DOI: 10.1002/pul2.12017

RESEARCH ARTICLE

Pulmonary Circulation Open Access | PVRI Pulmonary Vascular Research Institute | WILEY

Pulsatile pulmonary artery pressure in a large animal model of chronic thromboembolic pulmonary hypertension: Similarities and differences with human data

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Funding information
None.

Abstract

A striking feature of the human pulmonary circulation is that mean (mPAP) and systolic (sPAP) pulmonary artery pressures (PAPs) are strongly related and, thus, are essentially redundant. According to the empirical formula documented under normotensive and hypertensive conditions (mPAP = 0.61 sPAP + 2 mmHg), sPAP matches ~160%mPAP on average. This attests to the high pulsatility of PAP, as also witnessed by the near equality of PA pulse pressure and mPAP. Our prospective study tested if pressure redundancy and high pulsatility also apply in a piglet model of chronic thromboembolic pulmonary hypertension (CTEPH). At baseline (Week-0, W0), Sham ($n = 8$) and CTEPH ($n = 27$) had similar mPAP and stroke volume. At W6, mPAP increased in CTEPH only, with a two- to three-fold increase in PA stiffness and total pulmonary resistance. Seven CTEPH piglets were also studied at W16 at baseline, after volume loading, and after acute pulmonary embolism associated with dobutamine infusion. There was a strong linear relationship between sPAP and mPAP (1) at W0 and W6 ($n = 70$ data points, $r^2 = 0.95$); (2) in the subgroup studied at W16 ($n = 21$, $r^2 = 0.97$); and (3) when all data were pooled ($n = 91$, $r^2 = 0.97$, sPAP range 9–112 mmHg). The PA pulsatility was lower than that expected based on observations in humans: sPAP matched ~120%mPAP only and PA pulse pressure was markedly lower than mPAP. In conclusion, the redundancy between mPAP and sPAP seems a characteristic of the pulmonary circulation independent of the species. However, it is suggested that the sPAP thresholds used to define PH in animals are species- and/or model-dependent and thus must be validated.

KEYWORDS
animal models, pathophysiology, pulmonary hypertension experimental, right ventricle function and dysfunction

INTRODUCTION

In pulmonary hypertension (PH), the chronic increase in pulmonary arterial (PA) pressure leads to progressive right ventricular (RV) remodeling from compensated hypertrophy to maladaptive dilatation and failure. Animal models of PH are increasingly used to gain insight into the pathophysiology of this devastating disease and to test new treatments.[1–4] The importance of precisely characterizing RV afterload has been stressed because increased RV afterload plays a key role in precipitating RV failure and death.[5] The mean PA pressure (mPAP) reflects the steady pressure component of RV afterload[6,7] and it is the fundamental metric to define PH and to calculate pulmonary resistance.[8] The pulsatile pressure component of RV afterload may be quantified by the PA pulse pressure, that is, the difference between systolic and diastolic PA pressure (PApp = sPAP − dPAP). The PApp quantifies the oscillation of the PA pressure waveform around mPAP. The PApp is mainly determined by PA stiffness and RV stroke volume[9] and the importance of increased PA stiffness in the pathophysiology[10–13] and prognosis[14,15] of PH has been stressed.

A striking hemodynamic feature of the human pulmonary circulation is that mPAP and sPAP are strongly related through a linear relationship (mPAP = 0.61 sPAP + 2 mmHg) documented over a wide range of clinical and hemodynamic conditions. This empirical equation may help improve the noninvasive estimation of mPAP from the echo-Doppler-derived sPAP.[16,17] The empirical equation also implies that sPAP is ~160% mPAP, while systolic aortic pressure is only 120% to 150% mean aortic pressure.[18] This illustrates the fact that the human pulmonary circulation is much more pulsatile than the systemic circulation, as also attested by two other features. First, the oscillatory power is twice as high in the right ventricle (25%–30% of total power) than in the left ventricle (10%–15%). Second, the pulse pressure is a higher proportion of mean pressure in the pulmonary artery than at the aortic level.[18,19] It has been proposed that PApp and mPAP have an almost 1:1 ratio[19] and this has been recently confirmed by our group in patients with pulmonary arterial hypertension and in normotensive subjects.[18]

Precise documentation of the potential similarities and differences between animal and human PA pressure waveform characteristics is lacking. This may be viewed as an important goal from the viewpoint of comparative physiology and also to improve the translation of experimental findings to the human disease pathophysiology. Indeed, PH screening in humans is often based on the indirect estimation of mPAP derived from sPAP assuming a fixed sPAP to mPAP relationship, and this remains to be critically evaluated in animal models.

The aim of our invasive study was to document the pulsatile component of PAP in a large animal model of CTEPH. In piglets studied both at baseline and following RV preload/afterload increases and inotropic support, we tested the following hypotheses: (1) that there was a strong linear relationship between sPAP and mPAP; (2) that the equation line was close to that documented in humans; and (3) that the PA pulsatile pressure approximately matches the mPAP value. Similarities and differences between our model and previous observations in humans were documented and the potential implications were discussed.

METHODS

Animals

Our institutional animal care committee approved all procedures that were performed according to institutional guidelines complying with national and international regulations. We included 6- to 8-week old, large White piglets (*sus scrofa*). The CTEPH model[20–23] was documented both at baseline and following acute volume loading and acute pulmonary embolism with inotropic support (Figure 1). The first group (group 1) consisted of 35 piglets, namely, 8 Sham and 27 CTEPH animals. Pulmonary hypertension was induced ($n = 27$) by left PA ligation followed by weekly embolization of right lower lobes arteries with embucrilate tissue adhesive for 5 weeks (W) as previously reported.[20] Right heart catheterization was performed in resting conditions at W0 and W6. The second group (group 2) consisted of 7 piglets with CTEPH induced similarly as in the first group. Right heart catheterization was performed at W16 under the three following conditions: (1) at baseline; (2) after volume loading (saline 60 ml/kg over up to 2 h); and (3) after acute pulmonary embolism associated with dobutamine infusion (5 μg/kg/min).[23]

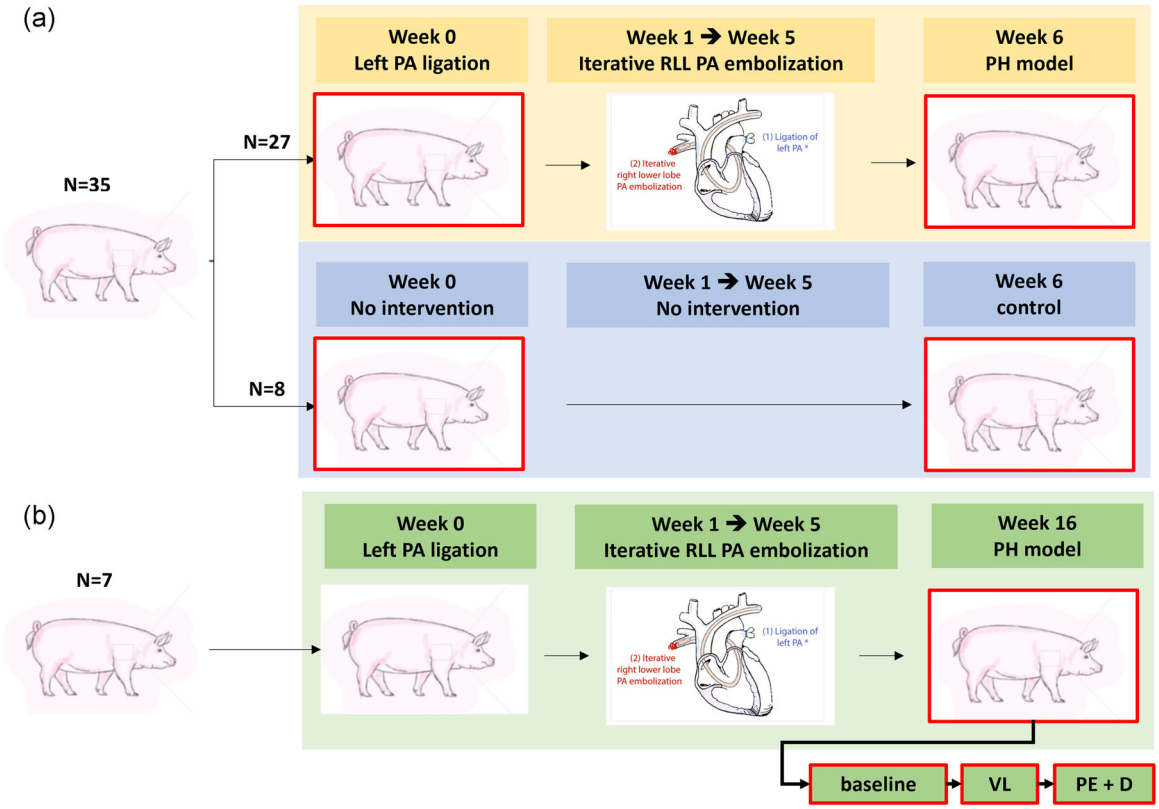

FIGURE 1 Study design. (a) In the first group of animals ($n = 35$), pulmonary hypertension was developed in 27 animals by left pulmonary artery (PA) ligation at Week 0 and weekly embolization of the right lower lobe (RLL) PA; the eight other animals underwent no intervention. Right heart catheterization was performed at Weeks 0 and 6 (red squares) in all animals. (b) In the second group of seven animals, pulmonary hypertension was induced similarly as in the first group. Right heart catheterization was performed at Week 16 under three conditions (red square) at baseline (rest), after volume loading with 60 ml/kg of saline infusion (VL) and after acute pulmonary embolism followed by dobutamine infusion at 5 μg/kg/min (PE + D)

Hemodynamic evaluation

All measurements were performed prospectively under general anesthesia.[22,23] In brief, after a 12-h fasting period, the animals received an intramuscular injection of Ketamine hydrochloride (30 mg/kg) for premedication. Then the animals received an intravenous bolus of fentanyl (0.005 mg/kg), propofol (2 mg/kg), and cisatracurium (0.3 mg/kg) intravenously through an ear vein and were intubate non-selectively with a 7-French probe. General anesthesia was maintained with inhaled 2% isoflurane, continuous infusion of fentanyl (0.004 mg/kg) and propofol (3 mg/kg). After general anesthesia induction, the animals were positioned on their backs. An 8-French sheath was placed into the jugular vein using the Seldinger method. The right heart catheterization was performed with a Swan-Ganz catheter to measure the pulmonary artery pressures (7 F; usable length 110 cm; Edwards Lifescience LLC). The disposable transducer was placed at the right atrial level (mid-chest) and connected to the workstation (Powerlab 16/35 and LabChart pro software v7.3.7; AD instrument). Live pressures (1000 Hz) were recorded on a personal computer. The fluid-filled catheter was well purged with saline to remove air bubbles so as to avoid pressure signal damping. The zero level was verified. The cardiac output (CO) was measured with a thermodilution technic with the Swan-Ganz catheter following the manufacturer's instructions (i.e., triplicate CO measurements with boli of 10 ml saline at 4°C). The heart rate (HR) was measured at the time of CO measurement; the stroke volume was calculated as the CO over HR ratio. The mPAP (time-averaged), sPAp, dPAP, and PApp were averaged out over five consecutive cardiac cycles. The time-averaged mPAP was measured as the time integral of the pressure versus time curve divided by the overall cycle length. In a subset of 12 resting piglets (five sham and seven with CTEPH), the peripheral systemic pressure was measured simultaneously with fluid-filled catheters. All the pressure measurements were performed during short periods of end-expiratory apnea. The total pulmonary resistance was calculated as mPAP/CO. The total PA compliance was estimated using the SV/PApp ratio,[13–15] and PA stiffness was defined as 1/PA compliance. To rule out the possibility of an

underestimation/overestimation of sPAP with fluid-filled catheters,[19] fluid-filled sPAP and high-fidelity right ventricular systolic pressure (Ventricath 207; Millar) were obtained within a few minutes time interval in a subset of 14 animals under 35 different experimental conditions. The right ventricular systolic pressure ranged from 21 to 105 mmHg. The mean ± SD bias between sPAP and the right ventricular systolic pressure was 0.5 ± 1.6 mmHg, which was deemed negligible (Figure S1).

Analysis of the link between the steady and pulsatile components of PA pressure

In healthy humans and in patients with precapillary PH, the steady and pulsatile components of PA pressure are tightly linked as follows:

- There is a strong linear relationship between mPAP and sPAP implying that the two measurements are essentially redundant.[16–18,24–30]
- Using a single-pressure model, an accurate and precise estimate of mPAP may be obtained from sPAP-only according to the following equation: mPAP = 0.61 sPAP + 2 mmHg (formula F1).[16–18] This empirical equation may be used in the echo-Doppler laboratory to estimate mPAP in humans.[31–33]
- The mPAP may be also estimated using various two-pressure models. At first approximation, PApp and mPAP have an almost 1:1 ratio: mPAP = PApp = sPAP − dPAP (formula F2).[18,19] There is also a proportional relationship among sPAP, mPAP and dPAP such that an accurate and even more precise estimate of mPAP may be obtained from sPAP and dPAP: $mPAP = \sqrt{sPAP \times dPAP}$ (formula F3).[24] mPAP may be also estimated by adding to dPAP either 0.33 PApp (mPAP = dPAP + 0.33 PApp, formula F4), or 0.41 PApp (mPAP = dPAP + 0.41 PApp, formula F5).[24]

We tested whether or not these features and various formulas also applied in our CTEPH model both at baseline and following RV preload/afterload increases. The accuracy of F1–F5 was also tested on the largest hemodynamic fluid-filled pressure database documented in patients with CTEPH by Madani et al.[34]

Statistical analysis

Given the nonnormal distribution of most hemodynamic data, they were expressed as median (interquartile range [IQR]). Comparison of paired data was performed using Wilcoxon signed-rank test. Spearman's correlation coefficient (ρ) was calculated. Regression lines were drawn using the least square method. The accuracy of every mPAP estimate (F1–F5) was quantified by calculating mean the bias, that is, the difference between the estimate (F) and the measured mPAP. As a measure of precision, the 95% limits of agreement (LOA) were used. The accuracy was deemed good (mean bias < 3 mmHg), moderate (3–5 mmHg), or mild (>5 mmHg). The SD of the bias quantified the precision of every estimate. The high-fidelity right ventricular systolic pressure and the filled method sPAP were compared using the Bland-Altman method. We used GraphPad Prism 9 (GraphPad Software, Inc). A p value <0.05 was considered significant.

RESULTS

Group 1

Sham ($n = 8$) and CTEPH ($n = 27$) had similar hemodynamics at W0 (Table 1). At W6, mPAP increased in CTEPH and remained unchanged in controls, while SV was unchanged in the two groups. In CTEPH, there was a three-fold increase in total pulmonary resistance and a 50% decrease in PA compliance (i.e., a two-fold increase in PA stiffness), while all remained unchanged in Sham. Pooled data demonstrated a strong linear relationship between mPAP and sPAP (mPAP = 0.80 sPAP − 1 mmHg, $n = 70$; $\rho = 0.973$; sPAP ranging from 9 to 64 mmHg) (Table 2 and Figure S2). The single-pressure model F1 resulted in an accurate and precise estimation of mPAP under normotensive conditions, namely, in Sham at W0 and W6 and in CTEPH at W0, while F1 underestimated mPAP by 3.9 mmHg on average under hypertensive conditions in CTEPH studied at W6 (Table 3). Using a two-pressure model, F2 was not verified as PApp was markedly lower than mPAP in all subgroups (Table 3). Formulas F3 and F5 consistently gave an accurate and precise estimation of mPAP in Sham and CTEPH and both at W0 and W6 (Table 3).

Group 2

In the seven piglets studied at W16, mPAP ranged from 23 to 81 mmHg. The median mPAP was 27 mmHg at baseline, 34 mmHg after volume loading, and 74 mmHg after acute pulmonary embolism associated with dobutamine infusion (Table 4). The PA compliance remained unchanged after volume loading and it decreased by 59% (PA stiffness increased by more than twofold) after acute pulmonary embolism plus dobutamine. Pooled data demonstrated a strong linear relationship between mPAP and sPAP (mPAP = 0.74 sPAP + 4 mmHg, $n = 21$; $r^2 = 0.986$, sPAP ranging from 31

TABLE 1 Demographic and hemodynamic characteristics of Sham and CTEPH piglets studied at week 0 and week 6 (group 1)

	Sham ($n = 8$)			CTEPH model ($n = 27$)		
	Week 0	Week 6	p	Week 0	Week 6	p
Weight (kg)	21.9 (20.2; 22.8)	26.7 (24.3; 28.6)	0.02	19.9 (17.8; 22.1)	25.8 (22.2; 29.2)	<0.01
BSA (m^2)	0.58 (0.55; 0.59)	0.66 (0.62; 0.69)	0.02	0.53 (0.50; 0.58)	0.64 (0.58; 0.70)	<0.01
sPAP (mmHg)	16.5 (14.3; 19.3)	17.0 (15.0; 18.75)	NS	17.0 (15.0; 20.0)	35.0 (31.0; 39.0)	<0.01
dPAP (mmHg)	7.5 (6.0; 10.0)	7.0 (5.3; 12.0)	NS	8.0 (7.0; 10.0)	20.0 (15.0; 24.0)	<0.01
mPAP (mmHg)	12.5 (9.75; 13.0)	12.0 (10.3; 16.0)	NS	13.0 (10.0; 14.0)	27.0 (23.0; 31.0)	<0.01
Papp (mmHg)	8.0 (5.0; 11.0)	9.0 (6.3; 10.1)	NS	8.0 (6.5; 10.0)	17.0 (12.0; 19.0)	<0.01
PApp/mPAP (−)	0.74 (0.40; 1.06)	0.73 (0.55; 0.92)	NS	0.75 (0.50; 1.00)	0.61 (0.52; 0.71)	<0.05
CO (L/min)	4.05 (2.95; 4.95)	3.05 (2.78; 4.25)	NS	3.50 (2.95; 4.05)	2.80 (2.4; 3.4)	<0.01
CI ($L/min/m^2$)	7.2 (5.6; 8.4)	6.7 (4.6; 8.5)	NS	7.6 (6.3; 10.0)	5.3 (4.5; 7.5)	<0.01
SV (ml)	34.5 (28.8; 49.8)	35.0 (26.8; 41.5)	NS	33.5 (25.5; 36.8)	33.0 (25.0; 40.0)	NS
SVi (ml/m^2)	58.0 (55.5; 84.5)	55.8 (38.8; 66.1)	NS	57.0 (50.0; 69.0)	51.0 (40.0; 62.0)	<0.01
HR (bpm)	103.5 (95.5; 119.5)	100.5 (84.0; 113.8)	NS	104.5 (95.8; 116.5)	92.0 (73.0; 105.0)	<0.01
PAC (ml/mmHg)	4.4 (3.0; 8.2)	4.4 (3.0; 6.6)	NS	3.9 (2.5; 5.7)	1.9 (1.4; 2.7)	<0.01
TPR (WU)	3.1 (2.5; 4.1)	3.3 (2.7; 4.8)	NS	3.5 (2.4; 4.3)	9.5 (7.4; 11.4)	<0.01

Note: Values indicated are median (interquartile range).

Abbreviations: BSA, body surface area; bpm, beats per minute; CI, cardiac index; CO, cardiac output; CTEPH, chronic thromboembolic pulmonary hypertension; dPAP, diastolic pulmonary artery pressure; HR, heart rate; mPAP, time-averaged mean pulmonary artery pressure; NS, not significant; PAC, total pulmonary arterial compliance estimated by using the SV/PApp ratio; PApp, pulmonary artery pulse pressure; sPAP, systolic pulmonary artery pressure; SV, stroke volume; SVi, indexed stroke volume; TPR, total pulmonary resistance; WU, wood units.

to 112 mmHg) (Table 2 and Figure S3). The single-pressure model F1 underestimated mPAP in all conditions and most importantly after volume loading and after acute pulmonary embolism plus dobutamine (Table 3). Using a two-pressure model, F2 was not verified as PApp was markedly lower than mPAP in all conditions and most importantly after volume loading and after acute pulmonary embolism (-Table 3). Formulas F3 and F5 consistently gave an accurate and precise estimation of mPAP at baseline, after volume loading, and after acute pulmonary embolism plus dobutamine (Table 3).

Pooled group 1 + group 2

When data from groups 1 and 2 were pooled together, there was a strong linear relationship between mPAP and sPAP ($n = 91$; $r^2 = 0.97$; sPAP ranging from 9 to 112 mmHg) (Table 2 and Figure 2). Assuming that the pressure intercept of the relationship was small enough to be neglected, the sPAP was 121% mPAP and mPAP was 83% sPAP and on average (Figure S4).

TABLE 2 Correlation matrix between the various pulmonary artery pressures for pooled data in group 1, in group 2 and in groups 1 and 2

	mPAP	sPAP	dPAP
Group 1 ($n = 70$)			
sPAP	**0.973**		
dPAP	0.937	0.874	
PApp	0.649	0.781	0.379*
Group 2 ($n = 21$)			
sPAP	**0.986**		
dPAP	0.985	0.948	
PApp	0.776	0.866	0.661
Group 1 and 2 ($n = 91$)			
sPAP	**0.987**		
dPAP	0.981	0.952	
PApp	0.737	0.823	0.610

Note: Spearman's ρ is indicated. Systolic (sPAP), diastolic (dPAP) and mean (mPAP, time-averaged) pulmonary artery (PA) pressures and PA pulse pressure (PApp). Each $p < 0.001$ except $^*p < 0.01$.

TABLE 3 A summary of the five empirical formulas for the estimation of mean pulmonary artery pressure (F1–F5), and the corresponding accuracy (mean bias) and precision (95% LOA) in group 1 and group 2

	0.61 sPAP + 2 (F1)	PApp (F2)	$\sqrt{sPAP \times dPAP}$ (F3)	dPAP +0.33 Papp (F4)	dPAP +0.41 Papp (F5)
Group 1					
Sham–W0, $n = 8$	0.1 (−3.5; 3.8)	−3.5 (−12.7; 5.7)	−0.6 (−2.2; 1.0)	−1.1 (−2.5; 0.3)	−0.4 (−1.8; 1.0)
Sham–W6, $n = 8$	0.1 (−3.8; 4.0)	−3.6 (−11.8; 4.6)	−0.5 (−4.8; 3.8)	−1.0 (−5.1; 3.1)	−0.4 (−4.1; 3.3)
CTEPH model–W0, $n = 27$	0.4 (−3.3; 4.1)	−3.3 (−11.7; 5.1)	−0.4 (−4.1; 3.3)	−0.9 (−4.2; 2.4)	−0.2 (−3.1; 2.7)
CTEPH model-W6, $n = 27$	−3.9 (−9.4; 1.6)	−11.6 (−26.3; 3.1)	−1.3 (−4.6; 2.0)	−2.6 (−5.5; 1.4)	−1.4 (−4.1; 1.3)
Pooled data, $n = 70$	−1.4 (−7.7; 4.9)	−6.6 (−20.3; 7.1)	−0.8 (−5.1; 3.5)	−1.6 (−5.9; 2.7)	−0.7 (−4.6; 3.2)
Group 2					
Baseline, $n = 7$	−5.1 (−10.2; 0)	−15.8 (−30.9; 0.7)	−0.8 (−2.8; 1.2)	−2.0 (−4.2; 0.2)	−1.1 (−2.7; 0.5)
Volume Loading, $n = 7$	−8.6 (−13.3; −3.9)	−24.1 (−36.3; −11.9)	−0.7 (−2.5; 1.1)	−2.2 (−3.8; −0.6)	−1.2 (−3.0; 0.6)
Acute embolism + dobutamine, $n = 7$	−14.2 (−22.4; 6.0)	−40.1 (−34.9; −11.3)	1.6 (−1.7; 4.9)	−1.7 (−4.4; 1.0)	0.6 (−2.3; 3.5)
Pooled data, $n = 21$	−9.3 (−18.7; 0.1)	−26.7 (−52.8; −0.6)	0.0 (−3.1; 3.1)	−2.0 (−4.2; 0.2)	−0.6 (−3.3; 2.1)

Note: Mean bias (95% LOA) is indicated. The bias was calculated as the difference between the mean pulmonary artery pressure estimated from each formula and the reference, time-averaged mean pulmonary artery pressure.

Abbreviations: CTEPH, chronic thromboembolic pulmonary hypertension; dPAP: diastolic pulmonary artery pressure. LOA, limits of agreement; PApp, pulmonary artery pulse pressure; PH, pulmonary hypertension; sPAP, systolic pulmonary artery pressure; W, Week.

Comparison of systemic and pulmonary pressure pulsatility

In the subset of 12 resting piglets having their peripheral systemic pressure simultaneously measured, the systolic arterial pressure over mean arterial pressure ratio was 1.33 (1.26; 1.40) (median [IQR]) and the sPAP over mPAP ratio was 1.27 (1.15; 1.40).

Analysis of the human CTEPH data published by Madani et al.[34]

The analysis of the 1.500 CTEPH patients investigated by Madani et al. confirmed that on average the single-pressure model F1 was reasonably accurate (<5% bias) (Table 5). The F2 model performed reasonably well such that the PApp/mPAP approached 1. The two-pressure model F3 was especially accurate.

DISCUSSION

The present study documented the relationship between the pulsatile and steady component of PA pressure in a piglet CTEPH model, both at baseline and following acute volume loading and acute pulmonary embolism with inotropic support. We confirmed the major redundancy of sPAP and mPAP previously documented in humans, but we observed lesser PA pressure pulsatility around the mean. The study may have implications regarding the translation of experimental findings into the pathophysiology of human disease. It is suggested that the sPAP-derived threshold used to diagnose PH may be species- and/or model-dependent in animals and, thus, must be validated before applying.

The PA pressure waveform reflects the integrated coupling between the right ventricle and pulmonary circulation. The contribution of enhanced PA pressure pulsatility to the pathophysiology and prognosis of PH is increasingly recognized in humans.[10–15,35] Animal models of PH are useful to gain insight into the pathophysiology of this devastating disease.[1–4] The piglet model of CTEPH combines a ligation of the left PA and weekly embolization of the right lower pulmonary lobe.[20–23] Although this model does not replicate the origin of the disease just the consequences of chronic pulmonary vascular obstruction, most aspects of the human CTEPH disease are reproduced in this model, as documented previously.[22] However, previous studies lacked precise documentation of PA pressure pulsatility.

Species independent characteristics of the pulmonary pressure and circulation

Some of the hemodynamic features previously documented in humans were extended to our model under

TABLE 4 Demographic and hemodynamic characteristics of the CTEPH piglets studied at Week 16 (group 2)

	Baseline (N = 7)	Volume loading VL (*N* = 7)	Acute embolism + dobutamine (PE + D) (*N* = 7)	Baseline versus VL (P)	Baseline versus PE + D (P)
Weight (kg)	34.4 (27.8; 37.0)	-	-		
BSA (m^2)	0.78 (0.67; 0.82)	-	-		
sPAP (mmHg)	32 (31; 39)	39 (35; 54)	91 (73; 101)	<0.05	<0.05
dPAP (mmHg)	20 (16; 24)	29 (25; 38)	63 (46; 66)	<0.05	<0.05
mPAP (mmHg)	27 (25; 29)	34 (31; 46)	74 (57; 80)	<0.05	<0.05
PApp (mmHg)	13 (8; 16)	11 (10; 15)	28 (19; 44)	NS	<0.05
PApp/mPAP	0.49 (0.28; 0.65)	0.32 (0.28; 0.36)	0.36 (0.32; 0.60)	<0.05	NS
sPAP/mPAP	1.26 (1.14; 1.32)	1.14 (1.13; 1.16)	1.25 (1.17; 1.37)	NS	NS
CO (L/min)	3.1 (2.4; 4.4)	4.2 (3.5; 5.4)	5.8 (5.0; 6.6)	<0.05	<0.05
CI ($L/min/m^2$)	4.4 (3.5; 5.6)	5.8 (5.1. 6.5)	7.6 (6.8; 9.4)	<0.05	<0.05
SV (ml)	47 (35; 49)	54 (38; 62)	43 (39; 57)	<0.05	NS
SVi (ml/m^2)	57 (54; 61)	75 (53; 81)	57 (47; 80)	<0.05	NS
HR (bpm)	71 (64; 96)	86 (74; 98)	130 (116; 140)	<0.05	<0.05
PAC (ml/mmHg)	3.4 (2.2; 6.5)	4.4 (2.8; 6.2)	1.4 (0.9; 3.0)	NS	<0.05
TPR (WU)	7.9 (6.7; 10.3)	8.9 (6.3; 11.6)	11.3 (10.1; 13.4)	NS	NS

Note: Values indicated are median (interquartile range). For abbreviations, see Table 1.

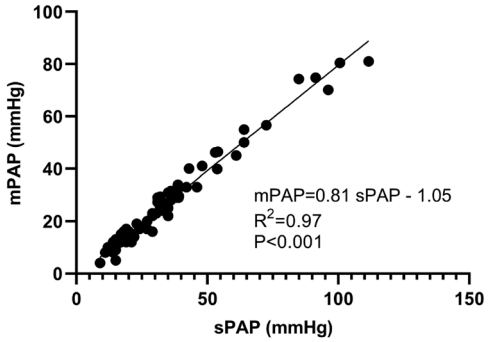

FIGURE 2 Linear relationship between mean pulmonary artery pressure (mPAP) and systolic pulmonary artery pressure (sPAP) in pooled groups 1 and 2 ($n = 91$ data points)

normotensive and hypertensive conditions, and thus these similarities may reflect species-independent characteristics of the pulmonary circulation. There was an unusually strong linear relationship between mPAP and sPAP with one pressure explaining 97% of the variability of the other (pooled data). This pressure redundancy has been first reported at rest in control subjects and in patients with precapillary PH (including CTEPH) or postcapillary PH studied at rest.[16,26] It has been subsequently confirmed[17,24–30,33] and it has also been extended to patients with precapillary PH performing supine exercise.[25] This unexpected property is a landmark feature of pulmonary circulation as it is not observed at the systemic counterpart in the aorta.[16,26] Syyed et al.[17] have confirmed such pressure redundancy ($r^2 = 0.99$) in freely moving rats and in horses studied at rest and during three bouts of exercise of increasing intensity. Overall, our study and previous literature suggest that the redundancy between mPAP and sPAP is independent of the species, loading conditions, and inotropic state.

Some authors have suggested that the mPAP versus sPAP linear relationship reflects the constancy or near-constancy of the time constant of the pulmonary Windkessel, as quantified by the pulmonary vascular resistance times PA compliance product.[36,37] This explanation has been recently ruled out because the value of the time constant of the pulmonary Windkessel is not constant but on the contrary more variable than mPAP in patients with either precapillary or postcapillary PH.[38] In other words, to continue to support the constancy or near-constancy of the time constant of the pulmonary Windkessel would be as unrealistic as supporting that mPAP is constant or near-constant in PH states.[38] One hypothesis may be that the redundancy between mPAP and sPAP is explained by the fact that changes in PA stiffness are primarily due to increases in mPAP under both experimental and clinical conditions.[10,12,38]

Another species-independent finding was that there was a proportional relationship among sPAP, mPAP, and dPAP.

TABLE 5 Invasive (fluid-filled) pulmonary artery pressures in patients with CTEPH (data from Madani et al.[34] and the corresponding mPAP and bias calculated using empirical formulas F1–F5

	Group 1 ($n = 1000$)	Group 2 ($n = 500$)
sPAP, mmHg	75.7	75.5
dPAP, mmHg	28.4	27.3
mPAP, mmHg	**46.1**	**45.5**
0.61 sPAP + 2 (F1)	48.2	48.1
Mean bias, mmHg (%)	2.1 (4.6)	2.6 (5.7)
PApp (F2)	47.6	48.2
Mean bias, mmHg (%)	1.5 (3.3)	2.7 (5.9)
$\sqrt{\text{sPAP} \times \text{dPAP}}$ (F3)	46.4	45.4
Mean bias, mmHg (%)	0.3 (0.7)	−0.1 (−0.2)
dPAP + 0.33 PApp (F4)	44.0	43.2
Mean bias, mmHg (%)	−2.1 (−4.5)	−2.3 (−5.0)
dPAP + 0.41 PApp (F5)	47.8	47.1
Mean bias, mmHg (%)	1.7 (3.7)	1.6 (3.5)

Note: All pressures and formulas (F) are means expressed in mmHg. The bias was calculated as the formula (F) minus mPAP difference and expressed in mmHg and as a percentage of the reference mPAP.
Group 1: Patients with CTEPH included between March 1999 and October 2006. Group 2: Patients with CTEPH included between October 2006 and December 2010.

Abbreviations: CTEPH, chronic thromboembolic pulmonary hypertension; dPAP, diastolic PA pressure; F, empirical formulas; mPAP, time-averaged mean pulmonary arter (PA) pressure; PApp, PA pulse pressure; sPAP, systolic PA pressure.

Indeed, an accurate and precise estimate of mPAP was obtained using mPAP = $\sqrt{\text{sPAP} \times \text{dPAP}}$ (F3). This formula has been initially validated in humans using high-fidelity pressure data[24] and has been subsequently confirmed.[37] Our retrospective analysis of the largest hemodynamic fluid-filled pressure database in patients with CTEPH by Madani et al.[34] here confirmed the high accuracy of F3 to estimate mPAP. Unlike the mPAP versus sPAP relationship, F3 also applies at the systemic counterpart in the aorta[39] and it may thus reflect a common biophysical property of central arteries when pressurized. The F5 formula (mPAP = dPAP + 0.41 PApp) also applied fairly well in our study.

Lesser PA pulsatility in piglet than in human CTEPH

In contrast, we documented a number of differences between our experimental findings and previous

observations made in humans. In normotensive and hypertensive humans, it is widely recognized that there is a high PA pulsatility, with the oscillatory power being twice as high in the right ventricle (25%–30% of total power) than in the left ventricle (10%–15%).[40,41] In practice, the high PA pulsatility is attested to by the fact that sPAP approximately matches 160% mPAP, and also that PApp and mPAP have an almost 1:1 ratio.[18,19] Pulmonary circulation was much less pulsatile in our piglet model both at baseline and in CTEPH animals. Indeed, the empirical equation F1 used in the echo-Doppler laboratory to estimate mPAP in humans, namely, mPAP = 0.61 sPAP + 2 mmHg,[16,24,27,33] did not apply and sPAP approximately matches only 120% mPAP. Furthermore, the F2 formula did not apply: the oscillation of PA pressure around the mean (PApp) was much lower than mPAP, and the discrepancy was even more marked at high prevailing mPAP. In humans, Milnor[19] has reported that PApp was very close to mPAP value on average.[19] This has been recently confirmed by our group in 981 incident untreated patients with pulmonary arterial hypertension (PAPp/mPAp = 0.95 [0.82–1.10], median [IQR]) and in 44 normotensive patients (PAPp/mPAp = 0.88 ± 0.23, mean ± standard deviation).[18] This, yet poorly referenced concept, seems a specific feature of the human pulmonary circulation, as it is not observed at the systemic counterpart, where aortic pulse pressure is most often markedly lower than mean aortic pressure.[19,42] The present study suggests that this property is also species-dependent as it was not observed in our piglet model. Finally, the possibility of an underestimation of sPAP with fluid-filled catheters was ruled out in a subset of piglets where high-fidelity right ventricular systolic pressure was obtained within a few minutes time-interval.

These findings are likely to reflect species-dependent characteristics of pulmonary circulation. It is admitted that PApp is grossly approximated by the product of RV stroke volume times PA stiffness (1/PA compliance).[13–15,35] In humans with precapillary PH including CTEPH, normal-to-low RV stroke volume together with markedly increased PA stiffness account for the abnormally high PApp.[9,13,35] Increased PA stiffness may be an underlying condition and a hallmark of patients with CTEPH which promotes the disease development and impacts the prognosis.[10–15] The lesser PA pulsatility we documented in piglets as compared to that of humans may be explained by lower stroke volume, or lower PA stiffness/higher PA compliance, or both. The higher PA compliance in piglets may be species-dependent. Although we cannot exclude the role of the young age of the animals, it is interesting to note that our formula F1 remains accurate in infants and children.[43]

Finally, our study suggests that pulsatility of the systemic circulation in piglets is close to that documented at the PAP level and is also within the same range as that documented in the human systemic circulation, but this point remains to be confirmed by other studies involving a larger sample

Limitations and implications

The present findings apply to animals free of flow obstacles (e.g., pulmonary stenosis). A low sPAP value was documented in one sham piglet and we cannot exclude the possibility that this was due to anesthesia (during which a transient vagal reaction may be observed), to hypovolemia or both. We studied pulmonary hemodynamics during short periods of end-expiratory apnea and it is likely that these conditions are close to the measurements performed in humans at end-expiration, although we cannot exclude the possibility that the study design may have slightly impacted on the differences observed between piglets and humans. The zero level was set at the mid-thoracic level as recommended by current guidelines.[44] However, we cannot exclude the possibility that 1/3 of the thoracic diameter below the anterior thorax surface would have been more accurate for assessment of the right atrial level.[45] Our animal model has used male, young piglets with a maximum of 16-weeks history of chronic PH. We previously showed that this model is an adaptive model of chronic CTEPH.[22] Therefore, the extension of our findings to symptomatic CTEPH beyond the “honeymoon” period may be limited. Conversely, translational aspects of our research to the early stages of CTEPH could be of interest and remains to be studied.

It is suggested that the echo-Doppler-derived sPAP thresholds used to diagnose PH in animals may depend upon the species under consideration. As an example, the old hemodynamic definition of PH (mPAP ≥ 25 mmHg) will correspond to an sPAP ≥ 38 mmHg sPAP in humans but only ≥32 mmHg in piglets. The new hemodynamic definition of PH (mPAP > 20 mmHg)[46] will correspond to a sPAP > 30 mmHg in humans but only >26 mmHg in piglet. It is thus suggested that the empirical equation used in the echo-Doppler laboratory to estimate mPAP from sPAP in humans (F1) must be validated in other animal species before use. The two-pressure formulas, and especially the geometric mean F3 and the F5 formulas may be viewed as utilitarian to cross-check the self-consistency of the echo-derived database when a full pressure set is sought (sPAP, dPAP, and mPAP), and this may help individualize major outliers in the overall pressure database. Finally, the

potential differences in PA stiffness in some animal species as compared to humans may have implications for translating experimental findings to the pathophysiology of the human disease.

In conclusion, the redundancy between mPAP and sPAP previously documented in human PH states seems a characteristic of pulmonary circulation which is independent of the species, loading conditions, and inotropic state. However, lesser PA pulsatility was documented in our piglet model as compared to human CTEPH, and it is thus suggested that the echo-Doppler sPAP thresholds used to define PH in animals are species-dependent and must be validated before applying. Finally, the F3 and F5 formulas may be viewed as utilitarian to cross-check the self-consistency of the sPAP, dPAP, and mPAP estimates obtained in the echo-Doppler laboratory.

ACKNOWLEDGMENT

No funding was received for this study.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest in the field (physiology).

ETHICS STATEMENT

Marie Lannelongue Hospital institutional animal care committee approved all procedures that were performed according to institutional guidelines complying with national and international regulations.

AUTHOR CONTRIBUTIONS

Study design and writing of the first draft: David Boulate and Denis Chemla. *Data acquisition*: David Boulate, Fanny Loisel, Mathieu Coblence, Bastien Provost, Alban Todesco, and Benoit Decante. *Calculations, discussion of the data, and correction of the first draft*: All authors.

REFERENCES

1. Maarman G, Lecour S, Butrous G, Thienemann F, Sliwa K. A comprehensive review: the evolution of animal models in pulmonary hypertension research; are we there yet? Pulm Circ. 2013;3:739–56.
2. Guihaire J, Bogaard HJ, Flécher E, Noly PE, Mercier O, Haddad F, Fadel E. Experimental models of right heart failure: a window for translational research in pulmonary hypertension. Semin Respir Crit Care Med. 2013;34:689–99.
3. Sztuka K, Jasińska-Stroschein M. Animal models of pulmonary arterial hypertension: a systematic review and meta-analysis of data from 6126 animals. Pharmacol Res. 2017;125:201–14.
4. Provencher S, Archer SL, Ramirez FD, Hibbert B, Paulin R, Boucherat O, Lacasse Y, Bonnet S. Standards and methodological rigor in pulmonary arterial hypertension preclinical and translational research. Circ Res. 2018;122:1021–32.
5. Lahm T, Douglas IS, Archer SL, Bogaard HJ, Chesler NC, Haddad F, Hemnes AR, Kawut SM, Kline JA, Kolb TM, Mathai SC, Mercier O, Michelakis ED, Naeije R, Tuder RM, Ventetuolo CE, Vieillard-Baron A, Voelkel NF, Vonk-Noordegraaf A, Hassoun PM, American Thoracic Society Assembly on Pulmonary Circulation. Assessment of right ventricular function in the research setting: knowledge gaps and pathways forward. An official American Thoracic Society research statement. Am J Respir Crit Care Med. 2018;198:e15–43.
6. Milnor WR, Conti CR, Lewis KB, O'Rourke MF. Pulmonary arterial pulse wave velocity and impedance in man. Circ Res. 1969;25:637–49.
7. Chemla D, Lau EM, Papelier Y, Attal P, Herve P. Pulmonary vascular resistance and compliance relationship in pulmonary hypertension. Eur Respir J. 2015;46:1178–89.
8. Maron BA, Brittain EL, Hess E, Waldo SW, Barón AE, Huang S, Goldstein RH, Assad T, Wertheim BM, Alba GA, Leopold JA, Olschewski H, Galiè N, Simonneau G, Kovacs G, Tedford RJ, Humbert M, Choudhary G. Pulmonary vascular resistance and clinical outcomes in patients with pulmonary hypertension: a retrospective cohort study. Lancet Respir Med. 2020;8:873–84.
9. Castelain V, Herve P, Lecarpentier Y, Duroux P, Simonneau G, Chemla D. Pulmonary artery pulse pressure and wave reflection in chronic pulmonary thromboembolism and primary pulmonary hypertension. J Am Coll Cardiol, 2001;200(37):1085–92.
10. Lammers S, Scott D, Hunter K, Tan W, Shandas R, Stenmark KR. Mechanics and function of the pulmonary vasculature: implications for pulmonary vascular disease and right ventricular function. Compr Physiol. 2012;2:295–319.
11. Delcroix M, Vonk Noordegraaf A, Fadel E, Lang I, Simonneau G, Naeije R. Vascular and right ventricular remodelling in chronic thromboembolic pulmonary hypertension. Eur Respir J. 2013;41:224–32.
12. Schäfer M, Myers C, Brown RD, Frid MG, Tan W, Hunter K, Stenmark KR. Pulmonary arterial stiffness: toward a new paradigm in pulmonary arterial hypertension pathophysiology and assessment. Curr Hypertens Rep. 2016;18:4.
13. Thenappan T, Prins KW, Pritzker MR, Scandurra J, Volmers K, Weir EK. The critical role of pulmonary arterial compliance in pulmonary hypertension. Ann Am Thorac Soc. 2016;13:276–84.
14. Mahapatra S, Nishimura RA, Sorajja P, Cha S, McGoon MD. Relationship of pulmonary arterial capacitance and mortality in idiopathic pulmonary arterial hypertension. J Am Coll Cardiol. 2006;47:799–803.
15. Gerges M, Gerges C, Pistritto AM, Lang MB, Trip P, Jakowitsch J, Binder T, Lang IM. Pulmonary hypertension in heart failure. Epidemiology, right ventricular function, and survival. Am J Respir Crit Care Med. 2015;192:1234–46.
16. Chemla D, Castelain V, Humbert M, Hébert JL, Simonneau G, Lecarpentier Y, Herve P. New formula for predicting mean pulmonary artery pressure using systolic pulmonary artery pressure. Chest. 2004;126:1313–7.
17. Syyed R, Reeves JT, Welsh D, Raeside D, Johnson MK, Peacock AJ. The relationship between the components of

pulmonary artery pressure remains constant under all conditions in both health and disease. Chest. 2008;133:633–9.
18. Chemla D, Boulate D, Weatherald J, Lau E, Attal P, Savale L, Montani D, Fadel E, Mercier O, Sitbon O, Humbert M, Herve P. Golden ratio and the proportionality between pulmonary pressure components in pulmonary arterial hypertension. Chest. 2019;155:991–8.
19. Milnor WR. Cardiovascular physiology. New York: Oxford University Press; 1990. p. 215.
20. Mercier O, Tivane A, Dorfmüller P, de Perrot M, Raoux F, Decante B, Eddahibi S, Dartevelle P, Fadel E. Piglet model of chronic pulmonary hypertension. Pulm Circ. 2013;3: 908–15.
21. Guihaire J, Haddad F, Noly PE, Boulate D, Decante B, Dartevelle P, Humbert M, Verhoye JP, Mercier O, Fadel E. Right ventricular reserve in a piglet model of chronic pulmonary hypertension. Eur Respir J. 2015;45:709–17.
22. Noly PE, Guihaire J, Coblence M, Dorfmüller P, Fadel E, Mercier O. Chronic thromboembolic pulmonary hypertension and assessment of right ventricular function in the piglet. J Vis Exp. 2015;105:L53133.
23. Boulate D, Amsallem M, Kuznetsova T, Zamanian RT, Fadel E, Mercier O, Haddad F. Echocardiographic evaluations of right ventriculo-arterial coupling in experimental and clinical pulmonary hypertension. Physiol Rep. 2019;7:14322.
24. Chemla D, Castelain V, Provencher S, Humbert M, Simonneau G, Herve P. Evaluation of various empirical formulas for estimating mean pulmonary artery pressure by using systolic pulmonary artery pressure in adults. Chest. 2009;135:760–8.
25. Chemla D, Castelain V, Hoette S, Creuzé N, Provencher S, Zhu K, Humbert M, Herve P. Strong linear relationship between heart rate and mean pulmonary artery pressure in exercising patients with severe precapillary pulmonary hypertension. Am J Physiol Heart Circ Physiol. 2013;305: H769–77.
26. Chemla D, Humbert M, Sitbon O, Montani D, Herve P. Systolic and mean pulmonary artery pressures: are they interchangeable in patients with pulmonary hypertension? Chest. 2015;147:943–50.
27. Steckelberg RC, Tseng AS, Nishimura R, Ommen S, Sorajja P. Derivation of mean pulmonary artery pressure from noninvasive parameters. J Am Soc Echocardiogr. 2013;26:464–8.
28. Miller WL, Grill DE, Borlaug BA. Clinical features, hemodynamics, and outcomes of pulmonary hypertension due to chronic heart failure with reduced ejection fraction: pulmonary hypertension and heart failure. JACC Heart Fail. 2013;1:290–9.
29. Handoko ML, De Man FS, Oosterveer FP, Bogaard HJ, Vonk-Noordegraaf A, Westerhof N. A critical appraisal of transpulmonary and diastolic pressure gradients. Physiol Rep. 2016;4: e12910.
30. Vanden Eynden F, Racapé J, Vincent J, Vachiéry JL, Bové T, Van Nooten G. The linear relationship between systolic pulmonary artery pressure and mean pulmonary artery pressure is maintained regardless of autonomic or rhythm disturbances. Respir Res. 2016;17:33.
31. Aduen JF, Castello R, Daniels JT, Diaz JA, Safford RE, Heckman MG, Crook JE, Burger CD. Accuracy and precision of three echocardiographic methods for estimating mean pulmonary artery pressure. Chest. 2011;139:347–52.
32. Bech-Hanssen O, Selimovic N, Rundqvist B, Wallentin J. Doppler echocardiography can provide a comprehensive assessment of right ventricular afterload. J Am Soc Echocardiogr. 2009;22:1360–7.
33. Amsallem M, Sternbach JM, Adigopula S, Kobayashi Y, Vu TA, Zamanian R, Liang D, Dhillon G, Schnittger I, McConnell MV, Haddad F. Addressing the controversy of estimating pulmonary arterial pressure by echocardiography. J Am Soc Echocardiogr. 2016;29:93–102.
34. Madani MM, Auger WR, Pretorius V, Sakakibara N, Kerr KM, Kim NH, Fedullo PF, Jamieson SW. Pulmonary endarterectomy: recent changes in a single institution's experience of more than 2.700 patients. Ann Thorac Surg. 2012;94: 97–103.
35. Chemla D, Weatherald J, Lau EMT, Savale L, Boucly A, Attal P, Jaïs X, Parent F, Girerd B, Simonneau G, Montani D, Humbert M, Sitbon O, Herve P. Clinical and hemodynamic correlates of pulmonary arterial stiffness in incident, untreated patients with Idiopathic pulmonary arterial hypertension. Chest. 2018;154:882–92.
36. Saouti N, Westerhof N, Postmus PE, Vonk-Noordegraaf A. The arterial load in pulmonary hypertension. Eur Respir Rev. 2010;19:197–203.
37. Kind T, Faes TJ, Vonk-Noordegraaf A, Westerhof N. Proportional relations between systolic, diastolic and mean pulmonary artery pressure are explained by vascular properties. Cardiovasc Eng Technol. 2011;2:15–23.
38. Chemla D, Berthelot E, Weatherald J, Lau E, Savale L, Beurnier A, Montani D, Sitbon O, Attal P, Boulate D, Assayag P, Humbert M, Herve P. The isobaric pulmonary arterial compliance in pulmonary hypertension. ERJ Open Res. 2021;7:00941-2020.
39. Chemla D, Antony I, Zamani K, Nitenberg A. Mean aortic pressure is the geometric mean of systolic and diastolic aortic pressure in resting humans. J Appl Physiol. 1985;2005(99): 2278–84.
40. Saouti N, Westerhof N, Helderman F, Marcus JT, Boonstra A, Postmus PE, Vonk-Noordegraaf A. Right ventricular oscillatory power is a constant fraction of total power irrespective of pulmonary artery pressure. Am J Respir Crit Care Med. 2010; 182:1315–20.
41. Chemla D, Castelain V, Zhu K, Papelier Y, Creuzé N, Hoette S, Parent F, Simonneau G, Humbert M, Herve P. Estimating right ventricular stroke work and the pulsatile work fraction in pulmonary hypertension. Chest. 2013;143: 1343–50.
42. Chemla D, Antony I, Zamani K, Nitenberg A. Mean aortic pressure is the geometric mean of systolic and diastolic aortic pressure in resting humans. J Appl Physiol. 2005;99:2278–84.
43. Chemla D, Lambert V, Losay J. Mean pulmonary artery pressure estimated from systolic and diastolic pulmonary artery pressure in children with congenital heart disease: an invasive study. Pediatr Cardiol. 2009;30:1137–42.
44. Rosenkranz S, Preston IR. Right heart catheterisation: best practice and pitfalls in pulmonary hypertension. Eur Respir Rev. 2015;24:642–52.

45. Kovacs G, Avian A, Olschewski A, Olschewski H. Zero reference level for right heart catheterization. Eur Respir J. 2013; 42:1586–94.
46. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, Williams PG, Souza R. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. Eur Respir J. 2019;53:1801913.

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How to cite this article: Boulate D, Loisel F, Coblence M, Provost B, Todesco A, Decante B, Beurnier A, Herve P, Perros F, Humbert M, Fadel E, Mercier O, Chemla D. Pulsatile pulmonary artery pressure in a large animal model of chronic thromboembolic pulmonary hypertension: similarities and differences with human data. Pulm Circ. 2022;12:e12017. https://doi.org/10.1002/pul2.12017