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PULSE TRANSIT TIME AND THE PULSE WAVE CONTOUR AS MEASURED BY PHOTOPLETHYSMOGRAPHY

THE EFFECT OF DRUGS AND EXERCISE

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ABSTRACT

Photoplethysmography (PPG) is a simple means of measuring the pulse wave in humans, exploitable for the purposes of timing the arrival of the pulse at a particular point in the arterial tree, and for pulse contour analysis. This thesis describes a methodology for measuring arterial pulse transit time (PTT) from cardiac ejection to pulse arrival at the finger. It describes the effect on PTT of drug and exercise induced changes in BP. The nature of the relationship between the PPG and arterial pressure is also examined, and the PTT technique extended to assessment of conduit vessel pulse wave velocity (PWV) during exercise.

PTT measured from ECG R-wave to PPG finger wave (rPTT) had a negative correlation (R^2=0.39) with systolic BP (SBP), unaffected by vasoactive drugs in some but not all persons. rPTT showed similar beat-to-beat variability to SBP, unaffected by drugs. rPTT correlated weakly with diastolic (DBP) and mean (MAP) pressure. Cardiac pre-ejection period (PEP) formed a substantial and variable part of rPTT (12% to 35%). Transit time adjusted for PEP (pPTT) correlated better with DBP (R^2=0.41) and MAP (R^2=0.45), than with SBP. The PPG wave tracked changes in the peripheral pressure wave. Drugs had little effect on the generalised transfer function (GTF) describing the association between arterial and PPG waves.

Strenuous exercise induced a large decrease in rPTT, mainly accounted for by decreases in PEP (53% of the total change in rPTT) and in transit time from aorta to distal brachial artery (33%). In contrast, minimal change in transit time from wrist to finger tip occurred with exercise.

Simultaneous ear-finger PPG signals were used to measure conduit artery PWV during exercise. Ear-finger PWV (PWV_{ef}) overestimated carotid-radial PWV throughout exertion (overall bias 0.81±1.05ms^{-1}, $p<0.001$), but the degree of difference remained constant. The increase in PWV_{ef} with exercise, was greater (1.18±0.54ms^{-1}, p=0.035) in healthy subjects with a positive cardiovascular family history compared to those without.

PPG enables analysis of the pulse contour during exercise, but estimation of the radial pressure wave from finger PPG by use of a GTF derived at rest, resulted in

inaccuracy following exertion. These effects were variable and relatively short-lived. Furthermore, a resting GTF used to determine central pressure from the peripheral wave, resulted in underestimation of SBP (-5.9±2.1mmHg) and central pressure augmentation index (-8.3±2.9%), which persisted for 10 minutes post-exercise.

rPTT had a negative linear association with SBP (R^2=0.94) during strenuous exercise, slightly stronger than during recovery (R^2=0.85). Differences existed in area-under-curve of the rPTT/SBP relationship between exercise and recovery, due to discrepancies in rate and degree of recovery of SBP and PEP. The linear relationship between the rPTT/SBP during exercise was affected by aerobic capacity, and the regression slope was less in the anaerobic compared to aerobic phase of exercise due to minimal change in PEP during anaerobic exertion. The correlation between rPTT/SBP did not change with prolonged aerobic exercise. Finally, measures of baroreflex sensitivity during exercise, were not significantly different between actual beat-to-beat SBP and SBP estimated using rPTT.

In conclusion, absolute BP cannot be reliably estimated by measurement of rPTT following administration of drugs and during exercise. However, rPTT may have a role in measuring BP variability and in the assessing exercise capacity. PPG may also be useful in determining the effects of exercise on arterial stiffness, and for estimating the pressure wave contour, although its use during exercise for the latter purpose must be treated with caution.

DECLARATION

This thesis has been written entirely by myself, and the substantial part of the work herein is my own. It has not been submitted previously for any other degree or professional qualification. Where data has been previously published, it has been done so with the approval of my supervisors, and the relevant articles appended to this thesis.

---------- 2009

Dr Rupert Alistair Payne

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ABBREVIATIONS

A1, A2	1st or 2nd pulse wave inflection
AAMI	Association for the Advancement of Medical Instrumentation
AC	Analogue current
AGE	Advanced glycation end-product
AIx	Augmentation index
ANOVA	Analysis of variance
AT	Anaerobic threshold
AUC	Area-under-curve
BEI	Baroreceptor effectiveness index
BMI	Body mass index
BP	Blood pressure
BRS	Baroreceptor sensitivity
DBP	Diastolic blood pressure
DC	Direct current
DN	Dicrotic notch
dZ/dt	First derivative of impedance
ECG	Electrocardiogram
EM	Electromechanical
FHx	Family history
GTF	Generalised transfer function
GTN	Glyceryl trinitrate
HDL	High density lipoprotein
HR	Heart rate
ITF	Individualised transfer function
LED	Light emitting diode
MAP	Mean arterial pressure
MET	Metabolic equivalent
MPC	Mechanophonocardiography
MRI	Magnetic resonance imaging
MRT	Mean response time

P1, P2	Pressure corresponding to 1st or 2nd pulse wave inflection
PEP	Pre-ejection period
PPG	Photoplethysmography
pPTT	Pulse transit time measured from end of pre-ejection period
PTT	Pulse transit time
PWV	Pulse wave velocity
RC	Respiratory compensation point
RER	Respiratory exchange ratio (VCO_2/VO_2)
rmANOVA	Repeated measures analysis of variance
RMSE	Root mean square error
rPTT	Pulse transit time measured from electrocardiogram R-wave
S1, S2	1st or 2nd inflection of WEP signal or first derivative of pressure
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SEVR	Subendocardial viability index
T1, T2	Time corresponding to 1st or 2nd pulse wave inflection
TT	Transit time
VO_2	Volume of oxygen used per unit time
WEP	Wideband external pulse
Z	Impedance

1 INTRODUCTION

1.1 BACKGROUND

The measurement of blood pressure (BP) in the twenty-first century is largely based on techniques developed in the nineteenth century. Current techniques using cuff occlusion were originally developed by Riva-Rocci (1896) and Korotkov described his eponymous sounds nine years later (1905).

BP is the most important haemodynamic parameter measured in everyday clinical practice. Numerous studies have shown a positive association between cardiovascular disease and raised BP (Lewington *et al*. 2002), and monitoring of BP is essential in the acute medical setting. However, BP is a dynamic phenomenon, varying from beat to beat, and affected in numerous ways such as by respiration, changes in posture, or exertion. A sphygmomanometer is not able to detect these rapid changes in pressure, taking as long as 1 minute to obtain a reading. Intra-arterial cannulation is usually agreed to be the gold standard for obtaining accurate continuous BP measurements, but is generally unacceptable due to its invasive nature. As a result, a number of alternative approaches have been used for non-invasively determining "beat-to-beat" pressure. Such measurements, if reliable, would be valuable in both a clinical and research setting. Techniques include finger blood-volume clamping (Peñáz 1973), arterial tonometry (Pressman & Newgard 1963) and adaptations of the occlusive arm-cuff sphygmomanometer (Tursky 1972).

It is, however, the use of the continuous measurement of pulse transit time (PTT) as a surrogate marker of BP that this thesis will focus on. Pulse transit time describes the time the arterial pressure wave takes to travel between different points in the vascular tree. The velocity of wave propagation is affected by pressure and arterial wall stiffness. This work describes the development of a methodology to measure PTT at a number of anatomical sites. It examines the effect of drugs and exercise on the relationship between BP and PTT. The signal analysis techniques described are also applied to the measurement of pulse wave velocity and quantification of the pulse wave contour, both of which have been shown to be valuable in the assessment of vascular function.

1.2 TECHNIQUES FOR MEASURING BLOOD PRESSURE

William Harvey (1578 – 1657) was the first person to describe the concept of the circulation of blood (Harvey 1628), but it was 200 years later before Jean Poiseuille (1797 – 1869) described the measurement of BP in humans (Poiseuille 1828). Following the development of the sphygmometer method of indirectly measuring BP (Hérisson 1834), significant progress was made in techniques for measuring BP and the pulse contour, providing a catalyst for much early research into human hypertension, including the proposal in the late 1800s of the concepts of a renal mechanism for hypertension and the idea of "essential hypertension". The methods for measuring BP that we still use today were developed around the turn of the century, with the development of the Riva-Rocci (1863 – 1937) cuff sphygmomanometer in 1896, and the description by Nikolai Korotkov (1874 – 1920) of the use of auscultation to determine systolic and diastolic pressure in 1905. Sphygmomanometer use was popularised in the early 20th century by the neurosurgeon Harvey Cushing (1869 – 1939) as a means of monitoring patients during anaesthesia, and continues largely unchanged a century later.

1.2.1 Intra-arterial invasive monitoring

Invasive monitoring of BP is generally regarded as the "gold standard", and is well established in clinical practice in high dependency and intensive care settings. In general, a catheter is inserted into either the brachial or radial artery, and connected via fluid filled manometer tubing to a pressure transducer positioned at heart level. Solid-state catheter-tip transducers may also be used to provide high fidelity pressure signals unaffected by the damping effects of the fluid filled tube, although are not generally used in a clinical setting. Invasive monitoring provides information about beat-to-beat BP variability and allows transient pressure changes to be identified. Furthermore, 24-hour direct continuous monitoring has been shown to be superior to indirect clinic measurements for risk stratification (Khattar *et al*. 2001). Despite the fact that intra-arterial monitoring is relatively safe (Scheer *et al*. 2002) and not as stressful as usually believed (Beamer & Shapiro 1973), it remains impractical for

general use, but does offer the opportunity to validate other non-invasive beat-to-beat techniques (O'Brien *et al*. 2002).

1.2.2 Sphygmomanometry

With cuff sphygmomanometry, the pressure required to collapse the brachial artery is determined by the use of an air-filled occluding cuff, stethoscope, and manometer. As the cuff is deflated from suprasystolic pressure, the pressure is noted at which the Korotkov sounds produced by the arterial pulse waves appear and disappear as flow in the artery increases. Sphygmomanometry and intra-arterial pressure are similar, but differences exist as the former detects changes in flow rather than pressure. An alternative to auscultation is oscillometry (Janeway 1904), which depends on the pressure in the cuff varying continuously with the cardiac cycle, and the amplitude of these pressure oscillations being maximal at the mean arterial blood pressure. An indirect measurement of systolic and diastolic blood pressure can be derived from the curve of oscillatory amplitude as a function of cuff pressure. Sphygmomanometry is less accurate and reproducible than direct blood pressure measurement. Inaccuracies may be due to equipment (manometer calibration, faulty valves, stethoscope), observer problems (digit preference, poor memory), or poor technique (inappropriate cuff size, manometer positioning, inflation/deflation rates) (Perloff *et al*. 1993). It is also unable to provide beat-to-beat measurement of pressure. Despite these points, sphygmomanometry is sufficiently accurate in most diagnostic and therapeutic circumstances.

1.2.3 Volume-clamping of digit arteries by servoplethysmomanometry

The Czech physiologist Peñáz described the technique of arterial volume clamping in 1973 (Peñáz 1973). This technique has now evolved into a commercially available system for continuous beat-to-beat blood pressure monitoring (Portapres, TNO Biomedical Instrumentation). An inflatable cuff with attached infrared photoplethysmograph is applied to the finger. The pressure in the cuff is rapidly adjusted to compensate for changes in intra-arterial pressure. The photoplethysmograph is used to assess the finger arterial volume. This volume clamps the artery at a set point equivalent to two-thirds of the maximal arterial

volume, thus resulting in zero transmural pressure. The cuff pressure therefore reflects absolute finger arterial blood pressure. An interface worn at the wrist controls air supply to the cuff, and relays the signals received from the cuff to the main components worn around the waist. There are two cuffs allowing measurement to be performed on adjacent fingers alternating at adjustable time intervals, and a mechanism is provided to compensate for the hydrostatic effects of raising and lowering the arm.

The method has been shown to correlate best with diastolic and mean intra-arterial blood pressure measurements. The accuracy of systolic measurements is less good, as variable amplification of systolic pressure occurs distally due to waveform reflection and dispersion. This, rather than device error, may account for the bias compared to invasively recorded brachial systolic pressure (Omboni *et al*. 1998), rather than the discrepancies being simply due to device inaccuracy. Peripheral vasomotor changes also cause error, primarily affecting systolic readings (Jagomagi *et al*. 2001; McAuley *et al*. 1997). If the cuff is applied incorrectly, calibration problems may occur, and no clearly defined method exists for determining the accuracy of cuff adjustment. Despite some of these potential problems, the technique has been widely used, particularly for the study of blood pressure variability (Gomez-Angelats *et al*. 2004) and baroreflex sensitivity (Gerhardt *et al*. 1999).

1.2.4 Arterial tonometry

Arterial applanation tonometry was first described in 1963 (Pressman & Newgard 1963). A superficial artery (usually the radial) is flattened (but not occluded) against bone using an inflatable cuff, and a piezo-resistive pressure transducer is located over the artery being studied. The circumferential tension is rendered negligible in the flattened segment of arterial wall, and so the intra-arterial pressure is perpendicular to this surface (Drzewiecki *et al*. 1983). The hold-down pressure required to applanate the vessel wall is adjusted so that the pulse pressure measured is maximised. The relative shape of the waveform is generally considered to closely approximate intra-arterial measurement, although some blunting of the early systolic phase of the tonometric waveform has been described (Sato *et al*. 1993). However

absolute pressure is difficult to determine, and the signal is generally calibrated on an intermittent basis using cuff sphygmomanometry. Errors can be due to difficulties with accurate and reproducible sensor positioning, motion artefact, and calibration problems (Matthys & Verdonck 2002). Studies have shown variable correlation of tonometry values and intra-arterial measurements, when examining differences in beat-to-beat variation, and mean, diastolic and systolic pressure values (Siegel *et al*. 1994; Weiss *et al*. 1996). Accuracy appears to be maintained during episodes of hypotension and hypertension (Kemmotsu *et al*. 1991; Sato *et al*. 1993). Perhaps of more interest in recent years, is the use of tonometry in order to enable the assessment of the pulse wave contour. So-called pulse wave analysis is described in more detail in section 1.4.3.

1.3 CLINICAL RELEVANCE OF BLOOD PRESSURE MEASUREMENT

1.3.1 Hypertension and hypotension

In 1914, the first large epidemiological studies conducted by the life insurance company Northwestern Mutual demonstrated that hypertension was associated with worse cardiovascular mortality (Fisher 1914), and this has been confirmed subsequently by numerous prospective studies (Lewington *et al*. 2002). Although diastolic BP was traditionally regarded as the more important risk factor, systolic BP has become recognised as a more powerful predictor of adverse cardiovascular outcome (Nielsen *et al*. 1995). Elevated BP also has relevance beyond simply its role in prognostication – hypertension may be a secondary sign of an underlying problem such as Cushing's disease or chronic kidney disease, and malignant or accelerated hypertension carries additional short-term risk, including stroke, cardiac failure and renal damage.

Low BP is also of importance. Although chronically low BP in the asymptomatic patient is associated with decreased cardiovascular risk (Robbins *et al*. 1982), symptomatic hypotension may cause considerable morbidity. There are numerous causes of low BP, including shock, hypovolaemia, vasodilatation, autonomic dysfunction, baroreceptor hypersensitivity and drug therapy. Beat-to-beat methods of

measuring pressure may be particularly valuable for facilitating the diagnosis of such problems, as they enable detection of rapid and transient falls in BP, which cannot be measured using sphygmomanometry.

1.3.2 Blood pressure variability

The BP measurements used for prognostic purposes have usually been "office" readings, which do not accurately reflect the dynamic nature of the cardiovascular system. Measurement of morning BP surges (Kario *et al*. 2003), and the degree of nocturnal dipping (Liu *et al*. 2003) have been shown to be of additional prognostic value. Variability of BP is associated with severity and progression of target organ damage (Parati *et al*. 1998; Sander *et al*. 2000), adverse cardiovascular events (Sander *et al*. 2000), and mortality (Kikuya *et al*. 2000). It may also be a risk factor that is independent of raised BP (Sander *et al*. 2000). There are both short and long term changes in BP. Most of the studies of pressure variability use sampling rates of every 15 minutes or more, but it has been shown that using intervals of this length, results differ significantly from those obtained by beat-to-beat monitoring, and the latter approach should probably be favoured (Di Rienzo *et al*. 1983; Parati *et al*. 1990).

BP beat-to-beat variability is characterised by spontaneous increases and decreases, which result in alterations in baroreceptor activity and reflex changes in heart rate. The degree to which changes in pressure are coupled to changes in heart rate through the baroreceptor mechanism – so-called baroreflex sensitivity (BRS) – is considered clinically important. Decreased BRS has been associated with increased cardiac sudden death and arrhythmia in myocardial infarction and heart failure (La Rovere *et al*. 1998; Mortara *et al*. 1997). It is also a sign of autonomic dysfunction in conditions such as diabetes, and again may be of prognostic use in such patients (Lawrence *et al*. 1997). Quantification of BRS can be carried out either by examining the effects of spontaneous fluctuations in BP, or by pharmacological or mechanical (e.g. valsalva) induced changes in BP (Parati *et al*. 2001). Beat-to-beat BP monitoring has employed finger volume-clamping, tonometry and invasive techniques (Chesterton *et al*. 2005; Oka *et al*. 2003; Pinna *et al*. 2000). PTT could

potentially be used as an alternative means of determining BRS, but no work has been published on this subject to date.

1.3.3 Drug effects

Drugs may have important effects on BP, due to alterations of vascular or cardiac function, fluid balance, or central regulation. The BP lowering effects may be of direct therapeutic benefit (i.e. antihypertensive agents) or a side effect (e.g. antiarrhythmic drugs, psychoactive medication). BP monitoring is essential to establish the appropriate dose regime of many drugs, and in the case of antihypertensive agents to also decide when to initiate therapy. Continuous BP measurement allows for more accurate pharmacodynamic evaluation of drugs, such as time of onset, peak, and physiological half-life. Furthermore, because the sphygmomanometer is only able to take a "snap-shot" of a dynamic phenomenon, continuous measurement reduces uncertainty over the actual degree of drug effect. Because increased BP variability has been associated with adverse outcome, the ability of certain drugs to blunt this variability, or alter other aspects of cyclical activity, may be of clinical benefit (Sirgo *et al*. 1988). Beat-to-beat BP recording is also of use in critically ill patients, not only for monitoring of dynamic pathophysiological processes, but for observing the effects of rapid pharmacological interventions, such as the use of inotropes or antiarrhythmic agents.

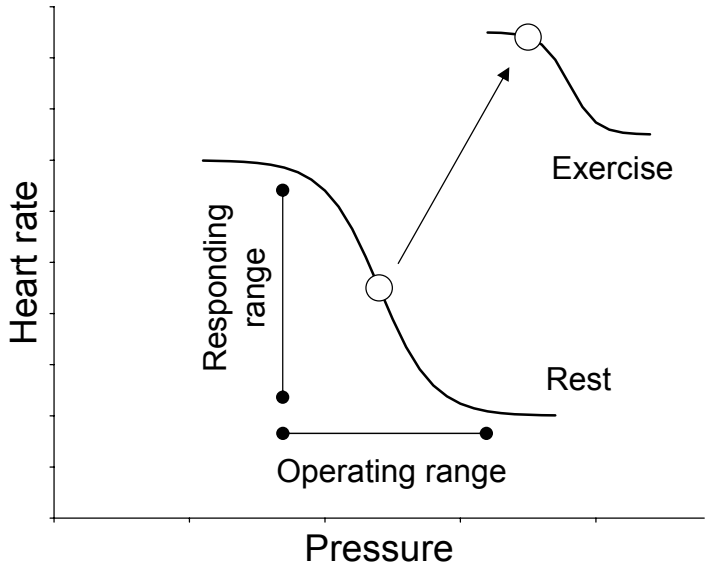
The effect of cardiovascular drugs is particularly relevant to the study of transit time and BP, as in order to use PTT as a marker of BP, one must assume a consistent relationship between the two variables. This is dependent on the intrinsic stiffness of the vessel wall and, when the electrocardiogram (ECG) is used for timing purposes, on cardiac contractility. Drug-induced changes in arterial stiffness and myocardial contractility, independent of pressure, may thus affect the ability of PTT to accurately predict BP.

1.3.4 Exercise blood pressure

BP is routinely measured during exercise testing. A failure of BP to increase appropriately is considered an indicator of myocardial ischaemia, and indeed rapid

detection of hypotension may be valuable in such circumstances. Furthermore, an exaggerated increase in pressure during exercise is associated with future development of hypertension (Manolio *et al*. 1994; Singh *et al*. 1999), correlates with the degree of left ventricular hypertrophy (Ren *et al*. 1985) and is considered a predictor of future cardiovascular events (Filipovsky *et al*. 1992; Sandvik *et al*. 1993). There is also evidence that trained athletes have an exaggerated BP response compared to sedentary individuals (Tanaka *et al*. 1996), presumably due to the greater cardiac output achieved by trained individuals (Clausen 1977).

Figure 1.1. *Effect of exercise on the baroreflex response curve*



Exercise causes decrease in both responding and operating ranges. Ranges are also shifted to higher values of heart rate and pressure. In addition, the operating point ○ on the curve shifts to a point of lesser gain. Adapted from Raven et al. 2006.

BP variability is also affected by exercise. It was previously thought that the baroreflex function was switched off during exertion, in order to allow parallel increases in both BP and heart-rate during exertion. However, it is now recognised that this is not the case. The baroreflex function can be described by a sigmoid curve, with minimal gain (change in heart rate) for pressures outwith a given operating range: that is, above a particular saturation point or below a particular threshold pressure. Dynamic measures of BRS (such as the sequence technique described in section 7.2.1) show reduced gain during exercise. This is because the arterial

baroreflex is reset in response to exercise, and the operating point on the baroreflex curve shifts to a point of lower gain, as shown in Figure 1.1 (Raven *et al*. 2006). Differences in BRS have also been observed between healthy sedentary individuals and athletes (Kingwell *et al*. 1995).

No standards exist for the measurement of BP during exercise. No accepted protocols exist for device validation during exercise. Intra-arterial monitoring is generally considered ideal, although clearly raises issues with respect to practicality and safety. Sphygmomanometry is therefore more commonly used, although is widely accepted to be inaccurate during exercise (Griffin *et al*. 1997). In general, significant underestimation of both systolic pressure (Gould *et al*. 1985; Rasmussen *et al*. 1985) and diastolic pressure (Kaijser 1987) occurs when compared to intra-arterial values. Furthermore, sphygmomanometry is unable to detect rapid changes in BP that can occur during recovery (Baum *et al*. 1992).

1.4 ARTERIAL STIFFNESS

Following left ventricular contraction, an arterial pressure pulse propagates distally through the arterial tree. The pulse wave is reflected at arterial branch points and the arteriolar bed – regions of impedance mismatch. The nature of pulse propagation and reflection, including amongst other characteristics the pulse wave velocity, is largely governed by the elastic properties of the arterial wall. The overall resulting pressure wave contour (a composite of incident and reflected waves) determines BP, and mean pressure is a key factor influencing wall stiffness. Arterial stiffness, pulse wave velocity and BP are therefore inextricably linked, and this is clearly of relevance with respect to use of pulse transit time as a marker of BP. The nature of the association between these parameters is discussed below.

1.4.1 Theoretical aspects of arterial stiffness

Classical elastic theory is often used to describe arterial mechanics. It relates the *force per unit area* that produces deformation of a body (stress, dyne.cm^{-2}), and the deformation described as the *ratio of deformation* to its original form (strain). An elastic modulus, *E* (Young's modulus), describes the relationship between the two.

Equation 1.1 $$E = \frac{stress}{strain} = \frac{\varsigma}{\varepsilon}$$

Hooke's law states that the deformation is proportional to the force applied. This does not hold for large forces, above the "elastic limit", and indeed assumes deformations are infinitesimal. Classical theory also assumes the material in question is homogeneous. However the arterial wall undergoes large deformations, and has an extremely heterogeneous form, composed as it is of a mixture of fibrous, elastic and extra-cellular matrix components. It has been shown that E increases exponentially with increased pressure according to

Equation 1.2 $$E = E_0 e^{\gamma P}$$

where E_0 is the zero pressure modulus, P is pressure, and γ is a coefficient dependent on the vessel type ($0.016 < \gamma < 0.018$) (Hughes *et al*. 1979). It is also worth noting that the properties of a truly elastic body are independent of the rate that stress is applied. The arterial wall is usually classed as *viscoelastic* however, where stiffness is higher if stress is applied rapidly. Despite this, Bergel found the elastic modulus to remain constant for frequencies of 2Hz and above (Bergel 1961b). Finally, the elastic behaviour of a material varies in different axes, unless the material is isotropic. The arterial wall approximates to this *in vivo* (Nichols & O'Rourke 1998).

Pulse wave velocity can be expressed in terms of pressure and volume change. The bulk modulus B is the ratio of compressive stress to relative volume change.

Equation 1.3 $$B = -\frac{\Delta P V_0}{\Delta V}$$

From Newton's work on sound wave velocity in air

Equation 1.4 $$c_0 = \sqrt{\frac{B}{\rho}}$$

where c_0 is wave velocity and ρ is fluid density. Substituting Equation 1.3 gives:

Equation 1.5 $$c_0 = \sqrt{\frac{V_0 \cdot \Delta P}{\rho \cdot \Delta V}}$$

This derivation was first made by Young in 1809 (Nichols & O'Rourke 1998) but is more often attributed to that described by Bramwell and Hill (1922). Alternatively the pulse wave velocity can be expressed in terms of the elastic modulus. The law of Laplace describes the circumferential tension T in a thin walled vessel with luminal radius R and under distending pressure P.

Equation 1.6 $$T = PR$$

The circumferential stress, ς, and strain, ε, are described by

Equation 1.7 $$\varsigma = \frac{PR}{h}$$

Equation 1.8 $$\varepsilon = \frac{2\pi\Delta R}{2\pi R}$$

where h is wall thickness. For a tube of length L, the change in tension ΔT can be shown to be

Equation 1.9 $$\Delta T = EhL \cdot \frac{\Delta R}{R}$$

and in terms of volume

Equation 1.10 $$\frac{\Delta V}{\Delta P} = \frac{2\pi R^3}{EhL - PR}$$

Substituting Equation 1.10 in Equation 1.5

Equation 1.11 $$c_0 = \sqrt{\frac{Eh}{2R\rho}}$$

for a single unit of length, with the mean value of P taken as 0. This is known as the Moens-Korteweg equation, first described in 1878. It assumes the wall is thin and the liquid incompressible. The Bergel correction takes into account wall thickness by

incorporating Poisson's ratio ν (the ratio of transverse to longitudinal strain) (Bergel 1960). This improves the accuracy of the velocity estimation:

Equation 1.12 $$c_0 = \sqrt{\frac{Eh}{2R\rho(1-\nu^2)}}$$

As pressure increases, *h* decreases and *R* increases, and so one would expect velocity to fall. However because the elastic modulus of the vessel increases with the distending pressure (Equation 1.2), the overall change is an increase in c_0. If Equation 1.2 is substituted in Equation 1.11:

Equation 1.13 $$e^{\gamma P} = \frac{2R\rho \cdot c_0^{\,2}}{hE_0}$$

then

Equation 1.14 $$P = \frac{1}{\gamma} \cdot \left[\ln(2R\rho) - \ln(hE_0) + \ln(c^2)\right]$$

If the first and second terms on the right side of Equation 1.14 change negligibly, then pressure is found to be related to the logarithm of pulse wave velocity.

Equation 1.15 $$P = K + \frac{2}{\gamma}\ln(c)$$

It is important to note that any physiological process which may result in a large change in pressure, such as neuro-hormonal changes during exercise, may also alter the zero pressure tonus E_0 of the vessel. This equation has been simplified further (Chen *et al*. 2000) to show a negative linear relationship between pressure and transit time *T*. However this assumes the change in pressure is small.

Equation 1.16 $$\Delta P = -\frac{2}{\gamma T_0}\Delta T$$

In summary, it can be seen that arterial stiffness is dependent on vessel size, wall thickness and distending pressure. Vessel stiffness is a key determinant of pulse wave velocity, as evident from the Equation 1.5 and Equation 1.11. Because stiffness

increases exponentially with pressure, it is therefore theoretically possible to use transit time to estimate pressure, due to the inverse linear relationship between the two parameters (Equation 1.16). In doing so, however, the assumption must be made that the intrinsic arterial wall stiffness (that is, the zero-pressure tonus, E_0) remains constant.

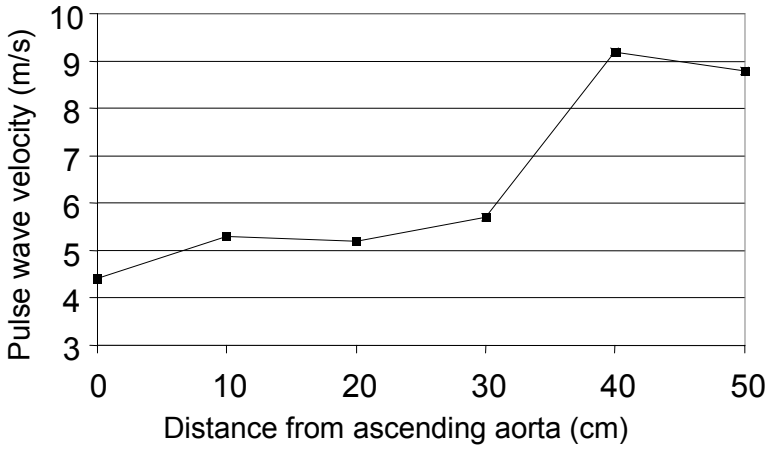
1.4.2 Physiological aspects of arterial stiffness and its association with blood pressure

Arterial stiffness is largely determined by two influences: firstly those related to the arteries themselves – wall structure and function, and lumen size; and secondly the mean distending arterial BP.

The arterial wall is composed of three concentric anatomical regions. The luminal tunica intima is thin and consists of a monocellular layer of endothelial cells surrounded by elastin and collagen. The endothelium has a key role in the modulation of vascular smooth muscle tone. These muscle fibres exist in well defined layers in the tunica media, together with structurally ordered collagen and elastin fibres. This layer forms the bulk of the vessel wall. The outermost region, the tunica adventitia, is largely collagenous and merges with surrounding connective tissue. The three regions are demarcated by the inner and outer elastic laminae.

Arterial stiffness does not remain constant in different points in the arterial tree (Latham *et al*. 1985), due to anatomical variations (Figure 1.2). Peripheral vessels have a greater collagenous component than central arteries, and so are relatively stiffer. Peripheral arteries are also narrower, resulting in an additional increase in pulse wave velocity. Disease processes such as diabetes, hypertension and atherosclerosis, can adversely affect both the intima and media (Nichols & O'Rourke 1998). Aging is also associated with degenerative structural changes (Carlson *et al*. 1970; O'Rourke *et al*. 1987), and a consequent increase in stiffness, despite the increased vessel diameter seen in older persons (Sonesson *et al*. 1993) which might be expected to decrease PWV.

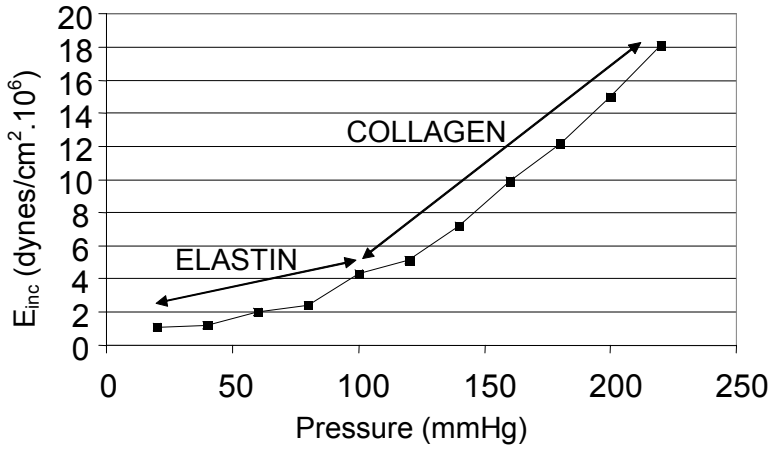
Figure 1.2. *Anatomical variation in pulse wave velocity*



PWV at different points in the human aorta (Latham et al. 1985).

In addition to these relatively "fixed" variations in stiffness, there are dynamic influences on wall elasticity. At low distending pressure, resistance to wall stretch is due largely to elastin, which is less stiff than collagen (Bergel 1961b; Dobrin & Canfield 1984). However, collagen fibres are recruited at higher pressures in a non-linear fashion (Figure 1.3). This occurs around 115mmHg in the canine thoracic aorta (Armentano *et al*. 1991). As discussed above, this results in an exponential increase in elastic modulus with increasing pressure (Hughes *et al*. 1979). Increasing pressure also results in vessel distension, which from the Moens-Korteweg equation might be expected to decrease pulse wave velocity. However, this effect is outweighed by the increase in elastic modulus.

Figure 1.3. *Effect of pressure on arterial wall stiffness*



Mean values of static incremental elastic modulus (E_{inc}) in the thoracic canine aorta (Bergel 1961).

Vascular smooth muscle also plays an important role in altering the elasticity of blood vessels (Dobrin & Rovick 1969). Contraction occurs predominantly circumferentially, and is associated with an increase in elastic modulus at any particular strain. Paradoxically, smooth muscle contraction leads to a decrease in elastic modulus when calculated against pressure. This is because contraction decreases the vessel radius, relaxing elastin fibres. The lower elastic modulus of elastin thus has a dominant effect over that of collagen fibres, and the stiffness of the contracted smooth muscle is unable to offset this (Dobrin & Rovick 1969). Different vessels exhibit different degrees of smooth muscle response to vascular mediators (Nichols & O'Rourke 1998). For instance, central elastic arteries respond less to vasodilators than muscular peripheral arteries, and arteriolar tone responds markedly to some agents (e.g. nitroprusside) but not others (e.g. nitroglycerin). Endothelial function is also important – acetylcholine usually causes vasodilatation, but induces constriction in denuded vessels (Furchgott & Zawadzki 1980).

Given that arterial stiffness is increased in patients with essential hypertension, and that arterial remodelling is a recognised feature of hypertension (Intengan & Schiffrin 2001), an important clinical question is whether increased arterial stiffness is fully accounted for by the increase in mean distending pressure, or whether there are intrinsic wall changes secondary to structural or functional effects. To examine

what effects the inherent properties of the wall have on arterial stiffness, it is necessary to make measurements under isobaric conditions. Calculation of pressure-compliance curves, with interpolation of stiffness at a given BP (Laurent *et al*. 1994), has suggested that the increase in arterial stiffness in hypertensive individuals is largely due to the increase in mean pressure. Stewart *et al* used a method for generating isobaric conditions using pharmacological interventions to adjust loading pressure, concluding that important differences exist in intrinsic wall stiffness between populations with normal or high BP, which account for the observed differences in pulse wave velocity (Stewart *et al*. 2006).

The different conclusions reached are probably accounted for by the methodologies employed. As the vessel wall is viscoelastic, luminal diameter at a given pressure is affected by the nature of the preceding pressure curve. The resulting compliance-pressure relationship therefore exhibits hysteresis, and this must be adjusted for when estimating isobaric compliance from such curves. This adjustment can lead to inaccuracy, and may mask important features of the curve such as differences existing due to truly different distending mean pressure.

The administration of a pharmacological agent creates its own problems, as it is difficult to be certain that the drug only affects BP and does not alter intrinsic wall stiffness. This issue extends beyond simply the use of drugs as a pharmacological tool. Changes in wall structure may occur over a relatively long period of time, due to direct effects of drugs such as advanced glycation end-product (AGE) crosslink breakers (Kass *et al*. 2001), or due to remodelling (Levy & Safar 1992). More rapid effects of drugs on vessel tone, independent of BP alteration, may occur due to direct action on underlying smooth muscle (Bulbring & Tomita 1987; Griendling *et al*. 1989) or via endothelial modulation (Furchgott 1983). There is indeed evidence that the therapeutic benefit of drugs such as ACE inhibitors may be due to direct effects on intrinsic wall stiffness rather than simply a reduction in BP (Tropeano *et al*. 2006). Measurement of drug-induced changes in BP using a surrogate marker such as transit time will therefore be prone to the effects of changes in intrinsic wall stiffness.

1.4.3 Pulse wave velocity and other measures of arterial stiffness

Arterial stiffness can be measured *in vivo* by various methodologies. Ultrasound and MRI both allow for the measurement of arterial diameter or cross sectional area throughout the cardiac cycle. Relating the change in vessel size (ΔS) to the change in intra-arterial pressure (ΔP), usually recorded by a transcutaneous pressure transducer, allows the compliance ($\Delta S/\Delta P$) or distensibility ($\Delta S/[\Delta P \cdot S]$) to be determined. By also measuring vessel wall thickness, it can be seen from Equation 1.10 that elastic modulus can also be determined. Both imaging modalities have the advantage of allowing assessment of vessel flow, in addition to assessing anatomical aspects of the vasculature. MRI allows assessment of inaccessible vessels such as the aorta, but is impractical in many circumstances, due to expense, scanner availability, the time consuming nature of the scans, and restrictions imposed by working within a strong magnetic field. Ultrasound is more widely available and cheaper, but is arguably more prone to inter-observer variability, and is limited to relatively superficial vessels.

Pulse wave velocity is probably the most widely used measure of arterial stiffness. The Moens-Korteweg equation (Equation 1.11) shows that PWV is related to the elastic modulus of the vessel wall. PWV is determined by timing the arrival of the pressure pulse at two points in the arterial tree, a known distance apart. Pressure measurements are either made sequentially, with synchronisation of the two pulses using ECG R-wave gating (e.g. SphygmoCor, AtCor Medical), or by simultaneous recordings (e.g. Complior, Artech Medical). Simultaneous measurement carries the advantage that it makes no assumptions of the constancy of the R-wave-to-pulse delay between each measurement site. The timing of the pulse can determined from either flow, volume or pressure. These approaches are discussed in more detail in section 1.5.1. PWV measurements have been used in more clinical studies of cardiovascular outcome than other modalities of assessing arterial stiffness. PWV is regarded as a robust and useful measure of compliance against which other measures of stiffness can be compared, although it is not a true "gold standard" (Woodman *et al*. 2005). It must be remembered, however, that the true PWV varies over the path

length studied, and values of PWV obtained are simply an average, potentially incorporating both muscular and more elastic vessels.

A third, albeit "indirect", approach is to measure the effect of arterial stiffness on the pressure pulse wave contour. Pressure waves are subject to attenuation as they travel distally (Bergel 1961a). Despite this, systolic pressure is usually higher in peripheral vessels than the aorta. This pulse amplification depends on pulse wave reflection. Partial reflection of the incident pressure wave occurs at multiple vascular bifurcations, where there are increases in vascular impedance (O'Rourke 1982). Of course, these reflected waves are also subject to attenuation. The result is marked differences in shape and amplitude at different points in the arterial tree, although the leading edge of the wave maintains its identity fairly well (Kapal *et al*. 1951; McDonald 1968). The shape of the pulse wave can be assessed in the time domain, by identifying the relative amplitudes of incident and reflected waves, the timing of different wave components, and the rate of decay of the diastolic portion of the pulse (Oliver & Webb 2003). Waveform decomposition, into incident and reflected parts, can also be performed, either by calculating the input impedance using a simultaneously recorded flow wave, or by estimating flow using a simple mathematical model (Westerhof *et al*. 2006). Less frequently, the wave is analysed in the frequency domain. However, changes in the frequency components of the pulse wave as it travels between two points can be assessed by computing a transfer function (Karamanoglu *et al*. 1993; O'Rourke 1970) – a mathematical description of the change in the magnitude and phase of different frequency components between the input and output signals of a system. This method is widely used in engineering sciences, but has been increasingly employed in clinical research allowing the estimation of central pressure waves – those that the heart directly interacts with in the proximal aorta – from waveforms recorded at more easily accessible peripheral sites such as the radial artery. Derived aortic waveforms obtained in this way have been shown to relate to cardiovascular outcome (Weber *et al*. 2004; Williams *et al*. 2006).

1.5 PULSE TRANSIT TIME

The association between arterial stiffness and BP, means that if a constant relationship can be assumed between the two variables, a measure of the former can potentially be used in conjunction with a simple calibration factor to predict the latter. Arterial stiffness lends itself to continuous beat-to-beat monitoring, through the measurement of the velocity of pulse wave propagation. Furthermore, in any given individual the distance over which the velocity is measured is constant, and so the pulse transit time – the time taken for the pulse to travel between the two distinct arterial sites – may be used without the need to measure path length.

1.5.1 Signal transduction

The site of pulse detection affects the choice of transducer required. Intravascular catheter-tip manometers are the gold standard for pulse transduction. A direct measurement of the pulse wave can be made, with no signal distortion due to fluid-filled tubing, and it is possible to precisely measure the distance between the two measurement sites. Latham *et al* (1985) carried out one of the first detailed studies of aortic PWV in humans, by using a special catheter fitted with six equally spaced (10cm) micromanometers.

Non-invasive methods are preferable, however, as risk and discomfort are minimised. Aortic pulse wave velocity can be measured by magnetic resonance imaging (Mohiaddin *et al*. 1993) or Doppler ultrasound (Lehmann *et al*. 1998), by timing the arrival of the arterial flow wave. Non-invasive pressure wave recording, using transcutaneous piezoelectric pressure transducers is considerably cheaper, quicker and easier to carry out, although is limited to relatively superficial vessels such as the femoral, brachial, carotid or radial arteries. Measurement at the latter two sites has the additional advantage of being possible to automate, either by use of a multisensor device that can optimise signal acquisition (e.g. Colin CBM7000) or by a clamp system that holds a single sensor over the required site (e.g. Complior). An alternative approach to pulse detection is photoplethysmography, which measures the blood volume wave, by monitoring changes in infrared light absorption by the tissues throughout the cardiac cycle (Millasseau *et al*. 2006). Such sensors are cheap, widely

available and relatively operator independent, although have disadvantages in the limited locations they can be positioned (usually digits or earlobe), and the fact that they may be prone to the variabilities in small vessel blood flow.

Most of the spectral power of the pulse wave form is <25Hz (Nichols & O'Rourke 1998), necessitating a minimal sampling frequency of twice this (the Nyquist criterion). With both invasive and non-invasive transducers, a digital sampling frequency of 100-200Hz is often used to avoid losing detail from the wave shape. However, up-sampling using mathematical interpolation is still required to improve the accuracy of transit time measurements.

1.5.2 Timing points on the waveform

In order to determine transit time, a point of identity must be determined on the travelling wave, and the velocity of this point used as a characteristic of the entire wave. However, the pulse wave contour varies throughout the arterial tree due to the effects of wave reflections and pulse wave amplification, resulting in different features on the waveform travelling with apparently different velocities. The foot of the waveform – where systole, and hence the steep leading edge of the wavefront begins – is least prone to the effects of wave reflections, as it occurs early and therefore maintains its identity in the propagated wave. The wave foot is therefore usually used for timing purposes, although its identification is not without its own difficulties. This is because the initial upstroke of the wave foot occurs relatively gradually over several milliseconds and may also be concealed by signal noise. Various approaches have been used to define the foot of the wave, including tangents intersecting with the signal baseline or diastolic slope (Laszt & Muller 1952) or the maximum of the second derivative (Chiu *et al*. 1991). The leading edge of the wave also maintains its identity relatively well, so other points on the rising limb have been used including the maximum first derivative (Greenfield, Jr. & Fry 1965), or a point that is a set percentage of the pulse height (Kapal *et al*. 1951).

1.5.3 Use of the ECG as a proximal timing point and the pre-ejection period

Although sensors can be placed with relative ease at distal locations on limbs or digits, it is more difficult to obtain continuous proximal waveform measurements. In order to circumvent this problem, the ECG Q or R wave can be used as a marker of cardiac ejection. The ECG has the advantages of being relatively tolerant of artefact, and simply to measure. However, this introduces the cardiac pre-ejection period (PEP) as an important confounding factor. PEP is known as a systolic time interval – a time period between two physiologically important points in the cardiac cycle – and is defined as the time between onset of electrical systole (the Q-wave) and the beginning of mechanical ejection (opening of the aortic valve). Use of the ECG also means that only specific timing points are available proximally – namely Q-wave or R-wave – rather than the choice of points on the distal pulse contour.

PEP is determined by preload, afterload and contractility (Nakamura *et al*. 1983), so transit time measured from the ECG is a composite measure comprised of cardiac and vascular components. PEP is primarily affected by cardiac contractility *in vivo* (Ahmed *et al*. 1972; Belz 1995), with increased contractility resulting in shortening of the isovolumic contraction time, and thus a decrease in PEP. However, all three influences on PEP are inextricably linked. A decrease in cardiac preload (such as that achieved with IV frusemide (Buch *et al*. 1980) or postural tilt (Stafford *et al*. 1970)), leads to a reflex decrease in contractility through the Frank-Starling mechanism, and a subsequent rise in PEP. An increase in afterload (e.g. with angiotensin (Belz 1995; Harris *et al*. 1967)) means the time it takes for left ventricular pressure to rise above aortic diastolic pressure is greater, and PEP is thus also prolonged. Although PEP is primarily a function of cardiac activity, it should nonetheless be noted that the association between BP and arterial stiffness, and the fact that BP is a function of both arterial and cardiac behaviour, means that arterial stiffness is still indirectly associated with PEP.

The effect of heart rate on PEP is complex and subject to debate (Li & Belz 1993; Spodick *et al*. 1984). Alteration in heart rate with pacing (Harris *et al*. 1967; Mertens *et al*. 1981) or vagal blockade (Harris *et al*. 1967; Kelman *et al*. 1981) does not

appear to affect the pre-ejection period. However some interventions which alter heart rate, can also affect PEP. This may be due to changes in other physiological parameters - for instance, adrenergic stimulation increases both heart rate and cardiac contractility. This is important, because it suggests that PEP cannot be compensated for by simply taking into account heart rate (Spodick *et al*. 1984).

1.5.4 Continuous transit time measurement

The main reasons for measuring beat-to-beat PWV or PTT is the potential for determining beat-to-beat blood pressure change. This has been the primary aim of most of the previous research in this area (Abenstein *et al*. 1993; Allen *et al*. 1981; Babchenko *et al*. 2000; Callaghan *et al*. 1986; Carruthers & Taggart 1988; Chen *et al*. 2000; Contrada *et al*. 1995; Davies *et al*. 1993; Davies *et al*. 1994; Geddes *et al*. 1981; Gribbin *et al*. 1976; Heard *et al*. 2000; Hon & Fukushima 1992; Jennings & Choi 1983; Johnston *et al*. 1982; Lane *et al*. 1983; Lo & Johnston 1984; Lu *et al*. 1992; Marie *et al*. 1984; Newlin 1981; Nitzan *et al*. 2002; Obrist *et al*. 1979; Ochiai *et al*. 1999; Pollak & Obrist 1983; Pruett *et al*. 1988; Smith *et al*. 1999; Steptoe *et al*. 1976; Steptoe 1977; Steptoe 1978; Thomas 1955; Thomas 1965; Weiss *et al*. 1980; Weltman *et al*. 1964; Williams & Williams 1964). Because the correlation between blood pressure and transit time is expected to vary between individuals, a calibration procedure needs to be performed to allow BP to be derived from PTT in any particular individual. Subsequent BP measurement then relies on a linear relationship existing between the two variables.

Weltman *et al* (1964) were the first group to describe the measurement of transit time from the ECG. The radial pulse was detected by using a crystal microphone to differentiate the pressure wave. In this thesis, the term rPTT has been used to denote the time between R-wave peak and the start of the peripheral pulse. pPTT describes the vascular transit time, between the end of the pre-ejection period, and the start of the peripheral pulse. Weltman *et al* studied the effects of respiratory events, including hyperventilation and Valsalva manoeuvre, on the change in rPTT. Changes in arterial pressure were not directly measured, however, and like other early

researchers in this field (Williams & Williams 1964), the PEP was assumed to be constant for the duration of measurements.

The first comparisons of true vascular transit time (i.e. not confounded by the presence of PEP) with intra-arterial BP *in vivo* were carried out by Gribbin *et al* who achieved changes in BP by subjecting the arm to negative and positive external pressure (Gribbin *et al*. 1976). They found a linear correlation between PWV and mean arterial pressure (MAP), and also demonstrated that the correlation coefficient remained constant after re-testing several months later. Further work found linear correlations between the rPTT, and both mean (Steptoe *et al*. 1976) and systolic (Allen *et al*. 1981; Obrist *et al*. 1979) intra-arterial BP. BP changes were achieved in these studies using psychological and physical stresses, and amyl nitrite. However, true vascular transit time is reciprocally related to PWV, and so from a strict mathematical perspective, a linear relationship between both these parameters and BP appears unlikely. It is possible the use of rPTT rather than true vascular transit time may explain this paradox; alternatively, the relationships observed may have actually been slightly curvilinear.

Beta-blockade (intravenous propranolol) was also used to examine the effect of sympathetic activity on the transit-time/BP relationship (Obrist *et al*. 1979). Interestingly, despite minimal effects on systolic blood pressure (SBP), the correlation between SBP and rPTT was attenuated. Although PEP was not directly measured in this particular study, the authors suggested that this may be evidence for PEP contributing significantly to BP-induced changes in rPTT. An alternative but probably less likely explanation might have been drug induced changes in the intrinsic stiffness of the arterial wall, altering the true vascular transit time despite the pressure remaining unchanged.

Newlin and Levenson (Newlin 1981; Newlin & Levenson 1979) used a polygraph technique to measure PEP. They found that transit time measured between ECG and ear, was largely determined by changes in PEP which accounted for approximately 80% of the total time delay. The fact that the arterial component was still important was supported by the improved correlation with BP when the peripheral pulse sensor

was located more distally. The importance of the longer path length was upheld by other data too (Geddes *et al*. 1981; Obrist *et al*. 1979). Geddes *et al* studied transit time measured from the ECG in anaesthetised dogs, and found the femoral time correlated better with BP than the carotid, but also confirmed the non-linear nature of the relationship between BP and true vascular transit time (Geddes *et al*. 1981).

Transit time measured from the ECG has been used during exercise in a number of psychophysiology studies (Johnston *et al*. 1982; Lo & Johnston 1984; Marie *et al*. 1984). The principle aim of these studies was to study cardiovascular feedback, rather than the haemodynamic changes occurring during exercise. Carruthers and Taggart (1988) and Barschdorff and Erig (1998) have used ECG-transit time as a marker of BP during exercise. Porta and colleagues proposed a method of using short sequences of transit time and systolic BP change to estimate the relationship between the two variables during exercise (Porta *et al*. 2006).

The r^2 linear correlation coefficients of these studies for the relationship between BP and rPTT, varied from less than 0.25 to greater than 0.95. Better r^2 values were found when transit time was measured distally (Lane *et al*. 1983; Newlin 1981; Obrist *et al*. 1979), or in studies that compared true PWV with BP (Geddes *et al*. 1981; Gribbin *et al*. 1976). Importantly, the BP range achieved in psychophysiology studies may be restricted (Lane *et al*. 1983), and this must be considered if applying the technique to situations such as exercise or the administration of vasoactive drugs where larger BP variation might be expected. In addition, such interventions may change PEP markedly and so impact more noticeably on the ECG-transit time to BP relationship (Pollak & Obrist 1983).

More recently, rPTT has been used as a marker of BP change in a clinical setting, including intensive care (Heard *et al*. 2000), haemodialysis (Ahlstrom *et al*. 2005) and obstetrics (Sharwood-Smith *et al*. 2006). The methodology has even been incorporated into a toilet seat as a means of "non-intrusively" measuring BP (Kim *et al*. 2006)! All these studies have used photoplethysmography as a means of transducing the pulse peripherally. Work has been published detailing the effects of cold (Zhang & Zhang 2006), sensor contact force (Teng & Zhang 2006) and limb

position (Foo *et al*. 2005b) on PPG transit time values. Methods of PPG artefact reduction have also been described (Foo *et al*. 2004; Foo & Wilson 2006).

1.5.5 Transit time calibration

As discussed above, estimation of BP using transit time requires a calibration procedure to be performed. There is no agreed method of doing this, although several potential options exist. One method is to use hydrostatic changes in pressure. It is possible to induce a pressure differential in the arm by raising or lowering the hand. This pressure differential can be estimated from the hydrostatic effect of raising a column of blood through a known height. However, any change in rPTT will be purely due to changes in pPTT, and such a calibration procedure would be unhelpful in situations where PEP can be expected to vary. An alternative approach is measuring beat-to-beat variability in rPTT and BP at rest, the latter using an established method such as tonometry or finger volume-clamping, and to use this to generate a regression equation with which to estimate pressure. However, beat-to-beat variability is relatively small compared with changes that may occur during exercise or in response to vasoactive drugs, and the resulting calibration slope will therefore be subject to considerable uncertainty. A third method is to use an intervention such as exercise or drugs to induce haemodynamic changes, and to use the corresponding values of BP and rPTT to obtain a calibration slope. Where measurements can be expected to be undertaken in similar repeated circumstances (e.g. multiple exercise tests), then such a procedure might be reasonable. Of course, such a process would be relatively time consuming, and the risks of additional exercise tests or drug administration may be difficult to justify. However, use of relatively brief and low intensity exercise to induce only a small BP change (but greater than spontaneous beat-to-beat changes) may be an option. A final approach might be to use a fixed calibration factor for all individuals, or a variable calibration factor adjusted for simple subject characteristics (e.g. age, sex, height/arm length).

1.6 AIMS

From the discussion above, it is evident that it would be attractive to use pulse transit time measured from the ECG as a marker of BP. It may be of particular value in situations where continuous BP monitoring is favoured over sphygmomanometry. This includes circumstances where cardiovascular drugs may be employed, or during exercise. However, it is possible that variations in the relationship between transit time and BP may occur under such conditions, potentially compromising the accuracy and reliability of such an approach. As a consequence of making the signal recordings necessary to determine transit time, it is also possible to examine the pulse wave contour and to calculate pulse wave velocity. Both these techniques may be of value in evaluating arterial stiffness. Importantly, although various approaches to ECG and pulse signal analysis are already published in the literature, there is no established system, commercial or otherwise, which combines these techniques in order to provide the ability to measure beat-to-beat transit time coupled with the flexibility to perform additional signal recordings and analyses.

The aim of this work was to develop a robust and flexible methodology for measuring pulse transit time and other cardiovascular parameters, and to use this to explore the effects of vasoactive drugs and exercise on the nature of the relationship between BP and transit time, and on arterial stiffness and the pulse waveform.

Principle hypothesis:

- **Pulse transit time will vary independently of blood pressure following administration of vasoactive drugs and after exercise**

Further specific hypotheses were that:

- Different vasoactive drugs will have dissimilar effects on each of the separate cardiac and vascular components of pulse transit time. Specifically:
  - GTN will increase pPTT and not affect PEP
  - Angiotensin II would decrease pPTT and not affect PEP
  - Noradrenaline would decrease pPTT and decrease PEP

- Salbutamol would increase pPTT and decrease PEP

- Exercise will result in dissimilar effects on the different cardiac component and anatomically distinct vascular components of pulse transit time. Specifically:
  - PEP will decrease
  - Vascular transit time will decrease more in larger arteries than smaller arteries
- Photoplethysmography can be used for timing the arrival of the pulse wave, and can thus be used as a simple means of determining conduit artery PWV during exercise.
- Changes in the intra-arterial waveform induced by vasoactive drugs, can be measured by simple, operator-independent, non-invasive methods. Specifically:
  - Infra-red digit photoplethysmography
  - Wideband external pulse recording during sphygmomanometer cuff inflation
- Exercise and vasoactive drugs will alter the nature of the relationship of the pulse wave between distinct points in the arterial tree, as determined through changes in the vascular transfer function. Specifically:
  - A peripheral-to-central transfer function estimated at rest, will underestimate proximal (central) pressure augmentation following exertion
  - The finger-to-peripheral (radial) arterial transfer function will be minimally affected by exercise or vasoactive drugs

2 METHODOLOGY

2.1 ETHICAL APPROVAL AND SUBJECT RECRUITMENT

All protocols were approved by the Lothian local research ethics committee, and conformed to the requirements of the Declaration of Helsinki (World Medical Association 2004). A standard consent form was used to obtain written informed consent from all participants. Subjects were all healthy, non-smoking volunteers recruited from the local community, with no history of cardiovascular disease or other clinically significant condition, and taking no regular medications.

2.2 ANALOGUE SIGNAL RECORDING

Studies involving intra-arterial pressure measurement employed custom hardware to perform measurements at run-time. 30-second recordings of raw waveform data from photoplethysmograph and arterial transducers were recorded at various points during these studies, for subsequent offline analysis. Signals were digitised at 200 Hz and 12 bit resolution using a custom analogue-digital converter, and recorded using custom software written by myself in LabVIEW 6.1 (National Instruments). ECG timing data was embedded in the photoplethysmograph (PPG) waveform data. These data were synchronised with bioimpedance data obtained from the serial output of the NCCOM3 Cardiodynamic monitor (BoMed Medical Systems, see below).

Subsequent studies used a multi-channel analogue-digital converter to record physiological waveforms, with subsequent off-line signal analysis using a custom-written LabVIEW program. Both ADInstruments (MacLab/400 12 bit, 200 Hz) and National Instruments (E-series 6036E PCMCIA; 16 bit, 1 kHz) systems were used.

A digital third-order low pass Butterworth filter was applied to all signals to remove noise prior to waveform contour analysis. A 40 Hz frequency cut-off was used for ECG signals and 20 Hz for pressure, volume and bioimpedance waveforms. All signals recorded at lower frequencies were up-sampled to 1 kHz using the standard approach of cubic spline interpolation before further processing.

2.3 MEASUREMENT OF PULSE TRANSIT TIME

Pulse transit time (PTT) was measured between the QRS complex of the ECG, and the pulse arrival at the finger or other distal pulse. The term rPTT is used to denote the time between R-wave peak and the start of the peripheral pulse. pPTT describes the vascular transit time, between the end of the pre-ejection period, and the start of the peripheral pulse. Thus, we have the equivalence *rPTT=PEP+pPTT*. A suffix (e.g. "finger") has been used where required to distinguish different transit time measurement sites respectively, although has been omitted where only one recording site was used.

Studies involving intra-arterial pressure measurement employed custom-built hardware developed by the University of Edinburgh School of Electronics and Engineering. The equipment comprised ECG and PPG signal recording hardware, and made transit time calculations at run-time. Although capable of functioning autonomously, the system was interfaced by a serial port to a personal computer for data logging and control purposes. To allow for more detailed signal processing, later studies recorded raw ECG and PPG signals for off-line analysis and computation of transit time.

2.3.1 ECG recording

Single channel ECG recording was used for all studies, using a standard 3-lead configuration (Lead II) connected using standard 5cm disposable solid-gel electrodes. Numerous methods have been used for R-wave detection, concentrating on the ability to detect complexes in the presence muscle noise, baseline wander and electrical interference (Friesen *et al*. 1990). Dual or multi-channel ECG recording is advantageous, particularly during exercise, as the redundancy in such systems can be used to improve QRS complex detection and arrhythmia and ectopic beat analysis, even when artefact is present (Dotsinsky & Stoyanov 2004; Kaiser & Findeis 1999). Lead-switch algorithms have also be used to continuously select the "best" lead (Kaiser & Findeis 1999). Multi-lead systems are more complex, however, requiring increased computer power and more complex hardware. As the majority of studies were to be conducted in healthy subjects, in whom ectopic beats and arrhythmias

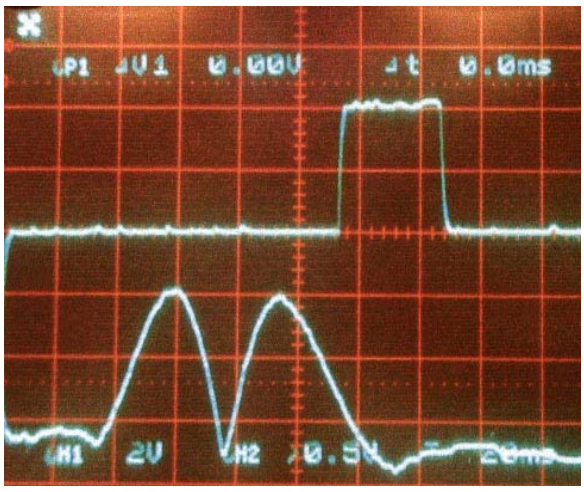
were anticipated to be relatively unusual, it was felt that the complexities of implementing a multi-channel system were not justified by the benefits.

Two different algorithms were used for detection of the ECG R-wave. These are detailed below, including a comparison of their accuracy. Accurate detection of the R-wave was crucial to the precise identification of other key points on the other pulse waveforms, and all timing measurements were made relative to the peak of the R-wave. The Q and S waves were also identified relative to the R-wave.

2.3.1.1 Real-time ECG QRS detection

A custom ECG system was used for detection of the R-wave for studies involving intra-arterial recordings. The ECG signal was recorded using the Lead II configuration. The signal was passed through a differential amplifier, additional gain stage with low-pass 1st order filter, 5th order low-pass filter, and full wave rectifier. Overall gain was around 1000, and the band pass was 5-23 Hz. This output signal was then digitised at 1 kHz (8 bit). The output signal consisted of a double hump, with a sharp minimum between the two peaks corresponding to a fixed period of 75 ms after the R-wave maximum (Figure 2.1).

Figure 2.1. *Analogue processing of ECG signal*



Bottom trace shows output signal after analogue pre-processing stage (photograph of oscilloscope screen). Sharp minimum between two humps corresponds to R-wave, delayed by a fixed period of 75 ms. Top trace shows timing pulse, the upstroke of which corresponds to 40 ms after the occurrence of the second hump which is used to confirm presence of preceding minimum and hence R-wave.

The first hump was identified from the 4-point moving average as 8 consecutively increasing samples followed by a similar period of decreasing samples. The

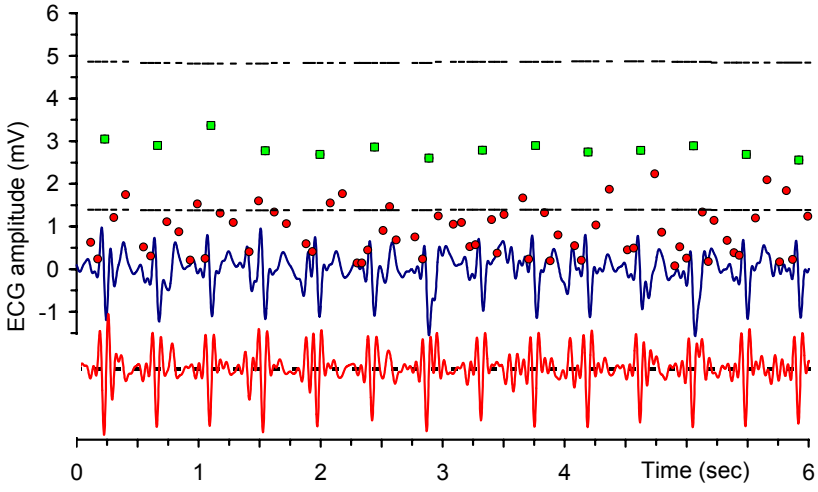
minimum was then found from un-averaged samples. The second hump was then identified as for the first. The difference between each peak and the minimum was required to exceed a predefined threshold. A minimum period of 256 ms was required to have elapsed since the previous detection.

Outlying beats were removed using a multi-pass filter, based on outlying values (greater than four standard deviations of the 16-second centred mean) of transit time (three-pass) and heart rate (single-pass).

2.3.1.2 Off-line ECG QRS detection

Subsequent studies employed a commercial ECG monitor (LifePulse LP15A, HME Ltd), with direct sampling of the analogue output signal. This provided the advantages of allowing timing of other ECG time points (e.g. Q wave), and functioned relatively independently of lead position (although was optimised for Lead II).

Figure 2.2. *Example of offline ECG detection*



Blue line, ECG signal in mV; red line, first derivative of ECG signal in arbitrary units (dotted line represents zero); broken lines represent QRS amplitude threshold limits (in mV); dots represent all possible ECG spikes, based on substantial positive deflections of first derivative; green dots represent those the algorithm identifies as likely R-wave candidates, red dots represent other dismissed deflections. See text for details.

Details of the algorithm used for ECG R-wave identification are given in Appendix 1, with an example shown in Figure 2.2. Briefly, the ECG signal was digitally filtered,

and a threshold for R-wave detection determined by calculating the running maximum of the first derivative of the signal. R-wave deflections were identified from positive-to-negative crossing points on the first derivative, occurring in close proximity to points at which the first derivative exceeding 30% of the threshold, and meeting various predefined criteria. The timing of these QRS complexes was used as the initial timing point of the cardiac cycle for all other measurements, including recalculating the ECG QRS timings. All ECG data was subject to a two-pass filter, with pulses lying outwith 3 standard deviations of the 5-second median average heart rate being automatically rejected.

2.3.1.3 Reliability of ECG QRS detection

As the real-time algorithm was dependent on specific analogue pre-processing, it was not possible to evaluate it using a formal ECG test database. The accuracy was therefore studied in 8 healthy males (median age 23) at rest and during an incremental exercise test, the same as that described in section 7.2.1. Results are shown in Table 2.1. Accuracy was estimated by comparing actual R-wave detections with the number expected based on mean heart rate, and using a 16-second centred median filter to determine outliers. This demonstrated a sensitivity of around 90% and positive predictive value of around 99%. It should be noted that the system employed to make real-time measurements used transit time to filter outlying data, rather than only heart rate. These sensitivity/positive predictive value results are therefore influenced by transit time measurement rather than simply ECG detection. However, as transit time measurement relies on ECG timing, these sensitivity/positive predictive value results still act as a guide to the reliability of the R-wave detection. Importantly, though, this approach may fail to detect the algorithm misidentifying different points close together in the same QRS complex (e.g. where there is an RSR pattern and there is variability in which R-wave is selected, or in situations where the upstroke of a large S-wave is selected in preference to a small R-wave), although larger beat-to-beat errors will be picked up. Further, it is not possible to determine any overall consistent bias relative to the actual R-wave.

In addition, 30-second samples of waveform data recorded from each exercise stage were studied. The number of missed or excess beats was counted manually using the

PPG wave as a reference. As only a time stamp marking the occurrence of the R-wave was recorded, rather than the raw ECG waveform, it was not possible to precisely evaluate the timing accuracy of the ECG detection, or the ability to detect ectopic beats. Sensitivity and positive predictive value assessed in this way were very high (≥99.9%, see Table 2.1). This probably reflects selection bias introduced by taking specific care over the 30-second recording interval to minimise movement artefact and thus optimise signal quality.

Table 2.1 *Real-time ECG algorithm*

	Sensitivity (%)		Positive predictive value (%)	
	Direct observation	Estimate	Direct observation	Estimate
Rest	99.9	90.9	99.9	98.7
Exercise	100.0	90.3	100.0	98.8

The reliability and accuracy of the off-line QRS detection system was tested against the Massachusetts Institute of Technology – Beth Israel Hospital (MIT-BIH) arrhythmia database (Moody *et al*. 1993). This database consists of 48 thirty-minute 2-channel ECG recordings. QRS complexes have been independently annotated by two or more cardiologists, and the database is widely used as standard test material for evaluation of arrhythmia detectors. Only the upper channel data (usually lead II) from this database was used for evaluation of the off-line algorithm. An estimate of the sensitivity of the off-line algorithm during exercise was made from data gathered from 10 healthy (7 male, mean age 36 years) individuals, by comparing the actual number of R-wave detections with the number expected based on the median heart rate. Measurements were made before, during and after a 1-minute incremental bicycle ergometer (Lode Rehcor) maximal exercise test as described in section 2.9.1.1. Selected 2 minute periods of data were analysed from pre-defined time periods: 2-4 minutes before exercise, initial 2 minutes of exercise, 2 minutes exercise centred around the anaerobic threshold (based on the estimate described in section 2.9.1), final 2 minutes of exercise, and 1-3 minutes after stopping exercise.

Table 2.2. *Off-line ECG algorithm*

		Sensitivity (%)	Positive predictive value (%)
MIT test database			
All test ECGs		82.2	83.2
Selected ECGs		97.6	98.3
Estimated reliability			
Rest		97.0	97.6
Exercise	Mild	96.0	96.9
	Anaerobic threshold	98.9	98.8
	Peak	98.6	99.7
Recovery		96.6	96.9

Results are shown in Table 2.2. The sensitivity and positive predictive value for the off-line algorithm, based on the MIT-BIH database, were 82.2% and 83.2% respectively. On assessment of individual ECG records, this appeared to be due to particularly poor QRS identification during arrhythmias, ectopics, and QRS complexes with unusual morphology. This is unsurprising, as the algorithm was purposely optimised to cope with noise due to muscle and movement artefact, in subjects with normal sinus rhythm, and was not designed to monitor arrhythmias. Indeed, when the MIT-BIH data set was limited to ECGs which excluded large numbers (over 5 minutes) of ectopics, periods of arrhythmia (e.g. atrial fibrillation), and abnormal QRS morphology (e.g. bundle branch block), sensitivity and positive predictive value were found to be similar to that estimated during exercise. The estimates of sensitivity during exercise and rest were substantially higher using the off-line algorithm than the estimates for the real-time algorithm ($p<0.001$ and $p=0.001$ respectively), although positive predictive value was no different ($p=0.59$ and 0.34 respectively). There were no differences in sensitivity or positive predictive value across varying levels of exercise ($p=0.54$ and 0.33 respectively by ANOVA).

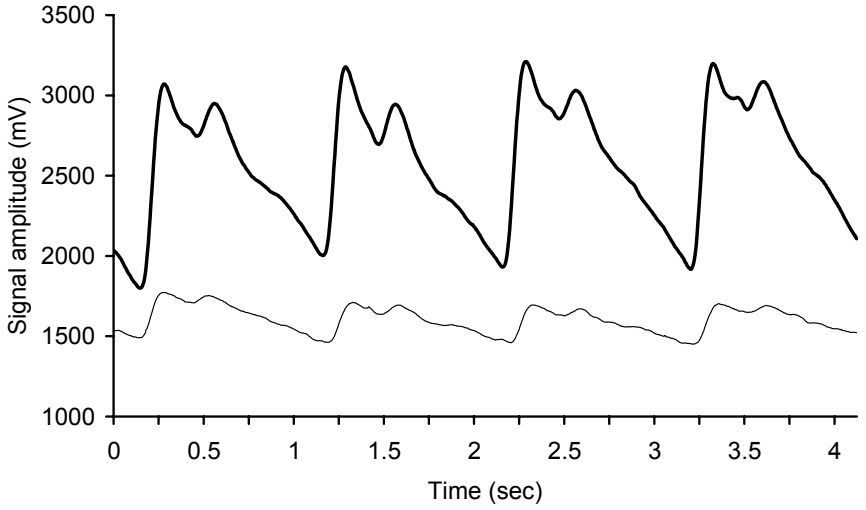
2.3.2 Photoplethysmography

Photoplethysmographic pulse monitoring was originally described in the 1930s (Hertzman & Spealman 1937). The technique relies on the absorption of light by soft tissues and blood, and measures either the reflected or transmitted light intensity by using a phototransistor. The precise mechanism underlying the changes in the

photoplethysmograph signal remains uncertain. Changes in blood volume throughout the cardiac cycle would appear important, and vessel diameter correlates with the PPG signal (Greenwald *et al*. 1997). It is possible that soft tissue movement related to underlying arterial pulsation may also be involved, and this is suggested by work demonstrating the ability to detect a pulsatile signal at sites overlying large vessels but not elsewhere (with the exception of the relatively vascular digits and ear lobe) (Loukogeorgakis *et al*. 2002). Regardless of the exact nature of the PPG signal, it is nonetheless a function of the underlying cardiac cycle. The start of the finger volume pulse (i.e. the foot of the leading edge of the wave) can therefore be expected to correspond to the start of the arterial pressure wave, although other points (e.g. the wave peak) may be not be simultaneous. The technique has therefore been used for timing purposes by a number of investigators (Allen & Murray 2002; Babchenko *et al*. 2000; Greenwald *et al*. 1997; Heard *et al*. 2000; Loukogeorgakis *et al*. 2002).

For all studies, the pulse volume wave was detected on the dominant index finger using a custom-made infrared transmission photoplethysmograph. A similar device was used at the ear. The signal current through the sensing phototransistor fluctuated over approximately 0.5μA, with a large DC component. The AC coupling had a long time constant ($\tau_c \approx 100$s) to reduce signal distortion, with DC levels restored rapidly after large signal artefacts by microcontroller intervention. A current-to-voltage converter was used with a conversion factor of 1V/μA, followed by voltage gain (×3) stage, 5^{th} order low-pass filter and 1^{st} order ripple reduction filter (τ_c=2 ms). Signal output amplitude varied between 0 and 5V. To accommodate low frequency variations in pulse amplitude, which varies with factors such as temperature and altered blood flow, the infra-red LED current was variable between 1 and 31mA, with greater current resulting in greater signal amplitude. A reflective transducer was similarly tolerant of motion artefact, but provided poor signal strength when extremities were cold (see Figure 2.3), to a degree which rendered it unusable in some circumstances. It was therefore decided to use the transmission device.

Figure 2.3. *Comparison of reflection and transmission photoplethysmography*



Simultaneous recording of finger pulse using transmission (bold line) and reflection (thin line) photoplethysmography, with equivalent LED current.

Both the algorithms used to identify the pulse wave leading edge were highly dependent on accurate identification of the preceding ECG R-wave. Both could wrongly identify marked positive artefact deflections as a pulse leading edge. All transit time data was therefore subjected to filtering, detailed below.

2.3.2.1 Real-time pulse wave leading edge detection

For studies employing arterial-line monitoring (Section 2.8.3), detection of the pulse wave leading edge was performed by custom hardware. The signal was digitised at 200 Hz and 12-bit resolution. The signal was analysed using a 3-point moving average. Following detection of the R-wave, the wave leading edge was considered to be the first occurrence of 35 ms consecutively increasing samples of minimum amplitude 0.156V. The maximum point was subsequently considered reached after 35 ms consecutively decreasing samples. The wave baseline was taken as the lowest point between the preceding maximum and the start of the leading edge. Transit times were calculated from the R-wave to the steepest point on the leading edge (maximum first derivative), and the point at which a tangent through the steepest point intersected the baseline. Transit time results were returned within 35 ms of the wave maximum being reached. A variety of pre-specified artefact rejection criteria were used. These included (for the finger) transit time values <100 ms or >496 ms,

pulse maximum occurring within 165 ms of the ECG, nonsensical timing points (e.g. maximum preceding minimum), and the maximum slope being too steep (>12.45 V/s).

2.3.2.2 Off-line pulse wave leading edge detection

For studies not employing arterial line monitoring, detection of the pulse wave leading edge was performed offline using a custom LabVIEW program.

The leading edge of the waveform was taken as the first point at which the first derivative exceeded 50% of its maximum over the associated R-R interval, in association with a signal amplitude of over 60% of the maximum. The start and end of the leading edge was defined as the associated negative-to-positive and positive-to-negative deflections. Within these limits, transit time was calculated from the R-wave to the maximum first and second derivatives, and intersecting tangents through these two points and the wave baseline. Waveforms were rejected if timing points did not make sense (e.g. maximum second derivative occurring after maximum first derivative), wave length outwith the range 250 ms to 2000 ms (irrespective of R-R interval), and episodes where signal amplitude remained unchanged over a 300 ms period (suggesting signal saturation or voltage clamping).

2.3.2.3 Reliability of pulse wave leading edge detection algorithms

The reliability of the real-time algorithm was determined in 8 subjects as referred to in section 2.3.1.3. Sensitivity was estimated as the number of transit time measurements after filtering expressed as a percentage of total number of R-wave detections (i.e. prior to filtering). Positive predictive value was estimated as the number of transit time measurements after filtering expressed as a percentage of the total number of transit time measurements. Sensitivity was not calculated relative to the number of R-wave detections after filtering, due to the decision to filter the ECG samples using corresponding transit time data, rather than heart rate data (any transit time value would therefore have a one-to-one association with an R-wave detection, misleadingly implying a “sensitivity” of 100%). Results are shown in Table 2.3.

Table 2.3. *Real-time pulse wave algorithm*

		Sensitivity (%)	Positive predictive value (%)
Rest		90.8	91.5
Exercise	Mild	93.5	94.1
	Moderate	93.7	94.0
	Strenuous	90.3	92.0
Recovery		96.1	96.3

Table 2.4. *Off-line pulse wave algorithm*

		Sensitivity (%)	Positive predictive value (%)
Rest		98.5	98.6
Exercise	Mild	97.4	98.4
	Anaerobic threshold	95.1	96.5
	Peak	94.7	95.9
Recovery		98.9	99.8

An estimate of the reliability of the off-line algorithm was made in 10 healthy individuals as described in section 2.3.1.3. Sensitivity and positive predictive value were estimated based on actual R-wave detections at rest and at different exercise phases. Results are shown in Table 2.4.

The sensitivity of the off-line algorithm tended to be higher than that of the real-time algorithm during exercise and recovery ($p=0.065$), and was significantly higher during rest ($p<0.001$). Positive predictive value was significantly higher for the off-line algorithm both during exercise/recovery ($p<0.01$) and at rest ($p<0.001$). Although there was an apparent overall trend for the sensitivity and positive predictive value of both algorithms to decrease during exercise, this was not statistically significant (ANOVA: $p=0.27$ and 0.45 respectively for real-time algorithm; $p=0.76$ and $p=0.66$ for off-line algorithm).

2.3.3 Conclusion

Although the real-time ECG sensitivity was somewhat less than ideal (down to 90.9%), the system was developed along similar lines to techniques employed by the heart-rate monitor manufacturers, with a view to being practical and implemented in a standalone measurement system, and positive predictive value was still high

(≥98.7%). The offline algorithm improved on sensitivity (≥96.0%), with the benefit of an adaptive R-wave detection threshold that would respond to varying signal strength and noise levels. Although still less sensitive than some commercially available systems (which may exceed 99%), it nonetheless offered good predictive power during exercise (≥96.9%). Pulse wave leading edge detection was highly dependent on accurate R-wave detection for both systems. The two methods were both considered satisfactory, although the more complex algorithm employed offline resulted in improved detection. Importantly, the ability of the algorithms to determine the peak of the R-wave and different points on the wave leading edge within 1 ms accuracy, was crucial to accurate transit time measurement, particularly over the short time periods (~30 ms) examined in some studies. Overall, when used for making beat-to-beat measurements over a reasonably long time periods, these levels of ECG and pulse wave detection reliability still allow a considerable proportion (>90%) of pulse wave measurements to be made accurately.

2.4 MEASUREMENT OF PRE-EJECTION PERIOD

The gold standard for non-invasive measurement of the pre-ejection period (PEP) is echocardiography. It is possible to directly visualise aortic valve motion in M-mode, and thus calculate PEP as the time between Q-wave and valve opening. However echocardiography is not suitable for continuous measurements and other non-invasive approaches have been developed instead. The usual method of determining systolic time intervals (including PEP) is by simultaneously recording the electrocardiogram, phonocardiogram (using a microphone to detect the second heart sound, S2), and carotid pulse wave. The method is sometimes known as mechanophonocardiography. This technique was first described by Katz and Feil in 1923 (Katz & Feil 1923), and popularised by Weissler et al in the 1960s (Weissler *et al*. 1969). Positioning of the carotid transducer and microphone are critical as both are prone to movement artefact. Erroneous results can be minimised by careful set up and averaging results. The technique has been employed by many groups, and has been used successfully in exercising subjects (van der Hoeven *et al*. 1973; van der Hoeven *et al*. 1977).

Bioimpedance measurement techniques were described back in the 19th century (White *et al*. 1990) but their present use as a means of determining cardiac function is attributed to work originally carried out by Kubicek (Kubicek *et al*. 1966), and elaborated upon by Bernstein (Bernstein 1986) and Sramek. The technique involves measuring the impedance (*Z*) to a high frequency alternating current passed between electrodes located at the neck and thorax. Changes in *Z* reflect changes in aortic blood flow, and particular points on the first derivative of the impedance cardiogram (*dZ/dt*) have been related to the opening and closing of the aortic valve (Lababidi *et al*. 1970). With respect to systolic time interval measurement, bioimpedance has been compared favourably to both mechanophonocardiography and echocardiography (Stern *et al*. 1985; Thomas 1992), and to invasive techniques (Rasmussen *et al*. 1975). Bioimpedance has been used during exercise, and the observed changes in *dZ/dt* reflected expected variations in systolic time intervals (Gollan *et al*. 1978). An unsuccessful comparison with MPC has been attempted during exercise (Thomas 1992), although more recently a small study suggested good correlation between impedance and MPC measures (Ono *et al*. 2004).

It was felt that bioimpedance was better suited for the purposes of beat-to-beat measurement of PEP, and potentially more reliable than MPC during exercise. PEP was therefore measured using an NCCOM3 Cardiodynamic monitor (BoMed Medical Systems). 5cm solid-gel disposable electrodes were placed in accordance with the manufacturer's instructions. The NCCOM3 device itself has been compared favourably with both echocardiography (Kerkkamp & Heethaar 1999) and mechanophonocardiography (Thomas 1992).

The NCCOM3 device determines systolic time intervals internally, and uses these to calculate cardiac output and other indices. The values are output in digital format every single beat, or as a 16-beat average. It also has analogue outputs (-8 to +8V) for the AC component of the impedance waveform (Z) and the first derivative (dZ/dt).

2.4.1 Calculation of PEP

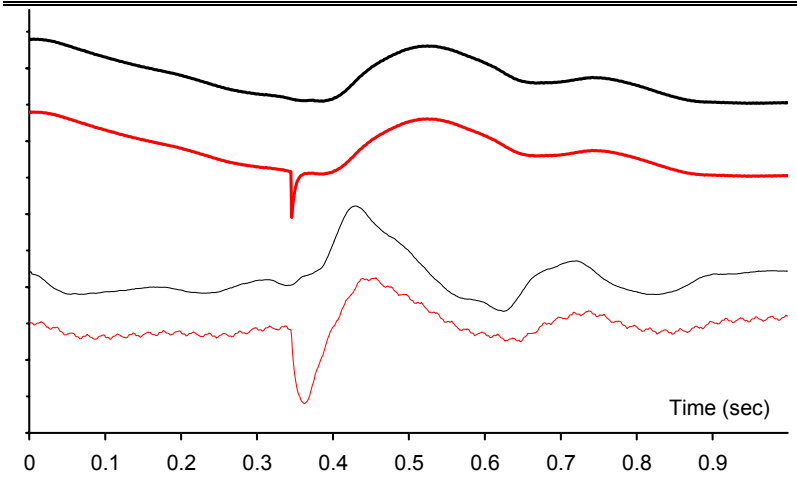
2.4.1.1 Real-time calculation of PEP by NCCOM3 device

Studies employing arterial line monitoring utilised PEP values obtained directly from the NCCOM3 device. Although this equipment uses systolic time intervals internally, the digital output does not include the PEP value. PEP was thus computed from the other available cardiac parameters, and was therefore subject to the integer rounding of the variables from which it was calculated: $PEP_{(ms)} = (60000/HR) \times STR \times ER$, where HR is heart rate in beats per minute, STR is systolic time ratio, and ER is ejection ratio.

2.4.1.2 Off-line calculation of PEP from dZ/dt signal

For other studies, PEP was measured by analysis of the NCCOM3 analogue output signal. The impedance signal was found to be subject to distortions corresponding to the internal detection of the ECG. This consisted of a large spike, of approximately 10 ms duration. The time constant introduced by analogue differentiation resulted in a broad spike of up to 100 ms, which distorted the analogue dZ/dt output. The impedance signal was therefore used directly, with spikes identified as changes in the first derivative outwith 4 standard deviations of the mean. The spike distortions were removed by cubic spline interpolation (Figure 2.4). The signal was filtered (20 Hz low-pass filter) and differentiated (dZ/dt) prior to analysis. The peak of the dZ/dt signal was identified as the maximum value within 170 ms of the ECG. The time to maximal slope of the dZ/dt signal, and the initial negative-to-positive crossing of the first derivative of dZ/dt, were identified. The amplitude of this latter point was also used as a baseline with which the time of intersecting of a tangent through the maximal slope of dZ/dt was calculated. The negative-to-positive crossing point is generally regarded as the “B-point”, and coincides with the end of PEP, but the other three points (peak, maximum slope, intersecting tangent) were also calculated to determine which was the most robust measurement.

Figure 2.4. *Removal of spike artefacts from impedance signal*



Bold lines are impedance (Z) signal, thin lines are first derivative of impedance (dZ/dt). Red lines represent original NCCOM3 sampled analogue output, black lines represent signal following filtering and differentiation.

2.4.2 Accuracy of PEP measurement

2.4.2.1 Reliability of real-time PEP detection by NCCOM3

Detection rates of PEP were evaluated in 8 healthy male subjects (section 2.3.1.3). PEP values were excluded if lying outwith 3 standard deviations of the running 16-second centred median. Sensitivity was assessed as the number of recorded PEP values as a percentage of the total number of measured transit time values. Results are shown in Table 2.5. There was an apparent overall trend for sensitivity to decrease with increasing exercise effort but this was not statistically significant (p=0.34 by ANOVA).

Table 2.5. *Real-time NCCOM3 PEP detection*

		Sensitivity (%)	Positive predictive value (%)
Rest		76.6	88.4
Exercise	Mild	77.2	96.7
	Moderate	68.4	92.9
	Strenuous	58.3	92.4
Recovery		71.1	88.9

2.4.2.2 Reliability of off-line PEP detection

The off-line analysis algorithm used to measure PEP was assessed by continuous bioimpedance and ECG recording in 10 healthy volunteers during exercise, as described in section 2.3.1.3. A two-pass filter was used to remove those detected values lying outwith 3 standard deviations of the running 20-second centred median.

Sensitivity was estimated as the number of valid PEP values after filtering expressed as a percentage of the total number of successful R-wave detections. Positive predictive value was estimated as the number of PEP values after filtering expressed as a percentage of the total number of PEP values. Changes in the delay between different time points was examined at all exercise stages.

Table 2.6. *Off-line PEP detection*

	Rest	Exercise	Early recovery
Sensitivity (%)			
B-point	77.7	54.2	64.8
Intersecting tangent	83.0	52.8	55.5
Maximum slope	84.3	57.8	69.3
Waveform peak	81.8	55.0	58.2
Positive predictive value (%)			
B-point	86.9	67.4	71.5
Intersecting tangent	97.7	81.1	79.0
Maximum slope	92.4	69.2	74.1
Waveform peak	99.1	84.7	82.7

Results are shown in Table 2.6. For clarity, exercise data are presented averaged over the three workloads. Statistical significance was determined by ANOVA, with post hoc analysis employing the Bonferroni correction for multiple comparisons. Over all three exercise intensities, there was a trend for sensitivity and positive predictive value of all time points to be less during strenuous exertion, but this was not statistically significant. However, overall sensitivity and positive predictive value of all time points were reduced during exercise relative to rest and recovery ($p<0.01$). Sensitivity of different time points did not vary significantly, either overall or by varying exercise intensity, but positive predictive value was better using the intersecting tangent and dZ/dt wave peak time points ($p<0.01$).

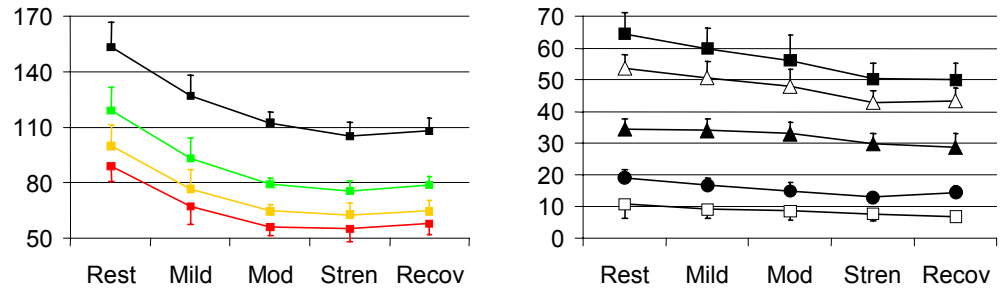
2.4.2.3 Relationship between different dZ/dt time-points

The relationship between the time points on the dZ/dt waveform, as measured by the off-line algorithm, was assessed using the data referred to in section 2.4.2.2. Results are shown in Figure 2.5. All bioimpedance time points decreased with exercise. The delay between all three pairs of adjacent time points decreased significantly over the exercise period although the maximum change was small (B-point to intersecting tangent: -4.1 ms, p=0.037 by ANOVA; intersecting tangent to maximum slope: -6.2 ms, p<0.001; maximum slope to dZ/dt peak: -10.9 ms, p=0.002). The time between the dZ/dt peak and the B-point and intersecting tangent time points decreased significantly with exercise (-14.4 ms, p<0.001 and -10.9 ms, p<0.001 respectively).

2.4.2.4 Comparison of PEP calculated by NCCOM3 and off-line algorithm

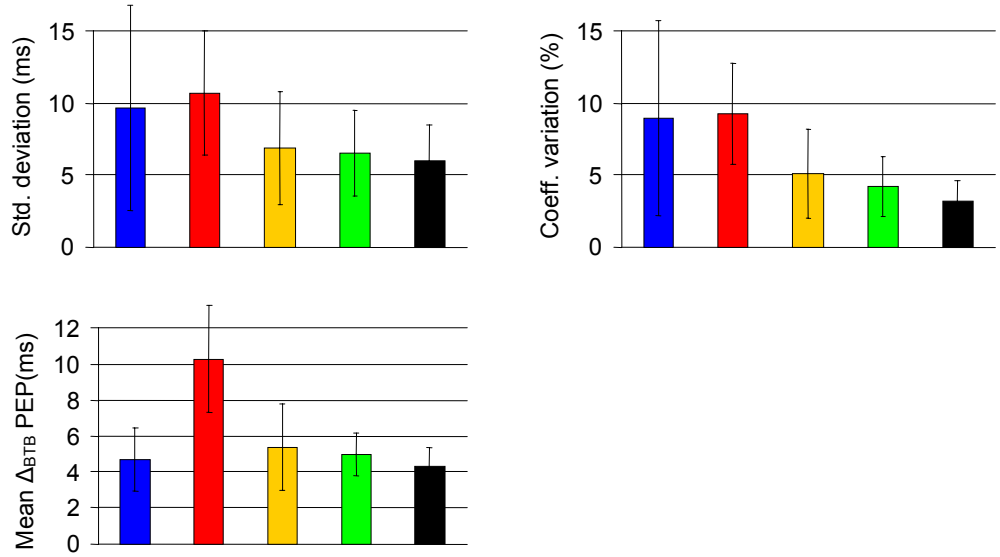
NCCOM3 PEP values were compared with those determined using the off-line dZ/dt analysis algorithm in 6 healthy individuals (4 male; median age 32 years). Resting ECG and bioimpedance waveform data were recorded simultaneously for 2 minutes with NCCOM3 PEP values. Mean values of PEP were calculated over this period. Waveforms were analysed from ECG Q-wave to the 4 time points on the bioimpedance waveform. Variability in PEP was assessed in terms of standard deviation (*SD*), coefficient of variation (*V*) and the mean beat-to-beat change (Δ_{BTB}).

Figure 2.5. *Relative timing of different dZ/dt points*



Y-axes = time in ms. Left: Change (mean±SD) in bioimpedance waveform measurements with exercise. ■ *true B-point;* ■ *intersecting tangent;* ■ *maximum slope;* ■ *dZ/dt peak. Right: Change (mean±SD) in delay between various bioimpedance waveform time points with exercise.* ■ *B-point and dZ/dt peak;* △ *intersecting tangent and dZ/dt peak;* ▲ *maximum slope and dZ/dt peak;* ● *intersecting tangent and maximum slope;* □ *B-point and intersecting tangent.*

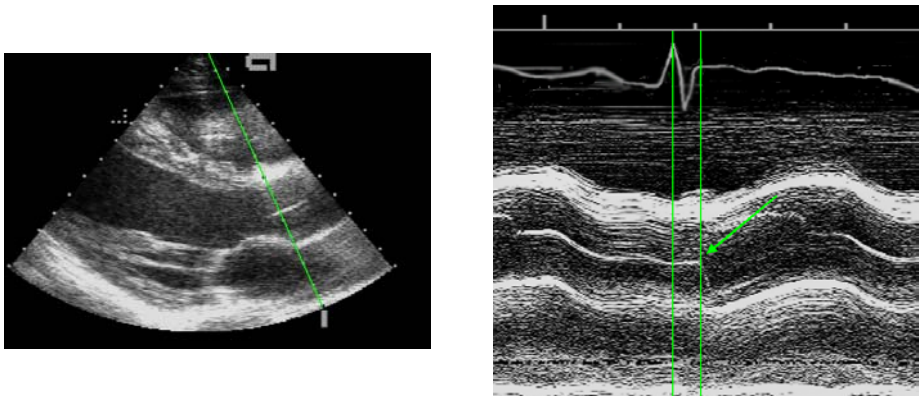
Figure 2.6. *Variability of different PEP measures*



■ *NCCOM3; waveforms:* ■ *true B-point;* ■ *intersecting tangent;* ■ *maximum slope;* ■ *dZ/dt peak.*

Results are shown in Figure 2.6. Mean values of NCCOM3 PEP were not significantly less than measures made using the waveform B-point (-5.1 ms, limits of agreement (2 SD) -20.9 to 9.0 ms, p=0.11), with other waveform measurements giving higher values (p<0.01) than the NCCOM3. No significant differences in *SD* and *V* were seen between NCCOM3 and the true B-point (p=0.47 and p=0.83 respectively). There was a trend for both *SD* and *V* to be lower for the other three waveform time points compared to the NCCOM3 PEP, and indeed this difference approached statistical significance for *V* (p=0.06, 0.06 and 0.05 for intersecting tangent, maximum slope and wave peak respectively). Δ_{BTB} was notably greater for values of PEP calculated to the true B-point on the waveform, than NCCOM3 (p<0.001), but for the other time points did not vary significantly from NCCOM3 values.

Figure 2.7. *Visual identification of PEP using echocardiography*



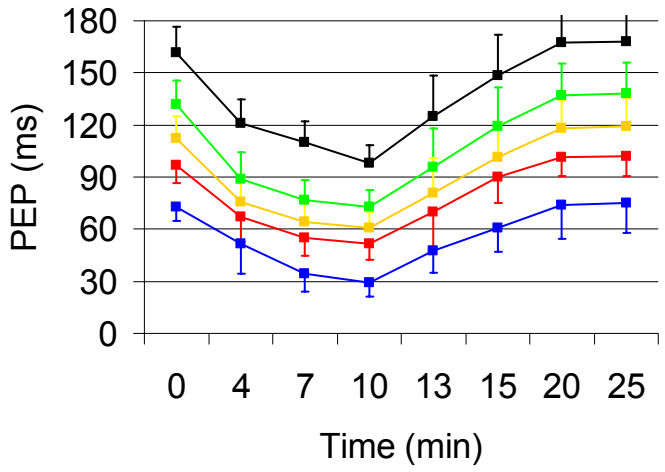
Left, parastermal 2-D long-axis view of aortic valve; right, M-mode view of aortic valve, with opening of valve shown by arrow.

2.4.2.5 Comparison of bioimpedance and echocardiography during exercise

Bioimpedance measurement of PEP was compared against measurements obtained by transthoracic echocardiography during exercise. 6 healthy males were studied (median age 22 years) at rest, at three points during exercise, and at four points (1, 4, 9, 14 minutes) following exercise. Exercise was based on a modified STEEP protocol (see section 2.9.1.2) with three 3-minute incremental workloads (median of 45, 90 and 180 watts) performed on a Kettler ergometer. Measurements were carried out in the last minute of each exercise phase.

Echocardiography was performed by a cardiologist using an Acuson 128XP/10c ultrasound device (Figure 2.7). Subjects continued pedalling throughout exercise measurements, and were encouraged to maintain trunk stability to improve image acquisition. Images of the aortic valve opening were captured in M-mode using the parasternal long-axis view, and recorded using a DT3152 MACH Series frame grabber (Data Translation, Inc.) with CVI Acquisition v1.5 software (Information Integrity, Inc.). PEP was measured manually from R-wave of ECG to the point of valve leaflet parting, by a single observer blinded to subject and experimental phase. The R-wave was considered easier to identify from the images than the Q-wave, and bioimpedance measurements were therefore also made relative to the R-wave. The timing resolution of echocardiography was 4 ms. 10 cardiac cycles were measured for each experimental time point.

Figure 2.8. *Change in PEP measures with exercise*



Time shows rest (0 min), three stages of exercise (peak at 10 min), and four points during recovery.
■ Echocardiography; waveforms: ■ true B-point;
■ intersecting tangent; ■ maximum slope; ■ dZ/dt peak.

Baseline heart rate was 80±12 bpm, increasing to 157±20 bpm at peak exercise, and remaining non-significantly elevated (88±20 bpm, $p=0.21$) at end of recovery. PEP results are shown in Figure 2.8. Echocardiogram measurements of PEP were significantly lower overall ($p<0.001$) than those made using the bioimpedance B-point (23±12 ms) and intersecting tangent (35±13 ms). There was no significant change in bias between echocardiogram and bioimpedance measurement points at different points during exercise or recovery ($p=0.60$ by ANOVA). There was a strong overall linear correlation between echocardiogram and bioimpedance intersecting tangent measures ($r=0.88$, $p<0.001$). A Bland-Altman plot (Bland & Altman 1986) comparing these two measures is given in Figure 2.9.

Figure 2.9. *Bland Altman plot comparing echocardiography and bioimpedance PEP*

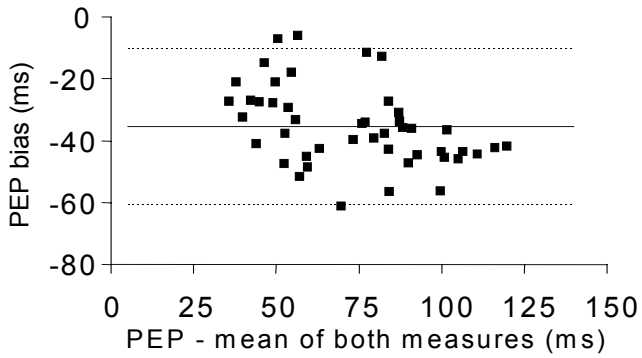


Chart compares echocardiographically determined PEP, with PEP determined using the intersecting tangent algorithm to determine the B-point on the bioimpedance dZ/dt wave. Lines show mean bias±1.96SD (95% limits of agreement).

2.4.3 Conclusion

Sensitivity for both the NCCOM3 built-in PEP measurements, and those obtained using the offline algorithm, were similar. The sensitivity of both systems became impaired during exercise. The positive predictive value for the NCCOM3 device remained relatively constant, but that of the offline analysis system decreased with exercise. Both systems showed similar variability in beat-to-beat measures, although this was reduced for certain bioimpedance timing points. The principal disadvantage of the NCCOM3 system was that it employed a separate ECG detection algorithm (i.e. the internal NCCOM3 system), and differences between the timing of the R-wave as determined by the NCCOM3 and detection by the separate custom algorithm, remained unknown. Accurate synchronisation of NCCOM3 and other beat-to-beat measurements was also technically difficult. The offline signal processing overcame these difficulties. It also demonstrated a reduction in variability, although positive predictive power during exercise was impaired.

Comparison of the different algorithms suggested relative constancy of the delay between different timing points. The intersecting tangent timing point was therefore selected as the value which came closest to the true B-point, whilst providing

satisfactory sensitivity and positive predictive value. This time point was therefore used for calculation of PEP in all other studies.

Validation of bioimpedance against echocardiography during exercise has not been hitherto described in the literature. A strong correlation was found between the two measures, although there was significant bias and wide limits of agreement. Part of this bias is due to the use of an intersecting tangent, rather than the "true" B-point. However, the bias may also have been affected due to uncertainty over the measurement delay between physiological ECG signal and display of the ECG waveform, and the delay between actual mechanical cardiac activity and display of the 1D M-mode ultrasound image data. Unfortunately the Acuson ultrasound device manufacturer was unable to provide this information, and although clinically unimportant, differences in these two delays may have nonetheless existed. The wide limits of agreement may also reflect inaccuracy in determining time-points on the echocardiogram images, as time resolution was limited to 4ms, and identification of the exact R-wave peak or point of valve separation on images could not always be made with single-pixel confidence. Regardless, bioimpedance showed similar changes in response to exercise to those seen with echocardiography. Furthermore, the relatively small measures of beat-to-beat variability with bioimpedance are consistent with satisfactory timing precision.

2.5 NON-INVASIVE ARTERIAL PRESSURE PULSE RECORDING AND ANALYSIS

2.5.1 Applanation tonometry

Applanation tonometry was used to non-invasively record the arterial pressure waveform. Applanation arterial tonometry was first described in 1963 (Pressman & Newgard). A superficial artery is flattened (but not occluded) against bone. The hold-down pressure required to applanate the vessel wall should be such that the pulse pressure measured by an overlying transducer is maximised. The circumferential tension is rendered negligible in the flattened segment of arterial wall, and thus the pressure perpendicular to this surface represents that within the arterial lumen. Accurate measurement of absolute pressure is not possible due to overlying soft

tissue, the thickness of the vessel wall, and the difficulty optimising hold-down pressure (particularly with a hand-held tonometer). The waveform is therefore usually calibrated using systolic/mean and diastolic BP values obtained by sphygmomanometry.

A hand-held high-fidelity micromanometer (SPT-301; Millar Instruments) coupled to a custom signal amplifier (similar to that used for the photoplethysmograph signal), was used to record the pulse waveform at peripheral sites, including the radial, brachial, carotid and femoral arteries. All tonometer measurements were made by a single, trained investigator.

An automated multi-array tonometer (CBM-7000; Colin Medical) was also used for continuous monitoring at the radial artery. This was used in accordance with the manufacturer's instructions. The device uses an array of 15 transducers, and automatically adjusts the position of the array over the artery to optimise signal strength. A wrist splint was used over the dorsal aspect of the wrist to minimise movement. The Colin device utilises an oscillometric cuff for pressure calibration, but this function was deactivated for all studies, as the signal was utilised for contour and timing analysis only (absolute pressure is irrelevant in this context) and cuff inflation can disrupt other distal contralateral arm measurements.

2.5.2 Wideband external pulse recording

Wideband external pulse (WEP) monitoring was first described in 1988 by Blank and colleagues as an alternative non-invasive technique for evaluating the arterial pressure pulse (Blank *et al*. 1988). By using a broad bandwidth sensor placed over the brachial artery under the distal edge of a sphygmomanometer cuff, they described changes in the externally recorded arterial waveform as a function of cuff pressure. They noted that, at suprasystolic cuff pressures, the resulting waveform exhibited a typical shape consisting of three peaks and two troughs, although the nature of these contour features was not examined further.

In Chapter 3, the suprasystolic WEP signal was recorded using two adjacent 1.5cm diameter piezoelectric sensors (frequency range 0.1 to >1000 Hz) placed beneath the

distal edge of a blood pressure cuff directly over the axis of the brachial artery (Pulsecor). The distal sensor was positioned centred 1cm from the cuff edge. No differences were subsequently found between proximal and distal sensors, and data are therefore only reported for the distal sensor. Measurements were made with the cuff temporarily inflated to 30mmHg above systolic pressure. The waveform was recorded at 200 Hz, thus band-limiting the signal, using software developed by Ilixir Ltd (Auckland, New Zealand).

2.5.3 Pulse wave analysis

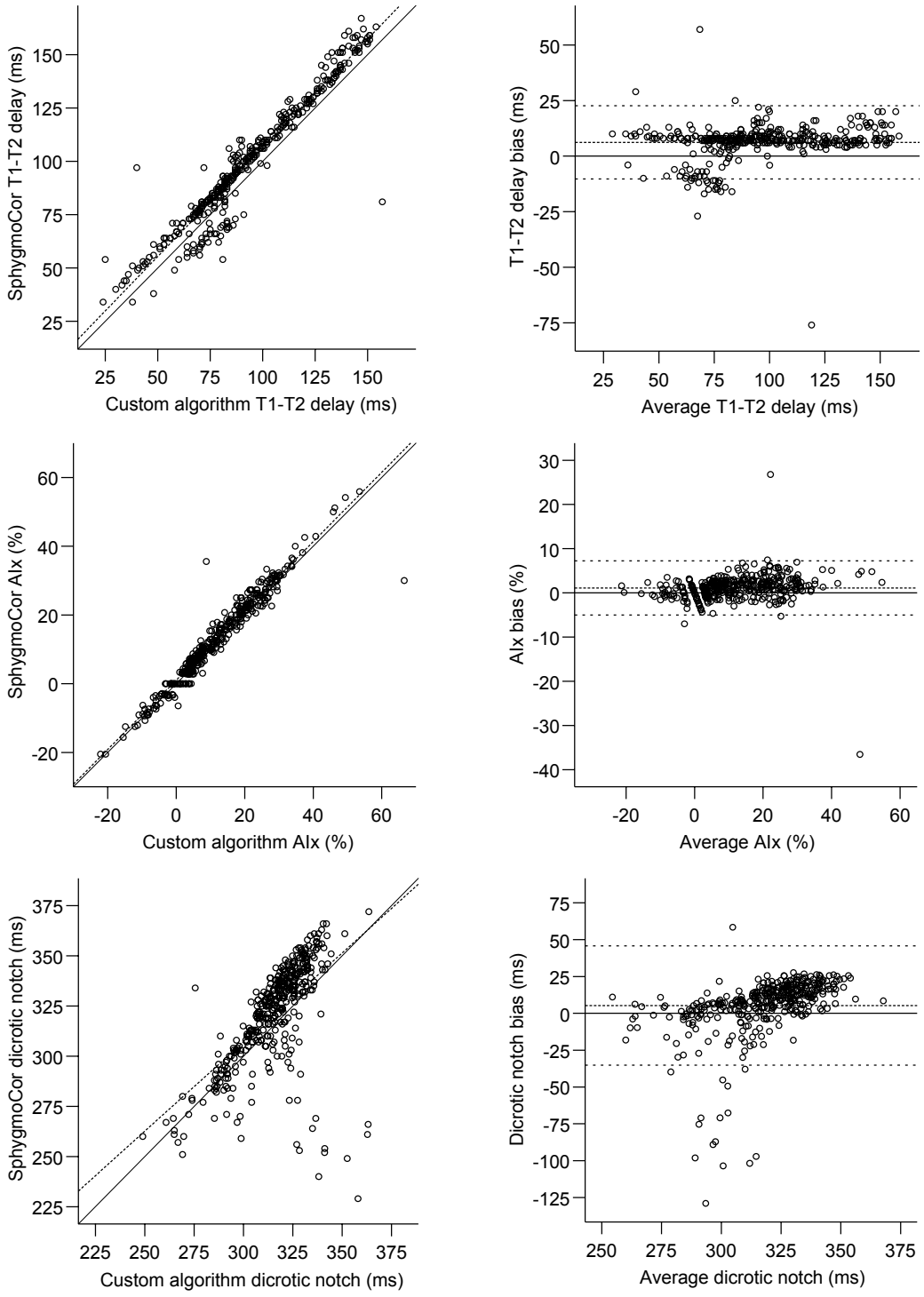
The contour of the pulse wave was analysed in the time domain by noting the timing and relative pressures of different inflections and turning points on the waveform in question. A minimum of 8 waveforms were ensemble averaged, after normalising for pressure but not for time. Timing of the different points was calculated relative to the start of the pulse, which was taken as the intersection of a tangent through the point of the maximum second derivative with the absolute pulse baseline. The overall difference between waveforms recorded at different sites was calculated by taking the root mean square error (RMSE) of the two signals, after synchronization using the offset between the peaks of the waves.

2.5.3.1 Arterial and photoplethysmograph pulse

The incident (P1) and reflected (P2) waves were identified on the peripheral pulse wave using the method described by Takazawa and colleagues, using the zero-crossing points of the fourth derivative (Takazawa *et al*. 1995). The dicrotic notch was identified as the first positive-to-negative zero crossing of the third derivative following the reflected wave. The hump in the diastolic portion of the wave was taken as the first positive-to-negative crossing of the first derivative, or in the absence of this point the first positive-to-negative crossing of the second derivative, following the dicrotic notch. Augmentation index was calculated as P2 expressed as a percentage of P1. Buckberg subendocardial viability index (SEVR) was calculated as the ratio of the area under the diastolic portion of the curve to the area under the systolic portion (Buckberg *et al*. 1972).

The custom algorithms used to determine P1, P2 and the dicrotic notch were compared against the unpublished algorithms used by the SphygmoCor system (AtCor Medical), by examining 400 waveforms analysed by the latter system. Waveforms had been recorded under a variety of circumstances, including administration of differing doses of GTN (Oliver *et al*. 2005), departmental validation work, and a student project examining the utility of pulse wave analysis in subjects with valvular heart disease (Neil Lachlan, University of Edinburgh, 2003). Comparisons were made of the delay between T1 and T2, augmentation index and time to dicrotic notch. There were strong correlations for measures of both T1-T2 delay and AIx ($R=0.96$ and 0.97 respectively, $p<0.001$). The correlation was somewhat less strong for dicrotic notch time ($R=0.60$, $p<0.001$). Mean bias (SphygmoCor value minus custom algorithm value) and 95% limits of agreement were +6.2ms (-10.3 to 22.7), +1.1% (-5.0 to 7.3) and +5.3ms (-35.2 to 45.8) respectively. Correlations and Bland-Altman plots are shown graphically in Figure 2.10. Similarity of AIx values was considered particularly good; the distinctive group of SphygmoCor AIx values of zero, reflects the rounding of exported AIx values performed by SphygmoCor. Of note, there was a small subset of T1-T2 values that were overestimated by the custom algorithm around 60-80ms. There was also a subset of dicrotic notch values which were overestimated by the custom algorithm, ranging over 275 to 375 ms. The clustering of outlying T1-T2 values is suggestive of a subtle difference in algorithm, perhaps due to cut-off limits employed the different systems. In contrast, the more variable nature of the outlying dicrotic notch values probably points towards the use of completely different algorithms, although may also reflect the difficulty often experienced trying to identify this point (Oppenheim & Sittig 1995). Given the lack of a "gold-standard" for identification of waveform features, the similarities between the custom system and a widely used commercial system were considered satisfactory to permit its use in subsequent studies.

Figure 2.10. *Comparison of pulse wave analysis systems*



Comparison of custom algorithm and SphygmoCor system for determining T1-T2 delay, AIx and time to dicrotic notch. Left hand panels show correlation, solid line is line of identity, broken line is linear regression. Right hand panels show Bland-Altman comparison, bias is SphygmoCor values minus custom algorithm values, broken lines show mean bias and 95% limits of agreement.

2.5.3.2 Wideband external pulse (WEP)

The WEP signal has been noted by others to have 3 principal waves (S1, S2, S3) (Blank *et al*. 1988). Preliminary visual inspection found the WEP signal to resemble the derivative of arterial pressure. The original WEP waveform (WEP_S) was therefore integrated to provide a derived pressure waveform (WEP_A), which was analysed as described above. The turning and inflection points on WEP_S were identified from the zero crossing points of the first through to third derivatives of this signal, in a similar manner to that described for arterial waveforms. The timing (T) and pressure (P) was noted at all 3 corresponding points, in addition to the trough between S1 and S2. The Pulsecor system also estimates compliance (expressed in mmHg/ml) from a first-order linear equation (Blank 1996), based on a natural logarithm of the ratio of amplitudes of P_{S1} and P_{S2} on the WEP_S waveform, but does not use measures of flow or volume.

2.5.3.3 Fourier analysis and transfer functions

The pulse signal was also examined in the frequency domain using Fourier analysis. A 10 second rectangular data window was analyzed to provide 0.1Hz frequency bins. Transfer functions (section 1.4.3) were computed by dividing the real fast Fourier transform of the response signal by that of the stimulus signal (strictly speaking an inverse transfer function) (Cerna & Harvey 2000):

$$H_{(stimulus \rightarrow response)} = P_{stimulus}(\omega) / P_{response}(\omega)$$

where $P(\omega)$ is the frequency domain of a complex harmonic signal, ω is the angular frequency, and a sinusoidal component is represented by $P=|x|e^{j\varphi}$, where $|x|$ is amplitude and φ is phase. Generalized transfer functions were computed by averaging the separate phase and gain components of the relevant individual transfer functions over 0 to 20 Hz. Prior to computing transfer functions, corresponding signals were normalised for amplitude with respect to one another, and synchronised at the maximal point of the first derivative of the wave-front to remove the phase shift due to the pulse transit time. For the work described in Chapter 3, signals were normalised to the same amplitude, as the pulse contour was of interest, rather than

ability to predict absolute blood pressure. For exercise work in Chapter 6, all signals were normalised to radial diastolic and mean pressure, as estimation of central systolic BP was of interest. In addition, a further radial-to-carotid GTF was computed in a similar manner from waveform data obtained using the commercially available SphygmoCor (AtCor Medical) system, by multiplying together the radial-to-aortic and aortic-to-carotid transfer functions employed by this device, so that

$$H_{(radial \rightarrow carotid)} = [P_{radial}(\omega)/P_{aortic}(\omega)] \times [P_{aortic}(\omega)/P_{carotid}(\omega)] = P_{radial}(\omega)/P_{carotid}(\omega)$$

As the derived aortic waveforms are a pure mathematical function of the measured peripheral signal, this allowed for an accurate reproduction of the two SphygmoCor transfer functions, relatively independently of the quality of signal recording.

2.6 MEASUREMENT DEVICE SIGNAL DELAY

The analogue signal processing that occurs in the majority of these medical devices, introduces a time delay between the physiological input signal, and the electronic analogue output signal which is digitised and recorded. This delay can vary between a few milliseconds and up to 70 ms, depending on the device, and it is therefore important to take this delay into account when determining the time difference between two waveforms recorded using different devices.

2.6.1 LifePulse ECG

The LifePulse LP15A signal delay was determined by connecting the device to a simulated ECG signal, and the delay measured using the software developed for transit time measurement. The mean delay was 5.7±0.4 ms. between the R wave peaks. The delay between Q wave nadirs was fractionally shorter at 5.2±0.5 ms. The delay between S wave nadirs was notably longer at 9.4±0.5 ms due to the time constant of the electronics. It was felt that it was therefore most appropriate to use the delay between R waves, as this was considered the most reliable timing point.

2.6.2 Photoplethysmograph and Millar tonometer amplifier

The photoplethysmograph and Millar tonometer amplifier delay was determined by the device manufacturer by passing a simulated pulse waveform to the device. The reported delay between the maximal slope of the input and output waveforms was found to be 48 ms.

2.6.3 NCCOM3 dZ/dt waveform

The NCCOM3 signal delay was determined by measuring the impedance across a specially designed electronic circuit. This circuit had a baseline resistance of 90Ω, which was increased by ~10Ω for a 200 ms period every second. A simulated 0-5V square wave was output simultaneously with the change in resistance. The delay between the leading edges of the simulator signal and the measured NCCOM3 impedance output signal was 7.5±0.7 ms.

2.6.4 Colin tonometer

It was not possible to pass a simulated signal to the Colin device in order to measure the input-output delay. The delay was therefore measured relative to the Millar tonometer using two approaches. Firstly, sequential measurements of the radial pulse, at exactly the same point on the skin surface, were made in a single individual, gated to the ECG R-wave. The foot-to-foot difference between wavefronts was determined using an intersecting tangent through the maximal wave slope, and the mean difference from the Millar tonometer found to be -6.9 ms. The second method involved simultaneously recording the Colin and Millar signals, with the Millar tonometer sequentially placed immediately proximal and immediately distal to the Colin transducer housing. The time delay between Colin and Millar signals was averaged for distal and proximal measurements, and found to be -8.8 ms. The average signal delay through the Colin device was thus estimated as 40.0 ms.

2.7 PULSE WAVE VELOCITY CALCULATION

Pulse wave velocity requires the pulse transit time and distance between two anatomical sites to be known. For all studies, values of transit time were based on

timing determined by using an intersecting tangent algorithm, except where stated otherwise. The intersecting tangent method identifies the wave start as the point at which a tangent through the steepest point of the wave front (maximum first derivative) intersects a horizontal baseline through the absolute minimum of the wave (Chiu *et al*. 1991).

Measurements requiring use of the Millar tonometer at two distinct anatomical sites were made sequentially, with timing gated to the ECG R-wave. A minimum of 10 seconds of data was used for sequential measurements. For velocity measurements made between different transducers (e.g. Millar to Colin, Colin to photoplethysmograph, etc.), simultaneous waveforms were used.

Distance was measured using a tape measure, along a straight line between sternal notch and waveform recording site. Measurements to points on the arm were made with the elbow and wrist straight, and the shoulder abducted at 90° with no extension or flexion. The sternal notch was used as the reference point for bioimpedance timing measurements. Ear measurements were made in a straight line to the ear lobe. Distance between two sites was taken as the difference in the two measurements from the sternal notch.

2.8 BLOOD PRESSURE MEASUREMENT

2.8.1 Manual sphygmomanometer

All manual sphygmomanometer blood pressure readings were made by the same experienced investigator for any particular study. Measurements were made using a calibrated mercury sphygmomanometer, in accordance with the guidelines laid down by the European Society of Hypertension (O'Brien *et al*. 2003). Blood pressure was read to the nearest 2mmHg, with diastolic BP taken as Korotkov phase V (disappearance), or as Korotkov phase IV (muffling) if phase V was indeterminate. In general, measurements were completed within 30 seconds of commencing cuff inflation. A manual sphygmomanometer was used in preference to an automated device, to improve reliability particularly during maximal exercise.

2.8.1.1 Reproducibility

5 blood pressure measurements were made every minute at rest in 20 healthy individuals. Average BP was 125/79mmHg. The average standard deviation of these five systolic and diastolic measurements were 3.9mmHg and 3.1mmHg respectively. Average coefficient of variation was 3.1% for systolic BP and 4.0% for diastolic BP.

2.8.1.2 Rounding of BP

98% of systolic BP values and 100% of diastolic BP values (from data in section 2.8.1.1) were found to end in an even digit, consistent with rounding of measurements to the nearest value adjacent to the mercury column. There was a tendency for a greater number of measurements than expected to finish in 0 (28% systolic, 40% diastolic; $p<0.01$ by Chi-Square test), and a lesser number to finish with 2 (12% systolic, 7% diastolic; $p<0.01$), suggesting a degree of bias toward rounding down (but not up) to a multiple of 10.

2.8.2 Automated sphygmomanometer

For studies where BP measurements were not required during exercise, and where a manual sphygmomanometer was not used, BP was recorded at the left brachial artery using a validated oscillometric sphygmomanometer (HEM705, Omron Healthcare).

2.8.3 Intra-arterial cannula

Intra-arterial pressure monitoring was carried out for all BP measurements described in Chapter 3, and for a single study as described in Chapter 7. A 20G 80-mm Vygon catheter was inserted under local anaesthesia (1% lidocaine) into the non-dominant radial artery, using the Seldinger technique. A splint was used to minimise wrist movement. The cannula was connected by fluid-filled (0.9% saline) semi-rigid tubing to a TruWave disposable transducer (Edwards Life Sciences) positioned level with the right atrium and connected to a Diascope 2 monitor (S & W Medical). The intra-arterial pulse was not used for calculation of transit time, as a significant delay was introduced by the use of a fluid filled catheter, and the constancy of this delay was difficult to ascertain.

Transducers were factory calibrated and exceeded AAMI standards for performance interchangeability, with a sensitivity of 5 $\mu V \cdot V^{-1} \cdot mmHg^{-1}$ ±1% and nonlinearity of the greater of ±1.5% or ±1mmHg. The natural frequency of the system was 40 Hz. A custom 12 bit 200 Hz digitiser was used to sample the analogue monitor output. Systolic BP and diastolic BP were taken as the maximum and minimum values of the waveform corresponding to the last measured R-wave, with mean pressure calculated as the integrated average over the corresponding pulse cycle.

2.8.4 Portapres

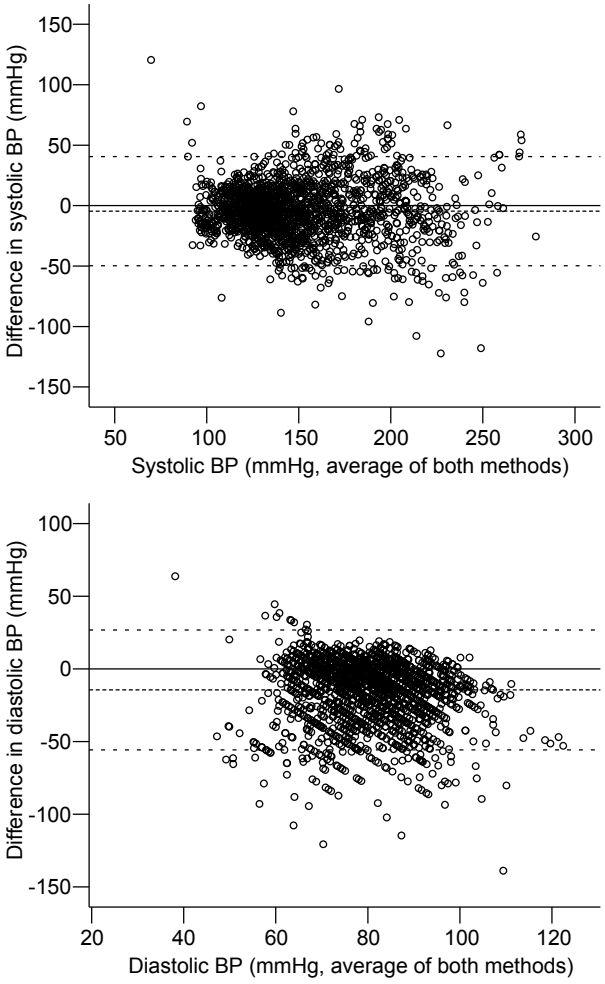
Non-invasive continuous finger blood pressure was measured using a Portapres device (TNO Biomedical Instrumentation). The technique of arterial volume clamping was originally described by the Czech physiologist Peñáz (1973). An inflatable cuff with attached infrared photoplethysmograph is applied to the finger. The pressure in the cuff is rapidly adjusted to compensate for changes in intra-arterial pressure. The photoplethysmograph is used to assess the finger arterial volume. This volume clamps the artery at a set point equivalent to two-thirds of the maximal arterial volume, thus resulting in zero transmural pressure. The cuff pressure therefore reflects absolute finger arterial blood pressure. A mechanism is provided to compensate for the hydrostatic effects of raising and lowering the arm. Only a single finger cuff was used (rather than switching repeatedly between dual cuffs), sized and positioned, on either the middle or ring finger, in accordance with the manufacturers instructions. The Portapres waveform analogue output was continuously recorded, and systolic, diastolic and mean pressure determined as for intra-arterial pressure.

2.8.4.1 Comparison with manual sphygmomanometry

Sphymomanometry has previously been compared with Portapres measurements during exercise. The main purpose of Portapres measurements was for assessment of BP variability, and a prospective comparison of the two methodologies was therefore not repeated. A retrospective analysis was performed, however, using measurements taken during the studies described in Chapter 7. In 2040 measurements made in healthy individuals before, during and after exercise, Portapres gave higher values of both systolic (4.6±23mmHg, $p<0.01$) and diastolic (14.5±21mmHg) pressure than

manual sphygmomanometer measurements (Figure 2.11). It is clear from the standard deviation of the difference, that limits of agreement between the measures were marked. The difference in diastolic measurements using the two techniques was skewed towards higher measures using the Portapres, and bias tended to become greater at higher pressures (Pearson coefficient -0.12 for mean BP versus difference in BP, $p<0.001$). These observations were not noted for systolic pressure.

Figure 2.11. *Bland-Altman plots comparing manual sphygmomanometry and Portapres*



Left chart, systolic BP; right chart, diastolic BP. Charts show mean bias±1.96SD (95% limits of agreement). Bias is difference relative to manual sphygmomanometry.

2.9 EXERCISE TESTING

Cycle exercise was performed in the semi-recumbent position. Subjects rested both arms on cushioned surfaces to either side of the ergometer to minimise movement, with elbows flexed and forearms horizontal. Pedals with straps were used for all studies. Three different ergometers were used for different studies – Comfort Cycle JPB 2000 (Johnson, UK), Kettler SX1 (Heinz Kettler GmbH & Co., Germany), and Lode Rehcor (Lode BV, Netherlands) with modification to enable pedalling in a semi-recumbent position (Figure 2.12).

Figure 2.12. *Modified Lode Rehcor cycle ergometer*

2.9.1 Exercise Protocols

Different exercise protocols were used depending on the study aims. At the end of exercise, pedalling was stopped completely with no "cool down" period, to improve the reproducibility of the transit time and blood pressure recovery slopes. Where unknown, the work/VO_2 slope was assumed to be 10 $mlO_2 \cdot min^{-1} \cdot W^{-1}$ (Wasserman *et al*. 2004), and physiological parameters were predicted from the following equations:

- Maximum heart rate = 220-a

where *a* is age in years.

- $VO_{2\text{-}MAX} = ((K1-(a\times K2)) \times (K3+A)) + B$

where *a* is age in years; *A* is predicted weight based on (h×K4)-K5), or average of predicted and actual weight if the latter is smaller; *B* is 6 × difference between predicted and actual weight, unless latter is smaller in which case *B*=0; *K1* to *K5* are 22.78, 0.17, 43.0, 0.65 and 42.8 respectively for females, or 50.72, 0.372, 0.0, 0.79 and 60.7 respectively for males (Wasserman *et al*. 2004).

- $VO_{2\text{-}AT} = VO_{2\text{-}MAX} \times (K1 + a \times K2)$

where *a* is age; *K1* is 0.3613 (female) or 0.400 (male); *K2* is 0.0026 (female) or 0.001 (male).

2.9.1.1 Maximal exercise protocol

Workload was increased in a linear fashion every 1 or 3 minutes, depending on the study. Increments were either every 3 minutes based on 15% of the predicted $VO_{2\text{-}MAX}$, or every minute based on the formula described by Wasserman, $Work_{watts/min}=((h\times s)-(150+(6\times w)))/100$, where *h* is height (cm), *w* is weight (kg), and *s* is either 12 (female) or 20 (male) (Wasserman *et al*. 2004). Workload increments were rounded to the nearest 5W in both cases. Exercise was continued to exhaustion, with subjects verbally encouraged to persevere as long as possible.

2.9.1.2 Modified STEEP protocol

The Standardised Exponential Exercise Protocol (STEEP) protocol was described by Northridge *et al* and uses exponential rather than linear workload increments (Northridge *et al*. 1990). It has the advantage of achieving a relatively wide range of workloads in a short time period, and is standardised for weight. The original bicycle STEEP protocol started at 2 METs and incremented workload by 15% of the previous stage every minute. (MET refers to metabolic equivalent, where 1 MET is equal to basal metabolic rate; estimation of corresponding workloads can be made

from standard equations). A modified protocol was used, where workload was only incremented every 3 minutes, but to levels based on a 25% increment every minute. This allowed measurements to be made in the last minute of every 3 minute period, once the exercise response had reached a plateau.

2.9.1.3 Heart-rate targeted protocol

Stages were based on a percentage of the predicted maximum heart rate (from the equation 220 minus age in years). Workload was incremented gradually (10W every 30s) until the target heart rate was achieved. This protocol has the advantage that assumptions about VO_2-MAX do not need to be made and subject's fitness has less impact on exercise duration.

2.9.1.4 Sub-maximal exercise

A 1 minute incremental protocol was used, as described in section 2.9.1.1, until workload corresponding to the anaerobic threshold was reached. The workload was maintained at this fixed value until the end of exercise, after which pedalling was stopped completely. Anaerobic threshold was either defined by previous maximal stress test, or predicted from the equation above.

2.9.2 Expired gas analysis

Expired breath gas analysis was performed using an Msx ErgoSpirometer System (Morgan Medical, UK). This device uses a continuous on-line quadrupole mass spectrometer to measure respiratory gas concentrations. The gas sampling rate is 30 ml/min with 50 Hz temporal resolution. The system has <1% stability per 24 hours and linearity <1%. The detection limit is 100 ppm and the accuracy ±0.2% for O_2 and CO_2. The gas delay time (i.e. the period between an instantaneous flow/volume event, and the corresponding gas analysis event) is fixed at 480 ms. The respiratory flow rate is measured using a turbine device connected to the end of a mouthpiece, and has a sensitivity of 2.2 ml per airscrew revolution.

The system was allowed to warm up for at least 2 hours prior to use. Calibration was two-way in accordance with the manufacturer's recommendations, and carried out

prior to each test. Turbine calibration was performed using a standard 3 litre syringe with 3 inspiratory and expiratory cycles, and considered acceptable if within the range ±20 ml of the reference standard. Gas calibration was performed by attaching the system to a mixed gas bottle (14.99% O_2, 5.02% CO_2, 4.99% Ar, balance N_2) and initiating the automatic calibration program. The delay time was in-built and not recalibrated.

Subjects breathed through a mouth-piece and wore a nose clip for the duration of exercise. Measurements were made at rest for 5 minutes immediately prior to and subsequent to pedalling.

Breath-by-breath data was centre-averaged over 8 breaths. $VO_{2\text{-}MAX}$ was taken as the peak value achieved during exercise. The V-slope method was used to determine the anaerobic threshold (AT) as previously described (Beaver *et al*. 1986). Briefly, the respiratory compensation (RC) point was first calculated by identifying the point which maximised the difference (at least 15%) between the linear regression slopes on either side of it on the VE/VCO_2 correlation plot. The RC point was taken as the intersection between these regression lines. The AT was then identified in a similar manner on the VCO_2/VO_2 correlation plot, but excluding data lying beyond the RC point. Where AT could not be identified using the V-slope method using plots of VE/VCO_2, VE/VO_2, RER, and end tidal CO_2 and O_2 as previously described (Wasserman 1984). $VO_{2\text{-}AT}$ was defined as oxygen uptake at the point at which AT was reached. A simple recovery model was calculated by calculating the mean response time (MRT) by use of a Levenberg-Marquardt algorithm to find the least squares set of coefficients of the best fit exponential decay curve, $VO_{2\text{-}T}=VO_{2\text{-}BL} -\Delta VO_{2\text{-}SS}\times[1-e^{-(T-\delta)/\tau}]$, where *T* represents time, *BL* is baseline, *SS* is steady state amplitude, δ is the delay and τ is the time constant, and $MRT=\delta+\tau$.

Transit time and other beat-to-beat data was matched to breath data by averaging over the corresponding breath period.

2.10 DRUGS

For the study described in Chapter 3, all drugs were made up after insertion of the arterial cannula. All drugs were administered via a 20G intravenous cannula sited in the antecubital fossa of the dominant arm. The rate of infusion was kept constant at 1 ml/min. All drugs were administered in 3 consecutive doses of 5 minutes each (total 15 minutes), with a 25 minute washout period following each drug. Drugs given were glyceryl trinitrate (GTN; Nitrocine, Schwarz; 0.1, 1, 4μg/kg/min), angiotensin II (Clinalfa; 2, 6, 12ng/kg/min), noradrenaline (Levophed, Abbott; 20, 60, 120ng/kg/min) and salbutamol (Ventolin, Allen and Hanburys; 0.4, 1.2, 2.4μg/kg/min). Drug order was not randomised. Salbutamol was given last due to its relatively long half-life. Dose ranges and washout periods were based on previous studies, and selected for their anticipated effects on BP and heart rate. GTN was selected for anticipated decreases in BP (Hargreaves & Muir 1992; Jiang *et al*. 2002). Noradrenaline and angiotensin II increase mean arterial pressure (MAP) to a similar degree, but the former also increases peripheral pulse pressure (Ramsay *et al*. 1992; Wilkinson *et al*. 2001). The expected response to salbutamol was a fall in diastolic pressure (DBP) and rise in systolic pressure (SBP) and HR (Gibson & Coltart 1971; Yacoub & Boyland 1973).

For the pilot work described in Chapter 4, GTN (Nitrocine, Schwarz) was diluted in water to give 50μg in 50μL. This was administered sublingually using a pipette. Dose was based on work previously conducted in the University of Edinburgh Clinical Pharmacology Unit (JJ Oliver, personal communication), which demonstrated that the selected dose of GTN would have minimal effects on blood pressure and conduit and central artery PWV, despite substantially reducing augmentation index (Oliver *et al*. 2005).

2.11 DATA ANALYSIS

Statistical analyses are described in detail in relevant chapters. SPSS (v12.5 and v14.0, SPSS Inc.) was used for most statistical analyses and graphing. Microsoft Excel (v9.0, Microsoft Corp.) was used for some statistical analyses (some t-tests and descriptive statistics) and for graphing data.

Data are generally presented in terms of mean ± standard deviation when describing the study population, and mean ± standard error when describing the accuracy of the mean. Evaluation of two comparable variables (e.g. actual and derived BP) is in terms of mean bias and limits of agreement, as described by Bland and Altman (1986). Comparison of different variables is generally by linear regression. Where beat-to-beat data are compared with single measurements of another variable (e.g. manual BP), the former is centre-averaged around the same time point. Assessment across multiple time points is by repeated measures analysis of variance (rmANOVA), with paired t-tests for post-hoc analysis where appropriate. Summary measures (e.g. maximum change from baseline), as described by Matthews *et al* (1990), are also used where appropriate, with comparisons using t-tests.

3 EFFECT OF VASOACTIVE DRUGS ON PULSE TRANSIT TIME AND THE PULSE WAVEFORM

3.1 INTRODUCTION

Beat-to-beat estimation of blood pressure using pulse transit time has many potential applications. One particular of area of value would be the assessment of rapid blood pressure changes in response to cardiovascular drugs, both in the clinical environment such as intensive care, and in a research setting. Furthermore, more widespread clinical use would involve measurements in patients taking medications for chronic cardiovascular conditions, such as hypertension, angina or heart failure. An understanding of the effects of drugs on the relationship between blood pressure and transit time is therefore essential. To date, little work has been published on the effects of vasoactive drugs on this measurement in humans. In particular, studies have not been carried out quantifying PEP or comparing rPTT with invasive BP measurement. This study used various vasoactive drugs to produce differing cardiac and vascular responses. The rationale was to compare changes in transit time measurements with the clinical "gold standard" for BP measurement, over a wide BP range and under different conditions of vascular tone and cardiac contractility.

As a consequence of pulse recording for transit time calculation, it is also possible to assess the pulse wave contour. The finger pulse wave (Millasseau *et al*. 2006) and blood pressure cuff, the latter which can be adapted for WEP recordings (Blank *et al*. 1988), are far more widely available in clinical practice than intra-arterial monitoring or non-invasive tonometry. These methodologies therefore provide an attractive means of recording the arterial pulse wave. As an extension of the transit time study, it was decided to investigate the utility of finger pulse and WEP recording as a means of evaluating the pulse waveform following cardiovascular drug administration.

It was hypothesised that the relationship between intra-arterial BP and rPTT would vary following the administration of different vasoactive drugs, due to differing effects on the vascular and cardiac components of rPTT, making rPTT an unreliable predictor of BP.

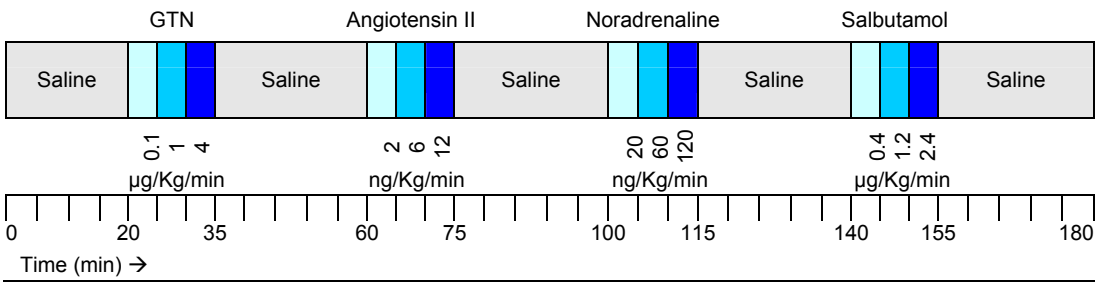
It was also hypothesised that the features of the suprasystolic WEP signal would be closely related to the shape of the invasively measured arterial pressure pulse. Furthermore, it was hypothesised that drugs would induce changes in the photoplethysmograph finger pulse, but also alter nature of the relationship between the finger and intra-arterial radial pulse.

3.2 METHODOLOGY

Studies were carried out in healthy men, aged 18 to 25, and performed in a quiet, temperature controlled (22±2°C) environment, after at least 1 hour of acclimatisation. Subjects were allowed a light breakfast not less than 4 hours prior to attending, and were requested to refrain from alcohol, caffeine, nicotine or medications for the preceding 24 hours. Studies were conducted with the subject lying supine.

Four drugs were administered as described in section 2.10. The experimental time course is shown in Figure 3.1. Drug order was not randomised, and doses and washout periods were based on published literature to produce consistent and predictable changes in blood pressure and arterial tone: a decrease in blood pressure was anticipated with GTN (Hargreaves & Muir 1992; Jiang *et al*. 2002); noradrenaline and angiotensin II were both expected to increase mean pressure, but the former was expected to cause a greater increase in pulse pressure (Ramsay *et al*. 1992; Wilkinson *et al*. 2001); salbutamol was predicted to cause a decrease in diastolic pressure due to peripheral vasodilatation, and a marked tachycardia and increase in systolic pressure due to positive chronotropic and inotropic effect (Gibson & Coltart 1971; Yacoub & Boyland 1973).

Figure 3.1. *Experimental time course*



Continuous real-time beat-to-beat measurements of intra-arterial BP (section 2.8.3), finger transit time (sections 2.3.1.1 and 2.3.2.1) and PEP (section 2.4.1.1) were made throughout the entire study protocol. Pulse wave velocity was calculated from the $pPTT_{finger}$ ($rPTT_{finger}$ minus PEP). Pulse waveform measurements, including Millar tonometry measurements, were recorded in the last 30 seconds of each experimental phase. Wide-band external pulse (WEP) measurements were made immediately before each drug and at the end of the highest dose (WEP measurements were discarded in one subject due to technical problems).

For beat-to-beat parameters, baseline was considered to be the 2 minutes immediately prior to each infusion period. Changes from baseline, and differences between each drug baseline, were assessed by analysis of variance (ANOVA). The relationships between BP and different measures of transit time were evaluated by linear regression. Regression slopes and intercepts, and Z-transformed Pearson correlation coefficients were compared for each drug infusion and washout period using repeated measures ANOVA. Beat-to-beat variability was assessed for SBP, rPTT and HR. Power spectra were calculated using a smoothed Lomb periodogram for all three variables for each individual drug dose. Coherence is analogous to correlation coefficient in the time domain, ranging from 0 (no coherence) to 1, and was computed over the frequency ranges 0.05Hz to 0.2Hz, and 0.2Hz to 0.4Hz. Comparison of coherence values was made by ANOVA.

As discussed in section 2.5.3.2, preliminary visual inspection of the waveforms revealed the WEP signal (WEP_S) to resemble the first derivative of arterial pressure (ART_S), and the WEP signal was thus mathematically integrated (WEP_A) for comparison with the directly recorded arterial pressure wave (ART_A). Equivalent timing and pressure parameters obtained by the two methodologies were compared by regression analysis (i.e. WEP_A with ART_A; WEP_S with ART_S). Bias for these measures was assessed by the Bland and Altman method (Bland & Altman 1986). Linear regression correlation coefficients between the difference and mean of both measures were computed to evaluate trends in bias over the measurement range. The overall difference between waveforms was assessed by root mean square error (RMSE).

PPG-to-radial transfer functions were computed as described in section 2.5.3.3. Individualised transfer functions (ITFs) were computed for each of the 13 experimental time points for each individual, and averaged to provide 13 dose specific, 4 drug specific, and 1 single overall generalised transfer functions (GTF_{dose}, GTF_{drug} and GTF_{all} respectively).

3.3 RESULTS

12 healthy, non-smoking men were studied. Subject characteristics are given in Table 3.1. All took regular non-competitive exercise. All were normotensive, with normal 12-lead ECGs, lipid profiles, serum biochemistry and blood count. Maximal change from baseline in transit time and blood pressure are given in Table 3.2, with dose response plotted in Figure 3.2. Changes in wave parameters are given in Table 3.3.

Table 3.1. *Subject characteristics (N=12)*

Age (years)	22 ± 1.7
Height (cm)	178 ± 6
BMI (kg/m^2)	23.6 ± 1.9
Resting brachial BP (mmHg)	126/75 ± 10.8/7.7
Heart rate (bpm)	63 ± 7
Total cholesterol (mmol/L)	4.1 ± 0.6
HDL cholesterol (mmol/L)	1.4 ± 0.3

Values are mean ± standard deviation or N(%).

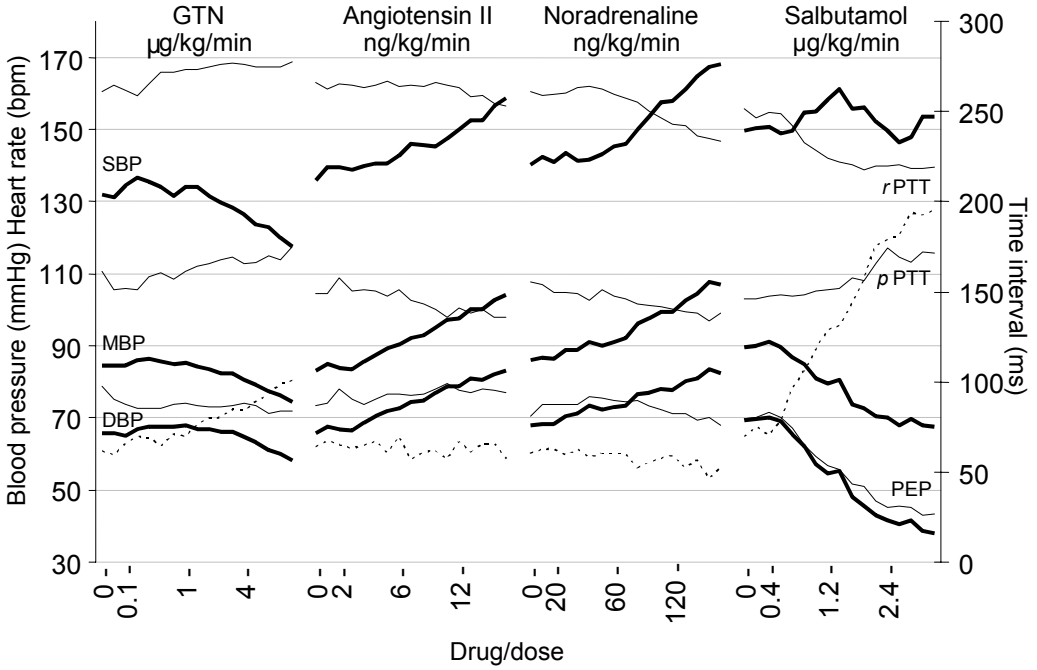
3.3.1 Transit time and blood pressure

3.3.1.1 Drug effect on transit time and blood pressure

GTN caused an increase in rPTT, pPTT and HR, and decreases in SBP, DBP and MAP. Angiotensin II and noradrenaline caused increases in SBP, DBP and MAP, and decreases in rPTT and pPTT. SBP and pulse pressure increases tended to be greater with noradrenaline than angiotensin II, but this difference was not statistically significant (p=0.11). The PEP and HR responses were variable between subjects, but overall both decreased with noradrenaline and did not change with angiotensin II. Despite the similar change in BP, decreases in PEP and rPTT were significantly greater with noradrenaline than with angiotensin II (p=0.005 and p=0.002 respectively). Salbutamol reduced DBP, MAP, rPTT and PEP, and increased HR and pPTT. The SBP response was varied, and overall, did not significantly change; 8

subjects had a significant increase in SBP, whereas 4 had a clear decrease. Baseline values of SBP, DBP, MAP and HR were not constant between drug phases ($p<0.05$ by ANOVA), due in particular to increases in all four parameters prior to salbutamol administration. This was mirrored by decreases in rPTT, pPTT and PEP.

Figure 3.2. *Blood pressure, heart rate and transit time responses*



Mean dose response graphs for BP (thick line), heart rate (broken line), pulse transit time and PEP (thin lines). Data averaged over 60s intervals for clarity.

3.3.1.2 Correlation between transit time and blood pressure

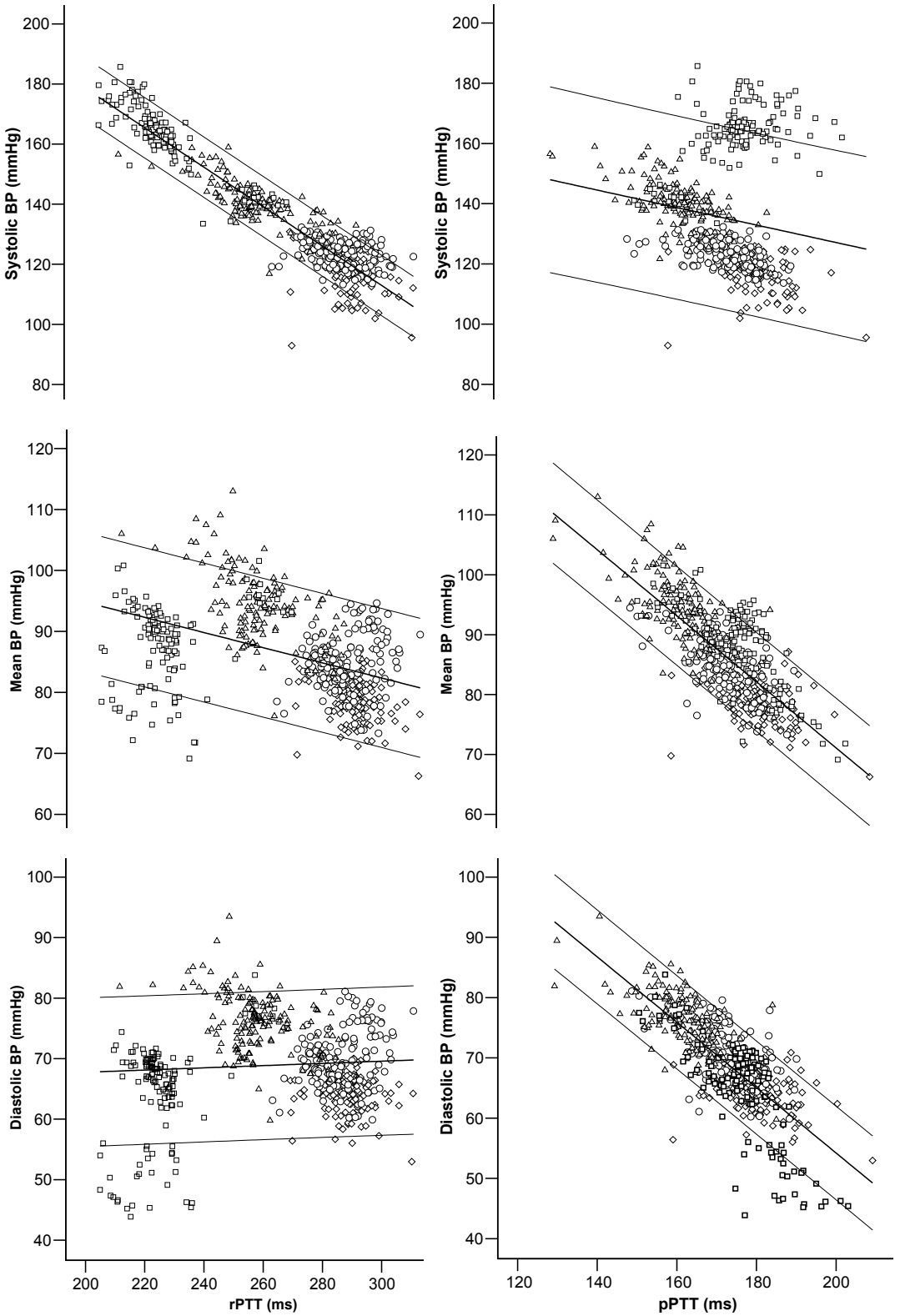
Correlations between BP and PTT are shown in Figure 3.3. rPTT had an inverse linear correlation with SBP (combined average across all subjects and drugs $R^2=0.39$). There was no difference in correlation coefficient ($p=0.88$) or slope ($p=0.69$) between different drugs by repeated measures ANOVA. rPTT was significantly ($p<0.01$) better correlated with SBP than with either DBP ($R^2=0.02$) or MAP ($R^2=0.08$). Also, rPTT showed differences in correlation with DBP ($p<0.001$) and MAP ($p<0.001$) between different drugs. pPTT was better correlated ($p<0.001$) with DBP ($R^2=0.41$) and MAP ($R^2=0.45$) than with SBP ($R^2=0.33$). Different drugs did not affect the correlations between pPTT and DBP ($p=0.11$), or pPTT and MAP ($p=0.39$). However, the pPTT/SBP correlation did differ between drugs ($p<0.01$).

Table 3.2. *Maximal change from baseline for different agents*

		rPTT *ms*	PEP *ms*	pPTT *ms*	Heart rate *bpm*	SBP *mmHg*	DBP *mmHg*	MAP *mmHg*
GTN	Baseline	260 (17)	95 (15)	164 (12)	63 (7)	134 (12)	66 (5)	85 (6)
	Maximum	276 (18)	92 (16)	184 (26)	77 (9)	122 (10)	61 (6)	77 (6)
	Change	15.3 (11.4)*	-3.5 (10.6)	20.6 (16.0)*	14.3 (5.2)*	-12.6 (8.1)*	-4.5 (5.0) †	-7.8 (4.9)*
Angiotensin II	Baseline	266 (19)	100 (15)	166 (23)	62 (8)	139 (13)	67 (6)	84 (6)
	Maximum	257 (23)	105 (19)	150 (21)	60 (10)	156 (13)	82 (7)	102 (8)
	Change	-9.2 (8.1)*	5.9 (11.5)	-15.2 (7.0)*	-2.6 (8.4)	17.5 (8.3)*	15.0 (4.4)*	18.1 (5.4)*
Noradrenaline	Baseline	261 (15)	95 (19)	165 (18)	61 (8)	141 (10)	68 (6)	86 (7)
	Maximum	238 (18)	87 (19)	151 (20)	57 (8)	164 (13)	81 (8)	104 (10)
	Change	-22.4 (10.5)*	-7.6 (10.1) †	-14.4 (7.6)*	-4.0 (6.4)*	22.9 (14.5)*	12.3 (5.5)*	17.4 (8.3)*
Salbutamol	Baseline	249 (16)	91 (19)	158 (21)	66 (10)	151 (12)	71 (9)	92 (9)
	Maximum	218 (18)	30 (12)	188 (16)	125 (13)	153 (25)	39 (9)	68 (11)
	Change	-32.5 (9.8)*	-62.2 (19.4)*	29.9 (14.2)*	59.1 (7.6)*	2.5 (19.0)	-31.7 (4.3)*	-23.3 (6.0)*

*Values are mean (SD) for all subjects. Significance * $p<0.01$, † $p<0.05$. Abbreviations as per text.*

Figure 3.3. *Linear regression analysis of the PTT and BP relationship in a typical subject.*



◇ *GTN,* ○ *angiotensin II,* △ *noradrenaline,* □ *salbutamol*

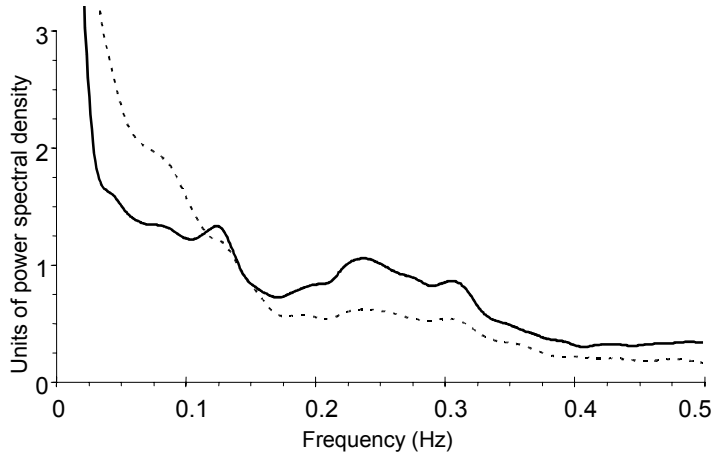
3.3.1.3 Accuracy of blood pressure prediction using transit time

The value of rPTT and pPTT as predictors of SBP and DBP respectively was assessed based on the assumption that it would be possible to obtain an ideal calibration slope for each individual equating to the average linear regression slope for all drugs. 95% limits of agreement for predicted versus actual BP were ±17.0mmHg (SBP/rPTT) and ±17.3mmHg (DBP/pPTT). Percentage predicted values falling within 5, 10 and 15mmHg of actual value (based on British Hypertension Society system for assessing BP measurement accuracy (O'Brien *et al*. 1993)) were 44%, 66% and 73% respectively for SBP, and 42%, 64% and 72% respectively for DBP.

3.3.1.4 Transit time and blood pressure variability

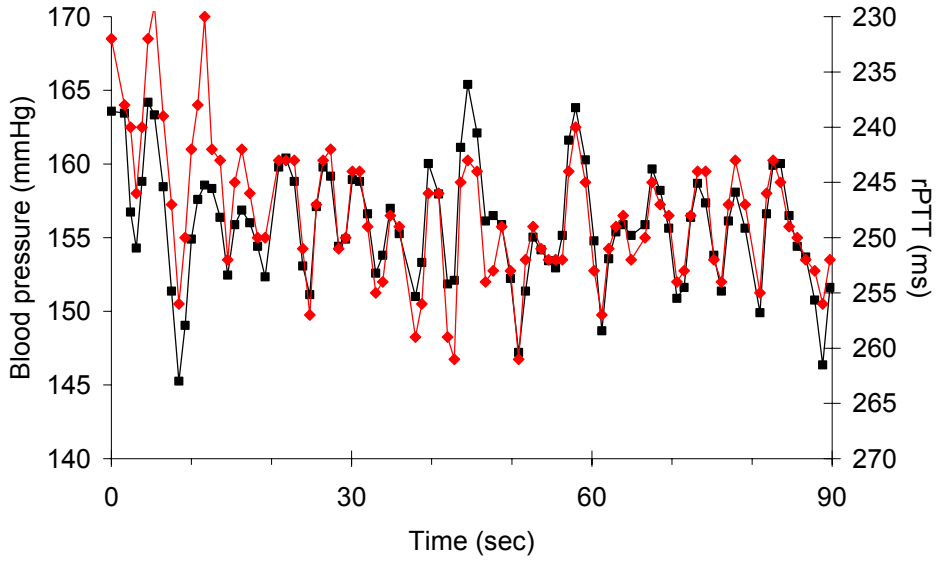
Average power spectra over all drugs are shown in Figure 3.4. An example of the similarity in SBP and rPTT variability is given in Figure 3.5. Mean coherence between SBP and rPTT variability was significantly ($p<0.001$) greater at both lower and higher frequencies (0.58±0.37 and 0.70±0.33 respectively) than coherence of HR and rPTT variability (0.46±0.41 and 0.52±0.38 respectively). There was no significant difference in coherence between HR and either rPTT or SBP, for lower ($p=0.33$) or higher ($p=0.16$) frequencies. Coherence was not significantly affected by drug type or dosage ($p=0.96$), and is shown in Figure 3.6.

Figure 3.4. *Average power spectra (all drugs)*



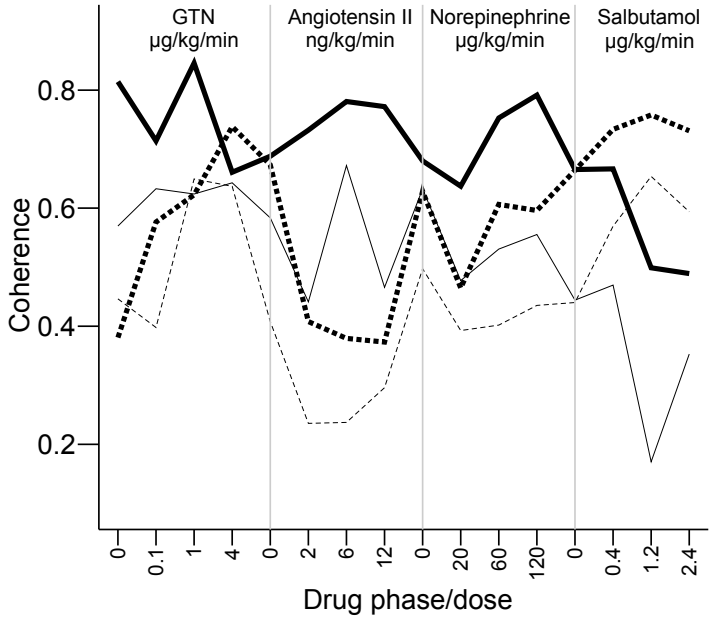
rPTT, solid line; SBP, dotted line.

Figure 3.5. *Example of beat-to-beat variability of SBP and rPTT*



Values are data from one individual recorded over 90 minutes during washout period prior to salbutamol. Black, systolic BP; red, rPTT. Note axis of rPTT has been reversed and re-scaled for clarity. Scaling is not based on formal regression analysis and is for sake of clarity only.

Figure 3.6. *Coherence between rPTT, SBP and HR variability*



Coherence between rPTT variability and SBP variability (heavy line), and rPTT and heart rate (thin line). Frequency band 0.05Hz to 0.2Hz is solid line, and 0.2Hz to 0.4Hz is dashed line.

Table 3.3. *Changes in waveform measures with individual drugs*

	GTN		Angiotensin II		Noradrenaline		Salbutamol	
Haemodynamic parameter	Before	End	Before	End	Before	End	Before	End
PWV (m/s)	5.3±0.6	4.8±0.8*	5.3±1	6.1±1.5*	5.2±0.7	5.9±1.3*	5.9±0.9	5±0.6*
Compliance (ml/mmHg)	1.7±0.2	2.2±0.1*	1.9±0.2	1.4±0.1*	1.8±0.2	1.5±0.2*	1.8±0.2	2.2±0.1*
WEP waveform								
T_{S1} (ms)	49±3	54±5*	51±5	51±5	49±5	47±4	48±4	43±3*
T_{S2} (ms)	249±21	266±53	253±17	215±14*	252±17	219±17*	234±21	174±44*
T_{S3} (ms)	362±28	388±48*	362±24	363±23	369±23	364±31	359±18	326±57
T_{S2}-T_{S1} (ms)	200±20	211±50	201±15	163±14*	202±14	172±16*	186±19	130±43*
P_{S1}/P_{S2} (%)	34±9	22±8*	30±10	37±11*	31±10	37±9*	33±9	22±18*
Intra-arterial waveform								
T_{A1} (ms)	109±14	121±12*	104±14	99±6	101±11	98±12*	98±12	81±14*
T_{A2} (ms)	237±16	247±24	229±15	213±8*	230±16	217±13*	223±17	190±17*
T_{A2}-T_{A1} (ms)	128±6	126±13	125±7	113±5*	128±8	118±5*	124±7	116±11
T_{DN} (ms)	319±16.6	330±46	321±25	306±10*	317±16	313±13	312±15	259±31*
AIx (%)	39±6	25±23	26±6	53±17*	29±7	52±14*	31±8	12±24
Root-mean-square error (RMSE)								
WEP_A & ART_A (mmHg)	14.2±5.4	17.2±18.3	13.3±5.3	17.7±5.0*	17.3±8.5	22.1±11.7	20.3±11.9	28.9±22.4
WEP_S & ART_S (mmHg/s)	39±13	49±18	42±16	42±12	53±30	58±38	59±38	139±74*
ART_A & GTF_{all} (mmHg)	9.0±3.3	5.5±3.4	5.5±4.7	7.4±5.8	6.2±3.8	8.1±4.4	7.8±2.6	16.3±8.8*

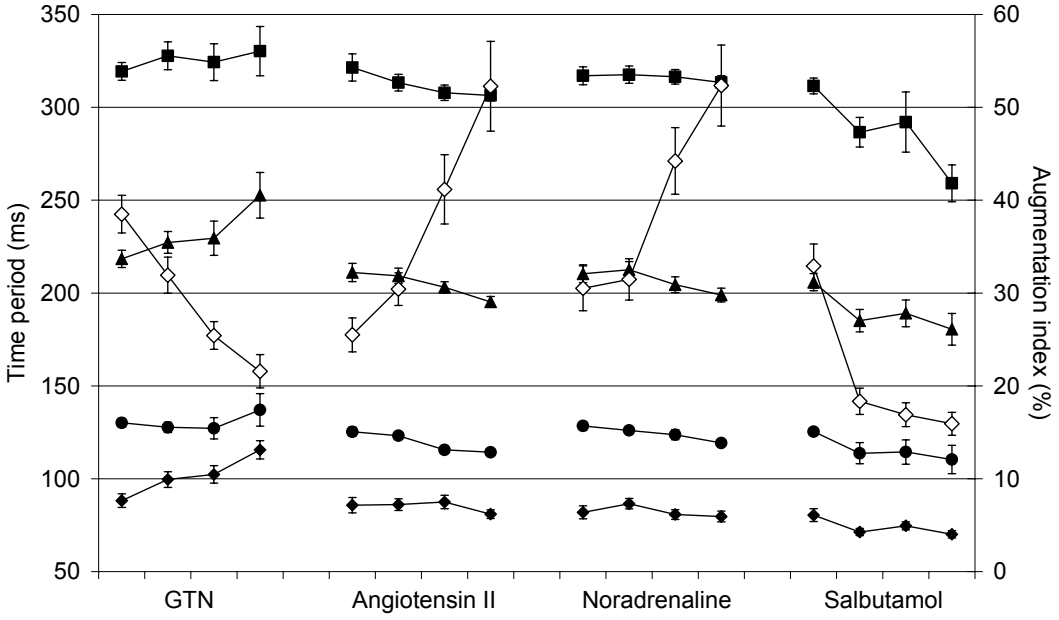
*Values are mean±SD. * indicates significant ($p<0.05$) change. Abbreviations as per text.*

3.3.2 Pulse wave analysis

3.3.2.1 Drug effect on wave parameters

Maximum changes in arterial (ART_A) and WEP_S wave parameters are shown in Table 3.3. Dose response for ART_A parameters is graphed in Figure 3.7. PWV, arterial AIx and WEP_S P_{S1}/P_{S2} ratio decreased with GTN and salbutamol, and increased with noradrenaline and angiotensin II. Compliance measured by WEP mirrored changes in PWV, with a negative correlation overall ($r= 0.44$, $p<0.05$). The WEP_S T_{S2}-T_{S1} time delay and arterial T_{A2}-T_{A1} time delay decreased with all drugs, except GTN. These changes were largely accounted for by a decrease in T_{S2} and T_{A2} respectively. However, T_{S1} and T_{A1} both decreased with salbutamol. Increases in arterial T_{A1} and T_{A2}, and in WEP_S T_{S1} and T_{S2} were observed with GTN, accounting for the lack of change in T_{A2}-T_{A1} or T_{S2}-T_{S1}.

Figure 3.7. *Changes in arterial pulse wave parameters over duration of experiment*



Values are mean ± standard error, at all 4 dosing periods. ◇ Augmentation index (%); ■ Time to dicrotic notch, T_{DN} (ms); ◆ T_{A1} (ms); ▲ T_{A2} (ms); ● T_{A2}-T_{A1} (ms). Time periods (solid symbols) on left axis.

The similarities between the WEP_S signal and the first derivative of the arterial waveform (ART_S) are demonstrated in

3.3.2.2 Relationship of WEP signal to first derivative of arterial pressure

Figure 3.8A, with the RMSE for the two signals shown at the bottom of Table 3.3. Bland-Altman plots are shown in Figure 3.10. The RMSE did not vary significantly between experimental phases, despite being notably greater at the end of salbutamol administration. Regression analysis confirmed a strong positive correlation between the two methods with respect to timing of different components of the waveform (see Table 3.4), although T_{S1}, T_{S2} and T_{S3} occurred consistently slightly earlier when measured by WEP_S compared to ART_S. There was also a trend for the bias to get progressively more negative at greater values of T_{S1} and T_{S3}, although the reverse was true for T_{S2}. Although no significant mean differences existed between methodologies in the T_{S2}-T_{S1} delay or the P_{S1}/P_{S2} ratio, there was nonetheless a statistically significant tendency for the bias to become increasingly positive at

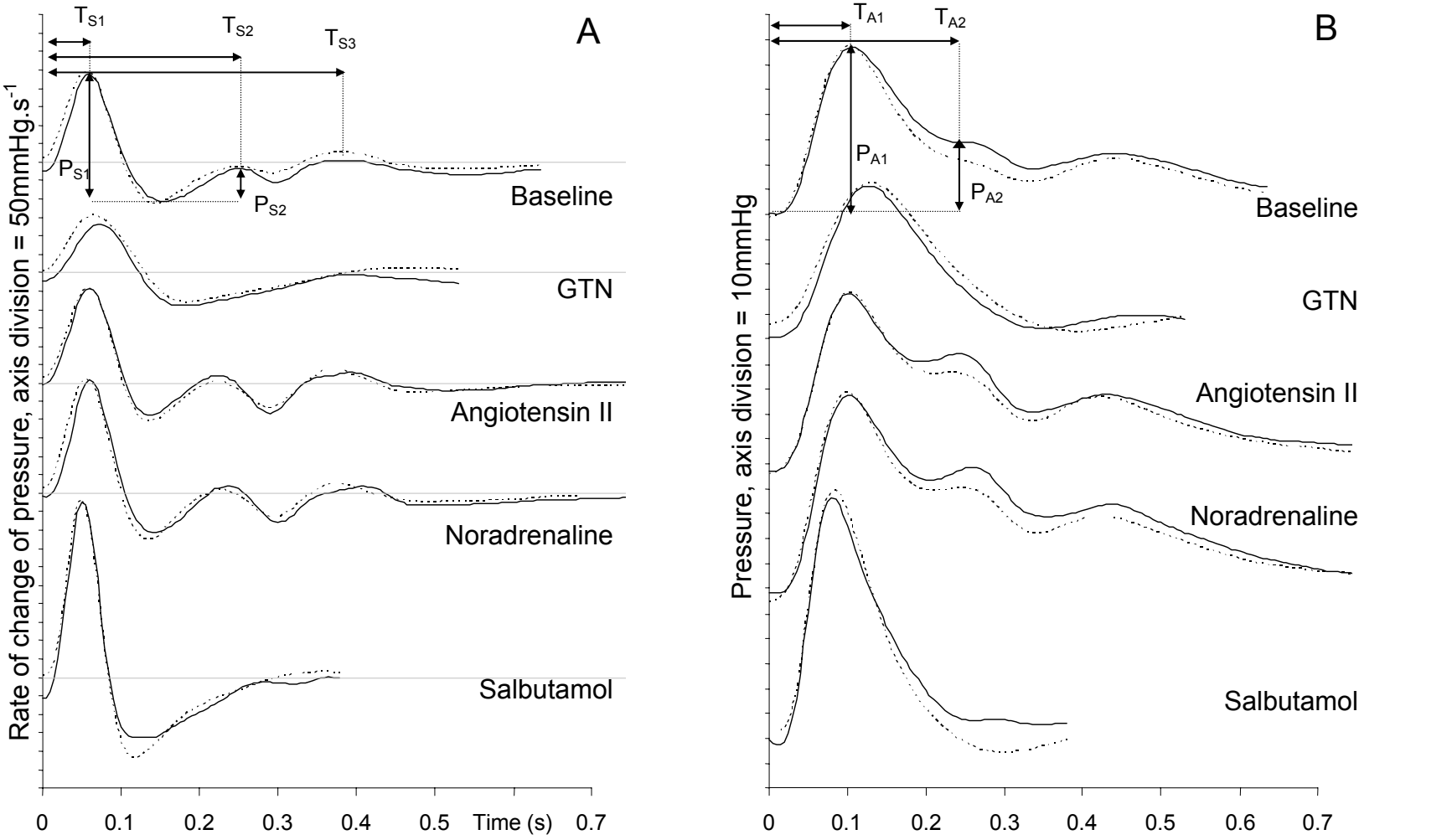
The WEP_S signal was integrated (WEP_A) to assess how accurately the arterial pressure wave contour could be estimated. Similarities in wave shape are shown in

greater values of T_{S2}-T_{S1} and P_{S1}/P_{S2}.

3.3.2.3 Arterial pressure wave estimation using WEP signal

Figure 3.8B. Bland-Altman plots are shown in Figure 3.11. RMSE values were large (Table 3.3), but statistically greater with angiotensin II only ($p<0.05$). The WEP_A signal appeared slightly damped relative to ART_A, although the timings of reflected waves were similar. Regression analysis (Table 3.4) confirmed that T_{A1} and T_{A2} occurred at similar times, although the bias of the T_{A2}-T_{A1} time delay became more positive with increasing values. AIx showed a consistent bias of 7±18% across the measurement range relative to intra-arterial measurements.

Figure 3.8. *Comparison of wave shapes obtained by WEP and intra-arterial monitoring*



A, average ART_S (solid) and WEP_S (dotted) signals; B, average ART_A (solid) and WEP_A (dotted) signals.

3.3.2.4 Comparison of transfer functions in the frequency domain

GTF_{all} and each GTF_{drug} are shown in Figure 3.9 (individual GTF_{dose} curves are not shown for the sake of clarity). GTFs were compared using the area under the curve (AUC) for magnitude and phase. No significant changes in gain were seen between any of the GTFs. Phase did vary significantly ($p<0.001$, rmANOVA), however, specifically due to an overall positive phase shift with higher doses of salbutamol.

3.3.2.5 Comparison of effect of transfer functions on derived pulse contour features

Overall, there were significant ($p\leq0.02$, rmANOVA) variations in T_{A1}, T_{A2} and the T_{A2}-T_{A1} difference using different GTFs. These variations were specifically accounted for by differences between GTF_{all} and GTF_{drug} ($p\leq0.005$), with no significant differences found between GTF_{drug} and GTF_{dose} ($p\geq0.85$). The degree of variation for T_{A1}, T_{A2} and T_{A2}-T_{A1}, was influenced by different drugs ($p\leq0.03$, rmANOVA), but not by the different doses ($p\geq0.08$). However, differences between GTF_{all} and GTF_{drug} were small for T_{A1} (-1.2±3.2ms, $p<0.001$), T_{A2} (+1.4±7.6ms, $p=0.01$) and T_{A2}-T_{A1} (+2.6±9.5ms, $p<0.001$). The effects of drugs on these differences were also small, with no clear pattern attributable to different agents: -2.0±5.9ms (angiotensin II) to 0.0±1.7ms (salbutamol) for T_{A1}; -2.1±3.8ms (GTN) to 3.7±8.1ms (salbutamol) for T_{A2}; -0.4±3.7 (GTN) to 4.2±14.5ms (angiotensin II) for T_{A2}-T_{A1}. No significant variation ($p\geq0.45$, rmANOVA) existed between GTFs for AIx, RMSE or T_{dn}.

3.3.2.6 Comparison of measured and derived waveforms

Due to the relatively small differences between GTFs, and the small variations in error introduced by different drugs, further comparisons have been made using waveforms derived using GTF_{all} only. Bias and limits of agreement between arterial measurements and derived waveforms are given in Table 3.4, with RMSE values shown in Table 3.3. Bland-Altman plots for the different comparisons are shown in Figure 3.12.

The degree of difference was significantly influenced by drug ($p \leq 0.027$, rmANOVA), but not by dose ($p \geq 0.13$). Specifically, GTF_{all} significantly ($p \leq 0.005$, *post hoc* paired *t*-test) overestimated AIx with GTN (+5.8±2.0%) and salbutamol (+6.8±1.4%), and T_{dn} with GTN (+10.9±3.2ms). GTF_{all} also overestimated ($p \leq 0.022$) T_{A2} and T_{A2}-T_{A1} for angiotensin II (+6.7±2.7ms, +8.9±2.1ms respectively), noradrenaline (+5.1±2.1ms, +4.5±1.5ms) and salbutamol (+22.4±5.3ms, +13.9±4.6ms). T_{A1} was underestimated during GTN administration (-5.3±2.2ms, $p=0.02$) and overestimated during salbutamol (+8.5±1.1ms, $p<0.001$), although this was not significant in the multivariate repeated-measures analysis.

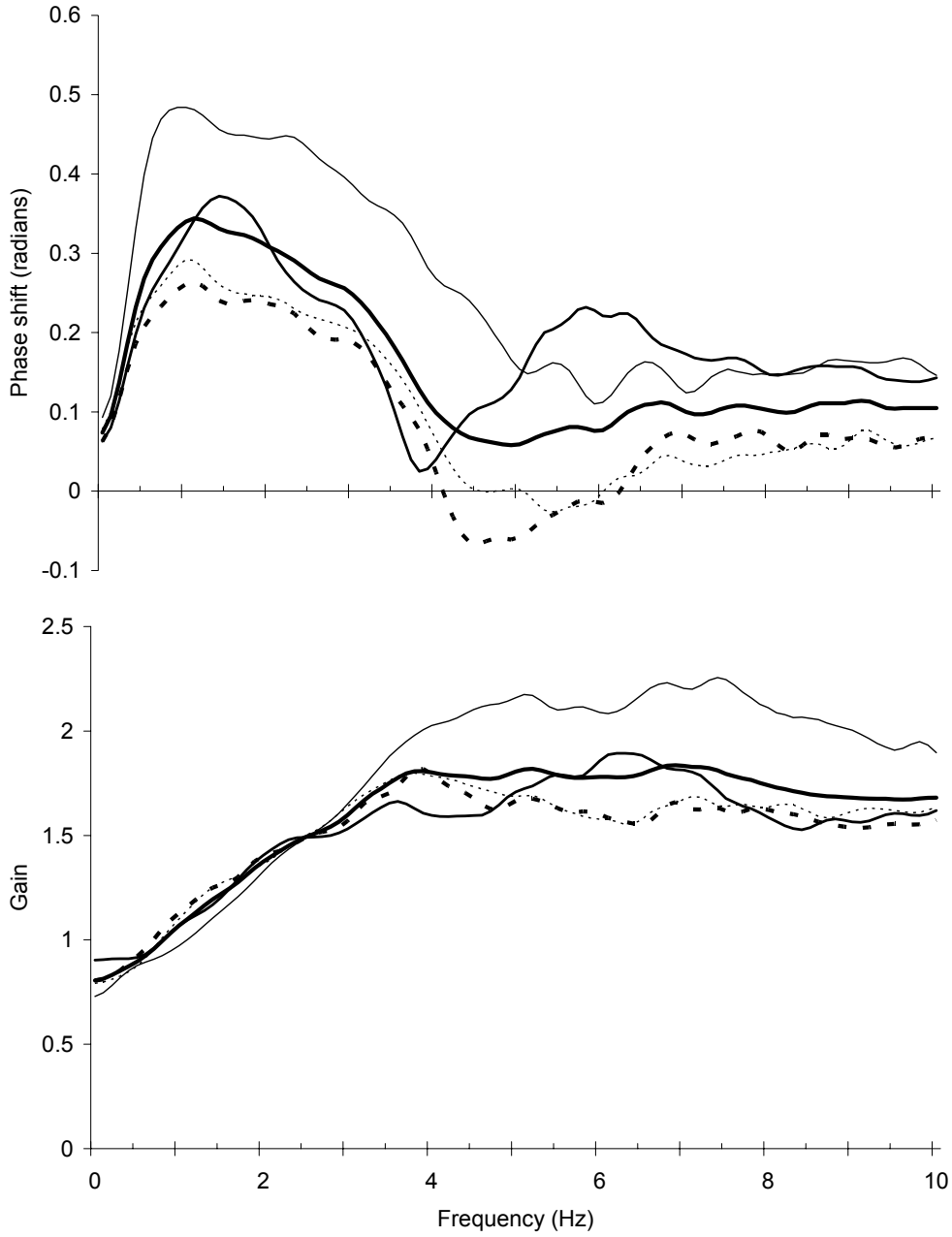
Overall, T_{A1}, T_{A2}, T_{DN} and AIx were all slightly overestimated by GTF_{all}, and derived and measured values were positively correlated (Table 3.4). There was no apparent correlation between derived and measured T_{A2}-T_{A1}, and the bias of 7.2±21.4ms was not statistically significant. There was a tendency for the bias to get significantly greater at increased values of T_{A1}, T_{A2} and T_{A2}-T_{A1}, and decreased values of AIx.

Table 3.4. *Comparison of different measured and derived waveform parameters*

Comparison	r for comparison	Mean bias (ms/%)	95% limits of agreement (ms/%)	r for bias
WEP_S - ART_S (Figure 3.10)				
T_{S1}	0.70‡	-13.6 ± 1.0‡	-30.7 – 3.5	-0.75‡
T_{S2}	0.73‡	-18.9 ± 2.9‡	-70.6 – 32.8	0.39‡
T_{S3}	0.68‡	-33.2 ± 3.6‡	-97.3 – 30.9	-0.27*
T_{S2}- T_{S1}	0.64‡	-5.3 ± 3.0	-59.4 – 48.8	0.52‡
P_{S1}/P_{S2} (%)	0.41‡	-0.6 ± 1.2	-22.0 – 20.8	0.52‡
WEP_A - ART_A (Figure 3.11)				
T_{A1}	0.39‡	-7.7 ± 1.9‡	-42.2 – 26.8	0.0
T_{A2}	0.61‡	-1.4 ± 2.6	-49.2 – 46.4	0.35†
T_{A2}- T_{A1}	0.61‡	1.4 ± 1.6	-27.0 – 29.8	0.71‡
AIx (%)	0.60‡	-7.2 ± 1.9‡	-41.3 – 26.9	0.0
ART_A - GTF_{all} (Figure 3.12)				
T_{A1}	0.68‡	-0.4 ± 0.9‡	-23.5 – 22.7	0.53‡
T_{A2}	0.29‡	-7.5 ± 2.0‡	-61. 6 – 46. 6	0.39‡
T_{A2}- T_{A1}	-0.11	-7.2 ± 1.6	-49.1 – 34.7	0.21†
T_{DN}	0.56‡	-4.8 ± 2.0*	-59.3 – 49.7	0.0
AIx (%)	0.65‡	-3.2 ± 0.9‡	-28.5 – 22.1	-0.19*

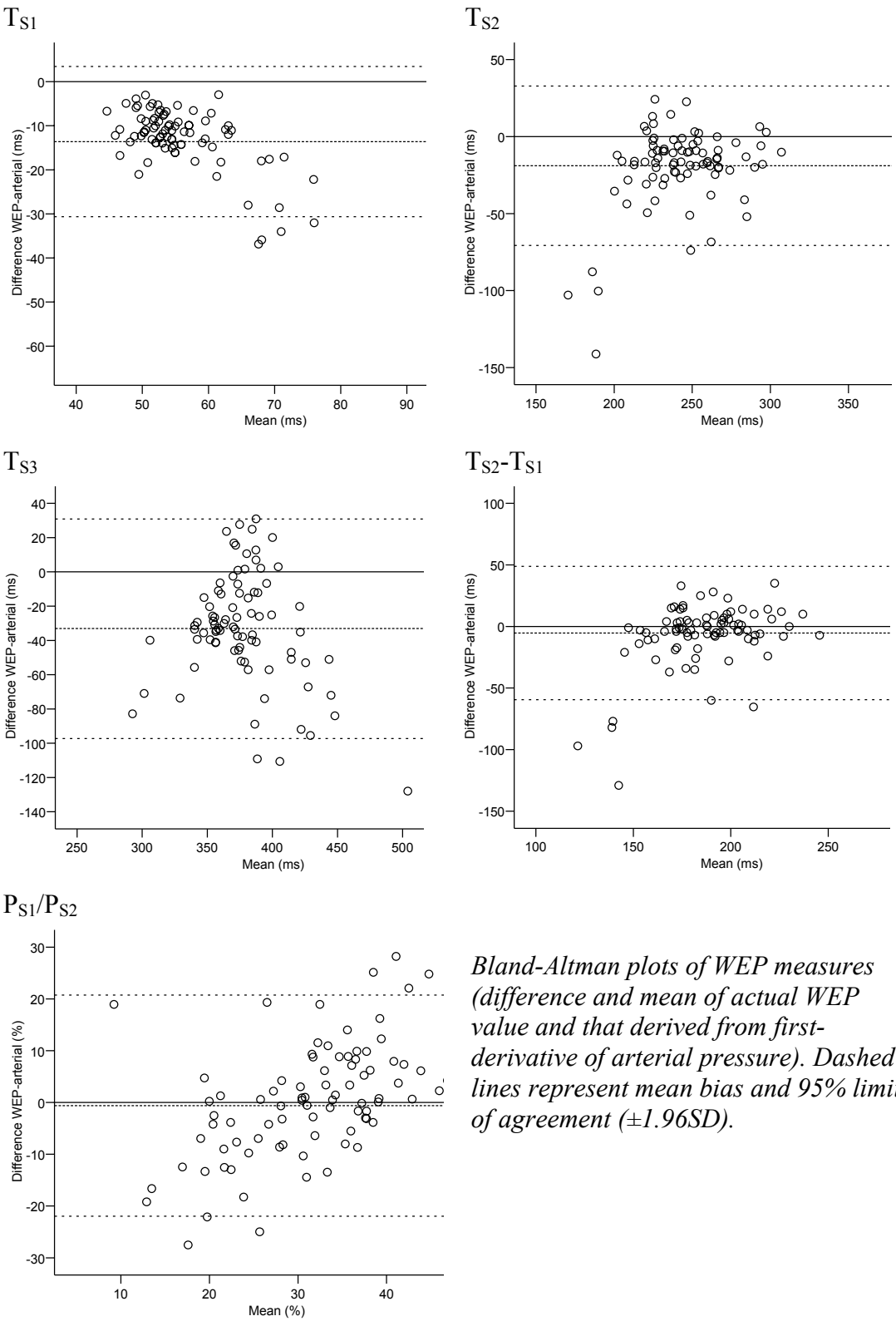
*"r for comparison" is Pearson coefficient for correlation between related parameters. "Mean bias" is mean±standard error of difference between the related parameters. "r for bias" is Pearson coefficient for correlation between difference and mean of related parameters. * $p<0.05$; † $p<0.01$; ‡ $p<0.001$. Abbreviations as per text.*

Figure 3.9. *Comparison of radial-to-finger generalised transfer functions*



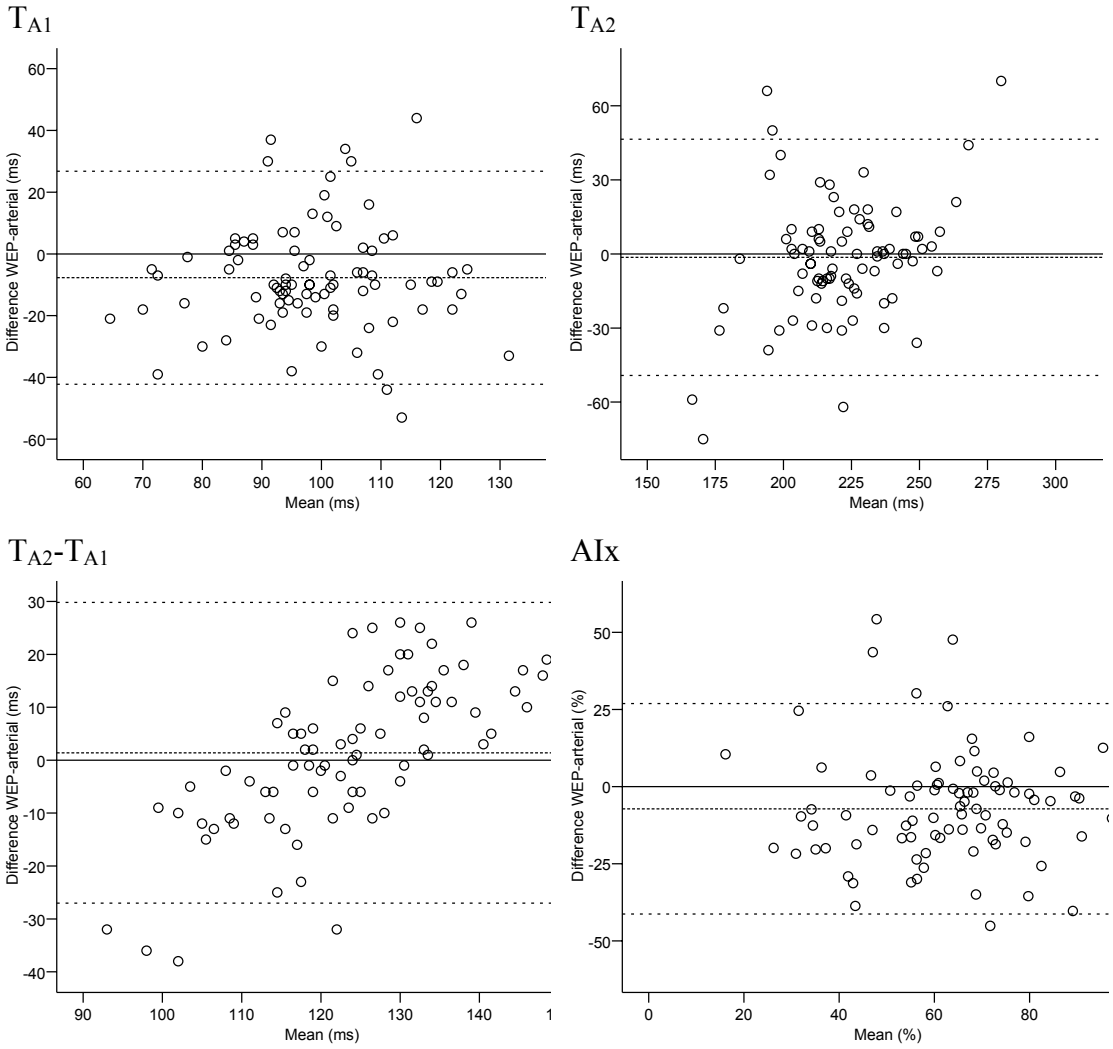
Lines represent GTF_{all} (heaviest, solid) and GTF_{drug} for GTN (moderate, solid), angiotensin II (moderate, dashed), noradrenaline (light, dashed). and salbutamol (light, solid).

Figure 3.10. *Comparison of wideband external pulse with first-derivative of arterial pressure*



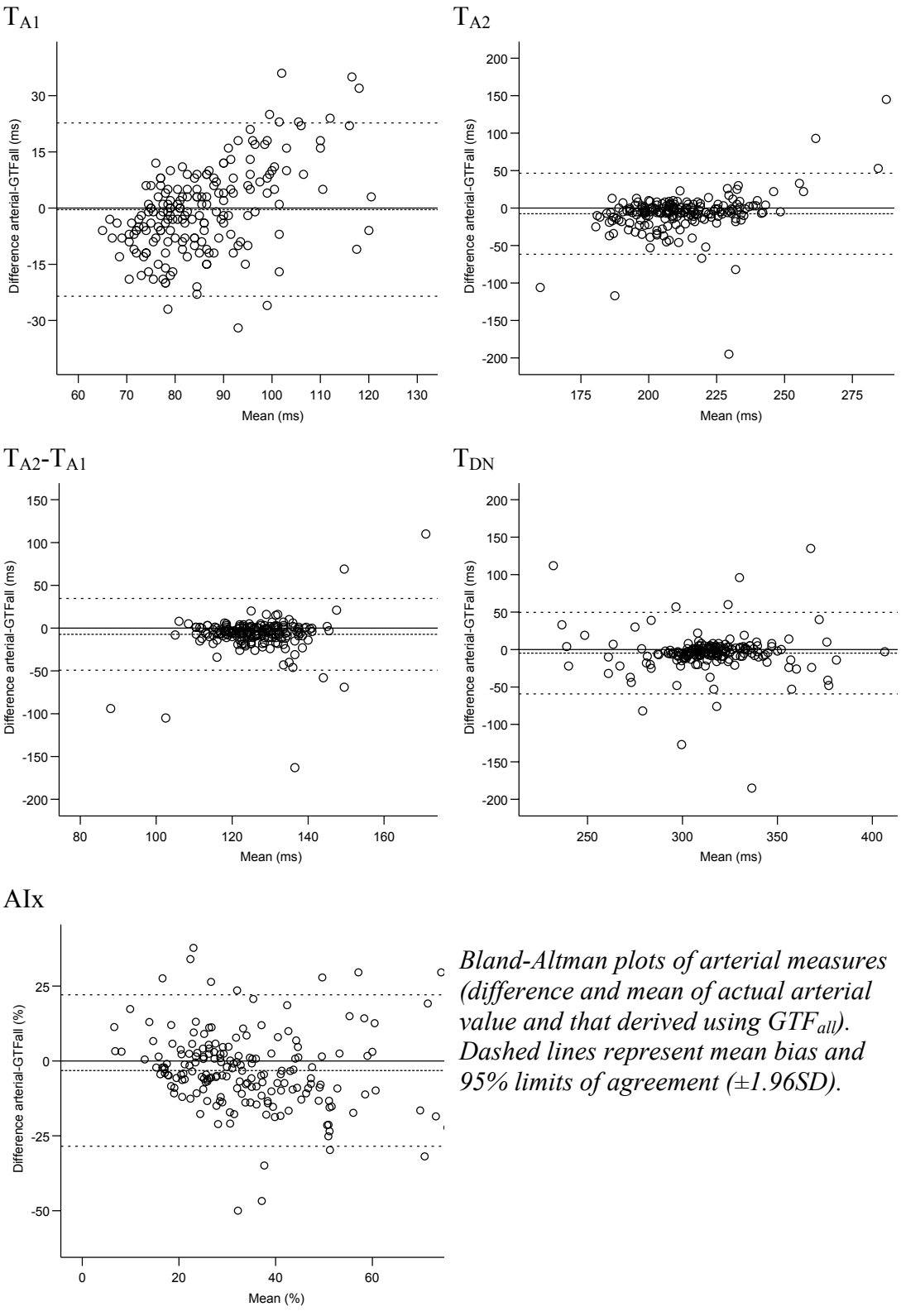
Bland-Altman plots of WEP measures (difference and mean of actual WEP value and that derived from first-derivative of arterial pressure). Dashed lines represent mean bias and 95% limits of agreement (±1.96SD).

Figure 3.11. *Accuracy of arterial measures derived using the wideband external pulse*



Bland-Altman plots of arterial pressure measures (difference and mean of arterial value derived by integrating WEP signal and actual measured value). Dashed lines represent mean bias and 95% limits of agreement (±1.96SD).

Figure 3.12. *Accuracy of arterial measures derived from photoplethysmography*



Bland-Altman plots of arterial measures (difference and mean of actual arterial value and that derived using GTF_{all}). Dashed lines represent mean bias and 95% limits of agreement (±1.96SD).

3.4 DISCUSSION

3.4.1 Effect of cardiovascular drugs on pulse transit time

The association between PTT and BP was studied extensively in the field of psychophysiology (Geddes *et al*. 1981; Gribbin *et al*. 1976; Lane *et al*. 1983; Steptoe *et al*. 1976; Weiss *et al*. 1980) in the 1970s and 1980s, and more recently by Ochiai *et al* (1999) and Chen *et al* (2000). rPTT has also been used to predict BP in a clinical setting (Heard *et al*. 2000). The current study is the first to examine simultaneously the effects of vasoactive drugs on rPTT, PEP and invasively measured BP in humans.

The expected haemodynamic changes occurred with all four drugs, although the SBP response to salbutamol was mixed. rPTT had a negative correlation with SBP, which was relatively unaffected by different drugs in the population as a whole. rPTT also appeared to be useful as a marker of SBP variability. However, DBP and MAP were weakly correlated with rPTT, although more strongly related to pPTT.

SBP is dependent on both vascular function and ventricular contraction, and so it is perhaps unsurprising that rPTT, a composite measure of both vascular and cardiac activity, is correlated with SBP. However, although in the study population as a whole the correlation between rPTT and SBP appeared relatively unaffected by drugs, this finding must be treated with caution. There were slight differences in the rPTT response between noradrenaline and angiotensin II, despite similar BP profiles. Furthermore, it should be noted that 4 subjects in this study had positive correlations between rPTT and SBP during the administration of salbutamol. This drug has positive inotropic and chronotropic β2 adrenergic effects, as well as causing peripheral arterial relaxation. Although a fall in PEP is associated with an increase in cardiac inotropy, this does not necessarily relate to an increase in SBP, as any potential pressure rise may be offset by decreases in pressure augmentation by reflected waves or changes in aortic stiffness (Nichols & O'Rourke 1998). It would therefore appear inappropriate to use rPTT as a predictor of SBP in all persons, particularly for assessing changes due to vasoactive drugs. Moreover, even using an idealised calibration slope, the limits of agreement between predicted and actual BP

were wide and would not meet the criteria of the British Hypertension Society, although similar inaccuracies have been described previously between sphygmomanometric and direct arterial pressure measurements (Brown *et al*. 1994; Turjanmaa 1989).

These data also show that PEP accounts for a substantial and variable proportion of rPTT, ranging from around 12% to 35%. A number of relatively recent studies have employed rPTT as a marker of vascular function (Bulpitt *et al*. 1999; Cameron *et al*. 2003), but this study demonstrates that the use of rPTT purely for the assessment of arterial stiffness is inappropriate and should be avoided, as PEP cannot be assumed to remain constant. Other devices such as the Colin VP-1000 (Colin Corporation, Japan), have eliminated PEP by utilising the phonocardiogram to time cardiac ejection. The phonocardiogram is regarded by many as the ideal way of determining systolic time intervals. The principal disadvantage, however, compared to bioimpedance, is that it requires accurate identification of two timing points rather than simply one: firstly, the end of cardiac ejection (the second heart sound); secondly, the left ventricular ejection period (measured by identifying the dicrotic notch using a proximal arterial pulse wave).

rPTT may nonetheless offer a potentially valuable means of detecting beat-to-beat changes in SBP. Indeed, with regular re-calibration to standard oscillometric BP as suggested by Chen *et al* (2000), rPTT offers the opportunity to assess BP variability and detect sudden or transient haemodynamic changes. BP and HR variability are considered to offer important insights into vasomotor activity, have been associated with clinical outcomes, including cardiovascular death, and may be used in assessment of autonomic neuropathy (Parati *et al*. 1995). Sympathetic modulation of BP alters the HR through the actions of the sinoaortic baroreflex; coherence between these two measures therefore reflects baroreflex activity (Lanfranchi & Somers 2002). rPTT shows beat-to-beat variability closer to that of SBP than HR, and therefore may have a role in the assessment of vasomotor control and BP variability.

pPTT, but not rPTT, was strongly inversely correlated with DBP and MAP. Furthermore, the correlation was inconsistent between pPTT and SBP. These findings are both consistent with the fact that arterial stiffness, and therefore vascular pulse transit time (i.e. pPTT), is dependent on MAP, rather than SBP. In many circumstances, SBP and DBP/MAP are positively associated with each other. This has led others to inappropriately use rPTT to predict both these variables (Carruthers & Taggart 1988; Heard *et al*. 2000), with DBP calculated following adjustment for HR. However, the divergent SBP and DBP/MAP responses to salbutamol in 8 subjects in this study have not been reported in previous published work in this field, and the current data suggest that rPTT cannot be used to predict DBP or MAP without a knowledge of PEP, regardless of the HR response.

This study has a few limitations. Baseline values of BP were not constant prior to each drug, tending to rise steadily over the course of the study, particularly after noradrenaline. Due to the short half-life of both pressor agents in particular, it seems unlikely that the rise in BP is entirely accounted for by direct drug effects. Randomising drug order was not carried out as the much longer half life of salbutamol necessitated its administration last, and it was not justifiable to carry out the separate elements of the study on different days, as this would have required repeated arterial cannulation. The washout periods were also kept relatively short, to minimise the duration of cannulation. Despite these points, the aim of the different drugs was to achieve a wide range of BP under varying conditions of vascular tone, and this was still achieved even if the haemodynamic effects of one drug had not completely resolved before the administration of the next. The use of fluid-filled manometer tubing introduces a degree of inaccuracy between pressure at the catheter tip, and that at the more proximal transducer. However, this discrepancy was constant between subjects, and fluid filled manometer tubes are nonetheless regarded as the "gold standard" in clinical practice. HR is a potential confounding factor (Lantelme *et al*. 2002) in the assessment of vascular stiffness and BP, although debate continues over whether reported increases in pulse wave velocity with HR are genuine (Hayward *et al*. 2002). Importantly however, the large change in HR seen with salbutamol does not affect the interpretation of DBP being more important than

SBP as a determinant of pPTT, because a high HR would, if anything, increase arterial stiffness and thus reduce vascular transit time.

3.4.2 Effect of drugs on the pulse wave contour

Pulse wave analysis is becoming increasingly popular as a means of assessing arterial stiffness. Elevated aortic AIx has been associated with increased mortality in end-stage renal disease (London *et al*. 2001), increased severity of coronary artery disease (Weber *et al*. 2004), presence of diabetes (Wilkinson *et al*. 2000) and hypercholesterolaemia (Wilkinson *et al*. 2002c), and increased age (Kelly *et al*. 1989). AIx has also been used in the assessment of endothelial function (Wilkinson *et al*. 2002a). However, applanation tonometry, the usual method of recording the waveform, can be difficult to perform, and methods requiring less user training and potentially cheaper technology are therefore attractive alternatives. Both sphygmomanometry and photoplethysmography (PPG) are already widely used in the clinical environment, the latter in the form of oxygen saturation monitors. The present study demonstrates that mathematical manipulation of the PPG and WEP waveforms can be performed to reproduce the signals measured using invasive arterial recording. The study also demonstrates that large haemodynamic changes due to vasoactive drug administration have little effect on the accuracy of the waveforms derived using either of these methodologies.

It was shown that the suprasystolic WEP signal resembles the first derivative of intra-arterial pressure and can, therefore, be used to estimate the arterial pressure wave. Time delays and measurements of reflected wave amplitude measured by WEP analysis, correlate with those obtained directly from the arterial signal, and similar changes occur with both techniques during administration of pharmacological agents. Although it is important to note that the RMSE was substantial with all drugs (4-5mmHg is the limit of accuracy of devices for recording arterial pressure (Millasseau *et al*. 2000)), and that this may therefore limit the role of suprasystolic WEP analysis as an accurate alternative to direct intra-arterial pressure recording or applanation tonometry, the WEP responses nonetheless tracked those of the arterial line and can thus still be considered a potentially useful means of evaluating

cardiovascular function. Furthermore, in addition to the obvious benefits of being non-invasive, the WEP system has the advantage that it has potentially far less operator dependency than tonometry and could be incorporated relatively easily into standard oscillometric sphygmomanometer devices.

It has been suggested by the manufacturers of Pulsecor that the S2-S1 delay is inversely related to PWV (Blank 1996); a similar relationship with PWV has been proposed for the time delay between systolic and diastolic peaks on the finger photoplethysmograph waveform (Millasseau *et al*. 2002). The current study found that the T_{S2}-T_{S1} delay, measured by both WEP_S and the first derivative of the arterial pressure pulse, decreased with all drugs except GTN, which caused a small non-significant increase. The T_{A2}-T_{A1} delay, measured from the arterial pressure pulse, also decreased with both pressor agents and salbutamol, albeit the latter not significantly. The T_{S2}-T_{S1} and T_{A2}-T_{A1} time delay findings were similar to each other, but not in line with either expected or measured PWV responses. Changes in the magnitude of reflected waves, due to changes in peripheral impedance mismatch, may affect the timing of apparent wave peaks and thus alter the apparent velocity of reflections. It can also be difficult to identify S2 in circumstances of marked vasodilatation and increased heart rate. These factors may in part explain the time delay findings described. GTN given in similar doses to those used in the present study has been shown to have only small effects on the finger pulse systolic-diastolic time delay, despite large changes in the relative amplitude of these wave components (Millasseau *et al*. 2003a), and thus inaccuracy in identifying S2 or A2 may have been particularly important with this drug. The decrease in PWV with salbutamol in this study is due to peripheral vasodilatation and a decrease in MAP, offsetting any potential increase as a result of tachycardia (Haesler *et al*. 2004). The corresponding fall in T_{S2}-T_{S1} (and to a lesser extent, T_{A2}-T_{A1}) is not consistent with this PWV change, and may also be explained by the factors described above. These findings were identified with both WEP and arterial line, and were consistent in all subjects, suggesting this is a genuine phenomenon. Regardless of the precise cause of these findings, it would, therefore, appear unwise to use these time delays as a surrogate marker of PWV.

The ratio of amplitudes of the original WEP_S signal showed changes similar to AIx and PWV. However, as pointed out by Millasseau and colleagues (Millasseau *et al*. 2003a), it is difficult to directly relate values obtained from the derivative of the pulse waveform to the biomechanical properties of the cardiovascular system. The ratio of amplitudes is also used by Pulsecor to obtain a measure of vascular compliance, and the values obtained in the current study correlate with measured PWV. It is important to note, however, that the currently unpublished mathematical function used to derive compliance is not validated and is based on small subject numbers (Blank 1996). Furthermore, the compliance value is an estimate only, as neither volume nor flow is known. The current study was not designed to validate the accuracy of the compliance values, and a measure of vascular function taken directly from the waveform was thus considered more relevant. In this respect, AIx is an established and useful marker of vascular function (Wilkinson *et al*. 2001), albeit not a direct measure of arterial compliance (Kelly *et al*. 2001). WEP-derived arterial pressure waves showed changes in AIx similar to those directly measured using the arterial line. As the correlation may have been inflated by pooling data across interventions known to alter AIx, baseline data was examined alone. This analysis revealed that the positive correlation persisted ($R=0.42$, $p<0.01$) with a similar degree of bias (-7.7±12.7%). The bias between the two methods probably reflects the damping of the WEP_A waveform, with a relatively smaller P_{A2} amplitude. It remains uncertain whether WEP signals can be used to evaluate central haemodynamics, although this would appear possible, given that radial AIx correlates closely with derived aortic AIx (Millasseau *et al*. 2003b).

Blank and colleagues described a similar appearance of the suprasystolic WEP pulse contour to that observed in the present study (Blank *et al*. 1988). Below systolic pressure, the suprasystolic signal became obscured, with the waveform taking on the intra-arterial pressure pulse contour as cuff pressure approached diastolic pressure. Below diastolic pressure, the signal diminished in size as it requires adequate coupling between the sensor surface and the skin. Although they did not compare the suprasystolic shape directly with intra-arterial pressure, they acknowledged that this waveform was probably still intrinsically related to the arterial pressure pulse, and may therefore contain clinically important information. This is supported by the

current study. In addition, Blank found that the suprasystolic signals had less high frequency energy than diastolic WEP signals, the latter correlating directly with intra-arterial pressure. This may explain the apparent damping of the arterial pressure signals derived from the suprasystolic WEP traces in the current study, and is presumably related to the effects of pulse transmission through the non-vascular upper limb tissues and inflated cuff. The principal advantage of using the suprasystolic WEP waveform, as opposed to the sub-systolic or diastolic signals, is that adequate coupling of the sensor to the skin is always present, and that a composite signal comprised of any diastolic component is avoided.

It is still not clear why the WEP signal resembles the derivative of the intra-arterial pressure wave. Occlusion of the brachial artery does not prevent the distal propagation of vibrations resembling the original pulse waveform through the air-filled cuff and non-vascular tissues. If the air-filled cuff is considered a low-impedance continuation of the artery, then a reflection would be expected to occur at the interface which would be subject to a 90 degree phase shift, effectively inverting it (Nichols & O'Rourke 1998). Assuming these two signals (normal and inverted) are of similar magnitude and slightly offset in time from one another, then the sum of the two amplitudes will be a function of the pressure gradient and thus resemble the first derivative. Alternatively, the signal may represent the effects of obstructed flow, generating waves similar to the flow wave which is closely related to the pressure gradient in peripheral vessels. However, these suggestions are purely speculative, and additional studies are required to understand the mechanics underlying generation of the suprasystolic WEP signal, and whether the signal is affected by non-vascular parameters, such as cuff material or size.

Use of the PPG waveform for pulse contour analysis is more widely recognised than use of the WEP signal (Dillon & Hertzman 1941; Morikawa 1967), and there has been a resurgence of interest largely thanks to the separate work of Takazawa (Takazawa *et al*. 1998) and Chowienczyk (Chowienczyk *et al*. 1999; Millasseau *et al*. 2000). Chowienczyk and colleagues have shown that the time to the first and second peaks of the pulse wave relate to arterial stiffness and endothelial function (Chowienczyk *et al*. 1999), and have used a transfer function applied to the PPG

waveform to obtain a corresponding radial waveform (Millasseau *et al*. 2000). Karamanoglu and Feneley have also used a transfer function to synthesis the aortic waveform from the finger (Karamanoglu & Feneley 1997). Nonetheless, the effect of large haemodynamic changes, as achieved in this study, on the relationship between PPG wave and the arterial pressure pulse, have not hitherto been described.

The haemodynamic changes resulting from drug administration did not appear to markedly affect the results obtained from different PPG-to-radial transfer functions. Others have also found that far smaller doses of GTN have had minimal impact on transfer functions, both PPG-to-radial (Millasseau *et al*. 2000) and radial-to-aortic (Karamanoglu *et al*. 1993; Pauca *et al*. 2001). The much larger doses of drugs given during this study might be expected to have particularly marked effects on smaller arteries, and thus potentially alter the PPG-to-radial transfer function. The constancy of the transfer function in this study is therefore somewhat unexpected, although may be explained by a tendency for these drugs to primarily affect higher frequency waveform components which contribute less to the overall spectral power of the signal (Nichols & O'Rourke 1998). Clearly, this study did not enable us to test whether the radial-to-aortic transfer function remains similarly constant, but it certainly adds weight to the argument that a peripheral-to-aortic GTF obtained from the finger can be utilised with equal acceptability in the assessment of vascular function during the administration of vasoactive drugs, as a GTF obtained from the radial artery.

A reasonably strong correlation was found between all waveform parameters obtained from both PPG and SphygmoCor signals. However, the limits of agreement were large, and significant bias existed for most parameters. Certainly the two methodologies cannot be directly interchanged, but both methods appear similarly able to track cardiovascular changes due to vasoactive substances. The doses of drugs given during this study were chosen to achieve large changes in blood pressure, and therefore the hypothesis tested is whether a generalised transfer function holds under haemodynamic extremes. However, it would be valuable to assess whether the PPG can also detect subtle differences in wave shape, such as those achieved by administration of low-dose vasoactive drugs, or found in different patient populations.

The use of fluid-filled manometer tubing for the measurement of arterial pressure is once again a weakness of this study, arguably more relevant in the context of pulse contour analysis, where the gold-standard is the intravascular catheter-tip solid-state transducer, than for measurement of pressure, where it is the regarded as the definitive clinical measure. Measurement error due to sub-optimal damping was minimised during the study by using a short tube length. The increased fundamental frequency of the heart rate during the administration of salbutamol might account for the greater RMSE observed between both WEP and PPG waveforms, and intra-arterial signals, following this drug. With respect to comparison of the brachial WEP signal with the radial intra-arterial wave, cannulation of the radial artery is safer than that of the brachial artery; and therefore ethically more acceptable. The resulting comparison of different anatomical sites may, therefore, partially account for the differences seen between actual and WEP-derived pressure signals, but is unlikely to influence the conclusions reached. As discussed above, the variability in baseline haemodynamic measurements prior to different drugs, does not prevent the study achieving the aim of comparing waveforms under widely varying pharmacologically-induced haemodynamic circumstances.

3.4.3 Conclusion

In conclusion, this study demonstrates that rPTT has a negative correlation with SBP, which although relatively unaffected by vasoactive drugs in some persons, is not reliable enough to enable rPTT to be a surrogate marker of SBP. Furthermore, the significant contribution of PEP to rPTT means that use of the latter parameter as a marker of purely vascular function should be avoided. However, rPTT may have a role in the assessment of BP variability and rapid pressure change. pPTT, rather than rPTT, is associated with DBP/MAP, and so the use of rPTT as a predictor of diastolic or mean pressure is inadvisable.

The study also shows that the supra-systolic WEP signal correlates strongly with the first derivative of the intra-arterial pressure wave. The PPG-to-radial transfer function is minimally affected by drugs. Both methods appear able to detect changes in the pulse waveform induced by vasoactive drugs similar to those measured by

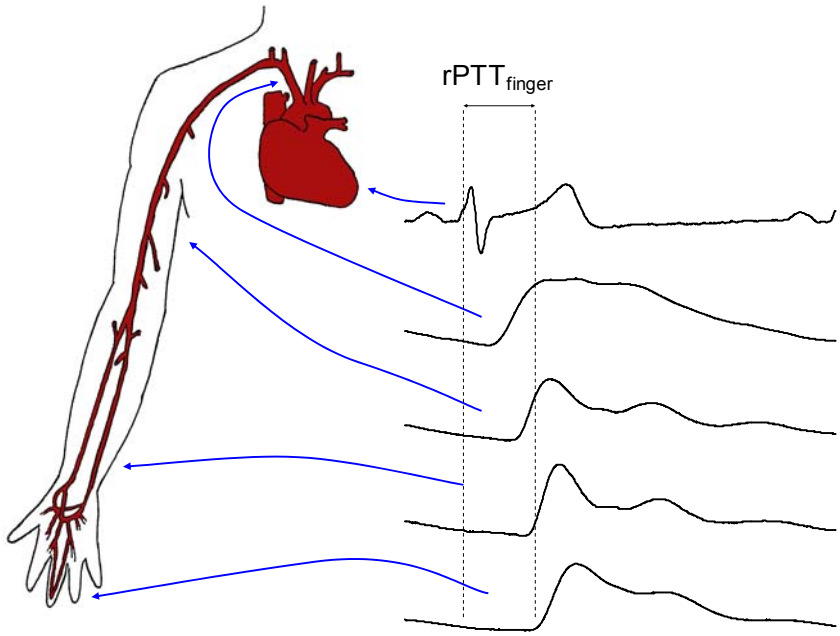
invasive monitoring. Further work is merited to investigate the true nature of the WEP signal, and whether either technique can be used in the study of cardiovascular physiology in disease states, including assessment of cardiovascular risk, disease severity and endothelial function (Safar *et al*. 2001; Wilkinson *et al*. 2002b).

4 EFFECT OF EXERCISE ON VASCULAR AND CARDIAC COMPONENTS OF PULSE TRANSIT TIME

4.1 INTRODUCTION

Estimation of beat-to-beat blood pressure using transit time during exercise has been attempted in the past (Barschdorff & Erig 1998; Carruthers & Taggart 1988), and is a potentially attractive approach as many physiological measurements become difficult during exertion due to movement artefact. Transit time measured from the ECG to finger is comprised of cardiac (pre-ejection period) and vascular components. The vascular path length incorporates a small segment of aorta, large and small conduit arteries, plus the smaller vessels in the hand and finger (Figure 4.1). Exercise results in large changes in vascular and cardiac function, but to date, the manner in which different components of transit time respond to exertion has not been described.

Figure 4.1. *Pulse arrival at different arterial sites*



It is well recognised that arterial stiffness varies throughout the arterial tree. Latham *et al* (1985) elegantly demonstrated that pulse wave velocity varies from proximal to distal aorta. Peripheral vessels have higher proportions of stiffer collagen and smooth muscle than central elastic arteries (Dobrin & Rovick 1969; Harkness *et al*. 1957),

reducing their distensibility (Armentano *et al*. 1995; Learoyd & Taylor 1966). Furthermore, it can be seen from the Moens-Korteweg equation that as peripheral vessels become narrower, pulse wave velocity will increase independent of the arterial wall elastic modulus (section 1.4.1).

Exercise is associated with release of a diverse range of vasoactive substances, such as catecholamines due to sympathetic activation, nitric oxide through changes in endothelial shear stress, and local metabolites from exercising muscle (Kingwell 2000). However, the mechanisms through which vasodilatation occurs may vary between arterial sites. For instance, initial neural stimulation and subsequently the accumulation of vasodilatory metabolites, may result in vasodilatation in microvessels. However, it is the pressure gradient resulting from these changes that induce upstream vasodilatation, due to flow-induced shear stress (Koller & Kaley 1991). Functional diversity can also be seen in the response to drugs – glyceryl trinitrate (GTN) has minimal effects on the aorta and on arterioles (Yaginuma *et al*. 1986), but causes marked arterial dilatation (Westling *et al*. 1984).

It was hypothesised that this structural and functional diversity would mean that changes in different components of transit time in response to exercise would not necessarily be proportional to one another.

Prior to work during exercise, a pilot study was conducted with the aim of establishing the feasibility of measuring transit time over a small distance (the radial artery at the wrist to the fingertip). GTN was used as a means of obtaining changes in conduit arteries, whilst having minimal effects on peripheral vascular resistance and therefore mean distending blood pressure (Yaginuma *et al*. 1986). Previous work by our department (JJ Oliver, personal communication) had suggested that the selected dose of GTN would have minimal effects on blood pressure and conduit and central artery PWV, despite substantially reducing augmentation index (Oliver *et al*. 2005). It was hypothesised that these changes in AIx may be due to alterations in smaller artery PWV, and may therefore be detectable in the hand, independent of larger conduit artery PWV.

4.2 METHODOLOGY

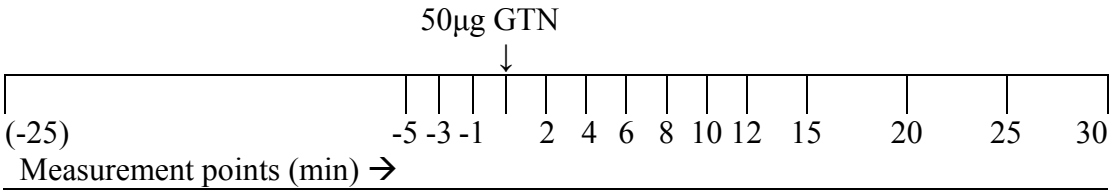
4.2.1 Pilot study of the effect of GTN of on radial-finger pulse wave velocity

9 subjects (2 male), mean age 29.2±5.5 years, participated in an unblinded, uncontrolled study of the effects of GTN on pulse wave velocity in the hand. None were on medications, and one female was a social smoker. Subjects rested supine for 20 minutes prior to measurements commencing. 30-second simultaneous recordings of carotid (Millar tonometer, section 2.5.1), radial (Colin CBM7000, section 2.5.1) and finger (photoplethysmography, section 2.3.2.2) waveforms were made. Wrist and finger measurements were made on the same side. Blood pressure was recorded using an oscillometric brachial sphygmomanometer on the contralateral arm. Distance measurements (for calculation of PWV) were made using straight-line measurements as described in section 2.7.

The study protocol is shown in Figure 4.2. Three baseline measurements were made, each 2 minutes apart, prior to GTN administration. 50µg GTN, made up to 50µL in water, was given sublingually using a pipette. Measurements were subsequently made every 2 minutes initially, and every 5 minutes thereafter.

Baseline values were taken as the average of the three pre-GTN measurements. Change over the course of the study was established by repeated measures ANOVA, with post-hoc analysis by paired t-test examining difference from baseline. Time to peak/trough was calculated by averaging time/magnitude of peaks for each individual.

Figure 4.2. *GTN pilot study protocol*



4.2.2 Effect of exercise on cardiac and vascular components of transit time

9 healthy subjects (4 female), mean age 25.8±5.4 years, were studied in a quiet, temperature controlled environment, following a 15-minute period of rest.

Subjects performed a 3-stage modified STEEP exercise protocol (section 2.9.1.2), using a Kettler SX1 semi-recumbent bicycle ergometer. Haemodynamic measurements were recorded at rest, in the last minute of each exercise phase, and at 2, 5, 10 and 15 minutes post-exercise.

Continuous recordings of bioimpedance (section 2.4.1.2), finger and ear photoplethysmograph waves (section 2.3.2.2), radial artery tonometer waveform (section 2.5.1) and ECG (section 2.3.1.2) were made. Brachial artery waveforms were recorded in the last minute of each experimental measurement phase, using a Millar SPT301 tonometer (section 2.5.1). Brachial blood pressure was measured manually at the same time as brachial waveforms, but in the contralateral arm (section 2.8.1). Signals were digitised at 1 kHz using a National Instruments E-series 6036E PCMCIA data acquisition card (section 2.2), and signal processing was performed offline.

Data were compared across time points using repeated measures ANOVA, with paired t-tests used *post hoc* to compare individual time points.

4.3 RESULTS

4.3.1 Effect of GTN on wrist-finger pulse wave velocity

Subject characteristics and baseline haemodynamic values are given in Table 4.1. Changes in certain parameters are shown graphically in Figure 4.3. No changes were seen in mean pressure ($p=0.18$ by repeat measures ANOVA), systolic ($p=0.67$) or pulse pressure ($p=0.16$) over the course of the study. Post-hoc analysis identified a small increase in systolic (1.9±2.1 mmHg, $p=0.025$) and pulse (3.8±2.8 mmHg, $p=0.004$) pressure at 4 minutes only. Heart rate increased after GTN ($p<0.001$), by an

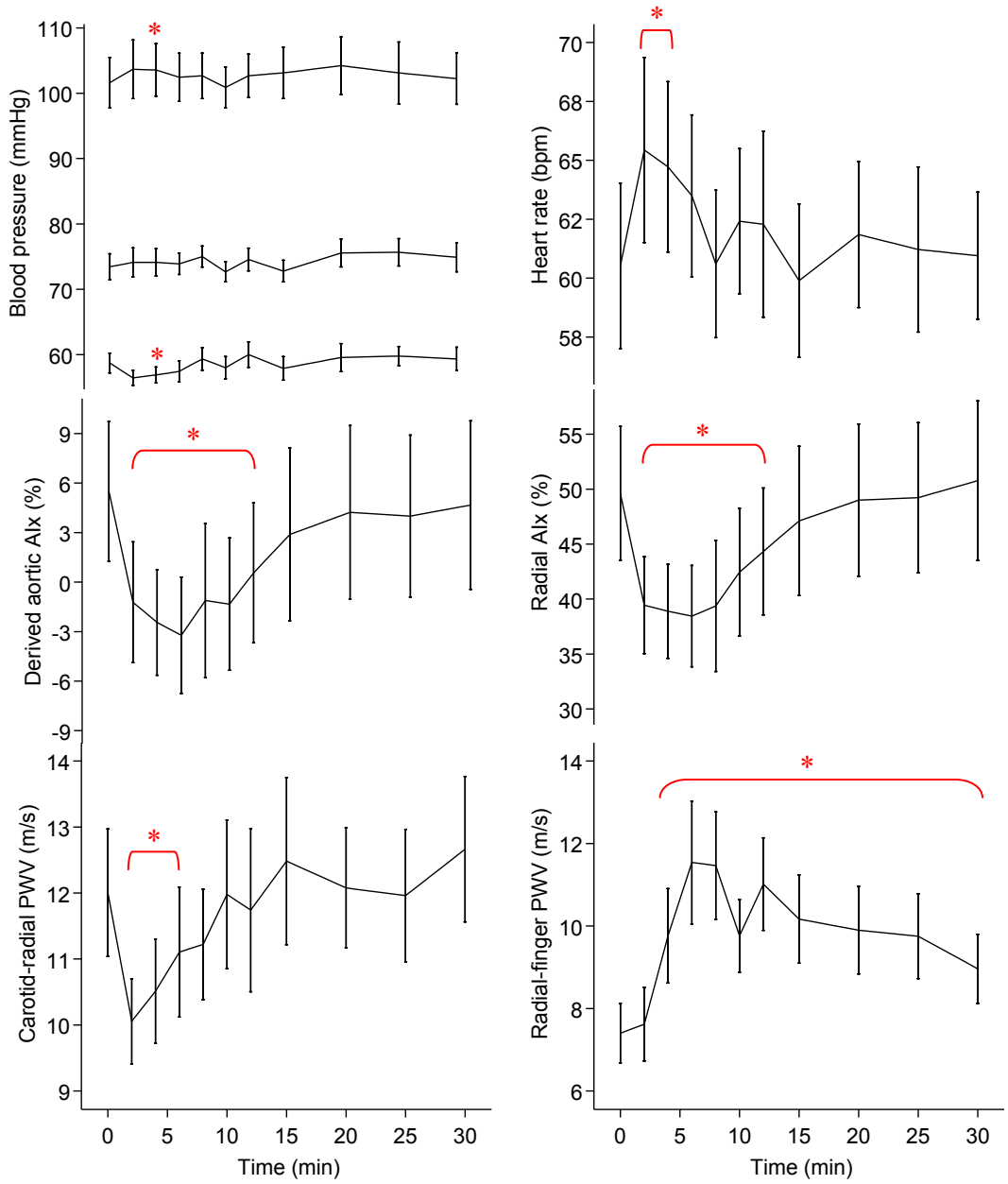
average of 6.8±4.7bpm at 5.8±6.2 minutes. Cardiac output and systemic vascular resistance did not vary during the experiment (p=0.34 and 0.23 respectively).

Central AIx decreased with GTN (p=0.001), by an average of -11.8±5.2 percentage points at 11.3±10.1 minutes. This change persisted after adjustment for HR (p=0.015). Peripheral AIx also decreased (p<0.001), by an average of -14.6±6.1 percentage points at 8.9±8.3 minutes. Carotid-radial PWV fell significantly (p<0.001), by an average of -2.1±1.1m/s at 4.3±4.7 minutes. Radial-finger PWV increased (p<0.001) following GTN, by an average of 5.2±3.2m/s at 8.2±2.7 minutes.

Table 4.1. *Subject characteristics and baseline haemodynamic values*

Parameter	Mean±SD, N(%)
Age (years)	29.5 ± 5.5
Male	2 (22%)
Smokers	1 (11%)
Height (cm)	167 ± 12
BMI (kg/m^2)	22.8 ± 4.4
Carotid-radial PWV (ms^{-1})	12.0 ± 2.9
Radial-finger PWV (ms^{-1})	7.4 ± 2.2
Radial AIx (%)	49.6 ± 18.3
Derived aortic AIx (%)	5.5 ± 12.7
Heart rate corrected aortic AIx (%)	-1.8 ± 10.4
Cardiac output (L/min)	7.9 ± 2.3
Heart rate (bpm)	60.5 ± 10.5
SVR	9.9 ± 2.6
SBP (mmHg)	101.6 ± 11.6
DBP (mmHg)	58.7 ± 4.5
Pulse pressure (mmHg)	42.9 ± 12.2
MAP (mmHg)	73.4 ± 6.0

Figure 4.3. *Haemodynamic responses to 50µg GTN*



*Values are mean±1SE. Time 0 is average of 3 baseline measurements made at -1, -3 and -5 minutes prior to administration of GTN. Blood pressure values are systolic, mean and diastolic. * shows points significantly ($p<0.05$) different from baseline by post-hoc paired analysis.*

4.3.2 Effect of exercise on cardiac and vascular components of transit time

Changes at peak exercise, and significance over the period of exercise are given in Table 4.2, and shown graphically in Figure 4.4.

4.3.2.1 Blood pressure and heart rate

BP increased from 104/67±13/11 (MAP 79±10) mmHg at rest, to 153/66±22/9 (MAP 94±7) mmHg at peak exertion. Diastolic BP did not change over the study. Heart rate increased from 63.6±8.1 to 120±18.3 bpm.

4.3.2.2 ECG Intervals

Baseline QR, RS and QS intervals were 28.3±15.6, 39.1±6.1 and 67.4±19.8 ms respectively. QR, RS and QS intervals did not vary with exercise (p=0.40, 0.45 and 0.48 respectively, rmANOVA).

4.3.2.3 Pulse waveform timing points

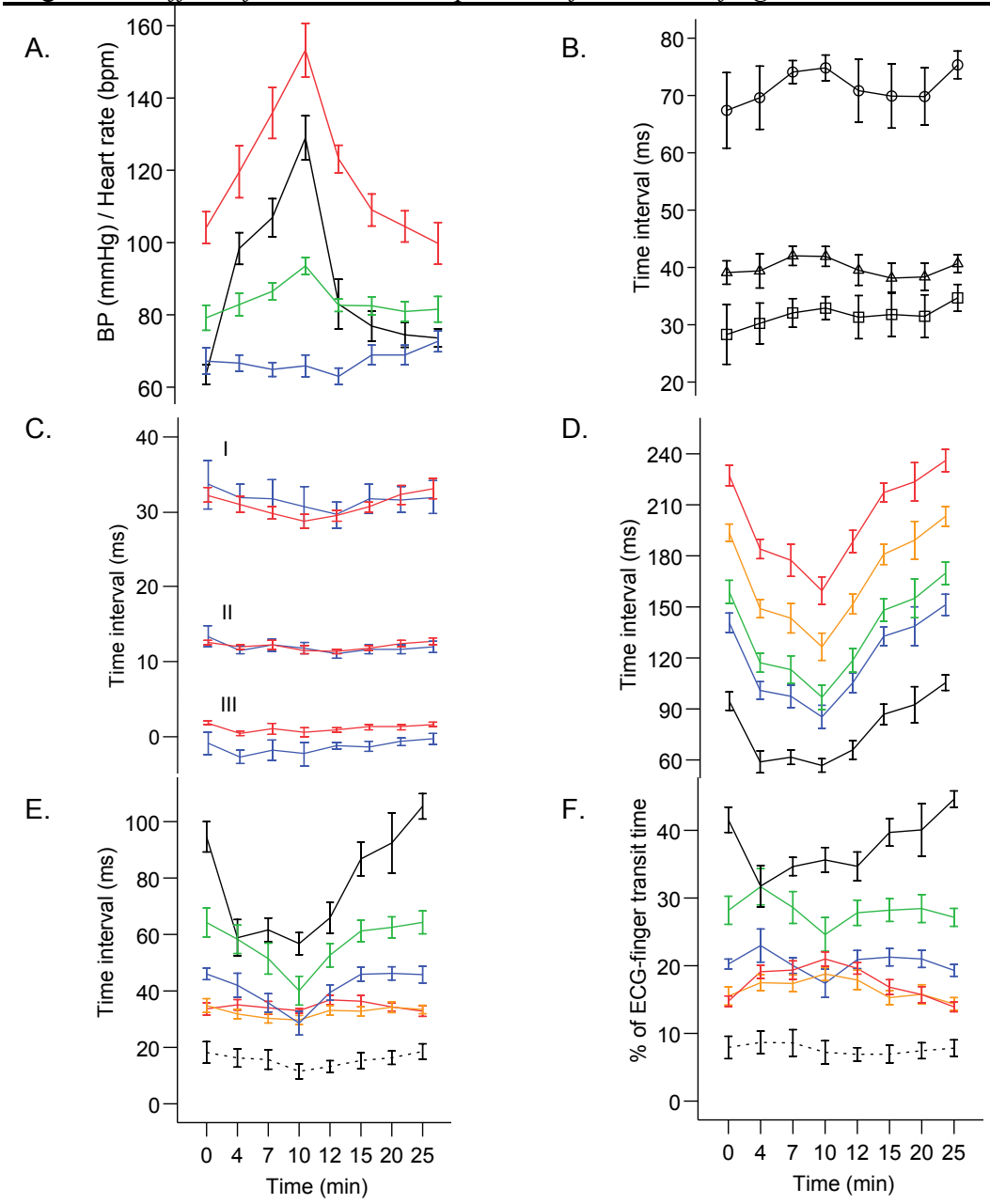
The time difference between the intersecting tangent of the maximum first derivative and intersecting tangent of the maximum second derivative, varied significantly over the experimental period for the finger wave (p=0.006, rmANOVA), decreasing significantly at peak exertion (p=0.029). Variation also occurred with the ear wave, although did not quite reach statistical significance (p=0.056). The time difference between the time of maximum second derivative, and the intersecting tangent of the maximum first derivative, was small (-2.3 to +1.9ms), and did not vary significantly for either finger (p=0.13) or ear (p=0.47) waveforms. The difference between the time of the maximum first derivative and the time of the maximum second derivative, varied significantly during the study for the finger wave (p=0.001), decreasing at peak exercise (p=0.009). No significantly difference was seen for this time delay with the ear wave (p=0.68). Of note, the magnitude of all three time differences was similar for both ear and finger waves, although the difference between maximum second derivative, and the intersecting tangent of the maximum first derivative, was marginally greater for the finger (2.5±2.9 ms, p<0.001). The variances of all three time intervals were considerably greater for the ear compared to finger waves.

Table 4.2. *Baseline and peak changes in timing parameters*

		Baseline	Peak	rmANOVA *p* value
ECG QRS-complex time intervals				
Q-R		28.3 ± 15.6	32.9 ± 6.0	0.40
R-S		39.1 ± 6.1	41.9 ± 5.2	0.45
Q-S		67.4 ± 19.8	74.8 ± 6.7	0.48
Pulse wave time intervals (ms)				
Int. tan. dP^2 – int. tan. dP^1	Finger	12.5 ± 0.9	11.6 ± 1.6*	0.006
	Ear	13.4 ± 4.3	11.8 ± 2.3	0.056
Int. tan. dP^1 – max. dP^2	Finger	1.9 ± 0. 7	0.6 ± 1.9	0.13
	Ear	-0. 9 ± 4.3	-2.3 ± 4.6	0.47
Max. dP^2 – max. dP^1	Finger	32.2 ± 2.8	28.7 ± 2.6†	0.001
	Ear	33.6 ± 9.6	30.6 ± 8.3	0.68
Transit time relative to R-wave (ms)				
dZ/dt int. tan.		94.6 ± 16.1	56.3 ± 10.5†	<0.001
Ear		140.7 ± 17.0	83.9 ± 18.5‡	<0.001
Brachial		158.9 ± 20.3	96.9 ± 20.3‡	<0.001
Radial		193.7 ± 15.1	122.0 ± 25.2‡	<0.001
Finger		227.3 ± 18.2	155.3 ± 24.9‡	<0.001
Time intervals (ms)				
dZ/dt int. tan. – ear		46.0 ± 6.1	27.6 ± 11.6†	<0.001
dZ/dt int. tan. – brach		64.2 ± 15.4	40.1 ± 20.3‡	<0.001
Ear – brachial		18.2 ± 11.4	11.5 ± 7.5*	0.019
Brachial – radial		34.8 ± 7.4	29. 7 ± 4.5	0.064
Radial – finger		33.6 ± 6.5	33.3 ± 2.5	0.094
Time intervals as % of $rPTT_{finger}$				
R-wave – dZ/dt int. tan		41.5 ± 5.6	36.5 ± 5.5	0.001
dZ/dt int. tan. – ear		20.2 ± 2.2	17.3 ± 5.6	0.12
dZ/dt int. tan. – brach		28. 2 ± 6.2	24.6 ± 7.1	0.093
Ear – brachial		7.9 ± 5.0	7.2 ± 4.9	0.66
Brachial – radial		15.5 ± 4.0	18.8 ± 2.7*	<0.001
Radial – finger		14.8 ± 2.3	21.9 ± 3.9‡	<0.001

*Values of various transit time measures at baseline and peak exertion. Values are mean±SD. Int. tan. dP^1/dP^2 and max. dP^1/dP^2 correspond to timing points using an intersecting tangent through, or the maximum point on, the first and second derivatives respectively. rmANOVA indicates significant change over entire experimental period. Significant change at peak exercise cf. baseline: *p<0.05; † p<0.01; ‡ p<0.001.*

Figure 4.4. *Effect of exercise on components of R-wave-to-finger transit time*



Changes in different components of transit time at rest (0 min), increasing exercise (4-10 min) and recovery (12-25 min). Values are mean ± standard error.

A. *BP and HR; red, SBP; green, MAP; blue, DBP; black, HR*

B. *Components of QRS complex; □ QR interval; △ RS interval; ○ QS interval*

C. *Difference between timing points on finger (red) and ear (blue) waves; I, maximum first and second derivatives; II, intersecting tangents of first and second derivatives; III, maximum second derivative and intersecting tangent of first derivative.*

D. *Timing of different components of transit time relative to ECG R-wave; black, bioimpedance B-point; blue, ear; green, brachial; orange, radial; red, finger.*

E. *Duration of different time interval components of transit time; black, R-wave to B-point; blue, B-point to ear; green, B-point to brachial; dashed black, ear to brachial; orange, brachial to radial; red, radial to finger.*

F. *As for E, but intervals expressed as percentage of total R-wave-to-finger time.*

4.3.2.4 Timing at Differing Vascular Sites

ECG R-wave to B-point (as determined by intersecting tangent), and ear, brachial, radial and finger (as determined by intersecting tangent of the maximum first derivative) transit times, varied over the course of the experiment ($p<0.001$, rmANOVA), decreasing from baseline at peak exercise for all parameters ($p\leq0.001$, paired t-test).

The time interval between bioimpedance B-point and pulse wave leading edge at the ear and brachial artery, in addition to the brachial-to-ear interval, showed a significant change over the course of the experiment ($p<0.001$, rmANOVA), with decreases from baseline to peak exertion ($p\leq0.02$). The brachial-radial transit time did varied slightly over the course of the experiment ($p=0.064$, rmANOVA), with a trend towards a small decrease by 4±5ms at peak exertion ($p=0.14$, paired t-test). The radial-finger transit time also showed no significant variation over the duration of the study ($p=0.094$, rmANOVA), although post-hoc analysis suggested a slight increase immediately in recovery (37±5 ms), relative to baseline (34±6 ms, $p=0.079$) and peak exercise (33±3 ms, $p=0.012$).

When expressed as a percentage of the overall r-wave-to-finger transit time, there were decreases in the proportional contribution of PEP ($p=0.001$), and B-point to ear ($p=0.12$) and brachial ($p=0.093$) intervals, no change in the ear-to-brachial interval ($p=0.66$), and increases in brachial-to-radial ($p<0.001$) and radial-to-finger ($p<0.001$) intervals.

4.4 DISCUSSION

4.4.1 GTN pilot work

This study demonstrated that GTN induced significant increases in PWV measured between the radial artery at the wrist and the finger. These findings were consistent across individuals, and detectable despite small changes in transit time in the order of 10ms, albeit a significant proportion of the baseline transit time of around 30ms.

The increase in radial-finger PWV was unexpected, as either vasodilatation or a fall in peripheral pressure – both potential effects of GTN – were anticipated to result in a decrease in this measure. The other haemodynamic changes found are supportive of a normal response to GTN. The changes in blood pressure and augmentation index were both in line with previous work (Oliver *et al*. 2005). Other than a small change at 4 minutes, the former did not significantly vary over the course of the study, and the latter (measured peripherally and derived centrally) decreased to the degree expected and returned to baseline by the end of the study. The fall in carotid-radial PWV was not in agreement with previous findings, although nonetheless consistent with the response that might be expected due to vasodilatation in the muscular arteries of the arm (Westling *et al*. 1984). Indeed, brachial artery dilatation in response to GTN is a well recognised phenomenon employed widely as an endothelial independent control for flow-mediated dilatation studies (Corretti *et al*. 2002). Of note, prior work carried out in our department utilised the commercial SphygmoCor (AtCor Medical) system, which employs sequential waveform recordings and uses a handheld tonometer at both sites. It is possible that the methodology used in the present study (simultaneous measurement of both waveforms, with an automated rather than hand-held tonometer at the wrist) improved the quality and accuracy of wave recordings, and thus the sensitivity for changes in carotid-radial PWV.

The time course of the radial-finger PWV change appeared to be similar to the changes in augmentation index, and both measures appear to lag marginally behind the conduit artery PWV and heart rate changes. They also show persistence after the normalisation of carotid-radial PWV has occurred. It is possible that this is accounted for by local changes in flow in the smaller peripheral vessels (such as those in the hand), resulting in a release over the next few minutes of vasoactive substances which mediate the changes observed, in contrast to the large conduit vessel where GTN has a direct, and therefore more rapid, action. Alternatively, the relatively earlier effects of GTN on proximal conduit vessels, or of increased heart rate effecting an alteration in viscoelastic arterial wall behaviour due to changes in the rate of change of pressure, may themselves result in a delayed release of vasoactive mediators. Such mediators may have downstream effects manifest in the changes in

augmentation index and radial-finger PWV. The similarity in time scales between augmentation index and radial-finger PWV responses suggests that changes in the former in response to GTN are due to the behaviour of small arteries, such as those in the hand, rather than larger conduit vessels.

Why an increase, rather than decrease, in radial-finger PWV? It is possible that the effects of blood viscosity – a determinant of PWV from the Moens-Korteweg equation – may be of relevance in this vascular region, with vasodilatation resulting in a decrease in viscosity and corresponding rise in PWV. Alternatively, dilatation of proximal feed arteries might be expected to result in increased downstream flow, regardless of constant mean systemic pressure and peripheral vascular resistance. It is possible that the increased flow results in direct mechanical distension of smaller distal vessels, accompanied by increased stiffness negating any possible local vasodilatation. The increase in local stiffness would be associated with an increase in PWV, despite mean distending pressure remaining constant.

Clearly, these suggestions are entirely speculative, although do point to the potential for intriguing further work in this area. Of course, this study did not have a placebo control, and it may be argued that some of the changes in radial-finger PWV (and in particular the fact that this measure remained elevated at the end of the monitoring period) may reflect environmental adaptation over the course of the study, such as warming of the hands. However, it would appear unlikely that this accounts for the relative rapidity of onset of the response, and the time course is highly suggestive of GTN being causative. Regardless of the lack of control, and the exact mechanism underlying the responses demonstrated, the study suggests that the technique employed is able to detect small changes in transit time in a local region, and may therefore be extended to the study the effects of exercise.

4.4.2 Effect of exercise on vascular and cardiac components of transit time

Most measurements of PEP commence at the Q-wave (unless absent), but for pulse transit time measurements, the R-wave is often used as its peak is relatively simpler to identify. This raises the question of whether the Q-R interval remains constant.

The current study found no significant change in Q-R interval with exercise. The Q-R interval was defined for the purposes of the present work as the period between nadir of Q-wave to peak of R-wave, and accounts for perhaps less than a third of the QRS duration. However, identification of the start of the Q-wave is actually more complicated than identification of the simple minimum of the Q-wave nadir. This is because it relies on accurately determining the ECG iso-electric line, and measuring the intersection of the start of the Q-wave with this line. This is particularly difficult if the iso-electric line is affected by baseline wander, as may occur during exertion. A similar problem applies to identifying the end of the S-wave. It has been shown that minimal change in the Q-R interval occurs with submaximal exercise (1.3 ± 2.7ms) (Goldberger & Bhargava 1983) or adrenaline (Mezzacappa *et al*. 1999), but there is little other published work that specifically discusses changes in the Q-R interval. Increases in QRS duration of 21ms were found in subjects with marked myocardial ischaemia during exercise (Michaelides *et al*. 1993), which probably equates to considerably less than 10ms change in Q-R interval, but such changes do not appear to occur in health.

The lack of change found in the present study may reflect the definition of Q-R described above (i.e. the use of the nadir rather than start of the Q-wave) resulting in a smaller Q-R time period that would be more difficult to identify any change in. It can probably be assumed that changes in Q-R interval will occur in proportion to changes in the overall QRS duration. However, the Q-S period, which forms a more substantial proportion of the true QRS duration, was also not found to change significantly. It can be concluded that exercise in healthy individuals does not significantly alter the individual QRS components, and that use of the R-wave rather than Q-wave is acceptable due to the constancy of the difference between them.

The intersecting tangent method was used to determine the timing point on the bioimpedance waveform, as this was established in the earlier methodological development to be the most robust measure of PEP, and varied minimally relative to other points on the waveform during exertion (section 2.4.2.3). It is worthwhile noting, however, that PEP is comprised of both the electromechanical (EM) delay, and the period of isovolumic contraction. The measurement of the pre-ejection

period rarely involves sub-dividing it into its component subintervals, and this was not possible during the present study. However, Martin *et al* (1971) used several different acute interventions to induce changes in ventricular function. They found that externally measured PEP (which includes the EM delay) had a close linear correlation with invasively determined actual isovolumic contraction time. Similar results were found by Mezzacappa *et al* (1999) with the administration of adrenaline. This suggests that most changes in PEP are due to variation in isovolumic contraction time.

Pulse wave velocity is frequency dependent; that is, the different harmonic components of the wave travel at different speeds. Measurements are therefore best made on high frequency parts of the waveform, where variations in apparent phase velocity are minimal. The foot of the wave is preferable as it is usually least affected by wave reflections (Hayward *et al*. 2002; Nichols & O'Rourke 1998), although the leading-edge can also be used. Accurately identifying the wave foot can be difficult, especially when there is a degree of baseline signal noise. The maximum first derivative (i.e. maximum rate of change) often occurs around halfway up the leading edge of the pulse wave (Greenfield, Jr. & Fry 1965). Identifying it is independent of finding the bottom or top of the wave, but it may be subject to changes in wave shape (Hayward *et al*. 2002). The peak of the second derivative (acceleration waveform) can be used to determine the foot of the pulse wave (Chiu *et al*. 1991). The intersecting tangent method uses a tangent drawn through a point on the leading edge, and determines the time at which this line intersects the baseline. The point on the leading edge is commonly taken as the maximum rate of change (Laszt & Muller 1952), although a tangent through the maximum second derivative also is described above. A number of different approaches have been compared (Chiu *et al*. 1991), and an intersecting tangent using the maximum first derivative is generally considered the most reliable time point.

The current study found a decrease in the time delay between the maximum first derivative and the maximum second derivative, and between the intersecting tangents of these two points, for the finger only, and is due to the steepening of the leading edge with increased cardiac contractility during exertion. There were similar trends

with the ear wave, and the lack of statistical significance echoes the greater variability in the pulse contour at this measurement site. This is particularly true of the middle part of the leading edge, as reflected in the high standard deviation of the difference between the timing of maximum first derivative and maximum second derivative. This variability is probably multifactorial, including variation in baseline perfusion of the pinna, varied blood flow increases in response to exercise, and inconsistent placement of the probe on the ear lobe. The proximity in time of the maximum second derivative and the intersecting tangent of the maximum first derivative accounts for the inability to detect any change in the interval between these two measures. The similarity in the overall time differences between ear and finger waves, reflects the relative consistency of the leading edge throughout the arterial tree (McDonald 1968; Nichols & O'Rourke 1998), in contrast to the later part of the waveform which is more prone to variability due to a combination of the effects of pulse wave amplification and wave reflections.

At rest, the R-wave-to-finger transit time was accounted for by around 42% PEP, 28% proximal vascular component (B-point to brachial pulse), and 15% for both brachial-to-radial and radial-to-finger intervals. There were decreases in the PEP and dZ/dt-to-brachial intervals of 38±22 and 27±12 ms respectively during exercise. The lack of change in distal transit time components corresponded to an increase in the proportion of R-wave-to-finger transit time accounted for by these intervals.

In vitro experiments have allowed the study of the independent effects of changes in preload, afterload and contractility on the pre-ejection period (Nakamura *et al*. 1983). An increase in preload reduces the PEP, whereas a rise in afterload prolongs the PEP. An isolated increase in contractility reduces PEP. *In vivo*, these parameters are inextricably linked, but PEP appears to be primarily affected by cardiac contractility (Ahmed *et al*. 1972; Belz 1995). An increase in contractility, as occurs during exercise, increases the rate of change of left ventricular pressure (*dP/dt*), and thus shortens isovolumic contraction and consequently the PEP. Exercise-induced peripheral arterial dilatation may also contribute to the fall in PEP.

With respect to vascular components of transit time, there was only a substantial decrease in the B-point-to-brachial interval. This arterial segment encompasses a short portion of aorta, subclavian artery and the majority of the brachial artery, varying between large elastic vessel proximally, and large muscular conduit vessel distally. It is probable that the decrease in transit time proximally is because of an increase in pulse wave velocity reflecting increased arterial stiffness due to increased mean distending pressure. Peripheral resistance is an important determinant of mean pressure, and dynamic exercise causes a decrease in peripheral resistance. However, mean pressure is also a function of cardiac work, and can therefore be increased during exercise due to catecholamine-mediated increased cardiac contractility and the subsequent rise in systolic pressure. It is possible to find a decrease in mean pressure during exercise when measured using sphygmomanometry. However, this can be due to underestimation of diastolic pressure by this technique, and intra-arterial pressure measurement during exertion often demonstrates an increase in diastolic BP (Palatini 1994).

Mean pressure remains relatively constant throughout the arterial tree, with the exception of the small resistance vessels, and this remains true during exercise (Rowell *et al*. 1968). It is therefore improbable that differences in mean pressure account for differences between the proximal pathway and, in particular, the brachial-to-radial component. Differences in pulse pressure between sites also appear unlikely to account for the lack of change in transit time in the brachial-to-radial segment. Pulse amplification occurs in the peripheries, and this effect is amplified by exercise (Rowell *et al*. 1968). An increase in pulse pressure results in a greater rate of change of pressure and, due to the viscoelastic nature of the arterial wall, a greater increase in stiffness. This would correspond to an increase in pulse wave velocity and corresponding decrease in transit time in this arterial segment, contrary to the observations made above. This suggests that it is differences in the change in intrinsic stiffness of the arterial wall in response to exercise that account for the discrepancies between arterial sites. Nitric oxide is a key regulator of vascular function during exercise (Kingwell 2000). Exercise increases blood flow, resulting in increased shear stress. This induces nitric oxide release by endothelial cells, and leads to vasodilatation. However, inhibition of basal nitric oxide has been shown to

have no effect on aortic PWV, other than through changes in mean distending pressure (Stewart *et al*. 2003). It is possible that a decrease in intrinsic wall stiffness, perhaps mediated by nitric oxide, occurs in the peripheries rather than centrally, offsetting any increase in stiffness due to the rise in mean distending pressure. This may account for the lack of change in transit time in the brachial-radial arterial segment, in contrast to the decrease observed proximally.

The lack of change in radial-to-finger transit time is perhaps unexpected given the large haemodynamic changes that occur with exercise, including thermoregulatory adaptations involving smaller blood vessels. Ostensibly, it might be concluded that the increase in stiffness resulting from the increase in mean arterial pressure with exercise, offsets the decrease in stiffness due to exercise-induced vasodilatation. Following exercise, mean pressure would fall, while vasodilatation persists, resulting in a prolongation of transit time. Although vasodilatation with GTN appears to cause the opposite effect, one explanation might be that exercise simply has a more profound effect at this arterial site, offsetting the effects of increased flow and pressure.

The mechanisms underlying these responses may have particular relevance for the study of exercise transit time in persons taking vasoactive drugs. Beta-blockers, for instance, can result in a prolongation in PEP following catecholamine stimulation, due to α-mediated vasoconstriction (Obrist *et al*. 1979); the usual response is PEP shortening due to β1 stimulation and increased contractility. Furthermore, peripheral vasoconstriction may result in changes in transit time in distal arterial segments. In contrast, calcium channel blockers may result in greater arterial vasodilatation. Furthermore, rate-limiting calcium channel blockers may also significantly reduce cardiac contractility and heart rate, again prolonging PEP. Nitrates and other nitric oxide donors may cause similar effects to those observed in the GTN pilot work on the radial-to-finger path. It is clear, therefore, that the composite nature of the $rPTT_{finger}$ measurement may be affected in a multitude of ways, and this may form the basis for future work.

4.4.3 Conclusion

In conclusion, it has been shown that the principal decrease in $rPTT_{finger}$ occurring during exercise is due to a shortening of the PEP and an increase in PWV in larger vessels. Although it has proven possible to measure changes in transit time between wrist and finger, changes in PWV in this anatomical region do not appear to contribute to overall changes in $rPTT_{finger}$ during exercise. Further work may be indicated to explore, perhaps using pharmacological tools, the reasons for these differences.

5 EXERCISE PULSE WAVE VELOCITY MEASUREMENT USING PHOTOPLETHYSMOGRAPHY

5.1 INTRODUCTION

Pulse wave velocity (PWV) is a widely used surrogate marker of arterial stiffness (Woodman *et al*. 2005). Numerous studies have now shown a positive association between PWV and adverse cardiovascular outcome. Central PWV has been correlated with cardiovascular mortality in end-stage renal failure (Blacher *et al*. 1999) and hypertension (Laurent *et al*. 2001), in addition to other unfavourable outcomes such as increased coronary events (Boutouyrie *et al*. 2002). Central PWV is increased in the presence of a number of cardiovascular risk factors, including age (Vaitkevicius *et al*. 1993), smoking (Zeiher *et al*. 1995), hypercholesterolaemia (Aggoun *et al*. 2000; Lehmann *et al*. 1992b) and type II diabetes (De Vriese *et al*. 2000; Lehmann *et al*. 1992a). Conduit artery stiffness would also appear to be of importance. Reduced peripheral artery compliance has been associated with atherosclerotic plaque burden in coronary artery disease (Syeda *et al*. 2003), increased lipoprotein (a) levels (Schillinger *et al*. 2002) (associated with endothelial dysfunction) and risk of cardiovascular events (Grey *et al*. 2003). Furthermore, augmentation index, which is dependent on both central and peripheral vascular stiffness, has also been associated with increased mortality in end-stage renal disease, independent of central PWV (London *et al*. 2001), and with risk of coronary artery disease (Weber *et al*. 2004). This supports the argument that peripheral vascular compliance may have an important association with cardiovascular outcome.

Importantly, these studies have involved the measurement of arterial stiffness at rest. However, arterial stiffness has particular relevance to exercise. Resting large artery stiffness, measured by various techniques including PWV, has been found to predict exercise tolerance in healthy subjects (Eugene *et al*. 1986), athletes (Kingwell *et al*. 1995; Tarnawski *et al*. 1994), and patients with heart failure (Bonapace *et al*. 2003) or coronary artery disease (Kingwell 2002). Aerobic exercise training has been shown to increase arterial compliance after 1 week (Cameron & Dart 1994).

Impaired exercise capacity has also been associated with reduced carotid artery distensibility in essential hypertension (Olsen *et al*. 2001). Furthermore, both absolute and relative increases in systolic blood pressure during exercise are recognised as better predictors of cardiovascular risk than resting blood pressure (Filipovsky *et al*. 1992; Mundal *et al*. 1994; Mundal *et al*. 1996). Increases in exercise diastolic pressure have also been associated with the presence of risk factors (Acanfora *et al*. 1991; Akhras *et al*. 1985), although have not been shown to be useful prognostically (Mundal *et al*. 1994).

BP and compliance are inextricably linked. The influence of resting arterial stiffness on exercise capacity, and the association of both resting stiffness and exercise BP with adverse cardiovascular outcome, suggests that changes in stiffness during exercise may be of importance. To date there is no established means of doing so, as most technologies are adversely affected by motion artefact. In section 4.3.2, it was shown that the radial-finger transit time is influenced minimally by exertion. It was decided to compare the use of photoplethysmography against applanation tonometry, as a means of measuring conduit vessel PWV during exertion. The technique was then applied in a small study comparing subjects with and without a family history of cardiovascular disease.

5.2 METHODS

5.2.1 Validation of exercise pulse wave velocity as determined by photoplethysmography

18 healthy, non-smoking subjects, aged 19 to 30 years, were studied in a quiet, temperature controlled environment, following a 20-minute period of rest. Volunteers were allowed a light meal not more than 1 hour prior to the study, and were asked to avoid vigorous exercise on the day of the study prior to participating.

Subjects performed a 3-stage exercise protocol, using a semi-recumbent bicycle ergometer (Kettler, SX1, section 2.9), with forearms supported horizontally at the level of the xiphisternum. Each exercise stage was heart rate targeted, lasting 3 minutes each, with target heart rates of 50-60%, 60-70% and 70-80%, as described in section 2.9.1.3. Haemodynamic measurements were recorded at rest, in the last

minute of each exercise phase, and at 3, 5 and 10 minutes post-exercise. Blood pressure was recorded using a manual sphygmomanometer (section 2.8.1). Pulse wave velocity was recorded using photoplethysmography at the right index finger and right ear lobe, and by applanation tonometry at the right carotid and radial arteries. All waveforms were recorded simultaneously using a MacLab/400 system (section 2.2).

Data were compared across time points using repeated measures ANOVA, with paired t-test used *post hoc* to compare individual time points.

5.2.2 Effect of family history of cardiovascular disease on exercise pulse wave velocity

Healthy, non-smoking male and female volunteers were recruited to participate in a maximal exercise test. Participants were allowed a light meal not more than 2 hours prior to the study. A positive family history of cardiovascular disease was defined as a 1st degree relative developing ischaemic heart disease, stroke, peripheral vascular disease or hypertension aged under 60 (males) or 65 (females) years of age.

Subjects performed a maximal exercise stress test, using a semi-recumbent bicycle ergometer (Kettler SX1 or Lode Rehcor, section 2.9.1.1). Haemodynamic measurements were made at rest, every 3 minutes throughout exercise and for the first 15 minutes of recovery. Blood pressure was recorded manually. Pulse wave velocity was measured as described above.

Comparisons were made between family history groups by unpaired t-test.

5.3 RESULTS

5.3.1 Validation of exercise pulse wave velocity as determined by photoplethysmography

Average subject age was 21.9±2.8 years. 9 subjects were male. Median workloads at the 3 exercise stages were 50W, 110W and 170W. Haemodynamic measurements are shown in Figure 5.1, and are discussed below.

Carotid-radial transit time (TT_{CR}) changed significantly from a baseline of 80.1±5.5ms over the experimental time course (p=0.029, repeated measures ANOVA). TT_{CR} was significantly (p<0.01, paired t-test) decreased from baseline at all exercise time points. Peak change occurred at stage 3 of exercise (68.2±8.4ms, p=0.001), and TT_{CR} had returned to baseline by 5 minutes recovery.

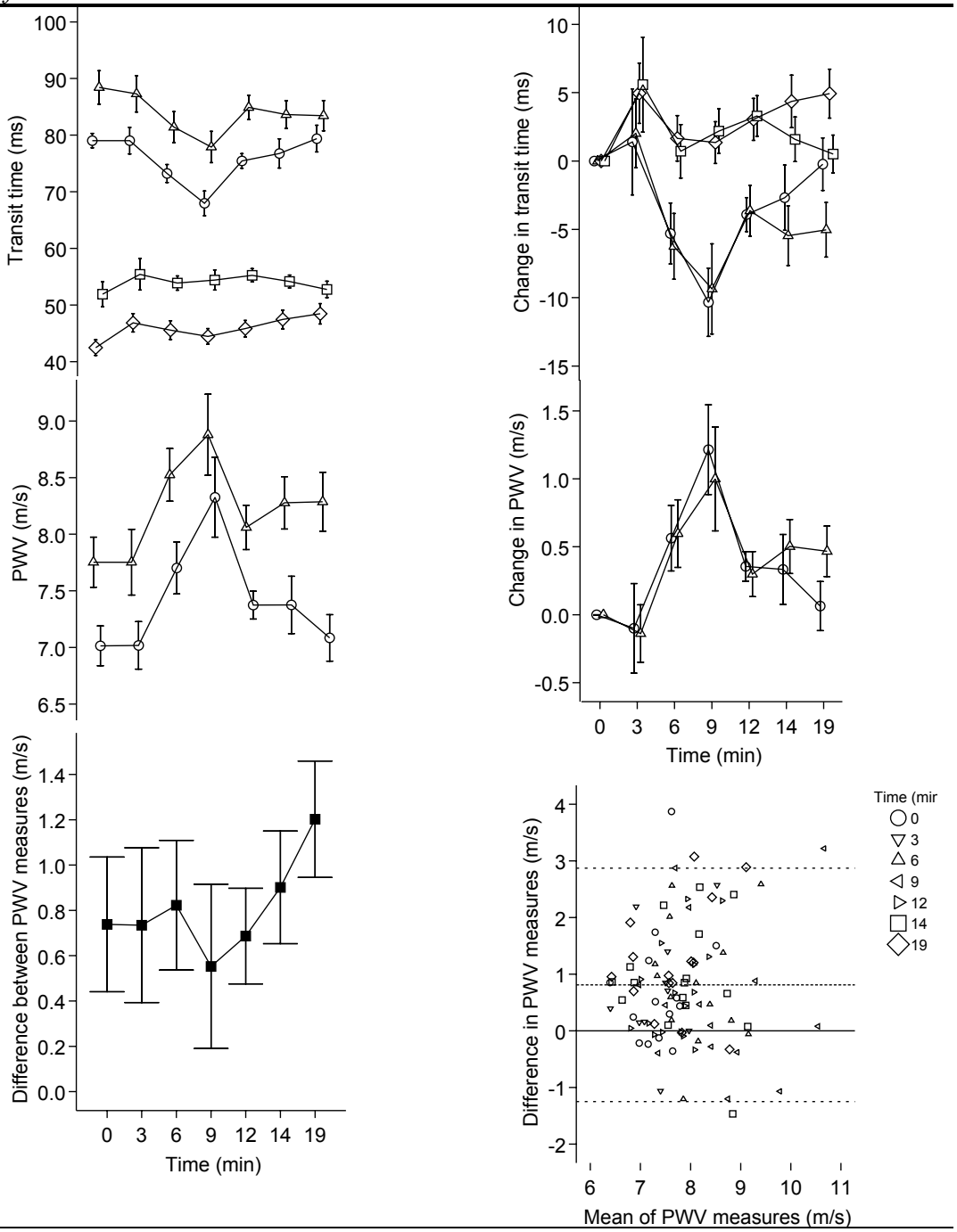
Ear-finger TT (TT_{EF}) also significantly changed from a baseline of 88.4±11.0ms over the course of the study (p=0.01, rmANOVA). TT_{EF} was significantly decreased at stages 2 (81.4±10.8ms, p=0.027) and 3 (78.3±11.1ms, p=0.016) of exercise, and remained depressed at 10 minutes recovery (82.9±9.4ms, p=0.027).

Carotid-ear (TT_{CE}) and radial-finger (TT_{RF}) transit time remained relatively constant over the period of exercise and recovery. However, although no significant changes in TT_{RF} were seen (p=0.12, rmANOVA), TT_{CE} showed slight variability over the study period (p=0.009, rmANOVA). Post-hoc analysis demonstrated that this was entirely accounted for by a small increase in TT_{CE} at the end of recovery, relative to baseline (+4.5±6.4ms, p=0.02).

TT_{EF} was significantly greater ($p \leq 0.022$) than TT_{CR} at all time points, with the exception of the end of recovery (p=0.06), the latter accounted for by a slight increase in TT_{CE}. However, the difference between these two TT measures did not vary over the course of the experiment (p=0.47, rmANOVA). Furthermore, the change from baseline in both TT_{EF} and TT_{CR} was not statistically different (p=0.12, rmANOVA) between the two methods.

Both PWV_{CR} and PWV_{EF} were significantly (p<0.05) increased at stages 2 and 3 of exercise. PWV_{CR} increased from 6.89±0.67ms^{-1} at rest to 8.29±0.63ms^{-1} at peak exercise (p=0.002). PWV_{EF} increased from 7.77±0.89ms^{-1} at rest to 8.85±1.42ms^{-1} at peak exercise (p=0.024). PWV_{CR} had returned to baseline after 5 minutes recovery, but PWV_{EF} was still elevated at 10 minutes recovery (8.29±0.94ms^{-1}, p=0.027).

Figure 5.1. *Effect of exercise on photoplethysmograph and tonometers measures of transit time*



Changes in different measures of transit time at rest (0 min), increasing exercise (3-9 min) and recovery (12-19 min). Values are mean ± standard error. Panels A to E, △ ear-finger; ○ carotid-radial; ◇ carotid-ear; □ radial-finger A*, absolute values of transit time.* B*, change from baseline of transit time.*C*, absolute values of PWV*. D*, change from baseline of PWV*. E*, difference between carotid-radial and ear-finger PWV*. F*, Bland-Altman plot comparing carotid-radial and ear-finger PWV; different symbols represent different experimental time points.*

Overall, PWV_{EF} was significantly higher than PWV_{CR} by an average of $0.81\pm1.05ms^{-1}$ ($p<0.001$, paired t-test). This difference was not significant at peak exertion (8.9 ± 1.3 vs. 8.3 ± 1.3 ms^{-1}, $p=0.15$), and was greatest at 10 minutes recovery ($1.2\pm1.0ms^{-1}$, $p<0.001$). However, the degree of difference between the two measures did not vary over the experiment ($p=0.42$, rmANOVA). Furthermore, the change from baseline did not vary significantly between PWV_{CR} and PWV_{EF} over the duration of the experiment ($p=0.20$, rmANOVA).

5.3.2 Effect of family history of cardiovascular disease on exercise pulse wave velocity

Subject characteristics are shown in Table 5.1. Subjects were well matched, although the negative family history group were on average slightly older. Haemodynamic parameters are shown in Table 5.2. There were no significant differences in baseline blood pressure (including mean pressure), heart rate or pulse wave velocity. Furthermore, no significant differences were found between these parameters at peak exercise. However, pulse wave velocity was slightly lower at rest and higher at maximum exertion in the positive family history group, resulting in a significantly greater ($+1.18 \pm 0.54$ ms^{-1}, $p=0.035$) increase in PWV in subjects with a positive family history.

Table 5.1. *Subject characteristics*

	Cardiovascular family history		
	Negative (N=24)	Positive (N=14)	*p* value
Male	13 (54%)	6 (43%)	0.28*
Age (years)	24.5 ± 2.6	22.8 ± 1.5	0.023
Weight (kg)	67.3 ± 8.4	65.4 ± 12.3	0.61
BMI (kg/m^2)	23.8 ± 4.1	22.0 ± 2.8	0.21
Peak workload (W)	234 ± 83	251 ± 94	0.42
BP (mmHg)	123/73 ± 16.5/7.6	120/74 ± 11.2/5.9	>0.51

*Values are mean ± standard deviation or N (%). * binomial test.*

Table 5.2. *Effect of cardiovascular family history on haemodynamic responses to exercise*

		Family history			
		Negative	Positive	Difference	p value
Blood pressure (mmHg)	Baseline	123/73 ± 16.5/7.6	120/74 ± 11.2/5.9	-3.4/+0.9 ± 5.2/2.5	>0.51
	Peak exercise	183/71 ± 30.9/13.7	179/77 ± 15.9/17.5	-4.6/+5.7 ± 8/5.4	>0.29
	Change	62/-1.5 ± 23.5/14.3	59/3.2 ± 14.9/18.6	-2.9/+4.7 ± 6.6/5.7	>0.41
Mean arterial pressure (mmHg)	Baseline	92 ± 7.3	94 ± 7.6	+2.4 ± 2.6	0.30
	Peak exercise	113 ± 19.1	121 ± 14.5	+7.9 ± 6.2	0.14
	Change	21.5 ± 18.1	26.8 ± 14.5	+5.3 ± 5.9	0.66
Heart rate (bpm)	Baseline	68 ± 16.3	73 ± 15.1	+5.7 ± 5.4	0.37
	Peak exercise	160 ± 22.2	169 ± 12.6	+8.9 ± 5.8	0.21
	Change	93 ± 22.3	96 ± 18.6	+3.1 ± 7.2	0.38
Pulse wave velocity (ms^{-1})	Baseline	11.3 ± 1.7	10.6 ± 1.6	-0.67 ± 0.57	0.25
	Peak exercise	13.1 ± 1.8	13.6 ± 2.3	+0.52 ± 0.68	0.46
	Change	1.8 ± 1.4	3.0 ± 1.9	+1.18 ± 0.54	0.035

Values are mean±SD. Difference is mean±SD between family history groups. Significance is calculated by unpaired t-test.

5.4 DISCUSSION

This study demonstrated that relative changes in PWV during exercise, measured using photoplethysmography at the finger and ear, closely matched changes in PWV determined by tonometry at the carotid and radial arteries. However, the former method consistently overestimated carotid-radial PWV. Furthermore, the limits of agreement between methods were substantial. As was noted in Chapter 4, there was minimal change in the distal radial-finger and carotid-ear components. The application of this methodology in a small population with and without a family history of cardiovascular disease, suggests that change in PWV may be positively associated with increased cardiovascular risk, independent of BP change.

5.4.1 Validation of ear-finger PWV

Previous work has been carried out using photoplethysmography to measure PWV. Greenwald first described the use of reflection PPG for timing the pulse wave at the radial and femoral arteries, comparing the technique against doppler and invasive aortic measurements (Greenwald *et al*. 1997). Loukogeorgakis *et al* (2002) used a reflection PPG probe placed over the radial and dorsalis pedis arteries, and found transit time values to compare favourably with those recorded using doppler ultrasound at the same sites (mean bias +8.6ms, 95% limits of agreement -27 to +45ms). They also found wrist-ankle transit time was similar to invasively determined aorto-iliac transit time (0ms, -22 to +22ms). Linear correlations with doppler and, to a lesser degree, invasive measurements were strong (r=0.95 and 0.83 respectively). More recently, Tsai and colleagues (2005) found that finger-toe PWV measured using PPG correlated with carotid-femoral tonometer PWV (r=0.67). They found both tonometry and PPG PWV gave higher values for hypertensive and dyslipidaemic subjects, compared with those without those risk factors. They did not compare measures in terms of bias/limits of agreement, which would seem appropriate given the different path lengths examined. The similarities found by both studies with aortic transit time is interesting due to the prognostic relevance of aortic PWV. It may suggest that arm and leg segments of the path length negate each other, making the PPG measures largely dependent on aortic (rather than conduit) stiffness. Foo and Lim have also described changes in PPG in response to changes in posture

but have not compared this with other recording modalities (Foo & Lim 2006). Nitzan *et al* found an increase in finger-toe PPG PWV with age, but did not compare the technique against other measures (2002). Other groups have used PPG for timing measurements. Allen and Murray measured rPTT for finger, ear and toe, and found that all decreased with age (2002). $rPTT_{finger}$ has also been used as a measure of compliance in diabetes (Cameron *et al*. 2003). However, these studies did not take into account pre-ejection period, and the former did not comment on the delay between different sites.

There have been few previous attempts at measuring PWV during exercise; this is undoubtedly due to the practical aspects of obtaining satisfactory signal recordings during movement. Although this hurdle can be theoretically overcome by the use of invasive catheter-tip pressure transducers, this solution clearly introduces new problems relating to safety, practicality and availability. The presence of a catheter in the femoral artery also restricts leg exercise. Alternatively, Siche *et al* described the measurement of PWV during exercise by doppler ultrasound at the subclavian artery and abdominal aorta (Siche *et al*. 1989b; Siche *et al*. 1989a). They found no significant differences in PWV at different stages of exercise with age or with presence or absence of hypertension, although PWV was significantly higher in hypertensive subjects during recovery (Siche *et al*. 1989b). This technique could potentially be extended to include the radial artery as a distal measurement site, allowing both central and conduit vessel PWV to be determined. The main hurdle is the expertise required to obtain good flow recordings – this can be a challenge at rest, and although the reproducibility was actually considered better during exercise (Siche *et al*. 1989a), the technical skill required cannot be underestimated. Other groups have described the measurement of PWV in the immediate post-exercise phase. Following acute dynamic exercise, Kingwell used whole body compliance (Liu *et al*. 1986) and both leg and aortic pulse wave velocity to demonstrate that arterial stiffness decreases in healthy subjects (Kingwell *et al*. 1997). Regional differences in the recovery response have also been demonstrated using a technique employing semi-occlusive blood pressure cuffs situated proximally and distally on both upper and lower limbs (Naka *et al*. 2003), with PWV in the latter showing a

greater decrease around 10 minutes post-exercise. However, neither of these techniques are particularly suited to use during exercise.

To date, only one other study has described the use of PPG to measure PWV during exercise. Nottin *et al* used a combination of Doppler ultrasound at subclavian and brachial arteries, and PPG at the finger, to determine upper limb PWV. They found subclavian-to-finger PWV to be reproducible (coefficient of variation 2.9%), although acknowledged the difficulties in obtaining satisfactory Doppler signal quality. They also attempted to measure subclavian-to-brachial PWV using sequential Doppler recordings, but the coefficient of variation was considerably greater (15.7%) (Nottin *et al*. 2006).

Exercise has been shown to have a minimal effect on the distal transit time components, meaning that change in the overall ear-finger transit time largely reflects changes in conduit vessel mechanics. Furthermore, the fact that the ear-finger path length is subtractive rather than sequential (i.e. they are both in opposite directions from one another), means that the distal components of transit time cancel each other out to a certain degree. Nonetheless, there are unresolved issues regarding the use of photoplethysmography for measurement of exercise PWV. One problem is the possible effect of skin temperature and environmental factors. With prolonged exercise, gradual changes in the radial-to-finger or carotid-to-ear time interval may occur that were not evident with the above study, independent of any change in conduit artery PWV. Furthermore, there appeared to be a slight increase in the difference between methods post-exercise. This may have reflected post-exercise vasodilatation primarily affecting the distal vessels – this potential lack of constancy in the distal arterial path could result in measurement inaccuracy. With respect to the methodology employed in the current study, the use of carotid artery tonometry is a limitation. Recording of accurate carotid signals proved extremely difficult at high work levels due to excessive motion artefact including both respiratory effort and trunk movement during pedalling. By using a 10-second recording time, this allowed for at least 20 pulse complexes to be captured, over around 3 respiratory cycles. The maximum slope of the pulse leading edge is moderately well preserved during exercise, and by use of an intersecting tangent through this point, timing points could

be identified with reasonable confidence. The relatively large number of pulse waves measured averages out further measurement error. Nonetheless, the rather large limits of agreement between the two techniques are mainly accounted for by variability in the timing of the carotid wave. Importantly, however, the differences between techniques remained similar at both high and low workloads, despite considerably less carotid artefact during less intense exercise. Although this is partially reassuring, it is not possible to discount the chance that bias introduced by poor quality carotid recordings masked genuine differences between methods. The considerable overall bias between methods means that it is not possible to simply substitute ear-finger measurements for those made by tonometry. However, the relative changes in ear-finger PWV mirrored changes in tonometry values quite accurately. Indeed, by calibrating the photoplethysmography technique using resting measurements of PWV obtained by tonometry, it may still be possible to estimate absolute values of conduit artery PWV.

Unfortunately, the technique is limited to measurement of PWV in an inactive limb. As demonstrated by Naka *et al*, regional differences in PWV occur in the recovery period (2003), and it is therefore probable that this extends to during exercise too. Limb movement results in excessive sensor artefact, and also results in a cyclical artefact related to repetitive body movements causing inertial blood flow. The latter artefact in particular completely obscures pulsation related to the cardiac cycle, preventing the sensor being placed on the toe during pedalling. This latter problem also limits the technique to treadmill or similar exercise, as movement of the hand and head during running are not compatible with satisfactory signal acquisition. Central PWV measurement is also not possible. However, coupling the ear measurement with bioimpedance, may allow for the aortic-carotid PWV to be determined, and this is a potential area for future work.

5.4.2 Finger-ear PWV as a marker of cardiovascular risk

The finding that young, healthy persons with a family history of cardiovascular disease, had a greater increase in ear-finger PWV than controls with no family history, is of potential importance as a means of predicting cardiovascular outcome. As discussed earlier, resting arterial stiffness is felt to offer prognostic information independent of resting blood pressure (Blacher *et al*. 1999; Boutouyrie *et al*. 2002; Grey *et al*. 2003; Laurent *et al*. 2001), and exercise blood pressure is felt to be a more powerful marker of cardiovascular risk than resting pressure (Filipovsky *et al*. 1992; Mundal *et al*. 1994; Mundal *et al*. 1996). Therefore, the hypothesis that exercise arterial stiffness may be of even greater use than these alternative measures, is of interest, and is supported by the present study. One key vascular mediator released during exercise is nitric oxide, and it is possible that impaired endothelial function in the higher risk population, was associated with a failure of the usual exercise-induced nitric oxide release, resulting in a greater increase in intrinsic wall stiffness despite similar increases in blood pressure in both groups. It is worthwhile noting, that although baseline values of PWV were not significantly different, there was a slight tendency for resting PWV to be lower in the positive family history group. This does raise the question of whether the study was underpowered to detect a genuine difference at baseline.

As the study was very small, the results must be interpreted with caution. A similarly sized study, showed differences in exercise BP between groups with and without a cardiovascular family history (Bond, Jr. *et al*. 1994), and indeed was the basis for powering the present study. The failure to find a change in blood pressure in the current work may simply represent the more heterogenous population studied. The population had a relatively narrow age range and all were healthy non-smokers, but both sexes were represented, and the diversity that this may have introduced could have obscured important differences. The age was slightly greater in the group with no family history, although if anything this might be expected to have reduced the difference in outcome measure. Unfortunately, lipid profiles could not be obtained on the majority of subjects due to technical problems.

5.4.3 Conclusion

It has been shown that relative changes in ear-finger PWV during exercise, reflect changes in conduit artery PWV measured by conventional tonometry. It is possible that this measure may be a useful marker of cardiovascular risk. There is the need to carry out further validation work on subjects with and without CV risk factors, during and after prolonged exercise, and in the presence of cardiovascular-active drugs. The utility of the technique as a method for risk stratification should also be studied in a larger population.

6 EFFECT OF EXERCISE ON THE ARTERIAL TRANSFER FUNCTION

6.1 INTRODUCTION

Exercise is an important aspect of everyday life, and peripheral blood pressure (BP) during exertion is recognised as a marker of cardiovascular risk (Filipovsky *et al*. 1992; Kurl *et al*. 2001; Mundal *et al*. 1996; Singh *et al*. 1999), independently of resting peripheral BP. From the pathophysiological perspective, central BP is likely to be more relevant than peripheral BP for the development of cardiovascular disease, and there is increasing evidence that resting central pressure may be a stronger predictor of vascular events and disease severity than brachial pressure (Roman *et al*. 2007). This may well also be true during exercise. Increased arterial stiffness augments central systolic pressure due to effects on wave reflections (Nichols & O'Rourke 1998). This results in greater pulsatile stress in the aorta, leading to structural changes, remodelling and atherogenesis (Nichols & O'Rourke 1998). It also increases left ventricular afterload and myocardial oxygen consumption (Kelly *et al*. 1992). Furthermore, the decrease in augmentation and the rapid decay in diastolic pressure, compromise myocardial perfusion which occurs predominantly during diastole (Watanabe *et al*. 1993). These factors are of particular importance during exercise, when large changes occur in cardiac work and vascular function. However, marked differences exist between central and peripheral BP (Kroeker & Wood 1955), due to systolic pressure amplification in the peripheral vessels, and these differences are amplified by exercise (Rowell *et al*. 1968). At present, however, there is no established way of measuring central BP during exertion.

To overcome the difficulties associated with invasive measurement of central pressure, transfer functions have been developed to derive the aortic pressure waveform from peripheral artery waveforms (Karamanoglu *et al*. 1993). A transfer function is a mathematical description of the change in the magnitude and phase of different frequency components between the input and output signals of a system (section 1.4.3). It can thus be used to estimate pressure waveforms in inaccessible arteries (e.g. the aorta) from more readily accessible sites (e.g. the radial artery). A

generalised radial-to-aortic transfer function (GTF) assumes that the properties of the upper limb arteries are virtually identical between individuals. The transfer function has indeed been shown to remain relatively constant in subjects with coronary heart disease (Chen *et al*. 1997), even after the administration of vasoactive drugs (Pauca *et al*. 2001; Segers *et al*. 2001; Soderstrom *et al*. 2002). Derived aortic waveforms obtained using this method have been shown to relate to cardiovascular outcome (Weber *et al*. 2004; Williams *et al*. 2006). Although this technique might therefore be seen as a potential method of determining central BP during exercise, most of the validation studies to date have been in males undergoing diagnostic cardiac catheterisation. Discrepancies in the transfer function have been demonstrated between men and women (Hope *et al*. 2002), diabetic and non-diabetic populations (Hope *et al*. 2004), and persons with and without coronary disease (Segers *et al*. 2000). The large increases in mean distending pressure and intrinsic arterial wall stiffness that occur with exertion might also be expected to alter the transfer function, with an increase in the velocity of higher frequency harmonics. This would result in greater convexity of the derived waveform, and a relatively lower systolic peak for any given diastolic and mean pressure. Using the finger pulse as an alternative means of predicting the pressure pulse contour is also attractive, due to the simplicity and widespread availability of photoplethysmography. However, marked changes in smaller arteries such as those in the hand occur during exercise, and these may be expected to significantly alter the transfer function under these circumstances.

The following study examined healthy volunteers undertaking moderately strenuous aerobic exercise, to address the hypothesis that a peripheral-to-central arterial transfer function derived at rest would underestimate central pressure immediately after exercise. It also studied the effects of exercise on the finger-to-radial transfer function, which not only has relevance to derivation of conduit artery pressure waveforms using the finger wave, but by extension of the prior hypothesis has a bearing on the estimation of central pressure.

6.2 METHODOLOGY

30 healthy male volunteers with variable exercise capacity, aged 18 to 31, were recruited. Studies were conducted in a quiet, temperature controlled (22±2°C) environment. Subjects avoided eating for 3 hours prior to the study.

Following an initial rest period of rest, baseline measurements of central (carotid-femoral) and conduit (carotid-radial) arterial pulse wave velocity were made in the supine position, as described in section 2.7. Subjects then transferred to a semi-recumbent cycle ergometer (SX1, Kettler) with all further measurements made in the sitting position. Central pressure was determined non-invasively at the carotid artery. Blood pressure, heart rate, carotid, radial and finger waveforms were recorded before, and immediately (within 1 minute) and 10 minutes after, aerobic exercise. A heart-rate targeted exercise protocol was used as described in section 2.9.1.3, until the heart rate reached 65-70% of the individual's estimated maximum. Pedal cadence was maintained between 70 to 100 rpm. Once the target heart rate was achieved, the associated workload was maintained for a further 15 minutes.

BP was recorded using an automated sphygmomanometer (section 2.8.2). PWV was measured by sequential 10 second-recordings of the radial, carotid and femoral artery waveforms using an SPT301 tonometer (section 2.5.1). Simultaneous non-invasive carotid, radial and finger waveforms were recorded using hand-held tonometry (SPT301), automated tonometry (CBM7000, Colin Medical) and photoplethysmography respectively, over around 30 seconds. Carotid waveforms were acquired on the right side with the subject looking straight ahead and minimising respiratory excursions to reduce movement artefact. Both forearms were supported at the level of the xiphisternum, with a splint positioned over the dorsal aspect of the right wrist to minimise movement during radial waveform recording. Signals were digitised at 1kHz using a DAQCard 6036E as described in section 2.2.

10-second simultaneous radial and carotid waveform segments were selected for the rest and immediate post-exercise phases, using visual inspection to ensure signal stability and to exclude artefact. Transfer functions were computed from these waveforms for each individual subject and both time points, as described in section

2.5.3.3, and averaged to obtain resting and exercise specific generalised transfer functions (GTF_{rest} and $GTF_{exercise}$) for both finger-to-radial and radial-to-carotid transformations. To address the potential for bias resulting from the application of a GTF derived from the same set of data as that to which it is subsequently applied, additional transfer functions ($GTF_{rest\text{-}2}$, $GTF_{exercise\text{-}2}$) were calculated using data from a subset (N=10) of the study population and applied to the remaining individuals. In addition, an independently generated radial-to-carotid GTF obtained from the SphygmoCor system ($GTF_{sphygmocor}$) was applied to all subjects' data. GTFs were applied to the relevant measured waveforms at each experimental time point. Measured and derived ensemble-averaged waveforms were analysed to obtain values of systolic BP, augmentation index, timing of the dicrotic notch (T_{DN}), maximum dP/dt, and the timing of incident and reflected waves (T_1 and T_2 respectively).

Results are expressed as mean ± standard deviation, and difference in means as mean difference ± standard error. Waveform measurements were compared using paired *t*-tests. The area under the curve for the 0 to 10 Hz phase and magnitude components of GTF_{rest} and $GTF_{exercise}$ were compared by paired *t*-tests.

6.3 RESULTS

Subject characteristics are shown in Table 6.1, and changes in peripheral haemodynamic variables in Table 6.2. Volunteers had normal resting BP, and normal resting central and conduit artery PWV. As expected, there were significant increases in heart rate, diastolic BP, and mean pressure. Systolic BP, AIx, maximum slope and dicrotic notch pressure also increased at all measurement sites. T_1 and T_{DN} decreased for at all measurement sites. T_2 decreased at the finger and carotid artery, but did not fall significantly at the radial artery. AIx and T_{DN} remained significantly different from baseline at 10 minutes recovery at all sites. SBP also remained significantly elevated after 10 minutes at the carotid artery and finger, but not the radial artery. Maximum slope remained significantly elevated at the radial artery only.

Table 6.1. *Subject characteristics (N=30)*

Age (years)	22.3 (2.4)	PWV (ms^{-1})	
Height (cm)	177 (9)	carotid–femoral	6.2 (1.1)
BMI (kg.m-2)	22.9 (2.6)	carotid–radial	8.2 (1.1)
FHx CVD (N, %)	10 (33%)	Resting HR (bpm)	72 (11.3)
Resting BP (mmHg)	119/69 (8.0/6.4)	Peak exercise work (W)	84 (18)

Values are mean (SD). BMI, body mass index; FHx CVD, family history of cardiovascular disease in first degree relative aged 65 years or less; PWV, pulse wave velocity, HR, heart rate.

Table 6.2. *Haemodynamic changes with exercise*

Variable	Rest	Immediately post-exercise	10 minutes post-exercise
Heart rate (bpm)	72 ± 11.3	131 ± 8.1‡	85 ± 11.9*
DBP (mmHg)	69 ± 6.4	89 ± 33.3†	75 ± 9.8†
MAP (mmHg)	86 ± 7.4	117 ± 29.4‡	91 ± 9.5†
Finger volume wave			
SBP (mmHg)	111 ± 11.7	167 ± 31.1‡	115 ± 11.6*
AIx (%)	32 ± 13.9	46 ± 13.0‡	39 ± 13.8†
Max. slope ($mmHg.s^{-1}$)	512 ± 125	1009 ± 370‡	474 ± 126
T_{DN} (ms)	278 ± 17	256 ± 20‡	263 ± 18†
T_1 (ms)	110 ± 8	101 ± 10‡	109 ± 8
T_2 (ms)	211 ± 16	200 ± 17‡	204 ± 17
Radial pressure wave			
SBP (mmHg)	119 ± 8.0	177 ± 30.2‡	121 ± 10.6
AIx (%)	57 ± 14.6	66 ± 13.2†	62 ± 12.3†
Max. slope ($mmHg.s^{-1}$)	705 ± 91	1292 ± 458‡	630 ± 140*
T_{DN} (ms)	269 ± 18	241 ± 23‡	253 ± 19‡
T_1 (ms)	88 ± 7	84 ± 8†	90 ± 7
T_2 (ms)	202 ± 13	198 ± 14	202 ± 12
Carotid pressure wave			
SBP (mmHg)	105 ± 10.3	157 ± 30.6‡	110 ± 11.9†
AIx (%)	18.9 ± 12.2	36 ± 14.8‡	29 ± 12.5‡
Max. slope ($mmHg.s^{-1}$)	477 ± 121	1038 ± 466‡	485 ± 139
T_{DN} (ms)	270 ± 17	240 ± 21‡	252 ± 19‡
T_1 (ms)	106 ± 15	87 ± 19‡	101 ± 19
T_2 (ms)	191 ± 16	173 ± 19‡	185 ± 17

*DBP, diastolic BP; MAP, mean arterial BP; SBP, systolic BP; AIx, augmentation index; T_{DN}, timing of dicrotic notch; T_1, timing of incident wave; T_2, timing of reflected wave; * $p<0.05$, † $p<0.01$, ‡ $p<0.001$, significant difference from rest.*

6.3.1 Radial-carotid generalised transfer function

Differences between measured carotid parameters, and carotid values derived from the radial artery (including those obtained using $GTF_{sphygmocor}$), are shown in Figure 6.1.

At rest, there were no significant differences between any measured and GTF_{rest}-derived parameters. $GTF_{sphygmocor}$ also showed no significant differences from measured values at rest, with the exception of T_{DN} which was overestimated by 9.9±1.7ms ($p<0.001$). In particular, GTF_{rest} and $GTF_{sphygmocor}$ were not significantly different from actual measured values of either SBP (0.0±0.9 mmHg, $p=0.96$; -0.2±0.9 mmHg, $p=0.83$ respectively) or AIx (0.4±2.8%, $p=0.9$; 2.1±2.8, $p=0.47$). $GTF_{exercise}$ overestimated SBP (2.5±1.0 mmHg, $p=0.02$), AIx (11.3±3.0%, $p=0.001$) and maximum dP/dt (89.9±21.3 $mmHg.s^{-1}$, $p<0.001$), and underestimated T_{DN} (-7.9±1.8 ms, $p<0.001$) and T_1 (-10.7±4.4 ms, $p=0.024$).

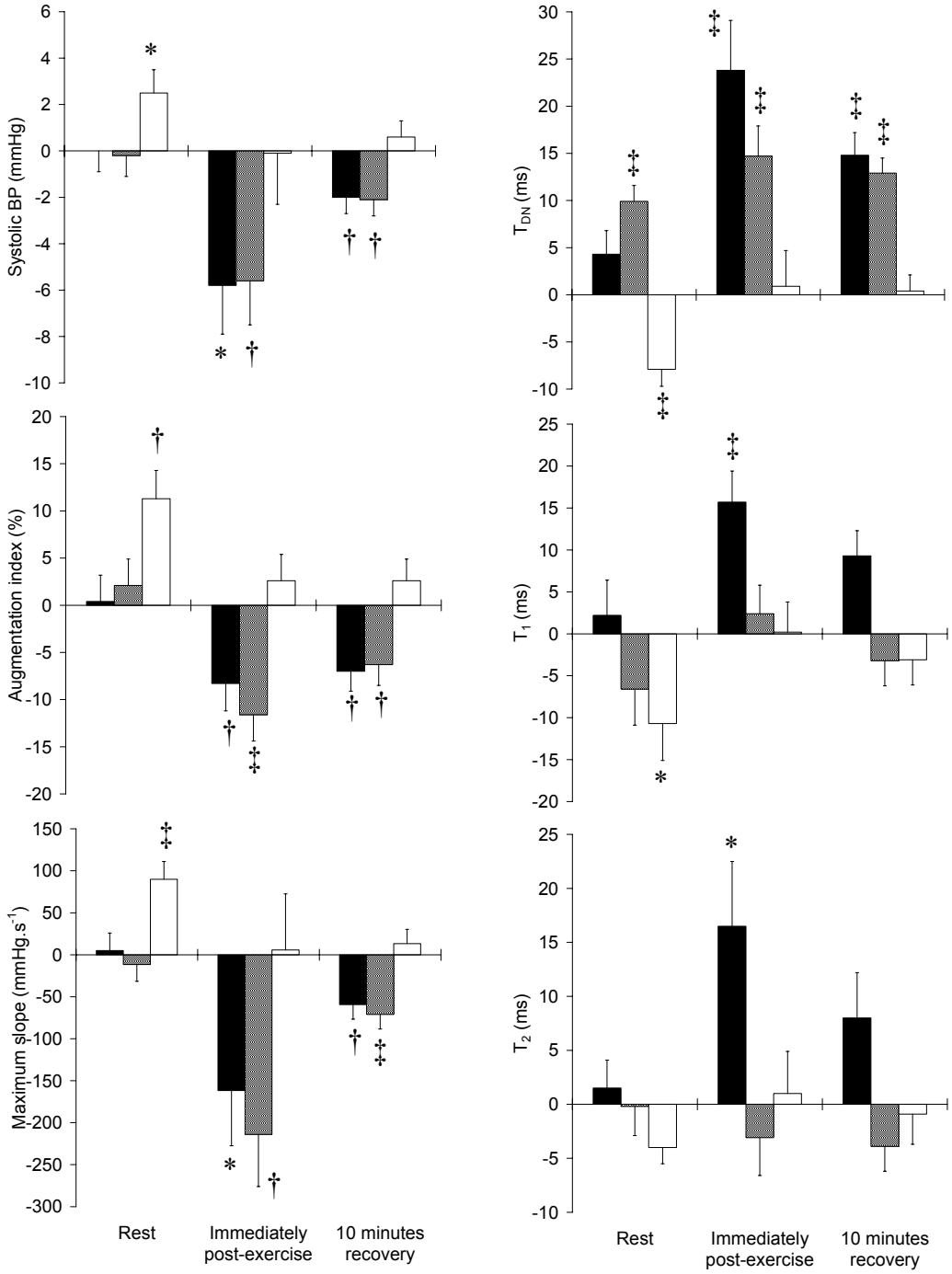
Immediately after exercise, GTF_{rest} and $GTF_{sphygmocor}$ significantly underestimated SBP (-5.8±2.1 mmHg, $p=0.01$; -5.6±1.9 mmHg, $p=0.007$ respectively), AIx (-8.3±2.9%, $p=0.008$; -11.6±2.8%, $p<0.001$) and maximum dP/dt (-161.8±65.6 $mmHg.s^{-1}$, $p=0.021$; -214±62 $mmHg.s^{-1}$, $p<0.001$), and significantly overestimated T_{DN} (23.8±5.3ms, $p<0.001$; 14.7±3.2ms, $p<0.001$). These errors persisted at 10 minutes, albeit to a lesser degree. T_1 and T_2 were overestimated by GTF_{rest} (but not $GTF_{sphygmocor}$) immediately post-exercise only (15.7±3.7ms, $p<0.001$; 16.5±6.0ms, $p=0.010$, respectively). Values of all parameters derived using $GTF_{exercise}$ demonstrated no significant bias either immediately or 10 minutes after exercise.

Similar findings were found using GTF_{rest-2} and $GTF_{exercise-2}$ to those described above. Carotid waves derived at rest using GTF_{rest-2}, or immediately or 10 minutes after exercise using $GTF_{exercise-2}$, showed no significant bias from measured values of AIx, SBP, maximum dP/dt, T_{DN} or T_1. GTF_{rest-2} underestimated AIx, SBP and maximum dP/dt, and overestimated T_{DN} and T_1 immediately (-15.4±4.5%, -7.5±2.3mmHg, -186±61$mmHg.s^{-1}$, 22.1±4.3ms, 15.0±5.9ms, respectively; $p\leq0.025$) and at 10 minutes post-exertion (-10.9±3.5%, -3.9±1.0mmHg, -92±24$mmHg.s^{-1}$, 22.9±3.4ms, 14.6±5.6ms; $p\leq0.022$). In contrast, $GTF_{exercise-2}$

overestimated AIx (14.1±5.4%, p=0.025), SBP (4.6±1.6mmHg, p=0.013) and maximum dP/dt (117±37mmHg.s^{-1}, p=0.009), and underestimated T_{DN} (-12.3±4.2, p=0.013) at rest. Exercise did not affect the estimation of T_1 by $GTF_{exercise\text{-}2}$, or estimation of T_2 by either $GTF_{rest\text{-}2}$ or $GTF_{exercise\text{-}2}$.

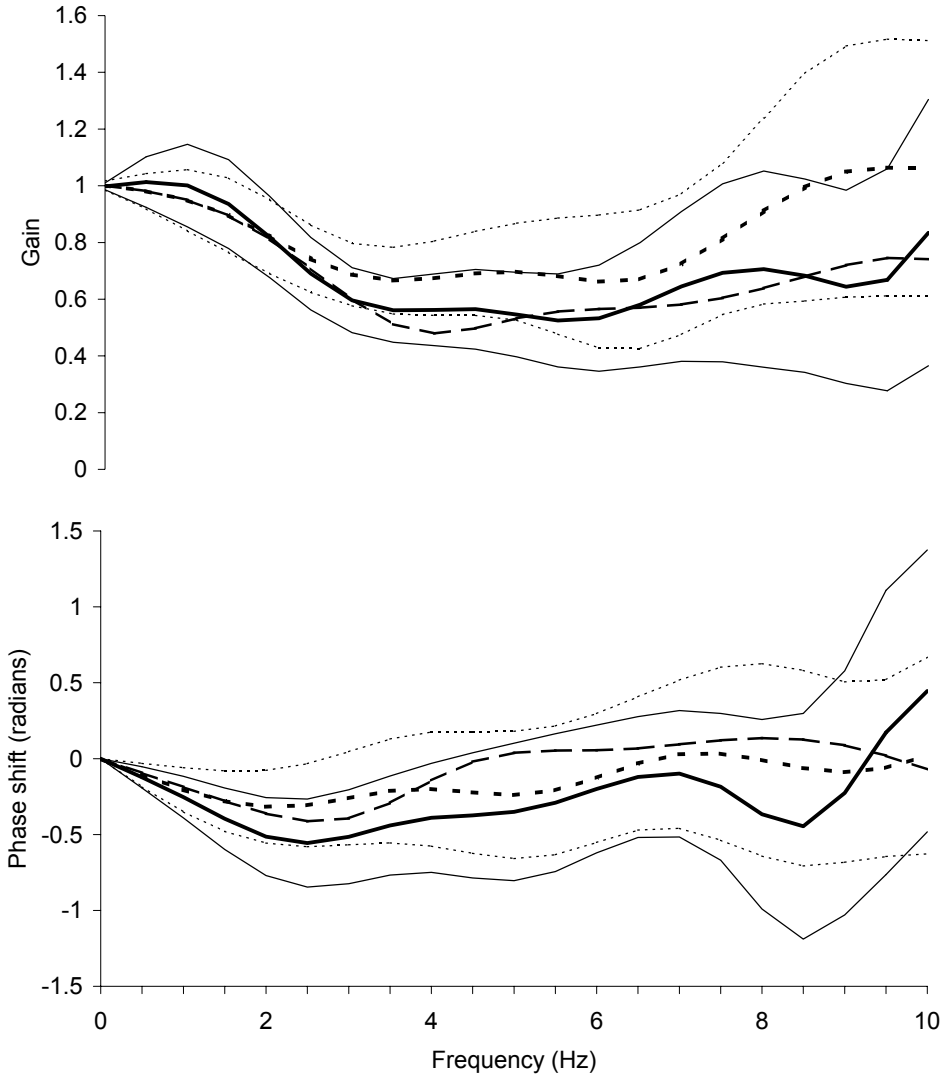
Radial-carotid GTF_{rest}, $GTF_{exercise}$, and $GTF_{sphygmocor}$ are shown in Figure 6.2. There was a significant decrease in gain (p=0.001) and a positive phase shift (p=0.012) in $GTF_{exercise}$ compared to GTF_{rest}, over 0 to 10 Hz.

Figure 6.1. *Difference in carotid parameters derived from radial wave, relative to actual measured carotid values*



*Values are mean±SE (N=30). Bars represent GTF_{rest} (black), $GTF_{sphygmocor}$ (grey) and $GTF_{exercise}$ (white). * p<0.05, † p<0.01, ‡ p<0.001, significant difference from measured value.*

Figure 6.2. *Radial-to-carotid transfer functions*



Top, gain; bottom, phase shift. Solid line, resting transfer function (GTF_{rest}); dotted line, exercise transfer function ($GTF_{exercise}$); dashed line, Sphygmocor-derived transfer function ($GTF_{sphygmocor}$). Heavy line is mean, thin lines are ±1 SD (SD not shown for $GTF_{sphygmocor}$ for clarity).

6.3.2 Finger-radial generalised transfer function

Differences between measured and derived (from the finger wave) radial parameters are shown in Figure 6.3.

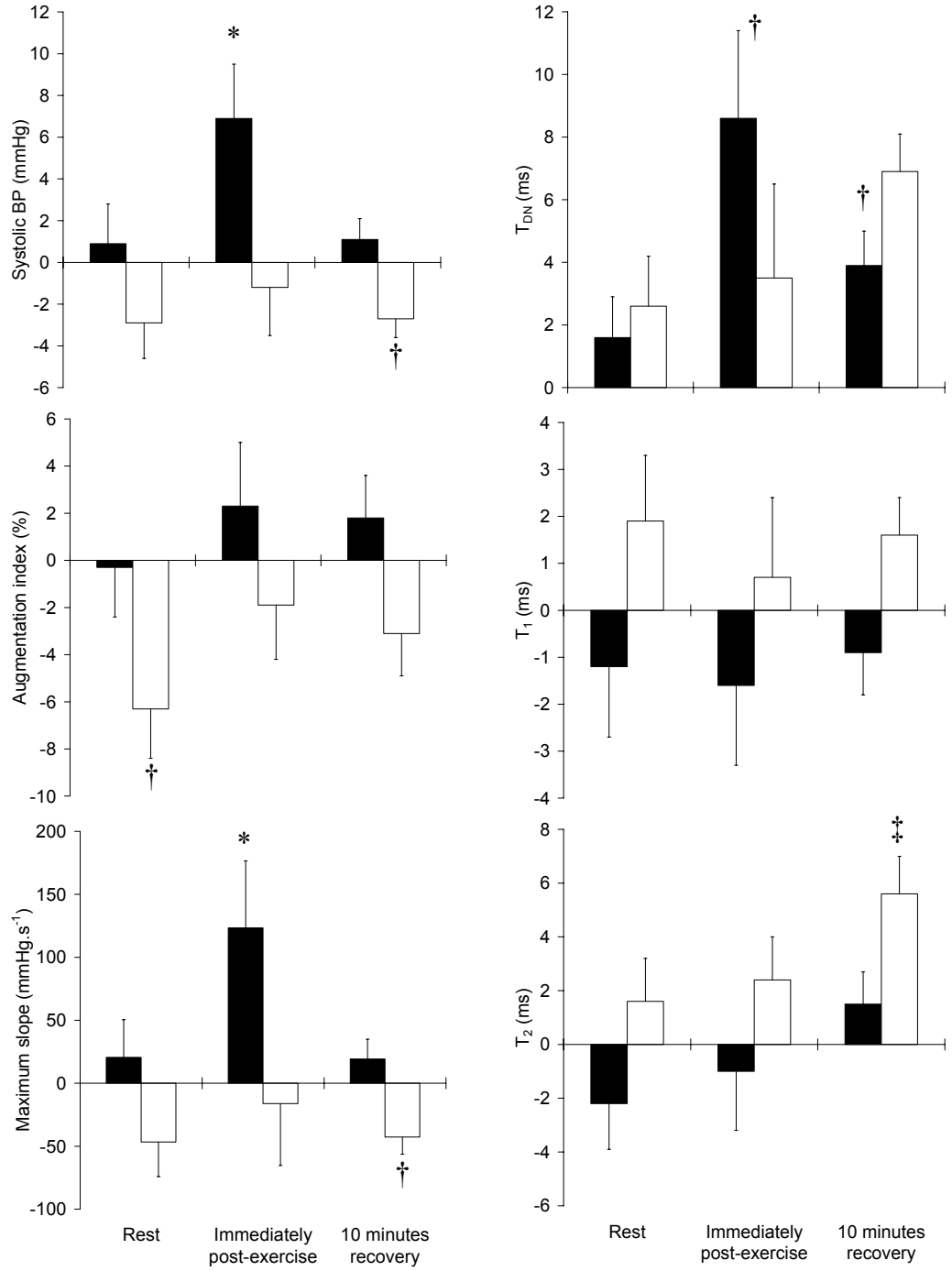
Radial values derived using GTF_{rest} were not significantly different from measured resting values for any parameter. However, GTF_{rest} significantly overestimated SBP (6.9±2.6 mmHg, p=0.015), maximum dP/dt (123.4±53.2 $mmHg.s^{-1}$, p=0.029) and T_{DN} (8.6±2.8 ms, p=0.005) immediately after exercise, although only T_{DN} remained significantly different at 10 minutes (3.9±1.1 ms, p=0.001).

$GTF_{exercise}$-derived waveforms showed a significantly lower AIx (-6.3±2.1%, p=0.005) at rest, but other parameters (SBP, maximum dP/dt, T_{DN}, T_1, T_2) were not significantly different from measured values. $GTF_{exercise}$ demonstrated no significant bias in any parameter immediately post-exercise. At 10 minutes recovery, however, $GTF_{exercise}$ underestimated SBP (-2.7±0.9 mmHg, p=0.007) and maximum dP/dt (-42.7±13.6 $mmHg.s^{-1}$, p=0.004), and overestimated T_2 (5.6±1.4 ms, p<0.001).

GTF_{rest-2} and $GTF_{exercise-2}$ did not reflect the findings with GTF_{rest} and $GTF_{exercise}$, with exercise not affecting the bias of either GTF_{rest-2} or $GTF_{exercise-2}$.

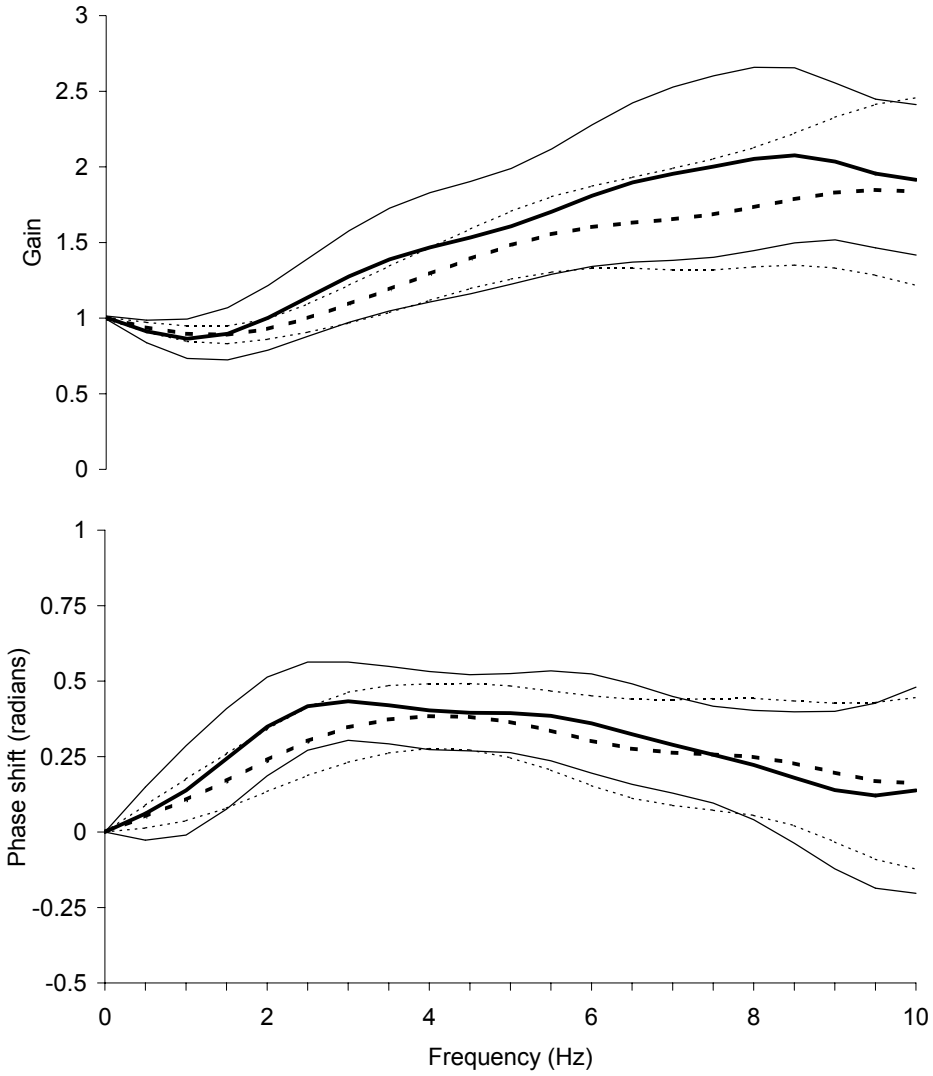
Finger-radial GTF_{rest} and $GTF_{exercise}$ are shown in Figure 6.4. There were no significant differences in gain or phase, as determined by AUC for <10Hz, between the two transfer functions.

Figure 6.3. *Difference in radial parameters derived from finger wave, relative to actual measured radial values*



*Values are mean±SE (N=30). Bars represent GTF_{rest} (grey) and $GTF_{exercise}$ (white). * $p<0.05$, † $p<0.01$, ‡ $p<0.001$, significant difference from measured value.*

Figure 6.4. *Finger-to-radial transfer functions*



Top, gain; bottom, phase shift. Solid line, resting transfer function (GTF_{rest}); dotted line, exercise transfer function ($GTF_{exercise}$); dashed line, Sphygmocor-derived transfer function ($GTF_{sphygmocor}$). Heavy line is mean, thin lines are ±1 SD (SD not shown for $GTF_{sphygmocor}$ for clarity).

6.4 DISCUSSION

The current study is the first to describe the effects of exercise on the peripheral-to-central and radial-to-finger transfer function in young, healthy adults. It demonstrates that if a peripheral-to-central arterial transfer function obtained at rest, is used to derive central waveform parameters immediately after exercise, various derived central pressure parameters will be biased. In particular, the key measures of central pressure load, central systolic BP and augmentation index, are underestimated after exercise. The effect of exercise on the peripheral-to-central transfer function is still present 10 minutes after stopping exercise. Although the use of an exercise-specific transfer function improves this estimation, it does not hold at rest. The effect of exercise on the finger-to-radial transfer function is less clear-cut, but appears to result in an overestimation of estimated systolic pressure with associated increase in the maximal leading edge slope, and an ensuing fall-off in pressure in diastole. Many of these changes appear to resolve within 10 minutes of cessation of exercise.

Peripheral-to-central transfer functions provide an attractive means of determining aortic pressure non-invasively, and their use during exercise is of particular interest as there are relatively few data on the effects of exertion on central haemodynamics. Exercise has marked effects on the stiffness of the arterial wall, both through an increase in distending mean BP (particularly at higher levels of exertion), and by effects on the intrinsic properties of the arterial wall due to the release of various local and systemic vasoactive mediators. Furthermore, increased heart rate is associated with an increased rate of change of pressure, and may therefore increase stiffness because of the viscoelastic nature of the arterial wall (Bergel 1961b). The change in gain and phase of the transfer function following exercise is probably accounted for by a combination of these factors. It is not entirely clear whether the resulting bias demonstrated in this study of -5.8 mmHg central systolic BP and -8.3% AIx, is great enough to be of clinical relevance. The discrepancies are similar to or greater than the differences in these parameters observed at rest in populations with and without cardiovascular disease (Weber *et al*. 2004), or resulting from different therapeutic agents (Asmar *et al*. 2001). The disagreement corresponds to an inaccuracy of only approximately 3.7% for SBP, although 11.1% of the overall

central SBP range. The relative error in AIx is considerably greater at approximately 23% of the peak AIx value. If the error across the populations remains relatively constant, then this is of less concern, although the advantage of using a GTF in the first place is somewhat defeated. It is also uncertain, however, whether drug treatment or other interventions such as exercise training, may alter the GTF response to exercise (and thus the degree of underestimation of central haemodynamics) in an unpredictable manner.

The finger volume wave has been used to derive the radial pressure wave at rest (Millasseau *et al*. 2000), including following administration of GTN. This has not previously been extended to exercise, although work has been done describing the relationship between finger pressure pulse and aortic pulse wave (Stok *et al*. 2006). The current study suggests that exercise results in changes in transfer function that generate a more convex wave contour. However, these changes are relatively short-lived. Furthermore, the variability in finger-to-radial transfer function gain, particularly at higher frequencies, is considerably greater than that for the radial-to-carotid transfer function, and differences in rest and exercise transfer function were not apparent from analysis in the frequency domain. It is therefore difficult to know whether or not these findings are of significant practical relevance. The timing of derived wave components was affected only minimally, whereas pressure was more markedly altered. This is reflected in the greater (albeit, not significantly) transfer function gain during exercise, with less variation in phase shift. Of note, although prediction of blood pressure during exercise using the volume wave is arguably of little use, systolic pressure estimation and the other wave parameters were used in the present study as ways of characterising the pulse shape, rather than as practical clinical measures. However, if it could be shown that the effects of exercise on the radial pulse contour have relevance in the context of cardiovascular risk prediction, then these findings may be of importance. In addition, the fact that exercise alters both radial-to-carotid and finger-to-radial transfer functions, may compound the inaccuracy of any attempt to derive central waveforms from the finger volume pulse.

Two other recent studies have examined the effect of exercise on the arterial transfer function. Sharman *et al* found no effect of exertion on the radial-to-aortic transfer

function (Sharman *et al*. 2006), whereas Stok *et al* found that increasing exercise led to an underestimate and more unreliable measurement of systolic pressure derived from the finger pressure pulse (Stok *et al*. 2006). Both studies used invasive measurement of aortic pressure in patients undergoing diagnostic coronary angiography. Important methodological issues may account for the different conclusions reached by these studies. The duration of exercise in the Sharman study was rather short at only 3 to 6 minutes, and included a protocol employing one-legged cycling. Furthermore, the heart rate responses were not particularly marked, at least in part due to beta-blockade in the majority of individuals. Although the blood pressure increases observed above were similar to those of Sharman, the exercise protocol in the current study was considerably longer and the intensity of workload more accurately gauged by the heart rate response; it is possible that the overall intensity of the exercise in the Sharman study was too low to have a significant effect on the intrinsic properties of the arterial wall, and thus no measurable effect on the transfer function. The work by Sharman was carried out in older subjects, many with proven coronary disease who were taking various cardiovascular drugs. Significant differences have been demonstrated in the resting radial-to-carotid transfer function between subjects with coronary disease and healthy volunteers (Segers *et al*. 2000). Age and vascular disease increase resting arterial stiffness, but also lead to diminished catecholamine-mediated inotropic, chronotropic and arterial vasodilatory effects in response to exercise (Nichols & O'Rourke 1998); this may well have blunted any change in transfer function in response to exercise. The presence of certain vasoactive drugs has been shown to have no effect on the resting GTF (Pauca *et al*. 2001; Segers *et al*. 2001; Soderstrom *et al*. 2002), but it is not known whether these too may have altered the arterial response to exercise, thus minimising the change in transfer function. Importantly, despite similar drug therapy in a comparable population, an effect of exercise on the transfer function was nonetheless demonstrated by Stok *et al*, suggesting that drug treatment, age and the presence of vascular disease was less likely to account for the findings of Sharman *et al*. Clearly, however, the transfer function developed by Stok and colleagues comprises both proximal and distal conduit arterial components, including the vasculature of the hand, and the question must therefore be raised as to

whether the differences in rest and exercise transfer function are explained by changes in larger conduit artery function, or whether they are simply due to effects on the finger-to-radial vascular segment. It is recognised that exercise can lead to inaccuracy of blood pressure measurement at the finger using the volume-clamp method employed by Finapres (Hildebrandt *et al*. 1991). Furthermore, the local effects of certain drugs may have resulted in changes in the finger pressure pulse that are not evident in the pulses of larger arteries. However, the current study suggests that, whereas the resting radial-to-carotid transfer function underestimates proximal pressure, the finger-to-radial transfer function overestimates it. It seems likely, therefore, that the overall change in finger-to-aortic transfer function demonstrated by Stok *et al* is indeed primarily determined by changes in proximal conduit artery function, rather than changes distally. It is worthwhile noting that the finger pressure pulse is not exactly the same as the volume wave, and a degree of caution must therefore be exhibited when using the findings of the current study to explain those obtained using the Finapres system. Nonetheless, both waveforms are closely associated due to the utility of photoplethysmography by both methodologies (Millasseau *et al*. 2000; Millasseau *et al*. 2006). Interestingly, although Sharman *et al* concluded that the resting GTF held following exercise, their published data nonetheless shows that the difference between derived and actual systolic BP was significantly greater ($p<0.001$ calculated from reported data using unpaired *t*-test) during exercise than at rest (-4.7±3.3 vs. -1.3±3.2 mmHg respectively). This group have published widely using the SphygmoCor system, so their interpretation of their findings may have been influenced by this conflict of interests.

The present study has some important limitations. First is the use of non-invasive carotid waveforms, rather than invasive recording of aortic pressure. The use of the carotid artery as a surrogate for central pressure is not ideal. However, the risks inherent in aortic catheterisation preclude its use in a young, healthy population. Furthermore, the carotid artery pulse contour has been used previously as a substitute for invasive central arterial measurements (London *et al*. 2001; Safar *et al*. 2002). The differences between aortic and carotid systolic pressure are less than 5mmHg (Karamanoglu & Feneley 1996). Discrepancies in stiffness at each site in healthy subjects are small (Paini *et al*. 2006), and differences between carotid and aortic

augmentation are minimal in young adults (Nichols & O'Rourke 1998). Second, the accuracy of manually recorded carotid waveforms was worse than the radial waveforms obtained using the automated tonometer during rest. This was quantified by differences in the coefficient of variation of pulse height (5.0±2.1% vs. 10.1±3.4%, radial and carotid respectively) and baseline (2.9±2.3% vs. 8.1±6.7%). No significant change was observed in the height variability following exercise, although baseline variability increased for both signals (5.1±3.8%, p=0.02 vs. 16.7±1.5%, p=0.01). Although sub-optimal waveforms might arguably have adversely affected the accuracy of the derived transfer function, the transfer function derived from the SphygmoCor system was not subject to such error and yet provided similar results. Carotid waveform inaccuracy may also have resulted in error in the actual and measured values of AIx at peak exercise. It is difficult to be certain whether such an error had a skewed distribution, but it seems more likely that this would result in greater variability rather than any mean bias. Third, it was assumed that diastolic and mean pressure remained constant between radial and carotid arteries, as absolute direct pressure measurement is not possible at the latter. It is recognised that diastolic pressure tends to decrease proximally by about 1-2mmHg (Pauca *et al*. 1992). However, this difference is small relative to the difference in systolic pressure, and calibration using this approach is considered generally acceptable (Nichols & O'Rourke 1998). Moreover, AIx is not dependent on absolute pressure, as it represents the relative amplitudes of incident and reflected wave components. Fourth, the timing of signal recording during this study is also worthwhile noting. During pilot work, it was not possible to obtain satisfactory and reliable carotid signal acquisition during exercise, and recordings were therefore made in the immediate post-exercise period to reduce movement artefact. It is possible that the rapid haemodynamic changes that occur in the immediate post-exertion period may account for the change in the transfer function, rather than the direct effects of exercise (Kingwell *et al*. 1997). It is unlikely that this issue will be resolved by non-invasive measurements (although radial and finger measurements would be possible), and it would be interesting to see whether significant changes in transfer function occur between peak exercise and immediate cessation of exertion. Fifth, GTF_{rest} and $GTF_{exercise}$ were derived from the same data that they were

subsequently applied to, potentially biasing the accuracy of each transfer function towards its associated exercise phase. Nonetheless, in the case of the radial-carotid GTF, the results given by $GTF_{sphygmocor}$ and by the use of a GTF derived from an independent subset of subjects, were not subject to such bias and yet yielded similar conclusions. In the case of the finger-radial GTF, it seems likely that the relatively small effect of exercise on the GTF, and the greater (*cf.* radial-carotid) inter-subject GTF variability, meant that the study was under-powered to detect differences between $GTF_{rest\text{-}2}$ and $GTF_{exercise\text{-}2}$. Finally, the limits of agreement between actual and derived measurements were fairly high, as evidenced by the large standard deviations of the difference. This is not entirely accounted for by variability in the carotid waveform quality, as limits of agreement were of similar magnitude for the finger-to-radial results. However, the aim of the present study was to establish whether overall bias existed between derived and actual measurements, rather than accuracy of the technique, and this does not affect the principal conclusions. There is arguably, of course, little point in predicting a proximal waveform from a distal site, when the former can be directly measured anyway. However, it is important to recognise that the use of the finger wave is attractive due to its simplicity and ease-of-use, and that mean bias in the derived carotid waveform may extend to error in derived aortic waveforms too.

6.4.1 Conclusion

The use of a generalised arterial transfer function for determining central pressure is an attractive alternative to invasive monitoring, with potential uses in both the clinical and research environments (Mackenzie *et al*. 2002; Oliver & Webb 2003). It has been increasingly employed in the assessment of cardiovascular risk (Weber *et al*. 2004; Williams *et al*. 2006) and vascular function at rest (Wilkinson *et al*. 2002a), despite increasing evidence that the GTF does not remain constant in all circumstances (Hope *et al*. 2002; Hope *et al*. 2004; Segers *et al*. 2000). Given the lack of data on the central arterial response to exercise, the use of a GTF in these circumstances is clearly appealing, potentially opening the gateway to larger clinical studies, examining amongst other things the effects of blood pressure lowering drugs on central exercise BP and the utility of this measure as a marker for cardiovascular

risk. The results of the current study suggest that significant underestimation of key central haemodynamic measures may occur when using a transfer function derived under resting conditions. The use of a resting GTF in such circumstances appears unwise; an exercise specific transfer function might be favoured, although this requires further investigation. Estimation of radial pressure from the finger wave also appears to be subject to inaccuracy, although these effects are more variable and short-lived.

7 EFFECT OF EXERCISE ON THE RELATIONSHIP BETWEEN PULSE TRANSIT TIME AND BLOOD PRESSURE

7.1 INTRODUCTION

Blood pressure measurement during exercise is considered a more powerful prognostic marker than resting BP (Filipovsky *et al*. 1992; Manolio *et al*. 1994; Ren *et al*. 1985; Sandvik *et al*. 1993), and may be an indicator of physical fitness (Tanaka *et al*. 1996). Measurement of BP during exercise is difficult however, due to the rapidity of pressure changes, underestimation with cuff sphygmomanometry, and motion artefact. Pulse transit time has been show previously to be linearly associated with systolic BP during exercise (Carruthers & Taggart 1988), and may therefore offer potential as a means on non-invasively estimating beat-to-beat exercise BP.

To date, however, the relationship between transit time and BP during exercise has not been examined in detail. In particular, differences between exercise and recovery phases, the effects of prolonged exercise compared with short, maximal exertion, and levels of fitness, have not been studied.

Three studies were carried out to address the hypothesis that it would not be possible to use transit time as a reliable measure of BP during exercise. The first study compared the association of pulse transit time with invasively measured BP, examining differences between correlations during and after exercise, beat-to-beat variability, and the potential for transit time use for baroreflex sensitivity monitoring during exertion. The second study examined the effects of prolonged sub-maximal exercise on the correlation between transit time and BP. The third study examined the effects of fitness on the correlation between the two variables, and the reproducibility of the technique.

7.2 METHODS

All studies were conducted in a quiet, temperature controlled (22±2°C) environment, following a 30-minute period of rest. Volunteers were allowed a light meal not less

than 4 hours prior to the study, and refrained from alcohol, caffeine, nicotine or medications for the preceding 24 hours.

7.2.1 Comparison of transit time and intra-arterial pressure

9 non-smoking male subjects, aged 18 to 25 years (22.6±1.4), were studied. Continuous real-time beat-to-beat measurements of intra-arterial BP (section 2.8.3), finger transit time (section 2.3) and PEP (section 2.4.1.1) were made throughout the entire study protocol. The arterial cannula was inserted prior to the rest period. Data was recorded as described in section 2.2.

5 minutes of baseline resting measurements were made prior to performing a 3-stage heart rate targeted exercise protocol, using a semi-recumbent bicycle ergometer (Comfort Cycle JPB 2000, Johnson, section 2.9), with forearms supported horizontally at the level of the xiphisternum. Stages lasted 4 minutes (heart rate <50%), 4 minutes (50-75%) and 2 minutes (>75%) respectively, as described in section 2.9.1.3. Measurements continued for 15 minutes following cessation of exercise.

Data were compared across time points using repeated measures ANOVA, with paired t-tests used *post hoc* to compare individual time points. Where specific experimental phases are given, data were either averaged across the entire phase, or averaged over a 2-minute period at the end of that phase, as specified in the results. Linear regression was carried out on beat-to-beat data, with comparison of correlation and regression coefficients made by paired t-test. Area-under-the-curve (AUC) was used to examine differences in correlation between exercise and recovery phases, using 15-second averaged data. The rate at which parameters returned to steady state during recovery was evaluated by fitting an exponential decay curve and calculating the mean response time. Frequency analysis was assessed for SBP, rPTT and HR. Power spectra were calculated using a smoothed Lomb periodogram for all three variables across the entire experimental period. Spectral coherence (see section 2.5.3.3) was computed over the frequency ranges 0.05Hz to 0.2Hz, and 0.2Hz to 0.4Hz. Baroreflex sensitivity (BRS) was examined using sequence analysis, with a sequence defined as a series of 3 consecutive beats during which systolic BP and the

R-R interval either increased or decreased continuously by at least 1mmHg/beat and 3ms/beat respectively. For each sequence, the slope of the regression line between SBP and R-R interval was calculated. Baroreflex effectiveness index (BEI) was calculated as the ratio of the number of valid sequences (as defined above) as a proportion of the total number of systolic BP sequences. BEI provides information about baroreflex function complementary to BRS, and reflects the effects of other mechanisms such as central inhibitory influences or non-baroreflex regulation at the sinus node level.

7.2.2 Effect of sub-maximal on exercise on transit time

8 non-smoking male subjects, aged 18 to 25 years (22.6±1.4), were studied. Continuous recordings were made of finger and ear PPG (section 2.3.2.1), Portapres pressure (2.8.4), ECG (section 2.3.1.2) and bioimpedance (section 2.4.1.1). Blood pressure was recorded manually (section 2.8.1). Signals were recorded as described in section 2.2, and timing measurements made offline.

5 minutes of baseline resting measurements were made initially. Subjects then carried out a sub-maximal exercise test, as described in section 2.9.1.4. At this point, exercise ceased completely and subjects were monitored for a further 15 minutes. Blood pressure was measured at each minute of baseline, incremental exercise and recovery, and every 5 minutes once steady workload was achieved.

Where comparisons with manual blood pressure were made, continuous waveform measurements were averaged over the 1 or 5 minutes (depending on frequency of measurements) centred around the corresponding BP time point. Trends across the sub-maximal phase, lasting from minute-15 to minute-60, were assessed by repeated measures ANOVA and by linear regression against time. Derived systolic BP was calculated using the correlation slope obtained by plotting rPTT and actual systolic BP at both rest and peak exercise (section 7.3.1.2). Baroreflex sensitivity and power spectra were also calculated during the constant workload phase for both Portapres and rPTT-derived SBP as described above (section 7.2.1).

7.2.3 Effect of fitness on the transit time – blood pressure relationship

46 subjects were studied undertaking a maximal stress test, as described in section 2.9.1.1. Haemodynamic measurements were made as described in section 7.2.2 above. Breath-by-breath expired gas analysis was carried out as discussed in section 2.9.2. "Fitness" was quantified using measures of oxygen uptake, workload, recovery time, heart-rate/work slope and usual level of physical activity.

Following 5 minutes of baseline resting measurements, subjects then carried out a maximal exercise test, continuing to exhaustion. Measurements were continued for 30 minutes post-exercise, with BP recorded every minute for the first 10 minutes, and 5 minutes thereafter. Statistical analyses were performed as for sections 7.2.1 and 7.2.2 above.

A sub-set of 16 individuals repeated the study after a variable period of time (1 to 12 weeks) to assess reproducibility with respect to transit time change and correlation slopes.

7.3 RESULTS

7.3.1 Comparison of transit time and intra-arterial pressure

Subject characteristics are shown in Table 7.1. Changes in arterial BP, transit time and PEP are shown in Figure 7.1, with baseline, maximal and final recovery values given in Table 7.2. There were significant ($p \leq 0.001$ by repeated measures ANOVA) changes in systolic, mean and diastolic pressure, heart rate, rPTT, pPTT and PEP over the course of the study. All three blood pressure parameters increased to peak exercise, and decreased below baseline levels during recovery. Heart rate remained increased at the 15 minutes recovery. rPTT, pPTT and PEP all decreased at peak exercise, but returned to baseline by 15 minutes recovery. PEP comprised 41% of rPTT at rest, falling to 29% at peak exertion.

Table 7.1. *Subject characteristics (N=9)*

Age (years)	22.6 ± 1.4
Height (cm)	176 ± 5
BMI (kg/m^2)	24 ± 1.9
Family history of CVD	1 (11%)
Total cholesterol (mmol/L)	4.1 ± 0.6
HDL cholesterol (mmol/L)	1.4 ± 0.3

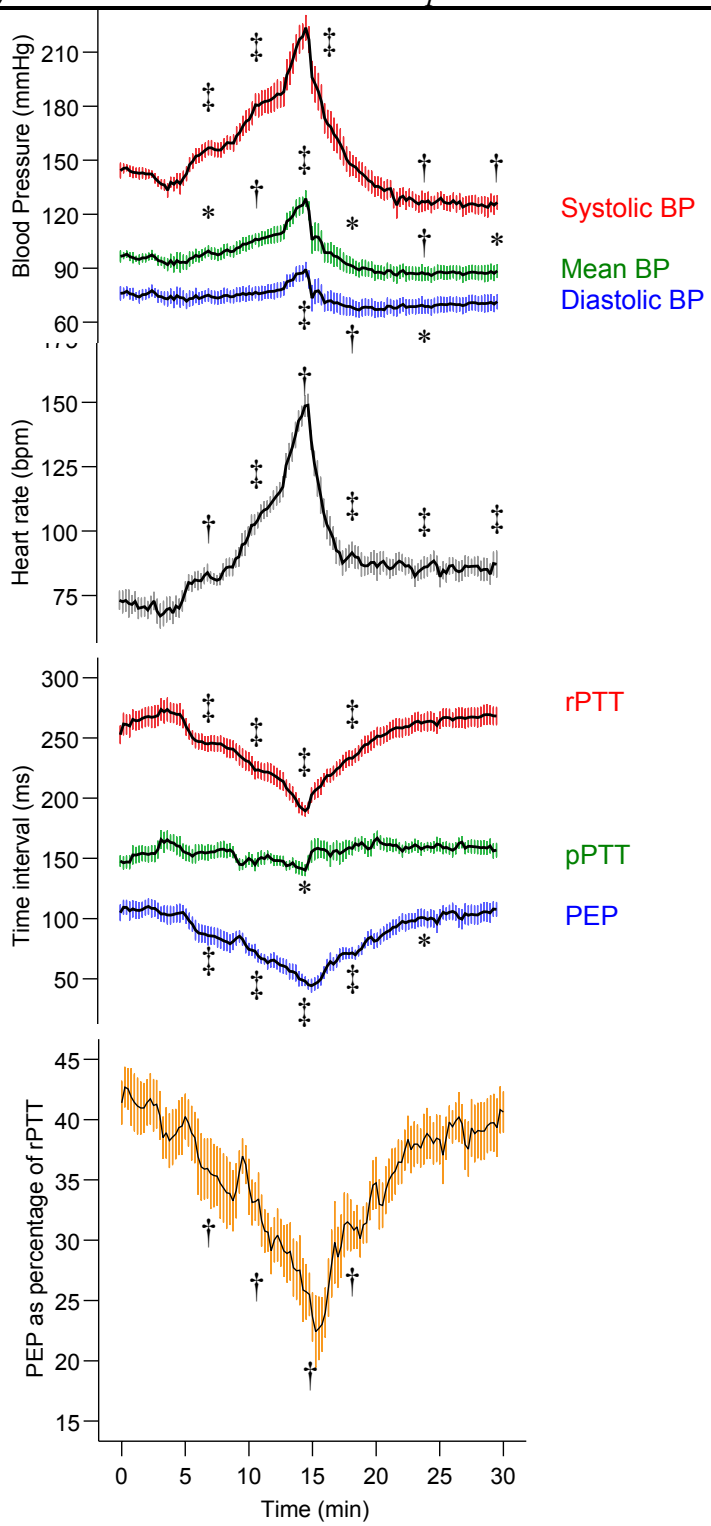
Values are mean ± standard deviation or N(%).

Table 7.2. *Effect of exercise on haemodynamic parameters*

Haemodynamic parameter	Rest	Peak exercise	End recovery
Systolic BP (mmHg)	142.0 ± 6.6	212.6 ± 21.0‡	125.4 ± 13.9†
Mean BP (mmHg)	95.3 ± 6.2	121.6 ± 13.4‡	87.6 ± 11.6*
Diastolic BP (mmHg)	75.0 ± 8.0	84.9 ± 12.0†	70.5 ± 12.3
Heart rate (bpm)	71.0 ± 10.7	140.6 ± 12.4‡	85.3 ± 11.8‡
rPTT (ms)	266.3 ± 23.0	196.3 ± 15.4‡	268.4 ± 22.7
pPTT (ms)	154.3 ± 18.1	137.7 ± 22.8*	158.7 ± 14.8
PEP (ms)	107.5 ± 16.0	55.1 ± 17.0‡	105.5 ± 18.6
PEP (% of rPTT)	41.0 ± 5.4	28.8 ± 9.8†	39.8 ± 5.6

*Values are mean ± SD. Rest values are mean of 5 minutes baseline. Peak exercise values are mean of entire 2 minutes of stage 3. End of recovery is average of final 2 minutes of recovery. * $p<0.05$; † $p<0.01$; ‡ $p<0.001$, significance relative to 5 minute baseline period.*

Figure 7.1. *Effect of exercise on intra-arterial blood pressure and transit time*



*Changes in different measures (from top) of blood pressure, heart rate, transit time (inc. PEP) and PEP as percentage of rPTT. Values are 15 second means ± 1 standard error. Significance is calculated from mean value of the 5 minute baseline, and is shown for average of last 2 minutes of each exercise phase, and for average of the 2 minutes at 5, 10 and 15 minutes recovery. * p<0.05; † p<0.01; ‡ p<0.001.*

7.3.1.1 Correlation between BP and transit time parameters

Linear regression and correlation coefficients describing the relationship between different components of PTT, and different BP parameters, are shown in Table 7.3. rPTT had a strong negative linear correlation with systolic BP (R=-0.97) during exercise, more so than with diastolic BP (R=-0.59, p=0.006) or mean BP (R=-0.87, p=0.022). rPTT also correlated more strongly with systolic BP (R=-0.92) during recovery, than with either diastolic BP (R=0.03, p<0.001) or mean BP (-0.69, p=0.001). Mean BP correlated marginally better with pPTT than did systolic BP (p=0.048) during recovery only, but the correlations were weak (R=-0.12 vs. -0.03), and otherwise there were no significant differences in the correlation between pPTT and any of the BP parameters during either exercise or recovery. rPTT correlated significantly better than pPTT with both SBP and MBP, for during both exercise (p=0.011 and 0.042 respectively) and recovery (p=0.001 for both). There were no significant differences in the strength of the correlation between diastolic BP and either pPTT or rPTT, during either exercise or recovery.

The strength of the SBP/rPTT correlation was slightly less during recovery (R=-0.92), although this difference was not statistically significant (p=0.12). Furthermore, no significant differences existed in slope (p=0.34) or intercept (p=0.13) of the SBP/rPTT relationship. DBP correlated better with rPTT during exercise compared with recovery (p=0.002), but otherwise no differences were found in the remaining BP/PTT correlations. Furthermore, no differences in the correlation gradient were found in any BP/PTT correlation between different experimental phases. The correlation intercept was greater during exercise for the rPTT/DBP (p=0.026) and rPTT/MBP (p=0.032) correlations only. Despite this, clear hysteresis was observed between exercise and recovery phases of the correlation between rPTT and all three BP parameters (Figure 7.2). This was confirmed by comparison of the area under the curve (AUC) of each experimental phase (p≤0.002 for each). The mean response time for SBP to reach steady state was less than that of rPTT (1.99±0.53 vs. 2.56±0.58 min, p=0.079), although there was no difference between rPTT and PEP (2.34±0.75 min, p=0.59). No significant difference in AUC was found between exercise and recovery, for the correlation between pPTT and any BP parameter. The

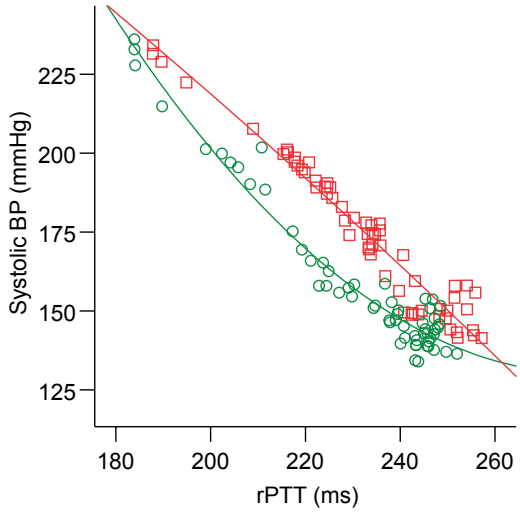
strength of the correlation between SBP and rPTT was not significantly different from that between HR and rPTT during exertion (p=0.44), but the former correlation was significantly stronger than the latter during recovery (p=0.037).

Table 7.3. *Regression and correlation coefficients for transit time / BP relationships*

		DBP	MBP	SBP	HR
Pearson correlation coefficient, R					
rPTT	Exercise	-0.59 ± 0.31	-0.87 ± 0.11	-0.97 ± 0.02	-0.96 ± 0.02
	Recovery	0.03 ± 0.53	-0.69 ± 0.3	-0.92 ± 0.08	-0.76 ± 0.10
pPTT	Exercise	-0.46 ± 0.32	-0.43 ± 0.44	-0.36 ± 0.5	-0.50 ± 0.30
	Recovery	-0.3 ± 0.21	-0.12 ± 0.4	-0.03 ± 0.49	-0.05 ± 0.39
Regression slope					
rPTT	Exercise	-0.14 ± 0.09	-0.37 ± 0.14	-1.02 ± 0.3	-1.00 ± 0.16
	Recovery	-0.05 ± 0.19	-0.24 ± 0.23	-0.9 ± 0.33	-1.42 ± 0.43
pPTT	Exercise	-0.31 ± 0.26	-0.5 ± 0.58	-0.98 ± 1.57	-0.32 ± 0.30
	Recovery	-0.19 ± 0.2	-0.16 ± 0.37	0.02 ± 1.21	-0.18 ± 0.65
Regression constant (intercept)					
rPTT	Exercise	109 ± 28	190 ± 37	405 ± 68	332 ± 25
	Recovery	79 ± 47	147 ± 54	360 ± 78	379 ± 45
pPTT	Exercise	124 ± 42	179 ± 83	315 ± 225	181 ± 30
	Recovery	97 ± 37	112 ± 57	128 ± 188	175 ± 58
Difference in AUC (ms.mmHg)					
rPTT		962 ± 612	790 ± 394	615 ± 417	-
pPTT		340 ± 2872	366 ± 1430	288 ± 686	-

Values are mean±SD of intra-individual correlations. AUC, area under curve (difference is exercise minus recovery).

Figure 7.2. *Example of hysteresis in the rPTT/systolic BP relationship between exercise and recovery phases*



Data in typical subject. Time points are 15 second averages for sake of clarity. □, exercise phase; ○, recovery phase. Regression lines shown are best-fit quadratic.

7.3.1.2 Estimation of systolic BP using rPTT

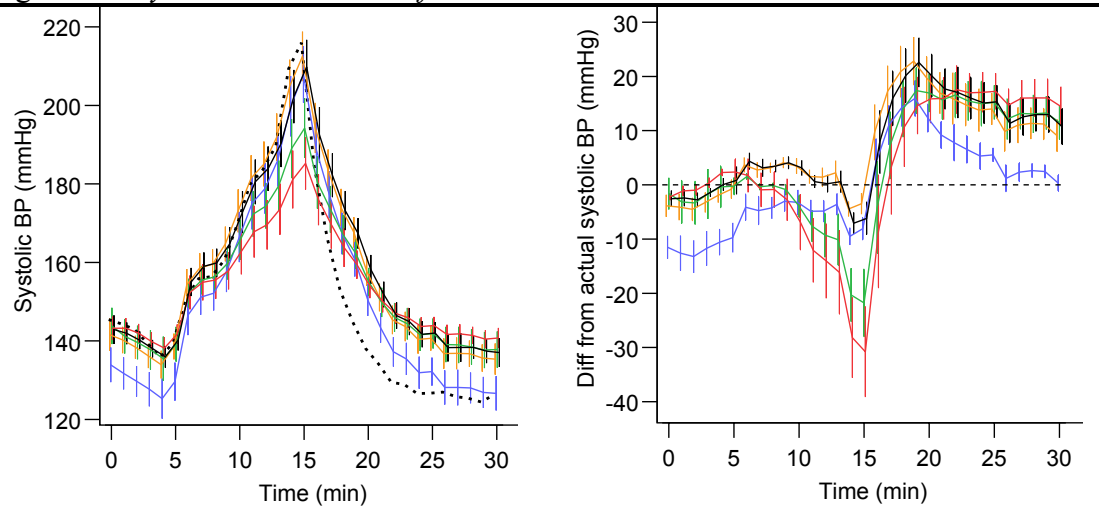
Linear regression coefficients for the exercise period, calculated from five different segments of data – entire exercise period alone, exercise and recovery periods combined, first 4-minute stage of exercise only, 5 minutes resting data alone, and a slope plotted between the 30 second average immediately prior to exercise and immediately prior to the cessation of exercise – are shown in Table 7.4. Systolic BP derived using these five regression equations (SBP_{ex}, $SBP_{ex\text{-}rec}$, SPB_{mildex}, SBP_{rest} and $SBP_{rest\text{-}peak}$ respectively), with differences from measured BP, are shown in Figure 7.3.

Table 7.4. *Regression coefficients for estimation of systolic BP using rPTT*

	SBP_{ex}	$SBP_{ex\text{-}rec}$	SPB_{mildex}	SBP_{rest}	$SBP_{rest\text{-}peak}$
Intercept	405 ± 68	413 ± 58	333 ± 76	298 ± 56	395 ± 73
Slope	-1.02 ± 0.27	-1.08 ± 0.26	-0.72 ± 0.28	-0.59 ± 0.19	-0.98 ± 0.33

Values are mean±SD of intra-individual comparisons.

Figure 7.3. *Systolic BP estimated from rPTT*

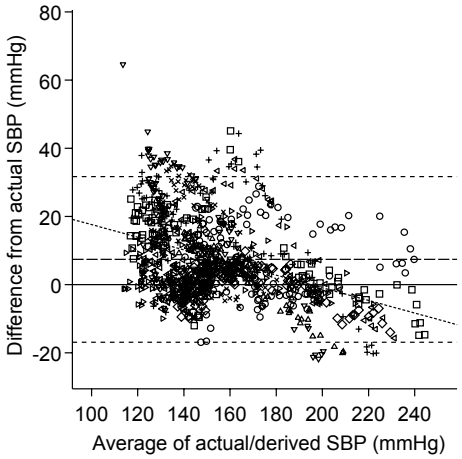


Left: Systolic BP estimated using rPTT, mean±1SE. Broken line indicates measured systolic BP. Right: Difference between estimated systolic BP and measured systolic BP, mean±1SE. Orange, SBP_{ex}; blue, $SBP_{ex\text{-}rec}$; green, SPB_{mildex}; red, SBP_{rest}; black, $SBP_{rest\text{-}peak}$..

Derived values were compared with actual systolic BP measured at baseline (average of 5 minutes), the final 2 minutes of each exercise stage, and the 2 minutes at 5, 10 and 15 minutes recovery. No significant differences existed between actual SBP and either SPB_{mildex} or SBP_{rest} at rest or during the first exercise stages. However, both considerably underestimated (>10mmHg) systolic BP at stages 2 and 3 of exercise both rest and peak BP, with considerable variability in the error. In contrast, during the recovery phase, both estimates of systolic BP were significantly ($p \leq 0.013$) higher by between 12 and 17mmHg than measured values at all time points. SBP_{ex} and $SBP_{rest\text{-}peak}$ underestimated by around 3mmHg ($p<0.01$) actual SBP at rest and stage 1, with no significant bias at stage 2 exercise. A significant underestimation persisted at peak exertion, albeit considerably less and with smaller limits of agreement (-4.0±3.3 mmHg, p=0.007 and –6.6±6.4 mmHg, p=0.014 respectively). Systolic BP was again overestimated during recovery, by around 21 mmHg at 5 minutes, but not significantly differently from SPB_{mildex} or SBP_{rest} later in recovery. $SBP_{ex\text{-}rec}$ gave a considerably greater error at rest than the other estimates, fell between the other four estimates during exercise, and gave substantially better estimates of BP in the latter two stages of recovery.

In summary, of the five different regression equations, SBP_{ex} and $SBP_{rest\text{-}peak}$ were considered to give the best overall estimates of actual peak exercise SBP, whilst differing only minimally from SPB_{mildex} and SBP_{rest} at rest and mild exercise. $SBP_{ex\text{-}rec}$ was not significantly less accurate at peak exercise, and gave an improved estimate of recovery BP, but underestimation of resting BP was not felt to favour its use for estimating systolic BP during the exercise phase. Comparison of SBP_{ex} and $SBP_{rest\text{-}peak}$ demonstrated minimal differences at all stages of exercise (–0.8±2.1 mmHg at rest, +1.2±1.5 mmHg at peak exercise, and –0.7±1.6 mmHg at 15 minutes recovery), and as the calculation of $SBP_{rest\text{-}peak}$ requires potentially only a single baseline and peak BP measurement, this simpler means of calibration was used for further work examining baroreflex sensitivity. A Bland-Altman plot comparing measured systolic BP and $SBP_{rest\text{-}peak}$ is shown in Figure 7.4. Of note, this demonstrates a significant tendency for bias to becoming increasingly positive with decreasing blood pressure (R=0.40).

Figure 7.4. *Bland-Altman plot comparing systolic BP and $SBP_{rest\text{-}peak}$.*

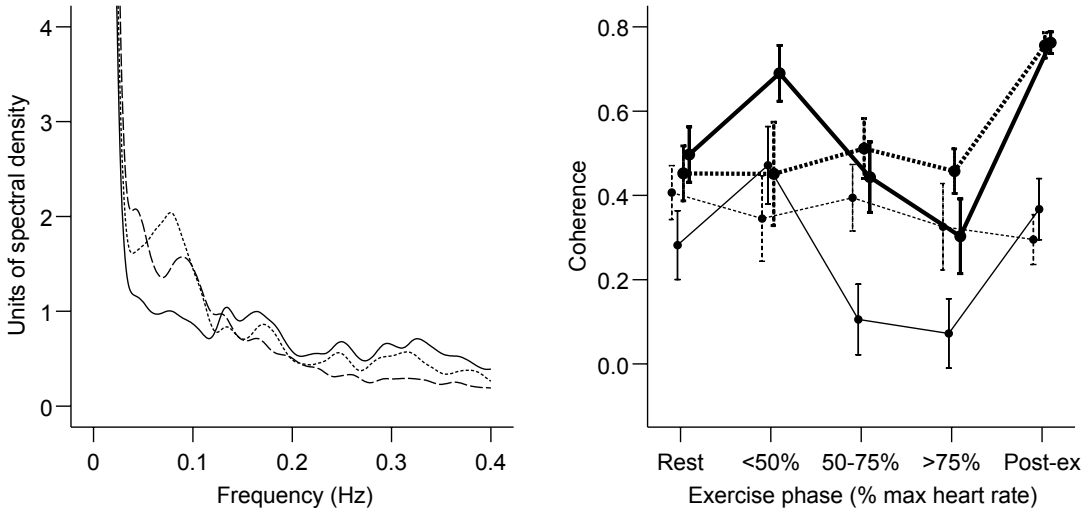


Broken horizontal lines show mean bias (predicted measurement minus actual) and 95% limits of agreement (mean±1.96SD). Zero reference line is shown as solid line. Broken diagonal line demonstrates correlation between bias and average of actual/derived values.

7.3.1.3 Estimation of beat-to-beat variability and baroreflex sensitivity using rPTT

Spectral power analysis of heart rate, systolic BP and rPTT are shown in Figure 7.5. Coherence between rPTT and systolic BP, and between rPTT and heart rate, is also shown in Figure 7.5. When individual experimental phases were examined, low frequency coherence (0.05 to 0.20 Hz) was significantly greater for rPTT/BP (compared with rPTT/HR) during recovery only ($p<0.001$), with no significant differences at other time points. When examined across the entire experimental period, rPTT/BP low frequency coherence was significantly higher than rPTT/HR coherence ($p<0.001$). High frequency coherence (0.20 to 0.40 Hz) was significantly higher ($p\leq0.015$) for rPTT/BP at mild and moderate exercise, and during recovery, with no differences found at rest or peak exertion. Overall, high frequency coherence was again higher ($p<0.001$) for rPTT/BP.

Figure 7.5. *Spectral power analysis and spectral coherence*

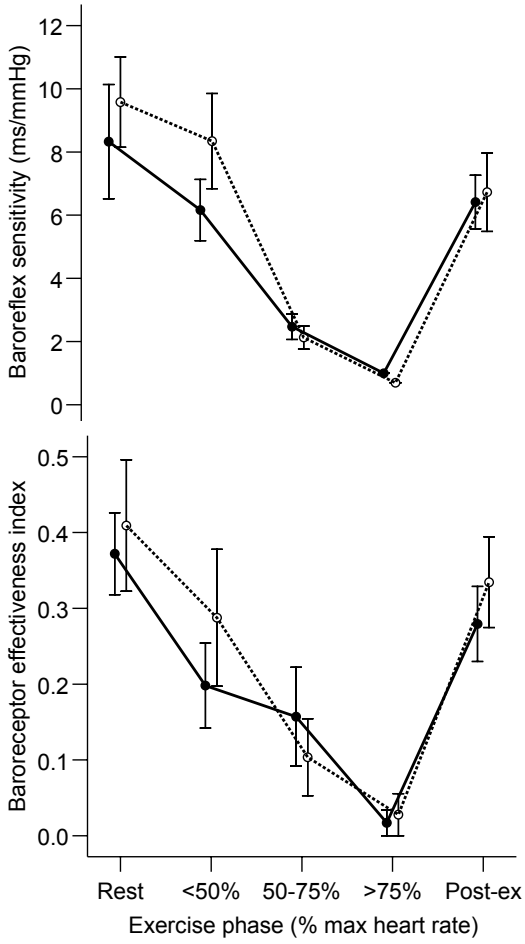


Left, *spectral power analysis of rPTT (solid line), measured systolic BP (dotted) and heart rate (dashed); values are mean across entire experimental time period.*

Right, *spectral coherence calculated over different experimental phases; thick lines represent coherence between systolic BP and rPTT, and thin lines, coherence between heart rate and rPTT; solid lines represent high frequency coherence (0.2-0.4Hz), and dashed lines, low frequency coherence (0.05-0.2Hz); values are mean±1SE.*

Baroreflex sensitivity, including baroreflex effectiveness index, is shown in Figure 7.6. There was not found to be any statistically significant difference between BRS, as quantified by both sequence regression slope and by BEI, when calculated using either actual SBP or derived pressure ($SBP_{rest\text{-}peak}$), at any of the 5 experimental time points.

Figure 7.6. *Baroreflex sensitivity and baroreflex effectiveness index*



Values are mean±1SE. Solid line, BRS derived from actual systolic BP values; dashed line, BRS derived from estimated systolic BP ($SBP_{rest\text{-}peak}$).

7.3.2 Effect of sub-maximal on exercise on transit time

Subject characteristics are given in Table 7.5. Baseline, sub-maximal (average during constant workload) and end-of-recovery BP, heart rate, rPTT, pPTT and PEP are shown in Table 7.6. Changes over the entire experimental time period are shown in Figure 7.7. SBP and HR increased at to peak exercise ($p<0.001$), whereas DBP, rPTT, pPTT and PEP all decreased ($p\leq0.001$). HR, PEP and rPTT were elevated at the end of recovery ($p=0.029$, 0.009 and 0.002 respectively), whereas other parameters were not significantly different from baseline. The increase in rPTT following exercise was therefore associated with an increase in PEP rather than any change in pPTT. The percentage contribution of PEP thus varied from 33.2% at baseline, 29.1% during exercise, and 36.8% post-exercise.

Table 7.5. *Subject characteristics (N=8, all male)*

Age (years)	28.2 ± 6.9
Height (cm)	181 ± 6
BMI (kg/m^2)	22.5 ± 1.9
BP (mmHg)	136/75 ± 12.6/7.6
Heart rate (bpm)	57.4 ± 7.2
$VO_{2\text{-}AT}$ (L/min)	2.44 ± 0.54
$VO_{2\text{-}MAX}$ (L/min)	3.94 ± 0.26
$Work_{AT}$ (W)	206 ± 41
$Work_{MAX}$ (W)	375 ± 36
Mean recovery response time (min)	3.72 ± 0.52
Average activity per week (hours)	8.0 ± 0.9
Competitive sport (≥national level)	6 (75%)

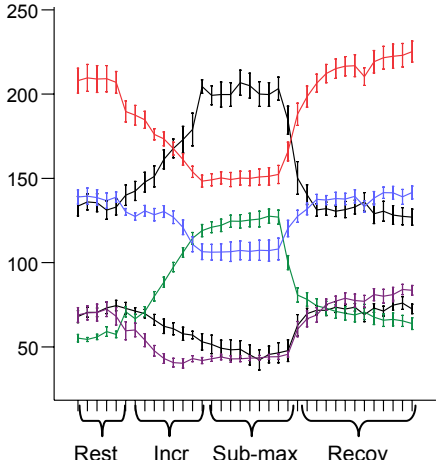
Values are mean ± standard deviation or N(%)

Table 7.6. *Baseline, sub-maximal exercise and recovery parameters*

	Baseline	Sub-maximal phase	End of recovery
Systolic BP (mmHg)	133.9±13.9	202±18.2	129.6±14.7
Diastolic BP (mmHg)	71.3±8.6	47.8±14.9	72.9±8.7
Heart rate (bpm)	56.2±5.5	124.1±10.8	66.4±10.3
rPTT (ms)	208±19.9	150.2±11.6	221.1±18.3
pPTT (ms)	138.6±11.2	106.9±14.7	139.7±10.7
PEP (ms)	69.4±12.5	43.3±4.3	81.5±9.8
PEP% (% of rPTT)	33.2±3.8	29.1±4.7	36.8±2.3

Values are mean ± 1SD.

Figure 7.7. *Haemodynamics changes during submaximal exercise*

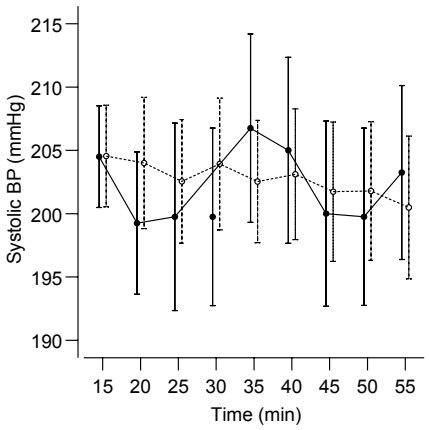


Time axis shows rest, 1-minute incremental, sub-maximal constant workload, and recovery phases. Measurement points are as detailed in text. Black, SBP and DBP (mmHg); green, heart rate (bpm); red, rPTT (ms); blue, pPTT (ms); purple, PEP (ms). Values are mean±1SE.

During the constant sub-maximal phase, HR had a tendency to increase with time (regression against time, R=0.24, p=0.043; rmANOVA, p<0.001). Both PEP and DBP showed variability over the sub-maximal phase (p=0.031 and 0.028, rmANOVA), but this was not related to time (R=0.12 and -0.18, p=0.31 and 0.14 respectively). SBP, rPTT and pPTT did not change during the sub-maximal phase.

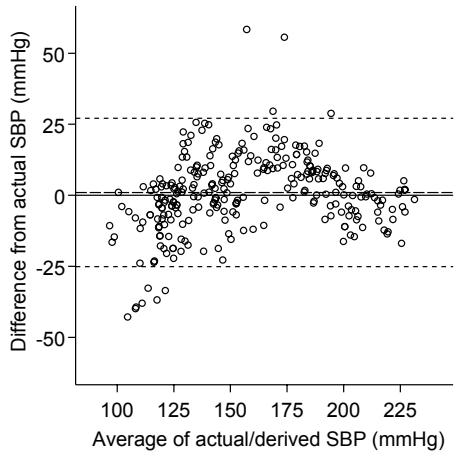
Derived systolic BP over the sub-maximal phase is plotted against actual SBP in Figure 7.8. The matching at the 15-minute time point is due to the use of this time as one of the points for calculating the regression slope used to derive SBP from rPTT. A Bland-Altman plot comparing derived and actual SBP over the entire study is given in Figure 7.9. Mean bias during the sub-maximal phase was +0.7±8.3 mmHg (p=0.45), and over the entire experimental period, +1.0±13.3 mmHg (p=0.20). Although there was a degree of variability in derived SBP over time (p=0.028, rmANOVA), this was not related to time (p>0.11, regression vs. time). In contrast, Portapres significantly underestimated SBP over the entire experiment (3.8±25.7 mmHg, p=0.014), and this was particularly so during the sub-maximal phase (-27±25.9 mmHg, p<0.001). Over the entire study, the percentage of derived SBP values falling within 5%, 10% and 15% of actual values (as based on British Hypertension Society grading) were 51%, 75.8% and 88.4%. The accuracy of SBP prediction improved when the sub-maximal phase alone was examined, with 77.8% of values within 5% and 20.8% within 10% accuracy.

Figure 7.8. *Systolic BP during sub-maximal exercise*



Values are mean±1SE. Solid dots and lines, actual SBP. Open dots and broken lines, SBP derived from rPTT.

Figure 7.9. *Bland-Altman comparison of actual and derived systolic BP*

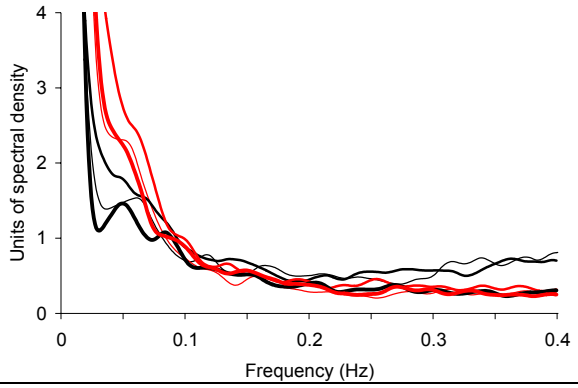


Broken lines show mean bias (derived minus actual SBP) and 95% limits of agreement, for entire experimental period.

Variability in baroreflex sensitivity was examined over the course of the sub-maximal phase. Baroreflex effectiveness index was very low, so the first and last 20 minutes of data were used, to increase the numbers of valid sequences available for comparison. BEI decreased from 0.02±0.05 to 0.005±0.02 (p=0.045, Mann-Whitney U), despite no change in the total number of SBP sequences observed (p=0.78). Initial BRS slope was 0.93±0.54, and did not change with exercise (p=0.12).

Power spectra for Portapres SBP and for SBP derived from rPTT, during the sub-maximal phase, are shown in Figure 7.10. Coherence was significantly greater in the 0.05 to 0.20 Hz frequency range (mean coherence 0.73±0.24) than the 0.20 to 0.40 Hz range (0.30±0.39). Although coherence, for both low and high frequency ranges, increased slightly from the initial 15 minutes (0.65±0.31 and 0.16±0.47 respectively) to the last 15 minutes (0.80±0.18 and 0.44±0.35), this change was not statistically significant (p=0.13 and 0.11 respectively). However, coherence between HR/rPTT (0.77±0.23 low frequency, 0.24±0.44 high frequency) and SBP/rPTT did not differ for low (p=0.23) or high (p=0.48) frequency ranges.

Figure 7.10. *Power spectra for systolic BP during sub-maximal exercise*



Red, Portapres systolic BP.
Black, systolic BP derived from $rPTT_{finger}$.
Thin, medium and heavy lines are first, second and third 15 blocks during sub-maximal exercise respectively.

7.3.3 Effect of fitness on the transit time – blood pressure relationship

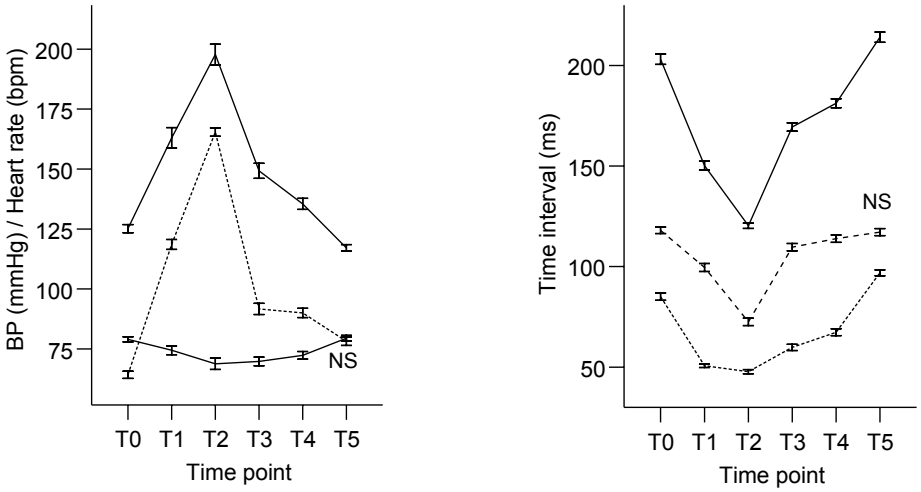
Subject details are given in Table 7.7, and responses to exercise are shown graphically in Figure 7.11.

Table 7.7. *Subject details (N=46)*

Male	31 (67.4%)
Age (years)	33 ± 8.7
Height (cm)	176 ± 10.4
BMI ($kg.m^2$)	23.0 ± 3.3
BP (mmHg)	126.4/80.0 ± 12.9/7.6
Heart rate (bpm)	64.4 ± 12.8
$VO_{2\text{-}AT}$ (L/min)	1.57 ± 0.62
$VO_{2\text{-}MAX}$ (L/min)	2.86 ± 0.93
$Workload_{AT}$ (W)	130 ± 54
$Workload_{MAX}$ (W)	270 ± 82
Mean recovery response time (min)	3.81 ± 0.40
Heart rate – work slope (W/bpm)	2.47 ± 0.82
Average activity per week (hours)	6.9 ± 4.8
Competitive sport (≥ national level)	11 (23.9%)

Values are mean±SD or N(%).

Figure 7.11. *Haemodynamic responses to exercise*



Values are mean±SE. Time points are, for T0 to T5 respectively, rest, anaerobic threshold, peak (VO2-max), mean-response recovery time, 5 minutes and 30 minutes post-exercise. Left chart from top, systolic BP, heart rate, diastolic BP; right chart from top, rPTT, pPTT, PEP. All time points show significant (p≤0.001) change from baseline (T0) with the exception of DBP and pPTT at time point T5 (marked NS).

The average regression slope of the rPTT/systolic BP correlation during exercise was -0.81±0.27 during exercise. There was a strong correlation between slope gradient and the change from baseline to peak exercise in systolic BP ($R=0.63$, $p<0.001$). However, no correlation existed between slope gradient and the change with exercise in rPTT ($p=0.29$), or its components PEP ($p=0.21$) or pPTT ($p=0.45$).

The slope was found to have a significant ($p<0.01$) positive correlation with various measures of exercise capacity, including $VO_{2\text{-}MAX}$, $VO_{2\text{-}AT}$, workload at peak exercise and anaerobic threshold, and the heart rate-work slope, although there was a large degree of co-linearity. In a multivariate linear model, incorporating the additional factors of age, sex, and height, $VO_{2\text{-}MAX}$ was the strongest predictor of slope gradient ($R=0.45$, $p=0.002$). However, this was largely due to the strong positive correlation between $VO_{2\text{-}MAX}$ and exercise systolic BP ($R=0.71$, $p<0.001$).

The linear regression equation for rPTT/SBP slope and $VO_{2\text{-}MAX}$, had the coefficients gradient=0.133 and intercept=-1.194. Given a difference in $VO_{2\text{-}MAX}$ of 1 standard deviation (0.93L/min), this corresponds to a change in the rPTT/SBP slope gradient of 0.124. As the offset of the rPTT/SBP slope can be calculated by use of resting values of rPTT and SBP, this would result in an underestimation of peak-exercise SBP of 10.1mmHg, based on the mean decrease in rPTT during exercise of 81.6ms. This corresponds to an error of 5.1%, given the average peak SBP of 196.9mmHg.

The slope of the SBP/rPTT correlation was significantly steeper during aerobic exercise, than after the anaerobic threshold was reached (-1.07±0.16 vs. -0.41±0.09, $p<0.001$). The strength of the correlation (as determined by Pearson coefficient) did not differ however ($p=0.25$). The rate of change of workload was constant throughout the entire exercise period, so changes in BP and rPTT were calculated relative to work. The BP/work slope was constant before and after exercise (0.26±0.13 vs. 0.26±0.12 mmHg/W, $p=0.95$), whereas the rPTT/work slope fell significantly during anaerobic exercise (-0.45±0.21 vs. -0.23±0.10 ms/W, $p<0.001$). The change in rPTT/work slope was due to a combination of minimal change in PEP post-anaerobic threshold (-0.31±0.18 vs. –0.02±0.05 ms/W, $p<0.001$), offset by a slight increase in the rate of change of pPTT (-0.15±0.09 vs. –0.20±0.08 ms/W,

p=0.006). The degree of change in rPTT/SBP correlation slope was unaffected by any of the above measures of exercise capacity (including $VO_{2\text{-}AT}$ and workload at anaerobic threshold) or by age, sex, height, or resting or exercise BP or heart rate.

The exercise and recovery SBP/rPTT correlations showed significant differences, as determined by AUC (472±729 ms.mmHg, p<0.001). rPTT and SBP had returned to a steady state by 30-minutes post-exercise, albeit 11.0±13.4 ms above and –7.6±8.6 mmHg below baseline respectively (p<0.001 for both). The systolic BP predicted from rPTT at 30-minutes post-exercise was not significantly different from the actual value (0.3±10.9 mmHg, p=0.85). However, SBP returned to steady state more rapidly than rPTT (mean response time 2.8±0.8 vs. 3.5±0.7 min respectively, p<0.001). The degree of difference in AUC between exercise and recovery was significantly negatively correlated with $VO_{2\text{-}MAX}$, the heart rate-work slope, and positively correlated with resting heart rate. The difference was also slightly greater in women (p=0.072). Multivariate analysis found $VO_{2\text{-}MAX}$ and heart rate/work slope to be independent predictors of the difference in AUC. Both $VO_{2\text{-}MAX}$ and heart rate/work slope had negative correlations with the difference in recovery rates between SBP (R=-0.26, p=0.09) and rPTT (R=0.38, p=0.009) with the recovery rate for SBP increasing with exercise capacity. However, neither of these measures of exercise capacity was correlated with the difference between measured and estimated SBP at 30-minutes post-exercise (p=0.43 for both).

Reproducibility of PTT and BP measurements was examined in 16 subjects who were studied on a second occasion, between 1 and 12 weeks following the first study. The baseline and peak measures of heart rate, SBP, rPTT, PEP, $VO_{2\text{-}MAX}$ and workload did not vary significantly between visits. Bias was -4.1±16.9 ms and 2.6±7.3 mmHg respectively for resting rPTT and SBP. At peak exercise, bias was 0.4±9.5 ms and -3.6±14.9 mmHg respectively. The corresponding coefficients of variation were 8.3%, 5.9%, 7.9% and 7.5% respectively. There was considerably greater variability in the correlation slope, although no significant bias between visits was observed (0.02±0.23, p=0.67). This corresponds to a potential error of ±31.5mmHg, based on a mean change in rPTT during peak exercise of 70ms. Coefficient of variation for regression slope was 28.2%.

7.4 DISCUSSION

rPTT has been used during exercise in a number of psychophysiology studies (Johnston *et al*. 1982; Lo & Johnston 1984; Marie *et al*. 1984). The principle aim of those studies was to study cardiovascular feedback, rather than the haemodynamic changes occurring during exercise. Carruthers and Barschdorff have also used rPTT as a marker of BP (Barschdorff & Erig 1998; Carruthers & Taggart 1988). Several groups have studied the changes in systolic time intervals, including PEP, that occur during exercise (Cardus & Vera 1974; Gollan *et al*. 1978; Martin *et al*. 1971; Sugiura *et al*. 1981; Thomas 1992). However, this is the first time that the relationship between exercise rPTT, PEP and blood pressure has been examined in detail.

A strong negative correlation SBP and rPTT was observed during exercise. rPTT correlated better with SBP than either DBP or MAP, whereas pPTT reflected changes in intra-arterial DBP. Furthermore, spectral coherence between HR, SBP and rPTT confirmed that rPTT was more closely associated with BP than HR. Importantly, however, these studies have demonstrated important discrepancies in the linear nature of the rPTT/SBP correlation which has important implications for use of transit time in the estimation of exercise BP.

7.4.1 Factors affecting the transit time – blood pressure relationship during exercise

Initially, it is worthwhile discussing important differences in the findings between the arterial-line study and the maximal stress test. Firstly, only minimal change was observed in pPTT at peak exercise in the arterial-line experiment (16.7±15.7 ms), compared to the maximal exercise test (45.5±13.5 ms). Secondly, whereas SBP fell to a steady-state recovery value below that of baseline during both experiments, rPTT returned to normal only during the arterial-line study. Why do these differences exist? It seems most likely that the exercise workload in the arterial-line study was significantly less than that corresponding to $VO_{2\text{-}MAX}$, and this is reflected in the lower maximal heart rates achieved in the arterial-line experiment. Furthermore, the maximum stage was only maintained for 2-minutes. Indeed, both the heart rate and fall in pPTT during the arterial-line study are actually similar to that observed at the

anaerobic threshold during the maximal stress test (-54.4±15.8 bpm and 18.0±12.6 ms respectively). At lower levels of exertion, any effect of an increase in systolic pressure on MAP is offset by a decrease in peripheral vascular resistance due to vasodilatation (Ekelund 1967). Increasing systolic pressure becomes the predominant determinant of MAP at higher workloads however, and the exponential association between distending pressure and arterial stiffness (Hughes *et al*. 1979) results in a corresponding decrease in pPTT. Most probably, the relative brevity and low-intensity of the final stage during the arterial-line study, meant that the changes in both MAP and pPTT were relatively small. MAP was not reported for the maximal test, as sphygmomanometry commonly underestimates diastolic pressure (Kaijser 1987). Indeed, the intra-arterial measurements clearly demonstrated an increase in DBP, whereas sphygmomanometry measurements showed a decrease. Finally, following maximal exercise, the greater post-exertion arterial dilatation reduces left ventricular afterload leading to a relative shortening of PEP (and therefore rPTT) compared to baseline values. An alternative explanation for these observations may be the differences in the methodology employed in each study. It is possible that, during the arterial line study, bioimpedance overestimated the decrease in PEP and hence masked any change in pPTT. Alternatively, the reverse may have been true of the custom bioimpedance algorithm used during the maximal exercise study. However, similarities between the two techniques as tested in the initial methodological development suggest this is unlikely to be the case. A final important issue is that the real-time signal processing system gave considerably higher values of transit time than the off-line analysis system. Unfortunately, it was not possible to directly compare the two systems. Both systems were able to detect small changes in transit time, and so it seems reasonable to make the assumption that the timing resolution was adequate for both. The off-line system was subject to considerable testing, including accurately determining hardware processing time delays and visual confirmation of time points, and its absolute timing accurate was therefore considered to be reliable. However, the real-time system could not be subject to such rigorous testing. The likeliest reason for the differences in timing are likely to be errors in the hardware signal processing delays, although subtle differences in the intersecting tangent algorithm may also have contributed. Unfortunately, as these

discrepancies only came to light after the decision to move to an off-line system had been made, it was not possible to explore them in more detail. Nonetheless, the relative changes in transit time recorded appear robust, and absolute measurements are probably subject to a constant error, thus not affecting the conclusions.

The rPTT/SBP correlation was slightly, albeit not significantly, weaker during recovery than during exercise, with no changes in regression slope. Hysteresis was observed between exercise and recovery phases. During both the experiments, SBP fell below pre-exercise baseline levels during recovery. However, rPTT reached a steady-state value greater than baseline only following maximal exercise, and indeed this corresponded to the lower-than-baseline value of SBP observed at this time. Despite this, hysteresis existed in the rPTT/SBP relationship in both studies, as in both experiments SBP returned to the steady-state recovery level more rapidly than did rPTT. Interestingly, the rate of change of SBP during recovery appeared inversely associated with exercise capacity, resulting in a rPTT recovery curve similar to that of SBP, thus reducing the hysteresis observed. This finding is perhaps not unexpected. Athletes are known to have marked resting left ventricular dilatation with superior mechanisms for increasing cardiac output (Clausen 1977), and a mismatch of stroke volume and isometric muscle effort occurs during recovery. This would result in a slower fall in BP in trained individuals corresponding to the slower increase in PEP (and thus rPTT), and hence increase the linearity of the rPTT/SBP recovery relationship.

Estimation of SBP from rPTT requires calibration of the system. Use of beat-to-beat rPTT and BP data gathered at rest is one option, and potentially a technique such as tonometry might be used to achieve resting BP measurements. Such an approach has the advantage of not requiring an initial exercise test to achieve the calibration. Despite this benefit, however, this approach resulted in an underestimation of peak-exercise pressure. Use of data from low intensity exercise is a compromise, with the advantage that only a brief calibration exercise test needs carried out and BP measurements may be obtained using a sphygmomanometer. Again, however, this resulted in underestimation of peak-exercise SBP. Unsurprisingly, use of rPTT and SBP data over a wide range resulted in a better estimation of peak SBP; the most

practical approach was to take measurements of BP and rPTT at rest and at peak exercise alone, and to assume linearity between these points. The decreased linearity between rPTT/SBP during recovery means that BP prediction will be subject to error. However, the degree of inaccuracy in recovery BP depends to a large degree on whether or not rPTT returns to a value relative to baseline that corresponds to the difference between baseline and recovery SBP. Of course, the intra-subject variability in regression slope was also considerable, and a single calibration has therefore the potential to result in large errors in estimated SBP.

The regression slope was primarily determined by the degree of change in SBP rather than the degree of change in rPTT. Furthermore, the regression slope was correlated with exercise capacity, due to the greater increase in SBP with increasing $VO_{2\text{-}MAX}$, a phenomenon which is well recognised (Tanaka *et al*. 1996). The variability in regression slope with $VO_{2\text{-}MAX}$, would correspond to an underestimation of peak exercise SBP if a single generalised regression equation was used, making individualised calibration essential. The regression slope also became less steep in the anaerobic phase of maximal exercise, due to a decrease in the rate of change of rPTT relative to SBP. This change was not influenced by exercise capacity. This difference was not observed during the arterial line study, presumably due to the lower level of exertion. This may also account for the steeper overall rPTT/SBP regression slope observed in the arterial line study, although differences in the BP measuring methodology may also be implicated, as sphygmomanometry tends to underestimate intra-arterial SBP during exertion. The decrease in rate of change of rPTT during the anaerobic phase was largely accounted for by minimal change in PEP at higher workloads. This probably corresponds to a marked increase in cardiac output (with corresponding increases in contractility causing a fall in PEP) due to vagal withdrawal upon initiation of exercise (Rowell 1991), whereas at higher exercise intensity first-order heterostatic reflexes mediated through sympathetic outflow increase heart rate and vasoconstriction (Palatini 1994).

The colinearity of many cardiovascular variables during a maximal exercise test may have potentially masked changes in the relationship between transit time and BP. So-called “cardiovascular drift” occurs during prolonged exertion. Peripheral vascular

resistance and MAP decline with long-lasting effort, whilst heart rate increases, and cardiac output remains relatively constant (Ekelund 1967; Palatini 1994). These changes appear largely related to cutaneous thermoregulatory mechanisms (Brengelmann 1983), although cardiac function may be affected (Douglas *et al*. 1987). BP is maintained through various mechanisms, including vasoconstriction of non-essential vascular beds, muscle chemoreflexes (Rowell 1991), and resetting of the arterial baroreflex to maintain a higher pressure (Walgenbach & Donald 1983). Despite these various changes, it was found that the accuracy of systolic BP prediction from rPTT remained relatively constant throughout a period of sustained sub-maximal exercise. Beat-to-beat accuracy, as assessed by coherence with Portapres values, also remained relatively constant during this period. The constancy of pPTT despite a presumed underlying decrease in MAP is suggestive of either a compensatory change in intrinsic wall stiffness, perhaps secondary to sympathetic tone, or possibly due to marked vasodilatation in the hand segment. The current work certainly suggests that transit time measurements can be used over periods of prolonged sub-maximal exertion, but more work needs to be done to elucidate the mechanisms involved. It would also be of interest to note the change in rPTT/SBP correlation if a further maximal exercise test were carried out following a period of prolonged sub-maximal exertion, as the decrease in peripheral vascular resistance limits the subsequent maximal BP response (Palatini *et al*. 1990).

Finally, SBP predicted from rPTT was shown to measure baroreflex sensitivity to a similar degree to intra-arterial beat-to-beat BP measurements. In addition, a slight fall in baroreflex effectiveness index (BEI) calculated from predicted SBP, occurred over prolonged sub-maximal exertion. The arterial baroreflex is set to a higher operating level during exertion in order to maintain an adequate BP (Raven *et al*. 2006). The slight decrease in BEI observed in the current work may reflect compensatory mechanisms in response to the cardiovascular drift discussed above.

7.4.2 Conclusion

In conclusion, there is a strong negative linear correlation between rPTT and SBP, which although constant during prolonged submaximal exercise, changes at higher

levels of exercise and is affected by exercise capacity. Furthermore, the correlation differs during recovery, with this too affected by both intensity of exertion and exercise capacity. Calibration is also essential, but one-off calibrations may be inadequate due to the large intra-individual coefficient of variation in regression slope. These factors mean that rPTT is not a reliable means of measuring absolute BP during exertion, although further work is merited to examine whether the technique can be used for the study of the effects of exercise on baroreflex sensitivity.

8 DISCUSSION

Elevated resting BP measured during clinical consultation is a powerful predictor of CV risk, and is usually measured using cuff sphygmomanometry. However measurement of BP is also of value in other less controlled circumstances, but the dynamic fluctuations in the cardiovascular system may mean that sphygmomanometry is unhelpful. Furthermore, there is increasing recognition that assessment of vascular function through the measurement of arterial stiffness may be of value beyond determination of BP alone. Pulse transit time measured between the ECG QRS complex and the peripheral photoplethysmograph waveform has been proposed as a potential surrogate marker of BP.

This thesis has described a methodology for the measurement of the pulse wave at various sites in the arterial system, using a variety of technologies. It has used these methods to examine the relationship between transit time and BP under two situations of particular importance – drug administration and exercise – and has also extended the techniques to the assessment of conduit artery pulse wave velocity and pulse contour analysis. This chapter reviews some of the important aspects of the methodology employed and the findings from the drug and exercise studies, and examines areas of possible future work.

8.1 A METHODOLOGY FOR MEASURING PULSE TRANSIT TIME

Digital processing has significant advantages over the analogue computing and manual techniques employed in the transit time work published around the 1970s. These include avoiding observer bias (a problem with manual measurements), time savings (particularly with respect to reanalysis of data using different algorithms and processing of large quantities of data), automatic identification of outliers, and the ability to use the recorded data for alternative purposes such as pulse wave analysis.

The initial real-time signal-processing system was developed by the University of Edinburgh School of Engineering and Electronics, in conjunction with Dr Ronald Mackie of Pulse Time Products (PTP), a company which develops heart rate

monitors for the consumer market. Although the real-time system had been thoroughly tested and commercially implemented, and indeed was optimised for the purposes of this thesis, it lacked flexibility, in particular with respect to integration with external signal measurement hardware and post-hoc data processing. Furthermore, although the ECG detection provided good temporal resolution (<1ms), it was felt that the sensitivity of the system, albeit adequate for the consumer market, could be substantially improved. The processing power of the system also limited the complexity and number of time points that could be measured on the leading edge of the pulse wave.

As no commercial system was available at the time that could perform the required analyses to address these issues, a custom system was developed using LabVIEW 6.1. LabVIEW (Laboratory Virtual Instrument Engineering Workbench) is a development environment based on graphical programming. It is designed to facilitate the design and implementation of test and measurement systems and is easily interfaced with external data acquisition hardware. In particular, LabVIEW provides a wide range of useful mathematical and signal processing tools, such as curve fitting and interpolation, Fourier transformation and digital filtering. It was used to create an off-line signal analysis system enabling automated timing analysis of various physiological signals, as well as analysis in the frequency domain and ensemble-averaging of a series of wave complexes in order to evaluate the pulse contour.

Most previously published work on transit time has reported results for the ECG-to-PPG delay, with the PPG signal recorded at the finger or occasionally the ear. The custom LabVIEW system demonstrated sensitivity and positive predictive values for both ECG and pulse wave algorithms of around 95 to 99%, even during strenuous exercise, with a timing resolution of <1ms. It also enabled the measurement of the arterial signal at other anatomical sites simultaneously, making it possible to determine the transit time in smaller segments of the overall path length, and to calculate the pulse wave velocity. Furthermore, it provided the ability to measure PEP. Many studies which utilise ECG-pulse transit time do not measure this systolic time interval, despite it contributing a significant amount to the overall transit time

period. Those studies that have measured PEP have generally employed a polygraph approach (Newlin 1981; Newlin & Levenson 1979), but it was felt that transthoracic bioimpedance would be more practical to implement on a beat-to-beat basis. Although validated at rest, the accuracy of the technique during exercise has been hitherto questionable (Ono *et al*. 2004; Thomas 1992), but a novel intersecting-tangent algorithm was developed which had satisfactory accuracy for the purposes of the subsequent studies. Bioimpedance measures changes in blood volume throughout the cardiac cycle, and therefore is influenced by both ventricular contraction and aortic distension. Aortic distension also varies along the length of the vessel, corresponding to propagation of the pressure wave. The interaction of these different factors means that accurately relating the overall bioimpedance waveform contour to the underlying physiological processes is not possible. Nonetheless, the start of the impedance wave (B-point) corresponds to the start of the cardiac cycle, regardless of the nature of the subsequent waveform, and the B-point has been shown to be the most well-defined and easily detected point on the impedance signal (Lababidi *et al*. 1970).

The use of external, commercially available hardware for signal acquisition introduces the important issue of the electronic delay between physiological input signal and analogue output signal. Although well recognised by electronic engineers, this phase lag is not usually acknowledged in medical literature. Although the error introduced might be assumed to be constant, it is of particular importance if measuring a short time interval with two different medical devices with different delays, where it may account for a substantial proportion of the overall period. Furthermore, the phase delay may fluctuate in certain commercial devices, presumably due to varying computational overheads (Foo *et al*. 2005a). Pilot work found this to be the case with the SphygmoCor system too, although it was not found to be a problem with any of the hardware employed in the studies described in this thesis. Finally, the delay may differ slightly between different points on the signal in question, and more importantly could theoretically be influenced by changes in the fundamental frequency of the input signal (i.e. heart rate). The phase delay must therefore be calculated for a specific timing point (e.g. R-wave *cf.* Q-wave), and the

assumption made that the spectral power of the higher frequency wave components that determine the aforesaid timing points remains relatively constant.

There is scope to improve on all the signal detection algorithms. The pulse wave and bioimpedance detection in particular, however, are adversely affected by the problems of movement artefact. Techniques for reducing motion artefact in PPG signals are described in the literature (Kim & Yoo 2006), and indeed a technique known as triaxial accelerometry has been used to reconstruct corrupted PPG signals in order to determine PTT (Foo *et al*. 2004). No means of artefact reduction for bioimpedance signals is described, although it is likely to be extremely challenging as it is not a small discrete anatomical region being measured. Nonetheless, overall the measurement system devised for this thesis was considered robust, flexible and easy to use, and should facilitate further work in this field.

8.2 EFFECT OF CARDIOVASCULAR DRUGS ON PULSE TRANSIT TIME

Many pharmacological agents have important effects on vascular function. In Chapter 3, the effects of haemodynamic disturbance with drugs was examined. Advantages of continuous measurement of BP during drug administration include improved evaluation of pharmacodynamic profiles of rapid acting agents, assessment of short term variability including baroreflex sensitivity, assessment of hypotension (which may be rapid onset or transient), and monitoring of acutely unwell patients. However, to be a reliable measure of BP, the relationship between transit time and this variable would have to remain constant; it was hypothesised that this would not hold under the influence of differing drugs, which may have diverse effects on cardiac contractility and the intrinsic stiffness of the arterial wall, independently of changes in BP. The work described in this thesis can be compared with other published work documenting the effects of vasoactive drugs on transit time.

Amyl nitrite is an obsolete nitric oxide donor, given by inhalation to treat angina, and has a similar mechanism of action to GTN. Despite having no major direct cardiac effects, it causes venodilation with a reduction in preload, and if a high enough dose is given, the resulting arteriolar dilatation will cause a fall in BP and afterload

(Sawayama *et al*. 1969). There may also be a baroreceptor-mediated increase in LV contractility (Talley *et al*. 1971). Both Steptoe (Steptoe *et al*. 1983) and Pollak (Pollak & Obrist 1983) found that amyl nitrite caused a transient fall in PEP in humans, which is consistent with the change expected based on mechanism of action. Although the former study did not compare this with BP change, the latter did find a positive correlation with SBP. Both studies also found an increase in pPTT, which would be expected due arteriolar dilatation and fall in MAP. However, Pollak found that rPTT increased, whereas Steptoe found it to decrease. The reason for this difference is unclear: both studies measured the distal pulse at a similar location (the radial artery), but neither document the BP changes that occurred, and the work by Pollak does not quantify the degree of PEP or pPTT change but merely the correlation with BP. Furthermore, due to the route of administration of amyl nitrite, the doses administered are unknown. The study described in Chapter 3 used a large dose of GTN to achieve a decrease in BP, and may be expected to have similar effects on transit time to amyl nitrite. Indeed, pPTT decreased, but PEP did not change (rPTT therefore also decreased). The reason for the constancy of PEP is uncertain. Indeed, Ochiai *et al* performed similar work in dogs, and found that GTN caused a decrease in PEP measured invasively. In the current study, it is possible that the decrease in preload causes a decrease in contractility (through the Frank-Starling mechanism) with a subsequent rise in PEP. This may have offset any fall in PEP due to the effects of a decrease in afterload: this might have occurred either due to a shortening of the isovolumic contraction time due to the reduction in aortic valve opening pressure, or secondary to baroreflex-mediated increased contractility. Interestingly, in earlier work, Steptoe found no change in rPTT in response to amyl nitrite, and proposed that the decrease in PEP may have offset the increase in pPTT, although PEP was not measured (Steptoe *et al*. 1976).

The effects of noradrenaline and angiotensin II have not been examined previously. Despite similar BP responses, noradrenaline achieved a greater decrease in PEP and rPTT compared to angiotensin II. Both drugs cause peripheral vasoconstriction resulting in increased BP and afterload with corresponding fall in pPTT, but positive inotropic effects of noradrenaline probably caused an additional decrease in PEP. Dobutamine, a β1-agonist, was used by Ochiai *et al* to achieve an increase in SBP

and MAP in dogs, resulting in a fall in PEP and pPTT due to positive inotropic effects (Ochiai *et al*. 1999). Ochiai also observed that phenylephrine, an α1-agonist, caused similar BP and pPTT changes, but an increase in PEP and rPTT. In contrast, Weiss observed that although MAP increased with phenylephrine, rPTT did not change, suggesting that the increase in PEP was not great enough to offset the decrease in pPTT (Weiss *et al*. 1980).

The response of transit time to salbutamol has not been studied either. However, isoproterenol, a non-selective β-agonist, has been used by Contrada and by Weiss (Contrada *et al*. 1995; Weiss *et al*. 1980). Both studies found a decrease in rPTT, although neither measured PEP. The variable increase in SBP and decrease in DBP observed by Contrada is similar to the changes described in Chapter 3 with salbutamol. Weiss reported a non-significant decrease in MAP with similar dosing, presumably because the increased SBP offset the fall in DBP. The measurement of PEP in the current study confirms that the changes in rPTT are due to a fall in PEP due to the positive inotropic response, offsetting the increase in pPTT secondary to a decrease in peripheral vascular resistance.

SBP is a function of both cardiac and arterial function, but similar values of SBP do not imply similar cardiac and arterial function. The findings of all the above studies suggest that changes in PEP do not reliably correlate with changes in SBP, regardless of any consistency in the relationship between MAP/DBP and pPTT, and hence rPTT cannot be used to predict SBP during administration of drugs with diverse cardiovascular effects. In addition, although no effect of drugs was found on the correlation between MAP/DBP and pPTT in the current study, potential changes in the intrinsic properties of the arterial wall independent of changes in distending pressure, mean that the constancy of the latter relationship should not be assumed either. Despite the problems with predicting absolute BP over a wide range of haemodynamic circumstances however, the coherence in the frequency domain between SBP and rPTT suggest that the latter measure may still have a role in the assessment of short-term BP variability.

8.3 PULSE TRANSIT TIME MEASUREMENT DURING EXERCISE

Although other studies have measured transit time during exercise, a detailed examination of the changes occurring during exertion has not been previously described.

Various timing points have been used in the past for measuring transit time. Given the large haemodynamic changes that occur during exercise, it was essential to establish whether the timing measurement point was important. Previous investigators have used either the Q-wave or the R-wave as a proximal timing point. Although the latter is easier to detect, the former corresponds more closely to the start of ventricular depolarisation. Nonetheless, the difference appeared relatively constant between all three points on the QRS complex. The leading edge of the PPG signal was also found to maintain its shape fairly well, although more variability was observed in the ear wave. The difference between measurement points remains relatively constant throughout exercise. The consistency of the leading edge is well recognised (McDonald 1968), although this has not been previously confirmed during exercise. The measurement points on the ECG and distal pulse would therefore not appear to be confounding factors, although clearly a constant timing offset will be introduced depending on the time points selected.

Transit time is usually treated as a single parameter, or occasionally broken down into a separate cardiac and vascular component. However, this has not been previously carried out during exercise, and moreover, the long vascular path length has generally be regarded as uniform. It has been shown here that not only does the change in PEP play a key role in determining exercise rPTT, but there are also substantial variations in the responses to exertion of the different arterial segments which make up the total path length. The reasons for these variations have not been examined here, although differing responsiveness to vascular mediators such as nitric oxide, and structural differences such as increased peripheral smooth muscle and vessel diameter, may be responsible. This is an important area for future work.

The relative constancy of radial-finger TT is useful, as it means that by simultaneously measuring PPG signals at ear and finger, the conduit artery pulse wave velocity can be determined during exercise. The current work suggests that this technique may have a role in cardiovascular risk stratification, and this offers the opportunity for further study. However, further validation work is merited first to determine whether the distal (e.g. radial-finger) vascular component remains constant in the presence of vasoactive drugs, vascular dysfunction (e.g. hypertension), and prolonged or exhaustive exercise. The use of bioimpedance also raises the question of whether the B-point can be used as a proximal timing point for the assessment of central PWV. The distal measurement point could potentially be the carotid artery (or indeed ear, assuming constancy of the carotid-ear segment). Alternatively, abdominal aortic flow could be measured as described previously (Siche *et al*. 1989b). Initial validation could be carried out against non-invasive tonometry or Doppler ultrasound measurements at rest, but validation during exercise would be more challenging, and ideally require invasive measurements.

The use of rPTT to predict absolute beat-to-beat systolic BP during exercise is attractive, and indeed a strong negative linear association between the two variables is apparent, and holds during prolonged submaximal exercise. However, at anaerobic levels of exertion, the rate of change of PEP slows, and the nature of the rPTT/SBP correlation changes. This results in an underestimation of peak SBP if rPTT is calibrated using data gathered at rest or during low-intensity work. It would be interesting to establish whether a clear inflection point exists in the rPTT/work slope or whether the decrease occurs gradually with increasing workloads. If an inflection point existed, it might be useful to see whether it corresponds to an identifiable physiological process, such as the anaerobic or lactate threshold. This would be of considerable interest in the sports science field, where this threshold is an important limiting factor in endurance exercise, and may be of value in guiding training. Furthermore, the nature of the rPTT/SBP correlation differs during recovery, and the nature of this difference is affected by the intensity of exertion and by exercise capacity. Recovery BP is of clinical relevance, although not commonly reported, and may be influenced by vascular dysfunction (Casiglia *et al*. 1994). Unfortunately, the error in rPTT-predicted SBP during recovery is considerable, and it is also possible

that differences in arterial function may influence this error. Prediction of SBP following exercise using this technique can therefore not be recommended.

8.4 PULSE WAVE ANALYSIS

It has also been possible to study what effect drugs and exercise have on the relationship between finger volume pulse and peripheral pressure pulse contours. The finger volume pulse has been used for the assessment of vascular function in aging (Millasseau *et al*. 2002), type II diabetes (Chowienczyk *et al*. 1999), and smoking (McVeigh *et al*. 1997), and to study the effects of various drugs (Millasseau *et al*. 2003a; Takazawa *et al*. 1998; Weinberg *et al*. 2001). However, only a limited work has been carried out to date comparing finger and peripheral artery pulses. Work by Allen and Murray have used neural networks to compare the signals (1999). Millasseau *et al* showed that the transfer function describing the relationship between the two signals remained relatively constant following the administration of GTN, or between hypertensive and normotensive individuals (Millasseau *et al*. 2000). The work in Chapter 3 demonstrated that even large haemodynamic disturbances due to pharmacological intervention have little impact on the transfer function. This is probably because the drugs had little effect on the lower frequency components of the waveform, which comprise the greater proportion of total spectral power. In Chapter 6 an effect of exercise on both the finger-to-radial and radial-to-carotid transfer functions was, however, detected. This seems likely to be due to the greater changes in BP, intrinsic stiffness and, possibly, heart rate, in comparison to the changes obtained with drugs. The more rapid normalisation of the distal transfer function following exercise, also suggests that the characteristics of this particular vascular domain are less affected by haemodynamic changes than larger conduit vessels.

The technique of photoplethysmography is still relatively infrequently used for the purposes of pulse wave analysis. It is clear, however, that the finger volume wave can be used to assess arterial function, and that both finger and peripheral arterial pulses are closely related and presumably influenced by similar vascular biomechanical properties. It would appear that use of the finger volume wave is an

entirely acceptable alternative to applanation tonometry, even in the presence of vasoactive drugs. Nonetheless, caution must be applied to the prediction of proximal arterial waveform measures or soon after exercise, although an interesting area for future work may be evaluation of how vascular dysfunction affects the impact of exercise on the transfer function.

8.5 CONCLUSIONS AND FUTURE WORK

Although the use of transit time as a predictor of absolute BP is questionable, the techniques described in this thesis may still have a role in assessment of cardiovascular function. Measurement of rPTT may be of value in the measurement of baroreflex sensitivity (BRS) and short-term BP variability, both of which have prognostic value (La Rovere *et al*. 1998; Parati *et al*. 1998; Sander *et al*. 2000). Although the present work describes assessment of spontaneous BRS, this could be extended to the administration of ephedrine, Valsalva manoeuvre or cold pressor test. It remains to be seen whether use of rPTT in any of these ways, including during exercise, can detect changes induced by therapeutic interventions or indeed have a role as a marker of cardiovascular risk. Photoplethysmography has also been shown to be useful in the measurement of pulse wave velocity during exercise. To date, little work has been done on arterial stiffness during exertion due to the difficulties in obtaining reliable measurements. The techniques described in this thesis need to be extended to larger cross-sectional studies and prospective trials, and the effects of interventions such as drugs and exercise training needs to be evaluated. Finally, pulse wave analysis using recording of the photoplethysmograph and WEP signals deserves more widespread use. Several studies have used PPG to examine arterial function, but there are no outcome studies to date, and the effects of therapeutic interventions are unknown. The WEP technique also shows potential in the assessment of the vascular function, with the advantage that it might be easily incorporated into standard sphygmomanometer devices, but a more detailed understanding of the true nature of the WEP signal is required before it can be applied more widely.

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APPENDIX 1

The threshold for R-wave detection was determined following digital filtering of the ECG signal using a 12^{th}-order 0.3 to 20 Hz band-pass Butterworth filter. The maximum first derivative of the ECG signal was determined for every 2 seconds of data, and the running 3-rank median calculated to provide a detection threshold. Potential ECG R-wave deflections were identified as positive-to-negative crossing points on the first derivative occurring within 128 ms of the first derivative exceeding 30% of this current threshold.

1. Do QRS complexes meet all the following three conditions?
    a. Q-R interval <50 ms
    b. R-S interval <50 ms
    c. sum of amplitudes of Q-nadir to R-peak, and R-peak to S-nadir, within 50-175% of the 5 second running median of this amplitude (this median amplitude is the "amplitude threshold")
2. Of these complexes, identify pairs where the R-R interval exceeds 50% of the 5 second running median, and exclude one of the pair which differs most in QR+RS amplitude sum from the corresponding amplitude threshold
3. The 5-second median R-R interval is recalculated, and step 2 repeated
4. If a gap exists between QRS complexes, of within 20% of an integer multiple of the median R-R interval, then it is possible that one or more QRS complexes have been wrongly excluded
    a. The QRS complex within 10% of the expected time (based on median R-R interval), which is closest in QR+RS amplitude sum to the amplitude threshold, and meets *either* criteria 1a *or* 1b, is reinstated
5. The timing of these QRS complexes was used as the initial timing point of the cardiac cycle for all measurements, including recalculating QRS timings

The limits given in 1a and 1b are defined by the nadir of the Q and S waves, and the sum of the two values is therefore less than the true QRS duration. All ECG data was subject to a two-pass filter, with pulses lying outwith 3 standard deviations of the 5-second median average heart rate being automatically rejected.

APPENDIX 2

The following papers have been published as a result of the work in this thesis, and are reproduced with permission of the respective publishers on the following pages:

Payne, R. A., Symeonides, C. N., Webb, D. J., Maxwell, S. R. 2006: Pulse transit time measured from the ECG: an unreliable marker of beat-to-beat blood pressure. *J Appl Physiol,* **100**, 136-41.

Payne, R. A. & Webb, D. J. 2006: Arterial blood pressure and stiffness in hypertension: is arterial structure important? *Hypertension,* **48**, 366-7.

Payne, R. A., Isnardi, D., Andrews, P. J., Maxwell, S. R., Webb, D. J. 2007: Similarity between the suprasystolic wideband external pulse wave and the first derivative of the intra-arterial pulse wave. *Br J Anaes,* **99**, 653-61.

Payne, R. A., Teh, C. H., Webb, D. J., Maxwell, S. R. J. 2007: A generalized arterial transfer function derived at rest underestimates augmentation of central pressure after exercise. *J Hypertens,* **25**, 2266-72.

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Pulse transit time measured from the ECG: an unreliable marker of beat-to-beat blood pressure

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Payne, R. A., C. N. Symeonides, D. J. Webb, and S. R. J. Maxwell. Pulse transit time measured from the ECG: an unreliable marker of beat-to-beat blood pressure. *J Appl Physiol* 100: 136–141, 2006. First published September 1, 2005; doi:10.1152/japplphysiol.00657.2005.— The arterial pulse-wave transit time can be measured between the ECG R-wave and the finger pulse (*r*PTT), and has been shown previously to have a linear correlation with blood pressure (BP). We hypothesized that the relationship between *r*PTT, preejection period (PEP; the R-wave/mechanical cardiac delay), and BP would vary with different vasoactive drugs. Twelve healthy men (mean age 22 yr) were studied. Beat-to-beat measurements were made of *r*PTT (using ECG and photoplethysmograph finger probe), intra-arterial radial pressure, PEP (using cardiac bioimpedance), and transit time minus PEP (*p*PTT). Four drugs (glyceryl trinitrate, angiotensin II, norepinephrine, salbutamol) were administered intravenously over 15 min, with stepped dosage increase every 5 min and a 25-min saline washout between agents. All subjects in all conditions had a negative linear correlation ($R^2 = 0.39$) between *r*PTT and systolic BP (SBP), generally constant between different drugs, apart from four subjects who had a positive *r*PTT/SBP correlation with salbutamol. The 95% limits of agreement between measured and *r*PTT-predicted SBP were ±17.0 mmHg. Beat-to-beat variability of *r*PTT showed better coherence with SBP variability than it did with heart rate variability ($P < 0.001$). PEP accounted for a substantial and variable proportion of *r*PTT (12–35%). Diastolic (DBP) and mean arterial BP (MAP) correlated poorly with *r*PTT ($R^2 = 0.02$ and 0.08, respectively) but better with *p*PTT (*r*PTT corrected for PEP, $R^2 = 0.41$ and 0.45, respectively). The 95% limits of agreement between measured and *p*PTT-predicted DBP were ± 17.3 mmHg. In conclusion, the negative correlation between *r*PTT and SBP is generally constant, even with marked hemodynamic perturbations. However, the relationship is not reliable enough for *r*PTT to be used as a surrogate marker of SBP, although it may be useful in assessing BP variability. DBP and MAP cannot be predicted from *r*PTT without correction for PEP. The significant contribution of PEP to *r*PTT means that *r*PTT should not be used as a marker of purely vascular function.

preejection period; pulse-wave transit time

MONITORING OF BLOOD PRESSURE (BP) in a clinical or research setting is often performed using techniques that evolved in the 19th century (31). However, traditional sphygmomanometry is unable to monitor the short-term dynamic variability that occurs with BP, and the invasive nature of arterial cannulation limits its use to critically ill patients. A noninvasive beat-to-beat measurement of BP would be extremely valuable. A number of approaches have been developed, including most notably finger blood-volume clamping (28) and arterial tonometry (29). Although some studies have suggested that there is reasonable accuracy with these systems (32, 40), the technology itself is generally expensive, cumbersome, and prone to movement artifact.

An alternative technique involves measuring the transit time of the pulse pressure wave through the arterial tree. Measurement of pulse transit time involves detecting the pulse arrival at two separate arterial sites. This can be achieved easily distally using infrared photoplethysmography, and differential pulse transit time measured between finger and toe, as detected by photoplethysmography, has indeed been shown to satisfactorily reflect changes in pulse-wave velocity measured by Doppler ultrasound (23). The ECG R-wave is often used as a proximal timing point because it is simple to detect and tolerant of motion artifact. However, importantly, there is a considerable delay between the onset of electrical cardiac activity and the start of mechanical ventricular ejection (22). This delay is comprised of both the electromechanical delay and the period of isovolumic contraction, and is referred to as the preejection period (PEP).

It has been suggested that, because a near-linear correlation exists between transit time measured from the R-wave (*r*PTT) and BP, *r*PTT might be used as a surrogate marker of pressure (9). The use of *r*PTT in this way was originally described in the 1950s (34), and considerable research was subsequently performed on its application in the study of cardiovascular feedback, mainly in the field of psychophysiology (9, 19, 33, 37). There have been several studies that revisited the technique in recent years, probably due to the increasing ease with which signal analysis can be carried out using modern technology (1, 7, 14, 26). However, little work has been published on the effects of vasoactive drugs on this measurement in humans. In particular, studies have not been carried out quantifying PEP or comparing *r*PTT with invasive BP measurement.

This study used various vasoactive drugs to produce differing cardiac and vascular responses. The rationale was to compare changes in transit time measurements with the clinical "gold standard" for BP measurement over a wide BP range and under different conditions of vascular tone and cardiac contractility. We hypothesized that the relationship between intra-arterial BP and *r*PTT would vary following the administration of different vasoactive drugs due to differing effects on the vascular and cardiac components of *r*PTT.

METHODS

Studies were carried out in healthy men, aged 18–25 yr, with no history of cardiovascular disease and taking no regular medications. Informed consent was obtained from all participants. The protocol

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was approved by the local research ethics committee and conformed to the requirements of the Declaration of Helsinki[5].

Studies were performed in a quiet, temperature-controlled (22 ± 2°C) environment after at least 1 h of acclimatization. Subjects were allowed a light breakfast not less than 4 h before attending and were requested to refrain from alcohol, caffeine, nicotine, or medications for the preceding 24 h. Studies were conducted with the subject lying supine. Continuous beat-to-beat measurements of BP, transit time, and PEP were made throughout the entire study protocol.

All drugs were administered via a 20-gauge intravenous cannula sited in the antecubital fossa of the dominant arm. The infusion rate was kept constant at 1 ml/min. After an initial 20-min 0.9% saline run-in period, four drugs were given, each for 15 min, with the dose increased every 5 min. A 25-min washout period followed each drug. Drugs given were glyceryl trinitrate (0.1, 1, 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$), angiotensin II (2, 6, 12 $ng \cdot kg^{-1} \cdot min^{-1}$), norepinephrine (20, 60, 120 $ng \cdot kg^{-1} \cdot min^{-1}$), and salbutamol (albuterol, 0.4, 1.2, 2.4 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Drug order was not randomized. Salbutamol was given last due to its relatively long half-life. Dose ranges and washout periods were based on previous studies and selected for their anticipated effects on BP and heart rate (HR) (10, 12, 15, 30, 38, 39). Glyceryl trinitrate was selected for anticipated decreases in BP (12, 15). Norepinephrine and angiotensin II increase mean arterial pressure (MAP) to a similar degree, but the former also increases peripheral pulse pressure (30, 38). The expected response to salbutamol was a fall in diastolic pressure (DBP) and a rise in systolic pressure (SBP) and HR (10, 39).

Intra-arterial pressure monitoring was used for all BP measurements. A 20-gauge 80-mm Vygon catheter was inserted under local anesthesia (1% lidocaine) into the nondominant radial artery using the Seldinger technique. A splint was used to minimize wrist movement. The cannula was connected by fluid-filled semi-rigid tubing to a TruWave disposable transducer (Edwards Life Sciences) positioned level with the right atrium and connected to a Diascope 2 monitor (S & W Medical). Transducers were factory calibrated and exceeded AAMI standards for performance interchangeability, with a sensitivity of 5 $\mu V \cdot V^{-1} \cdot mmHg^{-1}$ ±1% and nonlinearity of the greater of ±1.5% or ±1 mmHg. The natural frequency of the system was 40 Hz. SBP and DBP were taken as the maximum and minimum values of the waveform corresponding to the last measured R-wave, with mean pressure calculated as the average over the last pulse cycle. Custom hardware was used for determination of the transit time. The ECG was detected using a standard three-lead configuration (Lead II), with the signal sampled at 1 kHz. The pulse volume wave was detected on the dominant index finger using infrared transmission photoplethysmography, digitized at 200 Hz, with linear interpolation to 1-kHz accuracy. The time delay was calculated between R-wave peak and the base of the leading edge of the finger pulse wave. The pulse wave base was identified as the intersection of the tangent through the steepest part of the slope with the absolute minimum value of the pulse wave (8). PEP was determined from the B point of the first derivative of the transthoracic cardiac bioimpedance waveform, using an NCCOM3 Cardiodynamic monitor (BoMed Medical Systems). Bioimpedance has previously been validated for determination of systolic time intervals (17), and the NCCOM3 device has itself been compared favorably with both echocardiography (16) and mechanophonocardiography (35). Pilot work established that, over a 1-min resting period, the standard deviation of PEP measurements was 5.2 ms (mean 69 ms), with a mean beat-to-beat difference of 3.5 ms. The coefficient of variation in baseline PEP measurements was 8.9%. We consider these small variations in beat-to-beat PEP measurements obtained by our experimental technique to reflect satisfactory intrasubject reliability.

All data are expressed as means ± standard deviation (SD) unless otherwise stated. Changes from baseline, taken as the 2 min immediately before each infusion period, were assessed by ANOVA. Difference between each drug baseline was also compared by ANOVA. Measurements of transit time were taken both from ECG R-wave (*r*PTT) and from end of PEP (*p*PTT). The relationships between BP and different measures of transit time were evaluated by linear regression. Regression slopes and intercepts and Z-transformed Pearson correlation coefficients were compared for each drug infusion and washout period using repeated-measures ANOVA. Beat-to-beat variability was assessed for SBP, *r*PTT, and HR. Power spectra were calculated using a smoothed Lomb periodogram (18) for all three variables for each individual drug dose. Coherence is analogous to correlation coefficient in the time domain, ranging from 0 (no coherence) to 1, and was computed over the frequency ranges of 0.05–0.2 and 0.2–0.4 Hz. Comparison of coherence values was made by ANOVA.

RESULTS

Subjects were all healthy, nonsmoking men, age 22 ± 1.7 yr, who took regular noncompetitive exercise. Average height and weight were 178 ± 6 cm and 75 ± 4.8 kg, respectively. Resting oscillometric brachial BP was 126/75 ± 10.8/7.7 mmHg, and 12-lead ECGs were normal in all subjects. Total and high-density lipoprotein cholesterol were 158 ± 23 and 55 ± 13 mg/dl, respectively, with normal serum biochemistry and blood count. Maximal change from baseline in hemodynamic parameters are given in Table 1, with dose response plotted in Fig. 1. Glyceryl trinitrate caused an increase in *r*PTT, *p*PTT, and HR, and decreases in SBP, DBP, and MAP. Angiotensin II and norepinephrine caused increases in SBP, DBP, and MAP, and decreases in *r*PTT and *p*PTT. SBP and pulse pressure increases tended to be greater with norepinephrine than angiotensin II, but this difference did not achieve statistical significance ($P = 0.11$). PEP and HR responses were variable between subjects, but overall both decreased with norepinephrine and did not change with angiotensin II. Despite the similar change in BP, decreases in PEP and *r*PTT were significantly greater with norepinephrine than with angiotensin II ($P = 0.005$ and $P = 0.002$, respectively). Salbutamol reduced DBP, MAP, *r*PTT, and PEP and increased HR and *p*PTT. The SBP response was varied and, overall, did not significantly change; eight subjects had a significant increase in SBP, whereas four had a clear decrease. Baseline values of SBP, DBP, MAP, and HR were not constant between drug phases ($P < 0.05$ by ANOVA), due in particular to increases in all four parameters before salbutamol administration. This was mirrored by decreases in *r*PTT, *p*PTT, and PEP. The relationship between PTT and BP in a typical subject is shown in Fig. 2.

*r*PTT had an inverse linear correlation with SBP (combined average across all subjects and drugs $R^2 = 0.39$). There was no significant difference in correlation coefficient ($P = 0.88$) or slope ($P = 0.69$) between different drugs by repeated-measures ANOVA. *r*PTT was significantly ($P < 0.01$) better correlated with SBP than it was with either DBP ($R^2 = 0.02$) or MAP ($R^2 = 0.08$). Furthermore, *r*PTT showed significant differences in correlation with DBP ($P < 0.001$) and MAP ($P < 0.001$) between different drugs. *p*PTT was more strongly correlated ($P < 0.001$) with DBP ($R^2 = 0.41$) and MAP ($R^2 = 0.45$) than it was with SBP ($R^2 = 0.33$). The correlation between *p*PTT and DBP was unaffected by different drugs ($P = 0.11$). The same was true for *p*PTT and MAP ($P = 0.39$). However, the *p*PTT-SBP correlation was different between drugs ($P < 0.01$).

The value of *r*PTT and *p*PTT as predictors of SBP and DBP, respectively, was assessed based on the assumption that it would be possible to obtain an ideal calibration slope for each

Table 1. *Maximal change from baseline for different agents*

	*r*PTT, ms	PEP, ms	*p*PTT, ms	Heart Rate, beats/min	SBP, mmHg	DBP, mmHg	MAP, mmHg
GTN							
Baseline	260 (17)	95 (15)	164 (12)	63 (7)	134 (12)	66 (5)	85 (6)
Maximum	276 (18)	92 (16)	184 (26)	77 (9)	122 (10)	61 (6)	77 (6)
Change	15.3 (11.4)*	−3.5 (10.6)	20.6 (16.0)*	14.3 (5.2)*	−12.6 (8.1)*	−4.5 (5.0)†	−7.8 (4.9)*
Angiotensin II							
Baseline	266 (19)	100 (15)	166 (23)	62 (8)	139 (13)	67 (6)	84 (6)
Maximum	257 (23)	105 (19)	150 (21)	60 (10)	156 (13)	82 (7)	102 (8)
Change	−9.2 (8.1)*	5.9 (11.5)	−15.2 (7.0)*	−2.6 (8.4)	17.5 (8.3)*	15.0 (4.4)*	18.1 (5.4)*
Norepinephrine							
Baseline	261 (15)	95 (19)	165 (18)	61 (8)	141 (10)	68 (6)	86 (7)
Maximum	238 (18)	87 (19)	151 (20)	57 (8)	164 (13)	81 (8)	104 (10)
Change	−22.4 (10.5)*	−7.6 (10.1)†	−14.4 (7.6)*	−4.0 (6.4)*	22.9 (14.5)*	12.3 (5.5)*	17.4 (8.3)*
Salbutamol							
Baseline	249 (16)	91 (19)	158 (21)	66 (10)	151 (12)	71 (9)	92 (9)
Maximum	218 (18)	30 (12)	188 (16)	125 (13)	153 (25)	39 (9)	68 (11)
Change	−32.5 (9.8)*	−62.2 (19.4)*	29.9 (14.2)*	59.1 (7.6)*	2.5 (19.0)	−31.7 (4.3)*	−23.3 (6.0)*

Values are means (SD) for all subjects. GTN, glyceryl trinitrate; SBP, systolic blood pressure; DBP diastolic blood pressure; MAP, mean arterial pressure; *r*PTT, pulse transit time measured between the ECG R-wave and the finger pulse; *p*PTT, pulse transit time minus preejection period (PEP). *$P < 0.01$; †$P < 0.05$.

individual equating to the average linear regression slope for all drugs. The 95% limits of agreement for predicted vs. actual BP were ±17.0 mmHg (SBP/*r*PTT) and ± 17.3 mmHg (DBP/*p*PTT). Percentage-predicted values falling within 5, 10, and 15 mmHg of actual value [based on British Hypertension Society system for assessing BP measurement accuracy (25)] were 44, 66, and 73%, respectively, for SBP, and 42, 64, and 72%, respectively, for DBP.

Average power spectra over all drugs are shown in Fig. 3. An example of the similarity in SBP and *r*PTT variability is given in Fig. 4. Mean coherence between SBP and rPTT variability was significantly ($P < 0.001$) greater at both lower and higher frequencies (0.58 ± 0.37 and 0.70 ± 0.33, respectively) than coherence of HR and *r*PTT variability (0.46 ± 0.41 and 0.52 ± 0.38, respectively). There was no significant difference in coherence between HR and either *r*PTT or SBP for lower ($P = 0.33$) or higher ($P = 0.16$) frequencies. Coherence was not significantly affected by drug type or dosage ($P = 0.96$) and is shown in Fig. 4.

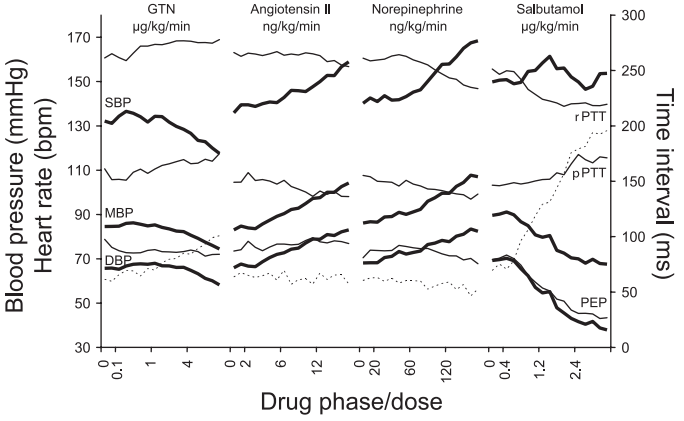

Fig. 1. Mean dose response graphs for blood pressure (BP; thick line), heart rate (HR; broken line), and pulse transit time (PTT) and preejection period (PEP) (thin lines). Data were averaged over 60-s intervals for clarity. See individual labels for separate BP and time components. SBP, systolic BP; DBP diastolic BP; MBP, mean BP; *r*PTT, PTT measured between the ECG R-wave and the finger pulse; *p*PTT, PTT minus PEP; bpm, beats/min.

DISCUSSION

The association between pulse transit time and BP was studied extensively in the field of psychophysiology (9, 11, 19, 33, 37) in the 1970s and 1980s, and more recently by Ochiai et al. (26) and Chen et al. (7). *r*PTT has also been used to predict BP in a clinical setting (14). The present study is the first to examine simultaneously the effects of vasoactive drugs on *r*PTT, PEP, and invasively measured BP in humans.

The expected hemodynamic changes occurred with all four drugs, although the SBP response to salbutamol was mixed. *r*PTT had a negative correlation with SBP, which was relatively unaffected by different drugs in the population as a whole. *r*PTT also appeared to be useful as a marker of SBP variability. However, DBP and MAP were weakly correlated with *r*PTT, although more strongly related to *p*PTT.

SBP is dependent on both vascular function and ventricular contraction, and so it is perhaps unsurprising that *r*PTT, a composite measure of both vascular and cardiac activity, is correlated with SBP. However, although in the study population as a whole the correlation between *r*PTT and SBP appeared relatively unaffected by drugs, this finding must be treated with caution. There were slight differences in the *r*PTT response between norepinephrine and angiotensin II, despite similar BP profiles. Furthermore, it should be noted that four subjects in this study had positive correlations between *r*PTT and SBP during the administration of salbutamol. This drug has positive inotropic and chronotropic β_2-adrenergic effects, as well as causing peripheral arterial relaxation. Although a fall in PEP is associated with an increase in cardiac inotropy, this does not necessarily relate to an increase in SBP, as any potential pressure rise

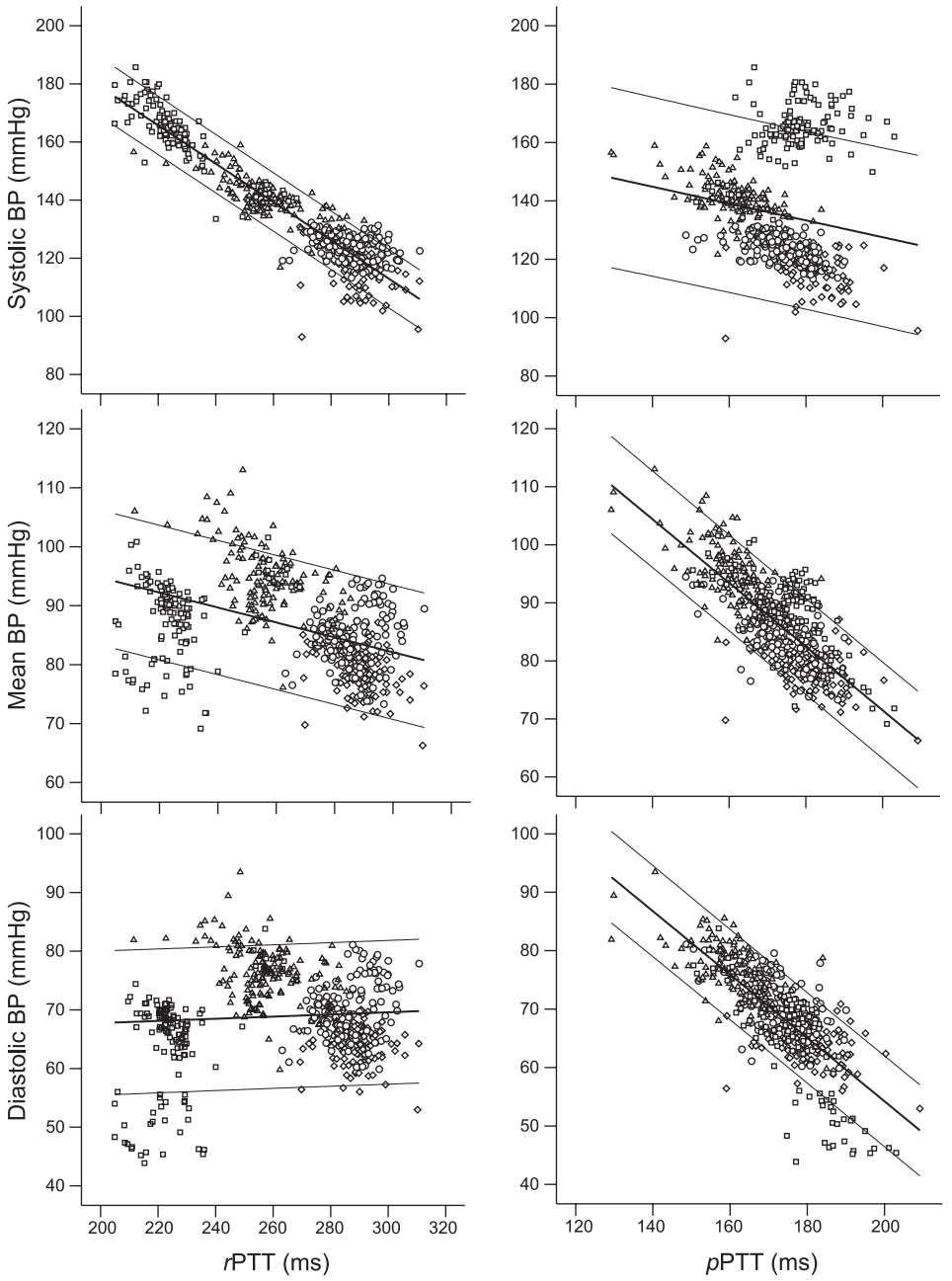

Fig. 2. Linear regression analysis of PTT and BP relationship in typical subject with glyceryl trinitrate (GTN; ◇), angiotensin II (○), norepinephrine (△), and salbutamol (□). Figure shows relationship between *r*PTT (*left*) and *p*PTT (*right*) with systolic (*top*), mean (*middle*), and diastolic (*bottom*) BP.

may be offset by decreases in pressure augmentation by reflected waves or changes in aortic stiffness (24). It would therefore appear inappropriate to use *r*PTT as a predictor of SBP in all persons, particularly for assessing changes due to vasoactive drugs. Moreover, even using an idealized calibration slope, the limits of agreement between predicted and actual BP were wide, although similar inaccuracies have been described previously between sphygmomanometric and direct arterial pressure measurements (2, 36).

These data also show that PEP accounts for a substantial and variable proportion of *r*PTT, ranging from ~12 to 35%. A number of relatively recent studies have employed *r*PTT as a marker of vascular function (3, 4), but this study demonstrates that the use of *r*PTT purely for the assessment of arterial stiffness is inappropriate and should be avoided, as PEP cannot be assumed to remain constant. Other devices, such as the Colin VP-1000 (Colin), have eliminated PEP by utilizing the phonocardiogram to time cardiac ejection. The phonocardiogram is regarded by many as the ideal way of determining systolic time intervals. The principal disadvantage, however, compared with bioimpedance, is that it requires accurate identification of two timing points rather than simply one: first, the end of cardiac ejection (the second heart sound); second, the left ventricular ejection period (measured by identifying the dicrotic notch using a proximal arterial pulse wave).

*r*PTT may nonetheless offer a potentially valuable means of detecting beat-to-beat changes in SBP. Indeed, with regular

recalibration to standard oscillometric BP as suggested by Chen et al. (7), *r*PTT offers the opportunity to assess BP variability and detect sudden or transient hemodynamic changes. BP and HR variability are considered to offer important insights into vasomotor activity, have been associated with clinical outcomes, including cardiovascular death, and may be used in assessment of autonomic neuropathy (27). Sympathetic modulation of BP alters the HR through the actions of the sinoaortic baroreflex; coherence between these two measures therefore reflects baroreflex activity (20). *r*PTT shows beat-to-beat variability closer to that of SBP than HR and, therefore, may have a role in the assessment of vasomotor control and BP variability.

*p*PTT, but not *r*PTT, was strongly inversely correlated with DBP and MAP. Furthermore, the correlation was inconsistent between *p*PTT and SBP. These findings are both consistent with the fact that arterial stiffness, and therefore vascular pulse transit time (i.e., *p*PTT), is dependent on MAP rather than SBP. In many circumstances, SBP and DBP/MAP are positively associated with each other. This has led others to inappropriately use *r*PTT to predict both these variables (6, 14), with DBP calculated following adjustment for HR. However, the divergent SBP and DBP/MAP responses to salbutamol in eight subjects in this study have not been reported in previous published work in this field, and the present data suggest that *r*PTT cannot be used to predict DBP or MAP without a knowledge of PEP, regardless of the HR response.

This study has a few limitations. Baseline values of BP were not constant before each drug, tending to rise steadily over the course of the study, particularly after norepinephrine. Due to the short half-life of both pressor agents in particular, it seems unlikely that the rise in BP is entirely accounted for by direct drug effects. Randomizing drug order was not carried out because the much longer half-life of salbutamol necessitated its administration last and it was not justifiable to carry out the separate elements of the study on different days, because this would have required repeated arterial cannulation. The washout periods were also kept relatively short to minimize the duration of cannulation.

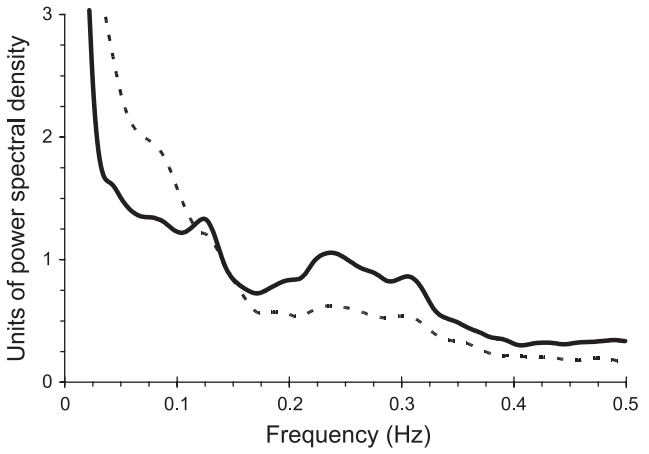

Fig. 3. Average power spectra (all drugs). Solid line, rPTT; broken line, SBP.

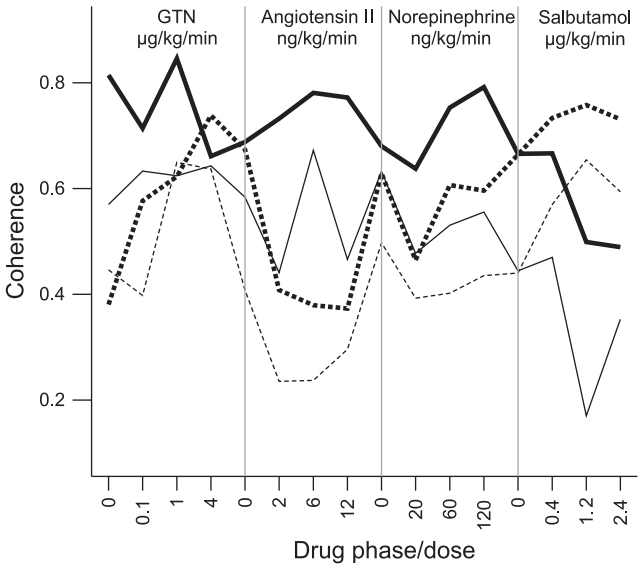

Fig. 4. Coherence between *r*PTT variability and SBP variability (thick line), and rPTT and heart rate (thin line). Frequency band of 0.05–0.2 Hz is solid line and 0.2–0.4 Hz is broken line.

Despite these points, the aim of the different drugs was to achieve a wide range of BP under varying conditions of vascular tone, and this was still achieved even if the hemodynamic effects of one drug had not completely resolved before the administration of the next. The use of fluid-filled manometer tubing introduces a degree of inaccuracy between pressure at the catheter tip and that at the more proximal transducer. However, this discrepancy was constant between subjects, and fluid-filled manometer tubes are nonetheless regarded as the gold standard in clinical practice. HR is a potential confounding factor (21) in the assessment of vascular stiffness and BP, although debate continues over whether reported increases in pulse-wave velocity with HR are genuine (13). Importantly, however, the large change in HR seen with salbutamol does not affect the interpretation of DBP being more important than SBP as a determinant of *p*PTT, because a high HR would, if anything, increase arterial stiffness and thus reduce vascular transit time.

In conclusion, this study demonstrates that *r*PTT has a negative correlation with SBP, which although relatively unaffected by vasoactive drugs in some persons is not reliable enough to enable *r*PTT to be a surrogate marker of SBP. Furthermore, the significant contribution of PEP to *r*PTT means that use of the latter parameter as a marker of purely vascular function should be avoided. However, *r*PTT may have a role in the assessment of BP variability and rapid pressure change. The association of *p*PTT with DBP/MAP means that use of *r*PTT as a predictor of diastolic or mean pressure is inadvisable.

GRANTS

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Arterial Blood Pressure and Stiffness in Hypertension
Is Arterial Structure Important?

Rupert A. Payne, David J. Webb

Increasingly, in recent years, the stiffness of large elastic arteries has been recognized as a major determinant of vascular function and cardiovascular risk.[1,2] The distally propagating arterial pressure pulse is reflected at arterial branch points (sites of impedance mismatch), and the velocity and magnitude of these reflections is determined by arterial stiffness. Whereas peripheral vascular resistance largely determines diastolic BP, central systolic BP and pulse pressure are influenced by the augmentation of aortic pressure because of wave reflections, as well as by the character of ventricular ejection. Increased stiffness leads to greater pulsatile stress and strain and may influence endothelial shear stress, contributing to remodeling and structural abnormalities of the blood vessel wall and to atherogenesis. An increase in aortic stiffness also results in an increase in left ventricular afterload and, consequently, myocardial oxygen consumption[3] and compromise of myocardial perfusion during diastole, particularly in the subendocardial region.[4] That central arterial stiffness is clinically relevant is evident from the studies showing a positive predictive value of aortic stiffness for cardiovascular risk in hypertension,[5,6] although the precise mechanism of this association remains unclear.

Arterial stiffness is largely determined by 2 influences: first, those related to the arteries themselves (wall structure and function and lumen size); and, second, the mean distending arterial BP. The main load-bearing components of the arterial wall are elastin fibers, stiffer collagen fibers, and vascular smooth muscle. Smooth muscle contraction results in increased arterial stiffness because of a decrease in lumen size and shifting of load onto stiffer collagen fibers. Increasing mean distending pressure causes a small increase in lumen size. However, transfer of stress from elastin to collagen fibers outweighs this effect, leading to an exponential increase in arterial stiffness with pressure. Given that arterial stiffness is increased in patients with essential hypertension and that arterial remodeling is a recognized feature of hypertension,[7] an important question has been whether increased aortic stiffness is fully accounted for by the increase in mean distending pressure or whether there are intrinsic wall changes secondary to structural or functional effects. Indeed, this may be of importance in selecting treatment for individual patients.

To examine what effects the inherent properties of the vessel wall have on arterial stiffness, measures such as compliance, distensibility, pulse wave velocity (PWV), or elastic modulus must be compared under isobaric conditions. Previous work has either used pressure-compliance curves with interpolation of stiffness at a given blood pressure (BP)[8] or has normalized transmural pressure by placing the arm in a pressurized air chamber.[9] However, in this issue of *Hypertension*, Stewart et al[10] describe a method for generating isobaric conditions using a pharmacological intervention that acutely normalized the loading pressure in hypertensive subjects, dispensing with some of the assumptions associated with other methods. Stewart et al[10] studied 20 subjects with treated but inadequately controlled essential hypertension and 20 matched normotensive controls. Acutely reducing mean arterial pressure in the normotensive subjects, using glyceryl trinitrate (GTN), caused a corresponding reduction in arterial stiffness, as quantified by carotid-femoral PWV (PWV_{CF}) and carotid distensibility. However, when mean pressure in the hypertensive patients was reduced to the baseline level of the normotensive individuals, there was no change in either PWV_{CF} or arterial distensibility. Furthermore, using angiotensin II to increase the mean arterial pressure of normotensive subjects to the baseline level of the hypertensive individuals, arterial stiffness increased but still remained lower in the normotensive subjects. These findings suggest that the increase in aortic stiffness seen in hypertensive patients is because of an increase in intrinsic wall stiffness rather than simply elevated BP and may also imply a degree of resistance to changes of distending pressure. Importantly, this is in contrast to results from experiments using alternative techniques, which suggest that the increase in arterial stiffness in hypertensive individuals is largely because of the increase in mean pressure.[8,9] The 2 questions one must surely ask are, first, why do these findings seem to disagree with the findings of others using different methodology, and second, what relevance might these observations have from a clinical perspective?

Estimation of isobaric compliance from pressure-diameter curves[8] requires important assumptions to be made, and it can be argued that the full pressure–diameter relationship should be considered rather than 1 value in the cardiac cycle. Because the vessel wall is viscoelastic, luminal diameter at a given pressure is affected by the nature of the preceding pressure curve. This results in the compliance–pressure relationship exhibiting hysteresis, which must either be "removed" or ignored to create a curve from which compliance

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can be calculated at any given BP. This curve-fitting procedure may result in a potentially large error in the estimate of compliance and, additionally, mask differences between 2 clearly distinct pressure–compliance loops. Furthermore, this approach disregards potential differences in the pressure–compliance loop that might exist at truly different distending mean pressures. Normalizing transmural pressure using mechanical means[9] removes the potential inaccuracy introduced by such mathematical assumptions. However, it ignores systemic hemodynamic differences, such as wave reflections, that may exist. It can also only be used to examine conduit vessels and not large central arteries. The administration of a pharmacological agent (in this case GTN) is, of course, not without problems either. Small doses of GTN do not seem to change BP or PWV_{CF}. It is possible, however, that the larger doses of GTN used in this study altered the intrinsic aortic wall stiffness independent of the reduction in mean BP. Furthermore, the aortic wall response to GTN may have differed between the hypertensive and normotensive groups. Alternatively, the duration of GTN administration may have been of sufficient duration to induce reflex neurohormonal responses to the hypotension induced and act in a counter-regulatory way to maintain a higher PWV_{CF} in the hypertensive patients: pressure-diameter relationships are captured within the pressure excursions of a single cardiac cycle and have the potential advantage that they are not subject to such unknown hemodynamic changes. This was a relatively small study, and the findings would benefit from confirmation, including studies in previously untreated patients. In addition, there is a lack of data describing the changes in arterial stiffness in response to acute BP lowering with drugs other than GTN, and this is an important area for future work. Nonetheless, use of pharmacological intervention to achieve isobaric conditions would seem more clinically applicable than previous methodology, given that PWV_{CF} can adversely affect central BP and cardiac function and is closely linked to cardiovascular risk.

Why are these findings potentially clinically relevant? Arterial stiffness is a risk factor for cardiovascular disease, independent of BP, and the study from Stewart et al[10] suggests that simply lowering BP may not necessarily be sufficient to address this important risk factor. Indeed, other work[2] has shown that antihypertensive treatment with angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and calcium-channel blockers reduces arterial stiffness, whereas thiazide diuretics have less favorable effects, and β-blockers have little impact. The study by Stewart et al[10] suggests that hypertensive remodeling is likely to be important, so any beneficial response is likely to take time to occur, either through direct effects on the arterial wall or because of reduced shear stress or pulsatile load. This would fit with recent work[11] showing that larger doses of perindopril increase distensibility while having no additional effect on BP, consistent with a direct effect on intrinsic wall stiffness. Some newer agents targeting endothelial dysfunction or those directly affecting arterial structure, such as advanced glycation end-product crosslink breakers,[12] may also offer promise in this area. Nevertheless, the failure of arterial stiffness to improve with thiazides, drugs with established morbidity and mortality advantages in hypertension, serves as a reminder that BP reduction per se remains of prime importance. More work is clearly indicated, using the powerful tools now available, to establish the mechanisms whereby chronic lowering of BP, using established and newer agents, reduces arterial stiffness and improves clinical outcome.

Disclosures

None.

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Similarity between the suprasystolic wideband external pulse wave and the first derivative of the intra-arterial pulse wave

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Background. Wideband external pulse (WEP) monitoring, using a broad bandwidth piezoelectric sensor located over the brachial artery under the distal edge of a sphygmomanometer cuff, can be used for evaluating the contour of the arterial pressure pulse wave. The pulse contour contains valuable information relating to cardiovascular function which may be of clinical use in addition to blood pressure measurements. The aim of this study was to compare the shape of the WEP signal during inflation of the cuff to suprasystolic pressure, with intra-arterial pressure waves, after the administration of vasoactive drugs.

Methods. Radial intra-arterial and suprasystolic WEP waveforms were recorded in 11 healthy men (mean 23 yr) before and at the end of infusion of glyceryl trinitrate, angiotensin II, norepinephrine, and salbutamol. Waveform similarity was assessed by comparing the timing and pressure of incident and reflected waves and by root mean square error (RMSE).

Results. The WEP signal was found to closely resemble the first derivative of intra-arterial pressure. The WEP signal could be used to derive an arterial pressure wave with minimal bias in the timing of incident [−8 (18) ms, mean (SD)] and reflected [−1 (24) ms] waves. Augmentation index was underestimated by WEP [−7 (18)%]. WEP also provided a measure of compliance which correlated with pulse wave velocity ($r=-0.44$). RMSE values after the administration of each of the four drugs mentioned earlier were 12.4 (3.8), 17.7 (5.0), 22.1 (11.7), and 28.9 (22.4) mm Hg, respectively. Changes in derived WEP signals were similar to those measured by arterial line with all drugs.

Conclusions. The suprasystolic WEP signals can be used to derive arterial pressure waves which, although not identical, track changes in the intra-arterial pulse wave induced by vasoactive drugs.

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The contour of the arterial pressure pulse wave contains valuable information about cardiovascular function and, although rarely used in clinical practice, is increasingly recognized as a valuable means of assessing vascular function.[1 2] After cardiac contraction, a pulse wave propagates distally through the arterial tree. This wave is partially reflected at arterial branch points, with the velocity and magnitude of the reflection dependent on arterial wall stiffness and the degree of impedance mismatch,[3] particularly at the arteriolar bed. (Impedance mismatch is essentially the change in vascular resistance that occurs at an arterial branch point.) The reflected wave augments the incident pressure wave, the latter also dependent on ventricular contraction and aortic stiffness, and thus determines the overall pulse wave shape. The ratio of pressure augmentation to pulse pressure is often referred to as augmentation index (AIx). Arterial stiffness is affected by many factors, including vessel wall structure,[4] vascular

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smooth muscle activity,[5] endothelial function,[6] and mean distending arterial pressure.[7] Increased arterial stiffness is a major contributing factor to elevated systolic and pulse pressures[2] and has been shown to be associated with target organ damage[8] and with increased mortality independent of blood pressure.[9 10] Furthermore, increased pressure augmentation, as a consequence of increased arterial stiffness, has been shown to be an important determinant of cardiac work and myocardial perfusion[11 12] and is associated with adverse cardiovascular outcome.[13 14] Analysis of the pulse shape can also be used for determining direct measurements or indirect estimates of cardiac function, such as left ventricular ejection time[15] or cardiac output,[16] respectively. At present, there is little evidence for an association between perioperative complications and admission blood pressures of $<$180/110 mm Hg.[17] An alternative means of preoperative risk stratification, such as pulse contour analysis, may therefore be more useful than blood pressure measurement alone, but the technique ideally needs to be simple and practical.

In clinical practice, monitoring of the pressure wave using intra-arterial methods is widespread in the setting of acute illness and anaesthesia. However, the invasive nature of this technique makes it unsuitable for most research studies or for general clinical use. Non-invasive applanation tonometry has been successfully used in a clinical research setting for pulse wave analysis,[18] but adequate practice and training are essential. Wideband external pulse (WEP) monitoring was first described in 1988 by Blank and colleagues[19] as an alternative non-invasive technique for evaluating the arterial pressure pulse. Using a broad bandwidth (0.1–2000 Hz) piezoelectric sensor placed over the brachial artery under the distal edge of a sphygmomanometer cuff, they described changes in the externally recorded arterial waveform as a function of cuff pressure. They noted that, at suprasystolic cuff pressures, the resulting waveform, transmitted through soft tissues and cuff material rather than the vasculature, exhibited a typical shape consisting of three peaks and two troughs, although the nature of these contour features was not examined further. It is an attractive technique, as it uses potentially simple and cheap technology, can be performed quickly, and requires minimal operator training, in comparison with other non-invasive assessments of arterial function such as tonometry[18] or vascular ultrasound.[20] This method has recently been incorporated into a commercial system (Pulsecor, Auckland, New Zealand) for the assessment of vascular function. We hypothesized that the features of the suprasystolic WEP signal would be closely related to the shape of the invasively measured arterial pressure pulse. We examined the relationship by recording both signals in healthy volunteers after the administration of vasoactive pharmacological agents to achieve large changes in the pressure contour through disturbance of blood pressure, the intrinsic properties of the arterial wall, and cardiac contractility.

Methods

Study population

The study was conducted at The University of Edinburgh's Clinical Research Centre in accordance with the principles of the Declaration of Helsinki and was approved by the local research ethics committee. Written informed consent was obtained from each volunteer. Eleven healthy men, aged 20–25 yr (mean 23) were enrolled. Exclusion criteria included taking any regular medication, contraindication to arterial cannulation or any of the study drugs, and the presence of cardiovascular or other significant illness. Subjects were studied after 4 h of fasting and 24 h of abstinence from caffeine, alcohol, and nicotine.

Measurement techniques

Arterial pressure was measured invasively at the non-dominant radial artery. A 20G 80 mm catheter (BP7-95 440, Vygon, Ecouen, France) was inserted under local anaesthesia (lidocaine 1%) using the Seldinger technique and connected by semi-rigid fluid-filled tubing to a disposable pressure transducer (TruWave, Edwards LifeSciences, Saint-Prex, Switzerland) positioned level with the right atrium. Transducers were factory-calibrated and exceeded AAMI standards for performance interchangeability, with a sensitivity of 5 $\mu V\ V^{-1}\ mm\ Hg^{-1}$ $\pm 1\%$ and a non-linearity of the greater of $\pm 1.5\%$ or ± 1 mm Hg. The natural frequency of the system was 40 Hz. Waveforms were recorded at 200 Hz using a custom amplifier and analogue–digital converter interfaced to LabVIEW 6.1 data-logging software (National Instruments, Newbury, UK).

The WEP signal was recorded using two adjacent 1.5 cm diameter piezoelectric sensors (frequency range 0.1 to $>$1000 Hz) placed beneath the distal edge of a blood pressure cuff directly over the axis of the contralateral brachial artery (Pulsecor). The distal sensor was positioned centred 1 cm from the cuff edge. No differences were subsequently found between proximal and distal sensors, and data are therefore reported for the distal sensor only. Measurements were made with the cuff temporarily inflated to 30 mm Hg above systolic pressure. The waveform was recorded at 200 Hz, thus band-limiting the signal, using software developed by Ilixir Ltd (Auckland, New Zealand). Signal processing was performed using MATLAB (R12) (The MathWorks Inc., Natick, MA, USA) and LabVIEW software.

Pulse wave velocity (PWV) was calculated determining the transit time of the pulse between the proximal aorta and finger, as previously described.[21] The proximal pulse wave was detected determining the B-point of the

transthoracic cardiac bioimpedance waveform recorded using an NCCOM3 Cardiodynamic Monitor (BoMed Medical Systems, Irvine, CA, USA). This point has been shown to correspond to the start of mechanical ventricular ejection.[22] The distal pulse was recorded simultaneously at the fingertip using infrared photoplethysmography. The start of the finger pulse wave was determined using an intersecting tangent algorithm, as described by Chiu and colleagues.[23] Photoplethysmography has been favourably compared with more established methods for the measurement of PWV.[24] The straight-line distance between the sternal notch and fingertip was used as a surrogate for the true vascular path length.

Waveform feature analysis

Waveforms were recorded during ~30 s intervals at each time point. The 30 s signal was then ensemble-averaged to provide a single representative waveform for each individual subject at each individual time point. A preliminary visual inspection found the WEP signal to resemble the first derivative of arterial pressure, dP/dt. The original WEP waveform (WEP_S) was therefore compared directly with the first derivative of the arterial pressure wave (ART_S) and was also integrated to provide an estimated pressure waveform (WEP_A) for comparison with the original intra-arterial signal (ART_A). WEP_S and WEP_A were normalized to the same amplitude range as ART_S and ART_A, respectively.

The WEP_S signal has been noted by others[19] to have three principal waves (S1, S2, S3). These were recognized using turning and inflection points identified from the zero crossing points of the first through to third derivatives of the signal in a similar manner to that previously described for arterial waveforms.[25] The timing (T) and pressure (P) were noted at all three corresponding points, in addition to the trough between S1 and S2. The WEP_A and ART_A signals were analysed by employing similar methods to identify the incident and first reflected waveforms (A1, A2) and using corresponding pressures to calculate AIx from the equation $100\times(P_{A2}-DBP)/(P_{A1}-DBP)$. These parameters are shown in Figure 1. The Pulsecor system also estimates compliance (expressed in mm Hg ml^{-1}) from a first-order linear equation on the basis of a natural logarithm of the ratio of amplitudes of P_{S1} and P_{S2} on the WEP_S waveform,[26] but does not use measures of flow or volume.

The overall difference between waveforms was calculated by taking the root mean square error (RMSE) of the

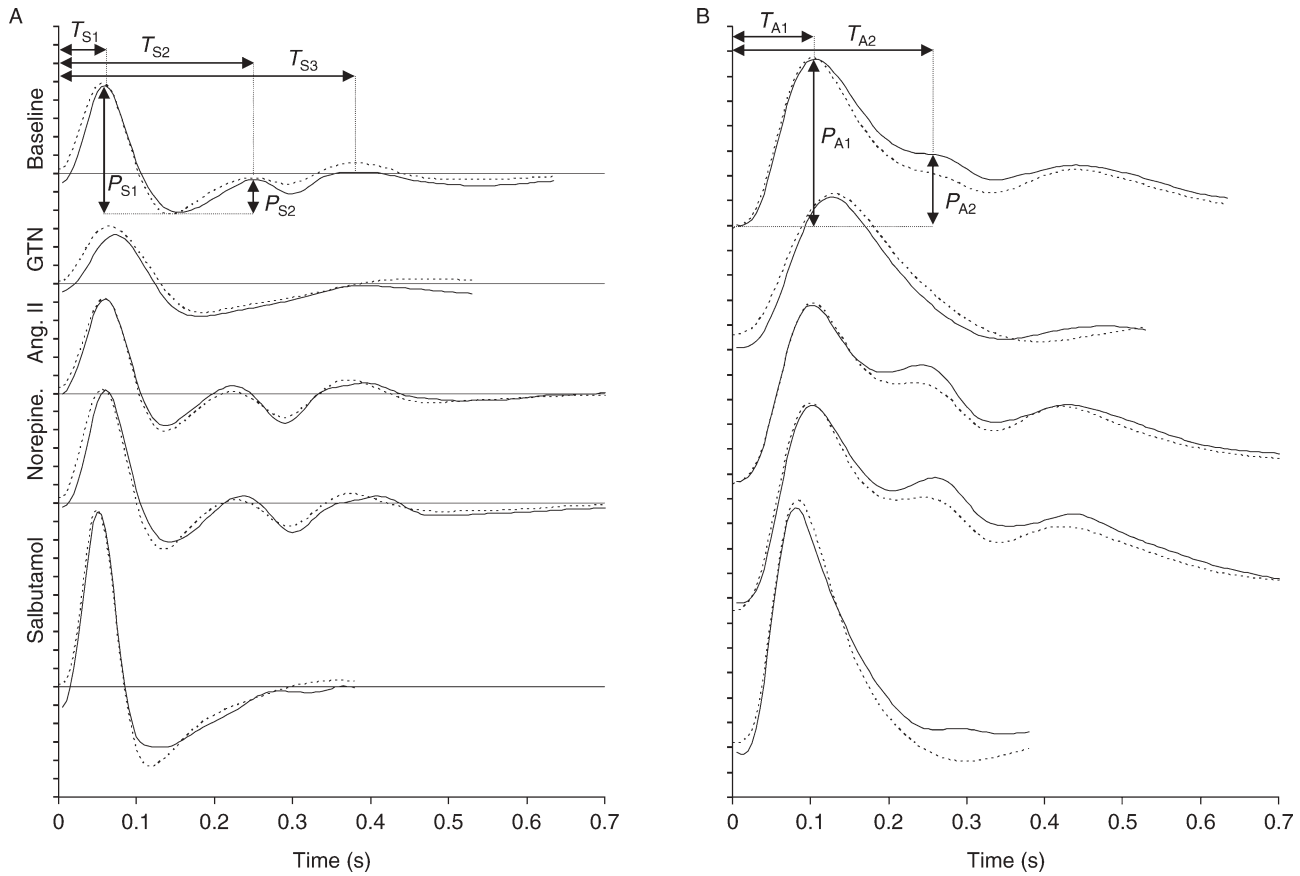

Fig 1 (A) Average ART_S (solid) and WEP_S (dotted) signals; y-axis divisions: 50 mm Hg s^{-1}. (B) Average ART_A (solid) and WEP_A (dotted) signals; y-axis divisions: 10 mm Hg.

two signals, after synchronization using the offset between the peaks of the WEP_S and ART_S waveforms.

Experimental protocol

All studies were conducted in a quiet, temperature-controlled environment [22 (2)°C], after 1 h acclimatization, with the subject in the supine position. After a saline 0.9% 20 min run-in period, four drugs were administered for 15 min, with the dose increased every 5 min. Drugs and doses were glyceryl trinitrate (GTN; 0.1, 1, 4 μg kg^{-1} min^{-1}; Nitrocine, Schwarz, Chesham, UK), angiotensin II (2, 6, 12 ng kg^{-1} min^{-1}; Clinalfa, Läufelfingen, Switzerland), norepinephrine (20, 60, 120 ng kg^{-1} min^{-1}; Levophed, Abbott, Maidenhead, UK), and salbutamol (albuterol; 0.4, 1.2, 2.4 μg kg^{-1} min^{-1}; Ventolin, Allen and Hanburys, Uxbridge, UK). All were administered i.v. at a constant rate of 1 ml min^{-1} through a 20G cannula sited in the antecubital fossa of the right arm. A 25 min washout period was allowed after each drug, with salbutamol given last because of its longer half-life. The order was not randomized, and doses and washout periods were based on published literature to produce consistent and predictable changes in arterial pressure and arterial tone: a decrease in arterial pressure was anticipated with GTN;[27] both norepinephrine and angiotensin II were expected to increase mean pressure, but the former was expected to cause a greater increase in pulse pressure;[28 29] salbutamol was predicted to cause a decrease in diastolic pressure because of peripheral vasodilatation, and a marked tachycardia and increase in systolic pressure because of positive chronotropic and inotropic effects.[30] Pulse transit time and arterial pressure data at each time point have been published elsewhere.[21] WEP and intra-arterial recordings were made immediately before each drug and at the end of the highest dose.

Statistical analysis

Data are presented as mean (SD). Changes in physiological parameters with each drug, relative to the respective baseline, were evaluated by paired *t*-tests. Changes in baseline between drugs were assessed by repeated measures analysis of variance (rmANOVA). The nature of the correlation between equivalent parameters obtained from WEP and intra-arterial signals was evaluated by linear regression analysis. Comparison of the different methodologies was expressed in terms of mean bias and limits of agreement, as recommended by Bland and Altman.[31] Linear regression correlation coefficients between the difference and mean of both measures were computed to evaluate any tendency for bias to increase or decrease over the measurement range. PWV is inversely proportional to compliance[32] and the nature of this correlation may vary between individuals; an average correlation coefficient was therefore computed for all individuals. Statistical analysis was performed using SPSS 12.0 (SPSS Inc., USA). *P*-values <0.05 were considered statistically significant.

Results

Changes in haemodynamic values are given in Table 1. Mean arterial pressure (MAP) and diastolic blood pressure (DBP) decreased with GTN and salbutamol, and increased with norepinephrine and angiotensin II. Systolic blood

Table 1 Changes in haemodynamics with individual drugs. Values are mean (SD). * indicates significant ($P<0.05$) change. Abbreviations are as per text

	GTN		Angiotensin II		Norepinephrine		Salbutamol	
	Before	**End**	**Before**	**End**	**Before**	**End**	**Before**	**End**
Haemodynamic parameter								
SBP (mm Hg)	130 (13)	119 (9)*	133 (17)	153 (13)*	137 (12)	162 (14)*	148 (12)	150 (24)
DBP (mm Hg)	63 (4)	57 (4)*	62 (7)	79 (7)*	65 (6)	79 (8)*	69 (7)	36 (6)*
MAP (mm Hg)	79 (5)	73 (4)*	76 (8)	99 (9)*	81 (7)	99 (10)*	88 (8)	66 (10)*
HR (beats min^{-1})	69 (10)	88 (12)*	68 (8)	62 (9)	68 (8)	63 (10)	73 (13)	136 (12)*
PWV (m s^{-1})	5.3 (0.6)	4.8 (0.8)*	5.3 (1)	6.1 (1.5)*	5.2 (0.7)	5.9 (1.3)*	5.9 (0.9)	5 (0.6)*
WEP waveform								
T_{S1} (ms)	49 (3)	54 (5)*	51 (5)	51 (5)	49 (5)	47 (4)	48 (4)	43 (3)*
T_{S2} (ms)	249 (21)	266 (53)	253 (17)	215 (14)*	252 (17)	219 (17)*	234 (21)	174 (44)*
T_{S3} (ms)	362 (28)	388 (48)*	362 (24)	363 (23)	369 (23)	364 (31)	359 (18)	326 (57)
$T_{S2}-T_{S1}$ (ms)	200 (20)	211 (50)	201 (15)	163 (14)*	202 (14)	172 (16)*	186 (19)	130 (43)*
P_{S1}/P_{S2} (%)	34 (9)	22 (8)*	30 (10)	37 (11)*	31 (10)	37 (9)*	33 (9)	22 (18)*
Compliance (ml mm Hg^{-1})	1.7 (0.2)	2.2 (0.1)*	1.9 (0.2)	1.4 (0.1)*	1.8 (0.2)	1.5 (0.2)*	1.8 (0.2)	2.2 (0.1)*
Intra-arterial waveform								
T_{A1} (ms)	109 (14)	121 (12)*	104 (14)	99 (6)	101 (11)	98 (12)*	98 (12)	81 (14)*
T_{A2} (ms)	237 (16)	240 (32)	229 (15)	213 (8)*	230 (16)	217 (13)*	223 (17)	190 (17)*
$T_{A2}-T_{A1}$ (ms)	128 (6)	126 (13)	125 (7)	113 (5)*	128 (8)	118 (5)*	124 (7)	116 (11)
AIx (%)	39 (6)	25 (23)	26 (6)	53 (17)*	29 (7)	52 (14)*	31 (8)	12 (24)
RMSE								
WEP_A and ART_A (mm Hg)	14.2 (5.4)	12.4 (3.8)	13.3 (5.3)	17.7 (5.0)	17.3 (8.5)	22.1 (11.7)	20.3 (11.9)	28.9 (22.4)
WEP_S and ART_S (mm Hg s^{-1})	39 (13)	49 (18)	42 (16)	42 (12)	53 (30)	58 (38)	59 (38)	139 (74)

pressure (SBP) increased with norepinephrine and angiotensin II, and decreased significantly with GTN only. Baseline values of SBP, DBP, and MAP varied between drugs ($P<0.05$ by rmANOVA), with an increase in all values exclusively before salbutamol. Heart rate at baseline did not vary between drugs ($P=0.33$).

PWV, ART_A AIx, and WEP_S P_{S1}/P_{S2} ratio decreased with GTN and salbutamol, and increased with norepinephrine and angiotensin II. Compliance measured by WEP mirrored changes in PWV, with a negative correlation overall ($r=-0.44$, $P<0.05$). The WEP_S $T_{S2}-T_{S1}$ time delay and ART_A $T_{A2}-T_{A1}$ time delay decreased with all drugs, except GTN, where no change was seen. These changes were largely accounted for by a decrease in T_{S2} and T_{A2}, respectively. However, both T_{S1} and T_{A1} decreased with salbutamol. Norepinephrine caused a greater increase in SBP and pulse pressure than angiotensin II ($P<0.05$), but similar changes in MAP and other measures of vascular function.

The similarities between the WEP_S and ART_S waveforms obtained are shown in Figure 1A, with the RMSE for the two signals shown at the bottom of Table 1. The RMSE did not vary significantly between experimental phases, despite being notably greater at the end of salbutamol administration. Regression analysis confirmed a strong positive correlation between the two methods with respect to timing of different components of the waveform (Table 2), although T_{S1}, T_{S2}, and T_{S3} occurred consistently slightly earlier when measured by WEP_S compared with ART_S (Fig. 2). There was also a trend for the bias to become progressively more negative at greater values of T_{S1} and T_{S3}, although the reverse was true for T_{S2} (Fig. 2). Although no significant mean differences existed between methodologies in the $T_{S2}-T_{S1}$ delay or the P_{S1}/P_{S2} ratio, there was a statistically significant tendency for the bias to become increasingly positive at greater values of $T_{S2}-T_{S1}$ and P_{S1}/P_{S2} (Fig. 2).

Table 2 Comparison of different WEP and intra-arterial parameters. '*r* for comparison' is Pearson's coefficient for correlation between related parameters; 'Mean bias' is the mean difference between the related parameters; '*r* for bias' is Pearson's coefficient for correlation between difference and mean of related parameters. *$P<0.05$. Abbreviations are as per text

Comparison	*r* for comparison	Mean bias	95% Limits of agreement	*r* for bias
WEP_S-ART_S				
T_{S1}	0.70*	−14 (ms)*	−31.1 to 3.1 (ms)	−0.75*
T_{S2}	0.73*	−19 (ms)*	−70.0 to 32.0 (ms)	0.39*
T_{S3}	0.68*	−33 (ms)*	−97.7 to 31.7 (ms)	−0.27*
$T_{S2}-T_{S1}$	0.64*	−5 (28) (ms)	−59.9 to 49.9 (ms)	0.52*
P_{S1}/P_{S2}	0.41*	−0.6	−22.2 to 21.0	0.52*
WEP_A-ART_A				
T_{A1}	0.39*	−8 (ms)*	−43.3 to 27.3 (ms)	0.0
T_{A2}	0.61*	−1 (ms)	−48.0 to 46.0 (ms)	0.35*
$T_{A2}-T_{A1}$	0.61*	1 (ms)	−27.0 to 29.8 (ms)	0.71*
AIx	0.60*	−7 (%)*	−42.3 to 28.3 (%)	0.0

The WEP_S signal was integrated (WEP_A) to assess how accurately the arterial pressure wave contour could be estimated. Similarities in wave shape are shown in Figure 1B. RMSE values were large (Table 1) and statistically greater with salbutamol only ($P<0.05$). The WEP_A signal appeared slightly damped relative to ART_A, although the timings of reflected waves were similar. Regression analysis (Table 2) confirmed that T_{A1} and T_{A2} occurred at similar times, although the bias of the $T_{A2}-T_{A1}$ time delay became more positive with increasing values (Fig. 3). AIx showed a consistent bias of −7 (18)% across the measurement range relative to intra-arterial measurements (Fig. 3).

Discussion

This study is the first to describe the relationship between the contours of the suprasystolic WEP signal and the intra-arterial pressure wave, and the effect of vasoactive drugs on the former. We have shown that the suprasystolic WEP signal resembles the first derivative of intra-arterial pressure and can therefore be used to estimate the arterial pressure wave. Time delays and measurements of reflected wave amplitude measured by WEP analysis correlate with those obtained directly from the arterial signal, and similar changes occur with both techniques during the administration of pharmacological agents. Although it is important to note that the RMSE was substantial with all drugs (4–5 mm Hg is the limit of accuracy of devices for recording arterial pressure[33]), and that this may therefore limit the role of suprasystolic WEP analysis as an accurate alternative to direct intra-arterial pressure recording or applanation tonometry, the WEP responses nonetheless tracked those of the arterial line and can thus still be considered a potentially useful means of evaluating cardiovascular function. Furthermore, in addition to the obvious benefits of being non-invasive, the WEP system has the advantage that it has potentially far less operator dependency than tonometry and could be incorporated relatively easily into standard oscillometric sphygmomanometer devices and utilized in pre-admission screening or during preoperative management.

It has been suggested by the manufacturers of Pulsecor that the S2−S1 delay is inversely related to PWV;[26] a similar relationship with PWV has been proposed for the time delay between systolic and diastolic peaks on the finger photoplethysmograph waveform.[33] The current study found that the $T_{S2}-T_{S1}$ delay, measured by both WEP_S and the first derivative of the arterial pressure pulse, decreased with all drugs, except GTN, which caused a small non-significant increase. The $T_{A2}-T_{A1}$ delay, measured from the arterial pressure pulse, also decreased with both pressor agents and salbutamol, although the latter not significantly. The $T_{S2}-T_{S1}$ and $T_{A2}-T_{A1}$ time delay findings were similar to each other, but not in line with either expected or measured PWV

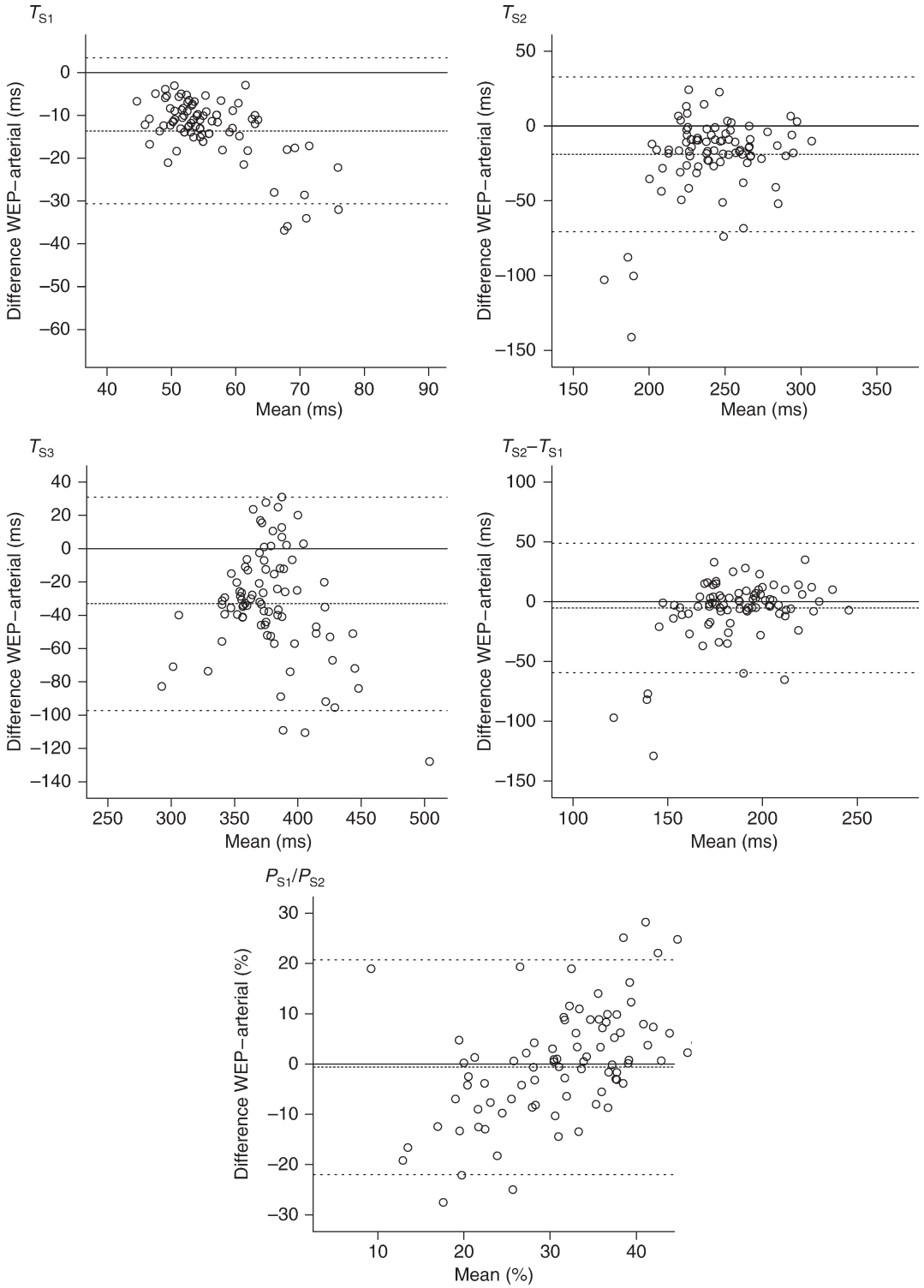

Fig 2 Bland–Altman plots of WEP measures (difference and mean of actual WEP value and that derived from first derivative of arterial pressure). Dashed lines represent mean bias and 95% limits of agreement (± 1.96SD).

responses. Changes in the magnitude of reflected waves, because of changes in peripheral impedance mismatch, may affect the timing of wave peaks and thus alter the apparent velocity of reflections. It can also be difficult to identify S2 in circumstances of marked vasodilatation and increased heart rate. These factors may in part explain the time delay findings described. GTN given in similar doses to those used in the present study has been shown to have only small effects on the finger pulse systolic–diastolic time delay, despite large changes in the relative amplitude

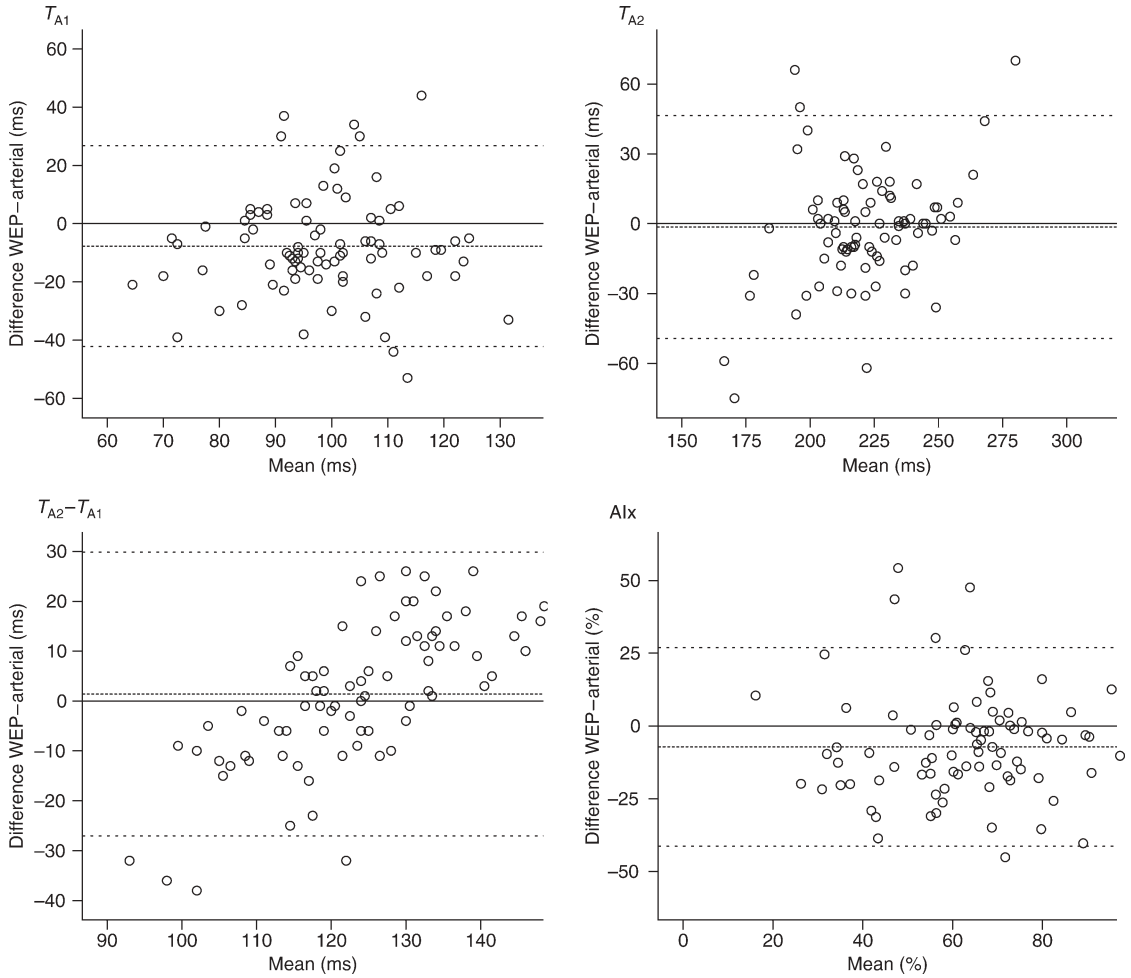

Fig 3 Bland–Altman plots of arterial pressure measures (difference and mean of arterial value derived by integrating WEP signal and actual measured value). Dashed lines represent mean bias and 95% limits of agreement ($\pm$1.96SD).

of these wave components,[34] and thus inaccuracy in identifying S2 or A2 may have been particularly important with this drug. The decrease in PWV with salbutamol in this study is because of peripheral vasodilatation and a decrease in MAP, offsetting any potential increase as a result of tachycardia.[35] The corresponding decrease in $T_{S2}-T_{S1}$ (and to a lesser extent, $T_{A2}-T_{A1}$) is not consistent with this PWV change and may also be explained by the factors described earlier. These findings were identified with both WEP and arterial line and consistent in all subjects, suggesting this is a genuine phenomenon. Regardless of the precise cause of these findings, it would therefore appear unwise to use these time delays as a surrogate marker of PWV.

The ratio of amplitudes of the original WEP_S signal showed changes similar to AIx and PWV. However, as pointed out by Millasseau and colleagues,[34] it is difficult to relate directly values obtained from the derivative of the pulse waveform to the biomechanical properties of the cardiovascular system. The ratio of amplitudes is also used by Pulsecor to obtain a measure of vascular compliance, and the values obtained in the current study correlate with measured PWV. It is important to note, however, that the currently unpublished mathematical function used to derive compliance is not validated and is based on small subject numbers.[26] Furthermore, the compliance value is an estimate only, as neither volume nor flow is known. The current study was not designed to validate the accuracy of the compliance values, and a measure of vascular function obtained directly from the waveform was thus considered more relevant. In this respect, AIx is an established and useful marker of vascular function,[29] although not a direct measure of arterial compliance.[28] WEP-derived arterial pressure waves showed changes in AIx similar to those directly measured using the arterial line. As the correlation may have been inflated by pooling data across interventions known to alter AIx, baseline data were examined alone. This analysis revealed that the positive correlation persisted (r=0.42, P<0.01) with a similar degree of bias [−7.7 (12.7)%]. The bias between the two methods probably reflects the damping of the WEP_A waveform, with a relatively smaller P_{A2} amplitude. It remains

uncertain whether WEP signals can be used to evaluate central haemodynamics, although this would appear possible, given that radial AIx correlates closely with derived aortic AIx.[36]

Blank and colleagues[19] described a similar appearance of the suprasystolic WEP pulse contour to that observed in the present study. Below systolic pressure, the suprasystolic signal became obscured, with the waveform taking on the intra-arterial pressure pulse contour as cuff pressure approached diastolic pressure. Below diastolic pressure, the signal diminished in size, as it requires adequate coupling between the sensor surface and the skin. Although they did not compare the suprasystolic shape directly with intra-arterial pressure, they acknowledged that this waveform was probably still intrinsically related to the arterial pressure pulse and may therefore contain clinically important information. This is supported by the current study. In addition, Blank found that the suprasystolic signals had less high frequency energy than diastolic WEP signals, the latter correlating directly with intra-arterial pressure. This may explain the apparent damping of the arterial pressure signals derived from the suprasystolic WEP traces in the current study and is presumably related to the effects of pulse transmission through the upper limb tissues and inflated cuff. The principal advantage of using the suprasystolic WEP waveform, as opposed to the sub-systolic or diastolic signals, is that adequate coupling of the sensor to the skin is always present, and that a signal comprised of any diastolic component is avoided.

It is still not clear why the WEP signal resembles the derivative of the intra-arterial pressure wave. Occlusion of the brachial artery does not prevent the distal propagation of vibrations resembling the original pulse waveform through the air-filled cuff and non-vascular tissues. If the air-filled cuff is considered a low-impedance continuation of the artery, then a reflection would be expected to occur at the interface, which would be subject to a 90° phase shift, effectively inverting it.[3] Assuming these two signals (normal and inverted) are of similar magnitude and slightly offset in time from one another, then the sum of the two amplitudes will be a function of the pressure gradient and thus resemble the first derivative. Alternatively, the signal may represent the effects of obstructed flow, generating waves similar to the flow wave which is closely related to the pressure gradient in peripheral vessels. However, these suggestions are purely speculative, and additional studies are required to understand the mechanics underlying generation of the suprasystolic WEP signal and whether the signal is affected by non-vascular parameters, such as cuff material or size.

The use of fluid-filled manometer tubing for the measurement of intra-arterial pressure is a weakness of this study. Invasive monitoring was selected in preference to tonometry, as there is no operator dependency. Measurement error because of sub-optimal damping was minimized during the study by using a short tube length. The increased fundamental frequency of heart rate during the administration of salbutamol might account for the greater RMSE observed between WEP and intra-arterial signals with this drug. Cannulation of the radial artery is safer than that of the brachial artery, and different arms were used for measurements owing to the loss of the ipsilateral radial pulse signal upon brachial cuff inflation. The resulting comparison of different anatomical sites may therefore partially account for the differences seen between actual and WEP-derived pressure signals but is unlikely to influence the conclusions reached. A further limitation of the study is the failure of haemodynamic responses to return to baseline after the administration of norepinephrine. The physiological changes were probably not directly because of the drug, as its half-life is very short, but rather due to a natural stress response to the prolonged study. Indeed there was a trend for arterial pressure to increase throughout the experiment. However, the failure of the response to return to baseline after norepinephrine did not prevent the study achieving the aim of comparing waveforms under widely varying pharmacologically induced haemodynamic circumstances.

In conclusion, the supra-systolic WEP signal appears to correlate strongly with the first derivative of the intra-arterial pressure wave and is able to detect changes in the pulse waveform induced by vasoactive drugs similar to those measured by invasive monitoring. The arterial pulse contour recorded using alternative methodologies has already been shown to be clinically relevant. Further work is therefore merited to investigate the true nature of the supra-systolic WEP signal, whether it can be used in the study of cardiovascular physiology in disease states, and to evaluate reproducibility in a larger population.

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A generalized arterial transfer function derived at rest underestimates augmentation of central pressure after exercise

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Objectives **Peripheral exercise blood pressure and resting central blood pressure are considered more relevant to cardiovascular health than resting peripheral blood pressure. Central exercise blood pressure may well be an even more useful measure, but there is no simple non-invasive means of determining it. The aim of the present study was to establish whether the estimation of central blood pressure from peripheral blood pressure using a transfer function derived at rest, would hold after aerobic exercise.**

Methods **Thirty healthy young men were studied before and immediately (<1 min) and 10 min after 15 min bicycle exercise at 65–70% of maximum heart rate. Simultaneous carotid and radial artery waveforms were recorded, and radial-to-carotid generalized transfer functions (GTF) were calculated using Fourier analysis for rest and immediately postexercise. Central systolic blood pressure (SBP) and augmentation index (AIx) were calculated for measured and derived waves.**

Results **The resting GTF underestimated central SBP and AIx immediately (-5.8 ± 2.1 mmHg, $P = 0.01$; -8.3 ± 2.9%, $P = 0.008$) and 10 min after (-2.0 ± 0.7 mmHg, $P = 0.008$; -7.0 ± 2.1%, $P = 0.003$) exercise. No significant bias was found between measured and derived (using resting GTF) carotid values at rest. The use of an exercise-specific GTF resulted in no specific bias immediately or 10 min after exercise, although it overestimated blood pressure and AIx at rest (2.5 ± 1.0 mmHg, $P = 0.02$; 11.3 ± 3.0%, $P = 0.001$).**

Conclusion **A peripheral-to-central arterial GTF derived at rest significantly underestimates key measures of central arterial pressure immediately after exercise, and pressure estimations may be improved by the use of an exercise-specific GTF. *J Hypertens* 25:2266–2272 © 2007 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: blood pressure, exercise, healthy volunteers, pulse wave analysis, transfer function

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Introduction

Exercise is an important aspect of everyday life, and peripheral blood pressure during exertion is recognized as a marker of cardiovascular risk [1–4], independently of resting peripheral blood pressure. At rest, central blood pressure has been shown to be a more important determinant of vascular function and cardiovascular risk than peripheral blood pressure [5], and this may well also be true during exercise. Increased arterial stiffness augments central systolic pressure by effects on wave reflections [6]. This results in greater pulsatile stress in the aorta, leading to structural changes, remodelling and atherogenesis [6]. It also increases left ventricular afterload and myocardial oxygen consumption [7], while compromising myocardial perfusion in diastole [8]. This is of particular importance during exercise, when large changes occur in cardiac work and vascular function. Marked differences exist between central and peripheral blood pressure [9], however, as a result of systolic pressure amplification in the peripheral vessels, and these differences are amplified by exercise [10]. At present, however, there is no established way of measuring central blood pressure during exertion.

To overcome the difficulties associated with the invasive measurement of central pressure, a transfer function has been developed to derive the aortic pressure waveform from the radial artery waveform [11]. A transfer function is a mathematical description of the phase and magnitude change in different frequency components of a pulsatile phenomenon between two sites, and is widely used in engineering sciences. A radial-to-aortic generalized transfer function (GTF) assumes that the properties of the upper limb arteries are virtually identical between individuals. The transfer function has indeed been shown to remain relatively constant in individuals with coronary heart disease [12], even after the administration of vasoactive drugs [13–15]. Derived aortic waveforms obtained using this method have been shown to relate to cardiovascular outcome [16,17]. Although this technique might therefore be seen as a potential method of determining central blood pressure during exercise, most of the validation studies to date have been in men undergoing diagnostic cardiac catheterization. Discrepancies in the transfer function have been demonstrated between men and women [18], diabetic and non-diabetic populations

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[19], and individuals with and without coronary disease [20]. The large increases in mean distending pressure and intrinsic arterial wall stiffness that occur with exertion might also be expected to alter the transfer function, with an increase in the velocity of higher frequency harmonics. This would result in greater convexity of the derived waveform, and a relatively lower systolic peak for any given diastolic and mean pressure.

We studied healthy volunteers undertaking moderately strenuous aerobic exercise, to address the hypothesis that a peripheral-to-central arterial transfer function derived at rest would underestimate central pressure immediately after exercise.

Methods

Subjects

Thirty healthy young male volunteers with variable exercise capacity were recruited. Subjects had no past history of cardiovascular or other significant illnesses, and were taking no regular medications. Studies adhered to the principles of the Declaration of Helsinki. Studies conformed to institutional ethical requirements and volunteers provided informed consent.

Study protocol

After an initial period of rest, baseline measurements of central (carotid-femoral) and conduit (carotid-radial) arterial pulse wave velocity (PWV) were made in the supine position. Subjects then transferred to a semi-recumbent cycle ergometer (SX1; Heinz Kettler GmbH and Co., Ense-Parsit, Germany) with all further measurements (including baseline arterial wave recordings) made in the sitting position. Central pressure was determined non-invasively at the carotid artery. Blood pressure, heart rate and carotid and radial artery waveforms were recorded before, and immediately (within 1 min) and 10 min after, aerobic exercise. All waveform measurements were made by a single investigator (C.H.T.). An initial workload of 50 W was set for all subjects, and pedal cadence maintained between 70 and 100 rpm. Workload was increased by 10 W every 30 s until the heart rate reached 65–70% of the individual's estimated maximum, calculated as 220 minus age in years. Once the target heart rate was achieved, the associated workload was maintained for a further 15 min.

Pulse wave velocity and blood pressure

Blood pressure was recorded at the left brachial artery using a validated oscillometric sphygmomanometer (HEM705; Omron Healthcare, Bannockburn, Illinois, USA). PWV was measured by sequential 10-s recordings of the radial, carotid and femoral artery waveforms using a hand-held tonometer (SPT301; Millar Instruments, Inc., Houston, Texas, USA), gated to the R-wave of the electrocardiogram (LifePulse LP15; HME Ltd., South Mimms, Herts, UK). PWV was calculated using the intersecting tangent of the maximum first derivative for waveform timing purposes [21], and straight-line distance measurement between the sternal notch and waveform recording sites.

Pulse waveform acquisition

Simultaneous non-invasive carotid and radial artery waveforms were recorded using hand-held (SPT301) and automated (CBM7000; Colin Medical Instruments Corp., Komaki City, Japan) tonometers, respectively, over approximately 30 s. Carotid waveforms were acquired on the right side with the subject looking straight ahead and minimizing respiratory excursions to reduce movement artefact. Both forearms were supported at the level of the xiphisternum, with a splint positioned over the dorsal aspect of the right wrist to minimize movement during radial waveform recording. Signals were digitized at 1 kHz using a 16-bit analogue–digital converter (DAQCard 6036E; National Instruments Corp., Austin, Texas, USA) and stored for off-line analysis.

Calculation of transfer functions and pulse wave analysis

Signals were analysed using custom software written in LabVIEW 6.1 (National Instruments). Ten-second simultaneous radial and carotid waveform segments were selected for the rest and immediate postexercise phases, using visual inspection to ensure signal stability and to exclude artefact. Signals were smoothed using a five-point moving average. Radial and carotid waveforms were synchronized by identifying the wave foot using the intersecting tangent, to remove the phase shift introduced by the transit time between the two signals. Radial signals were normalized to brachial diastolic and systolic pressure, with mean pressure computed using numeric integration of the waveform. Carotid signals were then normalized assuming a constant diastolic and mean pressure throughout the arterial tree (i.e. equivalent to radial values) [5]. Transfer functions were computed from these waveforms for each individual subject and both time points, by dividing the Fourier transform of the input (radial) signal by that of the output (carotid) signal [22]: $H_{(\text{radial}\rightarrow\text{carotid})} = P_{\text{radial}}(\omega)/P_{\text{carotid}}(\omega)$, where $P(\omega)$ is the frequency domain of a complex harmonic signal, ω is the angular frequency, and a sinusoidal component is represented by $P = |x|e^{j\varphi}$, where $|x|$ is amplitude and φ is phase. GTF (GTF_{rest} and GTF_{exercise}) were computed by averaging the relevant individual transfer functions over 0–20 Hz. Derived carotid waveforms were obtained by applying both GTF_{rest} and GTF_{exercise} to radial waveforms recorded at all three experimental timepoints. A similar procedure was carried out using a subset of 10 individuals to compute the GTF ($GTF_{\text{rest-2}}$ and $GTF_{\text{exercise-2}}$), and applying these GTF to the remainder of the study population. A further radial-to-carotid GTF ($GTF_{\text{sphygmocor}}$) was computed in a similar manner from waveform data obtained using the

Table 1 Subject characteristics ($N = 30$)

Variable	Mean ± SD
Age (years)	22.3 ± 2.4
Height (cm)	177 ± 9
BMI (kg/m^2)	22.9 ± 2.6
Family history CVD (*N*, %)	10 (33%)
Resting BP (mmHg)	119/69 ± 8.0/6.4
Pulse wave velocity (m/s)	
Carotid-femoral	6.2 ± 1.1
Carotid-radial	8.2 ± 1.1
Resting heart rate (bpm)	72 ± 11.3
Peak exercise workload (W)	84 ± 18

BP, Blood pressure; BMI, body mass index; Family history CVD, cardiovascular disease in first degree relative aged 65 years or less.

commercially available SphygmoCor (AtCor Medical Ltd., West Ryde, New South Wales, Australia) system, by multiplying together the radial-to-aortic and aortic-to-carotid transfer functions employed by this device, so that $H_{(\text{radial}\rightarrow\text{carotid})} = [P_{\text{radial}}(\omega)/P_{\text{aortic}}(\omega)] \times [P_{\text{aortic}}(\omega)/P_{\text{carotid}}(\omega)] = P_{\text{radial}}(\omega)/P_{\text{carotid}}(\omega)$. As the derived aortic waveforms are a pure mathematical function of the measured peripheral signal, this allows for an accurate reproduction of the two SphygmoCor transfer functions, relatively independently of the quality of signal recording. It also provides a further resting GTF obtained using waveform data independent of that recorded during the study.

Radial, carotid and derived-carotid waveforms were all analysed in a similar manner. Ten-second waveform signals were ensemble-averaged by synchronizing waves at the maximum first derivative. The augmentation index (AIx) was calculated as a measure of wave reflection and arterial stiffness, expressed as the difference in amplitude between incident and reflected waves, as a percentage of pulse pressure. Component waves were identified by finding the relevant zero-crossing points on the fourth derivative of the waveform as described previously [23].

Statistical analysis

Data were analysed using SPSS v14.0 (SPSS Inc., Chicago, Illinois, USA). Results are expressed as mean ± standard deviation, and difference in means as mean difference ± standard error. Waveform measurements were compared using paired *t*-tests. The area under the curve for the 0–10 Hz phase and magnitude components of GTF_{rest} and $GTF_{exercise}$ were compared by paired *t*-tests.

Results

Subject characteristics are shown in Table 1 and changes in peripheral haemodynamic variables in Table 2. Volunteers had normal resting blood pressure, and normal resting central and conduit artery PWV. As expected, there were significant increases in heart rate (58.9 ± 11.9 bpm, $P < 0.001$), diastolic blood pressure (20.3 ± 33.7 mmHg, $P = 0.005$), mean arterial pressure (31.7 ± 29.0 mmHg, $P < 0.001$), and radial systolic blood pressure (SBP; 57.5 ± 31.1 mmHg, $P < 0.001$) and AIx (7.8 ± 11.1, $P = 0.002$) immediately after exercise. Measured carotid SBP and AIx also both significantly increased (52.0 ± 30.3 mmHg and 16.9 ± 12.9%, respectively, $P < 0.001$ for both). All parameters remained significantly elevated ($P \leq 0.02$) above resting values at 10 min recovery, with the exception of radial SBP ($P = 0.23$).

Differences between measured and derived carotid parameters are shown in Fig. 1. There was no significant difference between carotid artery SBP obtained using GTF_{rest}, and measured values, at rest (difference 0.0 ± 0.9 mmHg, $P = 0.96$). GTF_{rest} significantly underestimated carotid artery SBP immediately after exercise (−5.8 ± 2.1 mmHg, $P = 0.01$), however, and at 10 min postexercise (−2.0 ± 0.7 mmHg, $P = 0.008$). Conversely, an exercise-specific transfer function, $GTF_{exercise}$, gave significantly higher values of carotid SBP at rest (2.5 ± 1.0 mmHg, $P = 0.02$), but there was no bias immediately postexercise (−0.1 ± 2.2 mmHg, $P = 0.96$) or at 10 min recovery (0.6 ± 0.7 mmHg, $P = 0.37$). Similar results were found for carotid AIx: GTF_{rest} underestimated carotid AIx immediately postexercise (−8.3 ± 2.9%, $P = 0.008$) and at 10 min (−7.0 ± 2.1%, $P = 0.003$), but not at rest (0.4 ± 2.8%, $P = 0.90$); $GTF_{exercise}$ demonstrated no significant bias immediately postexercise (−2.6 ± 2.8%, $P = 0.37$) and at 10 min recovery (2.6 ± 2.3%, $P = 0.27$), but overestimated resting (11.3 ± 3.0%, $P = 0.001$) carotid AIx.

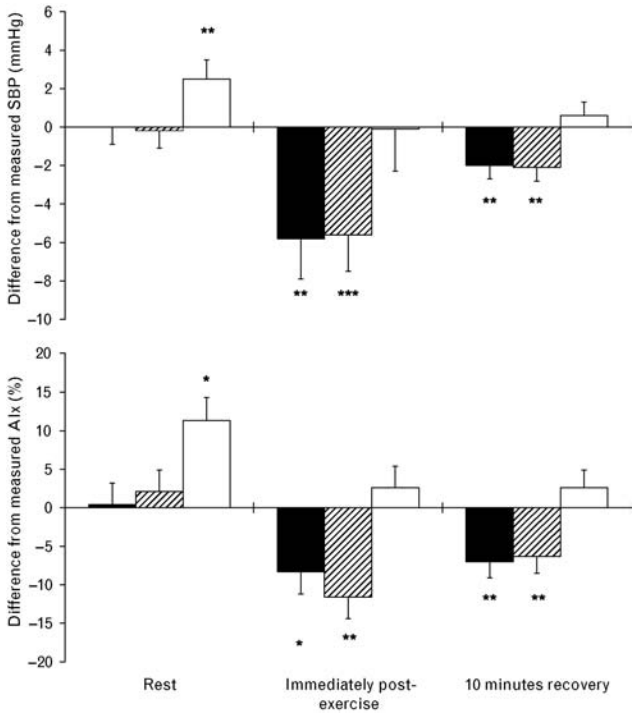
In order to address the issue of potential bias resulting from the application of a GTF derived from the same set of data as that to which it is subsequently applied, transfer functions calculated from a subset of the study population ($GTF_{rest\text{-}2}$, $GTF_{exercise\text{-}2}$) were applied to the wave data

Table 2 Haemodynamic changes with exercise

Variable	Rest	Immediately postexercise	10 Min postexercise
Heart rate (bpm)	72 ± 11.3	131 ± 8.1***	85 ± 11.9*
DBP (mmHg)	69 ± 6.4	89 ± 33.3**	75 ± 9.8**
MAP (mmHg)	86 ± 7.4	117 ± 29.4***	91 ± 9.5**
Radial SBP (mmHg)	119 ± 8.0	177 ± 30.2***	121 ± 10.6
Measured carotid SBP (mmHg)	105 ± 10.3	157 ± 30.6***	110 ± 11.9**
Radial AIx (%)	57 ± 14.6	66 ± 13.2**	62 ± 12.3**
Measured carotid AIx (%)	18.9 ± 12.2	36 ± 14.8***	29 ± 12.5***

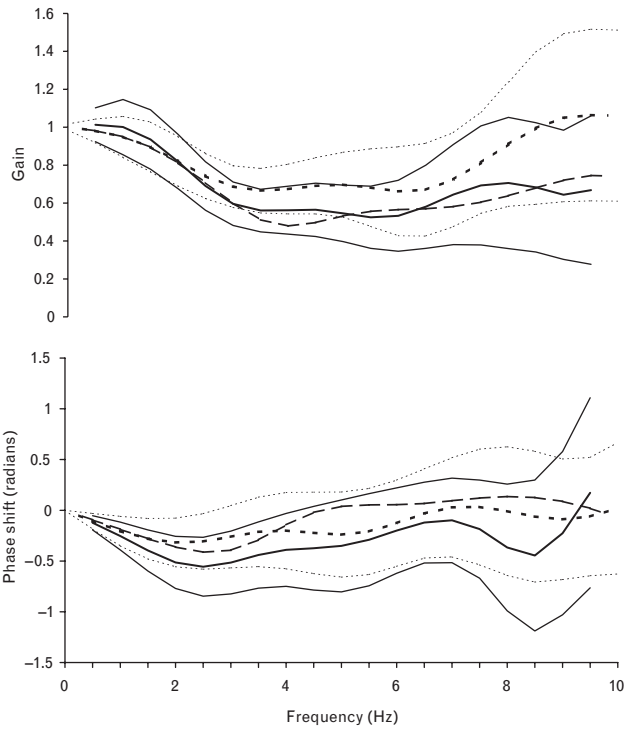
AIx, Augmentation index; DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, significant difference from rest value.

Fig. 1



Difference in derived carotid systolic blood pressure (SBP, top) and carotid augmentation index (AIx, bottom), compared with actual measured carotid values. Values are mean ± SE ($N = 30$). Bars represent GTF_{rest} (black), $GTF_{sphygmocor}$ (diagonal hatching) and $GTF_{exercise}$ (white). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, significant difference from measured value.

Fig. 2



Transfer functions, shown as gain and phase shift for the relationship between radial and carotid arteries. Solid line, resting generalized transfer function (GTF_{rest}); dotted line, exercise transfer function ($GTF_{exercise}$); dashed line, Sphygmocor-derived transfer function ($GTF_{sphygmocor}$). Heavy line is mean, thin lines are ± 1 SD (SD not shown for $GTF_{sphygmocor}$ for clarity).

obtained from the remaining individuals, and a resting GTF obtained independently from the SphygmoCor system was applied to the entire study population. Similar findings to those described above were obtained using $GTF_{rest\text{-}2}$ and $GTF_{exercise\text{-}2}$: AIx and carotid SBP were overestimated at rest when derived using $GTF_{exercise\text{-}2}$ ($14.1 \pm 5.4\%$, $P = 0.025$; 4.6 ± 1.6 mmHg, $P = 0.013$, respectively), and underestimated at peak exercise when derived using $GTF_{rest\text{-}2}$ ($-15.4 \pm 4.5\%$, $P = 0.005$; -7.5 ± 2.3 mmHg, $P = 0.007$). Differences between actual values of both AIx and carotid SBP, and those derived using $GTF_{rest\text{-}2}$, persisted at 10-min recovery ($10.9 \pm 3.5\%$, $P = 0.008$; 3.9 ± 1.0 mmHg, $P = 0.002$). No significant bias was observed between actual and derived measures of either AIx or SBP when derived using the GTF appropriate to that experimental timepoint. $GTF_{sphygmocor}$ gave similar findings to GTF_{rest}, underestimating both carotid SBP and AIx immediately postexercise (-5.6 ± 1.9 mmHg, $P = 0.007$; $-11.6 \pm 2.8\%$, $P < 0.001$, respectively) and at 10 min recovery (-2.1 ± 0.7 mmHg, $P = 0.004$; $-6.3 \pm 2.2\%$, $P = 0.009$), but demonstrating no bias at rest (-0.2 ± 0.9 mmHg, $P = 0.83$; $2.1 \pm 2.8\%$, $P = 0.47$).

GTF_{rest}, $GTF_{exercise}$, and $GTF_{sphygmocor}$ are shown in Fig. 2. There was a significant decrease in gain ($P = 0.001$) and a positive phase shift ($P = 0.012$) in $GTF_{exercise}$ compared with GTF_{rest}, over 0–10 Hz.

Discussion

The current study is the first to describe the effects of exercise on the peripheral-to-central arterial transfer function in young, healthy adults. It demonstrates that both central SBP and AIx, important measures of aortic pressure dependent on the effects of arterial stiffness, are underestimated after exercise when a transfer function derived at rest is used. The use of an exercise-specific transfer function improves this estimation, but does not hold at rest. The effect of exercise on the transfer function is still present 10 min after stopping exercise.

Transfer functions provide an attractive means of determining aortic pressure non-invasively, and their use during exercise is of particular interest as there are relatively few data on the effects of exertion on central haemodynamics. Exercise has marked effects on the stiffness of the arterial wall, both through an increase in distending mean blood pressure (particularly at higher levels of exertion), and by effects on the intrinsic properties of the arterial wall as a result of the release of various

local and systemic vasoactive mediators. Furthermore, increased heart rate is associated with an increased rate of change of pressure, and may therefore increase stiffness because of the viscoelastic nature of the arterial wall [24]. The change in gain and phase of the transfer function after exercise is probably accounted for by a combination of these factors. It is not entirely clear whether the resulting bias demonstrated in our study of −5.8 mmHg SBP and −8.3% AIx is great enough to be of clinical relevance. The discrepancies are similar to or greater than the differences in these parameters observed at rest in populations with and without cardiovascular disease [17], or resulting from different therapeutic agents [25]. The disagreement corresponds to an inaccuracy at peak exercise of only approximately 3.7% for SBP, although 11.1% of the overall central SBP range. The relative error in AIx is considerably greater at approximately 23% of the peak AIx value. If the error across populations remains relatively constant, then this is of less concern, although the advantage of using a GTF in the first place is somewhat defeated. It is also uncertain, however, whether drug treatment or other interventions such as exercise training may alter the GTF response to exercise (and thus the degree of underestimation of central haemodynamics) in an unpredictable manner.

Two other recent studies have examined the effect of exercise on the arterial transfer function. Sharman *et al.* [26] found no effect of exertion on the radial-to-aortic transfer function, whereas Stok *et al.* [27] found that increasing exercise led to an underestimate and more unreliable measurement of systolic pressure derived from the finger pulse. Both studies used invasive measurements of aortic pressure in patients undergoing diagnostic coronary angiography. Important methodological issues may account for the different conclusions reached by those studies. The duration of exercise in the study by Sharman *et al.* [26] was rather short at only 3–6 min, and included a protocol employing one-legged cycling. Furthermore, the heart rate responses were not particularly marked, at least partly as a result of beta-blockade in the majority of individuals. Although the blood pressure increases were similar between our study and that of Sharman *et al.* [26], the exercise protocol in our study was considerably longer and the intensity of workload more accurately gauged by the heart rate response; it is possible that the overall intensity of the exercise in the study by Sharman *et al.* [26] was too low to have a significant effect on the intrinsic properties of the arterial wall, and thus no measurable effect on the transfer function. The work by Sharman *et al.* [26] was carried out in older subjects, many with confirmed coronary disease who were taking various cardiovascular drugs. Significant differences have been demonstrated in the resting radial-to-carotid transfer function between individuals with coronary disease and healthy volunteers [20]. Age and vascular disease increase resting arterial stiffness, but also lead to diminished catecholamine-mediated inotropic, chronotropic and arterial vasodilatory effects in response to exercise [6]; this may well have blunted any change in transfer function in response to exercise. The presence of certain vasoactive drugs has been shown to have no effect on the resting GTF [13–15], but it is not known whether these may also have altered the arterial response to exercise, thus minimizing the change in transfer function. Importantly, despite similar drug therapy in a comparable population, an effect of exercise on the transfer function was nonetheless demonstrated by Stok *et al.* [27] suggesting that drug treatment, age and the presence of vascular disease was less likely to account for the findings of Sharman *et al.* [26]. It should, however, be noted that exercise causes marked peripheral vasodilatation and can lead to an inaccuracy of blood pressure measurement at the finger using the volume-clamp method employed by Finapres [28]. Furthermore, the local effects of certain drugs may result in changes in the finger pulse that are not evident in the pulses of larger arteries. It might therefore be argued that some of the inaccuracies in central pressure estimation found by Stok *et al.* [27] may be accounted for by error resulting from use of the Finapres, although our own work suggests that their findings are at least partly accounted for by changes in the conduit vessel properties in response to exercise. Interestingly, although Sharman *et al.* [26] concluded that the resting GTF held after exercise, their published data nonetheless show that the difference between derived and actual SBP was significantly greater ($P < 0.001$ calculated from reported data using an unpaired *t*-test) during exercise than at rest (-4.7 ± 3.3 versus -1.3 ± 3.2 mmHg, respectively).

The present study has some important limitations. First is the use of non-invasive carotid waveforms, rather than invasive recording of aortic pressure. The use of the carotid artery as a surrogate for central pressure is not ideal. The risks inherent in aortic catheterization, however, preclude its use in a young, healthy population. Furthermore, the carotid artery pulse contour has been used previously as a substitute for invasive central arterial measurements [29,30]. The differences between aortic and carotid systolic pressure are less than 5 mmHg [31]. Discrepancies in stiffness at each site in healthy individuals are small [32], and differences between carotid and aortic augmentation are minimal in young adults [6]. Second, the accuracy of manually recorded carotid waveforms was worse than the radial waveforms obtained using the automated tonometer during rest. This was quantified by differences in the coefficient of variation of pulse height ($5.0 \pm 2.1\%$ versus $10.1 \pm 3.4\%$, radial and carotid, respectively) and baseline ($2.9 \pm 2.3\%$ versus $8.1 \pm 6.7\%$). No significant change was observed in the height variability after exercise, although baseline variability increased for both signals ($5.1 \pm 3.8\%$, $P = 0.02$ versus $16.7 \pm 1.5\%$, $P = 0.01$). Although suboptimal waveforms

might arguably have adversely affected the accuracy of the derived transfer function, the transfer function derived from the SphygmoCor system was not subject to such error and yet provided similar results. Carotid waveform inaccuracy may also have resulted in error in the actual measured values of AIx at peak exercise. It is difficult to be certain whether such error had a skewed bias, but it seems more likely that this would result in greater variability but not necessarily any overall mean bias. Third, we assumed that diastolic and mean pressure remained constant between radial and carotid arteries, as absolute direct pressure measurement is not possible at the latter. It is recognized that diastolic pressure tends to decrease proximally by approximately 1–2 mmHg [33]. This difference is, however, small compared with the difference in systolic pressure, and calibration using this approach is considered generally acceptable [6]. Moreover, AIx is not dependent on absolute pressure, as it represents the relative amplitudes of incident and reflected wave components. Fourth, the timing of signal recording during this study is also worthwhile noting. During pilot work, we were unable to obtain satisfactory and reliable carotid signal acquisition during exercise, and recordings were therefore made in the immediate postexercise period to reduce movement artefact. It is possible that the rapid haemodynamic changes that occur in the immediate postexertion period may account for the change in the transfer function, rather than the direct effects of exercise [34]. It is unlikely that this issue will be resolved by non-invasive measurements, and it would be interesting to see whether significant changes in transfer function occur between peak exercise and the immediate cessation of exertion. Fifth, GTF_{rest} and $GTF_{exercise}$ were derived from the same data that they were subsequently applied to, potentially biasing the accuracy of each transfer function towards its associated exercise phase. Nonetheless, the results given by $GTF_{sphygmocor}$ and by the use of GTF derived from only a subset of the subjects, are not subject to such bias, but yielded similar conclusions. Finally, the limits of agreement between measurements were fairly high, as evidenced by the large standard deviations of the difference, probably secondary to the variability in the carotid waveform quality. The aim of the present study was, however, to establish whether overall bias existed between derived and actual measurements, rather than the accuracy of the technique, and this does not affect our principal conclusion. After all, there is arguably little other use for predicting carotid waveforms from the radial artery, when the former can be directly measured anyway; more important is that the mean bias in derived carotid waveform values may also extend to derived aortic waveforms.

In conclusion, the use of an arterial GTF for determining central pressure is an attractive alternative to invasive monitoring, with potential uses in both the clinical and research environments [5,35]. It has been increasingly employed in the assessment of cardiovascular risk [16,17] and vascular function at rest [36], despite increasing evidence that the GTF does not remain constant in all circumstances [18–20]. Given the lack of data on the central arterial response to exercise, the use of a GTF in these circumstances is clearly appealing, potentially opening the gateway to larger clinical studies, examining among other things the effects of blood pressure-lowering drugs on central exercise blood pressure and the utility of this measure as a marker for cardiovascular risk. The results of the current study suggest that a significant underestimation of key central haemodynamic measures may occur when using a transfer function derived under resting conditions. The use of a resting GTF in such circumstances should therefore be treated with caution; an exercise-specific transfer function might be favoured, although this requires further investigation.

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