prominent gaining ground outside of a clinical setting for the individual monitoring of health. This is namely due to the high prevalence of cardiovascular complications such as hypertension, as reported by the World Health Organisation (WHO, 2017). Typically BP is measured non-invasively with a pneumatic cuff, although as mentioned in Chapter 1, this parameter holds the limitations of being discontinuous and inconvenient. Due to the discontinuity these techniques they have the inability to track transient changes on a beat-to-beat basis that could provide important physiological information which may alter prognosis or interventions. Additionally, moving away from cuff-based techniques could provide greater convenience and practicality, such as seen in wearable devices like Fitbits for the measurement of heart rate (HR) and physical activity.

Advancements in technology, such as the miniaturisation of components, enhancement of microprocessors, wireless capabilities, and the capacity to store large data, has provided a platform for the measurement of physiological parameters, including BP, to shift and progress into more convenient and continuous measurements. Compared to invasive and cuff-based techniques, there are numerous approaches with the capacity to estimate BP non-invasively without the aid of a cuff (Table 2.1). PWV, as discussed in Section 2.1, is one approach with the capacity to resolve the apparent problems associated with cuff-based methods by estimating BP through a

physiological relationship. However, prior to the application of using PWV to estimate BP the relationship between the two parameters requires further investigation.

Table 2.1: Outline of methods used for the assessment of blood pressure.

Method	Approach	Examples	Measurement	Advantages	Limitations
Invasive					
	Intra-arterial	Solid-state pressure catheter	Blood pulsations detected by a force transducer on the tip of the catheter.	True beat-to-beat BP.	Invasive and restricted to surgical settings.
Noninvasive					
	Cuff-based	Oscillometric and manual auscultation	Measures physiological markers during the deflation of a pneumatic cuff that has occluded the artery.	Non-invasive, well-validated, and easy to measure.	An estimation, discontinuous, and inconvenient.
	Non-cuff based	SeismoWatch and BPro	Measures physiological signals to estimate BP through mathematical relationships.	Non-invasive and potential to be continuous.	An estimation, relies on assumptions, and requires calibration.

2.1 Pulse wave velocity

2.1.1 Background

PWV is parameter of cardiovascular health that is considered the 'gold standard' for the assessment of arterial stiffness (Laurent et al., 2006). It is defined as the speed of the pressure wave along an arterial segment that is generated due to the ejection of blood from the left ventricle into the aorta. This pressure wave travels in the arterial wall throughout the vascular system and is equal to the travelled distance divided by the time required for the pulse wave to travel that distance (pulse transit time, PTT) (Eq. 2.1). The speed at which this pulse wave travels is dependent on multiple factors, primarily involving the viscoelastic properties within the arterial wall (Learoyd and Taylor, 1966). These viscoelastic properties are predominantly

influenced by the ratio of collagen and elastin fibres whereby collagen fibres will contribute towards tensile strength and the elastin towards vessel distensibility (Roach and Burton, 1959). This means that a higher percentage of collagen will equate to a stiffer artery and consequently a faster PWV, and vice versa.

$$PWV = \frac{distance}{transit\ time} \tag{2.1}$$

Arterial stiffness may furthermore be influenced by the type of artery and the pressure loading exhibited against the arterial wall (BP). For example, muscular arteries, such as the brachial artery, have a higher composition of collagen fibres and when an increasing load is applied to the arterial wall the collagen fibres will begin to take the load and the artery will consequently become stiffer (Dobrin, 1978). In comparison, elastic arteries, such as the descending aorta, have a lower composition of collagen fibres, thus when a larger load is applied the artery will not become as stiff due to the minimal presence of collagen. Additionally, smooth muscle may differentially load the collagen and elastin fibres (Dobrin and Rovick, 1969). This differential loading can influence the contractile mechanics and elasticity of the artery and should be taken into account, especially considering the regional arterial differences with respect to the abundance of smooth muscle.

2.1.2 Relationship with stiffness and blood pressure

The collagen and elastin fibres are consequently important constituents of blood vessels that contribute, in part, to the apparent stiffness of the artery. As described by the Moens-Korteweg equation (Eq. 2.2) (Korteweg, 1878; Moens, 1878), arterial stiffness, as a product of multiple factors, can be mathematically related to wave speed, and in turn PWV. In this equation wave speed ($c,\ m/s$) is a function of wall thickness ($h,\ mm$), the modulus of elasticity ($E,\ mPa$), the density of blood (ρ , 1060 kg/m^3), and the vessel radius ($r,\ cm$).

$$c = \sqrt{\frac{Eh}{2\rho r}}$$
$$c \approx PWV \tag{2.2}$$

The modulus of elasticity is in reference to the arterial wall and is relative stiffness. However, as this relationship is built on a theoretical basis it must be brought to attention that the relationship between stiffness and PWV only holds true under the following assumptions: the ratio between the thickness of the luminal wall and the radius of the lumen remains relatively constant; the tube is uniform; and the fluid within the tube is viscous and incompressible. Evidently, under standard vascular conditions these assumptions do not consistently remain true at all times which leads to questioning the accuracy of this relationship.

As PWV is a parameter of arterial stiffness, and arterial stiffness is pressure dependent, PWV is therefore a potential candidate to continuously estimate BP non-invasively without the aid of a cuff. The feasibility of this becomes apparent through defining the relationship between stiffness and PWV which tends to build upon the Moens-Korteweg equation. Two notable equations to define this relationship are those described by Bramwell and Hill (1922) (Eq. 2.3) and Hughes et al. (1979) (Eq. 2.4). The key difference between the two highlighted relationships is that the Bramwell-Hill equation is theoretical whilst the Hughes equation is purely empirical. However, it is important to note that the relationship between stiffness and pressure is not exclusive to a single equation, although other equations are less established.

To further elaborate, the Bramwell-Hill relationship is based on the strain that the artery experiences following a change in pressure. In accordance with Young's modulus (Young, 1809) strain is a factor of stiffness and therefore, from a fundamental perspective, the equation relates stiffness to PWV. In this equation (Eq. 2.3) PWV is factor equal the luminal area (A, mm^2), the change in pressure (ΔP, $mmHg$), the density of blood (ρ , 1060 kg/m^3), and the change in luminal area (ΔA, mm^2). Although, as evident in the Moens-Korteweg equation (Eq. 2.2), for

this relationship to remain true it is assumed that the ratio of the vessel's distensibility remains relatively constant. This becomes a limitation of not just the described relationship but those which relate BP to stiffness. Conversely, whilst the Hughes equation is not built on a theoretical foundation is has proven useful in the estimation of BP (Chen et al., 2003; Ma et al., 2018). Compared to the Bramwell-Hill equation, the modulus of elasticity ($E,\ mPa$) is constituted by the modulus of elasticity at zero pressure ($E_0,\ mPa$), the material coefficient of the artery (α , $\approx 0.017\ mmHg^{-1}$), and pressure ($P,\ mmHg$). To summarise, in order to estimate BP from PWV it is essential to quantify the underlying relationship, which may be achieved through the described equations. Throughout this thesis, this BP/PWV relationship is described as the calibration of PWV used to estimate BP, otherwise known as the 'calibration factor'.

$$
\begin{aligned}
PWV &= \frac{1}{\sqrt{\rho \times distensibility}} \\
distensibility &= \frac{strain}{\Delta P} \\
&= \frac{\Delta A}{A\,\Delta P} \\
\therefore PWV &= \sqrt{\frac{A\,\Delta P}{\rho\,\Delta A}}
\end{aligned}
\tag{2.3}
$$

$$
E = E_0\, e^{\alpha\, P} \tag{2.4}
$$

2.1.3 Calibration

Although PWV seems to be a potential candidate for the estimation of BP, the calibration of the BP/PWV relationship has proven to be an issue leading to inaccuracies and unreliable estimations. As expressed by Mukkamala et al. (2015), "the greatest challenge is calibration". This calibration is referring to the scaling of PWV (m/s) to units of pressure ($mmHg$). In order to manage this hurdle studies have undertaken numerous approaches attempting to accurately

calibrate this relationship. These include using population-based look-up tables and undertaking both one-point and two-point calibration approaches (Vermeersch et al., 2008), with varying techniques to attain these points. Notably, there are limited studies that perform calibration with multiple points (more than two). Whilst these approaches may be considered as more ideal the process would become cumbersome and give rise to further complexities due to the requirement of additional interventions and their respective physiological responses. Other studies have even proposed 'calibration-free' methods (Kachuee et al., 2015) with some incorporating machine learning into their techniques (Khalid et al., 2018). There has been encouraging results with different techniques, however, the methods undertaken are either too complex, inconvenient, or have resulted in a large standard deviation in their estimations, despite producing a reasonable average (Sharma et al., 2017). As a result the calibration of this relationship needs to be investigated and refined in order to successfully and reliably estimate BP. Keeping in mind that PTT is inversely proportional to PWV, as expressed by Eq. 2.1, PTT has been equally be explored for the estimation of BP. This relies on the assumption that the distance between the sites of measurement remains fixed. As a result the concepts expressed pertaining to PWV may therefore be considered interchangeable with respect to issues surrounding its measurement and calibration.

2.1.3.1 Frequency

The frequency of this calibration is another important factor which has been extensively investigated (Muehlsteff et al., 2006; Poon et al., 2008; Young et al., 1995). Specifically, this refers to how often the relationship needs to be re-calibrated in order to maintain a reliable and accurate relationship. Re-calibration is inherently required due to the nature of drifting and physiological changes of varying BP under certain conditions (Shaltis et al., 2005). Despite this, single initial calibration approaches have been explored whereby an array of strategies were undertaken. These

strategies produced mixed results with some displaying only 29% of readings within 5 mmHg and others producing up to 70% of readings within 5 mmHg (Sola et al., 2013). This 5 mmHg threshold has been established as a good indicator for device validation (OBrien et al., 2010) as the larger the difference the greater the possibility of mislabelling an individuals BP and cardiovascular profile (Handler, 2009). Regarding periodic calibration (re-calibration) techniques, Poon et al. (2008) have expressed that re-calibration may be required within every 60 heart beats for 95% of readings to fall within 9.4 mmHg. On the other hand, Chen et al. (2000) researched a 5 minute calibration interval producing accurate results with an error of 10%, and Cattivelli and Garudadri (2009) yielded SBP and DBP readings that had a standard deviation of less than 7.8 mmHg following a 1 hour calibration interval. With respect to the approach, it must be noted that there is an unavoidable trade off in terms of the frequency and practicality of calibration. In conjunction with this, the importance of properly quantifying this relationship becomes a critical component for the development of cuffless BP estimation.

2.1.3.2 Standard techniques

Previous research has proposed population-based averages for calibration in order to characterise the relationship between BP and PTT and thus estimate BP (Poon and Zhang, 2005). This ideally provides a generic calibration for the BP/PTT relationship for certain populations. However, this method of calibration lacks in its reliability to accurately estimate BP from PTT (Butlin et al., 2018; Chen et al., 2009). This is due to PWV, and hence PTT, being affected by multiple factors, such as HR, age, and stress (Avolio et al., 1983; Tan et al., 2016), which vary greatly between individuals and thus leads to a possible source of error. Even though these papers do show a reasonable estimation of BP (R^2=0.89), the issue is that there is a large standard deviation present ($\pm$19.8 mmHg), especially with increasing BP (Gesche et al., 2012). This results as the approach is limited in its capacity such that using a generic population-based table will only

work on average for the one population type. Consequently, the BP output is not reflective of our highly diverse population, especially individuals that lay either side of the normal population average, which are typically those who require the greatest attention.

As highlighted, calibration is not restricted to population-based approaches. One-point calibration is an alternate approach that typically involves assigning a measured PWV to a simultaneously acquired brachial BP measurement (Butlin et al., 2018). Extrapolating a curve from this one point would lead to inaccuracies as the relationship is non-linear, it relies on assumptions, and PWV may be influenced by factors which do not necessarily affect BP. Alternatively, the standard calibration approach uses two points to define the relationship. However, it must be noted that even though two points define a line they do not define a curve and therefore assumptions are still required. This method involves attaining pairs of BP and PWV values following the perturbation of BP via an intervention. This is easily achieved following the administration of pharmaceutical agents, such as a vasodilator like nitroglycerin (Liao et al., 2011). Whilst this may be convenient, it is not practical and may also confound the BP estimation as vasoactive agents directly affect smooth muscle which alters in composition throughout the arterial tree. Other notable interventions to perturb BP include exercise (Petrofsky and Lind, 1975), a mental arithmetic test (Sleight et al., 1978), a Valsalva manoeuvre (Parati et al., 1989), or a cold pressor test (Mourot et al., 2009). These interventions can alter BP by 10-50 mmHg, however, such interventions are not necessarily controlled and may vary greatly depending on the individual. Drawing from the literature cited above, it is most probable that the BP to PWV relationship is subject-specific and needs to be identified as such to accurately and reliably estimate BP.

2.1.3.3 The hydrostatic effect

An alternative approach to calibrate the BP/PWV relationship involves a postural change. This approach is promising as it is controlled, practical, does not require the administration of a

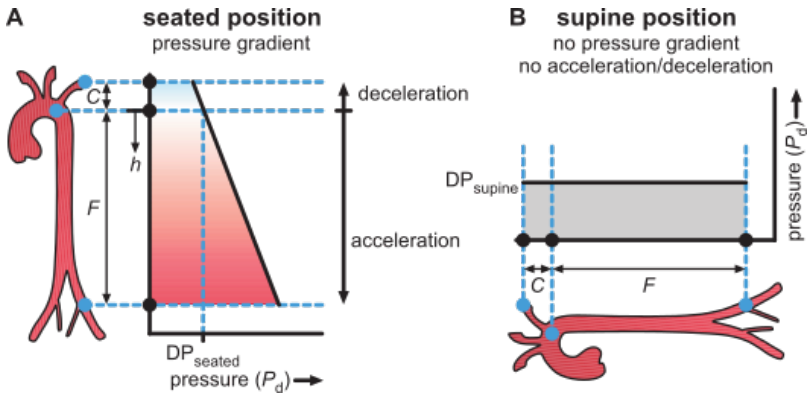

Figure 2.1: Hydrostatic pressure effect exhibited across the carotid (C) to femoral (F) arterial path length in a seated position **(A)** and in a supine position **(B)**. This diagram also displays the gradient effect of hydrostatic pressure in a seated position, such that the hydrostatic pressure may be subtracted when above the heart and added when below. Permission: https://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=f8a49646-0798-4bae-bd3d-118fee3b2932, paper: (Tan et al., 2016).

pharmaceutical agent, and is subject-specific. This entails either simply manipulating the entire body's position (i.e. seated to supine), or even just elevating the arm with respect to the level of the heart. This utilises the hydrostatic effect whereby the systemic pressure will be affected by gravity with respect to the vertical distance from the heart (Fig. 2.1). The hydrostatic pressure (P_H) is described in Equation 2.5 whereby it is a factor of the density of blood (ρ , 1060 kg/m^3), gravity (g , 9.81 m/s^2), and the height of the fluid column (h, m). This method of calibration has previously been explored by Poon and Zhang (2007) and Butlin et al. (2015b) where both respective hydrostatic interventions produced a significant change. The change observed by Poon and Zhang (2007) was a change in PTT within the arm (range $\approx$ 0.6-1.0 m/s/cm), whereas Butlin et al. (2015b) observed a change in carotid-femoral PWV (cfPWV) (1.9$\pm$1.3 m/s) and BP (brachial diastolic systemic pressure change 11$\pm$6 mmHg).

$$P_H = \rho g h \tag{2.5}$$

However, the hydrostatic effect, as expressed by equation 2.5, merely describes the hydrostatic pressure at one specific point. In reality, the effect that the hydrostatic pressure exhibits on the arterial system will be contingent to the height in the fluid column with respect to the level of

the heart. If measured above the beating heart the hydrostatic pressure will have a negative effect, whereas, if it is below it will have a positive effect. This concept has been highlighted by Butlin et al. (2018) whereby an average of the sum of the hydrostatic pressures experienced at various levels across the carotid-femoral pathway was taken. In conjunction with a difference in PWV from a seated to supine position they individually quantified the relationship between PWV and BP. This concept may be highly applicable to smart devices, although as with other calibration techniques, sources of error and the repeatability of this approach still require further investigation.

2.1.3.4 Alternative approaches

The last approaches which attempt to solve this calibration issue are those which are said to be 'calibration-free'. These approaches are not strictly calibration-free but are rather approaches that may incorporate look-up tables or mathematical models that involve attaining other physiological measurements, such as arterial distension. As recorded by Kachuee et al. (2015), BP estimation with the aid of machine learning in a calibration-free environment produced poor BP estimations. In accordance with the British Hypertension Society guidelines, the DBP estimation achieved a grade B whilst the mean arterial BP (MAP) fell into grade C. These results are far from satisfactory especially when the SBP estimation was not even satisfactory to achieve a grade C. Conversely, the study undertaken by Nabeel et al. (2018) produced results that are within an acceptable range with minimal error (root mean squared error: 8.3 mmHg), although, to attain signals required more time and expensive equipment. Regardless of whether it is calibration-free these methods still acquire physiological signals, which if not necessarily mathematically modelled, still theoretically entail calibration in order to estimate BP. This issue may only be investigated after the PWV has been recorded which may be achieved through a variety of methods, as discussed in Section 2.1.4.

2.1.4 Methods of measurement

With any physiological parameter it is important to attain a clean, reproducible, and clinically relevant signal. PWV is no exception and may be obtained through various methods. Irrespective of the chosen method the general procedure involves acquiring a proximal and distal arterial waveform along with the distance that the wave traverses. That being said if the distance remains constant the transit time may be used to estimate BP without the need of this distance. These waveforms are are most commonly measured via photoplethysmography (PPG) or tonometry-based techniques. However, as discussed in Section 2.1.4.1 and Section 2.2, the recording of the arterial waveform is not limited to these two techniques alone. Once a proximal and distal waveform are acquired the transit time may then be calculated based on fiducial markers on the waveforms. There are multiple factors and approaches that may be used to attain the signal, the fiducial markers, and the distance, which may influence the resulting PWV or transit time value.

2.1.4.1 Pulse wave acquisition techniques

There are an array of techniques which may be implemented to attain a pulse waveform. More utilised and established techniques include PPG, tonometry, piezoelectric, ultrasound, and ballistocardiography (Table 2.2). Of these techniques PPG and tonometry waveforms have been extensively research due to the high fidelity and easy acquisition of their respective signals. PPG is an optical technique which detects changes in blood volume, via an absorbance or reflective mode sensor. This can be used in anatomical locations where any blood flow is present, i.e. the skin, and more commonly the arteries within the fingers. However, it is highly susceptible to artefacts, mainly in the form of motion (Ram et al., 2012). Additionally, even though PPG is affected by the respiratory effect, this is something which may be dealt with by filtering processes. Other studies have indicated that PPG is also influenced by ambient temperature, posture, and the relaxation effect (the delay between a stressor and the response) (Lindberg and Oberg, 1991).

Despite PPG being a feasible and practical approach there are inherent limitations which must be considered.

Table 2.2: Waveform acquisition techniques to attain an arterial waveform measurement for the estimation of blood pressure.

Technique	Measurement	Sensor	Device Examples
Photoplethysmography	Blood volume	Pulse oximeter	Finapres
Tonometry or piezoelectric	Pressure waveforms	Force transducer	SphygmoCor XCEL, PulsePen, Complior
Ultrasound	Arterial wall distension	Ultrasound transducer	Dupplex doppler ultrasound device (GE 6P)
Ballistocardiogram	Movement of the body with respect to ventricular systole	Accelerometer	Weighing-scale
Seismocardiogram	Thoracic vibrations that are associated with the heartbeat	Accelerometer	SeismoWatch

Tonometry is another non-invasive technique that involves applanation of a relatively superficial artery (e.g. carotid or radial artery) to measure a pressure waveform generated from each pulse. Devices such as SphygmoCor XCEL (Jatoi et al., 2009) have used this technique in combination with a brachial cuff-based BP calibration to estimate BP. This approach is practical and may be measured at numerous anatomical locations (carotid, femoral, radial) with high fidelity. Although, similar to PPG, these signals are also vulnerable to the same artefacts and depending on the anatomical location they may require greater operator skill to attain a reasonable signal (Mukkamala et al., 2015). This technique further requires a continuous and even contact pressure, and overtime the accuracy of this measurement in a continuous setting may decrease (Peter et al., 2014). These are all considerable factors that are relevant to the underlying biophysical mechanisms associated to the measurement of PWV. A similar technique is the acquisition of piezoelectric signals, being similar to tonometry, incorporating a pressure mechanotransducer. However, this sensor measures the electrical potential across the arterial wall with regards to the mechanical energy (the pulse wave) (McLaughlin et al., 2003). Furthermore, it is subject

to the same complications associated with tonometry along with complexities arising from the algorithms applied which results in the question of suitability of such a technique.

Ultrasound is a technique for evaluating arterial vasculature by using sound waves to measure arterial wall distension caused by the pulse wave. This distension waveform is then calibrated and used in a mathematical relationship for the estimation of BP. This can be achieved solely by ultrasound (Beulen et al., 2011) or in conjunction with other physiologically obtained signals, i.e. PPG (Nabeel et al., 2018). As present with other pulse acquisition techniques the calibration of this waveform is still problematic (Vermeersch et al., 2008). Whilst this technique may require a high level of operator skill to obtain a reasonable signal this may be removed with systems that automatically track the arterial wall. Although ultrasound may produce waveforms and results similar to tonometry (MAP difference = 6.2% or 2.8±1.8 mmHg) (Kips et al., 2010) the use of this technique for cuffless BP estimation may be considered too expensive or impractical due to the nature of the device required for measurements.

Ballistocardiography is an alternative technique which provides a beat-to-beat pulse waveform generated from slight movement within the body. The generation of this movement arises in response to the movement of mass (blood) through the vessels following the ejection of blood from the ventricles. This is subject to limitations such as movement of the individual, or mechanical vibrations that are unavoidable and not experienced in other techniques (Pinheiro et al., 2010). A similar, less common technique that also utilises an accelerometer is the use of seismocardiogram signals. These are based on vibrations associated with the heartbeat and have typically been used to attain a proximal arterial waveform (Carek et al., 2017). The waveform generated has been described in the literature as complex, outdated, subject to noise (Zanetti and Tavakolian, 2013), and may also vary greatly depending on the placement of the sensor. Both the techniques mentioned above have not been compared in-depth with other approaches or extensively researched which makes them not ideal candidates for the estimation of BP, although,

they cannot be dismissed as alternative techniques. To summarise, within the literature there are mixed opinions surrounding the use of each of these techniques despite some promising results. For the sake of practicality, repeatability, and reliability tonometry-based and PPG-based approaches may be considered as ideal candidates for cuffless estimation of BP.

2.1.4.2 Arterial segment

The arterial segment in which these waveforms are measured is another aspect which must be considered. This is of concern as depending on the arterial segment, and the type of arteries measured in this segment (i.e. muscular or elastic), PWV will vary (Mitchell et al., 2010; Tillin et al., 2007). This concept is further supported by Lee and Park (2009) where they measured a difference in PWV of 7.07±1.48 m/s, 8.43±1.14 m/s, and 8.09±0.98 m/s between an aortic, arm, and leg segment, respectively. Waveform shape also differs travelling from proximal to distal locations, due to the reflection of the previous waveforms, and this may need to be accounted for in the calculation of PTT. A clinically interesting arterial segment to study is the carotid-femoral segment. Non-invasive PWV measurements can be made across this segment, which includes the aorta trunk. In comparison to other regions of measurement, such as carotid-radial and carotid-tibial, cfPWV is more predictive of cardiovascular events and all-cause mortality (Blacher et al., 1999; Mitchell et al., 2010; Pannier et al., 2005; Sutton-Tyrrell et al., 2005). This is partly owing to the functional and histological importance of elastic arteries. In conjunction with the clinical relevance of this arterial segment, as outlined, the carotid-femoral arterial segment provides a basis for further investigating PWV. This is additionally supported by the fact that these elastic arteries possess minimal smooth muscle. It is therefore less probable that this arterial segment is confounded by sympathetic activity, which may also influence the PWV measurement (Section 2.1.5). As a result, this solidifies the fact that the carotid-femoral segment

is of high clinical relevance, and is especially important in the estimation of BP as cfPWV may increase BP.

2.1.4.3 Transit time measurement

As noted, there are various fiducial markers which may be exploited to attain a transit time value. This has primarily been investigated for PPG or tonometrically derived arterial waveforms. As expressed in the literature (Millasseau et al., 2005), the diastolic foot obtained by the intersecting tangents method is considered as the best approach for the measurement of transit time, which has been validated against intra-arterial measurements (Chiu et al., 1991; Gao et al., 2016). The fiducial point is located by lines ("tangents") fitted to the late diastole and early systole segments of the waveform (Fig. 2.2). Other approaches, as investigated by Hemon and Phillips (2016), include the maximum value on the curve (systole), the minimum value (diastole), the peak of the first or second derivative of the waveform, and diastolic patching, which involves comparing multiple patches of the diastolic component of the waveform rather than a single point. These approaches all produced averages that closely agreed with the cardiac period, although, the root mean squared error was inconsistent as depicted by the variation. The intersecting tangents method produced the best correlation with invasive transit time (Gaddum et al., 2013) along with the least variation (Hemon and Phillips, 2016). The slightly larger variation present with other approaches may be attributable to factors affecting the regions on the waveform where the markers are present, such as wave reflection or phase noise (Hermeling et al., 2007). Wave reflection does not have a large impact on the diastolic foot, thus the foot is a relatively consistent and reliable marker for calculation of transit time across arterial segments.

Another method to attain a measurement of transit time is coupling an electrocardiogram (ECG) signal with a distal arterial waveform. The R-peak of the ECG provides a simple and rhythmic marker of the commencement of systole, and thus the pulse. The fiducial marker on the distal

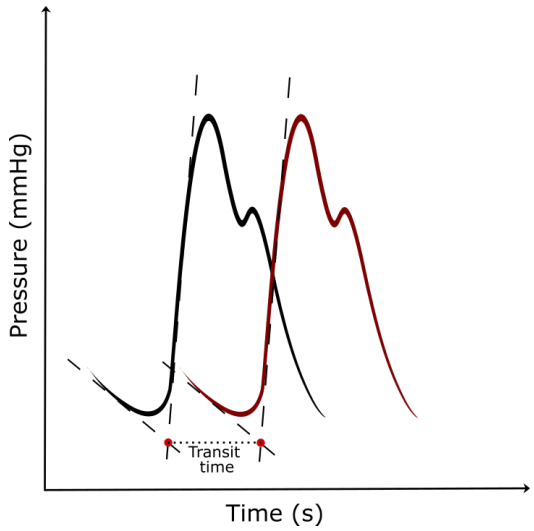

Figure 2.2: The location of a fiducial point on the respect waveform, associated with the diastolic foot, as calculated by the intersecting tangents method. The difference between the two fiducial points is the transit time.

arterial waveform is still attained by the same approaches discussed in the previous paragraph whilst the waveforms are obtained by the same techniques described in Section 2.1.4.1. The time difference between the ECG marker and the arterial waveform marker is known as pulse arrival time (PAT). PAT is calculated as the addition of PTT and the pre-ejection period (PEP), where PEP can be defined as the time required for ventricular ejection to occur from the onset of the generation of the electrical signal (Li and Belz, 1993). Although this method may be considerably simple and reproducible, it introduces a potential confounding factor in the transit time measurement. That is, rather than calculating PTT, this method actually calculates PAT, which fundamentally has a drawback of including the PEP. PEP becomes a confounding factor of the transit time as it is influenced by numerous other factors, such as mental and physical stressors, and these changes are not necessarily directly correlated to BP (Peter et al., 2014). It also varies non-linearly with HR and BP, introducing another unknown variable in the BP/PWV relationship. As a result, this additional time period, if not accounted for, will confound the transit time measurement for the purpose of BP estimation (Zhang et al., 2011).

2.1.4.4 Arterial path length measurement

The distance the pulse wave travels is another component of PWV which has conflicting views regarding its measurement (Van Bortel et al., 2012). This becomes important when using population-based calibration methods and carotid-femoral distances due to the indirect pathway. In comparison, this measurement becomes trivial in arterial segments which are more direct, such as the brachial to radial artery. Including catheter-based methods, tracing the arterial path length via imaging techniques, such as magnetic resonance imaging (MRI), are the only methods to provide the true arterial path length. These approaches are impractical and expensive for use in routine PWV or BP measurements, which leads to the requirement of distance being estimated by other approaches. Alternative approaches that are simple and practical involve body surface measurements or regression equations based on anthropomorphic measurements. One carotid-femoral body surface measurement uses 80% of the direct distance measured between the two sites palpated (Mattace-Raso et al., 2010). Another common approach subtracts the distance from the location of the palpatable carotid pulse to the suprasternal notch, from the distance between the suprasternal notch and the location of the palpatable femoral pulse (subtraction method) (Butlin and Qasem, 2016). Regression methods on the other hand use an equation primarily incorporating the individuals height multiplied and/or divided by a certain factor to predict the arterial path length (Filipovsk et al., 2010; Weber et al., 2009). Regardless of the approach undertaken, there are still discrepancies evident which have typically been investigated in the carotid-femoral path length.

These discrepancies may arise as a result of the approach undertaken to estimate the arterial path length. When compared to distances attained by an MRI the subtraction method produced distances which aligned best with the reference measurement (mean difference: 0.1 ± 2.0 m/s), despite slight over or underestimation within certain age groups (Weber et al., 2015). Keeping in mind that these results are for the carotid-femoral segment there seems to be variability

present within the literature. A contributing factor to these variable results may be related to the fact that within some individuals the arterial path length becomes progressively tortuous with age (Hutchins et al., 1977). This arterial tortuosity also becomes an issue in regression-based approaches. Even though regression-based approaches may be convenient and remove the likelihood for errors to arise with distance measurements, they rely on the correlation between height and arterial path length. This correlation will be affected by tortuosity and furthermore may not hold true among all individuals. Conclusively, depending on the method of BP estimation, the arterial path length measurement may become a determining factor in the estimation of BP and is a factor which therefore requires standardisation.

2.1.5 Factors influencing pulse wave velocity

To further develop an understanding of PWV it is important to discuss the other physiological factors that may influence its measurement. As discussed previously (Section 2.1.1), one such factor is the viscoelastic properties which alters the stiffness of the artery and thus the PWV. These histological properties can be influenced by age and BP (Tanaka et al., 2001) which are both factors that can induce structural and functional abnormalities, likely resulting in an increase in the abundance of collagen fibres with a reduction in elastin fibres, thus increasing stiffness and PWV. The abundance of smooth muscle is another histological feature that can influence PWV. The smooth muscle receives sympathetic input from the autonomic nervous system and when stimulated will cause constriction of the artery and a change in stiffness, independent of the BP distending that artery. As reported by Swierblewska et al. (2010), muscle sympathetic nerve activity was found to be independently and positively correlated with PWV. Individuals who had a faster PWV evidently had increased muscle sympathetic nerve activity compared to normal individuals (30±10 vs. 18±11 bursts/min, p=0.01) (Swierblewska et al., 2010). The degree of sympathetic activity and whether it is short-term or long-term stimulation may further induce

varying effects on the BP (Boutouyrie et al., 1994). Sympathetic activity therefore contributes in part to vascular remodelling resulting in an apparent stiffening of the artery. Considering that smooth muscle is a prominent histological feature in muscular arteries, it can be stated that the effect in which sympathetic stimulation will have on the stiffness of the artery will vary in accordance with the arterial segment. Although there has been research undertaken, further quantification of the influence that the sympathetic activity has is still required, especially for the extent of the effect on anatomically different arterial segments, should PWV be used to estimate BP.

2.2 Alternate methods of cuffless estimation of blood pressure

Cuffless and non-invasive estimation of BP is not restricted to its relationship with PWV, as noted previously and highlighted in Table 2.3. Although, the majority of these methods, similar to PWV, still require obtaining an arterial signal provided there is a measure of the transit time (PTT). As alluded to assuming that the distance remains constant PTT may consequently be used to estimate BP through its inverse relationship with BP. Typically this can be achieved by measuring a proximal and distal arterial signal or coupling an ECG signal with a distal signal for PAT calculation. The techniques used to attain these arterial signals are the same as those discussed in Section 2.1.4.1. An alternative cuffless approach to estimate BP without attaining a measurement of transit time involves performing an analysis of the characteristics of the arterial waveform, which can be measured locally at a single site (Samria et al., 2014). Between these potential approaches it is important that it is both repeatable and practical for an accurate estimation of BP.

Table 2.3: A comparative table highlighting the alternative methods in estimating blood pressure.

Method	Description	Strengths	Limitations
Transit time	Measures the transit time between two arterial waveforms (PTT) or the R peak on and ECG and a distal arterial waveform (PAT).	Quick, practical, reproducible, and subject-specific. Still provides numerous arterial vasculature data.	Calibration. Discrepancies in techniques used.
Arterial waveform analysis	Uses features on an arterial waveform that are related to BP.	Requires only one measurement that can be achieved simply.	Complex mathematical associations. Signal noise.

2.2.1 Methods of pulse acquisition for transit time measurement

Similar to PWV, transit time measurements may be attained by the same techniques displayed in Table 2.2. Rather than requiring a distance measurement of the arterial path length, transit time merely requires two fiducial markers. These markers may be derived from either two arterial waveforms or from an arterial waveform coupled with an ECG signal. The same approaches expressed in Section 2.2.1 are undertaken to attain these markers. To estimate BP a mathematical relationship must be built. The inverse relationship that transit time abides by, more specifically PTT, also relies on mathematical assumptions just as PWV does. This method of measurement furthermore requires a calibration component to accurately define its relationship with BP and therefore the same difficulties arise as observed in PWV calibration. As highlighted by Sharma et al. (2017), numerous mathematical models have been investigated in the hopes to address the calibration issue regarding transit time, with multiple results displaying a high correlation between PTT or PAT and SBP (R^2=0.9573) (Ma, 2014). However, these results are inconsistent across the literature or produce a poor correlation with DBP (R^2=-0.38) (Wong et al., 2009). Furthermore, transit time may be influenced by similar factors as described in Section 2.1.5 and Section 2.1.4.1, such as temperature, viscoelastic properties, and wave reflection. Despite these limitations transit time measurements are a viable avenue for the estimation of BP, however, additional research is still essential.

2.2.2 Arterial waveform analysis

The use of arterial waveform features without the integration of numerous signals, including an ECG, has also been researched as a potential candidate to estimate cuffless BP non-invasively. These waveforms carry a lot of useful features, such as the augmentation index, pulse pressure, pulse amplification, and inflexion points, which when interpreted correctly can offer a strong representation of the cardiovascular system. This notion has been expressed by Samria et al. (2014) where they estimated BP by measuring a single PPG waveform without the aid of a transit time measurement. A good correlation between SBP and DBP with diastolic time was observed (R^2= -0.869 and R^2= -0.811, respectively), however, as evident and explicitly mentioned, the method currently holds inaccuracies. The concept of estimating BP from a single waveform has further been explored by applying complex mathematical equations (Pauca et al., 2001) or machine learning (Khalid et al., 2018). In essence these approaches are a method of calibration in an attempt to generate an estimation of aortic BP based on either a tonometrically derived radial pressure waveform or a PPG waveform. As the case with many studies, there were minimal differences with MAP and DBP (mean±SD: -0.4±1.9 mmHg and -0.6±2.0 mmHg, respectively), however, there were greater differences observed with SBP (-12.8±6.1 mmHg) (Kang et al., 2012). As noted, this was likely due to factors such as the underlying equations, signal noise, pulse pressure amplification, or convolution of the radial pulse. Although this concept does show potential the mentioned limitations need to be addressed, which are not necessarily present with the well-established PWV and transit time methods.

Liu et al. (2018) have further investigated the estimation of BP in the absence of a transit time measurement. This was achieved through acquiring a total of 21 waveform features that were extracted from a radial piezoelectric signal. These features were then incorporated into a variety of linear regression models and compared to brachial oscillometric BP and PTT-based methods. Evidently a multiparametric fusion model produced the best correlation with brachial

systolic and DBP (R=0.87 and R=-0.85, respectively) in comparison to a logarithmic PTT-based approach (R=0.83 and R=-0.80, respectively). Additionally, the multiparametric fusion model also had greater robustness after a follow-up period whilst having a stronger adherence to current guidelines as devised by the Advancement of Medical Instrumentation/European Society of Hypertension/International Organisation for Standardisation (AAMI/ESH/ISO) (Stergiou et al., 2018) (mean±SD SBP and DBP: 0.70±7.78 mmHg and 0.83±5.45 mmHg, respectively). Whilst these results appear auspicious, this method also inherits the limitations of a piezoelectric sensor, is performed in a supine position alone with no intervention, required a total of sixteen calibration measurements, and lastly was based on linear regression models. Accordingly, it is inconclusive as to whether this approach is the ideal candidate for the cuffless estimation of BP.

Moving away from typical clinical/medical-based devices, this concept of arterial waveform features for the estimation of BP has been extended to smartphone technology. A study by Matsumura et al. (2018) utilised this notion whereby they measured HR and modified normalised pulse volume, an arterial measure that reflects the vascular tone, to provide an estimate of BP. These values were measured using reflectance-mode PPG that had been built into the smartphone and also incorporated the aid of a mobile application (iPhysioMeter^{SM}). The device and application produced fair results compared to reference brachial BP measurements ($R > 0.70$), however, this correlation should ideally be stronger. In addition to this, the study was limited by the sample population, being young healthy Japanese men and women, and also by the intervention which these individuals underwent, a mental arithmetic test. There has been numerous other mobile applications which purport to estimate BP using a smartphone, although, as evident in a paper by Plante et al. (2016) caution should be taken as the output values weakly adhere to guidelines (British Hypertensive Society) and may easily be over or underestimated, depending on the group. In spite of the fact that this approach is highly relevant and applicable to our technological society the underlying components applied within these devices and applications requires further research and development.

2.3 Discussion

At present, there is a plentiful amount of studies associated with the cuffless estimation of BP (Table 2.4), however, these studies provide contrasting viewpoints with varying results. Variability in these results may partially arise from the fact that some of these studies are compared to brachial oscillometry rather than the gold-standard of cuff-based BP estimation (auscultation). As brachial oscillometric devices already have an inherent error (mean±SD) this further results in potential variability. This has led to inconclusiveness which is attributable to the insufficiency of research focused on one technique in particular. As apparent from the literature, PWV is one candidate that shows promise and has received a great amount of attention. Despite this attention, PWV thus far has been unsuccessful for the cuffless estimation of BP. This arises as a product of the numerous factors that may influence these BP estimations, as expressed throughout this chapter.

These factors range from the calibration of the relationship to the measurement of PWV. It is clear that certain approaches appear more feasible and accurate when undertaking these measurements. These more ideal approaches have been alluded to and include using the diastolic foot as a fiducial marker, employing the effect of hydrostatic pressure for calibration purposes, subject-specific approaches, using body surface measurements in the calculation of PWV, and using the carotid-femoral arterial site as a site of measurement. Although, it must be kept in mind that the alternative approaches discussed should not be dismissed. To summarise, it is of crucial importance to consider these factors when estimating BP as their collective influence may greatly impact the results.

2.4 Conclusion

Evidently there are numerous approaches within the literature investigating the estimation of BP without the aid of a cuff (Table 2.4). Conclusively, it is important that the chosen method, and its respective calibration, is practical and reproducible for a reliable estimation of BP. PWV and transit time-based approaches are potential candidates providing the greatest foundations. This comes as a result of the techniques employed requiring simpler and less complex mathematical expressions to define the relationship with BP in comparison to arterial waveform analysis. In particular, PWV provides a framework for the cuffless estimation of BP due to the ease of measurement and its well-established relationship with arterial stiffness. However, as highlighted, factors such as the measurement, calibration, or BP perturbations may influence the estimation. Calibration through the hydrostatic pressure effect shows promise in defining the BP/PWV relationship that is subject-specific. The following chapters will seek to provide further knowledge on the discussed notions. Once these limitations have been addressed PWV based techniques may pave the way for cuffless estimation of BP in smart devices.

Table 2.4: An overview of blood pressure estimation results based on the three major methods discussed.

Measurement	Reference	Technique(s)	Calibration	Compared to	Correlation	Accuracy (mmHg)
PWV	Chen et al. (2009)	PPG x 2	Subject-specific (brachial artery BP)	Intra-arterial catheterisation	SBP: R = 0.69, DBP: R = 0.82	DBP: 1.4±7.5
	Marcinkevics et al. (2009)	PPG and ECG	Not specified	Brachial oscillometry	SBP: R = 0929, MAP: R = 0.825	N/A
	Gesche et al. (2012)	PPG and ECG	Subject-specific (brachial artery BP)	Brachial oscillometry	SBP: R = 0.83	SBP: ±10.1
	Chen et al. (2012)	PPG x 2	Subject-specific (brachial artery BP)	Intra-arterial catheterisation	N/A	SBP: 2.16±6.23, DBP: -1.49±6.51
	Sola et al. (2013)	ICG, PPG, and ECG	Subject-specific (brachial artery BP and PTT)	Brachial oscillometry	ICC: MAP 0.78	MAP: 0.7±5.1
	Sanuki et al. (2017)	PPG and ECG	'Calibration-free' (machine learning)	Brachial oscillometry	SBP: R = 0.86	Mean error; SBP: 0.18±8.68
	Nabeel et al. (2018)	PPG x 2 and ultrasound	'Calibration-free' (postural)	Automated auscultatory	SBP: R = 0.79, DBP: R = 0.86	RMSE DBP: 8.3
	Stabouli et al. (2019)	Tonometry	Subject-specific (brachial artery BP)	Brachial oscillometry	SBP: R^2 = 0.875	SBP: -0.3±3.34
PTT/PAT	Poon and Zhang (2005)	ECG and PPG	Subject-specific	Brachial auscultatory	N/A	SBP: 0.6±9.8, DBP: 0.9±5.6
	Wong et al. (2009)	ECG and PPG	Subject-specific (exercise)	Brachial oscillometry	SBP: R = -0.87, DBP: R = -0.30	SBP: 0.0±5.3, DBP: 0.0±2.9
	Ma (2014)	ECG and PPG	Subject-specific (postural)	Brachial oscillometry	SBP: R^2 = 0.9607, DBP: R^2 = 0.7075	SBP: -0.2±2.4, DBP: 0.5±3.9
	Kachuee et al. (2015)	ECG and PPG	'Calibration-free' (machine learning)	The measurement itself	N/A	MAE; SBP: 12.38±16.17, DBP: 6.34±8.45

Table 4 continued...

	Ding et al. (2016)	PPG and ECG	Subject-specific	Finger cuff BP	SBP: R = 0.91, DBP: R = 0.88	SBP: -0.37±5.21, DBP: -0.08±4.08
	Thomas et al. (2016)	ECG and PPG	Subject-specific (postural)	Radial tonometry BP	SBP: R = -0.55	SBP: 8.88±2.30, DBP: 5.97±1.15
	Kachuee et al. (2017)	ECG and PPG	'Calibration-free'	The measurement itself	SBP: R = 0.59, DBP: R = 0.48	MAE; SBP: 11.17±10.09 DBP: 5.35±6.14
	Carek et al. (2017)	SCG, PPG, and ECG	Subject-specific	Finger cuff BP	DBP: R = 0.84±0.09	RMSE SBP: 4.8, DBP: 2.9
Arterial Waveform Features	Pauca et al. (2001)	Tonometry	Subject-specific	Intra-arterial catheterisation	N/A	SBP: -0.1±4.3, DBP: 0.6±1.7
	Kang et al. (2012)	Tonometry	Subject-specific (brachial artery BP)	Brachial oscillometry	SBP: R = 0.982	SBP: -12.8±6.1, DBP: 1.0±0.7
	Kurylyak et al. (2013)	PPG	Not specified	BP waveform	N/A	SBP: 3.80±3.46, DBP: 2.21±2.09
	Samria et al. (2014)	PPG	Subject-specific	Brachial oscillometry	SBP: R^2 = 0.5483, DBP: R^2 = 0.8486	RMSE SBP: ±3.521, DBP: ±3.285
	Plante et al. (2016)	PPG	Subject-specific	Brachial oscillometry	SBP: Spearman p = 0.44, DBP: Spearman p = 0.41	SBP: 12.4±10.5, DBP: 10.1±8.1
	Matsumura et al. (2018)	PPG	Not specified	Brachial oscillometry	SBP: R = 0.722, DBP: R = 0.708	SBP: 0.67±12.7, DBP: 0.45±8.6
	Liu et al. (2018)	Piezoelectric	Subject-specific	Brachial oscillometry	SBP: R = 0.87, DBP: R = 0.85	SBP: 0.7±7.78, DBP: 0.83±5.45
	Khalid et al. (2018)	PPG	'Calibration-free' (machine learning)	Brachial oscillometry	N/A	SBP: -0.1±6.5, DBP: -0.6±5.2

Accuracy: mean±standard deviation (mmHg); BP: blood pressure; DBP: diastolic BP; ECG: electrocardiogram; ICC: intraclass correlation coefficient; MAE: mean absolute error; MAP: mean arterial pressure; PAT: pulse arrival time; PPG: photoplethysmography; PTT: pulse transit time; PWV: pulse wave velocity; R = correlation coefficient; R^2: coefficient of determination; RMSE: root-mean-square error; SCG: seismocardiogram; SBP: systolic BP.

CHAPTER 3

The repeatability of a subject-specific calibration factor for cuffless estimation of blood pressure

This chapter quantifies the repeatability of a PWV to BP calibration factor that primarily uses changes in hydrostatic pressure to determine the calibration factor. The data collected and the analysis presented in this chapter is novel. The method of using hydrostatic pressure to obtain a subject-specific calibration factor for the estimation of BP draws upon previously published work:

- Butlin, M., Hathway, P. J., Kouchaki, Z., Peebles, K., & Avolio, A. P. (2015). A simplified method for quantifying the subject-specific relationship between blood pressure and carotid-femoral pulse wave velocity. In *2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)* (pp. 5708-5711). IEEE.
- Butlin, M., Shirbani, F., Barin, E., Tan, I., Spronck, B., & Avolio, A. P. (2018). Cuffless estimation of blood pressure: importance of variability in blood pressure dependence of arterial stiffness across individuals and measurement sites. *IEEE Transactions on Biomedical Engineering, 65*(11), 2377-2383.

3.1 Introduction

Conventional non-invasive BP measurements have the limitation of being discontinuous and inconvenient. Arterial PWV, a measure of arterial stiffness, is an alternative parameter that has the potential to eliminate these disadvantages through its fundamental relationship with BP (Section 2.1.2). However, to reliably estimate BP from PWV it is critical to accurately characterise the relationship between the two measurements (calibration). Due to issues such as drifting of the relationship, calibration techniques, human variability, and physiological influences (Shaltis et al., 2005), it has proven difficult to characterise the relationship between BP and PWV, which has hindered the progression of cuffless BP estimation. As discussed in Section 2.1.3, there are numerous approaches which may be undertaken to calibrate this relationship. These approaches vary from machine learning (Kachuee et al., 2015), to population-based look-up tables (Gesche et al., 2012), to requiring interventions to alter the BP (Poon and Zhang, 2007). A two-point calibration is one approach in particular requiring an intervention that shows promise in characterising this relationship. This involves measuring both BP and PWV under baseline conditions and following an intervention which alters the BP. However, there are numerous factors to consider with one being the fact that depending on the intervention performed the physiological BP changes will vary (Mukkamala et al., 2015), or the BP will not be very stable in turn hindering the accurate measurement of PWV and BP. Whilst some interventions may result in a large BP change these approaches may conversely induce more artefacts or be less practical. Thus selecting the correct approach is of importance when considering this calibration issue.

Postural changes, i.e. seated to supine, is one intervention that allows a relatively simple and controlled procedure to alter the BP and in turn may be used for calibration purposes. This intervention relies on the hydrostatic pressure effect (Section 2.1.3.3) whereby gravity will influence the BP across the arterial length. However, as PWV incrementally varies with incremental

increases in BP and as BP varies across the height of the fluid column, this calculation is not as simple as gravity multiplied by the height of the fluid column and the density of the blood. It is important to note that the degree of hydrostatic pressure on the arterial system will vary in accordance with the orientation of the arterial bed and the height of the blood in the fluid column. This will be discussed further in the methods section of this chapter. This concept has previously been investigated by Butlin et al. (2018) whereby brachial DBP and the hydrostatic pressure was observed to change on average by 4±7 mmHg and 27±2 mmHg, respectively, when moving from a seated to supine position. The benefits of this approach in particular allow for the generation of a calibration factor that is subject-specific (Butlin et al., 2015a), which, as evident in the literature, is important due to the nature of variability of human physiology. Despite these promising findings determining how well this intervention can estimate BP and whether this intervention is repeatable is necessary. Henceforth, the following chapter explores the repeatability of a subject-specific calibration factor incorporating the measurement of PWV following postural changes that may be utilised to estimate BP.

3.2 Methods

A total of 25 participants (28±11 years, 52% female) were recruited through Macquarie University and friends of the investigator. There was no exclusion criteria present for this study. Participants were not required to fast and were asked to refrain from smoking, exercise, and the consumption of alcohol and caffeine prior to the commencement of the study. One participant was under treatment for hypertension and all others were classified as healthy. All participants were included in the data analysis.

3.2.1 PWV measurement

PWV was measured across the carotid-femoral arterial segment. All measurements, including BP, were attained in accordance with the validated SphygmoCor XCEL device (AtCor Medical, Sydney, Australia) (Butlin and Qasem, 2016; Goodwin et al., 2007). The peripheral arterial waveforms were measured simultaneously by two different techniques. Applanation tonometry was used to acquire the carotid waveform by placing the sensor (force transducer) over where the carotid artery was palpated the strongest. A femoral cuff, using volumetric displacement, was placed around the upper thigh to attain the femoral waveform. The incorporation of a femoral cuff is contradictory to the primary aim of this thesis (investigation of *cuffless* estimation of BP). However, it is used based on the principle of investigating the concept, rather than the technique itself. This method of PWV measurement is a standard and reliable technique and through using a cuff it allows for an investigation of the PWV to BP calibration factor.

Waveform quality was assessed simultaneously during the measurement by the operator to ensure a valid and reliable transit time measurement. This was achieved by visually by observing the waveform shape and the strength including use of the immediate software generated feedback on signal quality, as well as confirmation of a high quality measurement as per the software algorithms post measurement. Transit time was calculated between fiducial markers of the foot of the carotid and femoral cuff waveform (t_{cuff-C}) from the same cardiac cycle (Eq. 3.1). This was performed by the SphygmoCor XCEL device using the intersecting tangents method, which involves lines ("tangents") fitted to the late diastole and early systole segments of the waveform to attain a fiducial marker of the diastolic foot of the waveform (Fig. 2.2). Arterial length was calculated by the subtraction method (Eq. 3.1) using distances measured from the body's surface using a hand held tape measure. Measurements were taken between the site of carotid palpation and the suprasternal notch (d_{S-C}), the suprasternal notch to the top of the femoral cuff (d_{S-cuff}), and the top of the femoral cuff to the centre fold of the leg where the femoral

pulse is able to be palpated (d_{F-cuff}). The distance d_{F-cuff} is used to adjust the measurement with the femoral cuff to be identical to that if cfPWV was measured using tonometry at the site of palpation of the femoral pulse (Butlin et al., 2013). As displayed by Equation 3.1, cfPWV is therefore equal to this change in distance divided by the transit time. The algorithm for calculating this PWV is built into the SphygmoCor XCEL system.

$$\begin{aligned} Transit\ Time &= t_{cuff-C} - k \cdot d_{F-cuff} \\ Distance &= d_{S-cuff} - d_{S-C} - d_{F-cuff} \\ \therefore cfPWV &= \frac{d_{S-cuff} - d_{S-C} - d_{F-cuff}}{t_{cuff-C} - k \cdot d_{F-cuff}} \end{aligned} \tag{3.1}$$

3.2.2 Calibration factor calculation

BP changes can be employed via a variety of methods. This study utilises the hydrostatic pressure effect, as implemented by a postural change, for the purpose of generating a subject-specific calibration factor (Butlin et al., 2015a). The hydrostatic pressure (P_H in Pascals) at the base of a column of blood (artery) is equal to the density of blood ($\rho = 1060$ kg/m^3) multiplied by gravity ($g = 9.81$ m/s^2) and the vertical height of the fluid column (h in metres). Consequently, the hydrostatic pressure will primarily depend on the vertical orientation of the arterial bed and the vertical height of the fluid column, which when altered, can produce a controlled change in BP. Due to the height of the fluid column influencing that hydrostatic pressure, there will be a gradient effect in the seated position that is not experienced in the supine position. This is important because PWV will inherently change in accordance with this gradient. The nature of this gradient has been expressed by Butlin et al. (2018) and will be adopted for the following study (Fig. 2.1).

Two assumptions are made for the premise of this relationship. The first being that for small changes in BP there is a linear relationship with PWV (Eq. 3.2) (Ma et al., 2018). The second is that the measurement of PWV using the foot-to-foot technique correlates to the PWV at DBP. This pressure is used as a reference considering the diastolic foot is used for transit time measurements. Depending on the height along the blood vessel the pressure experienced will differ (Eq. 3.3 and 3.4). In these equations 'a' is the inverse of the pressure sensitivity to PWV and 'b' is the intercept of the linear relationship. Both are considered as unknown variables.

$$\mathrm{PWV} = a \cdot P_{\mathrm{d}} + b \tag{3.2}$$

$$P_{\mathrm{d}} = P_{\mathrm{d,seated}} + P_{\mathrm{H}} \tag{3.3}$$

$$= P_{\mathrm{d,seated}} + \rho g h \tag{3.4}$$

$$\mathrm{PWV} = a\left(P_{\mathrm{d,seated}} + \rho g h\right) + b \tag{3.5}$$

$$= a\rho g h + aP_{\mathrm{d,seated}} + b \tag{3.6}$$

As the vertical distance is a contributing factor in the calculation of PWV, the equation can be re-phrased to equal the incremental distance (dh) divided by the incremental transit time (dt) (Eq. 3.7). Making transit time the subject of the calculation, transit time now becomes the summation of all the incremental changes (integral) in PWV and pressure with respect to height (Eq. 3.9-3.11).

$$\mathrm{PWV} = \frac{\mathrm{d}h}{\mathrm{d}t} \tag{3.7}$$

$$\mathrm{d}t = \frac{\mathrm{d}h}{\mathrm{PWV}} \tag{3.8}$$

$$t = \int_{h_2}^{h_1} \frac{1}{\mathrm{PWV}} \mathrm{d}h \tag{3.9}$$

$$= \int_{h_2}^{h_1} \frac{1}{a\rho g h + (aP_{\mathrm{d,seated}} + b)} \mathrm{d}h \tag{3.10}$$

$$= \frac{\ln\left(a\rho g h + aP_{\mathrm{d,seated}} + b\right)}{a\rho g}\Bigg|_{h_2}^{h_1} \tag{3.11}$$

As referenced to in Section 2.1.3.3, depending on whether the pulse wave is above or below the heart will influence the net pressure effect (Fig. 3.1). Assuming that the brachial DBP is measured in line with the heart and using this point at a reference anything above this level will be considered to have a reduced pressure in comparison to the brachial pressure (Eq. 3.12). Comparatively, the hydrostatic pressure will provide an additional pressure to any region below this reference point (Eq. 3.13). It must be noted that these additional or reduced pressures align with the vertical distance, thus in a supine position there is no difference.

$$t_{\mathrm{C}} = \int_{-C}^{0} \frac{1}{\mathrm{PWV}} \mathrm{d}h \tag{3.12}$$

$$= \frac{1}{a\rho g} \ln\left(\frac{aP_{\mathrm{d,seated}} + b}{a\left(P_{\mathrm{d,seated}} - \rho g C\right) + b}\right) \tag{3.13}$$

$$t_{\mathrm{F}} = \int_{0}^{F} \frac{1}{\mathrm{PWV}} \mathrm{d}h \tag{3.14}$$

$$= \frac{1}{a\rho g} \ln\left(\frac{a\left(\rho g F + P_{\mathrm{d,seated}}\right) + b}{aP_{\mathrm{d,seated}} + b}\right) \tag{3.15}$$

With reference to the change in pressure experienced in relation to being above or below the level of the heart, it is apparent that the transit time in a seated position is defined as the difference of the waveform travelling to the carotid site and the waveform that travels to the femoral site. As this transit time is an incremental summation with respect to the distending pressure and the hydrostatic pressure along the arterial vessel, the transit time can therefore be expressed as displayed in Equation 3.17.

$$t_{\mathrm{seated}} = t_{\mathrm{F}} - t_{\mathrm{C}} \tag{3.16}$$

$$\begin{aligned} &= \frac{1}{a\rho g}[\ln\left(a\left(P_{\mathrm{d,seated}} + \rho g F\right) + b\right) \\ &+ \ln\left(a\left(P_{\mathrm{d,seated}} - \rho g C\right) + b\right) \\ &- 2\ln\left(aP_{\mathrm{d,seated}} + b\right)] \end{aligned} \tag{3.17}$$

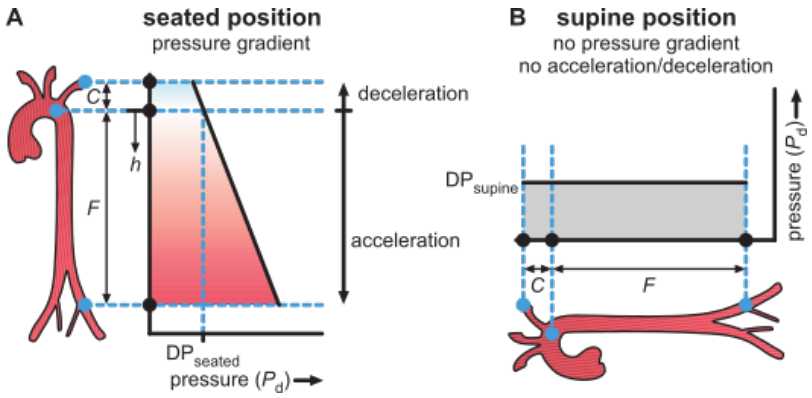

Figure 3.1: Hydrostatic pressure effect exhibited across the carotid (C) to femoral (F) arterial path length in a seated position **(A)** and in a supine position **(B)**. This diagram also displays the gradient effect of hydrostatic pressure in a seated position, such that the hydrostatic pressure may be subtracted when above the heart and added when below. Permission: https://s100.copyright.com/CustomerAdmin/PLF.jsp?ref=f8a49646-0798-4bae-bd3d-118fee3b2932, paper: (Tan et al., 2016).

As there is relatively no change in vertical height of the blood vessels in a supine position, there is essentially no change in the hydrostatic pressure as the arterial bed lies horizontally in the same plane. Consequently there is no incremental summation required for the calculation of PWV in a supine position (Eq. 3.2). The arterial pressure therefore remains relatively constant allowing a simple calculation of the supine transit time (Eq. 3.20). Following this, the change in arterial PWV can now be successfully calculated based on the incremental changes with respect to the hydrostatic pressure. This requires using Equation 3.17 and 3.20 to solve the two unknown variables and thus PWV.

$$t_{\text{supine}} = \frac{F - C}{\text{PWV}} \tag{3.18}$$

$$= \frac{F - C}{a \cdot P_{\text{d,supine}} + b} \tag{3.19}$$

Using this hydrostatic pressure equation an average hydrostatic pressure value across the arterial segment of interest was produced. Accompanied with the change in DBP following a postural change and the respective change in cfPWV a calibration factor for each individual was generated (Eq. 3.20). This calibration factor was calculated for each visit and is used later on in Chapter 5.

$$\begin{aligned} Calibration\ Factor &= \frac{DBP_{Seated} + Hydrostatic\ Pressure - DBP_{Supine}}{PWV_{Seated} - PWV_{Supine}} \\ &\approx \frac{\Delta BP}{\Delta PWV} \end{aligned} \tag{3.20}$$

3.2.3 Experimental protocol

Prior to the commencement of measurements participants provided informed consent and were then asked to refrain from talking throughout the study. Following 5 minutes of seated rest with their back supported and legs uncrossed brachial oscillometric BP, of the left arm at heart level, and cfPWV were measured in duplicate in the seated position (SphygmoCor XCEL). Cuff-size was based on a brachial circumference measurement and then using the respective cuff. SBP and DBP readings were required to be within ±5 mmHg of each other whereas cfPWV measurements were required to be within ±0.5 m/s of each other. Measurements were repeated until two consecutive readings fell within this criterion. Subjects were then asked to move into a supine position where BP and cfPWV measurements were then repeated in duplicate after an additional 5 minutes of rest. All measurements were repeated by the same operator across two separate visits approximately at similar times. The average duration of each visit was approximately 30 minutes.

3.2.4 Data and statistical analyses

All experimental data was collected with the SphygmoCor XCEL device under baseline conditions, by a single operator, in a temperature and noise-controlled environment. cfPWV was recorded and calculated based on handheld body-surface tape measurements and an average of a 20 second recording of the respective arterial waveforms. Data was extracted from the system and the calibration factor was calculated using a custom script written in the software 'R' before exporting the data for subsequent analysis in Microsoft Excel. Participant demographic data

was represented as the mean±SD (standard deviation). The calibration factor, change in PWV, and change in pressure were calculated in response to the postural change for each visit. The repeatability and agreement of the mentioned parameters was assessed between each visit through Bland-Altman plots and paired t-tests. Measurements were considered repeatable if they lay close to the line of unity in the Bland-Altman plots. The confidence intervals are described as limits (mean±1.96·SD) in the Bland-Altman. A p value less than 0.05 indicated significance.

3.3 Results

Participant baseline demographic data is highlighted in Table 3.1. The average HR, SBP, and DBP across both visits were within healthy ranges. Two participants had a slightly elevated BP which is evident from the range. One participant was taking antihypertensive medication. Table 3.2 displays the average changes in haemodynamic parameters following the postural change with respect to both visits. All parameters were significantly different between the seated and supine position, except for SBP. Notably there was a change in cfPWV of 1.9±0.7 m/s, DBP of 4±5 mmHg, and hydrostatic pressure of 27±2 mmHg. These changes form the inputs for the generation of the calibration factor.

Table 3.1: Participant baseline demographic data averaged across visit 1 and visit 2.

Parameter	Mean±SD	Range
Age (years)	28±11	19-69
HR (bpm)	72±10	50-93
Seated Brachial arterial blood pressure (mmHg)		
SBP	117±12	98-145
DBP	74±8	57-89

bpm: beats per minute; DBP: diastolic blood pressure; HR: heart rate (seated); SBP: systolic blood pressure; SD: standard deviation.

Table 3.2: Average changes in haemodynamic parameters following the postural change.

Parameter	Seated	Supine	Change
HR (bpm)	72±10	67±10*	6±6
SBP (mmHg)	117±12	116±10	1±8
DBP (mmHg)	74±8	69±7*	4±5
P_H (mmHg)	27±2	0±0*	27±2
Total Pressure (mmHg)	101±8	69±7*	32±6
cfPWV (m/s)	7.5±1.5	5.6±1.0*	1.9±0.7

bpm: beats per minute; cfPWV: carotid-femoral pulse wave velocity; DBP: diastolic blood pressure; HR: heart rate; P_H: hydrostatic pressure; SBP: systolic blood pressure.
$*$ indicates $p< 0.0001$, compared to seated position.

The measurements required to determine the calibration factor are displayed in Figure 3.2. This incorporates the change in pressure (DBP and hydrostatic) plotted against the change in PWV from a seated to supine position. Figure **A** and **B** represent the calibration factors generated from visit one and visit two, respectively. The dashed line represents the slope of the average calibration factor, assuming the intercept lies at zero-zero. From the first visit the mean calibration factor was 18.1±7.9 mmHg/m/s while for the second visit it was 19.5±7.0 mmHg/m/s. The dotted lines represent the calibration slopes for each individual and are colour-coded.

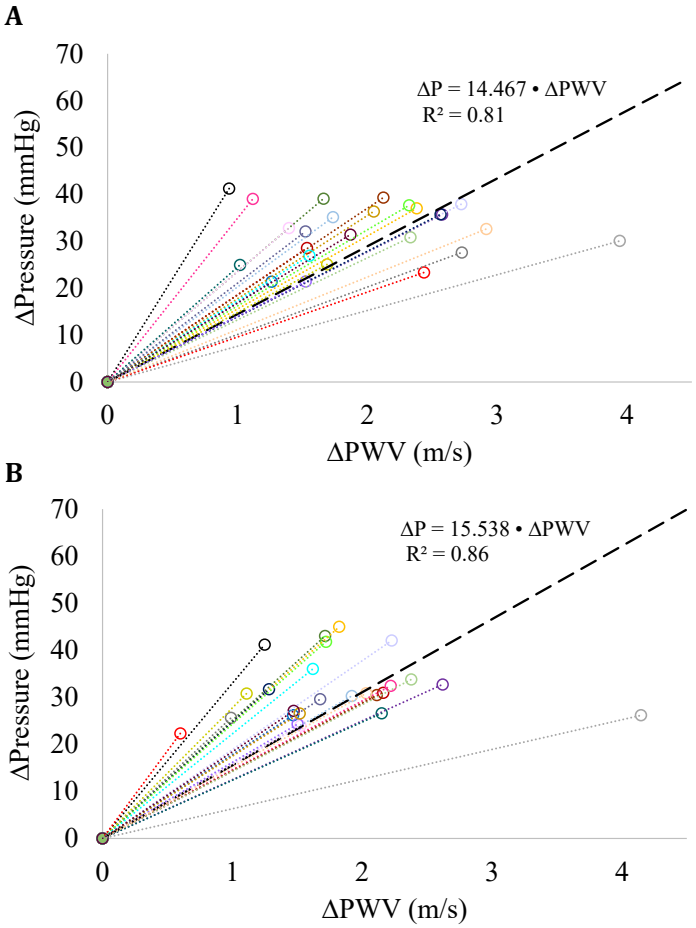

Figure 3.2: Measurements required to calculate the calibration factor from visit 1 **(A)** and visit 2 **(B)**. Each individual is represented by a specific colour. Dotted line: individual calibration slope. Dashed line: average of calibration slopes. (P: arterial pressure).

3.3.1 Repeatability of the calibration factor

The repeatability of the calibration factor was assessed between the first and the second visit using Bland-Altman plots (Fig. 3.3). Figure **A** depicts the calibration factor generated from the second visit on the y-axis whilst the x-axis depicts the calibration factor generated from the first visit. The dotted line represents the line of unity providing a visual representation of the agreement between the two measurements. The calibration factor ($\Delta BP/\Delta PWV$) for visit one and visit two were 18.1±7.9 mmHg/m/s and 19.5±7.0 mmHg/m/s, respectively. The difference between the two measurements was -1.4±9.8 mmHg/m/s and was not significantly different

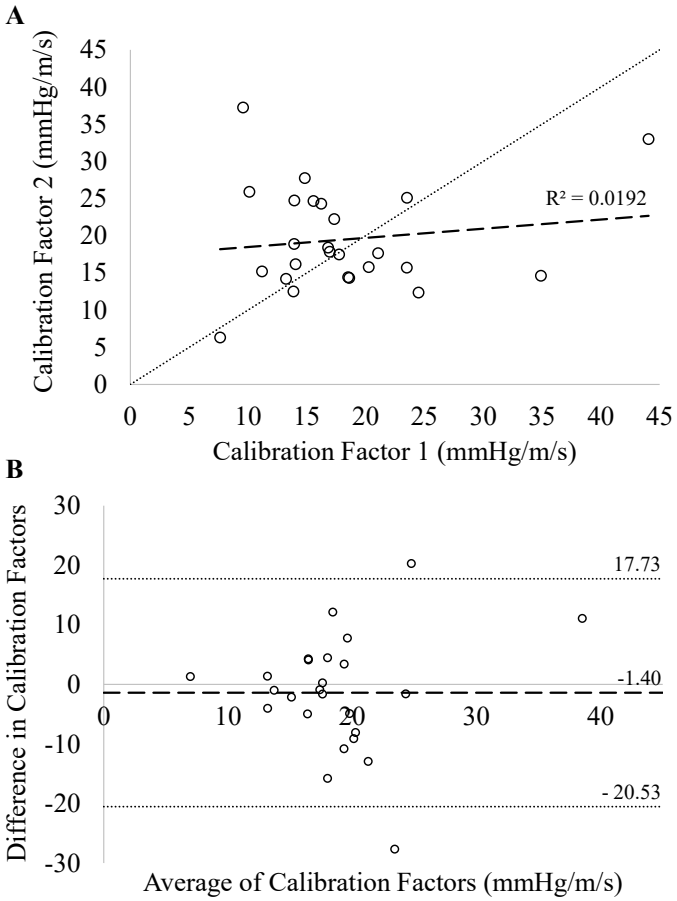

Figure 3.3: Bland-Altman analysis of calibration factor. **(A)** displays the repeatability of the calibration factor. The BP/PWV relationship from visit 1 was 18.1±7.9 mmHg/m/s and in visit 2 was 19.5±7.0 mmHg/m/s (difference -1.4±9.8 mmHg/m/s, p=0.48). Dotted line: line of unity. **(B)** represents the degree of agreement between the two slope measurements. Dashed line: mean difference. Dotted line: mean±1.96SD.

(p=0.48). However, the correlation between measurements from visit one and two across the cohort was low (R^2=0.0192, p=0.48). The agreement of the measurement between visit one and two was further assessed by comparing the difference in the calibration factors against the average of the calibration factors (Fig. **B**). The dashed line represents the average difference between the two measurements (-1.4 mmHg/m/s). The dotted lines represent an upper and lower limit in which points should fall in to be considered as having good agreement. The upper limit is equal to 18.1 mmHg/m/s whereas the lower limit is 20.9 mmHg/m/s. These lines are calculated by the mean±1.96·SD. Evidently there is variability present of the calibration factor despite there being no significant difference between visit 1 and visit 2 across the cohort.

3.3.2 Repeatability of the change in pressure and PWV

Due to the variability observed in the Bland-Altman plots of the repeatability of the calibration factor, further *post-hoc* analysis was undertaken. This involved performing a Bland-Altman analysis on the change in PWV (Fig. 3.4) and the change in pressure (Fig. 3.5). Average PWV for visit one and two was 2.00±0.70 m/s and 1.81±0.68 m/s, respectively (Fig. 3.4 **A**), whilst the coefficient of determination was R^2=0.1929. PWV appeared to have similar repeatability to that observed from the calibration factor. There was a root-mean-squared error of 41% in the measurement of PWV. PWV showed acceptable agreement between the two visits, although there was a reasonable amount of variability present (Fig. 3.4 **B**). The average difference between the two measurements was 0.19±0.73 m/s and was not significantly different between visits (p=0.21). The upper and lower 95% confidence interval limits were 1.6 m/s and -1.3 m/s, respectively.

Regarding the measurement of pressure, this included both the change in DBP and hydrostatic pressure. As observed in Figure 3.5 **A**, the points tended to be less scattered lying more uniformly along the line of unity. The average change in pressure for visit one and two was 32.1±5.9 mmHg and 31.7±6.5 mmHg. The correlation between the first and the second visit was greater for BP than PWV with the coefficient of determination for BP equal to 0.416. The change in pressure had upper and lower 95% confidence interval limits of 11.0 mmHg and -10.0 mmHg. The average difference between the change in pressures was 0.5±5.3 mmHg and was not significantly different (p=0.65). The change in pressure displayed a root-mean-squared error of 16%, less than that

observed in the measurement of PWV.

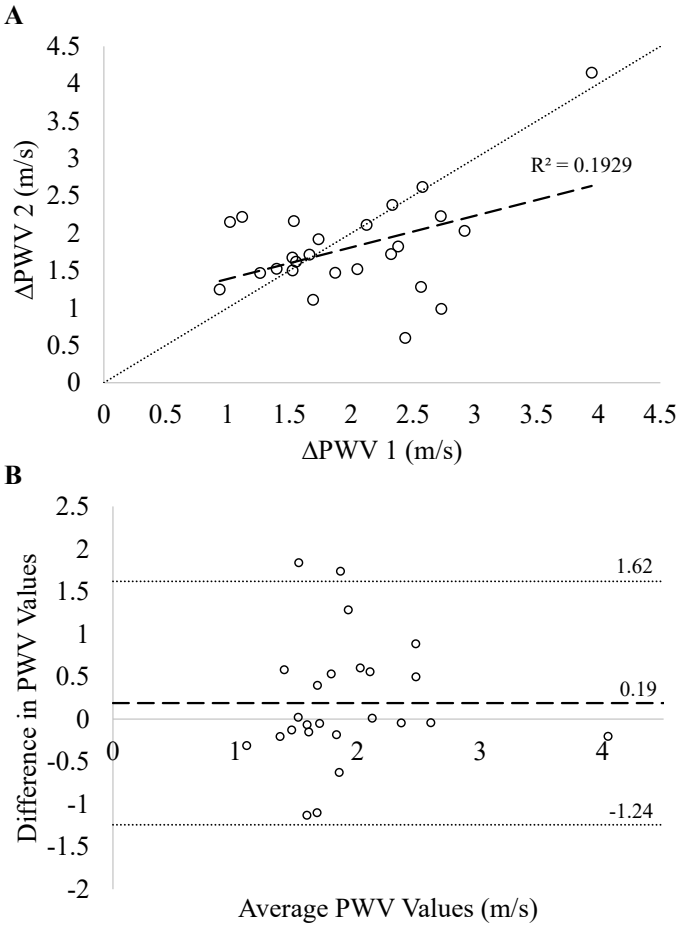

Figure 3.4: Bland-Altman analysis of PWV. **(A)** shows the repeatability of the PWV measurement. PWV measurements showed variability (difference 0.19±0.73 m/s, root-mean-square error 41%, p=0.21). Dotted line: line of unity. **(B)** depicts the degree of agreement between the two PWV measurements. The y-axis represents the change in PWV between visit 1 and visit 2 whilst the x-axis depicts the average of the PWV measurements made across both visits. Dashed line: mean difference. Dotted line: mean±1.96SD.

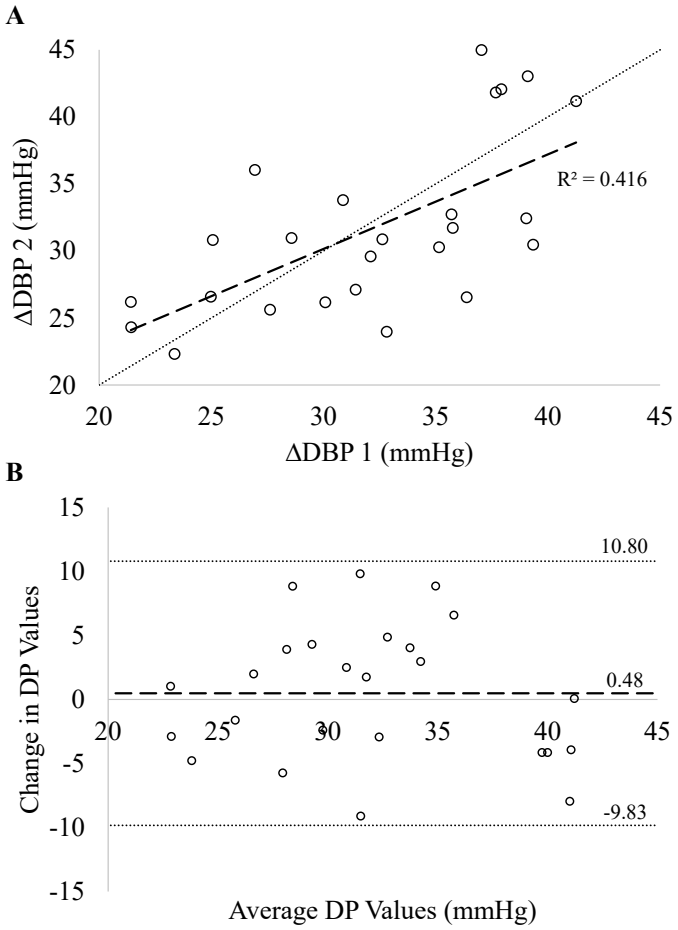

Figure 3.5: Bland-Altman analysis of the pressure change. **(A)** shows the repeatability of the pressure measurement between the 2 visits. Pressure change conveyed minimal variation (difference 0.5±5.3 mmHg, root-mean-square error 16%, p=0.65). Dotted line: line of unity. **(B)** depicts the degree of agreement between the two pressure measurements. The y-axis represents the change in pressure between visit 1 and visit 2 whilst the x-axis depicts the average of the pressure measurements made across both visits. Dashed line: mean difference. Dotted line: mean±1.96SD.

Additional analysis was undertaken to quantify variability in the PWV and DBP measurements between each visit and each posture (Fig. 3.6). Seated PWV measurements (Fig. **A**, p=0.41) were more variable than supine PWV measurements (Fig. **C**, p=0.17), as indicated by the coefficient of determination (R^2=0.7919 and R^2=0.9442, respectively). Supine PWV measurements tended to be slightly less scattered suggesting greater repeatability. Conversely, supine DBP measurements (Fig. **D**, p=0.85)) were more variable and less repeatable compared to seated DBP measurements (Fig. **B**, p=0.81)). DBP measurements appeared less repeatable, as apparent by the scatter. The coefficients of determination were R^2=0.6079 for Figure **(B)** and R^2=0.4873 for Figure **(D)**.

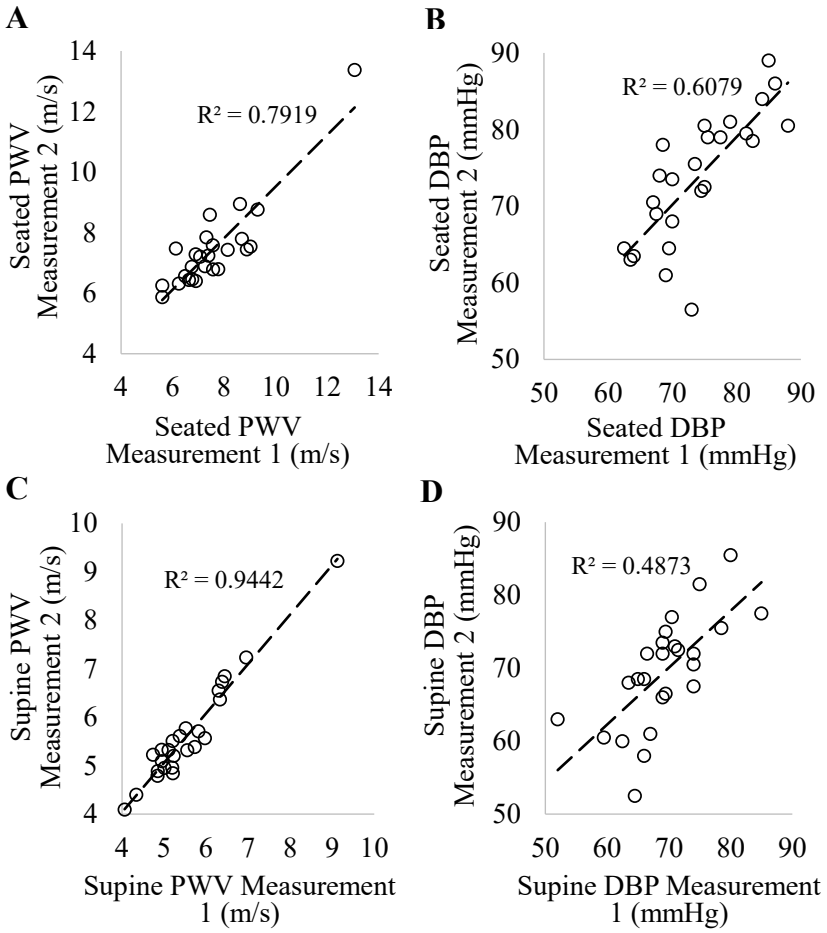

Figure 3.6: Bland-Altman analysis plot of the difference between seated pulse wave velocity (PWV) measurements from the second visit plotted against those from the first visit (R^2=0.7919) **(A)**. **(B)** plots the difference between seated diastolic blood pressure (DBP) measurements from the second visit plotted against those from the first visit (R^2=0.6079). **(C)** plots the difference between supine PWV measurements from the second visit plotted against those from the first visit (R^2=0.9442). **(D)** plots the difference between supine DBP measurements from the second visit plotted against those from the first visit (R^2=0.4873).

3.4 Discussion

This is the first study to investigate the repeatability of this calibration factor and one of the few studies investigating the hydrostatic pressure effect for the purpose of calibration. As anticipated the postural change induced a significant change in DBP (4±5 mmHg) and cfPWV (1.9±0.7 m/s). A similar magnitude of change was observed in previous studies with postural shifts invoking significant changes in DBP and cfPWV of 11±6 mmHg and 1.9±1.3 m/s (Butlin et al., 2015a), and 4±7 mmHg and 1.7±0.7 m/s (Butlin et al., 2018), with hydrostatic changes in pressure of 24

mmHg and 27±2 mmHg, respectively. These studies have produced significant systemic pressure changes, however, it must be noted that the study undertaken by Butlin et al. (2015a) used a postural change from a standing to supine position and furthermore used an over-simplified method to calculate the hydrostatic pressure that was not based on the incremental summation of changes with respect to height of the fluid column. While the hydrostatic pressure effects SBP and DBP equally, SBP did not significantly change following the postural change. This may be attributable to the dependency that SBP has on other factors that DBP does not. Despite this there was still a significant change in total pressure following the postural change which is what was required for the calculation of the calibration factor. HR also significantly decreased with the postural change ($p<0.001$). Acute increases in HR have been associated with increased cfPWV, as reported by Tan et al. (2016). An increase of 10 bpm increases cfPWV by 0.16-0.20 m/s. The change in HR in this study was 6±6 bpm. We can therefore calculate direct HR effect on cfPWV in this study to be in the range of 0.10 to 0.12 m/s. Given the average change in cfPWV from seated to supine was 1.9 m/s, the HR change might be considered to account for 5-6% of the cfPWV change, the remainder being most likely a pure BP effect.

Figure 3.2 highlights how the calibration factor for each individual is calculated. The variability in slopes suggests that there is great variability in the calibration factor across the population. As each person was their own control the only meaningful difference is that between the values from the first and second visit for that specific individual. This population variability was not of importance in this experiment but is investigated in a larger cohort later in this thesis (Chapter 5). This therefore excludes the potential of the data point on the far right of the graphs being considered as an apparent outlier. Whilst this point lies substantially distant from the cluster this may be attributable to the participants demographic data, in particular their older age and cardiovascular status.

Good repeatability and agreement was observed from the measurement of the calibration factor,

as assessed by the Bland-Altman plots (Fig. 3.3). The degree of repeatability was assessed by how close the data points lay along the line of unity (dotted line) (Fig. **A**). There was no significant difference (p=0.48) between the measurement of the calibration factor performed on the first and the second visit which strengthens the notion that the measurement is repeatable. The points tend to cluster around this line, however, as visually apparent there is variability present. In particular, there are two points which are exceedingly far from the line of unity. Whilst the coefficient of determination (R^2) is equal to 0.0192 and is well below what would be considered as an ideal value it is important to note that this statistical parameter does not provide a measure of the repeatability of the measurement, as expressed by Bland and Altman (1986). Upon further analysis, Figure **B** depicts the level of agreement of the measurement of the calibration factor. Whilst this graph does show agreement the variability observed from the previous figure is still present, as reflected by the scatter of the data points. It is difficult to determine whether the average difference in calibration factor values (-1.4±9.8 mmHg/m/s) falls within an acceptable range due to the lack of research on this method of calibration.

The disparity observed may be a product of the conditions in which the test was performed in (environmental or mental stress on the individual) or natural human variability. As tightly controlled as these factors may be there are unavoidable consequences of human research which may prove difficult in controlling but also reflect the real-world measurements of such a calibration factor. The variability may also be driven by error in the measurement itself, in the accurate determination of transit time, of distance measurements from surface markers, and of the blood pressure measurement with a brachial cuff. To expand upon this, Bland-Altman plots were generated for the change in pressure and change in PWV between the two visits. Figure 3.4 (**A** and **B**) illustrates that the difference in PWV between the two visits is a potential cause of this variability. Visually the data aligned more closely to the line of unity, but when the magnitude of the variability between the measurements and the line of unity is taken into account it indicates that PWV is a strong contributor to the observed between-visit variability in the calibration

factor. Whilst there was no significant difference in the change in PWV (p=0.21), 41% of the error in the calibration factor may be attributable to this change. A plausible explanation of this variability was established when the points were individually examined from the data set. It became apparent that there was a tendency of PWV values to vary more so in a seated position in comparison to a supine position (Fig. 3.6). Conversely, DBP values tended to be more variable in a supine position, which was an unexpected result considering it may be assumed that in a supine position an individual would be in a completely rested and stable state. This could be attributable to factors that effect BP greater than PWV following the postural manoeuvre and its respective change in hydrostatic pressure, such as the preload and afterload on the heart.

There are numerous studies which compare the repeatability of the PWV measurement (Grillo et al., 2018; Wilkinson et al., 1998; Yamashina et al., 2002). Despite the fact that these studies do not measure the change in PWV in accordance with a postural intervention they still provide a guideline on the repeatability and agreement of the PWV measurement. Yamashina et al. (2002) assessed the brachial-ankle PWV using a PPG-based apparatus to intra-arterial catheter measured aortic PWV. The intra-operator reproducibility was 10.0% with a strong correlation between the two measurements (R=0.87, p<0.01). Along with the method of measurement these results were recorded in anaesthetised participants undergoing cardiac angiography which becomes a limiting factor when comparing. In the study presented by Wilkinson et al. (1998) the repeatability of cfPWV was assessed from a single visit using the SphygmoCor apparatus using tonometry at both the carotid and femoral pulse sites. The within-operator differences were minimal (0.07±0.24 m/s, mean±SEM) and showed no significant difference (p=0.78) further supporting the repeatability of the PWV measurement. Conversely, the study performed by Grillo et al. (2018) measured cfPWV using the same SphygmoCor device (tonometry at both the carotid and femoral sites) under similar conditions to those performed in this study (two duplicate measurements by a single operator under baseline conditions in a controlled environment). cfPWV was found to have the greatest coefficient of variation (9.5%) and the largest confidence

intervals ($\approx \pm 2.99$ m/s, mean$\pm$1.96SD) in comparison to other methods of PWV measurement (PulsePen, Mobil-O-Graph, Complior, and BPLab). Whilst the measurement may still be deemed as repeatable, the agreement between and variability observed may be considered similar to that presented here, as reflected by the Bland-Altman plot. It must be taken into account that these measurements are all performed in a supine position, most likely by an experienced operator. This leads to another contributing factor regarding the inconsistency observed in the measurement of PWV, which may be aligned to the experience of the operator, and requires additional research.

Upon an analysis of the change in pressure between the visits, including DBP and hydrostatic pressure, the Bland-Altman plots displayed far stronger repeatability and agreement (Fig. 3.5) in comparison to the change in PWV and the calibration factor. Even though the data was still scattered it was evidently more tightly bound to the line of unity (Fig. **A**). The difference between the two measurements was minimal (0.5$\pm$5.3) which was not significant (p=0.65). It further displayed reasonable agreement with all data points falling within the limits of agreement (Fig. **B**). Additionally, the change in pressure only contributed to 16% of the error in the calibration factor variability. This supports the notion that BP can be altered in a controlled manner, which is practical for calibration purposes. A key limitation of the present study is the sample size. A *post-hoc* analysis revealed a sample size of 25 with a standard deviation of 7.45 had a statistical power of 67% to detect a 20% difference in the calibration factor, and a 23% power to detect a 10% difference in the calibration factor.

3.5 Conclusions

To conclude, as anticipated, the postural change induced a significant change in haemodynamic parameters including HR, DBP, P_H, and cfPWV. However, SBP was not significantly affected by the change in posture. Importantly, the calibration factor, on average, can be measured with good repeatability within an individual. Despite the promising results it was apparent that variability of the calibration factor was present. From an analysis of Bland-Altman plots regarding the change in PWV and change in pressure due to the postural manoeuvre, the variability present may be attributable to the change in PWV, rather than the pressure, as reflected by the relative error. In summary, this method of generating a subject-specific calibration factor does hold merit but still requires further investigation to properly quantify the relationship between PWV and BP, and in particular the variability observed in the measurement of PWV.

CHAPTER 4

Sources of error in the measurement of PWV

4.1 Introduction

Whilst it is established that cfPWV is a predictor of cardiovascular complications (Mitchell et al., 2010), there has been limited uptake of this parameter in a clinical setting (Gurovich and Braith, 2011). Despite its clinical relevance, this may partially be attributable to the variability in the repeatability of the cfPWV measurement, such as observed in Chapter 3. It is possible that this variability may be accounted for by errors within the measurement, such as the distance measured between arterial sites, and the operator's ability to acquire a reliable waveform for the determination of PTT. In conjunction with this variability the influence of inconsistencies in measurements additionally highlights the requirement of a subject-specific due to individual variability in cfPWV measurements. Although previous studies have proved helpful, they have predominantly focused on the accuracy and repeatability of PWV (Grillo et al., 2018; Lee and Park, 2009) using different techniques or waveform indices (Avolio et al., 2010) rather than the associated errors and the reliability.

While the relevance of cfPWV lies with its assessment of arterial stiffness it goes without saying that if this parameter is to be used for the purposes of BP estimation, such as described for the generation of a subject-specific calibration factor (Eq. 3.20), it is necessary to investigate the underlying biophysical mechanisms associated in the measurement of PWV. This involves

the potential sources of error. To quantify these discrepancies, the following chapter aims to examine the potential sources of error in the measurement of PWV. These sources of error will be investigated through assessing the variability in:

- The measurement of PWV;
- Operator experience;
- Waveform quality;
- Changing the arterial distance.

In understanding these potential sources of error in the measurement of cfPWV adjustments may be implemented to reduce any variability which will henceforth assist in the accurate characterisation of the calibration factor through increasing the reliability of measurements.

4.2 Methods

Fifteen participants (30±15 years, 80% female) were recruited through Macquarie University and friends of the investigator. For the purpose of this study no exclusion criteria was required. Participants were not required to fast and were asked to refrain from smoking, exercise, and the consumption of alcohol and caffeine prior to the commencement of the study. One participant was under treatment for hypertension. This study was not restricted to a particular time of the day. All other participants were deemed as healthy. Data analysis was performed on all participants recruited.

4.2.1 PWV measurements

All PWV measurements were taken using the SphygmoCor XCEL device (AtCor Medial, Sydney, Australia) from the carotid to femoral arterial segment, as previously described in Section 3.2.1. Measurements were split into three sections:

1. The accuracy of cfPWV, including PTT and distance measurements;
2. The effect of femoral cuff placement;
3. The effect of carotid pulse waveform quality.

The accuracy of cfPWV, PTT, and distance was assessed in a supine position by two operators with a difference in experience (experienced and inexperienced). This involved both operators performing repeated measurements of cfPWV, carotid-femoral PTT (cfPTT), and carotid-femoral distance to obtain a 'stable value', which from here on will be referred to as the 'true value'. PWV measurements assessing the effect of femoral cuff placement involved altering the arterial path length by re-positioning the femoral cuff to approximately 1 cm above knee. This extended path length incorporated an additional arterial segment that is more muscular in nature and is influenced by age and sympathetic activity in a different manner, compared to the aortic segment. Poor quality carotid waveforms were assessed by visually acquiring a noisy carotid signal that also had a low signal strength, as gauged by observing the waveform shape and the strength including use of the immediate software generated feedback on signal quality, as well as confirmation of a high quality measurement as per the software algorithms post measurement. All cfPWV, cfPTT, and distance measurements were taken under baseline conditions.

4.2.2 Experimental protocol

Participants first provided informed consent and undertook a general participant questionnaire. Individuals were asked to relax and refrain from speaking and sleeping throughout the duration of the experiment. Participants were fitted with a brachial and femoral cuff, placed on the left arm and upper thigh, respectively. A strip of tape was placed to mark the location of the femoral cuff due to the possibility of the cuff moving throughout the duration of the study as a result of repeated cuff inflation and deflation or from moving to a supine position. After 5 minutes of seated rest oscillometric brachial BP was recorded in the left arm (SphygmoCor XCEL). A total of three brachial BP recordings were performed. If the first two BP measurements had a difference of less than 7 mmHg a third measurement was performed (Parati et al., 1989). If this difference was greater than 7 mmHg measurements were repeated until there was agreement.

Participants were then asked to move to a supine position. HR (ECG lead II configuration) and BP (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands) were recorded continuously throughout the remainder of the experiment using PowerLab Acquisition System (ADInstruments, Dunedin, New Zealand). After 5 minutes of supine rest baseline BP measurements were repeated as previously described. An average of the last two measurements, regardless of their difference, was used for calibration of the SphygmoCor XCEL device. For the reliability part of the study, cfPWV was then measured a total of ten times by each operator, alternating operator between each measurement. One operator was experienced in the measurement of cfPWV (more than 10 year's experience) and the other operator had less experience (3 months experience at commencement of the study). The order (which operator took the first measurement) was randomised and operators were blinded to each other's measurements and their previous measurement. Each measurement was treated as a new recording such that new distance readings were acquired for each measurement.

Following the completion of the repeated cfPWV measurements the effect of femoral cuff placement and pulse waveform quality was assessed by the experienced operator. Duplicate cfPWV and cfPTT measurements following the movement of the femoral cuff to a lower thigh position were recorded. Duplicate cfPWV and cfPTT measurements were further repeated attaining a waveform of poor quality with the femoral cuff in its original position. After the final measurement was taken LabChart (PowerLab acquisition software) and the Finapres machine was stopped, all sensors were removed from the participant, and the output file from both the SphygmoCor software and LabChart were exported. On average this study required 1 hour and 30 minutes to complete the protocol.

4.2.3 Data and statistical analyses

BP was recorded by the oscillometric method. SphygmoCor XCEL cfPWV recordings were averaged over a 20 second period. BP and cfPWV data were extracted from the systems and converted into a usable comma-separated values file using a custom script written in the software 'R' before exporting the data for subsequent analysis in Microsoft Excel. Simultaneous time points from a 20 second snippet of the continuous HR, SBP, and DBP signals during the cfPWV measurements were extracted and averaged for each measurement, observer, and individual.

Assuming that there were minimal cardiovascular changes throughout the supine period of measurement, the 'true value' of HR, BP, cfPWV, and PTT was set as the average of all measurements taken by both operators within an individual. This is the principle of regression to the mean, whereby variability of multiple measurements, when averaged, converge on the mean. It was assumed in designing this experiment that twenty measurements (ten by each operator) would show fair regression to the mean, and therefore the average of these twenty measurements should represent the 'true value'. Averaging of measured distances from body surface markers

were similarly averaged. Data is represented using graphs that colour-code each individual and code operators (experienced operator = triangles, inexperienced operator = circles).

Ideal upper and lower limits for acceptable cfPWV, PTT, and distance measurements were set based on the value required to produce a 0.5 m/s difference in PWV. This was selected as a change of 0.5 m/s is deemed physiologically significant (Van Bortel et al., 2012; Vlachopoulos et al., 2010). Results from a lower thigh cuff and poor quality carotid pulse waveforms were compared to the 'true value' through Bland-Altman plots and paired t-tests. Measurements were considered repeatable if they lied close to the line of unity. Differences were considered statistically significant if p was less than 0.05. Participants baseline demographic data is represented as mean±SD, with BP and HR values attained from a seated position. All analyses were carried out using Microsoft Excel and SPSS 25.0 (IBM).

4.3 Results

Demographics of the study sample are provided in Table 4.1. One participant had hypertension with SBP on the margin of normotension and was on antihypertensive medication. Another participant had particularly low BP, as reflected by the lower SBP and DBP in the range. These values were considered normal for that particular individual and were not associated with cardiovascular complications. Table 4.2 highlights the average differences in HR, SBP, and DBP observed between operators throughout the experiment. For most participants this difference remained insignificant. However, for six participants there were significant changes between haemodynamic parameters within the individuals ($p<0.05$). SBP values between each operator had the most significant differences. Other than the difference in SBP observed in participant five (-9.18 mmHg) and nine (6.08 mmHg), the variances present were less than a magnitude of 5 bpm or mmHg for HR, SBP, and DBP.

Table 4.1: Participant baseline demographic data.

Parameter	Mean±SD	Range
Age (years)	30±15	17-69
HR (bpm)	75±8	58-88
Seated Brachial arterial blood pressure (mmHg)		
SBP	115±11	91-140
DBP	75±8	54-83

bpm: beats per minute; DBP: diastolic blood pressure; HR: heart rate (seated); SBP: systolic blood pressure; SD: standard deviation.

The difference between the cumulative average of HR, SBP, and DBP measurements for each operator compared to the average of all twenty measurements per individual ('true value') is displayed in Figures 4.1-4.3, respectively. By the fourth measurement HR had regressed within the range of ±5 bpm. The cumulative average of HR for one individual during the experienced operator's measurements remained around 4-5 bpm from the base regression line ('true value') and was significantly different when compared to the measurements taken during the same time frame to the of the inexperienced operator (p=0.04). SBP measurements were less consistent throughout the experiment whereby the cumulative averages did not regress within a range of ±5 mmHg until the final measurement. Two separate individuals had a distinctively elevated initial SBP value greater than 25 mmHg from the average of all twenty measurements. DBP values were slightly more variable, as evident by the reduction in regression, despite the decrease in magnitude of differences following successive measurements. However, the DBP readings all regressed within ±10 mmHg by the second measurement and ±5 mmHg by the seventh measurement. Only one DBP reading was observed outside of the range ±10 mmHg, being the first during the experienced operator's measurements. Whilst three individuals had a significant difference in DBP (Tab. 4.2) no individuals stood out in particular as the magnitude was less than 5 mmHg.

Table 4.2: Average difference in haemodynamic parameters between each of the experienced and inexperienced operators measurements.

Participant	Average difference between operators		
	HR (bpm)	SBP (mmHg)	DBP (mmHg)
1	0.16	2.54	0.27
2	1.34	-1.79	-1.86
3	0.23	-2.70	-1.06
4	1.59	-0.33	-0.24
5	0.07	-9.18*	-2.66*
6	4.24*	-1.17	-0.31
7	3.62*	2.76*	3.56*
8	-0.37	3.54*	-0.10
9	1.06	6.08*	4.29*
10	-0.13	-0.35	-3.89
11	-0.17	-1.84	-0.30
12	0.30	-2.41	-2.19
13	2.09*	2.60	2.05
14	0.69	-1.78	-2.62
15	-0.99	-2.62	-3.68*

bpm: beats per minute; DBP: diastolic blood pressure; HR: heart rate; SBP: systolic blood pressure.
$*$ indicates $p< 0.05$, compared between each operator.

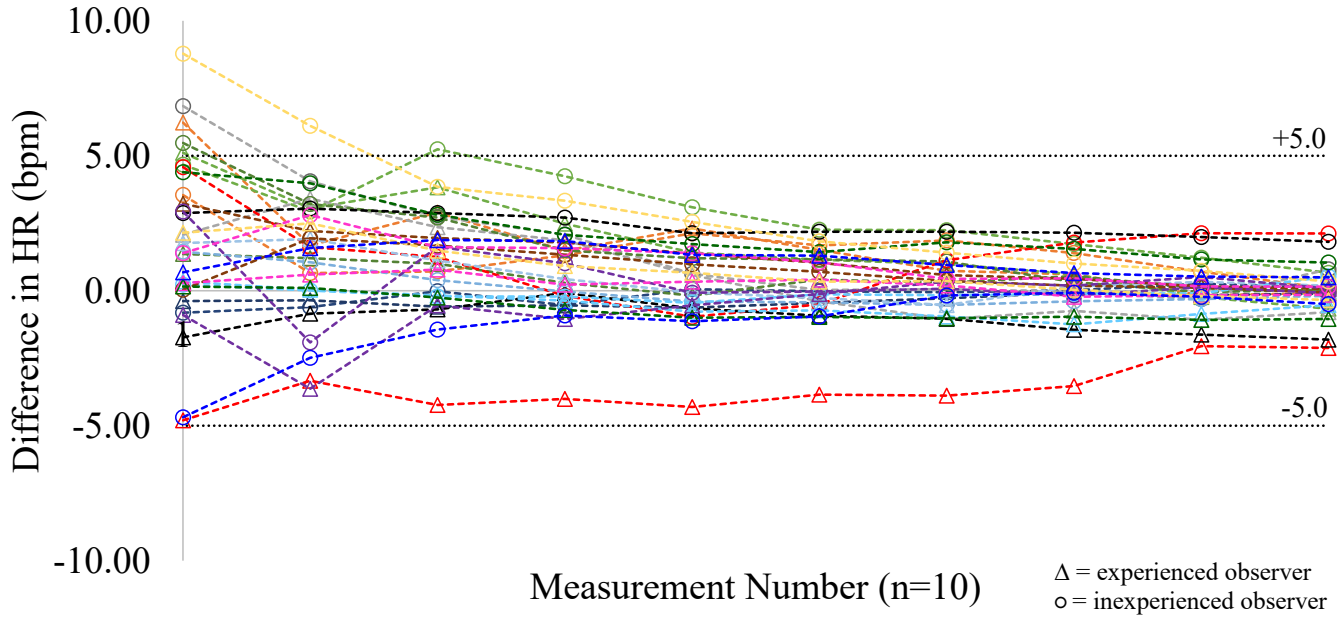

Figure 4.1: Difference between the cumulative average of simultaneous heart rate (HR) values against the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). Dotted line: upper (+5 bpm) and lower (-5 bpm) limits. Colours reflect a specific individual and markers represent the operator (circles = inexperienced operators measurements, triangles = experienced operators measurements).

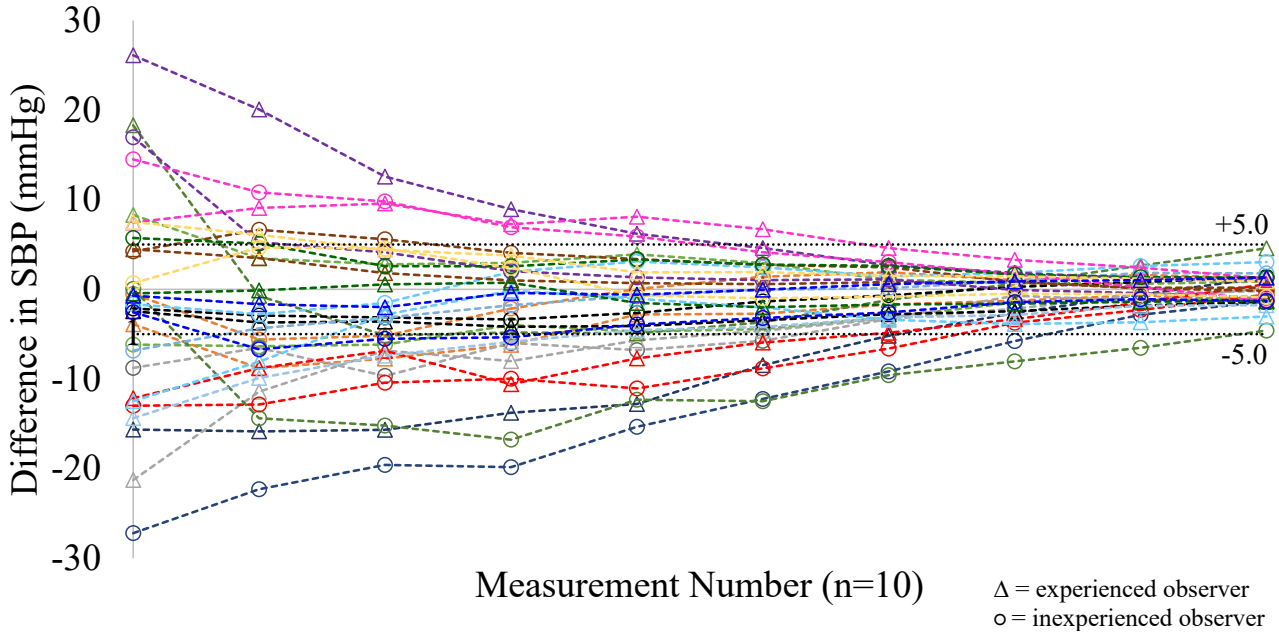

Figure 4.2: The difference between the cumulative average of simultaneous systolic blood pressure (SBP) measurements from the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). Dotted line: upper (+5 mmHg) and lower (-5 mmHg) limits. Colours reflect a specific individual and markers represent the operator (circles = inexperienced operators measurements, triangles = experienced operators measurements).

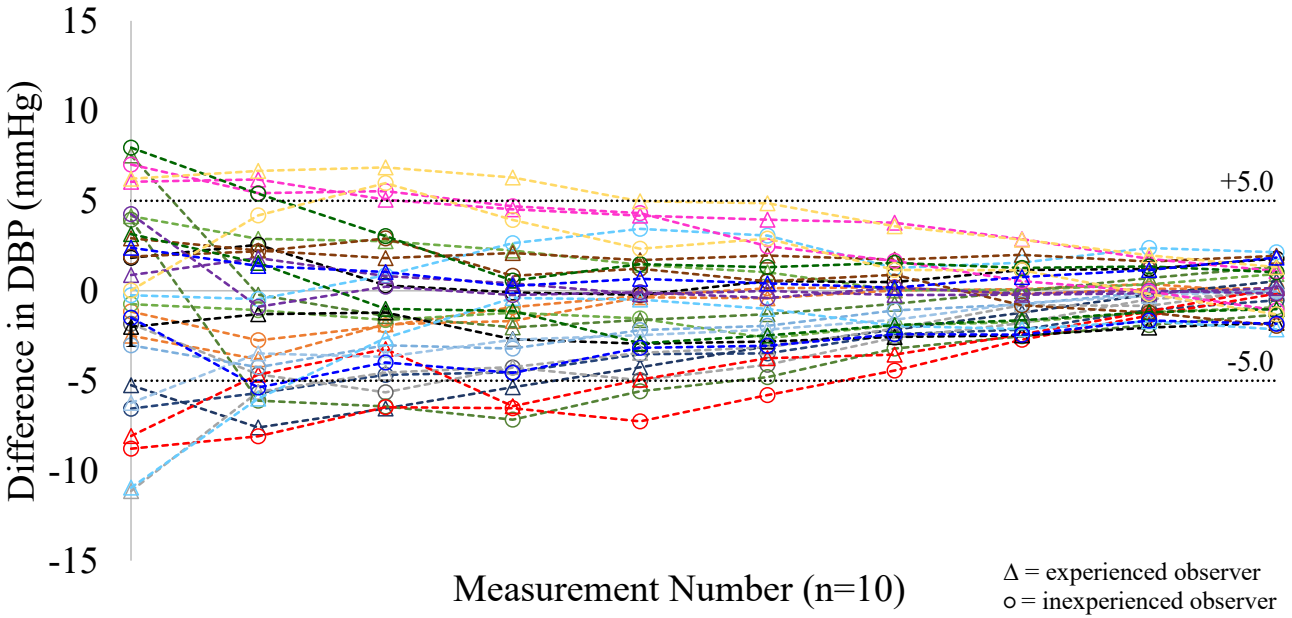

Figure 4.3: The difference between the cumulative average of simultaneous diastolic blood pressure (DBP) measurements from the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). Dotted line: upper (+5 mmHg) and lower (-5 mmHg) limits. Colours reflect a specific individual and markers represent the operator (circles = inexperienced operators measurements, triangles = experienced operators measurements).

4.3.1 Number of cfPWV measurements required to obtain an accurate cf-PWV measurement

Individual measurements and the cumulative average made between operators for each individual were not significantly different (p=0.25 and p=0.58, respectively). The cumulative difference in cfPWV values between each operator for each participant is compared in Figure 4.4. The repeated measure of cfPWV is assessed by the upper and lower limits and its regression towards the true value. These limits are represented by the dotted line and are equal to what is considered the physiologically important range of accuracy for cfPWV measurements to reside in, ±0.5 m/s. The cumulative average of cfPWV measurements had the tendency to regress towards the true cfPWV value, showing minimal difference upon successive measurements. The differences represent the cumulative average of successive measurements performed by each operator from the total cumulative average based on all twenty measurements from both operators for that specific individual. Each individual has a unique colour whilst each operator has a specific marker, with the experienced operator measurements being marked with triangles and the inexperienced operator measurements marked with circles. Three repeats were required for all cfPWV measurements to regress within this limit for both operators. The experienced operator's measurements (triangles) were all within the limits from the initial measurement, whilst two measurements for the inexperienced operator were slightly outside this limit (+0.64 m/s and +0.58 m/s), prior to their regression. An individual (light green line), as measured by the inexperienced operator, required five measurements to regress to a similar level to all other individuals but was within the ±0.5 m/s range within three measurements.

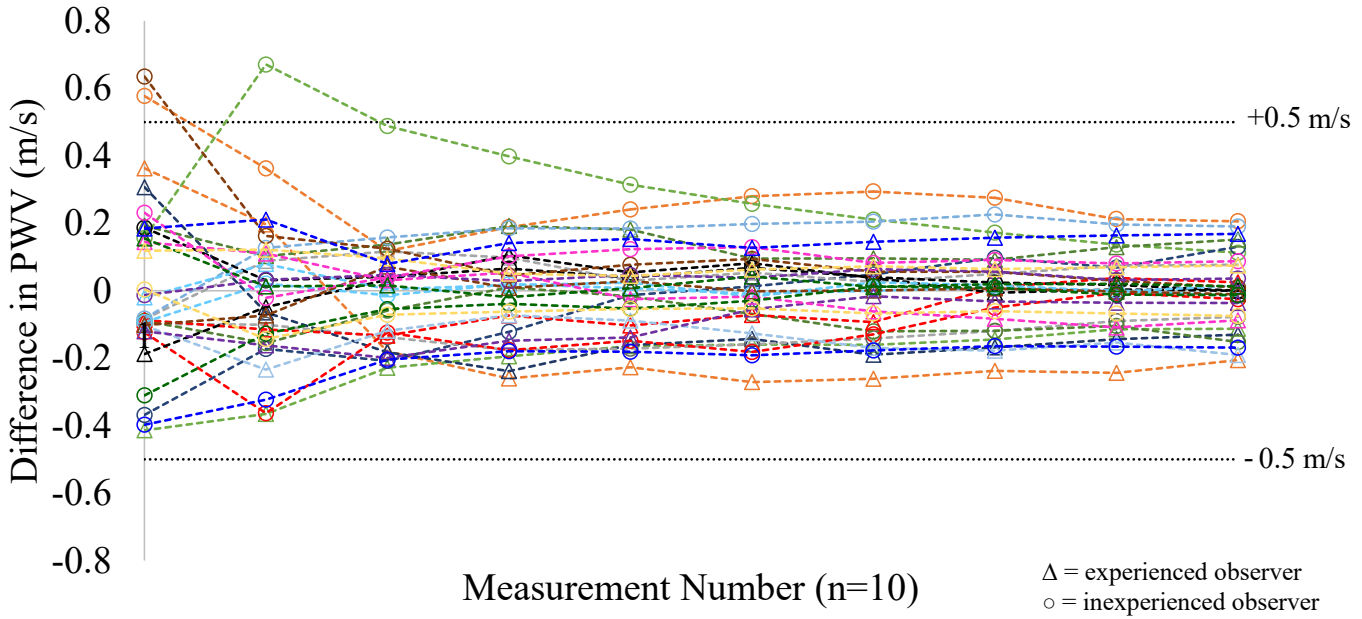

Figure 4.4: The cumulative average difference in successive carotid-femoral pulse wave velocity (cfPWV) measurements from the average of all twenty measurements for each individual (set to zero for the purposes of visualisation across the cohort). Participants are assigned a unique colour and each operator has a specific marker (circles = inexperienced operator, triangles = experienced operator). The upper and lower ideal range of accuracy are equal to 0.0±0.5 m/s (dotted lines).

4.3.2 Number of PTT and distance measurements required to obtain an accurate measurement

The accuracy of cfPWV was further analysed by breaking the measurement down into the measured components, that is, PTT and distance measurements. Figure 4.5 displays the difference of the successive cumulative average PTT values for each individual and operator against the total cumulative average of all twenty measurements made for that individual. An estimated change in ±6.69 ms (upper and lower limit) was required to change the PWV by ±0.5 m/s and was set as the acceptable accuracy of measurement. Upon the third repetition all measurements had regressed within the limit of ±6.69 ms. The first measurement for two individuals, as performed by the inexperienced operator, initially fell slightly outside the limit (+7.34 ms and -7.16 ms), however, by the second measurement all values were within the limits. The second measurement for an individual made by the experienced operator fell just outside of the upper limit (+6.87 ms), despite the first and third measurement being within the limits. Noticeably, measurements by the experienced operator for one individual (orange line) overshot the true value during the regression and then subsequently plateaued.

Measurements between operators were significantly different (p=0.03) whereas the differences between the cumulative average of each individual for each operator (i.e. an average of the operators ten measurements) was not (p=0.33). Comparatively, successive total distance measurements (Fig. 4.6) at an individual and cumulative average level were not significantly different between operators (p=0.14 and p=0.58, respectively). All distance measurements performed fell within the limit of ±39.3 mm (equivalent to average ±0.5 m/s cfPWV) from the initial measurement for both operators and regressed with less amount of measurements compared to PTT. Measurements further regressed within a range of ±20.0 mm by the fourth measurement. This limit was not reduced any further. There were no distinct differences between individuals.

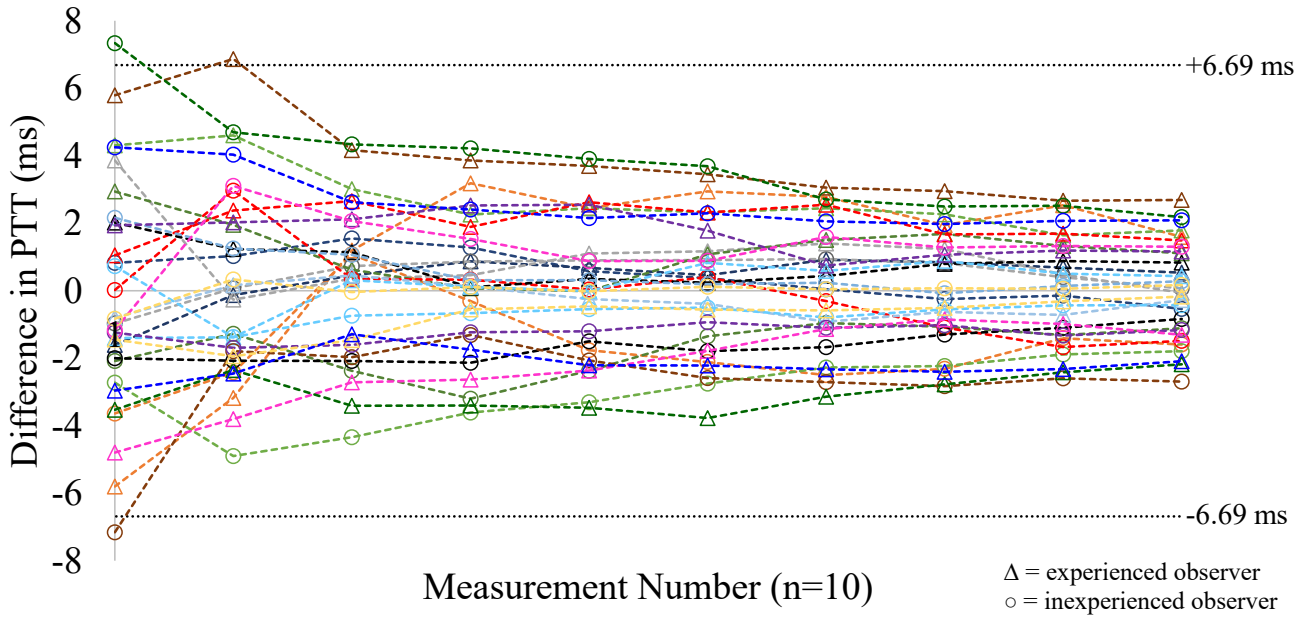

Figure 4.5: The difference in the cumulative average of pulse transit time (PTT) values per operator from the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). Individuals had their own respective colour whilst operators had their own marker. Circles indicated the inexperienced operators measurements and triangles indicated the experienced operators measurements. The upper and lower limit of acceptable accuracy is equal to the average change in PTT required to cause a 0.5 m/s change in pulse wave velocity (±6.69 ms).

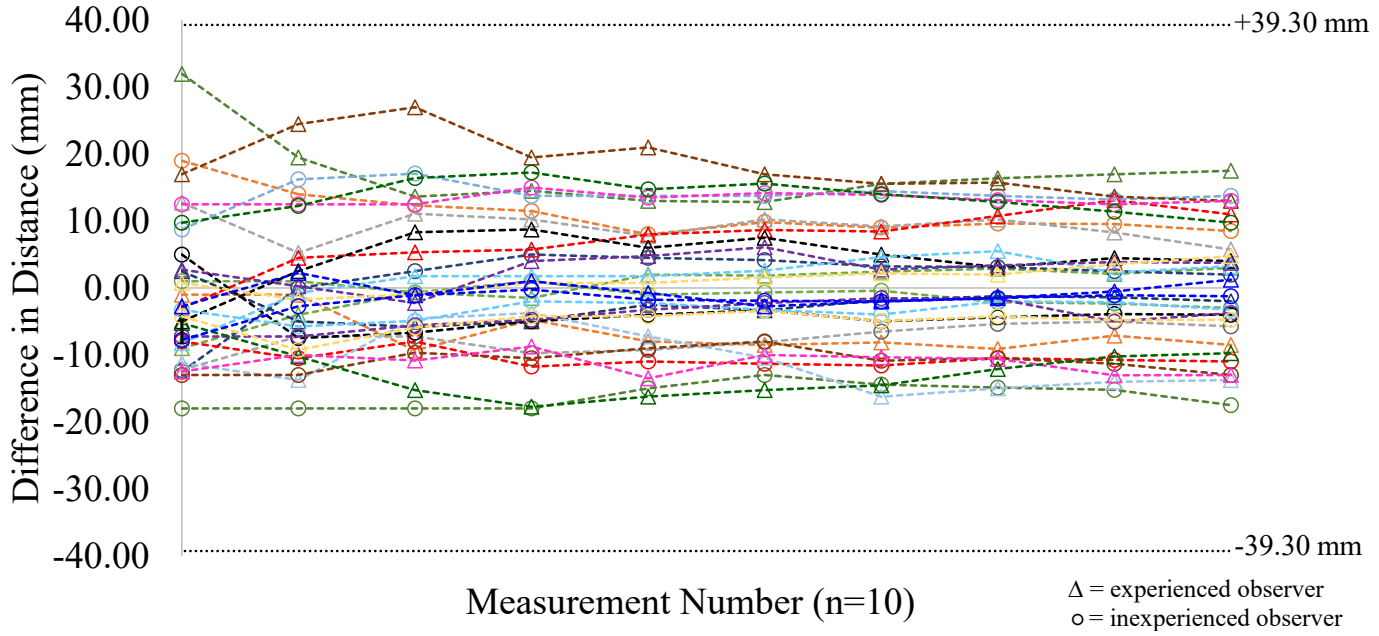

Figure 4.6: The cumulative average difference in the cumulative total distance values from the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). A colour is assigned to each individual and whilst a marker is assigned to each operator. Circles are indicative of the inexperienced operators measurements whilst triangles indicate the experienced operators measurements. The upper and lower limit of acceptable accuracy are equal to the average change in distance required to cause a 0.5 m/s change in pulse wave velocity (±39.90 mm).

The distances that constitute the total distance (suprasternal notch to carotid; suprasternal notch to cuff; femoral to cuff) were also assessed separately. Figure 4.7 is a plot of the cumulative average distance measurements between the suprasternal notch and where the carotid signal was attained. All carotid values regressed within ±10 mm by the second measurement where most readings remained relatively consistent thereafter. Figure 4.8 displays the cumulative average of differences in the measurement between the suprasternal notch to femoral cuff from the average of all twenty measurements for each individual. Apart from one individual (brown line), as made by the experienced operator, all measurements fell within ±20 mm from the first measurement. Every measurement converged towards the true value within ±11 mm by the tenth repeat. There was variability in the measurement until the fifth measurement when the regression line stabilised in comparison to the true value. Measurements made by the experienced operator (triangles) were typically on the positive side of the true value whereas measurements made by the inexperienced operator (circles) tended to fall below the true value.

All average cumulative distance differences made between the femoral cuff and the site where the femoral pulse is palpated (femoral) fell within the limit ±20 mm by the last repeat (Fig. 4.9). A minimal tendency was observed for the measurements to regress towards the true value, likely indicating that the two operators located the site of the femoral pulse as different locations. One individual (dark green line), as observed by both operators, remained relatively distant from the true distance value. Among the carotid and femoral distances the respective operators distance measurements laid distinctly on polar sides of the true value. The difference between each measurement by both operators from all three of the distances was significantly different ($p<0.05$).

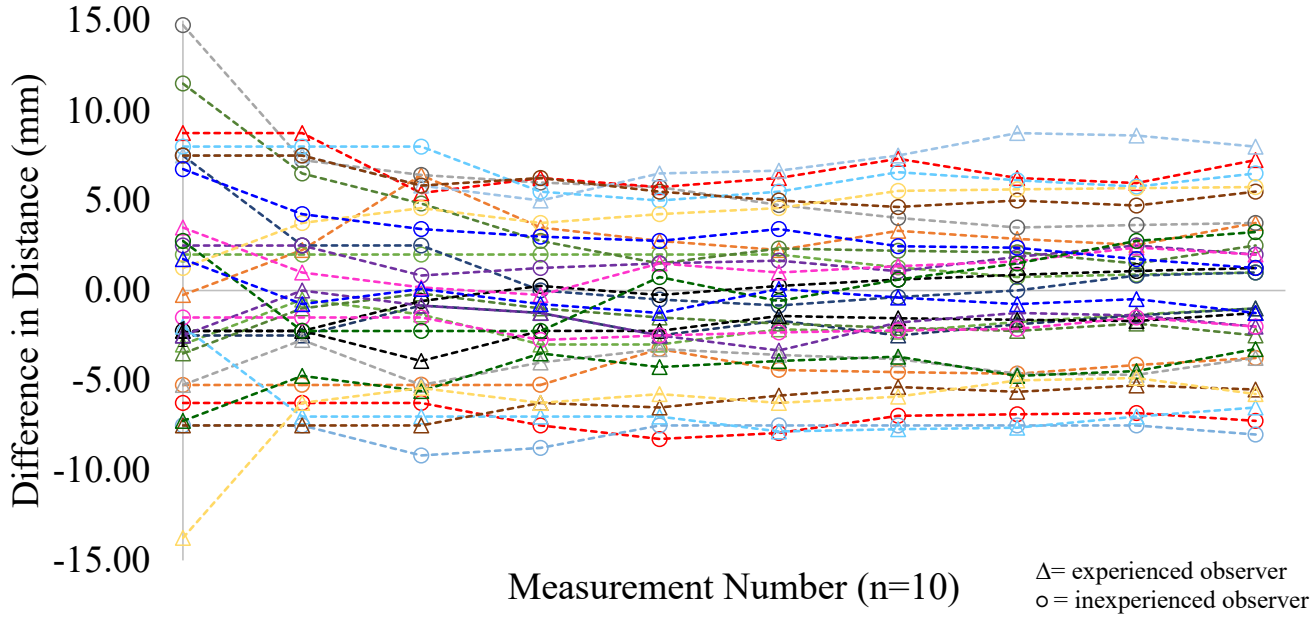

Figure 4.7: Average cumulative carotid to suprasternal notch (carotid) distance differences from the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). Each participant has a respective colour and each operator has a marker, with circles displaying the measurements from the inexperienced operator and triangles displaying those from the experienced operator.

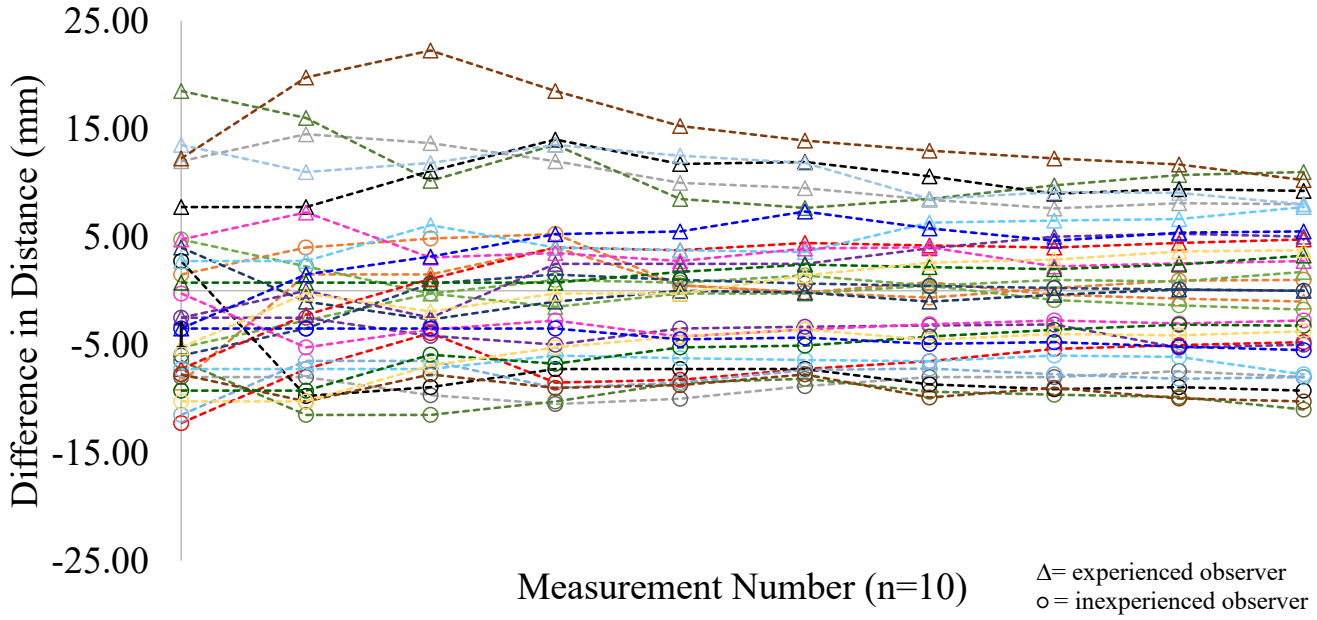

Figure 4.8: The difference in suprasternal notch-femoral cuff (cuff) average distance values from the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). Colours indicate each individual. Circles represent the inexperienced operators measurements whilst triangles represent the experienced operators measurements.

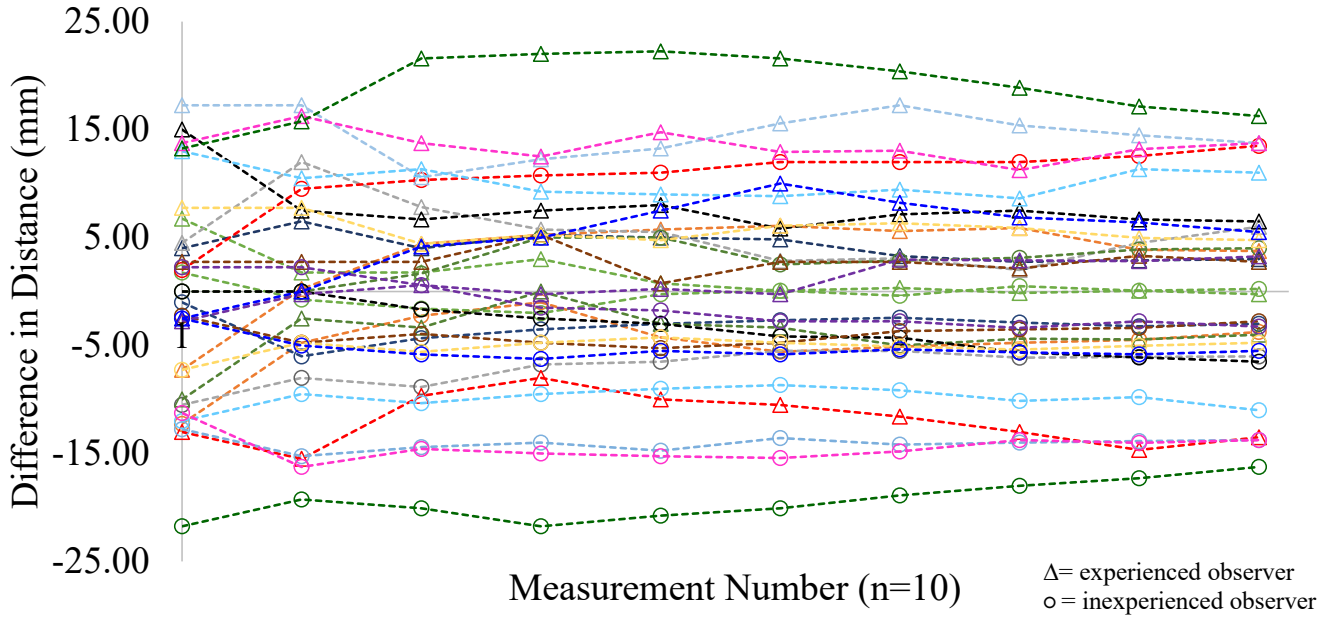

Figure 4.9: The cumulative average difference in femoral artery-cuff distances for each operator from the average of all twenty measurements per individual (set to zero for the purposes of visualisation across the cohort). Participants are reflected by a specific colour and operators by a specific marker (circles = inexperienced operator whilst triangles = experienced operator).

4.3.3 Effect of a lower positioning of the femoral cuff

Changing the position of the femoral cuff significantly increased the suprasternal notch to cuff distance by an average of 145±44 mm (p<0.0001), indicating the cuff was moved a significant distance down the thigh. cfPWV measured with a lower thigh cuff was compared to the average of the twenty measurements made during the analysis of repeated measurements with the cuff on the upper section of thigh ('true cfPWV', Fig. 4.10). Measurements were not significantly different between each other (p=0.08), were clustered between 5-6 m/s, and were in good agreement, as reflected by how close the points laid to the line of unity (dotted line, Fig. 4.10 **A**). Measurements between the two cuff positions were strongly correlated (R^2=0.9396) and had a low RMSE of 7%. There was strong agreement between the two measurements taken at the different cuff positions (Fig. 4.10 **B**). A mean difference of -0.18±0.37 m/s was observed. One individual fell just inside the lower end of the 95% confidence interval and was exceptional from the other study participants in that they had a higher cfPWV. This is the same individual observed in Figure 4.10 **A** with an elevated cfPWV and notably had a change in PWV of 0.90 m/s. Measurements were not biased to be either higher or lower with the different position of the femoral cuff.

Bland-Altman analysis of the differences in PTT for the different femoral cuff positions was also undertaken (Fig. 4.11). PTT values attained during the repeated measures were used to generate an average, 'true value'. This value was compared to the PTT values measured when the cuff was moved to the lower thigh region (Fig. 4.11 **A**). As observed with cfPWV measurements, PTT measurements were highly repeatable as they were tightly bound to the line of unity. These measurements were not significantly different (p=0.46), had a low RMSE (6%), and were highly correlated between each other (R^2=0.8834). The PTT for one participant was considerably faster (+12.55 ms) than the cluster of participants around 80 ms. Agreement between the PTT measurements was good. This was assessed by comparing the difference in measurements against the average of PTT measurements in conjunction with the upper and lower limit, +10.79 ms and

-8.84 ms, respectively (Fig. 4.11 **B**). There was a mean difference of 0.98±5.01 ms between PTT measurements attained with the different cuff positions. One individual had a PTT difference outside of the 95% confidence interval and was the same individual observed to have an elevated PTT from Figure 4.11 **A**. Another individual worth noting was observed to be on the border of the upper limit of the 95% confidence interval. Despite these two instances, collectively, PTT was still repeatable and displayed good agreement.

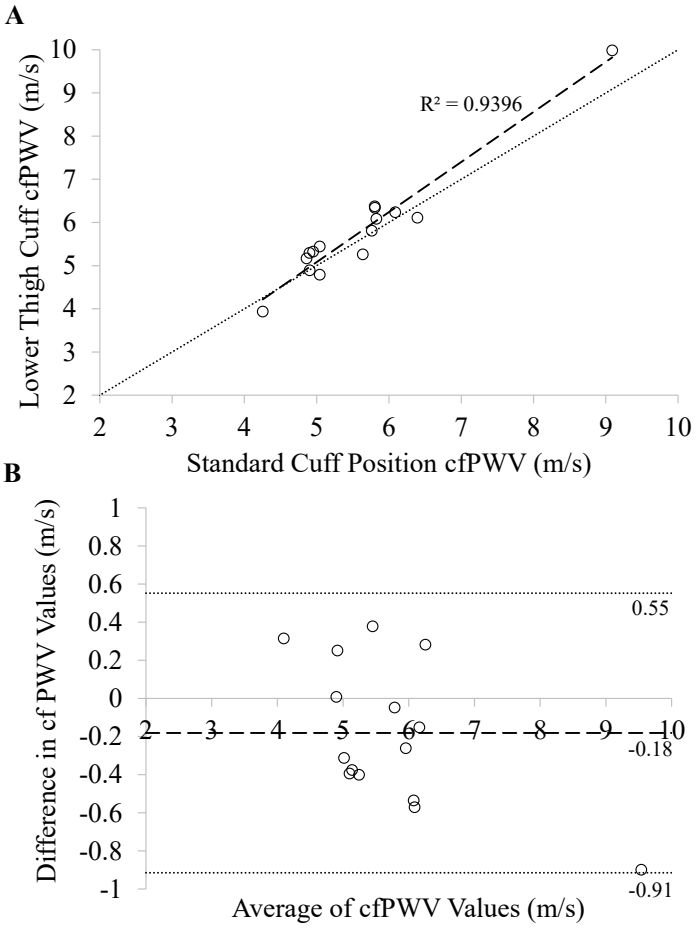

Figure 4.10: Bland-Altman analysis of cfPWV attained from a lower thigh cuff compared to the average of cfPWV with the cuff in the standard position ('true cfPWV'). **(A)** shows plots the values obtained with an upper thigh femoral cuff to values obtained with a lower thigh femoral cuff. PWV measurements showed minimal variability between the two cuff positions (difference 0.18±0.37 m/s, R^2=0.9396, root-mean-square error 7%, p=0.08). Dotted line: line of unity. **(B)** depicts the degree of agreement between the two PWV measurements. Dashed line: mean difference. Dotted line: mean±1.96SD.

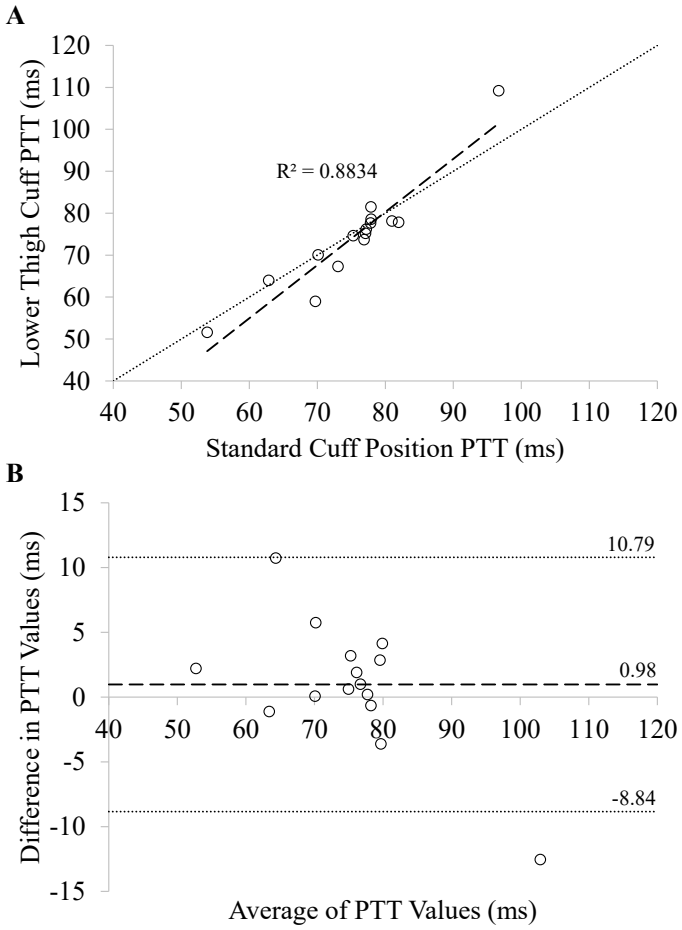

Figure 4.11: Bland-Altman analysis of PTT attained from a lower thigh cuff compared to the average of PTT with the cuff in the regular position. **(A)** shows plots the values obtained with an upper thigh femoral cuff to values obtained with a lower thigh femoral cuff. Variability of PTT measurements between the two cuff position was minimal (difference 0.98±5.00 ms, R^2=0.8834, root-mean-square error 6%, p=0.46). Dotted line: line of unity. **(B)** indicates the degree of agreement between the two PTT measurements. Dashed line: mean difference. Dotted line: mean±1.96SD.

4.3.4 Effect of attaining a poor carotid pulse waveform

The effect of attaining a poor carotid pulse waveform for the measurement of cfPWV was assessed with respect to the 'true cfPWV', the average of the twenty measurements made during the analysis of repeated measurements (Fig. 4.12). Figure 4.12 **A** depicts the measurements attained from a poor waveform compared to those attained with a good waveform. Measurements remained relatively close to the line of unity appearing to display minimal variability and little difference between the good and poor waveforms. The average cfPWV difference between the two waveforms

was 0.28±0.54 m/s with a RMSE of 11%. There was a good correlation between measurements (R^2=0.8038) whilst the differences between the two measurements were not statistically significant (p=0.06). The agreement between the two waveforms was good. One point sat outside the 95% confidence interval of +0.78 m/s, -1.34 m/s (Fig. 4.12 **B**). The mean difference between the cfPWV measured with good and poor waveforms was -0.28 m/s. The same participant that was observed to have an elevated cfPWV in the previous measurements was also evident in both Figure 4.12 **A** and 4.12 **B**. The cfPWV measured in this individual showed good repeatability and agreement. The participant below the 95% confidence interval in Figure **B** was observed to have an individual difference in cfPWV of -1.70 m/s whilst the individual nearing the edge of the 95% confidence interval had a difference in cfPWV of +0.71 m/s (Fig. **A**). Measurements were not biased to be either higher or lower with a poor carotid pulse waveform.

Poor waveform PTT measurements produced similar variability as evident with poor waveform cfPWV measurements (Fig. 4.13). The PTT measured from the poor waveforms was compared to the average of those measured with good waveforms (Fig. 4.13 **A**). There was acceptable repeatability as the points tended to lie close to the line of unity. The average differences in PTT measured between the two waveforms was 1.58±7.53 ms and were not significantly different (p=0.43). There was a RMSE of 9% with a reduction in the correlation between measurements compared to cfPWV measurements (R^2=0.7094). The agreement between the PTT attained by poor waveforms was reasonable (Fig. 4.13 **B**). Excluding one individual, all measurements fell within the 95% confidence interval (+13.19 ms to -16.35 ms). The individual that sat outside the confidence interval had a PTT difference of 21.82 ms.

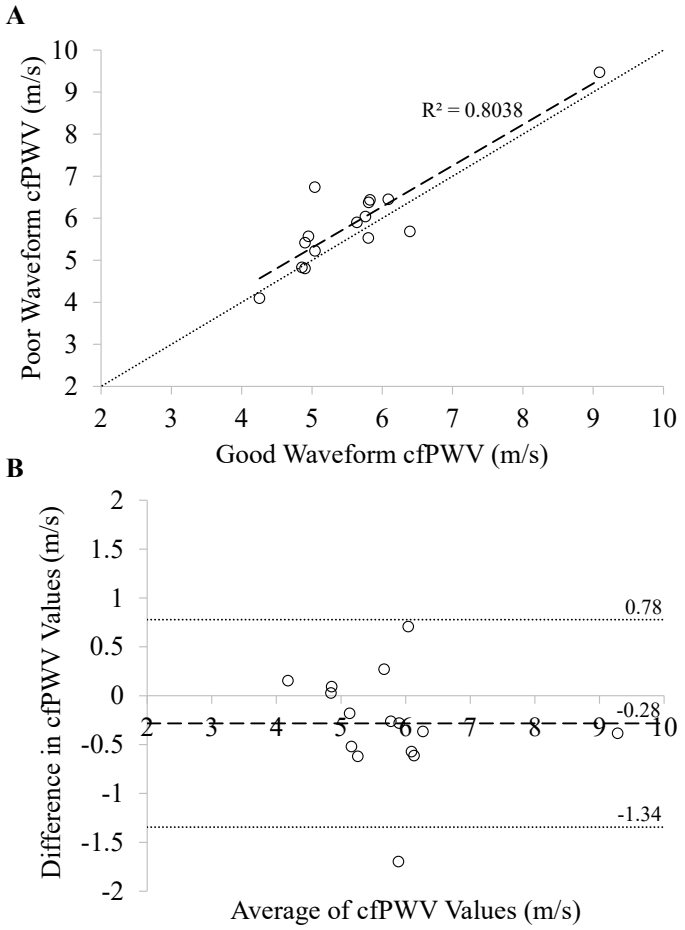

Figure 4.12: Bland-Altman analysis of carotid-femoral pulse wave velocity (cfPWV) attained from a poor carotid pulse waveform compared to the cfPWV obtained with a good carotid pulse waveform. **(A)** shows the agreement in cfPWV measurement. cfPWV measurements showed slight variability with a poor waveform (difference 0.28±0.54 m/s, root-mean-square error 11%, p=0.06). Dotted line: line of unity. **(B)** depicts the degree of agreement between the two cfPWV measurements. Dashed line: mean difference. Dotted line: mean±1.96SD.

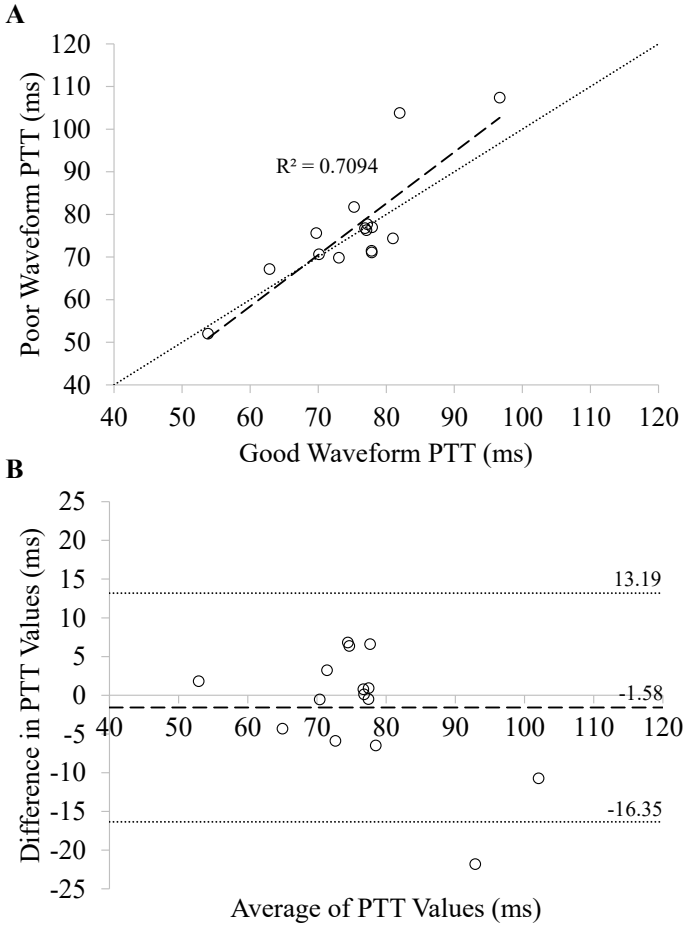

Figure 4.13: Bland-Altman analysis of pulse transit time (PTT) attained from a poor carotid pulse waveform compared to the PTT with a good carotid pulse waveform. **(A)** shows the agreement in PTT measurement. PTT measurements showed some variability with a poor carotid pulse waveform (difference 1.58±7.53 ms, root-mean-square error 9%, p=0.43). Dotted line: line of unity. **(B)** depicts the degree of agreement between the two PTT measurements. Dashed line: mean difference. Dotted line: mean±1.96SD.

4.4 Discussion

This is a novel study presenting findings on the reliability and potential sources of error in the measurement of cfPWV. The potential error in cfPWV also encapsulates the influence of distance and transit time measurements. These sources of error become physiologically important in the reliability of the measurement and thus when characterising the relationship between BP and PWV, or in this instance, the subject-specific calibration factor.

Continuous HR, SBP, and DBP did show inconsistency in some individuals both between each operator (Tab. 4.2) and measurements made throughout the duration of the study (Fig. 4.1-4.3). Considering the nature of physiological signals it is likely that these differences may merely be a product of natural variability. One could further speculate that these differences are a result of a subconscious judgement of the participant and who they are more comfortable with. As these measurements were performed under baseline conditions, it is unlikely that adopting similar approaches such as discarding the first measurement would greatly influence the results observed. Regardless, whilst some of these inconsistencies were statistically significant between operators it is important to consider the magnitude of these changes and whether they are physiologically significant. Aside from two individuals, these changes may be considered as acceptable as they were less than 5 bpm and 5 mmHg. In the two individuals where this was not the case there was a significant change in SBP greater than 5 mmHg but less than 10 mmHg. Upon revising the experiment notes and data there was no definite event to explain this occurrence.

HR measurements tended to regress to the true value more rapidly, followed by BP measurements. The regression of SBP appeared smoother in comparison to DBP despite the larger variability in differences observed in initial SBP measurements. HR and BP are both known to influence cfPWV (Nye, 1964; Tan et al., 2016), hence achieving stable conditions is necessary to reduce any confounding factors. Considering HR and most BP changes were not particularly physiologically meaningful, we can assume that these differences were not significant contributing factors to the variability observed in cfPWV measurements. A plausible explanation for the small fluctuations in BP may be explained by natural variability and transient temperature changes experienced within the individual altering the peripheral vasculature (Lindberg and Oberg, 1991). It is also possible that the variability in the Finometer Pro measurements itself did not reflect physiological BP variation (Silke and McAuley, 1998). This means that the variability observed in cfPWV is primarily driven by the measurement itself.

Assuming that the HR and BP remained reasonably stable throughout the experiment it provides reassurance that any changes observed are not biased by these haemodynamic parameters. A total of three successive measurements were required for all cfPWV measurements to regress within the ideal accuracy range of ±0.5 m/s. This has been deemed as the standard difference which PWV values should not exceed and thus provides an acceptable limit (Van Bortel et al., 2012; Vlachopoulos et al., 2010). Measurements undertaken by the experienced operator fell within the desired range upon the first measurement. For any operator the cfPWV may be considered reliable by the third measurement. Understandably, more measurements resulted in the measurements regressing closer to the true value and thus became more reliable. Although the regression line for individuals between both operators fell within the upper and lower limit the regression was still quite variable until the fifth repeat. This variability is likely to stem from either the PTT and/or distance measurements.

In using the notion of how much of a change in PTT or distance is required to create a change in PWV of 0.5 m/s, an upper and lower limit was calculated for both PTT and total distance, ±6.69 ms and ±39.3 mm respectively. Whilst PTT regressed within this limit following three measurements the regression lines compared to the true value per each individual were slightly more scattered within the first few measurements (Fig. 4.2). Additionally, these regressions lines did remain marginally more spread from the true PTT value. It is most probable that if there are inconsistencies among PTT measurements the quality of the waveform and where it is attained from are the predominant contributing factors. These factors are further expanded upon later in the discussion. Regarding the total distance, as attained by the subtraction method, repeated measurements regressed quite rapidly showing less spread amongst the individual regression lines (Fig. 4.6). Measurements made by both operators fell within the physiological limit from the first measurement. Such minimal error in subtraction-based distances has previously been reported by Weber et al. (2009). The overall distance may therefore be considered as reliable. In spite of these results it is important to consider the individual distances which constitute this total

distance, such that reducing the error in those measurements can further reduce the total error observed for cfPWV measurements.

Thinking about the location at which the measured arterial signals were attained we can reflect upon the anatomical diversity within individuals and take into consideration that both the femoral and carotid pulse can be palpated at multiple sites along those arteries, not fixed to one specific site. The right common carotid artery (the site of measurement) spans a considerable length of 22±2 cm vertically within the neck (Choudhry et al., 2016). As this can be palpated in numerous locations it is clear that discrepancies may arise between different observers. This is highlighted in Figure 4.7 whereby the distance between the suprasternal notch and site of carotid palpation was variable with a reasonable spread of regression lines from the true value for a small distance. Furthermore, it is probable that for some individuals the carotid site was palpated higher towards the mandible. Whilst this higher location may have provided a stronger pulse for measurement, the higher up the pulse is palpated the closer the pulse is to the carotid bifurcation and therefore it will be influenced more by wave reflection. This small difference in distance has been previously reported to have a significant impact on the pulsatility index (Gwilliam et al., 2009) which in turn influences the augmentation index, a property associated to PWV (Brown, 1999). These distance differences have also been explored in the literature whereby it was simulated that as distance discrepancies increase the error present in the PWV measurement increases in a non-linear fashion (Bolster et al., 1998). Whilst the region where the femoral artery is palpated does not necessarily bifurcate, the measured distance between the cuff and where the femoral pulse was palpated could likely be different between observers. Figure 4.9 highlights this concept where the femoral distance measurements were the most variable and the regression lines between operators and individuals were more spread. This alludes to the fact that although these distances measurements were not statistically different and did not significantly impact the PWV measurements as a whole they are most definitely a source of error, which should be noted when performing measurements for the calibration of PWV to BP.

Despite being the greatest distance among the individual distances, the suprasternal notch to femoral cuff distance was the least variable in comparison to femoral and carotid distances (Fig. 4.8). Whilst these measurements fell within distance limits, inconsistencies were still present. Although this was notable for all individual distances it may be said that when the magnitude of the differences is compared to the total length of the segment measured, the cuff distances possessed the least amount of error. It is somewhat expected that there are discrepancies in earlier distance measurements, however, what is important is the degree of regression of these measurements to the 'true value' which was noted to vary between individuals and operators. As visually observed, among the individual distances there was a general trend that the respective operators distance measurements would lie on opposing sides of the true value. Aligned to this, it may be stated that the discrepancies observed in the individual distance measurements counter-balanced each other in such a way that they did not have an overwhelming impact on the total distance and PWV. Supplementary to this, it should be noted that the distance measurement is prone to increase with age. This is especially prevalent for the aortic segment, due to the arteries becoming tortuous (Hutchins et al., 1977). This would lead to an underestimation of distance in an elderly population which would inherently underestimate cfPWV. Factoring this in and the variability observed from the distance measurements, this alludes to the concept of using height and age-based regression equations to estimate this arterial segment, as seen in Filipovsk et al. (2010). Establishing an accurate and reliable method would reduce the amount of distance measurements required whilst also factoring in potential tortuous changes in the arterial segment that occur with age. This would therefore potentially reduce the source of error that is apparent with these distance measurements thus resulting in a more uniform characterisation of the BP-PWV relationship.

A potential limitation regarding the above research, and more specifically the amount of measurements required to obtain an accurate measurement, is based on the underlying assumption mentioned in the Methods section. That is, the average of twenty measurements made by both

the experienced and inexperienced operator is considered as the 'true value'. The question then stands as to whether the average of the ten measurements made by the experienced operator would serve as a more reliable assessment of the 'true value'. Whilst this may be the case it cannot be said that all measurements made by the experienced operator are correct and that all measurements made by the inexperienced operator are inadequate. Subsequent to this the measurements made by the inexperienced operator would still regress towards a more definitive value following the succession of measurements. Taking this into considering, using the cumulative average of measurements made by both operators could in fact provide a better assessment of the 'true value', which is why it has been used for this particular study. Another possible limitation, which concerns all aspects of the study, is the sample population. As this research was performed in a relatively young and healthy cohort it may then be asked whether the results observed would be apparent in an older population or those with significant comorbidities. Considering that with age and certain comorbidities the arterial composition tends to stiffen this could result in reduced ability for PWV to vary, in turn resulting in a faster and perhaps smoother regression towards the 'true value'. Conversely, the ability to attain a reliable and accurate measurement of PWV may prove difficult due to the nature of measurement, which therefore may skew the results. These limitations require future studies to reliably assess the outcomes.

The relevance of including the change in femoral cuff position and attaining a poor waveform stems from the fact that these are individual components in the measurement of PWV. Consequently, if these components are measured incorrectly the changes may have the capacity to affect the PWV. This becomes significant in the cuffless estimation of BP, as achieved through the calibration factor investigated in Chapter 3. As visually represented in the Bland-Altman plots (Fig. 4.10-4.13) measurements can be considered both repeatable and agreeable with the cuff in a lower position or when acquiring a poor waveform. Additionally, there was no statistically significant change in cfPWV or PTT. Whilst these differences may not have been statistically significant there were some physiologically significant changes that can be considered clinically relevant. These

physiologically meaningful changes were derived from a 0.5 m/s change in PWV resulting in a 6.69 ms change in PTT, as previously established. Using these limits overlaid on the graphs highlights a few points which should be addressed.

A total of three individuals are of interest in Figure 4.10 which had differences in cfPWV greater than 0.5 m/s. The greatest difference observed after moving the cuff was 0.98 m/s. This apparent change may be distinguishable due to the age and the cardiovascular status (hypertensive) of the participant. Referring back to Chapter 2 it was noted that age can effect PWV. In conjunction with this, it has been cited in the literature that different arterial segments age at different rates (Avolio et al., 1983). This may therefore give reason to such a noticeable difference in cfPWV as the measurement incorporates the additional muscular arterial segment from the site of femoral pulse palpation to the cuff on the thigh. Regarding the other two individuals there were no distinguishable differences in BP, HR, or age which may have been considered as causative factors to this difference. In addition to this, it is not likely that the changes in distance are accountable for this difference either as there were individuals with both increased or decreased distances that did not have a physiologically significant change in cfPWV.

Interestingly, when the difference in PTT between cuff positions was assessed only two individuals had a difference outside of the established PTT range of ±6.69 ms. Furthermore, neither of these data points was accounted for by the individual that had such a distinguishable cfPWV difference. However, one of the individuals with a slightly elevated cfPWV (+0.57 m/s) was responsible for this change in PTT. The change in cuff position resulted in PTT increasing by 12.55 ms in one individual and decreasing by 10.72 ms in another. The decrease in PTT was an unexpected result considering that the length between the measured pulses had increased and thus should result in a slight increase in the PTT. These differences may consequently be the result of a transient stiffening of the arterial segment or possibly due to a slightly poorer quality waveform.

When assessing the impact of acquiring a poor waveform in comparison to a good waveform (Fig. 4.12 and 4.13) more differences in cfPWV and PTT arose. Whilst this did not influence the same individuals observed in changing the cuff position it did change the cfPWV by greater than 0.5 m/s in a total of six participants. The most notable change was an increase in cfPWV of 1.70 m/s. Although, as noted by the SphygmoCor XCEL system, one of the waveforms attained for this individual had an unsatisfactory quality. This skewed the results by most likely affecting the detection of the diastolic foot for the determination of PTT and consequently PWV. Typically this data point may therefore be considered as a potential outlier, however, this measurement may reflect occurrences that arise in an ambulatory setting. Of the remaining five participants with differences greater than 0.5 m/s another individual had a waveform of unsatisfactory quality and may consequently be judged in the same manner. Thus, a total of four out of the fifteen individuals may be considered to have a physiologically significant change in cfPWV when acquiring a poor waveform. For these individuals acquiring a poor waveform had the tendency to increase cfPWV.

When focusing on the change in PTT only two individuals exceeded the limit of ± 6.69 ms. These individuals were furthermore different to those with altered cfPWV measurements. Whilst one of the two individuals had a waveform attained with unsatisfactory quality the other individual did not. This individual had an increase in PTT of 21.82 ms. Regardless of whether it was PTT or cfPWV, a poor waveform had a larger physiological impact than altering the distance. Similar instances regarding a poor quality waveform have been reported by Sol et al. (2009) showing that techniques fail in repeatability when mild-to-severely noisy signals are attained. Additionally, when the waveform is of lower quality it is suggested to use the least square method to attain a fiducial marker for the purposes of PWV measurements (Gaddum et al., 2013). As for this experiment the intersecting tangents method, a foot-to-foot method, was used consistently throughout. When considering the difficulty in attaining a higher quality signal throughout the

population the occurrence of lower quality signals is probable and must therefore be accounted for in the future of BP estimation.

4.5 Conclusion

In conclusion, cfPWV measured by two operators only required three measurements in total to regress within the physiological limit of ±0.5 m/s towards the 'true PWV value'. Furthermore, this regression remained stable following the fourth measurement. Similarly, PTT measurements only required three measurements to fall within the upper and lower limit, whereas, total distance measurements, as made by the subtraction method, fell within the physiologically important range from the initial measurement. Whilst some successive measurements regressed quite rapidly towards the true value, others required more measurements prior to their stabilisation. This disparity became more evident upon assessing the individual distance measurements for the subtraction method. Femoral to cuff and carotid to suprasternal notch distances were more variable and possessed greater differences in distances from the true value. Notably, as only two cfPWV measurements were acquired for the generation of the subject-specific calibration factor in Chapter 3, the established variability from this chapter may be a contributing factor to the variability observed in the repeatability of the calibration factor. Additionally, a lower quality waveform had a greater tendency than a lower thigh cuff to influence the calculation of cfPWV and PTT. Whilst these differences were not statistically significant, some of these differences were physiologically significant. Caution should therefore be made when measuring cfPWV as these factors may have an underlying impact. To summarise, it is important to standardise the measurement of cfPWV as inconsistencies may influence the generation of the subject-specific calibration factor.

CHAPTER 5 Predictors of a subject-specific calibration factor

5.1 Introduction

Non-invasive and cuffless BP estimation by PWV is restricted in accurately characterising the relationship between BP and PWV (calibration). In order to address this limitation this thesis investigates a novel subject-specific calibration method (ΔBP/ΔPWV) that incorporates a postural change to generate a calibration factor (Eq. 3.20). As made evident in Chapter 3 and Chapter 4, individual variability in the repeatability of this calibration factor and in the measurement of cfPWV was present. In addition to this, it is important to further investigate and quantify the calibration factor to determine whether there is variability within the population as a whole. This purpose of this study can be summarised by the aims of this Chapter:

1. To determine whether the calibration factor is generalisable;
2. To establish if there are strong predictors of the calibration factor;
3. To assess what the calibration factor varies with across the population.

This would consequently provide a more detailed perspective of what can influence the calibration factor and whether there are variables, such as age, BP, or gender, that can be used as an alternative to generate the calibration factor, rather than individual measurements.

5.2 Methods

A total of 135 participants (49±23 years, 48% female) were recruited for this study through Macquarie University staff and students, friends of the investigator, and referrals from the Cardiology Clinic of the Macquarie University Hospital. No specific exclusion criteria was in place for the present study. Participants were not required to fast and were asked to refrain from smoking, exercise, and the consumption of alcohol and caffeine prior to the commencement of the study. All participants were included in the analysis and provided informed consent prior to the commencement of the study. Furthermore, this study was not restricted to a particular time of the day. The experimental protocol was approved by Macquarie Universitys Human Research Ethics Committee.

5.2.1 PWV measurements

All PWV measurements were taken as previously described in Section 3.2.1 using the SphygmoCor XCEL device (AtCor Medial, Sydney, Australia).

5.2.2 Calibration factor calculation

The subject-specific calibration factor was calculated as previously described in Section 3.2.2.

5.2.3 Experimental protocol

The protocol undertaken follows that performed in Chapter 3. All participants were subject to a participant questionnaire to gather information on their lifestyle factors and cardiovascular status. After the completion of the questionnaire participants were allowed 5 minutes of baseline rest in a supported seated position with their legs uncrossed. Brachial oscillometric BP, of the

left arm at heart level, and cfPWV were then measured in duplicate under baseline conditions (SphygmoCor XCEL). SBP and DBP were required to be within 5 mmHg of each other whilst cfPWV measurements were required to be within 0.5 m/s of each other. Measurements were repeated until two consecutive readings fell within this criterion. Subjects were then asked to move into a supine position. Following an additional 5 minutes of rest BP and cfPWV measurements were then repeated in duplicate under baseline conditions. An average of the baseline BP values for the respective posture was used to calibrate the respective cfPWV waveform. The average duration of the study was approximately 30 minutes.

5.2.4 Data and statistical analyses

All PWV and BP data was collected with the SphygmoCor XCEL device in a temperature and noise-controlled environment. Measurements were performed in either a seated or supine position. All measurements for an individual were performed by the same operator but operators varied between other participants. Oscillometric brachial BP was used to attain a BP reading whilst cfPWV measurements were an average of a 20 second recording of either the femoral or carotid waveforms. Data was extracted from the system and the calibration factor was calculated using a custom script written in the software 'R' before exporting the data for subsequent analysis.

A cross-sectional multivariate analysis on predictors of the subject-specific calibration factor using stepwise linear regression was performed. Predictors were selected on the basis of their clinical relevance and included age, height, weight, gender, supine cfPWV, and seated augmentation index, HR, SBP, and DBP. The strongest model, as determined by a statistical comparison between the predictive capacity of one model to another upon the addition or removal of variables in a stepwise manner, was used for further analyses. Durbin-Watson residuals were analysed following the stepwise linear regression. A value of 2 indicated no autocorrelation of the residuals. Values above 2 were deemed to have a positive autocorrelation whilst those below were deemed

to have a negative autocorrelation. Mahalanobis distances and chi-squared distributions were used to assess multivariate outliers. If values were less than 0.001, they were considered as a multivariate outlier and were advised to be removed. Individual plots for the predictors of the calibration factor were generated. All analyses were carried out using Microsoft Excel and SPSS 25.0 (IBM). Differences were considered statistically significant if p was less than 0.05. Participant demographic data is represented as the mean±SD (standard deviation).

5.3 Results

Participants' baseline demographic data were from a wide age range, with a large physiological range of HR and BP including normotensive and hypertensive individuals (Tab. 5.1). The majority of participants were considered normotensive, SBP: 90-140 mmHg and DBP: 60-90 mmHg. A total of 38 individuals were identified as hypertensive, as based on the guideline of SBP>140 mmHg and/or DBP>90 mmHg (Chalmers et al., 1999). There were only a few controlled hypertensives (SBP<140 mmHg and DBP<90 mmHg). In addition to this, one individual had tachycardia, as reflected by the elevated HR (>100 bpm) in a seated position (Brugada et al., 1991). Despite the average SBP for the population being slightly elevated (127±19 mmHg), along with DBP and HR, values were within physiologically healthy ranges. Upon moving from a seated to a supine position there was a statistically significant change in all haemodynamic parameters (p<0.0001, Tab. 5.2). As expected, the postural change from a seated to supine position produced a significant change in cfPWV (-1.8±0.8 m/s), DBP (-4±7 mmHg), and hydrostatic pressure (P_H, -27±2 mmHg). The change in cfPWV, along with the combined systemic and hydrostatic change in BP, was used for the generation of the subject-specific calibration factor (Eq. 3.20).

No autocorrelation was present between variables (values ≈ 2) and no multivariate outliers were highlighted from the cross-sectional multivariate analysis (values > 0.001). As established from the

Table 5.1: Participant demographic data.

Parameter	Mean±SD	Range
Age (years)	49±23	18-91
Height (cm)	169±10	145-199
Weight (kg)	70±16	37-126
HR (bpm)	70±11	49-121
Seated Brachial arterial blood pressure (mmHg)		
SBP	127±19	92-193
DBP	76±10	49-104

bpm: beats per minute; DBP: diastolic blood pressure; HR: heart rate; SBP: systolic blood pressure; SD: standard deviation.

Table 5.2: Average changes in haemodynamic parameters following the postural change.

Parameter	Seated	Supine	Change
HR (bpm)	70±11	65±10*	5±6
SBP (mmHg)	127±19	123±1*	4±8
DBP (mmHg)	76±10	72±8*	4±7
P_H (mmHg)	27±2	0±0*	27±2
Total Pressure (mmHg)	103±10	72±8*	31±7
cfPWV (m/s)	8.9±2.3	7.1±2.2*	1.8±0.8

bpm: beats per minute; cfPWV: carotid-femoral pulse wave velocity; change: seated - supine; DBP: diastolic blood pressure; HR: heart rate; P_H: hydrostatic pressure; SBP: systolic blood pressure.
$*$ indicates $p< 0.0001$, compared to seated position.

multivariate stepwise linear regression model (Tab. 5.3), weight (standardised β=0.247, p=0.003) and seated brachial DBP (β=0.244, p=0.004) were significant predictors of the calibration factor.

The cross-sectional model predicted 15% of the calibration factor variability (p<0.05, R^2=0.146) and had a standard error ($SD/\sqrt{n}$) of 12.000 (95% confidence interval range). The partial plots of the cross-sectional analysis are displayed in Figure 5.1. The calibration factor (ΔBP/ΔPWV, mmHg/m/s) is plotted against weight (Fig. **A**) and seated brachial DBP (Fig. **B**). There was a positive relationship between each variable and the calibration factor, such that as weight and DBP increase so does the calibration factor. The coefficient of determination for the respective

Table 5.3: Cross-sectional multivariate statistical analysis model predictors of the calibration factor.

Variable	Unstandardised β	SE	Standardised β	95% CI LB	UB	p
Weight (kg)	0.195	0.065	0.247	0.066	0.323	0.003
Seated brachial DBP (mmHg)	0.327	0.110	0.244	0.109	0.545	0.004

CI: confidence interval; DBP: diastolic blood pressure; LB: lower bound; SE: standard error; UB: upper bound.

variables are R^2=0.0887 and R^2=0.0874. Partial plots display a large amount of scatter.

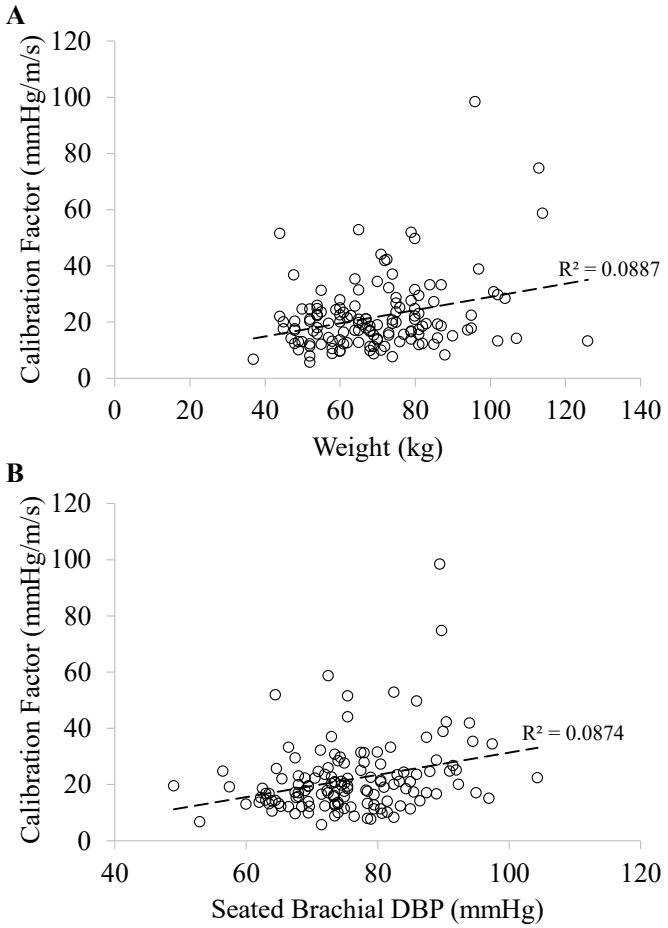

Figure 5.1: Cross-sectional multivariate statistical analysis partial plots of weight **(A)** and seated brachial diastolic blood pressure (DBP) **(B)**. **(A)** slope = 0.2345 and **(B)** slope = 0.3956. Dashed line: linear correlation between the calibration factor and the predictor variable (weight or seated brachial DBP).

5.4 Discussion

Undertaking a postural change in the present study successfully generated a subject-specific calibration factor. When the nine selected independent variables (age, height, weight, gender, supine cfPWV, and seated augmentation index, HR, SBP, and DBP) were computed in the stepwise linear regression model only two variables were identified as predictors of the calibration factor. Although weight (standardised β=0.247) and seated brachial DBP (β=0.244) were both predictors of the calibration factor, they both contributed minimally to the variability observed in the calibration factor, 8.9% (R^2=0.0887) and 8.7% (R^2=0.0874) respectively. This aligns to the second aim of this chapter such that the calibration factor cannot be predicted based on easily measured variables like age, height, weight, HR, and BP. Consequently, to generate a calibration factor individual measurements must be performed rather than using population demographics.

The partial plots of both predictors indicate a positive relationship between the calibration factor (Fig. 5.1). This is expected considering the pressure dependency of PWV. As pressure in the artery increases so too does the arterial stiffness due to the loading more collagen fibres and less elastic fibres (Wolinsky and Glagov, 1964). Consequently, aortic PWV is expected to increase with an increase in pressure. The pressure dependency of PWV can be extrapolated to this research such that the slope of BP plotted against PWV is how the calibration factor is derived. This pressure dependency has been examined in both animal models (Ng et al., 2012) and between healthy individuals (Gribbin et al., 1976). These studies have also shown the concept that as pressure within the artery increases the PWV also increases.

However, it is important to consider whether the slope (ΔPWV/ΔBP) produced remains the same between individuals of different cardiovascular statuses. This notion has been investigated by comparing the difference between the slopes in individuals with cardiovascular risk factors

such as hypertension (Gaddum et al., 2015) and haemodialysis patients (Shirai et al., 2006). In individuals possessing higher cardiovascular risk factors the slope between PWV and BP was not the same and became flatter, meaning that in these individuals as the blood pressure increases the change in arterial stiffness becomes less pronounced. This most likely comes as a result of the proteolytic degradation of elastin fibres with age, collagen cross-linking, and changes in vascular function (Wagenseil and Mecham, 2012). In the context of the calibration factor, by taking the inverse of this relationship (i.e. $\Delta BP/\Delta PWV$) it can be extrapolated that individuals with a greater cardiovascular risk are more likely to generate larger calibration factor values compared to healthy participants.

Complementary to this, it should be noted that both weight and seated brachial DBP are cardiovascular risk factors. As the data in this study includes individuals with an array of cardiovascular complications and these individuals are not separated from the healthy population in the analysis it becomes plausible as to why the correlations between the predictors are relatively low and why the partial plots are considerably flat. If the individuals were fragmented on the basis of their cardiovascular risk this study would lose a substantial amount of power and is therefore a limitation. Interestingly, the calibration factor was not highly predicted from other common variables, such as SBP or age, which may be considered as cardiovascular risk factors. It is reasonable to articulate that by separating the individuals based on their cardiovascular risk factors the potential variables associated with the calibration factor may become more prominent. With regards to the power of the study, to obtain a moderate effect size (f^2=0.15) a sample size of 55 participants (α=0.05, power of 80%, and nine predictors) would be required. *Post-hoc* analysis showed that the recruited sample size (n=135) provided a statistic power (1-β) of 99%. As such, the present study was not lacking in power.

In addition to the scatter present in Figure 5.1 **B**, it is important to consider the relationship of DBP with age. This refers to the anomaly that as age increases so does BP (systolic and

diastolic), however, around a certain age the DBP will begin to decrease whilst the SBP typically continues to increase (Miall and Lovell, 1967). This concept has been previously demonstrated by Landahl et al. (1986) whereby after the age of 50-60 years DBP began to decrease, whilst SBP did not. This is relevant to the results observed in the partial plot of seated brachial DBP. This pattern of DBP change is likely to have influenced the results by producing more scatter in the plot as these individuals would have had most likely had larger calibration factor values but lower DBP readings. Furthermore, DBP has also been shown to vary in accordance with cardiovascular risk (Landahl et al., 1986; Witteman, 1994). With respect to this, an alternate approach that may better characterise the calibration factor is by examining the presence of differences when assessed in conjunction with cardiovascular risk, such as observed in the Framingham study (Mitchell et al., 2010).

5.5 Conclusion

To conclude, the present study identified two significant determinants of the calibration factor, weight and seated brachial DBP. Notably, other common variables such as age or BP were not highly predictive determinants. As indicated by the coefficient of determination in the model and partial plots both variables were poor predictors. As a result, individual measurements incorporating a change in BP must be undertaken as demographic data and easily measured variables (e.g. BP) cannot be used to reliably predict the calibration factor. A plausible explanation for the disparity in results may be attributed to differences in PWV specificity between healthy individuals and those with a higher cardiovascular risk. Characterising the measurement of PWV under conditions involving BP changes may be advantageous in understanding the biophysical mechanisms associated with PWV, and consequently the calibration factor.

CHAPTER 6

Regional differences of PWV following a change in BP

6.1 Introduction

BP is a dynamic parameter that varies within every cardiac cycle and over subsequent cycles. This is especially true in an ambulatory setting where individuals are exposed to an assortment of stressors that have the capacity to change BP. Considering the natural regularity of BP changes and dependency of arterial stiffness (as measured by PWV) on BP (Lim et al., 2015; Zieff et al., 2018), it is important to factor in the effects that acute changes in BP may have on PWV across various arterial segments as these may influence the calibration factor. The cold pressor test is one intervention that can be implemented to cause acute changes in BP (Victor et al., 1987). This is achieved by the activation of afferent pain and temperature fibres. Upon receiving neural feedback the local sympathetic activity will increase accordingly and result in an increase in total peripheral resistance. Whilst sympathetic stimulation can cause venoconstriction in larger vessels, which indeed would increase venous return and thus cardiac output leading to an increase in BP, the reason total peripheral resistance increases BP is mainly due to vasoconstriction of arterioles and small arteries (Pfitzner, 1976). This occurs as when the luminal area of vessels decrease, pressure increases (pressure = force/area). This will inherently produce a systemic change in BP as BP is equal to cardiac output multiplied by total peripheral resistance. Referring back to the cold pressor test, this intervention has been shown to increase SBP and DBP by 14.7±10.4% and 19.1±14.6%, respectively (Mourot et al., 2009). In addition to this, Zygmunt and Stanczyk (2010)

have stated that an increase in DBP of 15 mmHg or greater is a normal physiological response. Although there are alternative methods to cause acute changes in BP, such as exercise (Marie et al., 1984) or postural changes (Poon and Zhang, 2007), these interventions make it difficult to acquire good measurements due to the nature of noise that is experienced in conjunction with these interventions. Thus, as the cold pressor test is relatively simple and movement free, it makes it an ideal intervention for the purposes of this study.

The aim of this study is the investigation of PWV, PTT, and PAT differences in different arterial segments (regional) following a change in BP, as made feasible by a cold pressor test.

6.2 Methods

Sixteen participants were recruited for the study through Macquarie University staff, students, and friends of the investigator. All participants were not required to fast and were asked to refrain from smoking, exercise, and the consumption of alcohol and caffeine prior to the commencement of the study. One participant was unable to complete the cold pressor intervention and was excluded from the study and all analyses. Therefore, a total of 15 participants (31±15 years, 67% female) completed the study and were included in the analyses. All participants provided informed consent. There was no designated exclusion criteria for the present study. Furthermore, this study was not restricted to a particular time of the day.

6.2.1 Arterial waveform measurements

Oscillometric brachial BP was measured using the SphygmoCor XCEL device (AtCor Medical, Sydney, Australia). PWV measurements were taken across the carotid-femoral arterial segment as previously described in Section 3.2.1 using the SphygmoCor XCEL device. All continuous measurements (ECG, radial arterial pulse waveform, finger PPG waveform, and finger BP

waveform) were captured using PowerLab acquisition system (ADInstruments, Dunedin, New Zealand). ECG was measured in lead II configuration to attain HR. The radial artery waveform was attained using a handheld tonometer by an experienced operator. Finger PPG waveforms were acquired using a reflectance mode PPG sensor whilst finger BP waveforms were measured using the Penaz technique (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). The Finometer PRO device uses feedback from a transmission mode PPG sensor to continuously inflate and deflate a pneumatic cuff placed around the finger to equal the pressure inside the arteries within the finger. As a result, it provides an estimation of finger BP that may be used to monitor BP in a continuous fashion.

6.2.2 Experimental protocol

Prior to the commencement of measurements participants were required to complete a questionnaire based on demographic data, lifestyle factors, and health status. Following 5 minutes of seated rest brachial oscillometric BP and cfPWV were measured in duplicate under baseline conditions by a single operator (SphygmoCor XCEL). Measurements were required to be within 7 mmHg and 0.5 m/s of each other, respectively, and were repeated until measurements fit this criterion. Participants then moved to a supine position and were fitted with ECG electrodes (lead II configuration), a finger PPG sensor on the fourth finger on the right hand (ring finger), and a finger BP cuff (Finapres PRO) on the right middle finger. The waveforms attained by these sensors were measured continuously throughout the remainder of the experiment. Following 5 minutes of baseline rest brachial BP (validated Microlife BP A100 monitor (Stergiou et al., 2006)) and cfPWV (SphygmoCor XCEL) were measured in duplicated using the same criterion from the seated position. Radial arterial pulse waveforms were simultaneously acquired in a continuous manner with a tonometer, and then for the remainder of the experiment. Upon the completion of baseline measurements the participant then submerged their right foot into icy

cold water (∼1°C). Following 2 minutes of the foot being submerged, brachial BP (Microlife BP monitor) and cfPWV were measured simultaneously in duplicate whilst the participants foot remained submerged. Upon completion of the final measurement the participant was able to remove their foot from the water which indicated the conclusion of this study. The duration of the study was approximately 20 minutes per individual.

6.2.3 Data and statistical analysis

All data were collected under standard laboratory conditions in a noise and temperature-controlled environment. cfPWV measurements were an average of a 20 second recording of both the femoral and carotid waveforms. SphygmoCor data was extracted and converted into a comma-separated values file using a custom script written in the software R before exporting the data for analysis.

Continuous HR, SBP, DBP, and PAT measurements (radial, finger PPG, and finger BP) were analysed using the LabChart 8 software (ADInstruments). HR was calculated as the frequency of RR intervals. SBP and DBP were calculated as the maximum and minimum points on the finger BP waveform, respectively. PAT was calculated as the difference between the R peak of the ECG waveform and the fiducial marker of the respective arterial waveform, as attained by the second derivative. The relationship between PAT (in seconds), as measured by radial tonometry, finger PPG, or finger BP, was determined by a linear mixed effects model. A random slope was specified in the model for the change in PAT with DBP and subjects were specified as the random intercept. To compare the difference between PAT-to-DBP slopes with the waveform types, an interaction term between DBP and waveform type was included in the final model. All data was analysed using Microsoft Excel, SPSS 25.0 (IBM), and R. Significance was classified as a p value of less than 0.05.

6.3 Results

Participants' age, HR, and seated brachial BP is summarised in Table 6.1. Average HR, SBP, and DBP were within a physiologically healthy range. One participant was taking antihypertensive medication and one was taking antipsychotic medication. On average, participants experienced a significant increase in SBP (mean±SD: 7.37±6.21 mmHg, $p<0.001$) and DBP (6.93±4.70 mmHg, $p<0.001$), as measured by the Microlife oscillometric BP device, following the cold pressor intervention (Fig. 6.1). The largest increase in SBP and DBP was 18.5 mmHg and 18.0 mmHg, respectively. However, one participant experienced a slight decrease in SBP (1.5 mmHg) despite an increase in their DBP (blue line).

Table 6.1: Participant demographic data.

Parameter	Mean±SD	Range
Age (years)	31±15	17-69
HR (bpm)	75±9	58-88
Seated Brachial arterial blood pressure (mmHg)		
SBP	119±10	108-140
DBP	76±5	67-83

bpm: beats per minute; DBP: diastolic blood pressure; HR: heart rate; SBP: systolic blood pressure; SD: standard deviation.

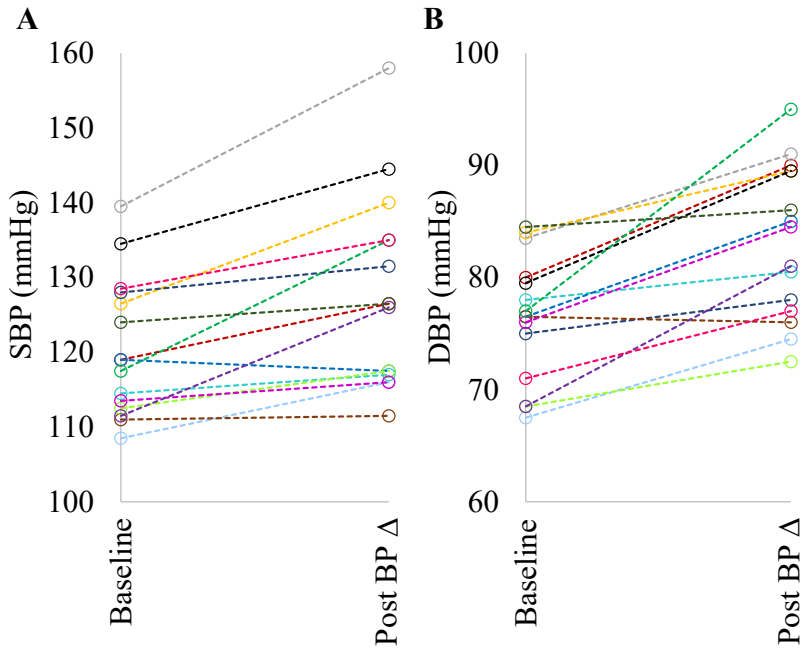

Figure 6.1: The change in oscillometric Microlife systolic blood pressure (SBP, $p<0.001$) **(A)** and diastolic blood pressure (DBP, $p<0.001$) **(B)** following the cold pressor intervention. Colours represent a specific individual.

Average baseline cfPWV and cfPTT for the study cohort was 6.11±1.20 m/s and 69.96±8.78 ms, respectively. One participant's cfPWV measurements post cold pressor intervention were classified as poor quality waveforms, as indicated by the SphygmoCor software algorithms post measurement. This participant was excluded from the remaining carotid-femoral arterial segment analyses to prevent skewing of the data. Following the increase in BP, cfPWV significantly increased (Fig. 6.2 **A**, 0.32±0.28 m/s, $p<0.001$) whilst cfPTT significantly decreased (Fig. **B**, 3.74±3.17 ms, $p<0.001$). The largest increase in cfPWV was 1.08 m/s and the largest decrease in cfPTT was 11.58 ms, as achieved by one individual. The average slope (DBP/cfPTT) for all participants was -0.42923 ms/mmHg.

Results from the linear mixed effects model are summarised in Table 6.2. Under baseline conditions PAT measured from the finger BP and finger PPG waveforms were not significantly different between each other ($p>0.05$). However, there was a significant difference between both finger BP PAT and finger PPG PAT were compared to radial arterial pulse PAT ($p<0.001$). Following the change in BP, PAT between the finger PPG and the finger BP waveforms were

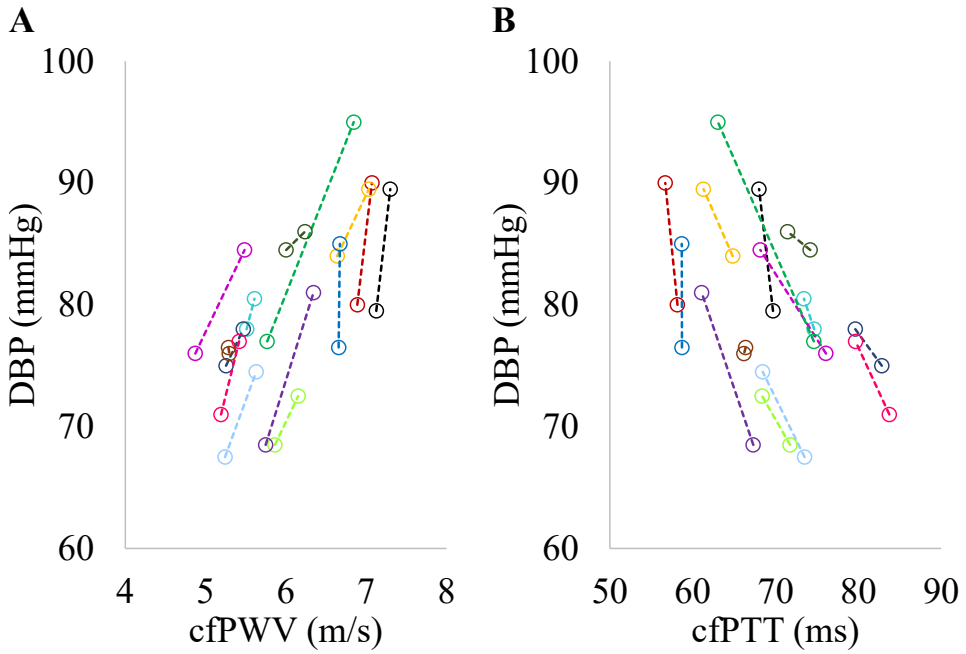

Figure 6.2: Change in carotid-femoral pulse wave velocity (cfPWV, $p<0.001$) **(A)** and carotid-femoral pulse transit time (cfPTT, $p<0.001$) **(B)** following a change in blood pressure (Post BP Δ). The average slope of cfPTT/DBP for all participants was -0.42923. Colours represent a specific individual.

significantly different from the radial arterial pulse waveform following the change in BP ($p<0.0001$ and $p<0.0001$, respectively). Finger PPG PAT was also significantly different from finger BP PAT ($p=0.0083$). As indicated by the slope value (-0.42247), finger BP PAT was most sensitive to the change in DBP in comparison to finger PPG PAT and radial PAT. Individual plots of transit time measurement types (radial = black, finger PPG = orange, and finger BP = blue) following a change in DBP are displayed in Figure 6.3. As observed with cfPTT, peripheral PAT types tended to decrease for all waveforms following an increase in DBP. The fits produced by the model varied with arterial waveform and between individuals. Due to the shorter arterial path length, radial PAT was inherently quicker than finger BP PAT and finger PPG PAT.

Table 6.2: Linear mixed effects model of the relationship between DBP and different PAT's.

Measurement	Slope (ms/mmHg)
Radial PAT	-0.09796*
Finger PPG PAT	-0.35376*
Finger BP PAT	-0.42247*

Standard error was equal to 0.134068 for all measurements. BP: blood pressure; DBP: diastolic blood pressure; PAT: pulse arrival time; PPG: photoplethysmography; S.E.: standard error. $*$ indicates $p< 0.01$, compared to each PAT.

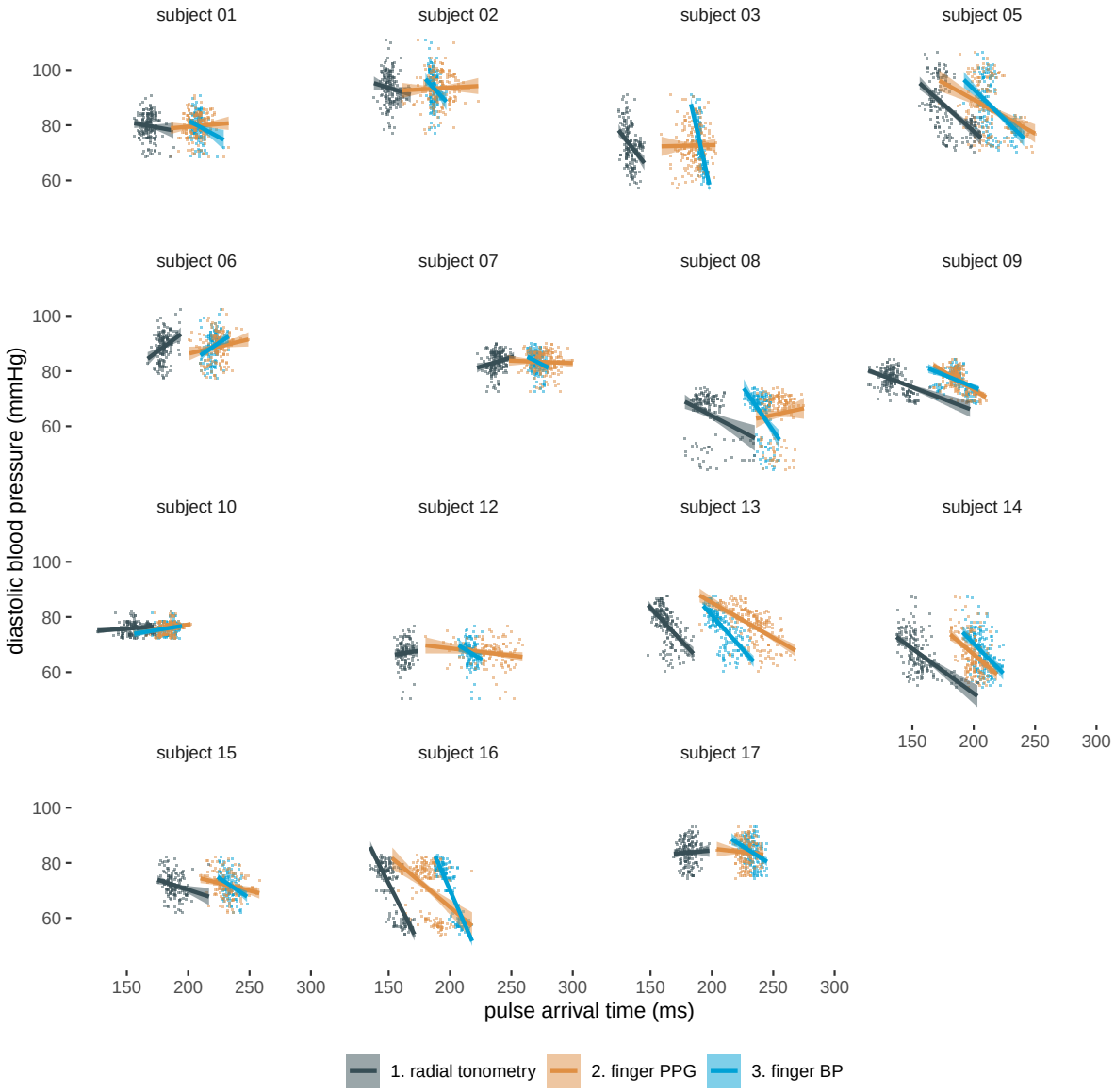

Figure 6.3: Individual plots of transit time (PAT) types following a change in diastolic blood pressure (BP). Black represents the radial tonometry transit time measurements, orange represents the finger photoplethysmography (PPG) transit time measurements, and blue represents the finger blood pressure (BP) transit time measurements. Measurements are calculated as the difference between the R peak of the electrocardiogram waveform and the fiducial marker for the respective cycle, as attained by the second derivative. All plots were generated based on the linear mixed effects model.

6.4 Discussion

On average, the cold pressor test successfully produced a significant increase in both SBP and DBP. Due to the dependency of arterial stiffness on BP (Zieff et al., 2018), a significant increase in cfPWV and decrease in PTT were also observed following the change in BP. One individual was excluded from the carotid femoral analysis due to acquired waveforms being unsatisfactory. When included in the analysis this individual skewed the data such that significance was lost following the BP change. It is likely that these measurements underestimated cfPWV and cfPTT, thus resulting in an apparent decrease in cfPWV and increase in cfPTT. This is not a realistic response and thus the removal of the individual for that specific analysis was warranted.

The linear mixed effects regression model demonstrated that the change in finger PPG PAT and finger BP PAT with a change in BP were significantly different from radial PAT. Additionally, the change in finger PPG PAT and finger BP PAT were significantly different with the change in BP. The difference in slopes between the finger PPG and BP waveforms was quite minimal, despite a significant difference. The physiological significance is therefore not substantial (approximately a 0.5 ms change for a 10 mmHg change in BP). When the finger BP and finger PPG waveform slopes were compared to the radial waveform slope a larger difference in magnitude was present. Considering the anatomical location of these waveforms these results are somewhat expected. Assuming that the arterial segments distances remained constant between measurements and considering that PAT is approximately inversely proportional to PWV (Eq. 2.1) it can be said that peripheral PWV, as measured by PAT, also increased. Notably, the individual plots produced for the relationship between DBP and PAT varied between each individual and between the transit time type (Fig. 4.7). These slopes appear abnormal due to the poor fit, and can not be explained by distance differences or waveform quality.

More importantly, the magnitude (slope) in which PWV increased or PAT decreased varied

between individuals and the arterial segment in which waveforms were measured. The difference in magnitude of these slopes suggests that cfPWV is more sensitive to changes in BP. In a previous study by Butlin et al. (2018) it was shown that a decrease in BP, as achieved by a postural change, exhibited a significant decrease in cfPWV but not carotid-radial or carotid-finger-volume PWV. Consequently this study supports the notion that cfPWV is a more sensitive arterial segment following changes in BP. This suggests that the difference in sensitivity is a necessary factor to consider between individuals. As discussed in the previous chapter (Chapter 5), sensitivity differences also arise when healthy individuals are compared to those with a greater cardiovascular risk (i.e. hypertension). These higher risk individuals are likely to be less susceptible to large changes in BP due to vasculature remodelling (Avolio et al., 1985). Such differences are common and has been shown to occur in diabetic (Kimoto et al., 2006) and hypertensive (Gaddum et al., 2015) individuals. Therefore, this should be accounted for when generating a calibration factor. Whilst the majority of individuals in this study were considered healthy, future research should factor in an individual's cardiovascular risk as this can influence the underlying biophysical mechanisms of arteries and thus individuals PWV.

As the change in PWV did significantly effect the more muscular arterial segments, it may be proposed that these segments could be used for the generation of the calibration factor. These arterial segments are more practical for measuring arterial waveforms and would consequently prove advantageous in the application of estimating BP in small devices, such as smartphones. However, as evident in the paper by Butlin et al. (2018), following a decrease in BP these more muscular arterial segments did not significantly decrease carotid-radial or carotid-finger-volume PWV. This may be attributable to the vascular structure of these muscular arteries such that they possess a higher composition of smooth muscle, and therefore, a greater influence that sympathetic activity may have (Dobrin and Rovick, 1969). As a result, this may lead to inaccuracies in characterising the relationship between BP.

Lastly, it is important to note that for the continuous measurements of radial arterial pulse waveform, finger PPG, and finger BP it was not PTT that was calculated but rather PAT. Strictly speaking this transit time measurement provides an additional time period (PEP) in which may vary differently under certain physiological circumstances, such as following a cold pressure test (Li and Belz, 1993). Despite some studies producing strong correlations (SBP: R^2=0.89 and DBP: R^2=0.78) and minimal BP variability (mean difference, SBP: -0.058 mmHg and DBP: -0.25 mmHg) (Mase et al., 2011), there is minimal consensus in the use of such a measurement in the estimation of BP, unless it has been accounted for (Sharma et al., 2017). This was a key limitation of the present study. Although this method is extremely practical, future research is required to explore these transit time differences following a BP change strictly using PTT.

6.5 Conclusions

In conclusion, an increase in DBP resulted in a significant increase in cfPWV and a significant decrease in cfPTT, radial PAT, finger PPG PAT, and finger BP PAT, on average. PAT, as measured at different arterial locations, was significantly different between each site, except for finger PPG and finger BP. The sensitivity of PAT measurements to DBP across different arterial segments varied, with cfPTT being the most sensitive to the change in DBP, as evident by the slope value. Whilst PWV was not directly measured for the peripheral arterial locations (radial, finger PPG, and finger BP), assuming that the PEP was minimal, that the distance remained constant, and through the inverse relationship of PAT/PTT to PWV, it can be said that PWV at these locations also increased. Along with the arterial segment, acute changes in BP should therefore be taken into consideration when quantifying the calibration factor as PAT/PTT and thus PWV can be influenced by different magnitudes across certain segments and BP's.

CHAPTER 7

Conclusions

Conventional BP measurements using a pneumatic cuff have been the gold standard of non-invasive BP estimation for over 100 years, despite being limited by their discontinuous and inconvenient nature. The use of the acute relationship between arterial stiffness and BP has been proposed previously as a plausible method of overcoming these limitations. This thesis presented an investigation of a novel approach to quantify the relationship between arterial stiffness and BP (the calibration factor). This involved investigating the underlying biophysical mechanisms in using the relationship between PWV and BP, as achieved by generating a subject-specific calibration factor that relied on a postural manoeuvre to induce a change in BP. With respect to the Aims of the thesis (Chapter 1), the main findings of the study are detailed below.

Aim 1 (Chapter 3): To quantify the repeatability of the subject-specific calibration factor for cuffless estimation of blood pressure.

The calibration factor displayed good repeatability, but with quantified variability inherent to the PWV measurement (Chapter 3). This variability has previously been observed in the repeatability of intra and inter-operator cfPWV measurements (Wilkinson et al., 1998), however, it has not been investigated beyond the measurement itself.

Aim 2 (Chapter 4): To examine potential sources of error in the measurement of pulse wave velocity.

Following an investigation of this inherent variability, Chapter 4 focused on quantifying the reliability of the cfPWV measurement and where the variability may be accounted for. On average, three successive measurements were required for the measurement to be considered reliable, as assessed based on the measurement regressing within the limit of ±0.5 m/s. Variability that was present was attributable to the waveform quality rather than the distance measurements. This suggests further standardisation of the measurement in order to increase its reliability and thus the repeatability of the calibration factor.

Aim 3 (Chapter 5): To establish predictors of the subject-specific calibration factor.

The calibration factor was poorly predicted by general demographics (Chapter 5). Evidently, weight and seated brachial DBP were the only two predictors, as determined by the standardised beta coefficients and the coefficient of determination. In a previous study by Butlin et al. (2018) the calibration factor was also poorly predicted. However, in addition to DBP and weight, the calibration factor was also correlated to age, HR and pulse pressure within this study, despite a smaller sample size. This may be due to the influence of cardiovascular risk on the relationship between BP and PWV. Therefore, this Chapter provides evidence as to why the calibration of BP to PWV must be measured in the individual and cannot be generalised across the population or calculated using look-up tables. This notion of BP estimation being subject-specific has previously been supported by Butlin et al. (2015a) and Mukkamala et al. (2015).

Aim 4 (Chapter 6): To investigate regional differences of pulse wave velocity following a change in blood pressure.

Including Chapter 5, this Chapter observed differences in arterial stiffness' dependency on BP in relation to the vascular site of measurement. Considering the variability of BP in an ambulatory setting, this Chapter provides insight into a more real-life scenario and how it may influence the estimation of BP through PWV. Identifying and quantifying these differences allows the assessment of whether more practical arterial segments are appropriate for the cuffless estimation of BP and whether the sympathetic activity needs to be considered when estimating BP.

In conclusion, this study furthers the understanding of the limiting factor in cuffless estimation of BP using arterial stiffness, which is the accuracy of the calibration factor. This was achieved through investigating the underlying biophysical mechanisms of PWV and its use in a subject-specific calibration method. The significance of this research is expressed through exploring a novel calibration method in order to address the limitations of conventional cuff-based BP measurements. This research has potential applications in both the clinical and commercial sphere whereby in understanding the relationship between BP and PWV the relationship may be accurately calibrated and implemented into devices and thus enhance health informatics. Considering that cuffless estimation of BP is a highly feasible concept, whilst this may not be the specific method used, the future of this field appears promising as it is merely a matter of when this research will universally be applied and integrated into smart devices.

CHAPTER 8

Future research

This thesis aimed to investigate the underlying biophysical mechanisms of a subject-specific calibration factor ($\Delta BP/\Delta PWV$). This did not extend to assessing the accuracy of this calibration factor in predicting BP. Considering that this is a novel approach and despite previous research (Butlin et al., 2018), the accuracy is still yet to be validated. These validation studies are essential in determining whether the calibration factor is useful, both in a clinical and commercial sphere. In addition to these validation studies, this calibration factor would ideally need to be integrated into a small consumer-friendly device enabling it to have the capacity to advance health informatics in an ambulatory setting.

Stemming from the concept of estimating BP in an ambulatory setting and the specificity of the calibration factor, it is important to consider the affects of changes in BP across different arterial segments. In terms of commercial applications, even though the carotid-femoral arterial segment provides the best estimate of cardiovascular risk (Mitchell et al., 2010), due to the nature of acquiring the respective waveforms this may not be considered ideal for the application in small devices. A more practical method may utilise an ECG and a peripheral waveform (finger PPG or radial tonometer), a method which has already been incorporated into small devices, such as in smart phones (iPhone, Apple) or watches (BPro G2). Whilst this does greatly improve convenience there are a few issues that arise. The first issue is that using an ECG waveform calculates the difference between the R peak of the ECG and a fiducial marker of a peripheral

waveform. As previously discussed in Chapter 2, Section 2.2.1, this approach calculates the PAT rather than PTT, which additionally includes the PEP. The PEP becomes a confounding factor as it varies in response to physiological factors which are not directly correlated to BP (Peter et al., 2014; Zhang et al., 2011). Thus future studies are required to prevent PEP from affecting the calibration factor and to determine whether the PEP can truly be accounted for.

Another issue revolves around transient changes in BP across different arterial segments. As PWV is BP dependent (Zieff et al., 2018) it is imperative to quantify how BP changes may affect the calibration factor in various anatomical locations. This may be studied through examining the influence of sympathetic activity on arterial stiffness, as performed in Chapter 6. As a result, this would determine whether more practical arterial segments are appropriate for the cuffless estimation of BP and whether the sympathetic activity needs to be considered when estimating BP. It would further provide insight into BP estimation in a more realistic scenario making the estimation more translatable. In conjunction with this, as the relationship between BP and PWV varies with respect to cardiovascular risk (Kimoto et al., 2006; Lim et al., 2015) undertaking studies to quantify the differences in the specificity of this calibration factor across different arterial segments in individuals with a higher cardiovascular risk (i.e. hypertension) would prove advantageous and aid in the development of the calibration and future devices for BP estimation.

Lastly, as concluded from Chapter 3, although the calibration factor was proven repeatable it is important to note that this study was only undertaken in a smaller sample size that was biased towards younger healthy university participants. This is an obvious limitation which may be addressed by performing further research with a larger and more diverse sample size. This limitation was also apparent in Chapter 4 and Chapter 6. With respect to the time frame of this project addressing this limitation would not have been feasible. To summarise, performing this future research would permit the advancement of cuffless estimation of BP by establishing an accurate, repeatable, and translatable approach progressing the future of health informatics.

APPENDIX A

Ethics approval for all research conducted in this thesis

This appendix contains the ethics approval for the research presented in Chapters 3, 4, 5 and 6.

Appendix A of this thesis has been removed as it may contain sensitive/confidential content

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