

Heart rate and blood pressure dependence of aortic distensibility in rats: comparison of measured and calculated pulse wave velocity

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Objectives: When assessing arterial stiffness, heart rate (HR) and blood pressure (BP) are potential confounders. It appears that the HR/BP dependences of pulse wave velocity (PWV) and distensibility are different, even though both assess arterial stiffness. This study aims to compare aortic PWV as measured using pulse transit time (PWV_{TT}) and as calculated from distensibility (PWV_{dist}) at the same measurement site and propose a solution to the disparity in dependences of PWV_{TT} and PWV_{dist}.

Methods: Adult anaesthetized rats ($n = 24$) were randomly paced at HRs 300–500 bpm, at 50 bpm steps. At each step, aortic PWV_{TT} (two pressure-tip catheters) and PWV_{dist} (pressure-tip catheter and ultrasound wall-tracking; abdominal aorta) were measured simultaneously while BP was varied pharmacologically.

Results: HR dependence of PWV_{dist} paradoxically decreased at higher levels of BP. In addition, BP dependence of PWV_{dist} was much larger than that of PWV_{TT}. These discrepancies are explained in that standard PWV_{dist} uses an approximate derivative of pressure to diameter, which overestimates PWV with increasing pulse pressure (PP). *In vivo*, PP decreases as HR increases, potentially causing a PWV_{dist} decrease with HR. Estimating the full pressure-diameter curve for each HR corrected for this effect by enabling calculation of the true derivative at diastolic BP. This correction yielded a PWV_{dist} that shows HR and BP dependences similar to those of PWV_{TT}. As expected, BP dependence of all PWV metrics was much larger than HR dependence.

Conclusion: Measured and calculated PWV have different dependences on HR and BP. These differences are, at least in part, because of approximations made in using systolic and diastolic values to calculate distensibility.

Keywords: arterial stiffness, blood pressure, distensibility, heart rate, pulse wave analysis, pulse wave velocity

Abbreviations: A, cross-sectional area; A_d, minimum (diastolic) cross-sectional area; A_s, peak (systolic) cross-sectional area; BIC, Bayesian information criterion; BP, blood pressure; d , distance between thoracic and abdominal pressure measurement sites; dp/dt_{max} , cardiac contractility, estimated as the maximum first derivative of the aortic arch blood pressure signal; FIR, finite impulse response; HR, heart

rate; k , finite impulse response filter width; MAP, mean arterial pressure; p , pressure; P , statistical P -value; $p_b(t)$, backward pressure wave used in wave separation analysis; $p_f(t)$, forward pressure wave used in wave separation analysis; PP, pulse pressure; PWV, pulse wave velocity; PWV_{dist}, distensibility-based pulse wave velocity based on linearized distensibility; PWV_{dist}, distensibility-based pulse wave velocity based on analytical pressure–cross-sectional area curve; PWV_{TT}, transit-time pulse wave velocity; $Q(t)$, triangular, estimated blood flow pattern used in wave separation analysis; RI, reflection index; RM, reflection magnitude; SD, standard deviation; SDC, supplemental digital content; SNP, sodium nitroprusside; t , pulse transit time; Z_c, characteristic impedance used in wave separation analysis; ρ , blood mass density

INTRODUCTION

Stiffness of the large arteries is an independent risk factor of all-cause mortality [1]. The two main methods for measuring arterial stiffness *in vivo* are pulse wave velocity (PWV) and distensibility. PWV is calculated by measuring the time difference of the passage of the blood pressure or flow wave between two points (e.g. carotid–femoral PWV). Distensibility is calculated from pressure and diameter measured at one arterial location. Pressure can be measured using cuff-based techniques in

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the case of the brachial artery, invasive pressure catheters in accessible vessels, or using filters or transfer functions to calculate pressure at one site (e.g. the aorta) by using measurement at another site (e.g. the brachial or radial artery) [2]. The required diameter measurements are commonly obtained using ultrasound wall tracking [3].

The American Heart Association recommendations for standardizing arterial stiffness research include that the intrinsic dependence of arterial stiffness on acute blood pressure and heart rate (HR) should be taken into consideration [4]. The acute effect of HR on carotid–femoral PWV, as a measure of large artery stiffness, has been studied in humans [5–12] with disparate results, largely because of the interaction between HR and blood pressure, which in turn affects arterial stiffness. Accounting for blood pressure reveals that an increase in HR causes a small increase in PWV (measured using the transit-time method) in humans [10], with controlled animal studies showing up to a 6% increase in aortic PWV over a 150 bpm increase in HR [13]. The acute effect of HR on carotid artery distensibility has been studied in rats [14,15] and showed a 15–43% decrease in distensibility with a 120 bpm increase in HR. No study has investigated if the disparity in distensibility and PWV changes with HR are because of anatomical location or the method of measurement.

In correcting arterial stiffness for blood pressure, controversy exists whether to use mean arterial pressure (MAP) [4], or DBP [16]; for a review, see reference [17]. It is also unclear whether this choice for MAP or DBP influences the obtained HR dependence.

The present study aims to quantify and explain the effect of HR and blood pressure changes on PWV and distensibility as measured at the same arterial location to investigate their disparity in HR dependence.

METHODS

Animal preparation

Adult Sprague–Dawley rats ($n = 24$, five female/19 male, obtained from the Animal Resource Centre, Perth, Australia) aged 12–17 weeks and weighing 446 ± 99 g (mean \pm standard deviation, SD) were studied. Anaesthesia was induced through intraperitoneal injection of urethane (1.3 g/kg in 10% w/w Ringer's solution) and was maintained by intravenous injections of the same solution as required. Electrocardiogram electrodes were connected to the rat's front paws (signal) and the right back paw (reference). Body temperature was maintained at 37 ± 1 °C using a heat mat and rectal temperature probe. Polyethylene tubes were placed into the left and right femoral veins for drug delivery. Atrial pacing was accomplished using a custom-made 2.7F bipolar catheter introduced into the right atrium through the right external jugular vein [13]. Pressure tip-catheters (Scisense 1.2F; Transonic Systems Inc., Ithaca, New York, USA or Millar 1.4F Mikro-Tip; Millar Inc., Houston, Texas, USA) were placed in the upper thoracic ($n = 12$) and abdominal aorta ($n = 24$) via the left carotid and femoral arteries to continuously measure blood pressure and determine transit-time PWV_{TT} ($n = 12$, including data from $n = 7$ described previously [13]). An ultrasound probe (13 MHz LA523 probe; MyLab70; Esaote Europe, Maastricht, The

Netherlands) was placed laterally on the shaved skin ($n = 17$) and held in place with an XYZ-micromanipulator. Probe location and rotation were manipulated to obtain a clear longitudinal fast B-mode image of the abdominal aorta immediately proximal to the pressure catheter most distal in the abdominal aorta.

All procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and the experimental protocol was approved by the Macquarie University Animal Ethics Committee.

Data acquisition

Blood pressures, electrocardiogram, and temperature were recorded continuously using a Power1401 data acquisition system interfaced through Spike2 software (Cambridge Electronic Design, Cambridge, UK) at a sampling rate of 2000 Hz. Pulse trains were generated using Spike2 and were used to trigger an isolated pulse stimulator (Model 2100; A-M Systems Inc., Sequim, Washington, USA; pulse duration and amplitude of 2 ms and 0.6–1.0 V, respectively) that was connected to the atrial pacing catheter. The distance between the two pressure recording sites was measured postmortem after dissection (while the catheters were still in place) by aligning a small suture along the artery and subsequently measuring the length of suture between the catheter sites.

Abdominal aortic blood pressure was additionally recorded on a separate computer (ART.LAB, Esaote) on which the ultrasound radiofrequency signal as output by the echo scanner was also recorded. Recording of abdominal blood pressure on both systems enabled post hoc beat-to-beat synchronization of the acquisitions on those systems.

Experimental protocol

To enable stable pacing even at low HRs, intrinsic HR was reduced through intravenous injection of a specific bradycardic agent [zatebradine (Sigma-Aldrich, Castle Hill, New South Wales, Australia; $n = 22$) or ivabradine (Servier Laboratories, Hawthorn, Victoria, Australia; $n = 2$); 1 mg/kg in 1 mg/ml of 0.9% w/w saline] to <300 bpm ($n = 20$) and, in four animals greater than 300 bpm but less than 350 bpm. Rats were then paced in a random sequence of HRs of 300, 350, 400, 450, and 500 bpm ($n = 20, 24, 24, 24,$ and 18, respectively). At each rate, blood pressure was raised with phenylephrine (30 μ g/kg per min intravenously) until MAP was greater than 130 mmHg and lowered with sodium nitroprusside (SNP, 30 μ g/kg per min intravenously) until MAP was less than 70 mmHg. During the return of blood pressure to baseline, when MAP crossed 70, 100, or 130 mmHg, ultrasound radiofrequency data in memory (~ 5 s) was saved for subsequent wall tracking. At these pressure points, a square wave zero signal was inserted into the abdominal blood pressure signal by means of an electronic switch, serving as a fiducial point for post hoc synchronization of ultrasound and blood pressure signals.

Data processing

Offline radiofrequency wall tracking was performed using ART.LAB software (Esaote). Diameter waveforms for all 14 recorded echo lines were exported together with the

abdominal pressure signal. These files were imported in a custom MATLAB-based program (MATLAB R2018a; MathWorks, Natick, Massachusetts, USA) where they were synchronized to the Spike-recorded pressure signals, after which blood pressure first and second derivatives were computed, and wave separation analysis was performed [details in Supplemental Digital Content (SDC) 1, <http://links.lww.com/HJH/B432>]. Beat detection and computation of minimum (diastolic), maximum (systolic), and mean values were performed separately for diameter and pressure signals. In the pressure signals, the diastolic foot was identified as the second derivative peak. As pressure and diameter were synchronized, data in detected beats in those signals could be easily matched. Pressure and diameter waveforms were all visually inspected; heart beats where the diameter waveforms showed artefacts because of ultrasound probe or body movement (e.g. in some respiratory cycles) were removed from further analysis.

Pulse transit time (t) was calculated as the time difference between the diastolic feet of the thoracic and abdominal pressure signals; PWV_{TT} was subsequently calculated as

$$PWV_{TT} = \frac{d}{t}, \tag{1}$$

with d the distance between thoracic and abdominal pressure measurement sites as measured post mortem.

Abdominal aortic distensibility was calculated using SBP and DBP, and peak (A_s) and minimum (A_d) abdominal aortic cross-sectional area (calculated from systolic and diastolic diameters as $A = \frac{1}{4}\pi d^2$):

$$\text{distensibility} = \frac{A_s - A_d}{A_d \cdot (\text{SBP} - \text{DBP})} \tag{2}$$

Distensibility was converted to (PWV_{dist}) using the Bramwell–Hill equation (Equation 3) for means of comparison to PWV_{TT} :

$$PWV_{\text{dist}} = \sqrt{\frac{1}{\rho \cdot \text{distensibility}}}, \tag{3}$$

with ρ the blood mass density, taken to be 1050 kg/m³. In order to obtain PWV_{dist} in metres per second, all preceding

equations were evaluated using values in SI units of metres (squared), seconds, and Pascals.

SBP, MAP, DBP, PWV_{TT} , PWV_{dist} , reflection index (RI), reflection magnitude (RM), and cardiac contractility (dp/dt_{max}) were obtained for each heartbeat. MAP was obtained by arithmetic averaging of the blood pressure waveform. Beat-to-beat data (i.e. one data point per heartbeat) were subsequently statistically analysed.

Statistical analyses

A detailed description of the statistical methods is provided in SDC 1 (<http://links.lww.com/HJH/B432>). Briefly, data were analysed on a beat-to-beat basis (total dataset of $n = 9142$ heartbeats) by means of multilevel linear modelling with blood pressure (either SBP, MAP, or DBP) or PWV (either PWV_{TT} or PWV_{dist}) as a function of HR (as a categorical variable), blood pressure (either MAP or DBP; quadratically, as a continuous variable), $\text{HR} \times \text{blood pressure}$ (interaction); and age. Similar models with HR as a continuous instead of a categorical predictor were also created to assess linear trends of blood pressure or PWV with HR. All reported blood pressure values are those that were measured abdominally. Note that in our statistical modelling approach, no blood pressure windows were used (contrary to our previous study [13]). Rather, PWVs were computed for each heart beat individually. Then, a quadratic relation was fit through these pressure–PWV points by means of multilevel modelling. This (analytical) relation can then be used to estimate PWV for any given pressure.

Calculation of pulse wave velocity from distensibility using analytical derivatives

The regular Bramwell–Hill equation as often used in clinical studies (Equation 3) uses an approximated diastolic-to-systolic compliance term ($\frac{A_s - A_d}{\text{SBP} - \text{DBP}}$; Equation 2). However, this compliance depends on the pulse pressure at the time of measurement (Fig. 1). As PWV_{TT} is based on the arrival time difference of the diastolic foot between two sites, PWV_{TT} depends on DBP. To obtain an equivalent PWV from a distensibility measurement, the Bramwell–Hill equation should be used based on the arterial compliance at diastole. In the present, experimental study, arterial diameter waveforms were measured at different pressures,

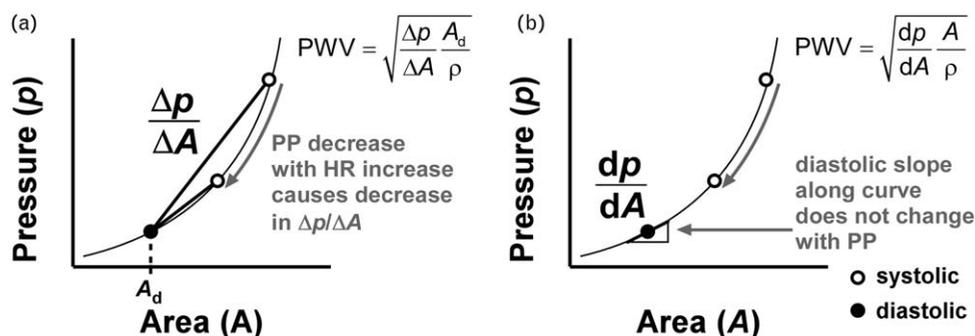


FIGURE 1 Two ways of calculating local pulse wave velocity (PWV) using the Bramwell–Hill equation from pressure and cross-sectional area measurements. (a) The standard Bramwell–Hill equation as often employed uses an approximated derivative of pressure (p) to area (A), ($\Delta p / \Delta A$). Due to the nonlinearity of the p – A curve, $\Delta p / \Delta A$ varies with pressure and $\Delta p / \Delta A$ is an overestimation of $\frac{dp}{dA}$ in the diastolic point. As pulse pressure (PP) is prone to decrease with a heart rate (HR) increase, a relative decrease in local pulse wave velocity with an increase in HR could result. This paradoxical effect can be overcome by estimating the pressure–area curve for each HR, and calculating the true derivative along this curve, at DBP. (b) p , blood mass density.

TABLE 1. Blood pressure and pulse wave velocity parameters corrected for mean arterial pressure

	Heart rate (HR)					HR dependence	
	300 bpm	350 bpm	400 bpm	450 bpm	500 bpm	Slope	P
Mean arterial pressure (MAP)							
70 mmHg							
SBP (mmHg)	96 ± 2	93 ± 2	92 ± 2	91 ± 2	89 ± 2	-3.6 ± 0.4	<0.001
DBP (mmHg)	53 ± 1	54 ± 1	56 ± 1	56 ± 1	57 ± 1	1.8 ± 0.2	<0.001
PWV _{TT} (m/s)	3.46 ± 0.15	3.46 ± 0.15	3.47 ± 0.15	3.47 ± 0.15	3.46 ± 0.15	0.01 ± 0.02	0.708
PWV _{dist} (m/s)	3.39 ± 0.14	3.36 ± 0.13	3.51 ± 0.13	3.60 ± 0.13	3.63 ± 0.13	0.15 ± 0.04	<0.001
100 mmHg							
SBP (mmHg)	136 ± 1	132 ± 1	130 ± 1	126 ± 1	124 ± 1	-5.7 ± 0.3	<0.001
DBP (mmHg)	77 ± 1	80 ± 1	81 ± 1	83 ± 1	84 ± 1	3.3 ± 0.2	<0.001
PWV _{TT} (m/s)	3.65 ± 0.14	3.70 ± 0.14	3.74 ± 0.14	3.84 ± 0.14	3.86 ± 0.14	0.12 ± 0.01	<0.001
PWV _{dist} (m/s)	3.92 ± 0.11	4.12 ± 0.11	4.31 ± 0.11	4.30 ± 0.11	4.44 ± 0.11	0.24 ± 0.03	<0.001
130 mmHg							
SBP (mmHg)	172 ± 2	169 ± 2	167 ± 2	164 ± 2	161 ± 2	-5.4 ± 0.3	<0.001
DBP (mmHg)	103 ± 1	105 ± 1	107 ± 1	108 ± 1	110 ± 1	3.4 ± 0.2	<0.001
PWV _{TT} (m/s)	4.22 ± 0.15	4.28 ± 0.15	4.36 ± 0.15	4.46 ± 0.15	4.53 ± 0.15	0.17 ± 0.02	<0.001
PWV _{dist} (m/s)	4.98 ± 0.13	5.00 ± 0.13	5.21 ± 0.13	5.23 ± 0.13	5.26 ± 0.13	0.16 ± 0.04	<0.001
MAP dependence							
Slope							
SBP (mmHg)	127 ± 2	126 ± 2	124 ± 2	122 ± 2	121 ± 2		
DBP (mmHg)	83 ± 1	85 ± 1	85 ± 1	87 ± 1	88 ± 1		
PWV _{TT} (m/s)	1.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	1.6 ± 0.2	1.8 ± 0.2		
PWV _{dist} (m/s)	2.6 ± 0.3	2.7 ± 0.3	2.8 ± 0.3	2.7 ± 0.3	2.7 ± 0.3		

Values denote mean ± standard error. SBP, DBP, transit time pulse wave velocity (PWV_{TT}), and distensibility-based pulse wave velocity (PWV_{dist}) as a function of mean arterial pressure (MAP, vertically) and heart rate (HR, horizontally). Note that values in each row are corrected to a fixed mean arterial pressure of 70, 100, or 130 mmHg. Hence, as expected, the decreasing pulse pressure with increasing heart rate leads to a decrease in SBP and an increase in DBP with heart rate. HR and MAP dependences are given in units of measurement per 100 bpm or per 100 mmHg, respectively. MAP dependences were calculated at 100 mmHg. All values are corrected for age. *P* denotes *P*-value for slope. For MAP dependence, all *P* < 0.001.

enabling construction of a pressure–area curve from the systolic and diastolic pressure and cross-sectional area points acquired at different MAP levels (Supplemental Methods in SDC 1, <http://links.lww.com/HJH/B432>). These curves can then be used to obtain analytical derivatives from pressure to area ($\frac{dA}{dp}$) at diastole, from which PWV can be calculated as

$$PWV_{dist,ana} = \sqrt{\left. \frac{dA}{dp} \right|_{p=DBP}} \cdot \frac{A_d}{\rho} \quad (4)$$

Note that pressure–area curves were fitted through beat-to-beat pressure–diameter points – we did not fit a pressure–area curve for each individual heartbeat.

RESULTS

Heart rate and blood pressure dependence of pulse wave velocity as a function of mean blood pressure

Table 1 shows the dependence of SBP, DBP, PWV_{TT}, and PWV_{dist} on HR as a function of MAP. Figure 2a illustrates the PWV dependences on HR. PWV_{TT}'s HR dependence increases with MAP (*P* < 0.001, 70 vs. 100 mmHg; *P* = 0.005, 100 vs. 130 mmHg; Table S1 in SDC 1, <http://links.lww.com/HJH/B432>). In contrast, PWV_{dist}'s dependence does not show a statistically significant increase, but does show a trend to increase from MAP = 70–100 mmHg (*P* = 0.070; Table S1, <http://links.lww.com/HJH/B432>). Figure 2b illustrates the PWV dependences on MAP, as a function of HR. MAP dependence of PWV_{TT} increases with HR (Table S2, <http://links.lww.com/HJH/B432>). PWV_{dist}'s dependence does not show a statistically significant increase.

Heart rate and blood pressure dependence of pulse wave velocity as a function of DBP

Table 2 shows the dependence of SBP, MAP, PWV_{TT}, and PWV_{dist} on HR as a function of DBP. Figure 3 illustrates the PWV dependences. Overall, the HR dependence of PWV is much lower in this case (i.e. as compared with MAP correction). PWV_{TT}'s HR dependence increases from DBP = 60 to 85 mmHg (*P* = 0.011; Table S3, <http://links.lww.com/HJH/B432>). PWV_{dist}'s dependence is equal for DBPs of 60 and 85 mmHg but decreases to loss of statistical significance (*P* = 0.686) at 110 mmHg (*P* = 0.023 for change from 85 to 110 mmHg; Table S2, <http://links.lww.com/HJH/B432>). Figure 3B illustrates the PWV dependences on DBP, as a function of HR. DBP dependence of PWV_{TT} did not change with HR. Dependence of PWV_{dist} on DBP, however, decreased with HR, though not significantly (Table S4, <http://links.lww.com/HJH/B432>).

Using an analytical derivative to calculate local pulse wave velocity

Figure 4 shows the effect of using an analytical derivative to calculate distensibility-based PWV (PWV_{dist,ana}, Eq. 4), which is facilitated by the availability of full pressure–area curves in this study (average curves as a function of HR are shown in Supplemental Figure S1 in SDC 1, <http://links.lww.com/HJH/B432>). Results using this method do not show the paradoxical decrease in HR dependence of PWV at high DBP as seen with the ordinary PWV_{dist} method. Instead, PWV_{dist,ana}'s blood pressure and HR dependences are very similar to those of PWV_{TT}.

Potential confounders

Five of the studied rats were females, whereas the other 19 were males. A separate statistical model was constructed to

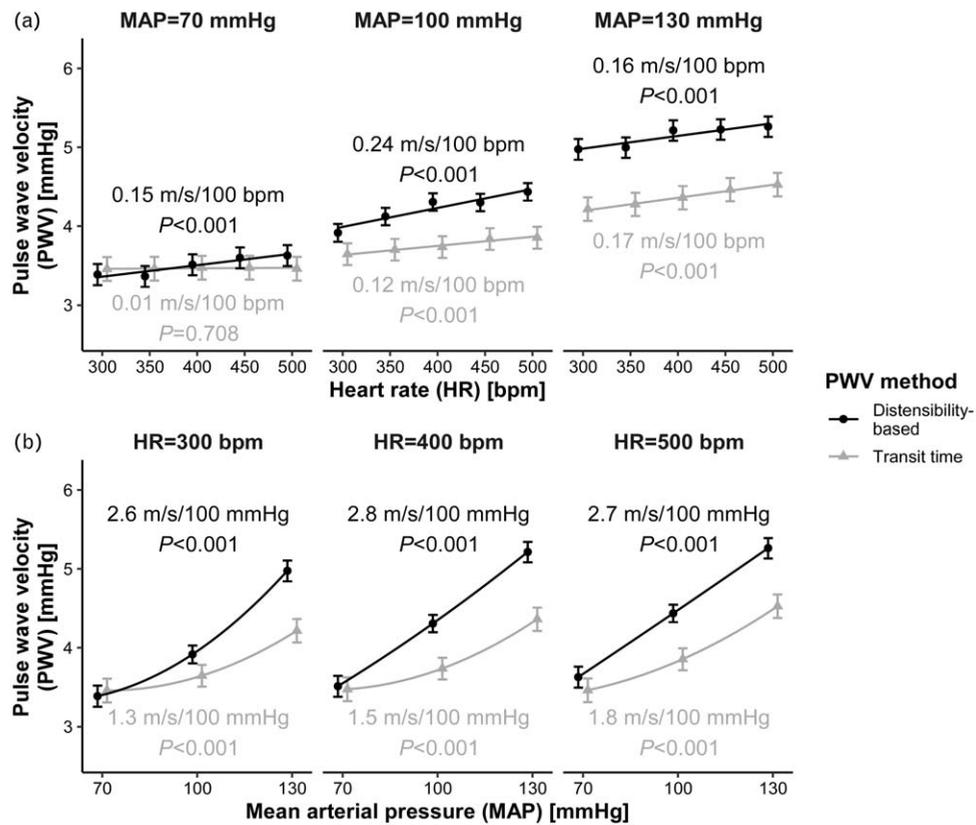


FIGURE 2 Dependence of transit time-based and distensibility-based pulse wave velocities (PWVs) on heart rate (HR, a) and mean arterial pressure (MAP, b). (a) PWV as a function of HR, for three values of MAP. (b) PWV as a function of MAP, for three values of HR. Blood pressure dependences are calculated for MAP = 100 mmHg. Points indicate mean ± standard error.

check for a potential influence of sex. The addition of sex as a predictor, with or without HR interaction, did not improve the statistical models (increased Bayesian information criterion, BIC). Rat weight may be another confounder.

However, its addition as a predictor also did not improve the models.

Age was found to significantly correlate with PWV_{TT} and PWV_{dist}. No age–HR or age–blood pressure interactions

TABLE 2. Blood pressure and pulse wave velocity parameters corrected for DBP

	Heart rate (HR)					HR dependence	
	300 bpm	350 bpm	400 bpm	450 bpm	500 bpm	Slope	P
DBP							
60 mmHg							
SBP (mmHg)	109 ± 2	102 ± 2	100 ± 2	96 ± 2	93 ± 3	-7.7 ± 0.5	<0.001
MAP (mmHg)	80 ± 1	77 ± 1	76 ± 1	74 ± 1	74 ± 1	-2.6 ± 0.2	<0.001
PWV _{TT} , m/s	3.49 ± 0.13	3.51 ± 0.13	3.53 ± 0.13	3.54 ± 0.13	3.54 ± 0.14	0.03 ± 0.01	0.023
PWV _{dist} , m/s	3.60 ± 0.14	3.62 ± 0.14	3.72 ± 0.14	3.77 ± 0.14	3.80 ± 0.14	0.11 ± 0.04	0.003
85 mmHg							
SBP (mmHg)	148 ± 2	140 ± 2	136 ± 2	130 ± 2	126 ± 2	-10.6 ± 0.5	<0.001
MAP (mmHg)	109 ± 1	106 ± 1	105 ± 1	103 ± 1	101 ± 1	-3.9 ± 0.2	<0.001
PWV _{TT} , m/s	3.79 ± 0.13	3.83 ± 0.13	3.85 ± 0.13	3.90 ± 0.13	3.91 ± 0.13	0.06 ± 0.01	<0.001
PWV _{dist} , m/s	4.32 ± 0.13	4.39 ± 0.12	4.54 ± 0.12	4.47 ± 0.12	4.56 ± 0.12	0.11 ± 0.03	0.001
110 mmHg							
SBP (mmHg)	181 ± 3	175 ± 3	172 ± 3	167 ± 3	162 ± 3	-9.2 ± 0.7	<0.001
MAP (mmHg)	137 ± 1	135 ± 1	134 ± 1	132 ± 1	130 ± 1	-3.7 ± 0.2	<0.001
PWV _{TT} , m/s	4.49 ± 0.14	4.47 ± 0.14	4.51 ± 0.14	4.55 ± 0.14	4.59 ± 0.14	0.06 ± 0.02	0.001
PWV _{dist} , m/s	5.51 ± 0.16	5.29 ± 0.16	5.45 ± 0.15	5.42 ± 0.15	5.38 ± 0.15	-0.02 ± 0.05	0.686
DBP dependence							
Slope							
SBP (mmHg)	144 ± 4	146 ± 4	144 ± 4	141 ± 4	139 ± 4		
MAP (mmHg)	117 ± 1	117 ± 1	116 ± 1	114 ± 1	112 ± 1		
PWV _{TT} , m/s	2.0 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.2		
PWV _{dist} , m/s	3.8 ± 0.4	3.3 ± 0.4	3.5 ± 0.4	3.3 ± 0.4	3.2 ± 0.4		

Values denote mean ± standard error. SBP, mean arterial pressure (MAP), transit time pulse wave velocity (PWV_{TT}), and distensibility-based pulse wave velocity (PWV_{dist}) as a function of DBP (vertically) and heart rate (HR) (horizontally). Note that values in each row are corrected to a fixed DBP of 60, 85, or 110 mmHg. Hence, as expected, the decreasing pulse pressure with increasing heart rate leads to a decrease in both SBP and MAP with heart rate. HR and DBP dependences are given in units of measurement per 100 bpm or per 100 mmHg, respectively. DBP dependences were calculated at 85 mmHg. All values are corrected for age. P denotes P-value for slope. For DBP dependence, all P < 0.001.

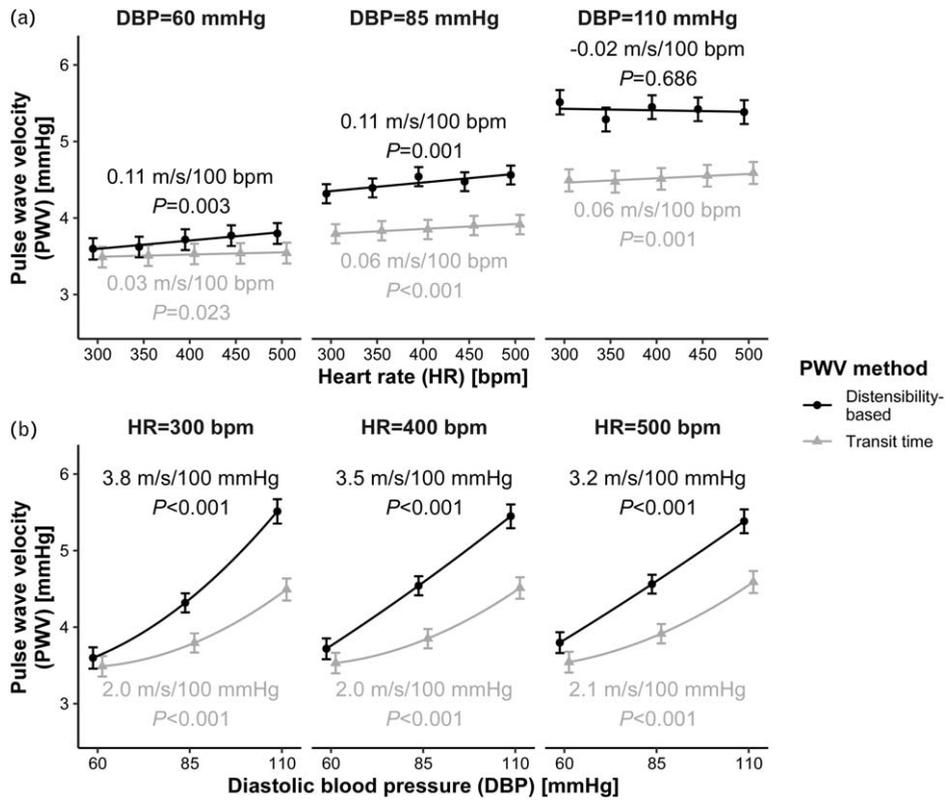


FIGURE 3 Dependence of transit time-based and distensibility-based pulse wave velocities (PWVs) on heart rate (HR, a) and DBP, b). (a) PWV as a function of HR, for three values of DBP. (b) PWV as a function of DBP, for three values of HR. Blood pressure dependences are calculated for DBP = 85 mmHg. Points indicate mean \pm standard error.

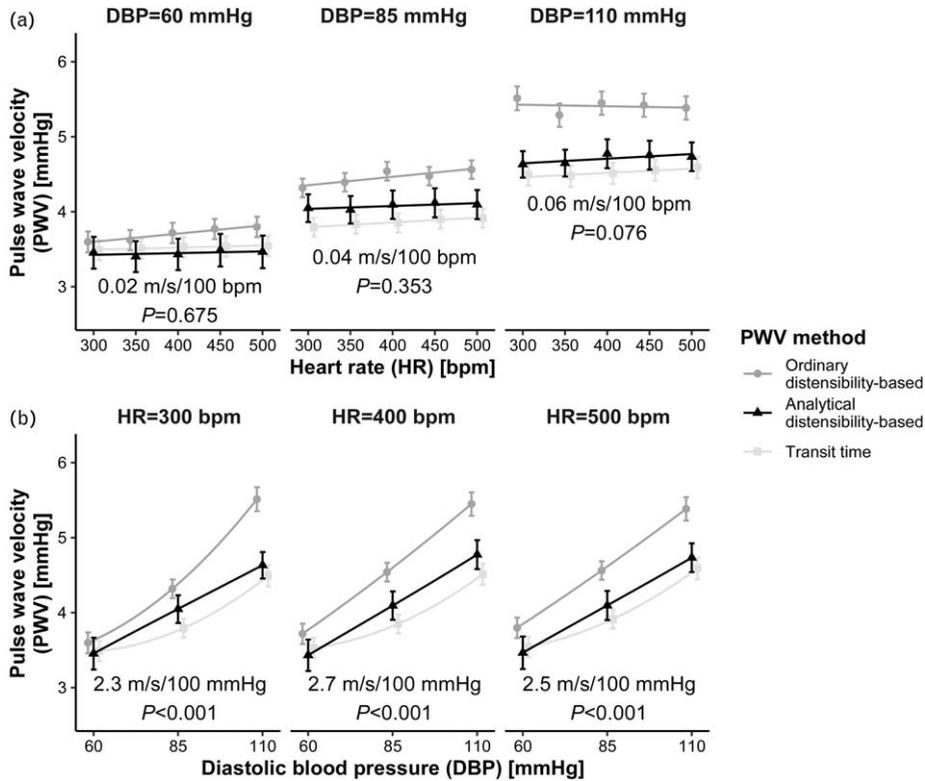


FIGURE 4 The effect of using an analytical derivative in calculating distensibility-based pulse wave velocity (PWV). Figure shows ordinary distensibility-based (PWV_{dist}) and transit time data in the background (dots and squares, as in Fig. 3), with analytical PWV_{dist} data (triangles) and P -values superimposed, as a function of heart rate (HR, a) and DBP (b). Note the similarity between analytical PWV_{dist} and transit time PWV, both in absolute and relative (HR and blood pressure dependence) terms. Blood pressure dependences are calculated for DBP = 85 mmHg. Points indicate mean \pm standard error.

were identified. Therefore, in our models, age only shifts the PWV–HR relationship as a whole but does not influence the slope of the HR/blood pressure dependence of PWV.

Wave reflection and contractility

Figure S2, <http://links.lww.com/HJH/B432> shows RI and RM as function of HR and blood pressure. Two aspects can be noted: first, with increasing blood pressure (i.e. progressively shifting from SNP infusion, to no infusion, to phenylephrine infusion), we observed a clear increase in RM and RI (Fig. S2b, <http://links.lww.com/HJH/B432>). Second, for a given DBP, RI/RM decreased with increasing HR (Fig. S2a, <http://links.lww.com/HJH/B432>).

Cardiac contractility, estimated as the maximum first derivative of the aortic arch blood pressure signal (dp/dt_{max}), showed a modest negative HR dependence at low blood pressure, gradually changing to a positive dependence at higher blood pressure (Fig. S3a, <http://links.lww.com/HJH/B432>). Blood pressure dependence of contractility (Fig. S3b, <http://links.lww.com/HJH/B432>) was negative at low HR (300 bpm), but the dependence gradually changes with increasing HR and does not reach significance at high HR (500 bpm).

Methodological aspects

PWV_{TT} computed using the intersecting tangent method showed an HR/blood pressure dependence very similar to that obtained using the second derivative method (Fig. S4, <http://links.lww.com/HJH/B432>). A sensitivity analysis of the effect of the finite impulse response (FIR) filter width (k) showed that enlarging k from 1.5 ms (as used in all analyses presented herein) to 4.5 ms modestly changed the HR and DBP dependence (Fig. S5; green curves do not overlap with blue curves, <http://links.lww.com/HJH/B432>). This discrepancy became slightly larger with increasing DBP (i.e. with a progressive shift from SNP infusion, to no infusion, to phenylephrine infusion). Decreasing k from 1.5 to 0.5 ms, however, had a negligible effect (Fig S5; red and blue curves overlap, <http://links.lww.com/HJH/B432>).

DISCUSSION

Principal findings

In this article, we present the largest animal study to date on the effect of HR and blood pressure on PWV_{TT} and the first animal study on the effect of HR on PWV_{dist} allowing comparison of arterial stiffness measurements using transit time and distensibility approaches. We showed that both PWV_{TT} and PWV_{dist} show an HR dependence, whether corrected for MAP or DBP. PWV_{dist} as commonly calculated differed in its relationship with HR compared with PWV_{TT}, which is explained by the method of calculation (Fig. 1).

Implications of heart rate dependence and mean arterial pressure vs. DBP correction

The HR dependence of PWV_{TT} and PWV_{dist} as obtained using MAP correction is of larger magnitude than when obtained using DBP correction. This can be explained as follows. PWV_{TT}, being estimated from the diastolic foot of

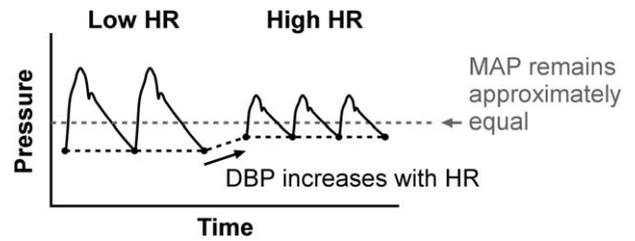


FIGURE 5 The significance of properly correcting transit time pulse wave velocity using DBP instead of mean arterial pressure (MAP). Changes in heart rate (HR) may induce substantial changes in DBP as DBP typically increases (and SBP decreases) because of the reduced stroke volume and resulting reduced pulse pressure with higher HR. If an MAP-based correction is employed, the heart beats in the figure and their PWVs are directly compared with each other. However, the beats at high HR have an elevated DBP, and therefore, an elevated transit time PWV, because of blood pressure alone. This leads to an increased, apparent HR dependence of transit time PWV when an MAP correction is employed.

the pulse wave [16,18–21], is determined by DBP. This is of particular relevance with respect to the HR dependence of PWV for the following reason (Fig. 5): assuming that cardiac output remains approximately equal among pacing settings, stroke volume will be inversely related to HR. Therefore, with an increase in HR, pulse pressure will decrease. Due to baroreflex regulation maintaining MAP at a steady level, this decreased pulse pressure will result in an increased DBP with increased HR. Therefore, PWV_{TT}'s dependence on DBP causes PWV_{TT} to increase with HR, an effect which is not corrected for if MAP is used for correction.

Implications of distensibility findings

We showed that, when using distensibility as calculated from SBP and DBP and cross-sectional area values to obtain PWV, the resulting measure (PWV_{dist}) shows a strong, nonlinear HR dependence. PWV_{dist} shows the highest dependence at low DBP, which vanishes at high DBP. This paradoxical effect is to a large extent explained by blood pressure changes that lead to artefactual changes in the (linearly approximated) PWV_{dist} (Fig. 1).

The linear approximation used in PWV_{dist} also explains why PWV_{dist} progressively diverges from PWV_{TT} with increasing DBP: With increasing DBP, pulse pressure also increases (as can be seen from the progressively bigger difference between SBP and DBP in Table 2). This increases the error that is made when estimating (diastolic) distensibility using the linearized diastolic-to-systolic slope (Fig. 1).

The method of calculation of distensibility and PWV_{dist} has important implications in comparison of clinical studies. For example, different trends in changes in aortic stiffness have been shown in cross-sectional studies, with PWV measurement (transit time) showing greatest changes (increases) in stiffness with age in the abdominal aorta [22] but distensibility measurement showing greatest changes (decreases) with age in the ascending aorta [23]. One of the contributors to this disparity may be the method of calculation of distensibility. In particular, distensibility and PWV_{dist} as commonly calculated (Eqs. 2–3; as also employed in [23]) shows a greater blood pressure dependence than PWV_{TT}. This, together with an increase in blood

pressure with age hence potentially explains why distensibility/PWV_{dist} show greater changes with age than PWV_{TT}. Correction according to Fig. 1 brings these two metrics closer in line.

In the present study, we were able to construct full pressure–area relationships, as measurements were taken at multiple levels of MAP. From these relationships, local PWVs based on the analytical pressure–area derivative could be computed. A similar approach has been adopted by Mangoni and Mircoli [14,15], and by Albaladejo *et al.* [24], who derived carotid distensibility from such curves by making use of true curve derivatives, permitting a true blood pressure-independent analysis of the dependence of distensibility on HR. Whereas Mangoni *et al.* [14] found a marked reduction in distensibility with HR, Albaladejo *et al.* [24] did not observe a change.

In routine clinical practice, it is not feasible to measure full pressure–area curves, as this requires sweeping a patient's blood pressure over a wide range. However, instead, an exponential pressure–area relationship could be assumed [25–27], which would enable calculation of an analytical derivative [28].

Diastolic foot detection method

In line with our previous study [13], PWV_{TT} was calculated by determining the time delay between the second derivative peaks of the proximal and distal pressure waveforms. This method has demonstrated accuracy similar to the intersecting tangent method [29], and yields nearly equal values [30]. Furthermore, in a previous study in patients, the foot detection method did not influence the finding of an HR dependence [5]. To confirm these findings, we re-estimated PWV_{TT} also using the intersecting tangent method; the observed differences between PWV_{TT} as estimated using both methods were indeed small (Fig. S4, <http://links.lww.com/HJH/B432>).

Possible explanations of the heart rate dependence of pulse wave velocity

The intrinsic mechanism that causes PWV to depend on HR remains to be accurately established. Hypothesized causes include: 1) confounding because of the foot detection algorithm, 2) pacing-induced changes in sympathetic tone, influencing vascular tone, 3) changes in wave reflection, or 4) visco-elasticity of the arterial wall. We will address these individually below. The reader is also referred to our previous works for discussions on this subject [10,13,31].

First, in the present study, we observed a very similar trend in HR dependence of transit-time PWV and distensibility-based PWV when calculated using an analytical derivative. Furthermore, we determined that using a different foot detection method (intersecting tangent instead of maximum second derivative) only minimally changed our results. This, together with the argumentation in the previous paragraph, excludes the influence of the foot detection algorithm as a cause for the observed HR dependence, which would only be applicable for PWV_{TT}; leaving hypotheses 2–4 intact.

Second, sympathetic tone has been shown to have no influence on stiffness in the large arteries following sympathectomy in rats [15,32].

Third, in the present study, we used SNP and phenylephrine to modulate blood pressure. The peripheral vasodilatory effect of SNP has previously been shown to decrease wave reflections [33], while phenylephrine-induced peripheral vasoconstrictions may increase wave reflections [34]. In addition, broad correlations have been demonstrated between wave reflection and PWV_{TT} [35] and PWV_{dist} [36], albeit showing different patterns of change with ageing [37]. To study the effects of wave reflections, hence, we estimated RI/RM and their relations to HR and blood pressure. As expected from the modulating effects of SNP/phenylephrine, with an increase in blood pressure (i.e. when shifting from SNP infusion, to no infusion, to phenylephrine infusion), we observed an increase in wave reflection. At given (fixed) blood pressures, however, wave reflection decreased with increasing HR, corroborating earlier simulation studies [38]. This effect became stronger with increasing blood pressure. PWV_{dist} (using diastolic as well as systolic points to linearly estimate dp/dA) strongly relies on determination of a systolic p – A point, which in turn will be influenced by wave reflections. Hence, the observed, paradoxical decrease in HR dependence of PWV_{dist} at higher DBP (Fig. 3a) could well be explained by changes in wave reflection (Fig. S2a, <http://links.lww.com/HJH/B432>).

Fourth, given that the previous three hypothesized causes seem unlikely, visco-elasticity is our primary hypothesized cause of the HR dependence of PWV. To further investigate, we estimated arterial dp/dt_{max} as a measure of contractility, noting that arterial dp/dt_{max} correlates reasonably with cardiac dp/dt_{max} [39,40]. With an increase in blood pressure, dp/dt_{max} showed an increase in HR dependence (from negative, to nonsignificant, to positive, Fig S3a, <http://links.lww.com/HJH/B432>). This aligns with our observed pattern of contractility changes with HR, which also shows an increase in HR dependence with blood pressure. This increasing dependence concurs with the observed increasing HR dependence of PWV. Indeed, dp/dt_{max} was also a significant predictor of PWV_{TT}. The observed pattern of contractility changes with blood pressure (Fig. S3b, <http://links.lww.com/HJH/B432>) can be explained as follows: At a low DBP, afterload is relatively low as arterial compliance is high (little recruitment of collagen). Combined with a low HR (which requires a large stroke volume), this results in a large pulse pressure and large dp/dt_{max} . As DBP increases, afterload will increase, contraction becomes more difficult, and dp/dt_{max} will decrease. As HR increases towards 500 bpm (Fig. S3b, middle and right panels, <http://links.lww.com/HJH/B432>), this relationship flattens, indicating a saturation effect. Overall, changes in dp/dt_{max} with HR were subtle (–10% and +16% with HR increases from 300 to 500 bpm at DBPs of 60 and 110 mmHg, respectively). The reader is reminded that we are modifying HR through pacing and not pharmacologically, the latter of which would be expected to have a more pronounced effect on dp/dt_{max} [39]. The effects of pacing on dp/dt_{max} , however, are inconclusive [41,42]. Note, also, that cross-sectionally, dp/dt_{max} has been shown not to correlate with HR [40].

Clinical significance of heart rate dependence

The HR dependence of PWV_{TT} when corrected for MAP is similar to what we observed previously (present study:

0.01–0.17 m/s/100 bpm; previously: no change to 0.17 m/s/100 bpm) [13]. The HR dependence of PWV when corrected for DBP is less pressure-dependent and generally smaller than when corrected for MAP. As explained above, DBP correction is most appropriate (considering the diastolic foot-detection transit time method), implying a (PWV_{TT}) dependence of 0.02–0.06 m/s/100 bpm.

When expressed as a relative change (%-change in PWV per %-change in bpm), this amounts to 0.003–0.005 %/%. In a previous study in humans, where we also employed DBP-based correction, larger relative changes of 0.07–0.18 %/%% were measured [10], though over the carotid–femoral trajectory and not over an ‘isolated’ abdominal aortic segment as in the present study. In both rats and humans, the absolute HR dependence is small compared with clinically attainable reproducibility levels. However, in studies involving large HR changes in many individuals (e.g. exercise studies), statistical differences between low-HR and high-HR conditions may be observed that can be (partially) attributed to the intrinsic HR dependence of PWV. Notably, the magnitude (and hence the clinical significance) of the blood pressure dependence of PWV is much larger than that of the HR dependence. Indeed, blood pressure correction is now commonplace in all major studies involving PWV [26,43]. Because of the magnitude of this dependence, blood pressure-corrected indices, such as cardio-ankle vascular index (CAVI) and CAVI₀ are becoming increasingly popular [28,44].

Limitations

In our study, urethane anaesthesia was used, which has been shown to potentially alter vascular tone [45]. However, randomization of the order of pacing HRs among rats ensured that any changes in level of anaesthesia over the time course of the experiments would not confound the result. The potential influence of urethane on blood pressure [46] was accounted for by actively controlling blood pressure using phenylephrine and SNP to ensure acquisition of data at distinct blood pressure values.

Furthermore, bradycardic agents (ivabradine and zatebradine) were used to lower HR before commencement of measurements. Both have been shown to elicit a ‘pure’ HR decrease, without modifying atrioventricular conduction, intraventricular conduction, or contractility; and without modifying blood pressure or showing vasoactive effects [47].

Vasoactive drugs were used to increase (phenylephrine) and reduce (SNP) blood pressure. A previous study [48] has shown that at the doses administered, phenylephrine altered rat aortic PWV_{TT} by 5% and SNP did not alter PWV_{TT} despite the same estimated concentrations of the vasoactive drugs altering aortic smooth muscle tension *ex vivo*. Although kinetics of drug absorption were not measured in that study, the kinetics are likely to be substantially different for an isolated aortic segment bathed in SNP or phenylephrine for a period of time to that of the *in vivo* aorta exposed to the same concentration of SNP or phenylephrine in blood for a short period of time. Regardless of the effect, the main comparison of the present study is between PWV_{TT} and PWV_{dist}. The arterial stiffness

conditions under which those parameters are compared were inherently identical.

Perspectives

In the present study, we showed that in rats, transit time PWV as well as distensibility-derived PWV show a small but statistically significant HR dependence of about 0.1 m/s/100 bpm; and PWV derived from arterial distensibility, when based on commonly used methods to approximate distensibility, shows an artefactually inflated blood pressure dependence as well as reduction in HR dependence with blood pressure. When a true derivative is used to calculate distensibility – as possible in this experimental setting – the distensibility-derived PWV shows a blood pressure- and HR dependence similar to transit time PWV.

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Conflicts of interest

There are no conflicts of interest.

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