Published in final edited form as:

Anesth Analg. 2016 May ; 122(5): 1280–1286. doi:10.1213/ANE.0000000000001215.

Right Ventricular Longitudinal Strain is Depressed in a Bovine Model of Pulmonary Hypertension

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The authors declare no conflicts of interests.

Reprints will not be available from the authors.

Preliminary results of this work were presented in abstract form at the American Heart Association 2012 Scientific Sessions.

Disclosures

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Attestation: Karsten Bartels approved the final manuscript. Karsten Bartels attests to the integrity of the original data and the analysis reported in this manuscript.

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Contribution: Kurt R. Stenmark secured funding for the study, contributed to the study design, conduct of the study, data collection, data analysis, and manuscript preparation.

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Abstract

Background—Pulmonary hypertension and resulting right ventricular (RV) dysfunction are associated with significant perioperative morbidity and mortality. Although echocardiography permits real-time, noninvasive assessment of RV function, objective and comparative measures are underdeveloped and appropriate animal models to study their utility are lacking. Longitudinal strain analysis is a novel echocardiographic method to quantify RV performance. Here, we hypothesized that peak RV longitudinal strain would worsen in a bovine model of pulmonary hypertension compared to control animals.

Methods—Newborn Holstein calves were randomly chosen for induction of pulmonary hypertension versus control conditions. Pulmonary hypertension was induced by exposing animals to 14 days of hypoxia (equivalent to 4,570 m above sea level or 430 mmHg barometric pressure). Control animals were kept at ambient pressure/normoxia. At the end of the intervention, transthoracic echocardiography was performed in awake calves. Longitudinal wall strain was analyzed from modified apical four-chamber views focused on the RV. Comparisons between measurements in hypoxic versus non-hypoxic conditions were performed using Student's t-test for independent samples and unequal variances.

Results—Following 14 days at normoxic versus hypoxic conditions, 15 calves were examined with echocardiography. Pulmonary hypertension was confirmed by right heart catheterization and associated with reduced RV systolic function. Mean systolic strain measurements were compared in normoxia-exposed animals (n=8) and hypoxia-exposed animals (n=7). Peak global systolic longitudinal RV strain after hypoxia worsened compared to normoxia (−10.5 % versus −16.1 %, p=0.0031). Peak RV free wall strain also worsened after hypoxia compared to normoxia (−9.6 % versus −17.3 %, p=0.0031). Findings from strain analysis were confirmed by measurement of tricuspid annular peak systolic excursion.

Conclusions—Peak longitudinal RV strain detected worsened RV function in animals with hypoxia-induced pulmonary hypertension compared to control animals. This relationship was demonstrated in the transthoracic echocardiographic four-chamber view independently for the RV

free wall as well as for the combination of the free and septal walls. This innovative model of bovine pulmonary hypertension may prove useful to compare different monitoring technologies for assessment of early events of RV dysfunction. Further studies linking novel RV imaging applications with mechanistic and therapeutic approaches are needed.

Introduction

Pulmonary hypertension and subsequent right ventricular (RV) dysfunction remain a significant challenge for the perioperative clinician.^{1–5} Indeed, in a retrospective study of pulmonary hypertension patients undergoing noncardiac surgery, 42 % suffered significant morbidity and 7 % died within 30 days postoperatively.⁶ Similarly, after coronary artery bypass surgery pulmonary hypertension was associated with a more than 2-fold increased mortality.⁷ Early intervention for treatment of RV dysfunction in the context of pulmonary hypertension is highly dependent on its prompt diagnosis. While echocardiography is a valuable tool to assess cardiac function, quantitative metrics to assess RV function are far less developed than for the left ventricle (LV).⁸ Accordingly, there is a great need to develop and validate echocardiographic measures in order to eventually enable rational echo-guided therapy of RV dysfunction.

Commonly, RV systolic function is described as normal versus mildly, moderately, or severely reduced based on qualitative visual impression.⁹ Reliance solely on such non-standardized assessments for RV systolic function are discouraged in recent guidelines.⁹ Alternative, quantitative approaches for estimation of RV function include RV fractional area change, RV ejection fraction, the rise of the RV to right atrial pressure gradient during systole (dP/dt), tricuspid annular plane systolic excursion (TAPSE), or the RV myocardial performance index (Tei-Index).¹⁰ Speckle tracking is a more recent technology, which permits quantification of regional myocardial strain. Peak longitudinal RV strain permits evaluation of RV contractile function in a quantitative fashion and independent of the ultrasound angle of incidence on the structure of interest.

Although declining RV strain has already been associated with poorer outcomes in clinical studies of pulmonary hypertension patients,¹¹ its utility to impact patient outcomes remains unproven.¹² Transformation of advanced RV echocardiographic imaging from a descriptive method into a tool to drive mechanism-based therapy will require large animal models that enable more seamless translation to clinical practice than is afforded by current rodent models.¹³ In a first step to establish such a model, we hypothesized that RV longitudinal systolic strain is depressed, reflecting declining RV performance, in a bovine model of hypoxia-induced pulmonary hypertension compared to control animals. In addition, we used cardiac magnetic resonance imaging (MRI) as a “gold standard” to determine RV systolic function in a second cohort of calves exposed to hypoxia versus normoxia.

Methods

Induction of pulmonary hypertension

All animal experiments were performed after Institutional Animal Care and Use Committee approval. Experiments conformed to the National Institutes of Health Guide for the care and

use of laboratory animals.¹⁴ Induction of pulmonary hypertension was performed as described previously.^{15,16} Briefly, 15 newborn male Holstein dairy calves were randomly chosen to be exposed to hypoxia or normoxia. Hypoxia was induced by nitrogen dilution or hypobaric hypoxia to the equivalent of 4,570 m above sea level or 430 mmHg for 14 days. Control animals were studied after spending 14 days at ambient pressure and oxygen levels.

Hemodynamic measurements

Hemodynamic assessments were made in awake animals as previously described.¹⁷ Briefly, for the hemodynamic measurements, an internal jugular venous introducer catheter was first placed with local anesthesia. Next, a balloon-tipped catheter was floated into the pulmonary artery via the introducer, and its position was confirmed through visual analysis of the transduced pressure waveform.

Echocardiography

Transthoracic echocardiography was performed in awake animals, manually restrained in lateral recumbency, with a General Electric Vivid 5 (General Electric Co., Cleveland, Ohio) echocardiography machine with concurrent three-lead electrocardiogram. To improve image quality, the animals were routinely shaved over the right and left chest. RV images were obtained using an apical RV-focused four-chamber view (Figure 1). Analysis of peak systolic RV strain was performed offline using a General Electric Echo Pacs® Version 3.0 software packet (Figure 2). Briefly, the RV endocardium was traced starting at the RV septal annulus, via the approximated RV apex, to the lateral annulus in the apical four-chamber view. Speckle tracking occurred in six segments of the RV myocardium and thickness of the tracked zone was adjusted as needed to include only myocardium. Negative strain values reflect tissue shortening/contraction, while positive strain values indicate tissue lengthening/relaxation. Hence, a less negative strain value indicates poorer RV systolic function compared to a more negative strain value.¹⁸ TAPSE¹⁹ was calculated from the maximum systolic displacement of the lateral basal segment of the tricuspid annulus.

Cardiac MRI

MRI examinations were performed in mechanically ventilated calves under general anesthesia with isoflurane (1.5–2 %). Subambient oxygen concentrations (10 %) were used for the pulmonary hypertension calves. A 1.5 Tesla GE Signa MRI scanner (General Electric Healthcare; Milwaukee, WI) was used. Cardiac functional imaging was performed with retrospective pulse gating, using segmented steady-state free precession (SSFP) technique and included a vertical long axis, horizontal short-axis, and short-axis stack. Typical scan parameters were Field-Of-View = 40–45 cm, slice thickness = 8 mm, Number of Excitations = 4 (free breathing), Echo Time/Repetition Time= 1.6/3.9, in-plane resolution 1.4–1.6 mm. Temporal resolution was 20–30 msec. RV volumes and ejection fractions were assessed via standard planimetry techniques using computer software (QMASS v.7.5, Medis Medical Imaging Systems, Netherlands).

Blinding and randomization

All echocardiographic assessments were made in duplicate by independent echocardiographers blinded to the hypoxia/normoxia group allocation. Analysis was performed in a random order by attaching a randomly generated number (Excel, Microsoft Co, Redmond, WA) to the animal ID# and then generating a list ordered according to the value of the associated random number.

Statistical analysis

An observed relative reduction of global RV longitudinal strain of 34.5% reported in a clinical study comparing pulmonary hypertension to control patients informed the sample size estimate.²⁰ For the primary outcome of this study (RV peak longitudinal strain) a sample size of 14 (n=7 per group), would yield 78% power to detect a 30% relative difference in strain and 95% power for to detect a 40% relative difference respectively.²¹ Continuous outcome variables for groups with n=3 were analyzed using unpaired Student's t-test with a two-tailed P-value of < 0.05 considered significant.²² For groups with n = 7, the Shapiro-Wilk test was used to assess normal distribution of residuals. Differences in means for continuous variables were assessed with Student's t-test for independent samples and unequal variances. The probability that a random observation from the hypoxia group was different than a random observation from the normoxia group was also assessed using the independent samples Mann-Whitney U test.²³ The Wilcoxon-Mann-Whitney P-values are exact.

For categorical variables, statistical significance was determined using Fisher's Exact Test. Correlation of mean pulmonary artery pressures with echocardiographic parameters was assessed using Pearson's correlation. Statistical significance was assumed at a two-tailed P-value of less than 0.05. Inter-rater reliability was assessed by Intraclass Correlation Coefficient (ICC) reporting confidence intervals (CI). SPSS® Version 23, IBM Corporation, Armonk, NY was used for statistical analysis.

Results

Exposure of newborn Holstein calves to two weeks of hypoxia compared to ambient oxygen levels has previously been shown to result in markedly reduced arterial oxygen content: In hypoxic versus normoxic calves means and standard deviations for systemic arterial oxygen partial pressures were 29 (5) mmHg versus 66 (4) mmHg, for oxygen saturation 56 (14) % versus 88 (4) %.¹⁷ Hypoxia-exposed calves in our study reliably developed pulmonary hypertension without significant tricuspid regurgitation. Right heart catheterization demonstrated a mean pulmonary artery pressure of 26 mmHg in normoxia compared to 111 mmHg in hypoxic animals (P=0.0003) as shown in Table 1. TAPSE was reduced in animals exposed to hypoxia (Table 1), consistent with RV dysfunction. Qualitative assessments of RV systolic function using conventional 2-dimensional echocardiography without quantitative measurements by two blinded echocardiographers are reported in Table 2. RV function was found to be qualitatively reduced in the hypoxic compared to the normoxic group (Rater 1: P=0.0427; Rater 2: P=0.0014). Septal flattening/distortion was more prevalent in the hypoxic group (P=0.0101 for both raters).

The results of RV strain analysis in normoxic and hypoxic animals are shown in Figure 3 and 4. Peak longitudinal RV strain, as evaluated from analysis of transthoracic echocardiographic images using a four-chamber view, was depressed in calves exposed to two weeks of hypoxia as compared to normoxic calves. This relationship held true both when analyzing combined peak systolic longitudinal strain in the free and septal wall of the RV (Figure 3), as well as for dedicated analysis of strain in the free wall only (Figure 4). Peak systolic strain rates in hypoxic versus normoxic animals were not statistically different: -1.8 (0.4)/s versus -1.5 (0.4) /s ($P=0.28$) for combined and -2.0 (0.4) /s versus -1.7 (0.5) /s ($P=0.18$) for free wall only analysis. Benchmark parameters of RV and LV function were assessed using cardiac MRI in a separate cohort of normoxia- and hypoxia-exposed animals ($n=3$ each), and results are shown in Table 3.

The Pearson correlation coefficient between RV strain and mean pulmonary artery pressure was 0.65 ($P=0.0086$) and 0.66 ($P=0.0076$) for combined and free wall-only strain respectively. The correlation coefficient between the average qualitative assessment of RV systolic function by the two raters and RV strain was 0.73 ($P=0.0020$) for combined and 0.68 ($P=0.0049$) for free wall-only strain.

Inter-rater reliability was assessed via ICC using a 98.3% CI to account for comparisons of three correlated endpoints: ICC=0.88 (98.3% CI: 0.53 to 0.97) for combined RV free and septal wall peak longitudinal strain, ICC=0.94 (98.3% CI: 0.78 to 0.98) for RV free wall peak longitudinal strain, and ICC=0.88 (98.3% CI: 0.53 to 0.97) for qualitative analysis of RV function. Conservatively applying ICC cutoffs to the lower limit of the 98.3% CI, we used the following scale for assessment of agreement: less than 0.4 indicates poor agreement between raters, 0.40–0.59 indicates fair agreement, 0.60–0.74 indicates good agreement, and 0.75–1.0 indicates excellent agreement. For RV free wall peak longitudinal strain agreement was excellent, for combined RV free and septal wall peak longitudinal strain as well as for qualitative analysis of RV function agreement was fair.

Discussion

In an established and well-validated bovine model of hypoxia-induced pulmonary hypertension, transthoracic echocardiography was successfully used to detect reduced RV function. In addition to conventional, qualitatively assessed echocardiographic RV systolic function assessments, peak systolic longitudinal strain was impaired in calves exposed to hypoxia.

Echocardiographic longitudinal strain encompasses all segments of the RV depicted in a four-chamber view as opposed to only quantifying the motion of the lateral annulus, as is usually done for TAPSE. In this sense, longitudinal RV strain analysis combines advantages of conventional 2-dimensional echocardiographic global RV function assessment using multiple segments with the quantitative measurement that is obtained from TAPSE. Our hypothesis that calves exposed to two weeks of hypoxia would exhibit depressed peak longitudinal RV strain was confirmed. Reduced RV ejection fraction found in anesthetized hypoxic animals using cardiac MRI is consistent with reduced systolic function detected from peak RV longitudinal strain analysis in awake animals. This work represents the

application of a novel quantitative echocardiographic approach to RV evaluation in an innovative large animal model of progressive pulmonary hypertension induced by exposure to chronic hypoxia.

Although the RV is commonly described as “crescent-” or “boot-shaped,” its structure is too complex to be condensed into a single attribute.⁸ Indeed, in an attempt to address the limited knowledge on quantitative assessment of RV function compared with LV function, the National Heart Lung and Blood Institute convened a working group to advance our knowledge on how to measure and detect RV dysfunction.²⁴ For the assessment of RV function, 2-dimensional longitudinal strain appears reproducible and feasible.²⁵ Strain is defined as the change in length of the myocardium over time compared to its baseline length at end-diastole.²⁵ Because the septum is often assumed to contribute mostly to LV systolic function, RV longitudinal strain can be derived from the free wall alone or from both the free wall and the septum.²⁶

Other large animal models to evaluate RV strain often rely on surgical banding of the pulmonary venous drainage²⁷ or pulmonary artery banding²⁸ to induce pulmonary hypertension. Aguero et al.²⁷ using a pig model of pulmonary vein banding via thoracotomy similarly found worsened RV longitudinal strain in the pigs with induced pulmonary hypertension. By contrast, our model used hypoxia-induced pulmonary hypertension and avoided confounding traumatic inflammatory responses commonly observed after even minor animal surgery.²⁹ Hence, this approach may be particularly translatable to patients with congenital heart disease, chronic obstructive pulmonary disease, or alveolar hypoventilation collectively arising from Group 3 pulmonary hypertension,³⁰ which is the most numerically abundant form of pulmonary hypertension.

Although worsened RV longitudinal strain has been associated with worse outcomes in pulmonary hypertension and heart failure, including in patients who underwent LV assist device surgery,^{31–34} the most sensitive and specific method for detection of RV dysfunction remains unknown. Our model is ideally suited to observe the early onset and temporal progression of RV failure that can be monitored using noninvasive indices of RV function such as RV longitudinal strain. Finally, this neonatal model will be uniquely relevant to study pediatric pulmonary hypertension, where altered hemodynamic load occurs in the developmental context of the transition of the RV to postnatal life.³⁵

Limitations

The strain measurements were performed in spontaneously breathing, awake animals. Given that echocardiographic strain measurements are load-dependent, positive pressure ventilation as was required for cardiac MRI may have altered loading conditions. In addition, normal strain values in humans and cows differ: A meta-analysis of strain measurements in children reported a normal value of –29.03% for RV global longitudinal strain.³⁶ This compares to –16.1% for combined and –17.3% for free wall peak longitudinal RV strain in normoxic neonatal calves in our study. Further, this study did not include all modalities for assessment of RV function. While we did measure pulmonary artery pressures and assessed RV systolic function qualitatively using 2-dimensional echocardiography, TAPSE, and cardiac MRI, we did not include measures such as radial strain,³⁷ myocardial performance index, or 3-

dimensional echocardiographic measurements to evaluate RV function. This indeed is a critical need and, therefore, will be the focus of a future study, where we will compare performance of different echocardiographic indices of RV assessment at different time points during induction of pulmonary hypertension. Our model is ideally suited for such a study because the exposure to hypoxia induces pulmonary hypertension gradually.

In conclusion, we found peak longitudinal RV strain obtained via transthoracic echocardiography to be depressed in a bovine model of induced pulmonary hypertension. Reflective of the RV response to higher pulmonary vascular resistance and elevated pulmonary artery pressures, longitudinal strain is an objective measure of RV function that is independent of the ultrasound beam angle of incidence. Therefore, it may be especially advantageous in the perioperative environment. Identifying the most sensitive and specific tools for assessment of RV function at an early stage of disease may prove useful when testing interventions to avoid or reverse RV failure.

Acknowledgments

The authors thank Dr. William Henderson, Ph.D., M.P.H., Professor, Department of Biostatistics and Informatics, University of Colorado School of Public Health for assistance with the statistical analysis.

Funding: This work was supported by National Institutes of Health (NIH) grants R01-HL114887, P01-HL014985, and R01-HL125827 to KRS.

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Figure 1. Transthoracic echocardiography in a bovine model of pulmonary hypertension
A two-week-old Holstein calf undergoing awake transthoracic echocardiography with concurrent three-lead electrocardiography in the left lateral decubitus position (this image is from an ongoing study using a General Electric Vivid 7 echocardiography machine). To improve echocardiographic image quality, hair has been shaved over the right and left chest.

Figure 2. Right ventricular (RV) strain analysis

Strain software (General Electric Echo Pacs® Version 3.0) tracks the RV myocardium in the four-chamber view. The upper left quadrant depicts the two-dimensional cine-loop with the tracked region of interest. The right side of the image shows the analysis window. Strain (extent of tissue deformation in %) is represented on the y-axis. Tissue shortening is assigned a negative strain value. Time is represented on the x-axis. Lines depicting longitudinal strain over time are color-coded according to the region of interest defined in the cine-loop image. The dotted line represents an average of all segments. The left lower quadrant depicts an M-mode heat map of the strain values depicted over time and according to their color-code. Segments in red reflect higher contractility. AVC = aortic valve closure.

Figure 3. Comparison of peak right ventricular (RV) longitudinal strain averaged from the RV free wall and septal wall in hypoxic and normoxic calves

In an RV-focused four-chamber view peak longitudinal RV strain was depressed in the hypoxic (n=7) compared to the normoxic (n=8) group (95% CI 2.6% to 8.6%). Statistical significance for continuous variables was determined using Student's t-test for independent samples and unequal variances. Shapiro-Wilk test to assess normal distribution of residuals revealed P=0.3437. The Mann-Whitney U test revealed P=0.037.

Figure 4. Comparison of peak RV longitudinal strain averaged from RV free wall-only segments (septal wall excluded) in hypoxic and normoxic calves

In an RV-focused four-chamber view peak longitudinal RV strain was depressed in the hypoxic (n=7) compared to the normoxic (n=8) group (95% CI 3.2% to 12.2%). Statistical significance for continuous variables was determined using Student's t-test for independent samples and unequal variances. Shapiro-Wilk test to assess normal distribution of residuals revealed P=0.2100. The Mann-Whitney U test revealed P=0.012.

Measurement of pulmonary artery pressures (PAP) and tricuspid annular plane systolic excursion (TAPSE).

Table 1

	Normoxia (n=8)	Hypoxia (n=7)	Student t P-value	Student t 95% CI	Mann-Whitney P-value
Systolic PAP (mmHg)	40 (9)	143 (51)	0.0015	56 to 150	0.0003
Diastolic PAP (mmHg)	15 (2)	82 (26)	0.0005	43 to 92	0.0003
Mean PAP (mmHg)	26 (4)	111 (31)	0.0003	57 to 115	0.0003
TAPSE (mm)	10.2 (2.7)	7.2 (2.0)	0.0289	-5.6 to -0.4	0.0289

Hemodynamic measurements were obtained using a pulmonary artery catheter in Holstein calves exposed to two weeks of normoxia or two weeks of hypoxia. Data are displayed as means with standard deviations in brackets. Shapiro-Wilk test to assess normal distribution of residuals revealed $P=0.0002$ for systolic PAP, $P=0.0008$ for diastolic PAP, $P=0.0013$ for mean PAP, and $P=0.7572$ for TAPSE measurements. Statistical significance for comparisons between means for continuous variables was determined using Student's t-test for independent samples and unequal variances. The Wilcoxon-Mann-Whitney U test P-value is shown too.

Table 2

Qualitative assessment of right ventricular (RV) systolic function.

Rater 1		RV systolic function			
		Normal	Mildly reduced	Moderately reduced	Severely reduced
	Normoxia (n=8)	6	2	0	0
	Hypoxia (n=7)	1	3	3	0
		Septal flattening/distortion			
		No	Yes		
	Normoxia (n=8)	7	1		
	Hypoxia (n=7)	1	6		
Rater 2		RV systolic function			
		Normal	Mildly reduced	Moderately reduced	Severely reduced
	Normoxia (n=8)	7	1	0	0
	Hypoxia (n=7)	0	4	3	0
		Septal flattening/distortion			
		No	Yes		
	Normoxia (n=8)	7	1		
	Hypoxia (n=7)	1	6		

Using conventional 2D transthoracic echocardiography RV systolic function and septal morphology was qualitatively assessed in duplicate by two blinded echocardiographers in Holstein calves exposed to two weeks of normoxia or two weeks of hypoxia. Statistical significance for RV systolic function was determined between two rows and three columns using Fisher's Exact Test. Rater 1: $P=0.0427$; Rater 2: $P=0.0014$. Statistical significance for septal flattening/distortion was determined between two rows and two columns using Fisher's Exact Test. Rater 1: $P=0.0101$; Rater 2: $P=0.0101$.

Table 3

Cardiac Magnetic Resonance Imaging

	Normoxia (n=3)	Hypoxia (n=3)	P-value	95% CI
RV stroke volume (ml)	88 (3)	55 (13)	0.0127	−54 to −12
RV ejection fraction (%)	59 (4)	32 (16)	0.0469	−54 to −1
RV cardiac output (l/min)	7.5 (0.3)	5.6 (0.9)	0.0305	−3.4 to −0.3
LV stroke volume (ml)	92 (3)	51 (21)	0.0287	−74 to −7
LV ejection fraction (%)	51 (9)	29 (10)	0.0401	−43 to −2
LV cardiac output (l/min)	7.8 (0.3)	5.1 (1.7)	0.0566	−5.4 to 0.1

Cardiac magnetic resonance imaging was performed to determine right ventricular (RV) and left ventricular (LV) systolic function as well as right- and left-sided cardiac output in anesthetized Holstein calves after two weeks exposure to normoxia or hypoxia. Data are displayed as means of the individual measurements with standard deviations in brackets. Statistical significance was determined using an unpaired Student's t-test with a two-tailed P-value < 0.05. These P-values are uncorrected P-values based on normal distributions and equal variance even though there are N=3 in each of the two groups, and the endpoints are correlated.