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Exercise stress echocardiography for the study of the pulmonary circulation

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ABSTRACT: Exercise stress tests have been used for the diagnosis of pulmonary hypertension, but with variable protocols and uncertain limits of normal.

The pulmonary haemodynamic response to progressively increased workload and recovery was investigated by Doppler echocardiography in 25 healthy volunteers aged 19–62 yrs (mean 36 yrs). Mean pulmonary artery pressure ($\bar{P}$pa) was estimated from the maximum velocity of tricuspid regurgitation. Cardiac output (Q) was calculated from the aortic velocity-time integral. Slopes and extrapolated pressure intercepts of $\bar{P}$pa–Q plots were calculated after using the adjustment of Poon for individual variability. A pulmonary vascular distensibility α was calculated from each $\bar{P}$pa–Q plot to estimate compliance.

$\bar{P}$pa increased from 14±3 mmHg to 30±7 mmHg, and decreased to 19±4 mmHg after 5 min recovery. The slope of $\bar{P}$pa–Q was 1.37±0.65 mmHg·min^{-1}·L^{-1} with an extrapolated pressure intercept of 8.2±3.6 mmHg and an α of 0.017±0.018 mmHg^{-1}. These results agree with those of previous invasive studies. Multipoint $\bar{P}$pa–Q plots were well described by a linear approximation, from which resistance can be calulated.

We conclude that exercise echocardiography of the pulmonary circulation is feasible and provides realistic resistance and compliance estimations. Measurements during recovery are unreliable because of rapid return to baseline.

KEYWORDS: Echocardiography, exercise stress test, pulmonary arterial compliance, pulmonary hypertension, pulmonary vascular resistance

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Pulmonary arterial hypertension (PAH) is currently defined by a mean pulmonary artery pressure ($\bar{P}$pa) of >25 mmHg, a left atrial pressure (Pla) ⩽15 mmHg and a pulmonary vascular resistance (PVR) of >3 Wood units [1]. Previous definitions included $\bar{P}$pa of >30 mmHg at exercise [2], but this has been abandoned because of uncertain limits of normal and unknown symptomatic relevance.

Recently, however, TOLLE *et al.* [3] reported on exercise-induced PAH as a new clinical entity, characterised by a sharp increase in $\bar{P}$pa of >30 mmHg as a cause of decreased exercise capacity. In that study, haemodynamic measurements were presented as log-log plots of $\bar{P}$pa as a function of oxygen uptake (V'_{O_2}), with plateau patterns typical of PAH and takeoff patterns representing a normal response. Since V'_{O_2} is related to cardiac output (Q), these patterns would appear at variance. Numerous studies have shown multipoint $\bar{P}$pa–Q plots to be best described by linear or slightly curvilinear approximations [4, 5]. REEVES *et al.* [6] modelled $\bar{P}$pa as a function of Q invasively measured in exercising normal volunteers, and indeed found a slight curvilinearity which they explained by the natural distensibility of the resistive pulmonary arterioles.

Even though the procedure has still not been validated, Doppler echocardiography is a recommended screening test for PAH [1, 2] and has been used in combination with exercise for the diagnosis of overt or latent PAH [7]. A systolic pulmonary artery pressure (Ppa,sys) of 40 mmHg is usually taken as the upper limit of normal [1, 2, 7], even though this value may be exceeded by exercising athletes [8].

The purpose of our study was to determine the feasibility of enhancing the methods and maximising the analyses of exercise-stress Doppler echocardiography for the study of the pulmonary circulation.

METHODS

25 consecutive healthy volunteers (12 females and 13 males) aged 36±14 yrs with a height and weight of 178±12 cm and 70±15 kg, respectively, were studied. Written informed consent was obtained from all volunteers. The study was

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approved by the ethical committee of the Erasme University Hospital (Brussels, Belgium). All participants led a healthy lifestyle, undertaking 2–5 h of exercise per week, but none were competitive athletes. Three additional young adult volunteers were excluded at initial screening on the basis of their morphology and difficulty in obtaining sufficient quality echocardiographic measurements at rest.

A standard echocardiographic examination was performed at rest, during exercise, and after 5, 10, 15 and 20 min of recovery. The workload was increased by 20 W every 2 min until the maximum workload tolerated because of dyspnoea and/or leg pain. Echocardiographic measurements were taken during the last minute of each workload. Heart rate (ECG lead) and blood pressure (sphygmomanometry) were recorded at baseline and during the last 15 s of each workload.

Doppler echocardiography was performed with a Vivid 7 ultrasound system (GE Ultrasound, Oslo, Norway) on a semi-recumbent cycle ergometer (model 900 EL; Ergoline, Bitz, Germany), as previously described [7]. The exercise table was tilted laterally by 20° to 30° to the left. Q was estimated from left ventricular outflow tract cross sectional area and pulsed Doppler velocity-time integral measurements [9]. $P_{pa,sys}$ was estimated from a trans-tricuspid gradient calculated from the maximum velocity (V) of continuous Doppler tricuspid regurgitation, as $4 \times V^2$+5 mmHg assigned to right atrial pressure [10]. $\bar{P}_{pa}$ was calculated as $0.6 \times P_{pa,sys}$+2 [11]. P_{la} was estimated from the ratio of Doppler mitral E flow-velocity wave and tissue Doppler mitral annulus flow E' early diastolic velocity, *i.e.* P_{la}=1.9+1.24 E/E' [12]. PVR was calculated as $(\bar{P}_{pa} - P_{la})/Q$.

The echocardiographic recordings were stored on optical disks and read by two blinded observers. The intra-observer variabilities were determined on random samples of 10 recordings of five successive measurements obtained in the same subjects at rest and at maximum exercise. Variability was calculated as SD divided by the mean. The inter-observer variabilities were calculated as 1.98 multiplied by the square root of the product of SD_1 by SD_1. SD_1 and SD_2 are the SD over the means of resting and maximum exercise measurements of $\bar{P}_{pa}$ and Q performed by the first and the second reader, respectively.

The linearity of $\bar{P}_{pa}$–Q curves was analysed qualitatively and quantitatively before and after applying the technique of POON [13] using pooled subject data. Linear regression was used to determine best fit slope and intercept minimising the sum of least square error. Following the study of TOLLE *et al.* [3], the slopes of two best fit straight line segments were determined for each $\bar{P}_{pa}$–Q curve after log-log transformation with a similar technique [14]. Finally, as previously reported, each multipoint $\bar{P}_{pa}$–Q plot was fitted to the equation:

$$\bar{P}_{pa} = \frac{\left[(1+\alpha P_{la})^5 + 5\alpha R_0 Q\right]^{\frac{1}{5}} - 1}{\alpha}$$

where R_0 is the total PVR at rest, to calculate the distensibility α as change in diameter per mmHg increase in transmural pressure [6].

Results are presented as mean±SD. The statistical analysis consisted of a repeated measure of ANOVA. When the F ratio of ANOVA reached a $p<0.05$ critical value, paired or unpaired modified t-tests were applied as indicated to compare specific situations [15].

RESULTS

Good quality signals were available at all levels of exercise in all the subjects. Taking into account the initial exclusion of three subjects, this corresponds to an 88% recovery rate. The intra-observer variabilities for $P_{pa,sys}$ and Q estimates were 4.3% and 4.0% at rest, and 8.2% and 7.7% at maximum exercise, respectively. The inter-observer variabilities of $P_{pa,sys}$ and Q estimates were 1.9% and 4.9% at rest, and 7.9% and 13.9% at maximum exercise, respectively. $\bar{P}_{pa}$ estimated by the two independent blinded observers were 13.3±2.2 mmHg and 13.5±2.8 mmHg at rest, respectively, and 31.8±6.9 mmHg and 30.8±7.3 mmHg at maximum exercise (p=NS), respectively. Mean Q estimated by the two independent blinded observers was 4.7±1.0 L·min^{-1} and 4.8±0.97 L·min^{-1} at rest, respectively, and 17.7±3.9 L·min^{-1} and 18.0±4.2 L·min^{-1} at maximum exercise (p=NS), respectively. Source Doppler tracings and derived $\bar{P}_{pa}$ and Q calculations for a representative subject are shown in figure 1.

The maximum achieved workload was 170±51 W. As shown in table 1, this was accompanied by a four-fold increase in Q and an increase in $P_{pa,sys}$ to >40 mmHg. At maximum exercise, 19 subjects had a $P_{pa,sys}$ of >40 mmHg and 14 subjects had a $\bar{P}_{pa}$ of >30 mmHg. Both $P_{pa,sys}$ and Q were markedly decreased after 5 min of recovery, but were still higher than at baseline. After 20 min of recovery, $P_{pa,sys}$ was back to baseline but Q remained slightly elevated. Exercise did not affect P_{la} or PVR.

The $\bar{P}_{pa}$–Q relationships fitted well to the distensibility equation, which imposed a curvilinear, convex, downward shape (fig. 2). Each $\bar{P}_{pa}$–Q plot was also well described by a linear approximation. The average slope was 1.37±0.65 mmHg·min^{-1}·L^{-1}, and the intercept was 8.2±3.6 mmHg, with a correlation coefficient R^2 of 0.92±0.06 (p=0.0018±0.005). After Poon's adjustment, the slope of a line best fit to pooled data was 1.32 mmHg·min^{-1}·L^{-1} and the intercept was 8.2 mmHg, with an R^2 value of 0.95 and $p<0.0001$ (fig. 3). The average distensibility coefficient α for all subjects was 0.017±0.018 mmHg^{-1}.

After log-log transformation of $\bar{P}_{pa}$ and Q, an inflection point could be discerned with a plateau pattern (slope before inflection point (m1) > slope after inflection point (m2)) in 11 subjects and an m1<m2 takeoff pattern in 14 subjects (fig. 4). These patterns were also seen in the $\bar{P}_{pa}$–Q relationships before log transformation. The patterns were not correlated to baseline PVR, slope of $\bar{P}_{pa}$–Q or maximum workload.

The relationships between workload and $P_{pa,sys}$ and Q were highly linear, with slopes of $P_{pa,sys}$ *versus* Q of 1.93±0.19 mmHg·min^{-1}·L^{-1} (R^2=0.92±0.06; $p<0.005$) and $P_{pa,sys}$ *versus* workload of 0.17±0.07 mmHg·W^{-1} (R^2=0.95±0.04; $p<0.001$).

DISCUSSION

Our findings demonstrate that exercise stress echocardiography is feasible for studying the pulmonary circulation and

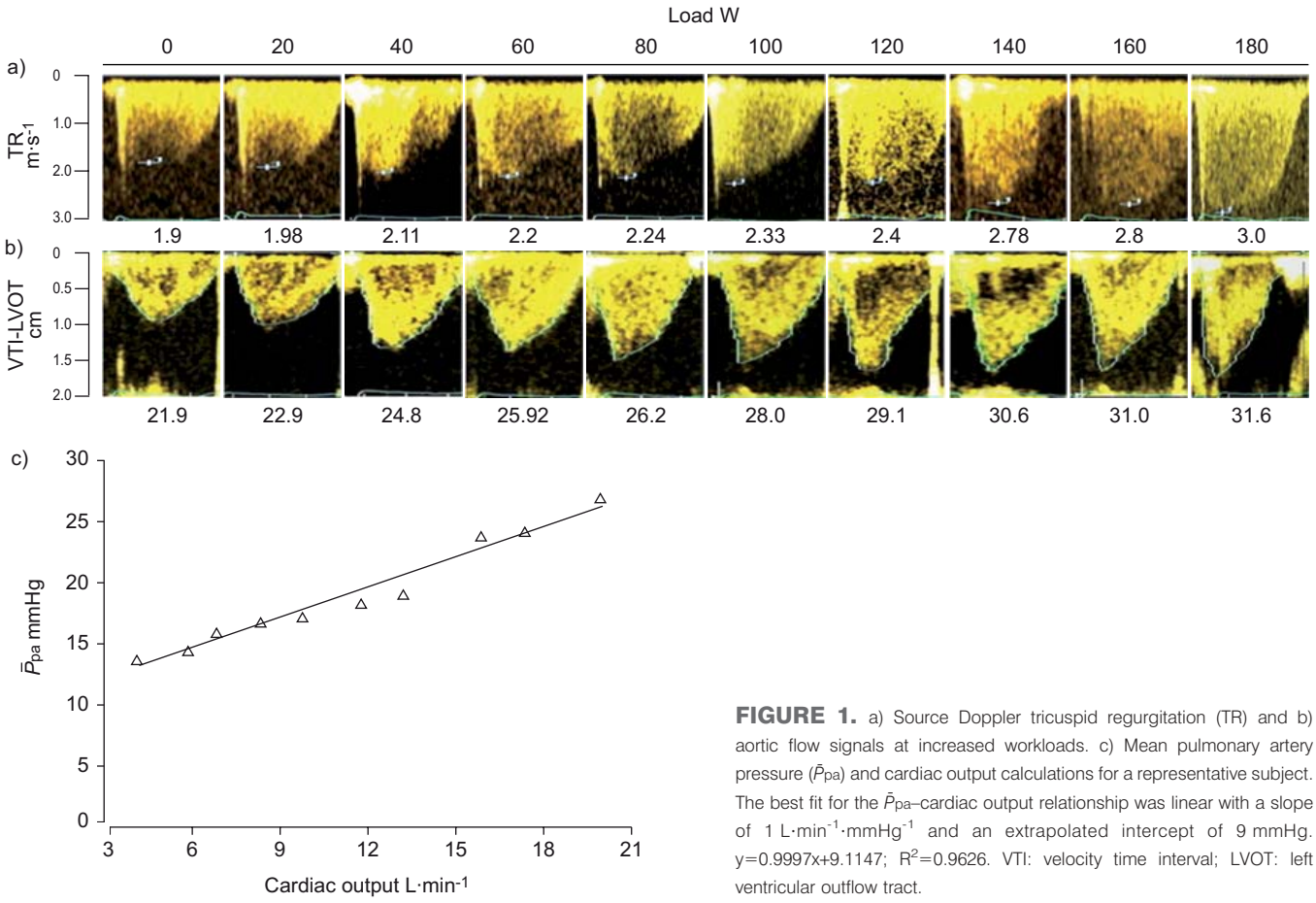

FIGURE 1. a) Source Doppler tricuspid regurgitation (TR) and b) aortic flow signals at increased workloads. c) Mean pulmonary artery pressure ($\bar{P}$pa) and cardiac output calculations for a representative subject. The best fit for the $\bar{P}$pa–cardiac output relationship was linear with a slope of 1 L·min^{-1}·mmHg^{-1} and an extrapolated intercept of 9 mmHg. y=0.9997x+9.1147; R^2=0.9626. VTI: velocity time interval; LVOT: left ventricular outflow tract.

provides realistic values compared to those obtained by invasive haemodynamic measurements.

The subjects in the present study exercised in a semi-recumbent position, in contrast to previous reports of upright exercise haemodynamics [3–5]. However, this would only affect the relationship between $\bar{P}$pa and Q at rest, probably by some degree of de-recruitment of the pulmonary resistive vessels at a lower Q in the upright position [4, 5, 16]. The relationship between $\bar{P}$pa and Q has been shown to be independent of body position during exercise because of increased Q and associated full recruitment of the pulmonary circulation [4, 5, 16].

TABLE 1 Haemodynamic measurements at rest, maximum exercise and during recovery in 25 normal subjects

Variables	Baseline	Maximum exercise	Recovery time		
			5 min	10 min	20 min
HR bpm	66±10	159±21*	92±14*	88±15*	79±13*
SBP mmHg	116±9	169±17*	121±11*	115±10	113±11
$P_{pa,sys}$ mmHg	19±5	46±11*	27±7*	24±6*	20±5
P_{la} mmHg	8±2	9±1	8±2	8±1	8±1
Q L·min^{-1}	4.7±1.0	18.0±4.2*	7.2±1.8*	6.4±1.8*	5.3±1.2*
PVR Woods units#	1.2±0.6	1.2±0.5	1.4±0.6	1.3±0.6	1.2±0.7

Data are presented as mean±SD. HR: heart rate; SBP: systolic blood pressure; $P_{pa,sys}$: systolic pulmonary artery pressure; P_{la}: left atrial pressure; Q: cardiac output; PVR: pulmonary vascular resistance. #: or mmHg·L^{-1}·min^{-1}. *: $p<0.05$ compared with baseline.

Exercise-induced PAH has recently been described as a clinical entity characterised by decreased exercise capacity and explained by an excessive increase in $\bar{P}$pa at exercise often accompanied by a decrease in right ventricular function [3]. In the study by TOLLE *et al.* [3], exercise measurements were presented as log-log plots of $\bar{P}$pa *versus* V'_{O_2}, with takeoff patterns in 14 out of 15 normal subjects and frequent plateau patterns (in 32 out of 78 patients) with exercise-induced PAH. The plateau pattern was associated with a greater reduction in exercise capacity and higher PVR. In the present study, V'_{O_2} was not measured and patterns of log $\bar{P}$pa *versus* log Q were analysed instead, but it is assumed that this should not affect the relationship as V'_{O_2} and Q are tightly correlated [3, 4]. We found that takeoff and plateau patterns were of approximate equally frequency in normal subjects and clearly shown in log-log transformed data. However, the patterns were unrelated to incremental resistance and workload, which leaves uncertainty as to their functional significance. Patterns could be produced in relation to nonlinear relationship between V'_{O_2} and Q at the

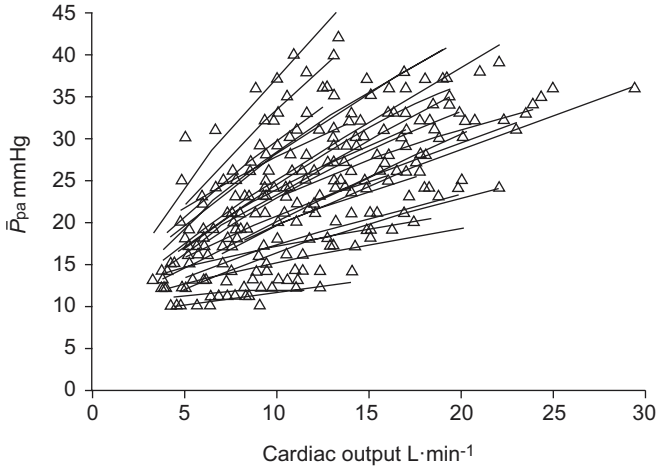

FIGURE 2. Mean pulmonary artery pressure ($\bar{P}_{pa}$) and cardiac output measurements at rest and at progressively increased workloads in 25 healthy subjects. By best fit to a simple model of pulmonary vascular distensibility, a slight curvilinearity with convexity to the pressure axis can be seen in $\bar{P}_{pa}$–cardiac output relationships.

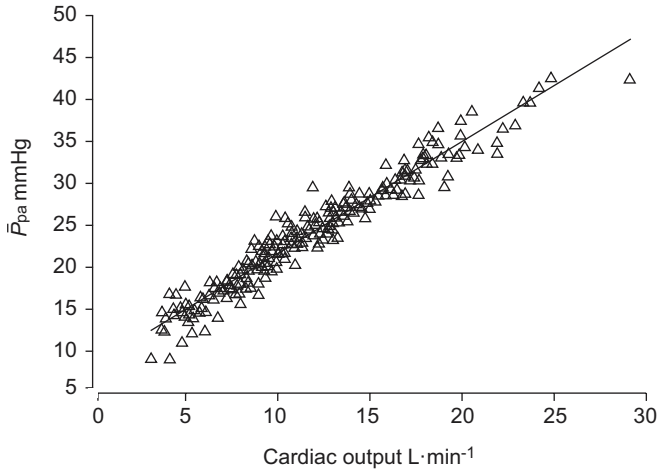

FIGURE 3. Poon-adjusted mean pulmonary artery pressure ($\bar{P}_{pa}$) as a function of cardiac output measurements at rest and at progressively increased workloads in 25 healthy subjects. The slope was 1.32 mmHg·min^{-1}·L^{-1} and the intercept was 8.2 mmHg.

highest level of exercise [17] and log-log transform enhancement [18]. There could also have been a problem of decreased accuracy of Q measurements by Doppler echocardiography at exercise in our study. However, no patterns were identified in previously reported *in vivo* or *in vitro* multipoint $\bar{P}_{pa}$–Q relationships [5, 18], and could not be identified in a recent study which confirmed the clinical relevance of exercise-induced pulmonary hypertension in scleroderma patients [19].

Pulmonary vascular pressure–flow relationships have been, until now, best described by a linear approximation, with an extrapolated pressure intercept either equal to or slightly higher than resting P_{la} [4, 5]. This is explained by the combined effects of resistance and distension. Previous analysis of invasive haemodynamic measurements during exercise in normal subjects showed a slope of $\bar{P}_{pa}$–Q of 0.94 ± 9.4 mmHg·min^{-1}·L^{-1} with an extrapolated pressure intercept of 8.2 ± 7.9 mmHg in 63 young adults, and a slope of 2.54 ± 0.77 mmHg min^{-1}·L^{-1} with a pressure intercept of 2.3 ± 5.4 mmHg in 14 older subjects [4]. The average slope of $\bar{P}_{pa}$–Q of 1.37 mmHg·min^{-1}·L^{-1} in our study in more middle aged subjects agrees with these previous invasive measurements.

Our results also support the notion that multipoint $\bar{P}_{pa}$–Q relationships are slightly curvilinear [5]. *In vitro* studies have shown that pulmonary arterial distensibility α is constant at a value of 0.02 mmHg^{-1} from one species to another, including humans [6]. In other words, on average and over the linear portion of the pressure–diameter curve, normal resistive pulmonary arteries distend by 2% of their initial diameter for each mmHg increase in transmural pressure. The same α-value of 0.02 mmHg^{-1} has been recalculated from invasive pressure and flow measurements [6]. Our noninvasive measurements allowed for the calculation of a distensibility α-coefficient calculation that was strikingly similar, which does not prove, but strongly supports, their validity. Given the complexity of the equation used to calculate α, such a close agreement would indeed be very unlikely to occur by chance.

The distensibility coefficient α has been shown by invasive studies to decrease with ageing and with chronic hypoxic exposure [6]. Log α was recently reported to be inversely correlated to pulmonary arterial pulse pressure as an independent prognostic factor in severe pulmonary hypertension [20]. The clinical relevance of noninvasive determinations of pulmonary arterial distensibility warrants further investigations.

Exercise-stress echocardiography in clinical practice is often limited to measurements of peak $P_{pa,sys}$ and workload. The present results underscore the importance of cardiac output measurements, which are essential for describing incremental resistance, considering that stroke volume and Q vary greatly at a given workload.

We calculated $\bar{P}_{pa}$ from $P_{pa,sys}$ on the basis of a previously validated equation derived from tight correlations demonstrated with invasive pulmonary haemodynamic measurements at rest and at exercise, with and without different types of pulmonary vascular disease [11, 21]. It is interesting that derived $\bar{P}_{pa}$ calculations still led to realistic pulmonary arterial distensibility estimates.

In the present study, P_{la} estimated by the E/E′ ratio did not increase with exercise, which seems to disagree with previous invasive studies [4, 5]. Previous validation studies compared E/E′ with wedged pulmonary artery pressure (P_{pa}) [12, 22]. Wedged P_{pa} measurements in these studies were actually performed with balloon-tipped pulmonary artery catheters, yielding balloon-occluded P_{pa}, as previously shown, to be an excellent surrogate measurement of P_{la} in zone III perfused lungs in a variety of pathophysiological circumstances [5]. However, estimation of wedged P_{pa} or P_{la} from E/E′ has been reported to suffer from a large confidence interval estimated by an SD of ±3.8 mmHg on the mean rest [12], and there is an absence of linear relationship between E/E′ and wedged P_{pa} at exercise [22]. These are important limitations to the estimation of P_{la} at exercise in our study, and may have accounted for the absence of the expected decreased in PVR. It is of interest that

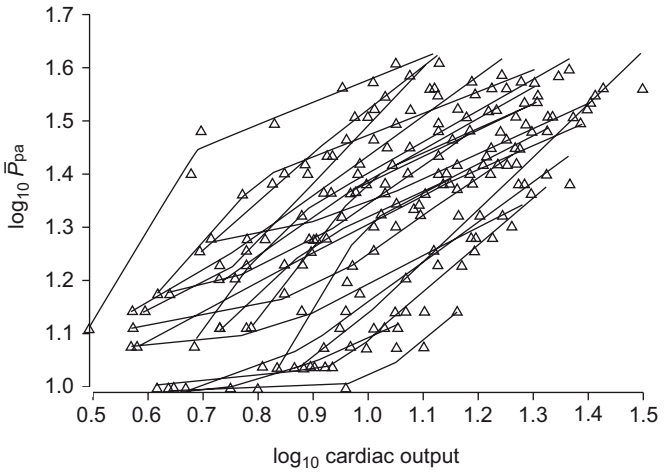

FIGURE 4. Log mean pulmonary artery pressure ($\bar{P}_{pa}$) as a function of log cardiac output measurements at rest and at progressively increased workloads in 25 healthy subjects. Takeoff and plateau patterns can be identified in 14 and 11 subjects, respectively.

this limitation did not affect distensibility calculations, in keeping with the notion of $\bar{P}_{pa}$ as the major determinant of pulmonary arterial compliance at the site of resistance [5]. However, more recent invasive haemodynamic measurements in physically fit normal subjects indicate that at the average workoad of 170 W and Q of 20 L·min^{-1} reached in our study, P_{la} may remain unchanged compared with supine resting baseline [16]. Absence of change or only slight increase in P_{la} would be undetected by changes in E/E′. Uncertainty on the estimation of P_{la} probably also contributed to the observed large confidence intervals on the distensibility α-calculations.

Exercise echocardiography takes time (on average 40 min per subject in our study), is technically demanding and requires considerable training and experience. This is why, in some centres, measurements are often not performed during exercise but instead immediately after exercise. Our finding that $P_{pa,sys}$ has a rapid but variable return to resting baseline suggests that this approach would have a poor reliability to characterise exercise-induced pulmonary hypertension.

Doppler echocardiography has been considered less than adequate for the study of the pulmonary circulation [1–3]. Concerns include poor quality control and technical demands, an excessive proportion of false positives and negatives as a screening tool for pulmonary hypertension [23], and sub-optimal recovery rates of tricuspid regurgitant jets, particularly in normal subjects or in patients with obstructive lung diseases [24]. However, a large scale collaborative European study recently demonstrated how exercise echo-Doppler measurements of $P_{pa,sys}$ can be used to identify normal and abnormal pulmonary vascular reactivity in family members of patients with idiopathic PAH, especially in carriers of the bone morphogenetic protein-2 mutation [25]. In that multicenter study, only 15% of subjects had inadequate tricuspid velocity signals. We have demonstrated that a high level of training and dedication allows for 88% of recovery of good-quality signals for the study of pulmonary haemodynamics in normal subjects, at rest and during exercise.

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STATEMENT OF INTEREST

None declared.

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REFERENCES

1 McLaughlin VV, Archer SL, Badesch DB, *et al.* ACCF/AHA 2009 expert consensus document on pulmonary hypertension: a report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents and the American Heart Association. *Circulation* 2009; 119: 2250–2294.

2 Galie N, Torbicki A, Barst R, *et al.* Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. *Eur Heart J* 2004; 25: 2243–2278.

3 Tolle JJ, Waxman AB, Van Horn TL, *et al.* Exercise-induced pulmonary arterial hypertension. *Circulation* 2008; 118: 2183–2189.

4 Reeves JT, Dempsey JA, Grover RF. Pulmonary circulation during exercise. *In*: Weir EK, Reeves JT, eds. Pulmonary Vascular Physiology and Physiopathology. New York, Marcel Dekker, 1989; pp. 107–133.

5 Naeije R. Pulmonary vascular function. *In*: Peacock AJ, Rubin LJ, eds. Pulmonary Circulation: Diseases and their Treatment. 2nd Edn. London, Arnold, 2004; pp. 3–13.

6 Reeves JT, Linehan JH, Stenmark KR. Distensibility of the normal human lung circulation during exercise. *Am J Physiol Lung Cell Mol Physiol* 2005; 288: L419–L425.

7 Grunig E, Janssen B, Mereles D, *et al.* Abnormal pulmonary artery pressure response in asymptomatic carriers of primary pulmonary hypertension gene. *Circulation* 2000; 102: 1145–1150.

8 Bossone E, Rubenfire M, Bach DS, *et al.* Range of tricuspid regurgitation velocity at rest and during exercise: implications for the diagnosis of pulmonary hypertension. *J Am Coll Cardiol* 1999; 33: 1662–1666.

9 Christie J, Sheldahl LM, Tristani FE, *et al.* Determination of stroke volume and cardiac output during exercise: comparison of two-dimensional and Doppler echocardiography, Fick oximetry, and thermodilution. *Circulation* 1987; 76: 539–547.

10 Yock PG, Popp RL. Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation. *Circulation* 1984; 70: 657–662.

11 Chemla D, Castelain V, Humbert M, *et al.* New formula for predicting mean pulmonary artery pressure using systolic pulmonary artery pressure. *Chest* 2004; 126: 1313–1317.

12 Nagueh S, Middleton K, Kopelen H, *et al.* Doppler tissue imaging: a noninvasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. *J Am Coll Cardiol* 1997; 30: 1527–1533.

13 Poon CS. Analysis of linear and mildly nonlinear relationships using pooled subject data. *J Appl Physiol* 1988; 64: 854–858.

14 Orr GW, Green HJ, Hughson RL, *et al.* A computer linear regression model to determine ventilatory anaerobic threshold. *J Appl Physiol* 1982; 52: 1349–1352.
15 Winer BJ, Brown DR, Michels KM, eds. Statistical Principles in Experimental Design. 3rd Edn. New York, Mc Graw-Hill, 1991; pp. 220–283.
16 Stickland MK, Welsh RC, Petersen SR, *et al.* Does fitness level modulate the cardiovascular hemodynamic response to exercise? *J Appl Physiol* 2006; 100: 1895–1901.
17 Wong YY, van der Laarse WJ, Vonk-Noordegraaf A. Reduced systemic oxygen extraction does not prove muscle dysfunction in PAH. *Med Sci Sports Exerc* 2008; 40: 1554.
18 Naeije R, Melot C. On exercise-induced pulmonary hypertension. *Circulation* 2009; 120: e283.
19 Kovacs G, Maier R, Aberer E, *et al.* Borderline pulmonary arterial pressure is associated with decreased exercise capacity in scleroderma. *Am J Respir Crit Care Med* 2009; 180: 881–886.
20 Blyth KG, Syyed R, Chalmers J, *et al.* Pulmonary arterial pulse pressure and mortality in pulmonary arterial hypertension. *Respir Med* 2007; 101: 2495–2501.
21 Syyed R, Reeves JT, Welsh D, *et al.* The relationship between the components of pulmonary artery pressure remains constant under all conditions in both health and disease. *Chest* 2008; 133: 633–639.
22 Talreja DR, Nishimura RA, Oh JK. Estimation of left ventricular filling pressure with exercise by Doppler echocardiography in patients with normal systolic function: a simultaneous echocardiographic-cardiac catheterization study. *J Am Soc Echocardiogr* 2007; 20: 477–479.
23 Arcasoy SM, Christie JD, Ferrari VA, *et al.* Echocardiographic assessment of pulmonary hypertension in patients with advanced lung disease. *Am J Respir Crit Care Med* 2003; 167: 735–740.
24 Fisher MR, Forfia PR, Chamera E, *et al.* Accuracy of Doppler echocardiography in the hemodynamic assessment of pulmonary hypertension. *Am J Respir Crit Care Med* 2009; 179: 615–621.
25 Grünig E, Weissmann S, Ehlken N, *et al.* Stress-Doppler-echocardiography in relatives of patients with idiopathic and familial pulmonary arterial hypertension: results of a multicenter European analysis of pulmonary artery pressure response to exercise and hypoxia. *Circulation* 2009; 119: 1747–1757.