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Preeclampsia and risk of maternal pulmonary hypertension at high altitude in Bolivia

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Abstract

Women with a history of preeclampsia (PE) have a greater risk of pulmonary arterial hypertension (PAH). In turn, pregnancy at high altitude is a risk factor for PE. However, whether women who develop PE during highland pregnancy are at risk of PAH before and after birth has not been investigated. We tested the hypothesis that during highland pregnancy, women who develop PE are at greater risk of PAH compared to women undergoing healthy highland pregnancies. The study was on 140 women in La Paz, Bolivia (3640m). Women undergoing healthy highland pregnancy were controls (C, $n = 70$; 29 ± 3.3 years old, mean ±SD). Women diagnosed with PE were the experimental group (PE, $n = 70$, 31 ± 2 years old). Conventional (B- and M-mode, PW Doppler) and modern (pulsed wave tissue Doppler imaging) ultrasound were applied for cardiovascular íííassessment. Spirometry determined maternal lung function. Assessments occurred at 35 ± 4 weeks of pregnancy and 6 ± 0.3 weeks after birth. Relative to highland controls, highland PE women had enlarged right ventricular (RV) and right atrial chamber sizes, greater pulmonary artery dimensions and increased estimated RV contractility, pulmonary artery pressure and pulmonary vascular resistance. Highland PE women had lower values for peripheral oxygen saturation, forced expiratory flow and the bronchial permeability index. Differences remained 6 weeks after birth. Therefore, women who develop PE at high altitude are at greater risk of PAH before and long after birth. Hence, women with a history of PE at high altitude have an increased cardiovascular risk that transcends the systemic circulation to include the pulmonary vascular bed.

Introduction

The leading cause of mortality in women is cardiovascular disease, accounting for a quarter of deaths in developed as well as low- to middle-income societies.[1] It is known that women with a history of pregnancy complications, such as preeclampsia, have more than double the risk of future cardiovascular disease compared to women with healthy pregnancies.[2,3] Therefore, diagnosis of complicated pregnancy offers a window of opportunity to identify and treat women at risk of future cardiovascular complications, with the hope of ameliorating the female mortality rate.

It is also established that pregnancy at high altitude is an independent risk factor for preeclampsia and increased maternal mortality.[4,5] This is a particular problem for Bolivia, where a quarter of its 11.6 million population lives in the cities of La Paz and El Alto above 3400 m or 11,000 ft. Together with other populations residing at altitude, this makes Bolivian women part of the largest single human group at risk for preeclampsia during pregnancy with an increased future risk of cardiovascular mortality. Preeclampsia in Bolivia, with an incidence as high as 14% in the city of La Paz, is the second greatest cause of maternal death.[6,7] It is estimated that 38% of maternal mortality in Bolivia stems from maternal deaths in La Paz and El Alto.[6,7] This comprises a maternal mortality rate in Bolivia of 346/100,000 live births, compared to just 7/100,000 in the UK.[8] Unsurprisingly rates for maternal mortality in Bolivia are the second highest in the Western hemisphere.[8]

While a link between preeclampsia and an increased risk of pulmonary arterial hypertension is established in sea-level pregnancies not only during pregnancy but also long after birth,[2,3] whether women who develop preeclampsia during highland pregnancy are also more likely to develop pulmonary arterial hypertension before and after pregnancy has not been studied. This is important as both preeclampsia and pulmonary hypertension independently contribute to

significant maternal mortality,[2,9] and the risk of both conditions are elevated at high altitude.[4,5] Therefore, highland women may be at much greater risk of preeclampsia and pulmonary hypertension, and both conditions may contribute to their much greater long-term cardiovascular risk and mortality. To address this, we carried out a prospective cohort study in Bolivia to test the hypothesis that during highland pregnancy, women who develop preeclampsia are at greater risk of pulmonary arterial hypertension compared to women undergoing healthy pregnancy at high altitude. The hypothesis was tested by determining, in the third-trimester, indices of right heart and pulmonary vascular function via echocardiography in Bolivian women undergoing healthy pregnancy and pregnancy diagnosed with preeclampsia. To determine if any differences persisted, assessments were repeated *ca.* 6 weeks after birth.

Methods

Data, materials and code disclosure statement

The data that support the findings of this study are available herein. Additional relevant data can be requested from the corresponding author.

Study cohort

Using a multi-institutional approach, women undergoing healthy pregnancies or pregnancies complicated by preeclampsia were recruited following their written consent from 5 hospitals with maternity units in La Paz. The institutions involved were the Hospital Materno-Infantil (Caja Nacional de la Salud), the Hospital de la Mujer, Hospital Nuestra Señora de La Paz, the Hospital Cotahuma, and the Centro de Salud Materno Infantil Tembladerani. Preeclampsia was diagnosed by obstetricians according to guidelines set by the Royal College of Obstetrics & Gynaecology in the UK and the American College of Obstetrics and Gynecology (Hypertension in Pregnancy Task Force, 2013).[10] These guidelines diagnose preeclampsia as new-onset hypertension (defined as a systolic blood pressure $\geq$ 140 mmHg and/or a diastolic blood pressure $\geq$ 90 mmHg), most often after 20 weeks of gestation and frequently near term. Abnormal blood pressure readings were usually accompanied by other symptoms including proteinuria, a low platelet count, impaired liver function, signs of kidney problems other than protein in the urine, pulmonary oedema and new-onset headaches or visual disturbances.

The inclusion criteria were that women should be highland residents for at least two generations, non-smoking mothers of normal BMI and no history of health complications carrying a singleton pregnancy and a maternal age between 18–45 years. The study recruited 208 pregnant women who satisfied the inclusion criteria at the time of enrolment. This initial cohort was divided into 110 control (C) and 98 preeclamptic (PE) women. Several women were lost during follow-up for reasons including change of mind, incomplete assessments, and infant death, to yield a final cohort of C ($n = 70$) and PE ($n = 70$) women.

Cardiovascular assessment

Maternal B-mode, M-mode, spectral pulsed-wave (PW) Doppler and spectral tissue Doppler imaging (PW-TDI) were applied at 35 (4.3) weeks of pregnancy and 6 (2.2) weeks after birth (median, IQR). One investigator (C.E.S.) performed all pre- and post-natal maternal ultrasound examinations using the same machine (VIVID, Model 2007, Digital Graphic Printer Up- D897). M-mode ultrasound was used for the assessment of left ventricular geometry and for right ventricular (RV) longitudinal contractility by measurement of the tricuspid annular plane systolic excursion (TAPSE).[11] B-mode imaging was performed for measurements of pulmonary artery size, RV and right atrial (RA) dimensions in systole and diastole. RV relative wall thickness was estimated as twice the RV free wall thickness divided by RV ventricular end-diastolic diameter. The PW Doppler technique was used to obtain Doppler signals from the RV outflow tract (RVOT) with measurements of peak velocity (Vmax), peak gradient and the velocity-time integral (VTI). The tricuspid regurgitation velocity (TR Vmax) and the RV-RA peak gradient were obtained for the calculation of the pulmonary artery pressure (PAP) by application of the modified Bernoulli equation 4x[TR Vmax]2+expected RA pressure.[12] Assessment of changes in vena caval collapse did not show any differences between groups. Therefore, a fixed RA pressure of 10 mmHg was used for the estimation of PAP in all women. Pulmonary vascular resistance was then estimated as $10 \times (\text{TR Vmax/VTIRVOT}) + 0.16$.[13,14] The PW-TDI technique was applied to derive RV myocardial time-intervals: isovolumetric contraction time (IVCT'), isovolumetric relaxation time (IVRT') and ejection time (FT'). These time-interval indices were then used to estimate the RV myocardial performance index (MPI'), calculated as MPI'=[(IVCT'+IVRT')]/ET'.[11] All echocardiographic measurements were performed following the American Society of Echocardiography guidelines for echocardiographic assessment of the right heart in adults and recommendations from the British Society of Echocardiography for the assessment of pulmonary hypertension.[11,12,14]

Maternal oxygen saturation and spirometry

At the same gestational age and post-partum period than echocardiography was performed, maternal oxygen peripheral saturation was measured by pulse oximetry (Criticare System INC, model 504-504P). Maternal respiratory function was determined by standard spirometry (Dymamic Spirometry, Microloop, Sensor Medics) following guidelines set by the American Thoracic Society and the European Respiratory Society.[15,16] The forced vital capacity (FVC), the forced expiratory volume in one second (FEV1), the FEV1/FVC ratio (the bronchial permeability index or the amount of air one can forcefully exhale from the lungs), and the forced expiratory flow at 50% (FEF50) and 75% (FEF75) of vital capacity were determined.

Statistical analyses

Wherever possible, scientists measuring outcomes were blinded to the maternal preeclampsia status by coding the patients. Data are presented as the mean ± SEM or SD. Distribution was verified with the Shapiro-Wilk test, and non-parametric data were log transformed to approximate a normal distribution where necessary. Data were analysed by Two-Way ANOVA with the Tukey *post hoc* test. For comparison of the percentage of cases with a TR Vmax > 2.8 m/s between Control and PE women, the Chi-Squared test and the Fisher's Exact test were used. For all comparisons, statistical significance was accepted when $P < 0.05$.

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Table 1. Maternal data of controls vs preeclamptic women before and after birth

Parameters	Before birth		After birth	
	Controls (n = 70)	Preeclampsia (n = 70)	Controls (n = 70)	Preeclampsia (n = 70)
MATERNAL AND INFANT CHARACTERISTICS				
Gestational or postnatal age at assessment, weeks	36 (3.3)	34 (5.0)*	6 (0.2)	6 (0.4)
Maternal age, years	29 (3)	31 (2)*		
Height, cm	154 ± 6	153 ± 6	154 ± 6	153 ± 6
Weight, kg	70.3 ± 9.0	73.8 ± 10.3	64.6 ± 9.2#	67.1 ± 10.7†
Maternal BSA, m^2	2.9 ± 0.2	2.9 ± 0.2	2.8 ± 0.2#	2.8 ± 0.3†
Maternal BMI, $kg.m^{-2}$	29.6 ± 6.2	31.5 ± 5.4	27.4 ± 4.2#	28.6 ± 5.1†
Gravidity, n	2.0 ± 0.1	2.4 ± 0.2		
Primiparous, %yes	10.3	17.2		
Infant gestational age, weeks			38.4 ± 2.1	35.2± 2.8*
Infant body weight at birth, g			3244 ± 422	2377 ± 863*
Infant body length, cm			49 ± 2	46 ± 5*
Infant sex (male), n (%)			40 (57%)	39 (56%)
Caesarean-section, n (%)	27 (39)	60 (85)*		
MATERNAL ECHOCARDIOGRAPHIC DATA				
CARDIAC GEOMETRY				
LV EDD, cm (PLAX)	4.5 ± 0.3	4.7 ± 0.64*	4.4 ± 0.2	4.7 ± 0.43*
LV ESD, cm (PLAX)	2.9 ± 0.3	3.0 ± 0.3*	2.9 ± 0.2	3.1 ± 0.3*
RV EDD, cm (PLAX)	1.4 ± 0.1	1.6 ± 0.2*	1.5 ± 0.2	1.6 ± 0.2*
RV ESD, cm (PLAX)	1.0 ± 0.1	1.1 ± 0.1*	1.0 ± 0.1	1.1 ± 0.1*
RV EDD, cm (4CHV)	2.3 ± 0.3	2.6 ± 0.2*	2.4 ± 0.3	2.6 ± 0.2*
RV EDL, cm (4CHV)	4.4 ± 0.2	4.7 ± 0.3*	4.3 ± 0.2	4.7 ± 0.3*
RV SI	0.53 ± 0.06	0.55 ± 0.05	0.55 ± 0.07	0.55 ± 0.04
RV wall thickness, cm	0.45 ± 0.05	0.44 ± 0.05	0.44 ± 0.05	0.45 ± 0.05
RV relative wall thickness (RWT), cm	0.39 ± 0.06	0.35 ± 0.05*	0.38 ± 0.06	0.35 ± 0.05*
RA ESD, cm	3.3 ± 0.3	3.6 ± 0.2*	3.3 ± 0.3	3.7 ± 0.3*
RA ESA, cm^2	1.6 ± 0.2	1.7 ± 0.2*	1.5 ± 0.2	1.7 ± 0.3*
Pulmonary artery (PA) dimension, cm	1.5 ± 0.1	1.7 ± 0.1*	1.5 ± 0.1	1.7 ± 0.1*
CARDIAC FUNCTION				
LV SF, %	35 ± 5	36 ± 3	35 ± 3	35 ± 3
LV EF, %	64 ± 4	65 ± 3	64 ± 3	64 ± 4
RV EF, %	63 ± 4	63 ± 3	63 ± 3	63 ± 3
TAPSE, cm	2.1 ± 0.2	2.4 ± 0.2*	2.1 ± 0.2	2.3 ± 0.2*†
RV MPI'	0.33 ± 0.10	0.35 ± 0.05	0.33 ± 0.01	0.36 ± 0.03*
PV Vmax, cm/s	0.95 ± 0.14	0.99 ± 0.12	0.96 ± 0.13	0.99 ± 0.14
PV mean PG, mmHg	1.99 ± 0.54	2.21 ± 0.59*	2.11 ± 0.60	2.20 ± 0.67
PV VTI, cm	19.4 ± 3.0	20.6 ± 3.2*	19.4 ± 2.8	20.0 ± 2.7

(*Continued*)

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Table 1. (*Continued*)

Parameters	Before birth		After birth	
	Controls (n = 70)	Preeclampsia (n = 70)	Controls (n = 70)	Preeclampsia (n = 70)
PULMONARY ARTERY PRESSURE				
TR Vmax, m/s	2.5 ± 0.2	2.9 ± 0.2*	2.5 ± 0.2	2.8 ± 0.3*†
RV-RA PG, mmHg	26 ± 3	35 ± 4*	26 ± 3	32 ± 6*†
Systolic PAP, mmHg	36 ± 3	45 ± 4*	35 ± 3	42 ± 6*†
PVR, mmHg·min/l	1.49 ± 0.22	1.61 ± 0.25*	1.47 ± 0.20	1.58 ± 0.21*

Data presented as mean ± SD or median (IQR). *, significant difference ($P < 0.01$) Preeclampsia *vs.* Controls before and after birth; †, significant differences within Preeclampsia group; #, significant differences within Control group. Abbreviations: BMI, body mass index; BSA, body surface area; EDD, end-diastolic dimension; EDL, end-diastolic length; EF, ejection fraction by Teicholtz method; ESA, end-systolic area; ESD, end-systolic dimension; LV, right ventricle; MPI', myocardial performance index by pulsed wave tissue Doppler; PA, pulmonary artery; PAP, pulmonary artery pressure; PG, peak gradient; PLAX, parasternal long axis view; RA, right atrium; RV, right ventricle; PVR, pulmonary vascular resistance; SI, sphericity index; SF, shortening fraction; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation; Vmax, maximal systolic velocities; VTI, velocity time integral; 4CHV, four-chamber view. RV-RA pressure gradient calculated by application of modified Bernoulli equation: 4 x $(TR\ Vmax)^2$.

Results

Maternal characteristics

Values for maternal age, height, weight, body mass index (BMI) and body surface area (BSA) were not different for control and PE women (Table 1). Similarly, gravidity and primiparity were similar in control and PE women (Table 1). Post-partum, all women lost weight, BMI and BSA (Table 1).

Infant characteristics

Relative to babies from control mothers, babies from PE mothers were born with a lower birth weight and shorter body length, by a greater incidence of Caesarean section, at an earlier stage of gestation (Table 1). The infant sex ratio was similar between groups (Table 1).

Cardiovascular assessment

Right ventricular and pulmonary geometry. Relative to controls, PE women had significantly greater values for RV end-systolic and end-diastolic diameter, and for RV end-systolic and end-diastolic length (Fig 1 A-D and Table 1). Relative to controls, PE women had significantly lower values for RV relative wall thickness (Fig 1 E and Table 1). Relative to controls, PE women had significantly greater values for RA end-systolic diameter and area (Fig 1 F & G and Table 1). Relative to controls, PE women had significantly greater values for pulmonary artery dimension (Fig 1 H and Table 1). Combined, the data show that relative to controls, PE women had enlarged RV and RA chamber sizes and pulmonary artery dimensions, and that these differences remained *ca.* 6 weeks after birth (Fig 1 A-H and Table 1).

Right ventricular and pulmonary artery function. Relative to controls, PE women had significantly greater values for the TAPSE and the maximal TR Vmax (Fig 2 A & B and Table 1). Combined, these data show that relative to controls, PE women had significantly increased RV global longitudinal contractility and blood flow to the lungs (Fig 2 A & B). Relative to controls, PE women also had significantly greater values for the RV to RA pressure gradient (RV-RA PG), the pulmonary valve velocity time integral (PV VTI), estimated mean PAP, estimated systolic PAP, and estimated mean pulmonary vascular resistance (PVR; Fig 2 C-F and Table 1). Most differences remained significant *ca.* 6 weeks after birth (Fig 2 A-F and Table 1). Values for TAPSE, TR Vmax, RA-RV PG and mean and systolic PAP were significantly lower in PE women after compared to before birth. However, these variables remained significantly elevated after birth relative to control women (Fig. 2 and Table 1). Mean estimated PAP values in control women were 35.8 ± 0.4 mmHg before birth and 34.9 ± 0.4 after birth. Mean estimated PAP values in PE women were 45.2 ± 0.5 mmHg before birth and 40.6 ± 0.9 after birth. The British Society of Echocardiography has provided guidelines for the estimation of pulmonary hypertension based on a flow chart that assesses the probability of pulmonary hypertension using values for the maximum TR Vmax and other echocardiographic indices of pulmonary hypertension. Values for the TR Vmax > 2.8 m/s coupled with two other positive echocardiography indices are classified as an intermediate to high probability of significant pulmonary hypertension in affected patients.[14] In our Bolivian study, the percentage of cases with a TR Vmax > 2.8 m/s with two other echocardiographic indices of pulmonary hypertension was greatly elevated in PE relative to Control pregnant women at high altitude before (80% *vs.* 6%) and ca. 6 weeks after (54% *vs.* 4%) birth (both $P < 0.05$).

Respiratory assessment

Relative to controls, PE women had significantly lower values for peripheral O_2 saturation (Fig 3 A). Relative to controls, PE women had significantly lower values for the FVC, the FEV1, the bronchial permeability index (FEV1/FVC), and the FEF50 and FEF75 (Fig 3 B-F). These differences, except FVC and FEF50, remained significant post-partum (Fig 3 A-F). In contrast, basal respiratory rate was similar between groups pre- and post-partum (Table 1). Values for peripheral O_2 saturation were lower in control and PE women after compared to before birth. Values for FEV1/FVC and FEF50 were lower in control women after compared to before birth (Fig. 3).

Discussion

In this prospective cohort clinical study in the high-altitude city of La Paz, Bolivia, the data show that relative to women undergoing healthy pregnancy at high altitude, women diagnosed with preeclampsia at high altitude had greater values for RV end-systolic and end-diastolic diameter and length. Women diagnosed with preeclampsia at high altitude also showed greater pulmonary artery dimension and maximal TR velocity, increased RV

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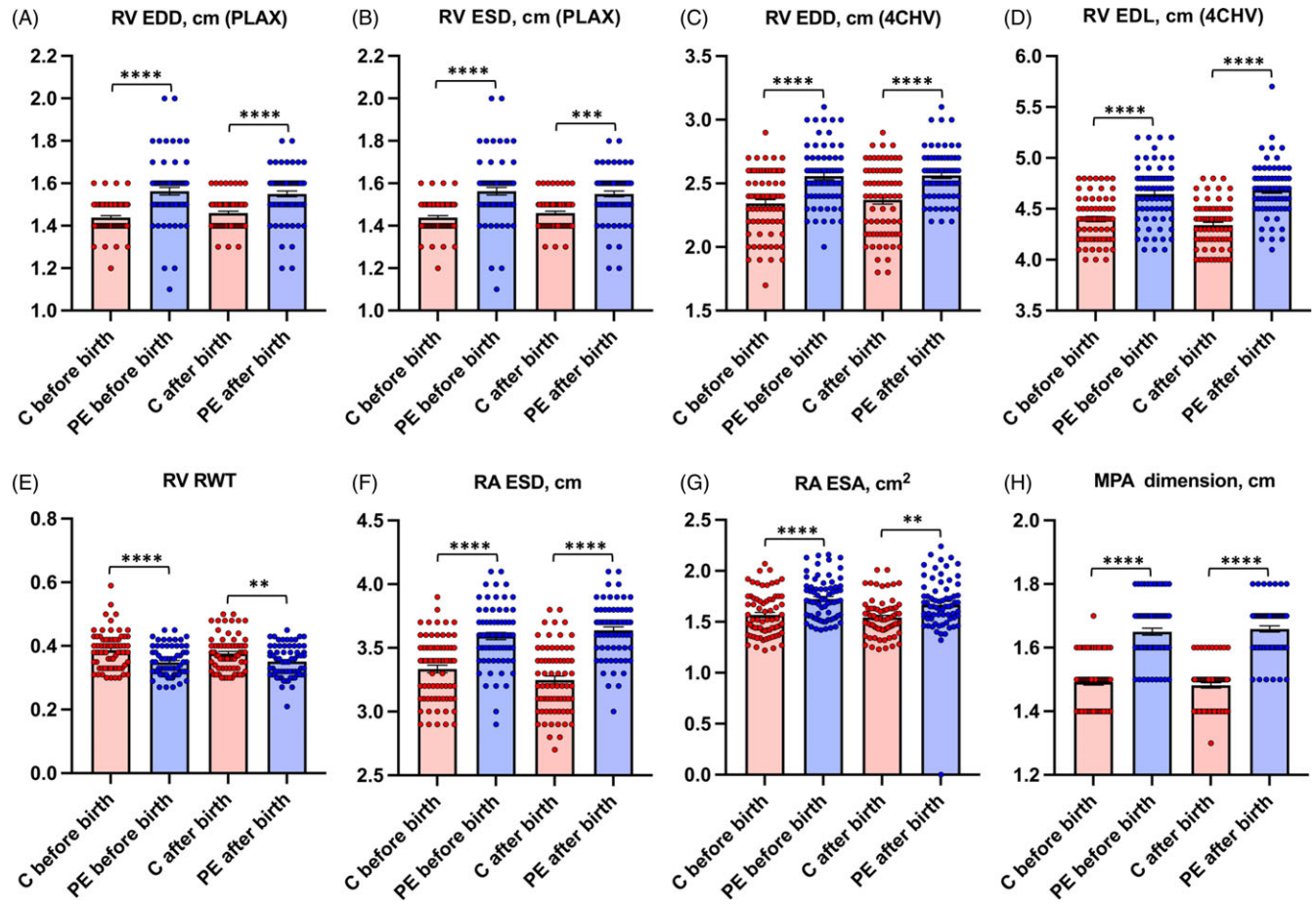

Figure 1. Maternal right heart geometry for controls and preeclamptic women. Column plots demonstrate significant perinatal alterations in right heart geometrical parameters in preeclamptic women [PE] *(in blue)* compared to healthy controls [C] *(in pink):* A. Right ventricular (RV) end-diastolic dimension (EDD) derived in the parasternal long axis view (PLAX); B. RV end-systolic dimension (ESD) obtained in PLAX; C. RV EDD obtained in the four-chamber view (4CHV); D. RV end-diastolic length (EDL) derived in 4CHV; E. RV relative wall thickness (RWT); F. Right atrial (RA) ESD; G. RA end-systolic area (ESA); H. Main pulmonary artery (MPA) dimension. Values are means ± SEM. The effect of preeclampsia was determined by two-way ANOVA. Significant differences between groups: ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$. RWT = (RV free wall thickness)^2/RV EDD. *Abbreviations:* EDD, end-diastolic dimension; EDL, end-diastolic length; ESA, end-systolic area; ESD, end-systolic dimension; MPA, main pulmonary artery; PLAX, parasternal long axis; RA, right atrium; RV, right ventricle; RWT, relative wall thickness; 4CHV, four-chamber view.

contractility and performance index. Women diagnosed with preeclampsia at high altitude also showed elevated estimated values for PAP and pulmonary vascular resistance. All outcomes measured in women diagnosed with preeclampsia at high altitude are consistent with pulmonary hypertension. Relative to women undergoing highland healthy pregnancy, women diagnosed with preeclampsia at high altitude also had lower values for peripheral oxygen saturation, forced expiratory flow and a reduced bronchial permeability index. All cardiorespiratory differences were independent of body weight, and most differences remained *ca.* 6 weeks after birth.

The present study was a multi-institutional clinical investigation, whose study population was pregnant women undergoing pregnancy at the high-altitude in the city of La Paz, Bolivia (3,625m; 11,893 ft). Patients were transferred from five different first, second and third-level health care centres with either a diagnosis of preeclampsia or normal pregnancy to undergo cardiorespiratory assessment at the Instituto Boliviano de Biologia de Altura (IBBA, 3,650m). All patients wished to participate in the study on a voluntary basis. All patients were born and had been permanent residents in La Paz for at least two generations.

The annular plane systolic excursion (TAPSE) is an established measure of global RV function, which describes load-dependent longitudinal contractility.[17,18] The TR Vmax is the most widely used variable by transthoracic echocardiography in patients with suspected pulmonary hypertension.[19] In presence of significant TR and normal contractility, TAPSE is increased.[18,19] An increase in the right ventricular-right atrial (RV-RA) pressure gradient is associated with pulmonary hypertension.[20] Pulmonary arterial hypertension results in increased RV afterload and increased tricuspid valve regurgitation.[21] Therefore, increased values for the pulmonary artery dimension, TR Vmax, TAPSE, together with enlarged RV and RA chamber sizes are all strongly indicative of pulmonary hypertension. The latter was further supported by calculation of a significant increase in the estimated PAP and in the pulmonary vascular resistance in Bolivian women diagnosed with preeclampsia at high altitude relative to those undergoing healthy pregnancies at high altitude. Values for the estimated mean PAP in women diagnosed with preeclampsia in our study were much greater than the elevated normal threshold for those living at high altitude as proposed by Leon Velarde *et al.*[22] In addition, according to Augustine *et al.*[14] and the flow chart provided in the British Society of Echocardiography guideline for the evaluation of pulmonary hypertension, calculation of the percentage of women with TR Vmax > 2.8 m/s coupled with two other echocardiography categories, also indicated a significantly greater probability of

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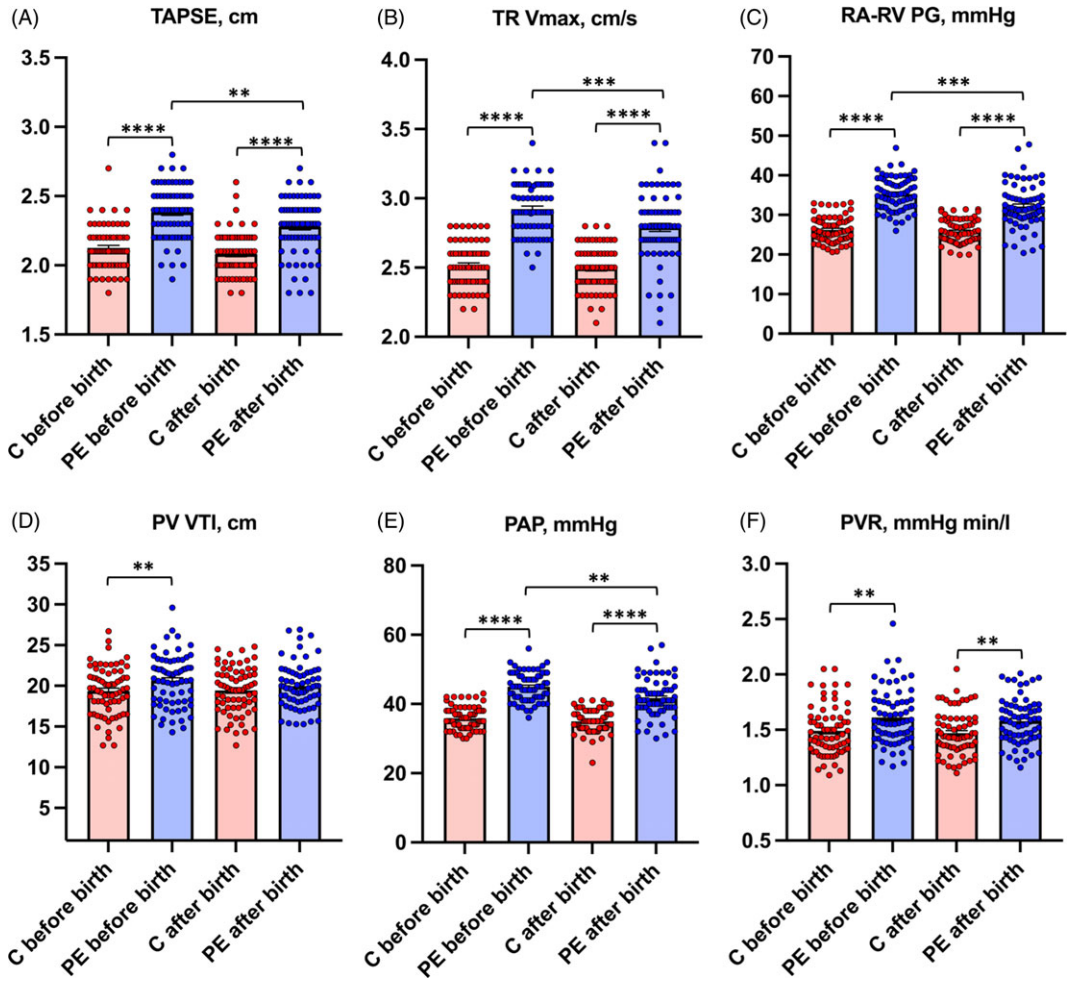

Figure 2. Maternal right heart function and indices of pulmonary hypertension for controls and preeclamptic women. Column plots demonstrate significant perinatal alterations in right ventricular functional indices in preeclamptic women [PE] *(in blue)* compared to healthy controls [C] *(in pink):* A. Tricuspid annular plane systolic excursion (TAPSE); B. Tricuspid regurgitation peak systolic velocity (TR Vmax); C. Right atrium to right ventricle peak gradient (RA-rV PG); D. Pulmonary valve velocity time integral (PV VTI); E. Mean pulmonary artery pressure (PAP); F. Pulmonary vascular resistance (PVR). Values are means ± SEM. The effect of preeclampsia was determined by two-way ANOVA. Significant differences between groups: ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$. *abbreviations:* PAP, pulmonary artery pressure; PG, peak gradient; PVR, pulmonary vascular resistance; PV, pulmonary valve; RA, right atrium; RV, right ventricle; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation; Vmax, maximal velocity; VTI, velocity time integral.

pulmonary hypertension in Bolivian women diagnosed with preeclampsia at high altitude relative to those undergoing healthy pregnancy at high altitude.

Measurements of forced expiratory volumes (FEV) and flow (FEF) are used in the diagnosis of obstructive pulmonary function.[23] The bronchial permeability index or FEV1/FVC ratio indicates how much air one can forcefully exhale. The FEV1 measures how much air one can exhale in one second, and the FVC measures the total amount of air one can exhale forcefully in one breath. A reduced value for FEV1 and for the FEV1/FVC ratio is indicative of increased airway resistance to expiratory flow.[23] Several studies have also confirmed increased peripheral airway obstruction in patients diagnosed with pulmonary hypertension.[24] Therefore, data in the present study also show that relative to women undergoing healthy highland pregnancy, women diagnosed with preeclampsia during pregnancy at high altitude are not only at greater risk of pulmonary hypertension but also of impaired peripheral airway function, leading to compromised oxygenation. The latter was again confirmed by measurement of significantly ílower values for the peripheral O_2 saturation in Bolivian women diagnosed with preeclampsia relative to those undergoing healthy pregnancies at high altitude. This is striking considering that some of the inclusion criteria for patients in this study were that women should be non-smoking mothers of normal BMI and no history of cardiovascular or respiratory health complications. Although many differences in respiratory function were statistically significant, absolute differences between groups were generally modest, and the long-term implications of these differences are unclear.

Previous studies in the USA, Saudi Arabia and Bolivia have revealed that the prevalence of preeclampsia is significantly increased during pregnancy at high altitude.[4,25,26]. Two studies of preeclampsia in Bolivia have hypothesised that vascular toxic factors may be released into the maternal circulation by the diseased placenta, and that these factors can pass the placental barrier and affect the circulation of the offspring, increasing their cardiovascular risk in later life.[27,28] Augmented oxidative stress and an angiogenic imbalance have been linked with preeclampsia during pregnancy at high altitude and pulmonary abnormalities in the newborn and young children.[27,28] Pre-clinical studies in

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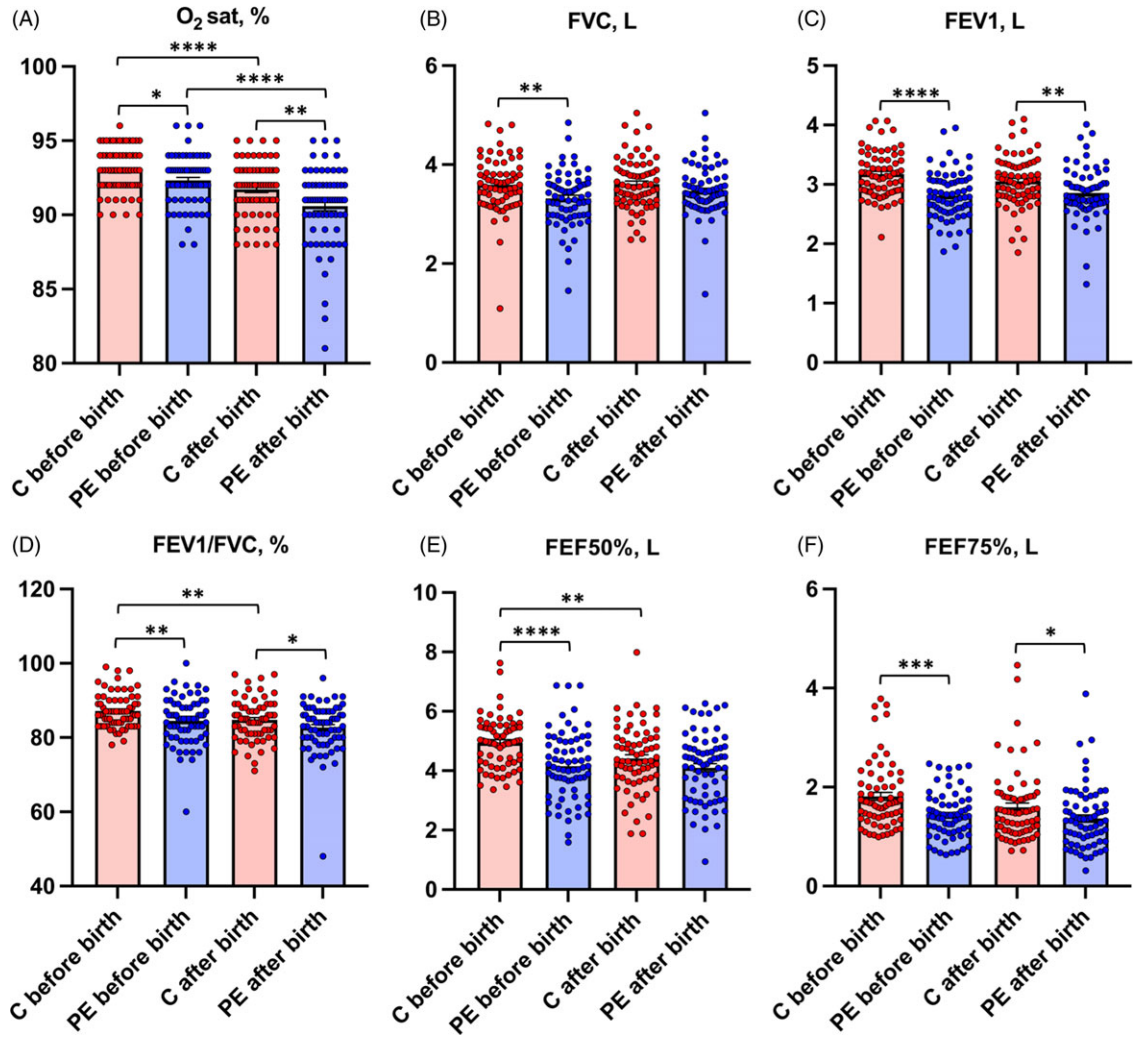

Figure 3. Maternal respiratory health for controls and preeclamptic women. Column plots demonstrate significant perinatal alterations in respiratory indices in preeclamptic women [PE] *(in blue)* compared to healthy controls [C] *(in pink):* A. Oxygen saturation (O_2 sat); refers to peripheral O_2 saturation; B. Forced vital capacity (FVC); C. Forced expiratory volume in 1 s (FEV1); D. FEV1 to FVC ratio (FEV1/FVC); E. Forced expiratory flow at 50% of vital capacity (FEF50%); F. Forced expiratory flow at 75% of vital capacity (FEF75%). Values are means ± SEM. The effect of preeclampsia was determined by two-way ANOVA. Significant differences between groups: ****, $p < 0.0001$; ***, $p < 0.001$; **, $p < 0.01$. *Abbreviations:* FEF, forced expiratory flow; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; L, litre; O_2 sat, peripheral oxygen saturation.

pregnant sheep have reported that pregnancy at high altitude also increases uterine vascular resistance and promotes maternal hypertension.[29] Studies in sheep, mice, rat and guinea pigs have also confirmed that it is the hypoxia of high-altitude pregnancy that promotes the adverse effects on the placenta and mother, since pregnant animals undergoing isobaric hypoxic pregnancy also show symptoms associated with preeclampsia.[30–34] One study in sheep undergoing isobaric hypoxic pregnancy reported evidence of increased placental vascular resistance, placental oxidative stress, placental activation of the unfolded protein response and an angiogenic imbalance in the maternal circulation.[34,35] The present study extends these ideas and reports the novel link between greater risk of preeclampsia and greater risk of concurrent as well as future pulmonary hypertension in the mother at high altitude. Similar placental and maternal vascular toxic factors may provide a mechanism that underlies this link. However, this needs to be confirmed in future investigations. A study of women with severe preeclampsia at sea level in Baltimore, Maryland, USA,[36] also showed higher estimated PAP compared to corresponding controls, consistent with the present finding of higher estimated PAP in women with preeclampsia in high altitude Bolivia. Interestingly, both control and pre-eclampsia groups in the present study in Bolivia had higher values of estimated PAP and cardiac output than preeclamptic women in the Baltimore study, which may be related to living at altitude.

The present study has several strengths and limitations. Strengths include that this was a prospective clinical study of cardiovascular as well as respiratory outcomes with follow-up after pregnancy. The follow-up data showed that significant differences between control and preeclamptic women remained at *ca.* 6 weeks after birth. The study was also multi-institutional and conducted in highland women of homogenous mestizo ethnic background who were residents at ~ 3625 m or 11,893 ft in La Paz for at least two generations derived from five maternity hospitals. Since an increase in body weight is related to an increase in cardiovascular risk, including pulmonary hypertension, matching control and preeclamptic women with similar values for body weight and body mass surface area in addition to gestational age at the prenatal ultrasound examination was also important. Another strength was that estimated PAP as well as pulmonary vascular resistance were

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calculated. Finally, the echocardiography measurements were performed by a single expert (C.E.S.) using the same machine and probe, and every outcome was the average of several measurements to satisfy intra-observer variability. The limitations of the study are just as important. They include the lack of pre-pregnancy measurements and longer-term follow-up with cardiorespiratory measurements past 6 weeks. The study also did not collect maternal plasma samples, which could have been used to give greater insight into underlying mechanisms.

In conclusion, the data in the present study support the novel hypothesis tested that during highland pregnancy, women who develop preeclampsia are at greater risk of pulmonary hypertension compared to women undergoing healthy highland pregnancy. Such information is important for exploring whether there are common underlying pathways between preeclampsia and future cardiovascular risk, and for designing public health prevention initiatives in women with an increased cardiovascular risk, particularly in those who are residents at high altitude. Since preeclampsia and maternal pulmonary hypertension are independent risk factors for maternal mortality, women undergoing pregnancy at high altitude should be counselled regarding the risks, and a multi-professional approach with expert care in pulmonary hypertension centres should be adopted to improve outlook and reduce risks. Such an approach will hopefully lead to a decrease in the alarming rates of maternal mortality in high-altitude countries, particularly in Bolivia.

Data availability statement. All data supporting the results are presented in the manuscript.

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Ethical approval. The study was carried out according to standards set by the Declaration of Helsinki and all procedures were approved by the Local Ethics Committee of the Bolivian Institute for High Altitude Biology (Consejo Técnico, Instituto Boliviano de Biología de Altura, Universidad Mayor de San Andrés, La Paz, Bolivia).

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