

## RESEARCH ARTICLE

# Divergent physiological responses in laboratory rats and mice raised at high altitude

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## ABSTRACT

Ecological studies show that mice can be found at high altitude (HA – up to 4000 m) while rats are absent at these altitudes, and there are no data to explain this discrepancy. We used adult laboratory rats and mice that have been raised for more than 30 generations in La Paz, Bolivia (3600 m), and compared their hematocrit levels, right ventricular hypertrophy (index of pulmonary hypertension) and alveolar surface area in the lungs. We also used whole-body plethysmography, indirect calorimetry and pulse oxymetry to measure ventilation, metabolic rate ( $O_2$  consumption and  $CO_2$  production), heart rate and pulse oxymetry oxygen saturation ( $p_{O_2,sat}$ ) under ambient conditions, and in response to exposure to sea level  $P_{O_2}$  (32%  $O_2=160$  mmHg for 10 min) and hypoxia (18% and 15%  $O_2=90$  and 75 mmHg for 10 min each). The variables used for comparisons between species were corrected for body mass using standard allometric equations, and are termed mass-corrected variables. Under baseline, compared with rats, adult mice had similar levels of  $p_{O_2,sat}$ , but lower hematocrit and hemoglobin levels, reduced right ventricular hypertrophy and higher mass-corrected alveolar surface area, tidal volume and metabolic rate. In response to sea level  $P_{O_2}$  and hypoxia, mice and rats had similar changes of ventilation, but metabolic rate decreased much more in hypoxia in mice, while  $p_{O_2,sat}$  remained higher in mice. We conclude that laboratory mice and rats that have been raised at HA for >30 generations have different physiological responses to altitude. These differences might explain the different altitude distribution observed in wild rats and mice.

**KEY WORDS:** Chronic hypoxia, Metabolism, Physiology, Respiration, Rodents

## INTRODUCTION

Physiological changes at high altitude are required to optimize the diffusion, transport and cellular utilization of oxygen and counteract the ambient low pressure of oxygen ( $P_{O_2}$ ). In endemic species that have been living for millions of years at altitude, physiological adaptations are thought to be linked to genetic selection, and are mostly characterized by a low or absent pulmonary hypertension (Tucker and Rhodes, 2001), elevated affinity of hemoglobin (Hb) for  $O_2$  (Storz et al., 2010a, 2009), higher density of micro-vessels and mitochondria surface area in the heart (Qi et al., 2008), left-ventricle hypertrophy that could lead to increased stroke volume (Pichon et al., 2013), and hematocrit (Hct) level that remains

within the sea level range (Monge and Leon-Velarde, 1991; Storz et al., 2010b). Contrastingly, some species originating from sea level have high Hct level, drastic pulmonary hypertension or hypertrophy of the heart when exposed to high altitude (Tucker and Rhodes, 2001), illustrating the fact that these species might have a genetic pre-disposition that would interfere with survival at high altitude and present a ‘genetic barrier’ for colonization of high altitude regions.

Interestingly, ecological reports from South America and New Zealand indicate that mice (*Mus musculus*) are present up to an altitude of 4000 m (Storz et al., 2007), while rats (*Rattus norvegicus*) are notably absent at high altitude (Anderson, 1997; Innes, 2005). In these two regions, rats and mice have been introduced over the last eight to five centuries by human migrations (Wilmschurst et al., 2008; see also Storz et al., 2007); accordingly, comparative studies between rats and mice might be useful to further understand the physiological responses at altitude in species that are not ‘high altitude native’, but might nonetheless have different success for life at altitude.

Over the past 20 years, we have been able to raise laboratory rats (Sprague–Dawley) for more than 30 generations in laboratory conditions at an altitude of 3600 m above sea level, in La Paz, Bolivia. These animals present elevated Hct and Hb levels, a right ventricular hypertrophy (a sign of pulmonary hypertension), an altered alveolar structure with enlarged airspaces in the lungs, and impaired respiratory control (Lumbroso et al., 2012). Considering that elevated Hct and pulmonary hypertension impair survival and lead to right heart failure (Lumbroso et al., 2012; Naeije and Manes, 2014; Storz et al., 2010b), this might explain why rats have not been able to establish stable colonies in high altitude regions under natural conditions.

In the present study, we asked whether laboratory mice with a similar history of life at high altitude have different physiological responses from rats. To this aim we compared arterial oxygen saturation, hematological, cardiac, ventilatory, metabolic and lung characteristics in adult mice and rats from a population that have been born and raised at an altitude of 3600 m (La Paz, Bolivia) since 1992. The study was performed in males and females to address whether sex-specific physiological differences would be present in adult mice and rats at high altitude.

## RESULTS

### Physiological parameters, hematology and lung architecture in high altitude rats and mice

#### Arterial oxygen saturation, heart rate and hematology

Under baseline conditions, rats and mice had oxygen saturation ( $p_{O_2,sat}$ ) values around 80%, without significant effect of species or sex (Fig. 1A). Mice had a lower right ventricular hypertrophy compared with rats ( $P<0.0001$  for species, Fig. 1B). Heart rate ( $f_H$ ) was higher in mice than in rats ( $P<0.0001$  for species,

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**List of symbols and abbreviations**

Epo	erythropoietin
$f_H$	heart rate
$f_R$	respiratory frequency
$F_{I_{O_2}}$	inspired $O_2$ fraction
HIF	hypoxic inducible factor
$L_m$	mean linear intercept
LV	left ventricle
$P_{I_{O_2}}$	partial pressure of inspired $O_2$
$P_{O_2}$	partial pressure of $O_2$
$\rho_{O_2, sat}$	pulse oxymetry $O_2$ saturation
RV	right ventricle
S	cardiac septum
$\dot{V}_{CO_2}$	$CO_2$ production rate
$\dot{V}_E$	minute ventilation
$\dot{V}_{O_2}$	$O_2$ consumption rate
$V_T$	tidal volume

Fig. 1C), but when the difference in body mass was taken into account by applying the allometric scaling factor ( $f_H$  in beats  $min^{-1}/mass$  in  $g^{-0.25}$  – see Materials and methods), mice had a lower mass-corrected  $f_H$  than rats ( $P<0.0001$  for species, Fig. 1D), and female rats had a lower mass-corrected  $f_H$  than male rats (Fig. 1D).

Compared with rats, high altitude mice showed lower Hct ( $P<0.0001$  for species, Fig. 1E) and Hb concentration ( $P<0.0001$  for species, Fig. 1F) values. Interestingly, the Hb value in mice was below the normal range of the sea level value (see Table 1) while Hct was slightly higher than the normal sea level range (Table 1). In rats, both Hct and Hb values were above the normal sea level range (Table 1). Compared with males, females of both species had lower

Hct ( $P<0.0001$  for sex, Fig. 1E), and female rats had lower Hb values than male rats (Fig. 1F).

**Minute ventilation, tidal volume and respiratory frequency**

Compared with rats, mice had a higher mass-specific minute ventilation ( $\dot{V}_E$ ), tidal volume ( $V_T$ ) and respiratory frequency ( $f_R$ ,  $P<0.0001$  for species, Fig. 2A–C). Female mice had a higher mass-specific  $\dot{V}_E$  ( $P=0.04$  for sex) and  $V_T$  ( $P=0.04$  for sex) than male mice (Fig. 2A,B). When corrected for body mass using standard allometric corrections, there was a significant effect of species ( $P<0.0001$ ) and sex ( $P=0.04$ ) for  $\dot{V}_E$ : male mice had a similar mass-corrected  $\dot{V}_E$  and  $f_R$  to male rats, but a higher mass-corrected  $V_T$  (Fig. 2D–F). Female mice had a higher mass-corrected  $\dot{V}_E$  and  $V_T$  compared with male mice (Fig. 2D,E). Mass-corrected  $f_R$  was lower in female rats compared with male rats (Fig. 2F), but there was no significant effect of species.

**Metabolic rate, respiratory exchange ratio and rectal temperature**

Compared with rats, mice had a higher mass-specific (Fig. 3A,B) or mass-corrected (Fig. 3C,D)  $O_2$  consumption ( $\dot{V}_{O_2}$ ) and  $CO_2$  production ( $\dot{V}_{CO_2}$ ) ( $P<0.0001$ ). The respiratory exchange ratio ( $\dot{V}_{CO_2}/\dot{V}_{O_2}$ ) was higher in rats than in mice ( $P<0.0002$  for species, Fig. 3E). Rectal temperature measured before the onset of respiratory and metabolic recordings was similar in rats ( $35.2\pm 0.2^\circ C$ ) and mice ( $35.2\pm 0.2^\circ C$ ). There was no effect of sex for metabolic variables.

**Lung volume, lung mass and lung architecture**

Compared with rats, mice had a higher mass-specific lung volume and lung mass ( $P<0.0001$  for species, Fig. 4). Because the allometric scaling parameter is 1 for lung volume and lung mass (see Materials and methods), mass-specific values allow direct comparison between species of different body mass. Representative lung images are presented in Fig. 5A for rats and Fig. 5B for mice: note the enlarged air spaces in rats compared to mice. The mean linear intercept ( $L_m$ ) was lower in mice than in rats ( $P<0.0001$  for species, Fig. 5C), which was reflected in higher mass-corrected relative and total alveolar surface area in mice ( $P<0.0001$  for species, Fig. 5C,D). There was no effect of sex for these variables.

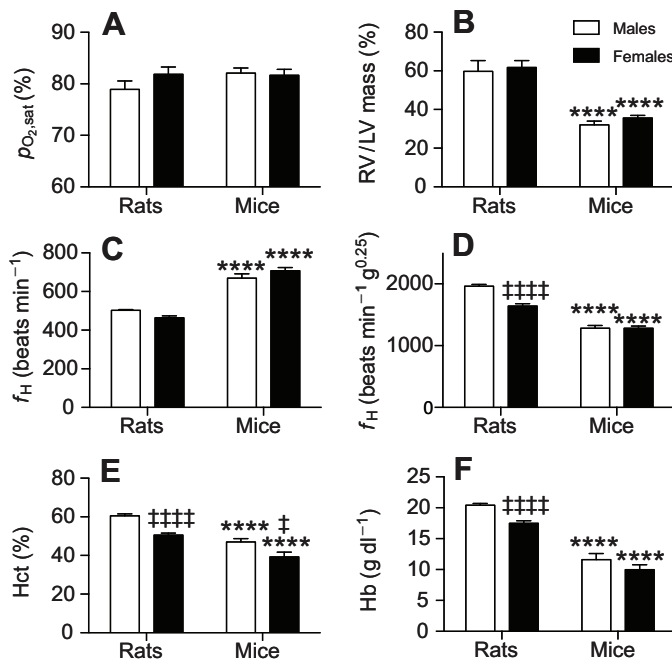
Because alveolar surface area is directly proportional to oxygen consumption (Tenney and Remmers, 1963), we also calculated the ratio of the total alveolar surface area to  $O_2$  consumption ( $m^2 ml^{-1} O_2$  consumed in 1 min). There was no significant effect of sex ( $P=0.58$  for sex) or significant sex $\times$ species interaction. The mean value was higher in mice than in rats (Fig. 6), and both values were higher than the expected sea level values (Table 1).

**Respiratory, metabolic and heart rate responses to changes in inspired  $P_{O_2}$  in high altitude rats and mice**

For the responses that were normalized to baseline values, there was no significant effect of sex; accordingly, males and females were pooled. At 12%  $O_2$  [corresponding to a partial pressure of inspired  $O_2$  ( $P_{I_{O_2}}$ ) of 60 mmHg – see Materials and methods], four rats (three males and one female) showed signs of distress, prolonged apneas and gasps within the first minute of exposure. The experiments were therefore stopped and data recorded at 12% (for rats and mice) are not presented – with the exception of rectal temperature. All mice supported exposure to 12%  $O_2$  without signs of distress, apneas or gasps.

 **$\rho_{O_2, sat}$  and  $f_H$** 

$\rho_{O_2, sat}$  was higher in mice than in rats upon exposure to sea level  $P_{I_{O_2}}$  (32%  $O_2$ ) and in moderate hypoxia (18%  $O_2$ ; Fig. 7A).  $f_H$



**Fig. 1. Hematological variables, right ventricular hypertrophy, heart rate and arterial saturation in high altitude rats and mice.** (A) Arterial  $O_2$  saturation ( $\rho_{O_2, sat}$ ), (B) right-to-left ventricle (RV/LV) mass ratio, (C) heart rate ( $f_H$ ), (D) mass-corrected  $f_H$ , (E) hematocrit (Hct) and (F) hemoglobin (Hb) in 2 month old male and female rats and mice living at high altitude for 30 generations. Values are means $\pm$ s.e.m. \*\*\*\* $P<0.0001$  mice versus rats; † $P<0.05$  and ††† $P<0.0001$  females versus males.

**Table 1. Comparison between values obtained at sea level and high altitude for selected variables in adult (3 months old) male rats or mice**

	Rats		Mice	
	Sea level	High altitude	Sea level	High altitude
Body mass (g)	333±21	232±9	27.3±0.8	13.7±0.9
Rectal temperature (°C)	36.8±0.2	35.2±0.2	35.6±0.3	35.2±0.2
Hematocrit (%)	41.2–47.3 <sup>a</sup>	60.5±1.1	39.0–42.5 <sup>b</sup>	47.0±1.7
Hemoglobin (g ml <sup>-1</sup> )	14.4–16.0 <sup>a</sup>	20.4±0.3	14.3–15.2 <sup>b</sup>	11.6±1.0
RV/(LV+S) (%)	27.9±0.6 <sup>c</sup>	59.7±5.6	~20 <sup>d</sup>	32.0±2.0
Heart rate (beats min <sup>-1</sup> )	250–400 <sup>e</sup>	503±4	500–700 <sup>f</sup>	669±23
Lung volume (×10 <sup>2</sup> ml g <sup>-1</sup> )	2.4±0.2	2.6±0.1	2.7±0.2	7.6±0.4
Lung mass (×10 <sup>2</sup> g g <sup>-1</sup> )	0.29±0.01	0.44±0.02	0.49±0.01	1.06±0.05
Total alveolar surface area (×10 <sup>2</sup> m <sup>2</sup> g <sup>-0.88</sup> )	0.99±0.05	0.87±0.04	1.04±0.13	3.41±0.34
O <sub>2</sub> consumption rate (ml min <sup>-1</sup> g <sup>-0.76</sup> )	0.144±0.003	0.064±0.001	0.149±0.01	0.109±0.006
Total alveolar surface area to O <sub>2</sub> consumption ratio (m <sup>2</sup> ml <sup>-1</sup> min <sup>-1</sup> )	~0.10	0.30±0.03	~0.11	0.47±0.05

Values are ranges (minimum–maximum) or means±s.e.m. All high altitude values are those obtained in the present study. Sea level values are unpublished data from our colony of Sprague–Dawley rats and FVB mice or are from the following references: <sup>a</sup>(Sharp and La Regina, 1998), <sup>b</sup>[http://www.informatics.jax.org/mgihome/other/mouse\\_facts1.shtml](http://www.informatics.jax.org/mgihome/other/mouse_facts1.shtml), <sup>c</sup>(Lumbroso and Joseph, 2009), <sup>d</sup>(Ciuculan et al., 2011), <sup>e</sup>(Giknis and Clifford, 2006), <sup>f</sup>(Sebastian et al., 2013). RV, right ventricle; LV, left ventricle; S, cardiac septum.

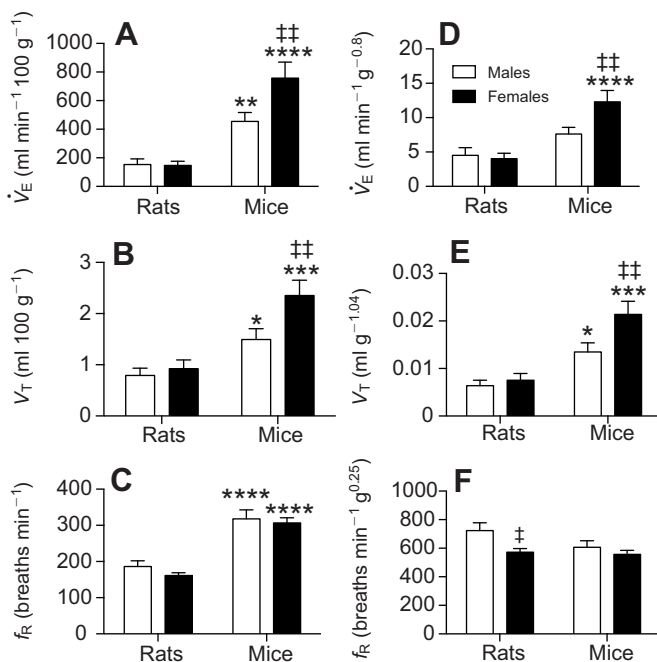
declined in rats when exposed to sea level  $P_{I_{O_2}}$ , but remained unchanged upon hypoxic exposure (Fig. 7B). In mice,  $f_R$  remained unchanged when exposed to sea level  $P_{I_{O_2}}$ , slightly declined upon hypoxic exposure to 18% O<sub>2</sub>, and returned to baseline levels at 15% O<sub>2</sub> (Fig. 7B).

#### $\dot{V}_E$ , $V_T$ , $f_R$ and metabolic rate

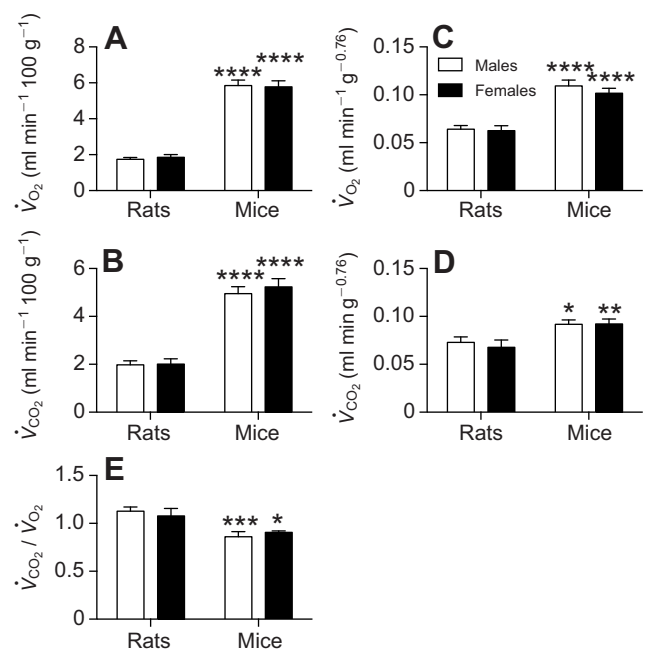
Exposure to sea level  $P_{I_{O_2}}$  reduced  $\dot{V}_E$  to a similar extent in mice and rats (Fig. 8A); however, rats had a more pronounced decline of  $V_T$  than mice (Fig. 8B), and contrastingly mice had a more marked decline of  $f_R$  than rats (Fig. 8C). Under moderate hypoxia (18%),  $V_T$  was higher in mice than in rats (the apparent drop of  $V_T$  at 18% O<sub>2</sub> versus 21% O<sub>2</sub> in rats is not significant).

In response to sea level  $P_{I_{O_2}}$ , there was no change of metabolic rate in either species (Fig. 9), but upon hypoxic exposure metabolic rate decreased in mice but not in rats: at 15% O<sub>2</sub>,  $\dot{V}_{O_2}$  and  $\dot{V}_{CO_2}$  had fallen by more than 30% in mice (Fig. 9A,B). Similarly, rectal temperature fell by about 2°C in mice (from 35.2±0.2°C to 33.1±0.2°C), but remained unchanged in rats (Fig. 9C).

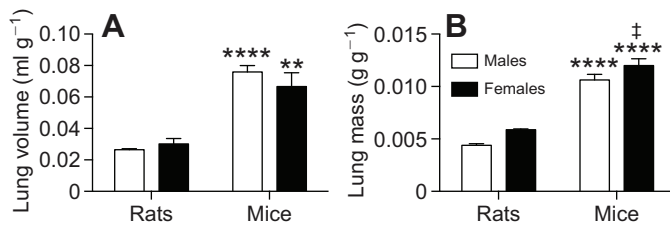
Under hypoxic conditions, rats did not increase  $\dot{V}_E$  and maintained their metabolic rate at the baseline level, while in mice metabolic rate fell during hypoxic exposure. To find out whether this pattern in rats was achieved by increasing O<sub>2</sub> extraction from pulmonary gas, we calculated the percentage of O<sub>2</sub> extraction (O<sub>2</sub> consumption/ $\dot{V}_E \times F_{I_{O_2}}$ ; Fig. 10A,B). There was a significant effect of species for this parameter ( $P=0.05$ ) and a significant species×hypoxia interaction ( $P=0.0009$ ). In



**Fig. 2. Ventilatory variables under baseline conditions (21% O<sub>2</sub>) in high altitude rats and mice.** (A,D) Minute ventilation ( $\dot{V}_E$ ), (B,E) tidal volume ( $V_T$ ) and (C,F) respiratory frequency ( $f_R$ ) in 2 month old rats and mice. A and B are mass-specific values; D–F are mass-corrected values; means±s.e.m. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  and \*\*\*\* $P<0.0001$  mice versus rats. † $P<0.05$  and †† $P<0.01$  females versus males.



**Fig. 3. Metabolic variables under baseline conditions (21% O<sub>2</sub>) in high altitude rats and mice.** (A,C) O<sub>2</sub> consumption rate ( $\dot{V}_{O_2}$ ) and (B,D) CO<sub>2</sub> production rate ( $\dot{V}_{CO_2}$ ) in 2 month old rats and mice. A and B are mass-specific values; C and D are mass-corrected values. (E) Respiratory exchange ratio ( $\dot{V}_{CO_2}/\dot{V}_{O_2}$ ). Means±s.e.m. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  and \*\*\*\* $P<0.0001$  mice versus rats.



**Fig. 4. Lung morphology variables in high altitude rats and mice.**

(A) Mass-specific lung volume and (B) mass-specific lung mass in 2 month old rats and mice. Values are means+s.e.m. \*\* $P<0.01$  and \*\*\*\* $P<0.0001$  mice versus rats. † $P<0.05$  females versus males.

male rats,  $O_2$  extraction increased in hypoxia (18% and 15%  $O_2$ ) compared with 32%  $O_2$ , while it remained unchanged in male mice (Fig. 10A). Female mice had a lower  $O_2$  extraction value in hypoxia than female rats (Fig. 10B), because mass-specific  $\dot{V}_E$  (Fig. 10C,D;  $P=0.002$  for sex,  $P=0.004$  for sex $\times$ species) and  $V_T$  (not shown) were higher in female mice than in male mice throughout the hypoxic exposure, while there was no effect of sex for mass-specific  $\dot{V}_{O_2}$  (Fig. 10E,F).

## DISCUSSION

We compared physiological responses in laboratory rats and mice that have been raised at 3600 m above sea level for about 30 generations. The key differences between these two species include higher erythrocytosis and elevated right ventricular hypertrophy (a sign of higher pulmonary hypertension) in rats compared with mice, and lower mass-corrected lung volume, alveolar surface area,  $V_T$  and  $\dot{V}_{O_2}$  in rats compared with mice. However, rats and mice had similar levels of  $p_{O_{2,sat}}$ .

The relevance of performing comparative studies between two species as an approach to understanding adaptive processes to a given environmental condition has been questioned (Garland and Adolph, 1994). In line with the limitations of this approach, this study was not designed to draw conclusions on genetic adaptation to altitude, but rather to illustrate physiological differences at altitude between two species that are known to have a different altitudinal range of distribution. As such, our results provide a relevant model of divergent physiological responses at high altitude, and might help to explain ecological reports suggesting that mice are more frequently found under natural conditions at high altitude than rats (Anderson, 1997; Innes, 2005; Storz et al., 2007).

To illustrate how rats and mice might differ in their responses to high altitude hypoxia, Table 1 presents a series of data obtained

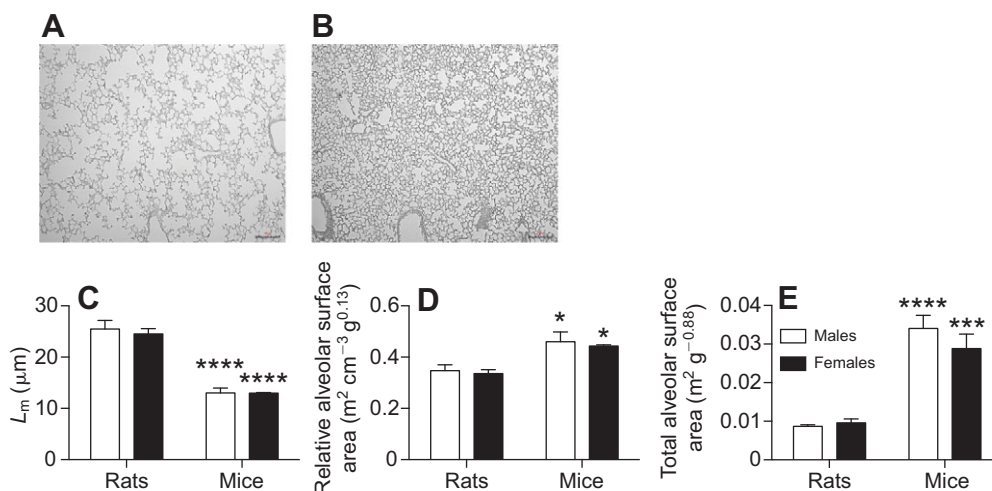
from the present high altitude study and from other studies at sea level, including some data obtained in our sea level laboratory in Sprague–Dawley rats and FVB mice (all adult males of similar age to the high altitude animals).

One should, however, keep in mind that we have not been able to precisely determine the identity of the mouse species from the high altitude colony. However, as they are descendants from a provider of standard laboratory mice, they should be *Mus musculus domesticus* (Yang et al., 2011), but we cannot exclude the possibility that they have been hybridized with local species such as *Mus musculus castaneus*, which (with *M. musculus domesticus*) have been identified among wild-caught mice in La Paz (Storz et al., 2007). Nevertheless, it remains unclear from the literature whether these groups of mice should be considered as distinct species or subspecies of *Mus musculus* (for a detailed discussion, see *Mus musculus* entry in Wilson and Reeder, 2005).

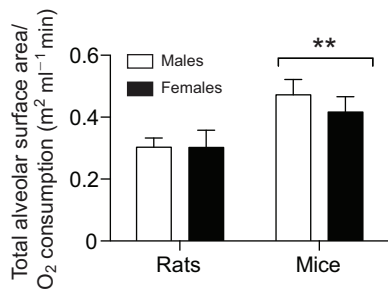
## Right ventricular hypertrophy, excessive erythropoiesis and elevated $f_H$ are present in high altitude rats but not in mice

In mice, the right-to-left ventricle ratio, an index of arterial pulmonary hypertension, was around 35%. While this is higher than values normally reported at sea level (around 20%; Ciuculan et al., 2011), it is much lower than the values reported in rats in our present and past (Lumbroso et al., 2012) studies. Protection against elevated pulmonary hypertension is common in species adapted to high altitude, which demonstrate thinner pulmonary vessel walls with a reduced number of muscular cells (Tucker and Rhodes, 2001). We did not perform specific analysis of the arterial wall structure in mice, but it is likely that they would also present this typical characteristic.

There was also a pronounced difference in Hct and Hb values between rats and mice, and similar  $p_{O_{2,sat}}$  levels. The enhanced Hct value in rats might be due to a more sensitive hypoxia-sensing system in the kidneys, which could include stabilization of the hypoxic inducible factor (HIF), expression of HIF regulatory proteins and/or synthesis of erythropoietin (Epo) (Franke et al., 2013). In addition, it is worth mentioning that the synthesis of Epo in the kidney is regulated by the glomerular filtration rate (Olsen et al., 2011) because the consumption of  $O_2$  in the kidneys (a major determinant of local  $P_{O_2}$ ) is tightly dependent on sodium reabsorption and glomerular filtration rate (Donnelly, 2001). Accordingly, a lower glomerular filtration rate and/or sodium reabsorption in mice compared with rats could contribute to lower Epo production and reduced Hct.



**Fig. 5. Lung architecture variables in high altitude rats and mice.** Typical image of the lung architecture obtained for rats (A) and mice (B). (C) Mean linear intercept ( $L_m$ ), (D) mass-corrected relative alveolar surface area and (E) mass-corrected total alveolar surface area in 2 month old rats and mice. Values are means+s.e.m. Scale bars in A and B, 50  $\mu$ m. \* $P<0.05$ , \*\*\* $P<0.001$  and \*\*\*\* $P<0.0001$  mice versus rats.



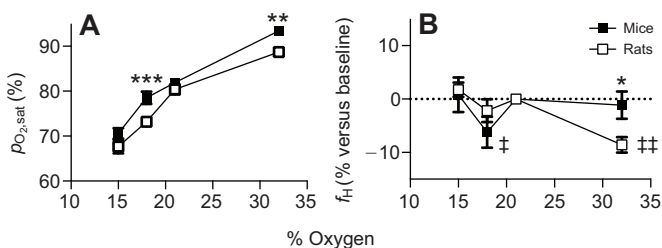
**Fig. 6. Total alveolar surface area to O<sub>2</sub> consumption ratio in high altitude rats and mice.** Values are means+s.e.m. \*\**P*<0.01 mice versus rats.

In male rats raised at altitude,  $f_H$  was around 500 beats  $\text{min}^{-1}$ , whereas normal sea level values of  $f_H$  are around 250–300 beats  $\text{min}^{-1}$  during daytime and 350–400 beats  $\text{min}^{-1}$  during night-time (Lemmer et al., 1993). However, it is not possible from our data to elucidate whether the elevated  $f_H$  effectively increases cardiac output or compensates for a reduced stroke volume. By comparison, while the  $f_H$  reported in mice under baseline conditions ( $670 \pm 20$  beats  $\text{min}^{-1}$  in males) is higher than normal sea level values at rest (500–600 beats  $\text{min}^{-1}$  daytime), it remains within the normal range of values recorded during the active phase (night-time 600–700 beats  $\text{min}^{-1}$ ) (Sebastian et al., 2013).

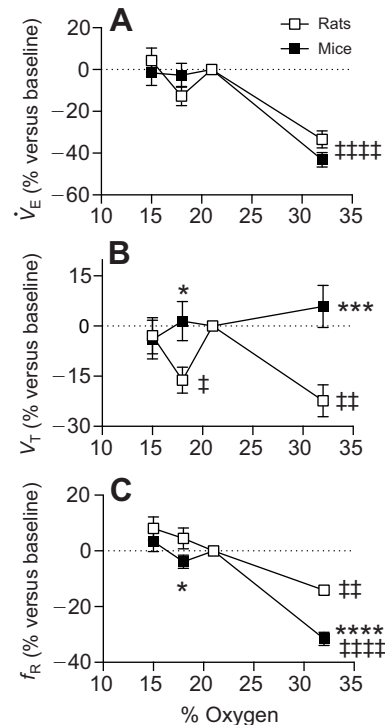
#### Reduced metabolic rate and elevated respiratory exchange ratio in high altitude rats versus mice

Compared with mice, rats had a reduced  $\dot{V}_{O_2}$  (either mass specific or mass corrected), which might help them to maintain the  $p_{O_{2,\text{sat}}}$  value despite the reduced mass-corrected alveolar surface area of the lungs. In several vertebrate species, reduced O<sub>2</sub> consumption rate is a key strategy of adaptation to hypoxia (Bickler and Buck, 2007). Subterranean species, such as naked mole rats that live in burrows under severe hypoxic conditions, have mechanisms of tolerance to hypoxia including low metabolic rate and reduced core body temperature (Nathaniel et al., 2012). Accordingly, rats raised under chronic hypoxia for several generations might have developed cellular mechanisms to reduce O<sub>2</sub> consumption and therefore help maintain elevated values of arterial  $p_{O_{2,\text{sat}}}$ . However, it should be emphasized that in rats this strategy is apparently not successful and does not allow them to overcome the drastic elevation of right ventricular hypertrophy and pulmonary hypertension.

Rats showed a higher respiratory exchange ratio ( $\dot{V}_{CO_2}/\dot{V}_{O_2}$ ) than mice. We previously reported elevated values in high altitude rats (Lumbroso et al., 2012). High values of the respiratory exchange ratio indicate that energy production is mostly accomplished by oxidation of glucose molecules, which is an effective way to optimize synthesis of ATP under low oxygen availability



**Fig. 7. Response to changes of inspired O<sub>2</sub> in high altitude rats and mice.** (A) Arterial oxygen saturation ( $p_{O_{2,\text{sat}}}$ ) and (B) heart rate ( $f_H$ ) in 2 month old rats and mice. Values are means+s.e.m. \**P*<0.05, \*\**P*<0.01 and \*\*\**P*<0.001 mice versus rats. †*P*<0.05 and ††*P*<0.01 versus baseline (21% O<sub>2</sub>).

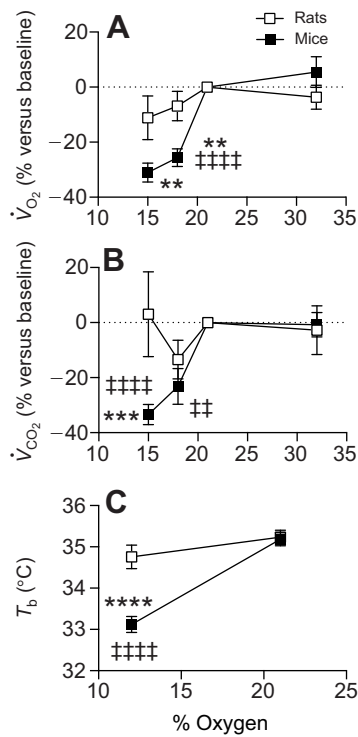


**Fig. 8. Response to changes of inspired O<sub>2</sub> in high altitude rats and mice.** (A) Minute ventilation ( $\dot{V}_E$ ), (B) tidal volume ( $V_T$ ) and (C) respiratory frequency ( $f_R$ ) in 2 month old rats and mice. Values are means+s.e.m. \**P*<0.05, \*\*\**P*<0.001, \*\*\*\**P*<0.0001 mice versus rats. ††*P*<0.01 and ††††*P*<0.0001 versus baseline (21% O<sub>2</sub>).

(Hochachka and Somero, 2002) as glucose oxidation produces the highest ratio of ATP synthesized for each molecule of O<sub>2</sub> consumed compared with other metabolites. Interestingly, deer mice (considered as being genetically adapted to altitude) maintain low values of  $\dot{V}_{CO_2}/\dot{V}_{O_2}$  and high levels of fatty acid oxidation under combined exposure to cold and hypoxic stress (Cheviron et al., 2012). This pattern probably helps to maintain elevated glycogen stores that can be used for bursts of intense exercise ('fight or flight' stress response). As fatty acid oxidation requires more O<sub>2</sub> than glycolytic pathways, it should be supported by physiological adjustments of the O<sub>2</sub> transport system.

#### Reduced lung volume and alveolar exchange surface area in high altitude rats versus mice

Compared with mice, rats had enlarged air spaces leading to lower mass-corrected relative alveolar exchange surface area. Combined with a reduced mass-corrected lung volume, this leads to a drastic difference of the estimated mass-corrected total lung exchange surface area between rats and mice. In mammals – with a range of body mass from 10 g (bat) to >1000 kg (whale) – lung alveolar surface area in m<sup>2</sup> is directly proportional to O<sub>2</sub> consumption in ml  $\text{min}^{-1}$ , with a scaling variable of 1 (Tenney and Remmers, 1963). Therefore, the ratio of alveolar surface area to O<sub>2</sub> consumption should be similar between two species, which is not the case when comparing rats and mice at altitude (see Fig. 6). However, if we compare the values obtained at high altitude with an estimation of expected sea level values (see Table 1), it is clear that, relative to O<sub>2</sub> consumption, the total alveolar surface area is enhanced in rats and mice living at high altitude. Yet, while in mice this was achieved by increasing the volume of the lungs and the total alveolar surface area, in rats this is possible only because  $\dot{V}_{O_2}$  is much lower at high

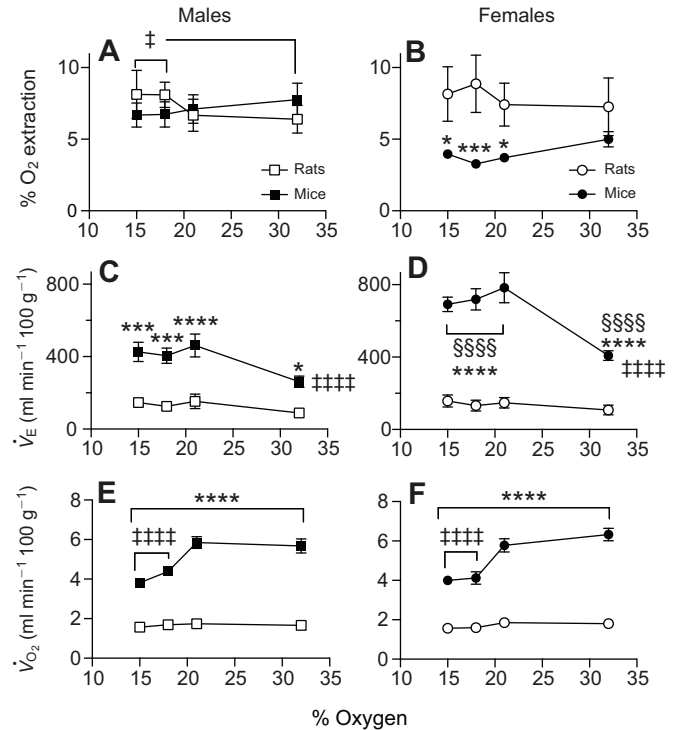


**Fig. 9. Response to changes of inspired O<sub>2</sub> in high altitude rats and mice.** (A) O<sub>2</sub> consumption ( $\dot{V}_{O_2}$ ), (B) CO<sub>2</sub> production rate ( $\dot{V}_{CO_2}$ ) and (C) rectal temperature (body temperature,  $T_b$ ) in 2 month old rats and mice. Values are means+s.e.m. \*\* $P<0.01$ , \*\*\* $P<0.001$  and \*\*\*\* $P<0.0001$  mice versus rats. †† $P<0.01$  and †††† $P<0.0001$  versus baseline (21% O<sub>2</sub>).

altitude than at sea level. This striking difference might be due to an ability of the lungs of mice to respond to hypoxia by increasing the gas exchange surface area, while these responses would not be present in rats, which therefore have reached a fragile equilibrium at altitude by reducing  $\dot{V}_{O_2}$ . Of note, compared with sea level rats, high altitude rats had an increase in the mass of the lungs but not in lung volume (see Table 1), probably as a consequence of neo-angiogenesis and hypertrophy of lung vessels.

#### Respiratory and metabolic responses to hypoxia in high altitude rats and mice

Chemoreflex function maintains adequate ventilation under chronic hypoxia, and is an important contributor to efficient adaptation to hypoxia (Dempsey et al., 2014; Joseph and Pequignot, 2009). At high altitude, a relevant approach to evaluate the basal activity of the peripheral chemoreceptors is to relieve the chronic hypoxic stimulus, which in the present study was achieved by exposure to sea level  $P_{O_2}$ . Rats and mice showed a similar decrease of  $\dot{V}_E$  in response to sea level  $P_{O_2}$ , suggesting similar sensibility of peripheral chemoreceptors to hypoxia, but this was achieved by a different pattern of response with a higher decrease of  $f_R$  being observed in mice compared with rats, and a decrease of  $V_T$  in rats but not in mice. This is a striking difference if we take into account that under baseline conditions the  $V_T$  of rats (either mass specific or mass corrected) is already smaller compared with that of mice. Hence, the chemoreflex drive in rats increases the  $V_T$ , which probably helps to maintain an elevated  $p_{O_2, sat}$  by optimizing ventilation of the lungs. Such differences between rats and mice probably arise from different neurochemical processes within the central respiratory pathways leading to differential translation of the peripheral chemoreceptor inputs into phrenic nerve activity. It is intriguing to report that HIF and HIF target genes appear to be key elements in the



**Fig. 10. Responses to changes of inspired O<sub>2</sub> in high altitude rats and mice.** (A,B) Percentage O<sub>2</sub> extraction, (C,D) minute ventilation ( $\dot{V}_E$ ) and (E,F) mass-specific O<sub>2</sub> consumption rate ( $\dot{V}_{O_2}$ ) in 2 month old male and female rats and mice. Values are means+s.e.m. § $P<0.05$  and §§§§ $P<0.0001$  females versus males. \* $P<0.05$ , \*\*\* $P<0.001$  and \*\*\*\* $P<0.0001$  mice versus rats. † $P<0.05$  and †††† $P<0.0001$  versus baseline (21% O<sub>2</sub>); in A, † $P<0.05$  versus 32% O<sub>2</sub>.

plasticity of the respiratory control system under chronic hypoxia (Kline et al., 2002; Pascual et al., 2001; Powell and Fu, 2008; Prabhakar and Semenza, 2012; Soliz et al., 2007); accordingly, species differences might be related to differential control of HIF or HIF-regulating proteins in rats compared with mice.

It is also noteworthy that mice are able to reduce metabolic rate and rectal temperature in response to hypoxia, while this response is not present in rats. This response is typically seen as being protective and contributes to the preservation of arterial oxygen pressure in hypoxia. This is an active process resulting from a reduction of the thermoregulatory set point, regulated within the preoptic hypothalamic nucleus (Barros et al., 2001; Steiner et al., 2002). It is possible to ask whether the drop in rectal temperature accounted for the fall in metabolic rate or whether there is evidence of metabolic suppression beyond that due to a resetting of the body temperature set point (Barros et al., 2001). We used a web-based calculator ([www.physiologyweb.com/calculators/q10\\_calculator.html](http://www.physiologyweb.com/calculators/q10_calculator.html)) and calculated that in mice, O<sub>2</sub> consumption would drop from 5.82 to 4.62 ml min<sup>-1</sup> 100 g<sup>-1</sup> with a drop of rectal temperature from 35.2 to 33.1°C and a  $Q_{10}$  of 3. As O<sub>2</sub> consumption was below these values, there is indeed evidence of metabolic suppression beyond that due to a resetting of the body temperature set point. The excess metabolic suppression is 1.37 ml O<sub>2</sub> min<sup>-1</sup> 100 g<sup>-1</sup> – almost 1/4 of the basal O<sub>2</sub> consumption of mice. The fact that this response is present in mice but not in rats indicates that mice are able to display protective responses to counteract further reduction of O<sub>2</sub> level, which is clearly another advantage at high altitude.

The high  $f_H$  observed in rats might also explain the fact that several rats were not able to withstand hypoxic exposure at 12% O<sub>2</sub>. At the

altitude of La Paz, this  $O_2$  level corresponds to an inspired  $P_{O_2}$  of 60 mmHg (or 7.8%  $O_2$  in inspired air at sea level), a severe level of hypoxia that could be close to the minimum  $O_2$  level necessary to maintain the function of the heart under already challenging conditions.

### Sex-specific effects in high altitude rats and mice

Females of both species showed lower Hct values than males, probably reflecting the inhibitory effect of estradiol on Epo synthesis (Mukundan et al., 2002). However, sex-specific effects on respiratory parameters were mostly present in mice, with females mice showing higher  $V_T$  and  $\dot{V}_E$  than male mice (either mass-specific or mass-corrected data). In rats, females had lower mass-corrected  $f_R$  than males, but  $V_T$  and  $\dot{V}_E$  were similar (either mass-specific or mass-corrected data). Higher values of  $\dot{V}_E$  and  $V_T$  in females compared with males likely reflects the respiratory stimulant effect of ovarian steroids (Joseph et al., 2002), and have been reported in our previous studies (Lumbroso et al., 2012). The fact that sex-specific effects were not reported in the present study in rats might be related to the reduced sample size (six males, six females), while we compared 17 males with 16 females in our previous study (Lumbroso et al., 2012).

### Conclusions

We conclude that adult laboratory rats and mice that have been raised for a similar period of time under conditions of chronic hypoxia at high altitude display divergent physiological responses. Interestingly, a previous genetic study showed that there is no adaptive modification of the Hb function in wild *M. musculus* caught in La Paz compared with other specimens at sea level (Lima, Peru) (Storz et al., 2007); therefore, it is tempting to speculate that the physiological responses observed in mice that have been bred in La Paz might explain the ability of this species to successfully withstand the high altitude hypoxic environment.

## MATERIALS AND METHODS

### Animals

We used adult (2–3 months old) male and female rats (six animals of each sex) and mice (10 animals of each sex) from different colonies. Mice were obtained from the Instituto Nacional de Laboratorios de Salud (INLASA, La Paz, Bolivia). These mice are descendants of a lineage of animals that were originally imported from France (IFFA-CREDO) 20–25 years ago. Because the genetic background of the mice was not available, we performed a complete genetic analysis on a sample of DNA extracted from the lungs of an adult male mouse, by using the background characterization offered by Charles River (St Constant, Québec, Canada). This analysis compares allele variations of 384 single-nucleotide polymorphisms of the unknown DNA with the DNA of 40 common inbred and F1 hybrid strains. The analysis indicated that the mice scored as 73.11% FVB, and that they are either a mix of FVB and two other strains, or an outbred strain.

Rats were Sprague–Dawley from the Instituto Boliviano de Biología de Altura (IBBA, La Paz, Bolivia). These animals were originally imported from France (IFFA-CREDO) in 1992, and constantly bred at the IBBA (for the present study, we used rats from the 30th generation).

Animals were housed under standard conditions, had access to food and water *ad libitum*, and were exposed to a 12 h:12 h light:dark cycle. After they were transported from INLASA to the IBBA, mice were left undisturbed for 1 week before starting the experiments. All protocols were reviewed and approved by the scientific committee of IBBA in Bolivia and are in concordance with the guidelines of the Canadian Council of Animal Care.

### Recording of ventilatory parameters, arterial $p_{O_2, \text{sat}}$ and $f_H$ in unrestrained, unanesthetized rats and mice at high altitude

The animals were placed in a whole-body flow-through plethysmograph chamber for mice or rats (Emka Technologies, Paris, France) that was

constantly flushed with fresh room air and previously calibrated by injecting a known volume of air (0.5 ml for mice, 5 ml for rats). The respiratory flow trace was recorded using a differential pressure transducer (ML141, ADInstruments, Colorado Springs, CO, USA). The flow of air through the chamber was set and continuously monitored at 200 ml  $\text{min}^{-1}$  (mice) or 1500 ml  $\text{min}^{-1}$  (rats) using a pump and gas flow restrictor/monitor (G265, Qubit systems, Kingston, ON, Canada). Inlet and outlet gases were alternately subsampled, directed toward a water pressure analyzer (RH-300, Sable Systems, Las Vegas, NV, USA), dried and directed to an oxygen/carbon dioxide analyzer (ML206 Gas Analyzer, ADInstruments) for respiratory gas analysis. All signals were directed toward a PowerLab acquisition interface for analog-to-digital conversion and storage on a computer running LabChart 5 software (ADInstruments).

Before each experiment, rectal temperature was measured, and the animal was weighed and then fitted with a limb sensor for continuous recordings of pulse oximetry capillary oxygen saturation ( $p_{O_2, \text{sat}}$ ) and  $f_H$  (MouseSTAT, Kent Scientific, Torrington, CT, USA). The animal was then placed in the chamber for a period of acclimation (10–15 min), and baseline recordings were initiated for 20 min, followed by acute exposure to 32%  $O_2$  – corresponding to sea level  $P_{O_2}$  – for 10 min. The animal was then exposed to graded levels of hypoxia (18%, 15% and 12%  $O_2$  for 10 min each). The mean barometric pressure in La Paz being around 490 mmHg, these percentage  $O_2$  values correspond, respectively, to a  $P_{iO_2}$  of 90, 75 and 60 mmHg. At sea level (barometric pressure 760 mmHg), these  $P_{iO_2}$  values would be equivalent to 12%, 10% and 8%  $O_2$  in inspired air, respectively. Rectal temperature was measured immediately at the end of the last hypoxic exposure.  $V_T$  was calculated from the integrated flow trace as previously described (Lumbroso and Joseph, 2009; Lumbroso et al., 2012), by using standard equations (Bartlett and Tenney, 1970). All values were obtained while the animal had a stable breathing pattern during baseline recordings and within the last 3 min of each condition.

Oxygen consumption ( $\dot{V}_{O_2}$ ) and  $CO_2$  production ( $\dot{V}_{CO_2}$ ) rates were calculated using the following equations (Lighton, 2008):

$$\dot{V}_{O_2} = \frac{\text{Flow} \times [(O_{2, \text{in}} - O_{2, \text{out}}) - O_{2, \text{out}} \times (CO_{2, \text{out}} - CO_{2, \text{in}})]}{1 - O_{2, \text{out}}}, \quad (1)$$

$$\dot{V}_{CO_2} = \text{Flow} \times \frac{[(CO_{2, \text{out}} - CO_{2, \text{in}}) - CO_{2, \text{out}} \times (O_{2, \text{in}} - O_{2, \text{out}})]}{1 - CO_{2, \text{out}}}, \quad (2)$$

where ‘Flow’ is the flow of air measured before entry into the chamber, ‘ $O_{2, \text{in}}$ ’ and ‘ $CO_{2, \text{in}}$ ’ are the gas fractions in the inflowing air (considered at 20.9% and 0.038%, respectively), and  $O_{2, \text{out}}$  and  $CO_{2, \text{out}}$  are the gas fractions measured in the outflowing line. The respiratory exchange ratio was calculated as  $CO_2$  production/ $O_2$  consumption.

### Hematological parameters, and dissection of hearts and lungs

A sample of blood was drawn from the tail. Hct was measured by microcentrifugation (Micro-MB centrifuge, International Equipment Company, USA) for 15–20 min, and Hb concentration was determined by using a Hemocue field spectrophotometer (Agelholm, Sweden). All samples were processed in duplicate for rats, and for mice if enough blood was obtained.

Following blood sampling, animals were anesthetized by an intraperitoneal injection (0.1 ml 100  $\text{g}^{-1}$  of body mass) of ketamine (87.5  $\text{mg ml}^{-1}$ ) and xylazine (12.5  $\text{mg ml}^{-1}$ ) then perfused through the left ventricle with ice-cold PBS (pH 7.2) at a constant pressure of 24  $\text{cmH}_2\text{O}$  for mice and 35  $\text{cmH}_2\text{O}$  for rats. The heart was quickly dissected and weighed. The atria were separated from the ventricles, then the right ventricle (RV) was cut from the left ventricle (LV, which was left with the cardiac septum, S). We weighed the ventricles separately (RV and LV+S) and these values were used to measure the ratio  $RV/(LV+S)$ , an index of right ventricular hypertrophy and pulmonary hypertension. In four males and four females of each species, after cardiac perfusion with PBS, a catheter was fixed in the trachea, and the lungs were inflated with 4% PFA for 30 min at a constant pressure of 2.3 kPa (or 24  $\text{cmH}_2\text{O}$ ), then dissected. The total volume of the inflated lungs was measured by liquid displacement, and they were kept in 4% PFA for 24 h at room temperature. The next day, the lungs were separated into left and right lung (for mice) and five lobes (for rats), which were dehydrated in alcohol (1 h in 65% alcohol solution, then 2 h in

each graded alcohol solution: 75%, 85%, 95% and 100%); alcohol was then replaced by xylol (two baths, 1 h each), and paraffin (two baths, 1 h each and a final overnight bath). The samples were embedded in paraffin and shipped to Québec city where they were processed to determine lung histology.

### Lung histology

Paraffin-embedded lungs were cut at a thickness of 5  $\mu\text{m}$  using a microtome (Jung RM2065 Leica Instruments GmbH, Germany). Sections were then mounted on glass slides and dried for 24 h in room air. Slices were deparaffinized in toluene baths (2 $\times$ 10 min), and re-hydrated by successive immersion in alcohol [100% ( $\times$ 2), 95%, 70%, 50%] and in water before being colored with Harris Hematoxylin solution (VWR International) for 3 min, rinsed in water for 1 min, exposed in acid-alcohol solution (five successive immersions in 1% HCl, 70% ethanol), washed with water for 1 min, dipped in Bluing Reactive RTU (VWR International) and then in water for 1 min. The slides were mounted in water-based mounting medium (Liquid Coverglass SHUR/Mount™, EMS, Hatfield, PA, USA). Images were captured using a Nikon eclipse E600 digital imaging system at a magnification of  $\times$ 100.

### Lung morphology

We randomly selected three non-overlapping images from each slide using three slides per animal and eight animals per group (four males and four females). The mean linear intercept ( $L_m$ ) was determined by overlapping a grid of 20 horizontal and vertical lines (189  $\mu\text{m}$  each) on each image and by counting the number of intersections with alveolar walls (Hsia et al., 2010). When a line crossed a vessel wall rather than an alveolar wall, it was counted as 0.5 intersection.  $L_m$  was calculated by using the following equation:  $L_m = (Nd)/m$ , with  $N$  being the number of lines (20),  $d$  the length of each line (189  $\mu\text{m}$ ) and  $m$  the number of intersections with alveolar walls. From  $L_m$  values, we calculated the relative alveolar surface area as  $S$  ( $\text{m}^2 \text{cm}^{-3}$ ) =  $4V/L_m$ , with  $V$  being the volume of one image (Hsia et al., 2010). An estimation of the total alveolar surface area was calculated as the product of the relative alveolar surface area and lung volume (measured by water displacement after fixation).

### Allometric scaling in rats and mice

To compare physiological and morphological values between control mice and rats, we used allometric scaling, a standard approach to compare animals of different size (Maina et al., 1989; Stahl, 1967). The allometric scaling variables ( $b$  in the equation below) were obtained by calculating the slope of a regression line fitted through a log–log plot of a parameter ( $x$ ) as a function of body mass ( $M$ ). To obtain an equation of the following form:  $x = aM^b$ . From this equation, we reported mass-specific variables with  $M^b$ . For the respiratory variables, we used the scaling variable calculated by Stahl (1967), which are: lung mass and lung volume,  $b=1$ ;  $\dot{V}_E$ ,  $b=0.8$ ;  $V_T$ ,  $b=1.04$ ;  $f_R$  and  $f_{IH}$ ,  $b=-0.25$ ;  $O_2$  consumption and  $CO_2$  production,  $b=0.76$ ). For relative and total alveolar surface area, we respectively used  $b=-0.13$  and  $b=0.88$  as reported for mammals by Maina et al. (1989). In the text, data corrected for the allometric scaling variables are referred to as mass corrected whereas data compared for body mass are referred to as mass specific.

### Sea level values

Expected sea level values for a selected series of variables are presented in Table 1. These values were either selected from the literature or were obtained from our colony of sea level Sprague–Dawley rats and FVB mice (A.J.-L. and V.J., unpublished results). Values of lung morphology were obtained by using the approach described above. These values are mostly informative, and we have not made statistical analysis to compare high altitude versus sea level values.

### Statistical analysis

We used GraphPad Prism 6.0c (for 2-way ANOVA and *post hoc* analysis) and JMP 11.0 (for 2-way ANOVA with repeated measures) for statistical analysis. All values are reported as means $\pm$ s.e.m., and the significant  $P$ -value was set a 0.05.

For the Hct, right ventricular hypertrophy and lung morphology data, we first performed 2-way ANOVA with species and sex as grouping variables. When significant effects or a significant interaction between species and sex appeared, a *post hoc* analysis was performed (Fisher's LSD).

For variables measured at different levels of  $P_{I_{O_2}}$  we used a MANOVA model (in JMP) with species and sex as grouping variables and  $P_{I_{O_2}}$  as the repeated term. When no significant effect of sex, or significant interactions between sex and species or sex and  $P_{I_{O_2}}$  appeared for these values, data from males and females were pooled. When significant species effects appeared, a *post hoc* analysis was performed for each  $P_{I_{O_2}}$  level (Fisher's LSD) to determine the effects of species, or for each group to determine the effects of  $P_{I_{O_2}}$  level.  $P$ -values are reported in the figures with the following general pattern: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  and \*\*\*\* $P < 0.0001$ , respectively.

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### Competing interests

The authors declare no competing or financial interests.

### Author contributions

V.J., M.G., R.S. and A.J.-L. developed the concepts and designed the experiments. A.J.-L., G.V., M.G. and I.V. performed the experiments. A.J.-L. and V.J. analyzed the data and prepared the figures. V.J. and A.J.-L. prepared or edited the manuscript prior to submission.

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