



Respiratory Nitric Oxide and Pulmonary Artery Pressure in Children of Aymara and European Ancestry at High Altitude*

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Invasive studies suggest that healthy children living at high altitude display pulmonary hypertension, but the data to support this assumption are sparse. Nitric oxide (NO) synthesized by the respiratory epithelium regulates pulmonary artery pressure, and its synthesis was reported to be increased in Aymara high-altitude dwellers. We hypothesized that pulmonary artery pressure will be lower in Aymara children than in children of European ancestry at high altitude, and that this will be related to increased respiratory NO. We therefore compared pulmonary artery pressure and exhaled NO (a marker of respiratory epithelial NO synthesis) between large groups of healthy children of Aymara (n = 200; mean ± SD age, 9.5 ± 3.6 years) and European ancestry (n = 77) living at high altitude (3,600 to 4,000 m). We also studied a group of European children (n = 29) living at low altitude. The systolic right ventricular to right atrial pressure gradient in the Aymara children was normal, even though significantly higher than the gradient measured in European children at low altitude (22.5 ± 6.1 mm Hg vs 17.7 ± 3.1 mm Hg, p < 0.001). In children of European ancestry studied at high altitude, the pressure gradient was 33% higher than in the Aymara children (30.0 ± 5.3 mm Hg vs 22.5 ± 6.1 mm Hg, p < 0.0001). In contrast to what was expected, exhaled NO tended to be lower in Aymara children than in European children living at the same altitude (12.4 ± 8.8 parts per billion [ppb] vs 16.1 ± 11.1 ppb, p = 0.06) and was not related to pulmonary artery pressure in either group. Aymara children are protected from hypoxic pulmonary hypertension at high altitude. This protection does not appear to be related to increased respiratory NO synthesis. (CHEST 2008; 134:996–1000)

Key words: children; high-altitude dwellers; hypoxia; nitric oxide; pulmonary hypertension

Abbreviations: NO = nitric oxide; ppb = parts per billion

An increase in pulmonary artery pressure is a hallmark of the physiologic response to ambient lack of oxygen. It occurs very rapidly after exposure to high altitude. In certain subgroups of subjects, this physiologic response is exaggerated^{1–5} and may predispose to high-altitude pulmonary edema.^{2–5}

In chronic hypoxic states, sustained pulmonary vasoconstriction and/or remodeling of the pulmonary vasculature⁶ may lead to persistent pulmonary hypertension and its associated complications. Consistent with this concept, invasive studies^{7,8} in small numbers of high-altitude native children in the Andes showed pulmonary hypertension. However,

long-term exposure to hypoxia in high-altitude populations may have resulted in adaptation patterns that may confer protection against hypoxic pulmonary hypertension, but the underlying mechanism is not clear.

In this regard, there is evidence that nitric oxide (NO) synthesized by the respiratory epithelium plays a role in the regulation of pulmonary artery pressure under hypoxic conditions.^{9,10} In rabbits, inhibition of alveolar epithelial NO synthesis increases the pulmonary artery pressure response to hypoxia.¹⁰ In healthy subjects, short-term exposure to hypoxia increases exhaled pulmonary NO (a marker of NO

release in the distal airways).¹¹ In subjects prone to high-altitude pulmonary edema, the hypoxia-induced stimulation of exhaled NO is impaired and contributes to the exaggerated altitude-induced pulmonary hypertension that characterizes these subjects.^{11,12} Exhaled NO was found to be higher in high-altitude dwellers from Tibet and ethnic Aymara from Bolivia than in low-altitude residents from the United States.¹³ Moreover, exhaled NO explained part of the variation of pulmonary artery pressure at high altitude in Tibetans.¹⁴ Based on these data, we hypothesized that pulmonary artery pressure will be lower in Aymara children than in children of European ancestry living at high altitude because the increased respiratory epithelial NO synthesis may help to maintain pulmonary artery pressure within normal limits. To test this hypothesis, we measured pulmonary artery pressure and exhaled NO in large groups of healthy children of Aymara and European ancestry permanently living at the same high-altitude location. To gain additional insight, we also studied a group of European children living at low altitude.

MATERIALS AND METHODS

Study Design

Two hundred Aymara children (105 boys, 95 girls) born at high altitude (3,600 to 4,000 m; mean \pm SD age, 9.5 ± 3.6 years; range, 1 to 17 years) were included in the study. Both parents of all children had typical Aymara surnames and self-identified themselves as Aymaras. We also studied 77 children of European ancestry (42 boys, 35 girls) born or having lived for at least 2 years at high altitude (age, 9.7 ± 3.7 years; range, 2 to 16 years); 8 of them were born in La Paz, and the others had lived there for 5.0 ± 3.6 years (range, 2 to 14 years). In addition, we studied 29 white children (16 boys, 13 girls) born and living at low altitude (450 m; age, 8.8 ± 2.6 years; range, 4 to 13 years). All children were healthy and free of physical or psychological infirmity. The Aymara children were studied either in El Alto ($n = 146$), a suburb of La Paz located at 4,000 m, or at the Instituto Boliviano

de Biología de Altura ($n = 54$) in La Paz (3,600 m). The white children living at high altitude were all studied in La Paz, and those living at low altitude were examined at the University Hospital in Bern, Switzerland (450 m). The experimental protocol was approved by the institutional review board on human investigation of the University of San Andres, La Paz, Bolivia, and the University of Lausanne, Switzerland. The parents gave written informed consent, and one of them was present during the examination.

Doppler Echocardiography

Transthoracic Doppler echocardiography was performed in all children to rule out structural heart disease. To measure pulmonary artery pressure, echocardiographic recordings were obtained with a real-time, phased-array sector scanner (Acuson Cypress; Siemens, Germany) with an integrated color Doppler system and transducers containing crystal sets for two-dimensional imaging (3.6 MHz or 6.0 MHz) and for continuous-wave Doppler recording (2.15 MHz or 3.6 MHz). The recordings were stored on magneto-optical disks for offline analysis by an investigator who was unaware of the subject identity. All reported values represent the mean of at least three measurements. After tricuspid regurgitation had been localized with Doppler color flow imaging, the peak flow velocity of the transtricuspid jet was measured with the use of continuous-wave Doppler, and the pressure gradient between the right ventricle and the right atrium was calculated by use of the modified Bernoulli equation.^{5,15} At this study altitude, a tricuspid regurgitation jet of sufficient quality could not be obtained in $< 5\%$ of this healthy pediatric population. Right ventricular to right atrial pressure gradient measurements are the standard method for the noninvasive estimation of pulmonary artery pressure both in adults¹⁶ and children,¹⁷ and have been validated against invasive measurements at high altitude.¹⁵ The intraobserver and interobserver variabilities for the right ventricular to right atrial pressure gradient measurements were $5.1 \pm 4.6\%$ and $6.0 \pm 8.6\%$, respectively ($n = 30$).

In the largest published series¹⁶ of echocardiographic estimations of pulmonary artery pressure in healthy children, the mean right ventricular to right atrial pressure gradient at low altitude was 16.4 ± 4.0 mm Hg (95% confidence interval, 8.6 to 24.2 mm Hg) in girls, and 17.2 ± 4.6 mm Hg (95% confidence interval, 8.2 to 26.2 mm Hg) in boys. Based on these data, we considered a right ventricular to right atrial pressure gradient > 26.2 mm Hg to indicate pulmonary hypertension.

Exhaled NO

Exhaled NO was measured at 3,600 m in 49 Aymara children and 58 children of European ancestry living at high altitude and in 21 European children living at low altitude. Measurements were made in the sitting position with a hand-held electrochemical analyzer (NIOX MINO; Aerocrine; Solna, Sweden)¹⁹ using standard quality criteria.²⁰ The built-in flow control of this device keeps exhalation at 50 mL/s regardless of patient skill. The reported values are the mean of two determinations that varied one from another by $< 10\%$. In a group of 37 subjects, the intraindividual variability of exhaled NO was 0.3 ± 3.7 parts per billion (ppb).

Arterial Oxygen Saturation and Heart Rate

Transcutaneous arterial oxygen saturation and heart rate were measured at a fingertip with a pulse oximeter (OxiMax N-595; Nellcor; Pleasanton, CA).

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Statistical Analysis

Data were analyzed with Statview 4.5 (Abacus Concepts; Berkeley, CA) and the JMP software package (SAS Institute; Cary, NC). Statistical analysis included analysis of variance for between-group comparisons and Fisher protected least significant difference test for analysis of mean differences between groups. Relations between variables were analyzed by calculating Pearson product-moment correlation coefficient. A value of $p < 0.05$ was considered to indicate statistical significance. Unless otherwise indicated, data are expressed as mean \pm SD.

RESULTS

Figure 1 shows that the systolic right ventricular to right atrial pressure gradient in the Aymara children studied at high altitude was normal but significantly higher than the gradient measured in European children at low altitude (22.5 ± 6.1 mm Hg vs 17.7 ± 3.1 mm Hg, $p < 0.0001$), and also roughly 5 mm Hg higher than the mean value reported in the largest series of children studied at low altitude.¹⁶ In contrast, in children of European ancestry studied at high altitude, the systolic right ventricular to right atrial pressure gradient was 33% higher than in the Aymara children (30.0 ± 5.3 mm Hg vs 22.5 ± 6.1 mm Hg, $p < 0.0001$), a value that was above the upper limit of the 95% confidence interval (26.2 mm Hg) reported for children at low altitude.¹⁶ Twenty-five percent of the Aymara children and 75% of the white children had pulmonary artery pressures > 26.2 mm Hg at high altitude. The pulmonary artery pressures in the 8 white children born at high altitude and the 69 white children not born at high altitude were comparable (29.8 ± 4.7 mm Hg vs 30.1 ± 5.4 mm Hg, $p = 0.86$). There was a direct relationship between age and pulmonary artery pressure in Aymara children ($r = 0.26$, $p < 0.001$) and white children ($r = 0.44$, $p < 0.001$).

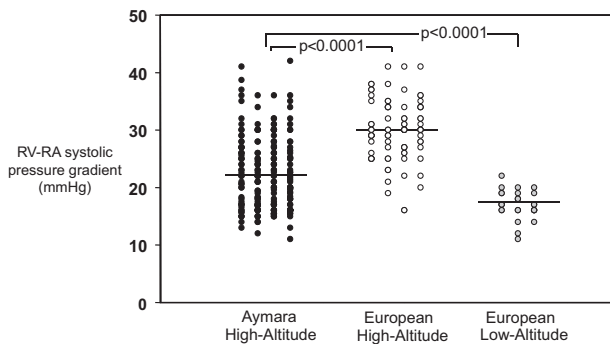


FIGURE 1. Resting right ventricular (RV) to right atrial (RA) systolic pressure gradient in healthy Aymara and white children living at high altitude (3,600 to 4,000 m) and in white children living at low altitude (450 m). Horizontal lines represent mean values.

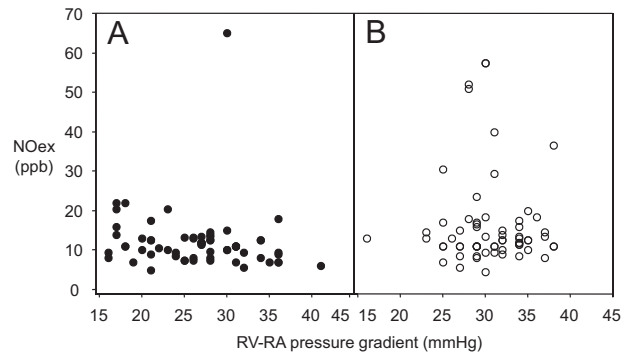


FIGURE 2. Relationship between respiratory NO (NO_{ex}) and the right ventricular to right atrial systolic pressure gradient in healthy Aymara children (left, A; $r = 0.095$, $p = 0.52$) and children of European ancestry (right, B; $r = 0.015$, $p = 0.91$) living at high altitude (3,600 to 4,000 m). See Figure 1 for expansion of abbreviations.

In the high-altitude studies, arterial oxygen saturation was significantly lower in the subgroups of Aymara children examined at 4,000 m ($89.7 \pm 3.1\%$) or at 3,600 m ($92.7 \pm 2.4\%$) than in the children of European ancestry who were all studied at 3,600 m ($93.6 \pm 2.0\%$; $p < 0.001$ and $p = 0.03$, vs Aymaras at 4,000 m and 3,600 m, respectively). Heart rate, another factor that may influence the right ventricular to right atrial pressure gradient, was similar in Aymara and white children (86 ± 14 beats/min vs 85 ± 15 beats/min, $p = 0.87$).

At high altitude, exhaled NO tended to be higher in children of European than of Aymara of ancestry (16.1 ± 11.1 ppb vs 12.4 ± 8.8 ppb, $p = 0.06$). There was no relationship between pulmonary artery pressure and exhaled NO at high altitude either in Aymara children ($r = 0.095$, $p = 0.52$) or in white children ($r = 0.015$, $p = 0.91$) [Fig 2]. At low altitude, exhaled NO in European children tended to be higher than at high altitude (20.5 ± 7.9 ppb vs 16.1 ± 11.1 ppb, $p = 0.08$), and there was no relationship between exhaled NO and pulmonary artery pressure ($r = 0.14$, $p = 0.55$).

DISCUSSION

These data represent the first comparisons of echocardiographic estimations of pulmonary artery pressure and measurements of exhaled NO between large groups of healthy children of Aymara and European ancestry permanently living at the same high-altitude location. The major new finding was that in children of European but not of Aymara ancestry, long-term high-altitude exposure was associated with mild pulmonary hypertension, as evidenced by a roughly 33% higher right ventricular to

right atrial pressure gradient, a value that was above the 95% confidence interval reported for children at low altitude.¹⁶ The protection from hypoxic pulmonary hypertension in Aymara children was not related to increased respiratory NO synthesis because exhaled NO tended to be lower in Aymara than in white children, and there was no relationship between pulmonary artery pressure and exhaled NO in the two groups.

In Aymara children, pulmonary artery pressure was within normal limits at high altitude, although the values were roughly 5 mm Hg higher than those measured in European children at low altitude in the present studies and the values reported in a large series of children studied at low altitude. The mechanism by which Aymara children are protected from altitude-induced pulmonary hypertension is not clear yet. It was not related to better arterial oxygenation because arterial oxygen saturation was lower in Aymara than children of European ancestry. The lower oxygen saturation in the Aymara children was not only related to the study location 400 m higher, where the majority of them were examined, because Aymara children who were studied at the same altitude as white children also had lower arterial oxygen saturation. This suggests that hypoxemia in Aymara children was related to relative hypoventilation at rest.²¹ In line with this concept, arterial oxygen saturation at rest was also found to be lower in adult Aymaras than in acclimatized Europeans.²² The lower arterial oxygen saturation in Aymara children could suggest that the present studies may underestimate the magnitude of their protection against hypoxic pulmonary hypertension.

There is abundant evidence that not only NO synthesized by the vascular endothelium, but also NO produced by the respiratory epithelium plays a role in the regulation of pulmonary vascular tone during hypoxia in both animals and humans.^{10,11,23} Respiratory NO synthesis has been reported to be increased in adult Aymara high-altitude dwellers when compared with low-altitude residents of non-Aymara origin studied at low altitude,¹³ and has been suggested to represent a protective mechanism against hypoxic pulmonary hypertension. In line with this hypothesis, increased respiratory NO synthesis attenuates hypoxic pulmonary hypertension in adult Tibetans.^{14,24} The present findings provide no evidence for this mechanism to be operational in Aymara children because they had the lowest exhaled NO concentration of all the groups we studied. In healthy adult Europeans, short-term high-altitude exposure increases respiratory NO.¹¹ In this study, exhaled NO tended to be lower in children of European ancestry living at high than in those living at low altitude, suggesting that chronic

high-altitude exposure does not stimulate respiratory NO synthesis in these children.

Earlier studies in small groups of subjects suggested that at high altitude, in contrast to low altitude,¹⁶ pulmonary artery pressure decreases gradually from birth to adolescence in healthy children.²⁵ The present data do not support this assumption because we found a significant positive relationship between age and the right ventricular to right atrial pressure gradient in these large groups of Aymara and white children. These findings suggest that in children living at high altitude, a slight increase of pulmonary artery pressure with age represents a physiologic phenomenon that occurs independent of ethnicity.

The present results challenge the long-held concept that healthy children living at high altitude in the Andes invariably display pulmonary hypertension.^{7,8,25} This concept was based mainly on invasive studies performed at an altitude of 4,540 m in Peru, showing a mean systolic pulmonary artery pressure of 58 mm Hg in a small group (n = 7) of 1- to 5-year-old children, and a mean value of 41 mm Hg in a group (n = 25) of 6- to 14-year-old children.⁷ The discrepancy between these earlier and the present findings could be related to differences in the altitude of the study location or ethnicity because our data indicate that ethnicity is an important determinant of pulmonary artery pressure in children at high altitude. The latter explanation seems unlikely, however, because an echocardiographic study²⁶ reported normal values for a surrogate marker of pulmonary artery pressure in a large group of Peruvian children studied at 4,100 m. We cannot rule out that the discrepancy could be related to differences in the technique used to measure pulmonary artery pressure, but we have found that at high altitude, the mean difference between echocardiographic estimates and invasive measurements of systolic pulmonary artery pressure was < 1 mm Hg in adults¹⁸; and such estimates also correspond closely with direct measurements in children.²⁷ In the earlier studies, there is no information regarding arterial oxygenation or the presence of cardiopulmonary malformations. In the present study, we were careful not to include children with cardiac malformations or cardiopulmonary disease.

The present data represent the first measurements of pulmonary artery pressure in a large group of children of European ancestry permanently living at an altitude where major high-altitude cities are located. While further studies are needed to determine the long-term evolution of pulmonary artery pressure in children permanently living at high altitude, our findings suggest that in healthy children and adolescents living at

3,600 m, pulmonary hypertension is mild and appears to be well tolerated.

In conclusion, we found that in Aymara children living at high altitude, pulmonary artery pressure was normal and lower than in children of European ancestry permanently living at the same altitude. Protection from hypoxia-induced pulmonary hypertension may represent a specific high-altitude adaptation pattern of Aymara children. In contrast to what might have been expected, this protection does not appear to be related to increased respiratory NO production.

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