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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 4,000 m) while rats are absent at these altitudes, and there is 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 (3,600 m) and compared their hematocrit levels, right ventricular hypertrophy (index of pulmonary hypertension), and alveolar surface 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 (SpO_2) under ambient conditions, in response to exposure to sea level PO_2 (32% O_2 = 160 mmHg - 10 minutes), and hypoxia (18 and 15% O_2 = 90 and 75 mmHg – 10 minute 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 to rats, adult mice had similar levels of SpO_2, but lower hematocrit and hemoglobin levels, reduced right ventricular hypertrophy, and higher mass-corrected alveolar surface, tidal volume and metabolic rate. In response to sea level PO_2 and hypoxia, mice and rats had similar changes of ventilation, but metabolic rate decreased much more in hypoxia in mice, while SpO_2 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.

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 (PO_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 for O_2 (Storz et al., 2010a; Storz et al., 2009), higher density of micro-vessels and mitochondria surface in the heart (Qi et al., 2008), left-ventricle hypertrophy that could lead to increased stroke volume (Pichon et al., 2013), and hematocrit 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 exaggerated hematocrit 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 4,000 m of altitude (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 (Wilmshurst 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 3,600 m above sea level, in La Paz, Bolivia. These animals present an elevated hematocrit and hemoglobin levels, a right ventricular hypertrophy (sign of pulmonary hypertension), an altered alveolar structure with enlarged airspaces in the lungs, and impaired respiratory control (Lumbroso et al., 2012). Considering that an elevated hematocrit 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 than rats. To this aim we compared arterial oxygen saturation, hematological, cardiac, ventilatory, metabolic, and lungs characteristics in adult mice and rats that have been born and raised at an altitude of 3,600 m (La Paz, Bolivia) since 1992. The study has been performed in males and females to address whether sex-specific physiological differences would be present in 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 SpO_2 values around 80%, without significant effect of species or sex (Fig. 1A). Mice had a lower right ventricular hypertrophy compared to rats (P for species <0.0001, Fig. 1B). Heart rate was higher in mice compared to rats (P value for species<0.0001, Fig. 1C), but when the difference in body weight was taken into account by applying the allometric scaling factor (heart rate in bpm / weight in $g^{-0.25}$ – see material and method), mice had lower mass-corrected heart rate compared to rats (P for species <0,0001, Fig. 1D), and female rats had lower mass-corrected heart rate than male rats (Fig. 1D).

Compared to rats, high altitude mice showed lower hemoglobin concentration (P for species <0.0001, Fig. 1E), and hematocrit (P for species <0.0001 Fig. 1F) values. Interestingly, the hemoglobin value in mice was below the normal range of the sea-level value (see table 1) while hematocrit was slightly higher than the normal sea-level range (table 1). In rats, both hematocrit and hemoglobin values were above the normal sea level range (table 1). Compared to males, females of both species had lower hematocrit (P for sex <0.0001, Fig. 1E), and female rats had lower hemoglobin values than male rats (Fig. 1F).

Minute ventilation, Tidal volume, and Respiratory Frequency.

Compared to rats, mice have higher mass-specific minute ventilation, tidal volume, and respiratory frequency (P for species<0.0001, Fig. 2 A-C). Female mice have higher mass-specific minute ventilation (P for sex = 0.04) and tidal volume (P for sex = 0.04) than male mice (Fig. 2 A-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 minute ventilation: male mice had similar mass-corrected minute ventilation and respiratory frequency than male rats, but higher mass-corrected tidal volume (Fig. 2D-F). Female mice had higher mass-corrected minute ventilation and tidal volume compared to male mice (Fig. 2D-E). Mass-corrected respiratory frequency was lower in female rats compared to male rats (Fig. 2F), but there was no significant effect of species.

Metabolic rate, respiratory exchange ratio, and rectal temperature.

Compared to rats, mice had higher mass-specific (Fig. 3A-B) or mass-corrected (Fig. 3C-D) O_2 consumption and CO_2 production (P<0.0001). The respiratory exchange ratio was higher in rats compared to mice (P for species <0,0002, Fig. 3E). Rectal temperature measured before the onset of respiratory and metabolic recordings was similar in rats (35.2 ± 0.2 °C) and mice (35.2 ± 0.2 °C). There was no effect of sex for metabolic variables.

Lung volume, lung weight, and lung architecture.

Compared to rats, mice have higher mass-specific lung volume and lung weight (P for species <0.0001, Fig. 4 A-B). Since for lung volume and lung weight, the allometric scaling parameter is 1 (see methods), mass-specific values allow direct comparison between species of different body weight. 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 (Lm) was lower in mice compared to rats (P for species <0.0001, Fig. 5C), which was reflected in higher mass-corrected relative and total alveolar surfaces in mice (P for species <0.0001, Fig. 5 C-D). There was no effect of sex for these variables.

Because alveolar surface is directly proportional to oxygen consumption (Tenney and Remmers, 1963), we also calculated the ratio of the total alveolar surface to the O_2

consumption (m^2/ml O_2 consumed in 1 minute). There was no significant effect of sex (P for sex = 0.58) or significant sex x species interaction. The mean value was higher in mice compared to rats (Fig. 6), and both values are higher than the expected sea level values (table 1).

Respiratory, metabolic and heart rate responses to changes in inspired PO_2 in high altitude rats and mice.

For the responses normalized to baseline values there was no significant effect of sex, accordingly males and females are pooled. At 12% O_2 (corresponding to a PiO_2 of 60 mmHg – see material and method), 4 rats (3 males and 1 female) had signs of distress, prolonged apneas, and gasps within the 1st 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.

Arterial oxygen saturation and heart rate.

SpO_2 was higher in mice compared to rats upon exposure to sea level PiO_2 (32% O_2, Fig. 7A) and in moderate hypoxia (18% O_2, Fig. 7A). Heart rate declined in rats when exposed to sea level PiO_2, but remained unchanged upon hypoxic exposure (Fig 7B). In mice, heart rate remained unchanged when exposed to sea level PiO_2, slightly declined upon hypoxic exposure to 18% O_2, and returned to baseline levels at 15% O_2 (Fig 7B).

Minute ventilation, tidal volume, respiratory frequency, and metabolic rate.

Exposure to sea level PiO_2 reduced minute ventilation to a similar extent in mice and rats (Fig. 8A), however rats had a more pronounced decline of tidal volume than mice (Fig. 8B), and contrastingly mice had a more important decline of respiratory frequency than rats (Fig. 8C). Under moderate hypoxia (18%) tidal volume was higher in mice compared to rats (the apparent drop at 18% O_2 in rats is not significant compared to 21% O_2).

In response to sea level PiO_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_2, VO_2 and VCO_2 had fallen by more than 30% in mice (Fig. 9A and 9B). 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 increased minute ventilation and maintained their metabolic rate to the baseline level, while in mice metabolic rate fell during hypoxic exposure. To ask whether this pattern in rats is achieved by increasing O_2 extraction from pulmonary gas, we calculated the percentage of O_2 extraction (O_2 consumption / Ve x FiO_2 - Fig. 10A and B). There was a significant effect of species for this parameter (P=0.05) and a significant species x hypoxia interaction (P=0.0009). In males rats, O_2 extraction increased in hypoxia (18 and 15% O_2) compared to 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), due to the fact that mass-specific ventilation (Fig 10C and 10D – ANOVA P value for sex = 0.002 : sex x species = 0.004) and tidal volume (not shown) is higher in female mice than male mice throughout the hypoxic exposure, while there is no effect of sex for mass-specific VO_2 (Fig. 10E and 10F).

DISCUSSION

We compared physiological responses in laboratory rats and mice that have been raised at 3,600 meters above sea level for about 30 generations. The key differences between these two species include higher erythrocytosis and elevated right ventricular hypertrophy (sign of higher pulmonary hypertension) in rats compared to mice, and lower mass-corrected lung volume, alveolar surface, tidal volume and O_2 consumption in rats compared to mice. However rats and mice have similar levels of SpO_2.

The relevance of performing comparative studies between two species as an approach to understand 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 illustrate physiological differences at altitude in 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 easily 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, the 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 SD rats and FVB mice (all adult males of similar age than 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 descendent from a provider of standard laboratory mice they should be M musculus domesticus (Yang et al., 2011), but we cannot exclude that they have been hybridized with local species such as M 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 if these groups of mice should be considered as distinct species or subspecies of Mus musculus (For a detailed discussion, see "Mus Musculus" entry in Mammals Species of the World 2005).

Right ventricular hypertrophy, excessive erythropoiesis and elevated heart rate 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% (Ciuclan 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 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 an important difference of hematocrit and hemoglobin values between rats and mice, and similar SpO_2 levels. The enhanced hematocrit 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 PO_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 to rats could contribute to lower Epo production and reduced hematocrit.

In male rats raised at altitude heart rate was around 500 bpm, whereas normal sea level values of heart rate are around 250-300 bpm during daytime, and 350-400 bpm during nighttime (Lemmer et al., 1993). However, it is not possible from our data to elucidate whether the elevated heart rate effectively increases cardiac output or compensates for a reduced stroke volume. By comparisons, while the heart rate reported in mice under baseline conditions (670 +/- 20 bpm in males) is higher than normal sea level values at rest (500-600 bpm daytime), it remains within the normal range of values recorded during the active phase (nighttime 600-700 bpm) (Sebastian et al., 2013).

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

Compared to mice, rats had a reduced O_2 consumption rate (either mass-specific or mass-corrected), which might help them to maintain SpO_2 value despite the reduced mass-corrected alveolar surface of the lungs. In several vertebrate species, reduced O_2 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_2 consumption and therefore help maintains elevated values of arterial SpO_2. However, it should be emphasized that in rats this strategy is apparently not successful and does not allow overcoming 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 (Hochachka and Somero, 2002) since glucose oxidation produces the highest ratio of ATP synthesized for each molecules of O_2 consumed compared to 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). Since fatty acid oxidation requires more O_2 than glycolytic pathways, it should be supported by physiological adjustments of the O_2 transport system.

Reduced lung volume and alveolar exchange surface in high altitude rats versus mice. Compared to mice, rats had enlarged airspaces leading to lower mass-corrected relative alveolar exchange surface. Combined with a reduced mass-corrected lung volume this leads to a drastic difference of the estimated mass-corrected total lung exchange surface between rats and mice. In mammals - with a range of body weight from 10 g (bat) to > 1000 kg (whale) - lung alveolar surface in m^2 is directly proportional to O_2 consumption in ml/min, with a scaling variable of 1 (Tenney and Remmers, 1963). Therefore the ratio of alveolar surface to O_2 consumption should be similar between 2 species, which is not the case when comparing rats and mice at altitude (cf. 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, relatively to O_2 consumption, the total alveolar surface is enhanced in rats and mice living at HA. Yet, while in mice this was achieved by increasing the volume of the lungs and the total alveolar surface, in rats this is possible only because O_2 consumption rate is much lower at HA 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, while these responses would not be present in rats, which therefore have reached a fragile equilibrium at altitude by reducing O_2 consumption rate. Of note, in high altitude rats compared to sea level rats (see table 1) there is an increase in the mass of the lungs but not in the volume, it is likely the consequence of neo-angiogenesis and hypertrophy of lung vessels.

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

Chemoreflex function helps maintaining 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 is achieved by exposure to sea level PO_2. Rats and mice had similar decrease of minute ventilation in response to sea level PO_2 suggesting similar sensibility of peripheral chemoreceptors to hypoxia, but this was achieved by a different pattern of response with a higher decrease of respiratory frequency being observed in mice compared to rats, and a decrease of tidal volume in rats but not in mice. This is a striking difference if we take into account that under baseline conditions the tidal volume of rats (either mass-specific or mass-corrected) is already smaller compared to mice. Hence, the chemoreflex drive in rats increases the tidal volume, which probably helps to maintain an elevated SpO_2 by optimizing ventilation of the lungs. Such differences between rats and mice probably rely on different neurochemical processes within the central respiratory pathways leading to differential translation of the peripheral chemoreceptors inputs into phrenic nerve activity. It is intriguing to report that HIF and HIF target genes appear to be key elements in the 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 to 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 preserve 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 account for the fall in metabolic rate or if is there evidence of metabolic suppression beyond that due to a resetting of Tb set point (Barros et al., 2001). We used a web-based calculator

(www.physiologyweb.com/calculators/q10_calculator.html) and calculated that in mice O_2 consumption would drop from 5.82 to 4.62 ml/min/100g with a drop of rectal temperature from 35.2 to 33.1°C and a Q10 of 3. Since O_2 consumption was below these values, there is indeed an evidence of a metabolic suppression beyond that due to a resetting of Tb set point. The excess metabolic suppression is 1.37 ml O_2/min/100g – almost 1/4th of the basal O_2 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_2 level, which is clearly another advantage at high altitude.

The high heart rate observed in rats might also explain the fact that several rats were not able to withstand hypoxic exposure at 12% O_2. At the altitude of La Paz, this O_2 levels corresponds to an inspired PO_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 an already challenging condition.

Sex specific effects in high altitude rats and mice.

Females of both species showed lower hematocrit values than males, probably reflecting the inhibitory effect of estradiol on Epo synthesis (Mukundan et al., 2002). However, sex specific effects on respiratory parameters where mostly present in mice, with females mice showing higher tidal volume and minute ventilation than male mice (either mass-specific or mass-corrected data). In rats, females had lower mass-corrected respiratory frequency than males, but tidal volume and minute ventilation were similar (either for mass-specific or mass-corrected data). Higher values of minute ventilation and tidal volume in females compared to males likely reflect 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 (6 males, 6 females), while we compared 17 males to 16 females in the previous study (Lumbroso et al., 2012).

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 haemoglobin function in wild *Mus musculus* caught in La Paz compared to 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.

MATERIAL AND METHODS

Animals

We used adult (2-3 months old) male and female rats (6 animals each sex) and mice (10 animals each sex) from different breed. Mice were obtained from the Instituto Nacional de Laboratorios de Salud (INLASA, La Paz, Bolivia). These mice are descendent of a lineage of animals that have been originally imported from France (IFFA-CREDO) 20-25 years ago. Since 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 to 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 2 other strains, or an outbred strain.

Rats are Sprague Dawley from the Instituto Boliviano de Biologa de Altura (IBBA, La Paz, Bolivia). These animals have been 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-libidum, and were exposed to a 12:12h light/dark cycle. Once transported from INLASA to the IBBA, mice were left undisturbed for 1 week before starting the experiments. All protocols have been 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 oxygen saturation, and heart rate 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/min (mice), or 1500 ml/min (rats) using a pump and gas flow restrictor/monitor (G265, Qubit systems, Kingston, ON, Canada). Inlet and outlet gases were alternatively subsampled, directed toward a water pressure analyzer (RH-300, Sable System, Las Vegas, NV), dried and directed to an oxygen/carbon dioxide analyzer (ML206 Gas analyzer, ADInstruments) for respiratory gases analysis. All signals were directed toward a PowerLab acquisition interface for analog-to-digital conversion and storage on a computer running the LabChart software 5 (ADInstruments).

Before each experiment, rectal temperature was measured and the animal were weighed, and equipped with a limb sensor for continuous recordings of pulse oximetry capillary oxygen saturation (SpO_2) and heart rate (MouseSTAT – Kent Scientific, Torrington, CT, USA). The animal was then placed in the chamber for a period of tranquilization (10 - 15 minutes), and baseline recordings were initiated for 20 min, followed by acute exposure to 32% O_2 – corresponding to the sea level PO_2 – for 10 min. The animal was then exposed to graded levels of hypoxia (18, 15, 12% O_2 – 10 min each). The mean barometric pressure in La Paz being around 490 mmHg, these O_2% correspond respectively to a partial pressure of inspired O_2 of 90, 75, and 60 mmHg. At sea level (barometric pressure=760 mmHg), these PiO_2 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. Tidal volume 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 minutes of each condition.

Oxygen consumption and CO_2 production rates were calculated using the following equations (Lighton, 2008):

411 $$O_2 consumption = \frac{flow \times \left[\left(O_{2_{in}} - O_{2_{out}} \right) - O_{2_{out}} \times \left(CO_{2_{out}} - CO_{2_{in}} \right) \right]}{\left(1 - O_{2_{out}} \right)}$$

412 $$CO_2 production = flow \times \frac{\left[\left(CO_{2_{out}} - CO_{2_{in}} \right) - CO_{2_{out}} \times \left(O_{2_{in}} - O_{2_{out}} \right) \right]}{\left(1 - CO_{2_{out}} \right)}$$

413 where "flow" is the flow of air measured before entry into the chamber, "O_2in" and "CO_2in"
414 are the gas fractions in the inflowing air (considered at 20.9% and 0.038%, respectively),
415 and "O_2out" and "CO_2out" are the gas fractions measured in the outflowing line. The
416 respiratory exchange ratio was calculated as CO_2 production / O_2 consumption.
417
418 *Hematological parameters, dissection of hearts and lungs.*
419 A sample of blood was drawn from the tail. The hematocrit was measured by
420 microcentrifugation (Micro-MB centrifuge – International Equipment Company, USA) for
421 15-20 minutes, and hemoglobin concentration was determined by using the Hemocue field
422 spectrophotometer (Agelholm, Sweden). All samples were processed in duplicates for rats,
423 and mice if enough blood was obtained.
424 Following blood sampling, animals were anesthetized by an intraperitoneal injection (0.1
425 ml/100g of body weight) of ketamine (87.5 mg/ml) and xylazine (12.5 mg/ml) then
426 perfused through the left ventricle with ice-cold PBS (pH 7.2) at a constant pressure of 24
427 cmH_2O for the mice and 35 cmH_20 for the rats. The heart was quickly dissected and
428 weighted. The atria are separated from the ventricles. Then the right ventricle (RV) was cut
429 off from the left ventricle (LV - left with the cardiac septum - S). We weighted the ventricles
430 separately (RV and LV+S) and these values were used to measure the ratio of RV/(LV+S), an
431 index of right ventricular hypertrophy and pulmonary hypertension. In 4 males and 4
432 females of each species, after cardiac perfusion with PBS, a catheter was fixed in the
433 trachea, the lungs were inflated with 4% PFA for 30 minutes at a constant pressure of 24
434 cmH_2O, then dissected. The total volume of the inflated lungs was measured by liquid
435 displacement, and they were kept in 4% PFA for 24 hours at room temperature. The next
436 day, the lungs were separated into left and right lung (for mice) and 5 lobes (for rats),
437 which were dehydrated (1 hours into 65% alcohol solution, then 2 hours in each graded
438 alcohol solution - 75%, 85%, 95% and 100%), alcohol was then replaced by xylol (2 baths, 1

hour each), and paraffin (2 baths, 1 hour each and a final overnight bath). The samples were included in paraffin and shipped to Québec city where they were processed to determine lung histology.

Lung histology.

Paraffin embedded lungs were cut at 5 µm thick using a microtome (Jung RM2065 Leica instruments GmbH, Germany). Sections were then mounted on glass slides and dried for 24h in room air. Slices were deparaffinized in toluene baths (2 x 10 min), and re-hydrated by successive immersions in alcohol 100% (x2), 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 (5 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). The images were captured using a Nikon eclipse E600 digital imaging system at a magnification of 100 X.

Lung morphology.

We randomly selected 3 non-overlapping images from each slide using 3 slides per animal and 8 animals per group (4 males and 4 females). The Mean Linear Intercept (Lm) was determined by overlapping a grid of 20 horizontal and vertical lines (189 µm 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. Lm was calculated by using the following equation: $Lm = (N.d)/m$, with N being the number of line (20), d the length of each line (189 µm), and m the number of intersections with alveolar walls. From Lm values, we calculated the relative alveolar surface as $S (m^2/cm^3) = 4V/Lm$, with V being the volume of one image (Hsia et al., 2010). An estimation of the total alveolar surface was calculated as the product of the relative alveolar surface 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) are obtained by calculating the slope of a regression line fitted through a log-log plot of a parameter (x) as a function of body weight (M). This allow to obtain an equation of the following form: $x=aM^b$. From this equation, we expressed and compared mass-specific variables reported to M^b. For the respiratory variables we used the scaling variable calculated by Stahl (Stahl, 1967), which are: lung weight and lung volume (b=1), minute ventilation (b=0.8), tidal volume (b=1.04), respiratory frequency and heart rate (b=-0.25), O_2 consumption and CO_2 production (b=0.76). For relative and total alveolar surface we respectively used (b=-0.13) and (b=0.88) as reported for mammals by Maina (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 have been obtained from our colony of sea level sprague-dawley rats and FVB mice (unpublished results). Values of lung morphology have been obtained by using the approach described above. These values are mostly informative, and we have not made statistical analysis to compare high altitude vs. 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 mean ± s.e.m., and the significant P value was set a 0.05.

For the hematocrit, right ventricular hypertrophy, and lung morphology data, we first performed 2-way ANOVAs 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 PiO_2 we used a MANOVA model (in JMP) with species and sex as grouping variables and PiO_2 as the repeated term. When no significant effect of sex, significant interactions between sex and species, or sex and PiO_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 PiO_2 level (Fisher's LSD) to determine the effects of species, or for each group to determine the effects of PiO_2 level. P values are reported in the figures with the following general pattern: *, **, ***, and **** are used to report $P< 0.05$, 0.01, 0.001, and 0.0001, respectively.

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Authors contribution: VJ, MG and AJL developed the concepts and designed the experiments. AJL, GV, MG and IV performed the experiments. AJL and VJ analyzed the data and prepared the figures. VJ and AJL prepared or edited the manuscript prior to submission.

	Rats		Mice	
	Sea level	High altitude	Sea level	High altitude
Body weight (g)	333±21	232±9	27.3±0.8	13.7±0.9
Rectal temp. (°C)	36.8±0.2	35.2±0.2	35.6±0.3	35.2±0.2
Hematocrit (%)	41.2 - 47.3 (a)	60.5±1.1	39.0 - 42.5 (b)	47.0±1.7
Hemoglobin (g/ml)	14.4 - 16.0 (a)	20.4±0.3	14.3 - 15.2 (b)	11.6±1
RV/(LV+S) (%)	27.9±0.6 (c)	59.7±5.6	~20 (d)	32.0±2.0
Heart rate (bpm)	250 - 400 (e)	503±4	500 - 700 (f)	669±23
Lung volume (ml/g)x10²	2.4±0.2	2.6±0.1	2.7±0.2	7.6±0.4
Lung weight (g/g)x10²	0.29±0.01	0.44±0.02	0.49±0.01	1.06±0.05
Total alv. surface ($m^2/g^{0.88}$)x10²	0.99±0.05	0.87±0.04	1.04±0.13	3.41±0.34
O_2 consumption ($ml/min/g^{0.76}$)	0.144±0.003	0.064±0.001	0.149±0.01	0.109±0.006
Total alv. surface to O_2 consumption (m^2/ml/min)	~0.10	0.30±0.03	~0.11	0.47±0.05

Table 1: Comparison between values obtained at sea level and high altitude in adult (3 months old) male rats or mice for selected variables. Values are ranges (min – max) or mean ± SEM. All high altitude values are those obtained in the present study. Sea level values are unpublished data from our colony of SD rats and FVB mice or are from the following references: (a): (Sharp and La Regina). (b) http://www.informatics.jax.org/mgihome/other/mouse_facts1.shtml, (c) (Lumbroso and Joseph, 2009), (d) (Ciuclan et al., 2011), (e) (Giknis and Clifford, 2006), (f) (Sebastian et al., 2013).

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FIGURE LEGEND

Figure 1. Hematological variables, right ventricular hypertrophy, heart rate and arterial saturation in high altitude rats and mice. (A) arterial saturation (SpO_2 – %), (B) right-to-left ventricle ratio (%), (C) heart rate (bpm), (D) mass-corrected heart rate ($bpm/g^{-0,25}$), (E) hematocrit (Hct – %), and (F) hemoglobin (Hb – g/dl), in 2 months old males and females rats and mice living at high altitude for 30 generations. Values are ± s.e.m.

****: P<0.0001 mice vs. rats.

°, °°°°: P<0.05 and <0.0001 females vs. males.

Figure 2. Ventilatory variables under baseline condition (21% oxygen) in high altitude rats and mice. (A, D) minute ventilation ($\dot{V}_E$ – ml/min/100g and $ml/min/g^{0,8}$), (B, E) tidal volume (V_T – ml/100g and $ml/g^{1,04}$), (C, F) respiratory frequency (fR – breaths/min and $breaths/min/g^{-0,25}$), in 2 months old rats and mice. (A, B) are mass-specific values, and (D, E, F) are mass-corrected values. Values are ± s.e.m.

*, **, ***, ****: P<0.05, <0.01, <0.001 and <0.0001 mice vs. rats.

°, °°: P<0.05 and <0.01 females vs. males.

Figure 3. Metabolic variables under baseline condition (21% oxygen) in high altitude rats and mice. (A, C) O_2 consumption ($\dot{V}_{O_2}$ – ml/min/100g and $ml/min/g^{0,76}$), (B, D) CO_2 production rate ($\dot{V}_{CO_2}$ – ml/min/100g and $ml/min/g^{0,76}$) in 2 months old rats and mice. (A, B) are mass-specific values, and (C, D) are mass-corrected values. (E) Respiratory exchange ratio ($\dot{V}_{CO_2} / \dot{V}_{O_2}$). Values are ± s.e.m.

*, **, ***, ****: P<0.05, <0.01, <0.001 and <0.0001 mice vs. rats.

Figure 4. Variables of lung morphology in high altitude rats and mice. (A) mass-specific lung volume (ml/g), and (B) mass-specific lung weight (g/g) in 2 months old rats and mice. Values are ± s.e.m.

**, ****: P<0.05, and <0.0001 mice vs. rats.

°: P<0.05 females vs. males.

Figure 5. Variables of lung architecture in high altitude rats and mice. Typical image of the architectural lungs obtained for rats (A) and mice (B). (C) mean linear intercept (Lm – µm), (D) mass-corrected relative alveolar surface ($m^2/cm^3/g^{-0.13}$), (E) mass-corrected total alveolar surface ($m^2/g^{0.88}$) in 2 months old rats and mice. Values are ± s.e.m.

Scale bars on A and B = 50 µm

*, ***, ****: P<0.05, <0.001 and <0.0001 mice vs. rats.

Figure 6. Total alveolar surface to O_2 consumption (m^2/ml/min) in high altitude rats and mice. Values are ± s.e.m.

**: P<0.01 mice vs. rats.

Figure 7. Response to changes of inspired O_2 in high altitude rats and mice. (A) Arterial oxygen saturation (SpO_2 – %), (B) heart rate (% vs. baseline) in 2 months old rats and mice. Values are ± s.e.m.

*, **, ***: P<0.05, <0.01 and <0.001 mice vs. rats.

†, ††: P<0.05 and <0.01 vs. baseline (21% O_2).

Figure 8. Response to changes of inspired O_2 in high altitude rats and mice. (A) minute ventilation, (B) tidal volume, and (C) respiratory frequency (all as % vs. baseline) in 2 months old rats and mice. Values are ± s.e.m.

*, ***, ****: P<0.05, <0.001 and <0.0001 mice vs. rats.

††, ††††: P<0.01 and <0.0001 vs. baseline (21% O_2).

Figure 9. Response to changes of inspired O_2 in high altitude rats and mice. (A) O_2 consumption ($\dot{V}_{O_2}$ – % vs. baseline), (B) CO_2 production rate ($\dot{V}_{CO_2}$ – % vs. baseline), and (C) rectal temperature (°C) in 2 months old rats and mice. Values are ± s.e.m.

, *, ****: P<0.01, <0.001 and <0.0001 mice vs. rats.

††, ††††: P<0.01 and <0.0001 vs. baseline (21% O_2).

Figure 10. Responses to changes of inspired O_2 in high altitude rats and mice. (A-B) % O_2 extraction, (C-D) minute ventilation, (E-F) mass-specific O_2 consumption rate ($\dot{V}_{O_2}$ – ml/min/100g) in 2 months old, male and female rats and mice. Values are ± s.e.m.

°°°°: P<0.05 and <0.0001 female vs. male.

*, ***, ****: P<0.05, <0.001, and 0.0001 mice vs rats.

†, ††††: P<0.05, and <0.0001 vs. baseline (21% O_2) – in A †: P<0.05 vs. 32% O_2.

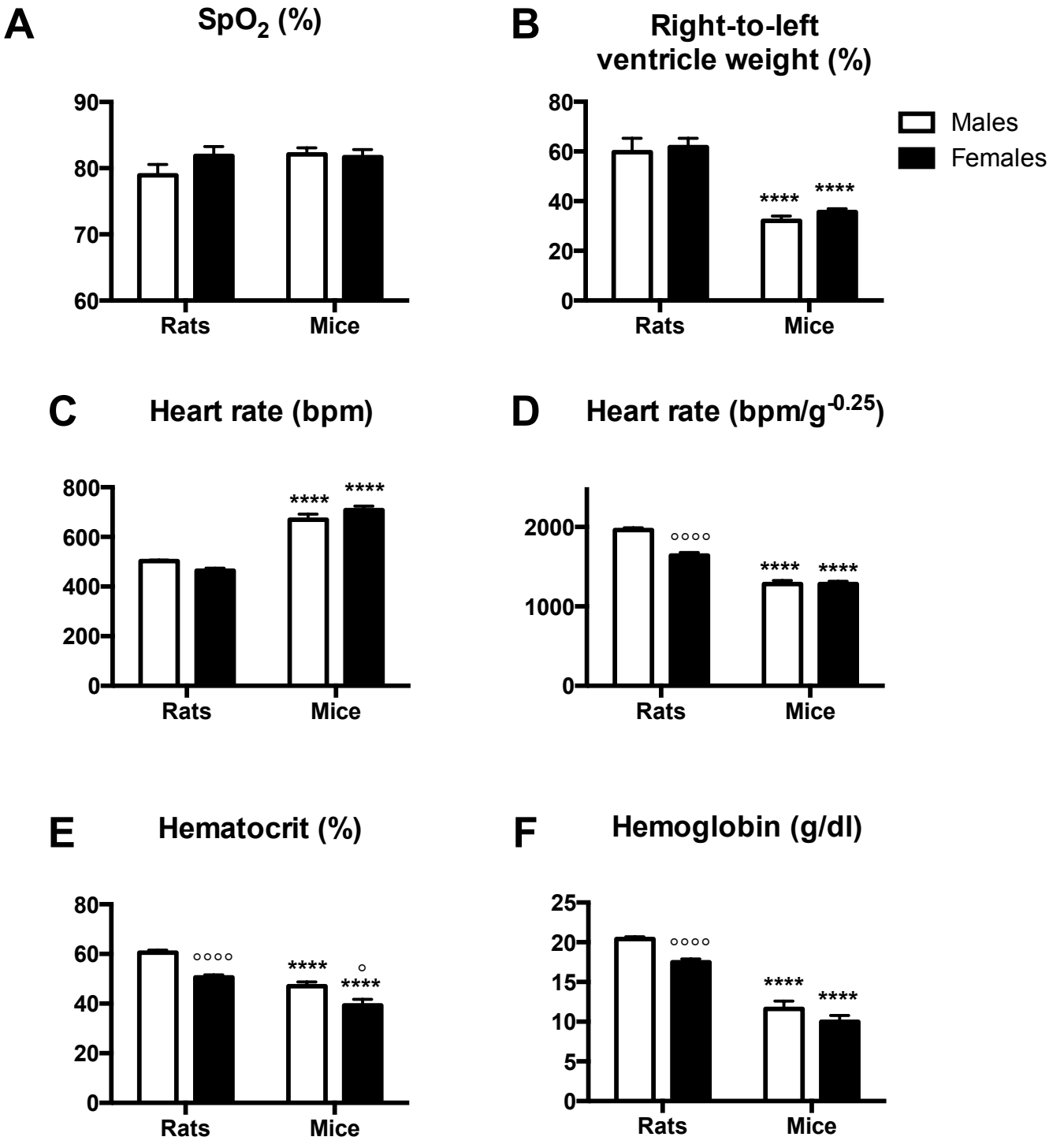

A
SpO2 (%)
90
80
70
60
Rats
Mice
B
Right-to-left ventricle weight (%)
80
60
40
20
0
****
****
Rats
Mice
Males
Females
C
Heart rate (bpm)
800
600
400
200
0
****
****
Rats
Mice
D
Heart rate (bpm/g-0.25)
2000
1000
0
°°°°
****
****
Rats
Mice
E
Hematocrit (%)
80
60
40
20
0
°°°°
****
°
****
Rats
Mice
F
Hemoglobin (g/dl)
25
20
15
10
5
0
°°°°
****
****
Rats
Mice

Figure 1

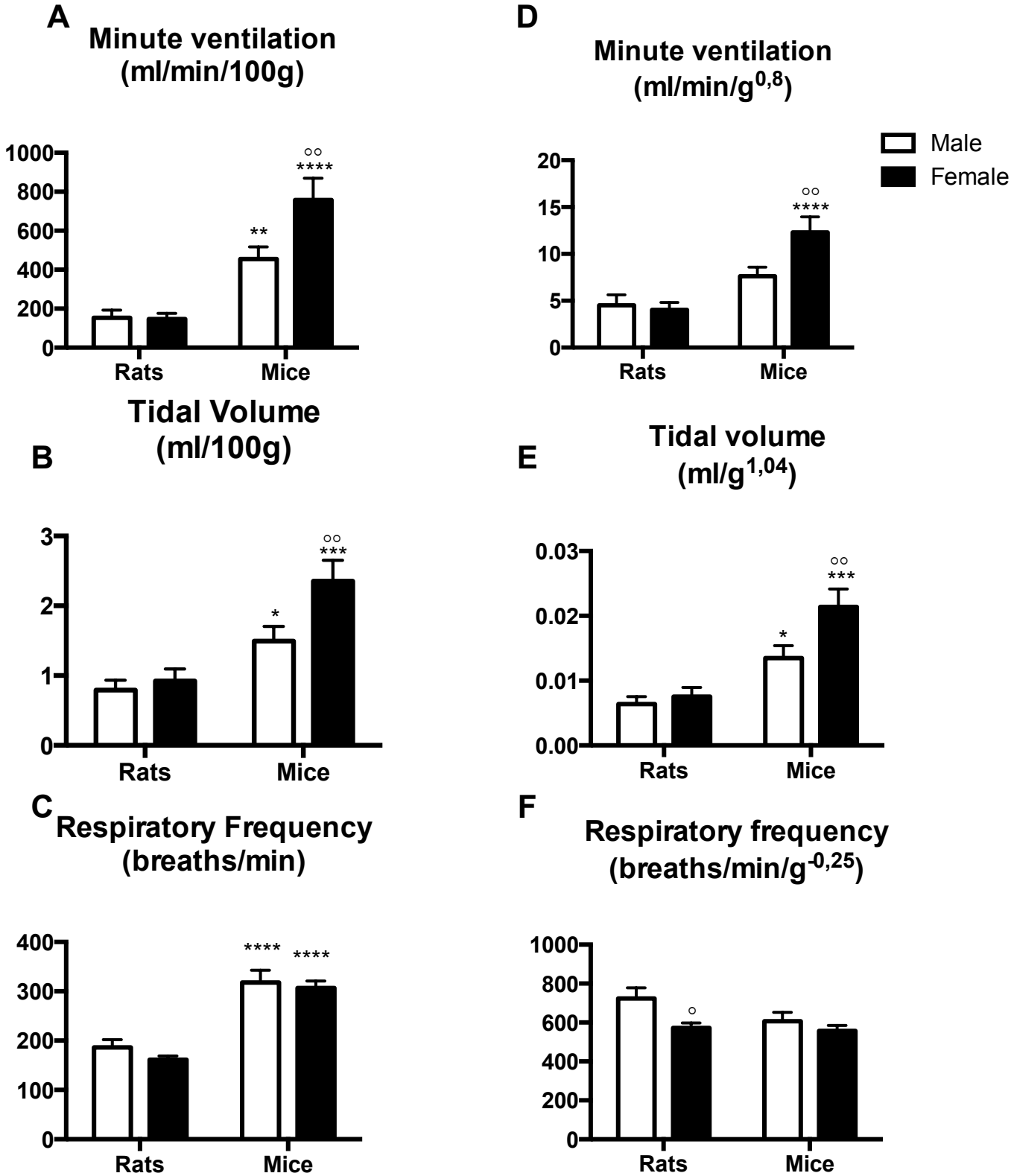

A
Minute ventilation
(ml/min/100g)
1000
800
600
400
200
0
**
○○
****
Rats
Mice
D
Minute ventilation
(ml/min/g0,8)
Male
Female
20
15
10
5
0
○○
****
Rats
Mice
B
Tidal Volume
(ml/100g)
3
2
1
0
*
○○
***
Rats
Mice
E
Tidal volume
(ml/g1,04)
0.03
0.02
0.01
0.00
*
○○
***
Rats
Mice
C
Respiratory Frequency
(breaths/min)
400
300
200
100
0
****
****
Rats
Mice
F
Respiratory frequency
(breaths/min/g-0,25)
1000
800
600
400
200
0
○
Rats
Mice

Figure 2

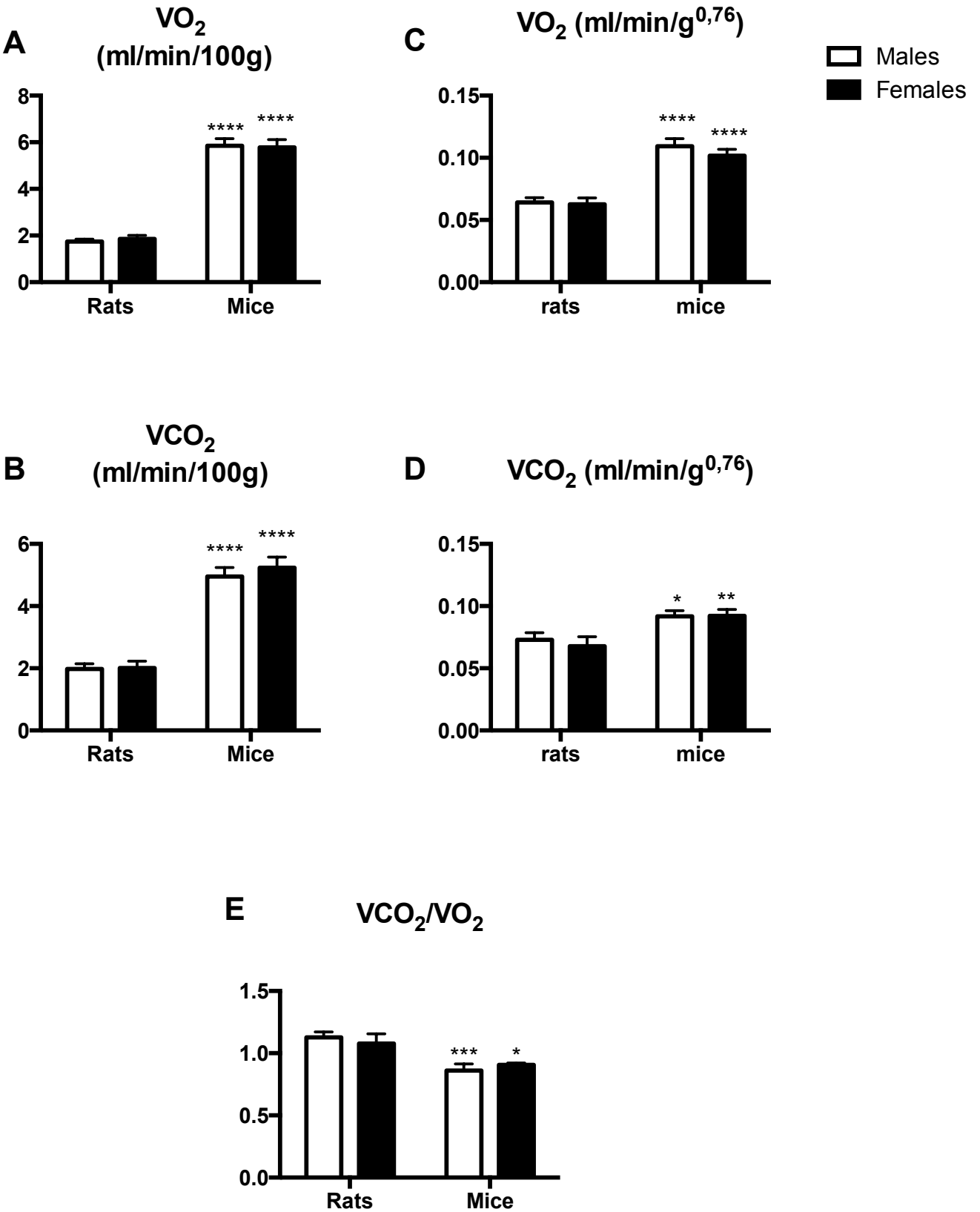

A
VO_2 (ml/min/100g)
8
6
4
2
0
****
****
Rats
Mice
C
VO_2 (ml/min/$g^{0,76}$)
0.15
0.10
0.05
0.00
****
****
rats
mice
Males
Females
B
VCO_2 (ml/min/100g)
6
4
2
0
****
****
Rats
Mice
D
VCO_2 (ml/min/$g^{0,76}$)
0.15
0.10
0.05
0.00
*
**
rats
mice
E
VCO_2/VO_2
1.5
1.0
0.5
0.0
***
*
Rats
Mice

Figure 3

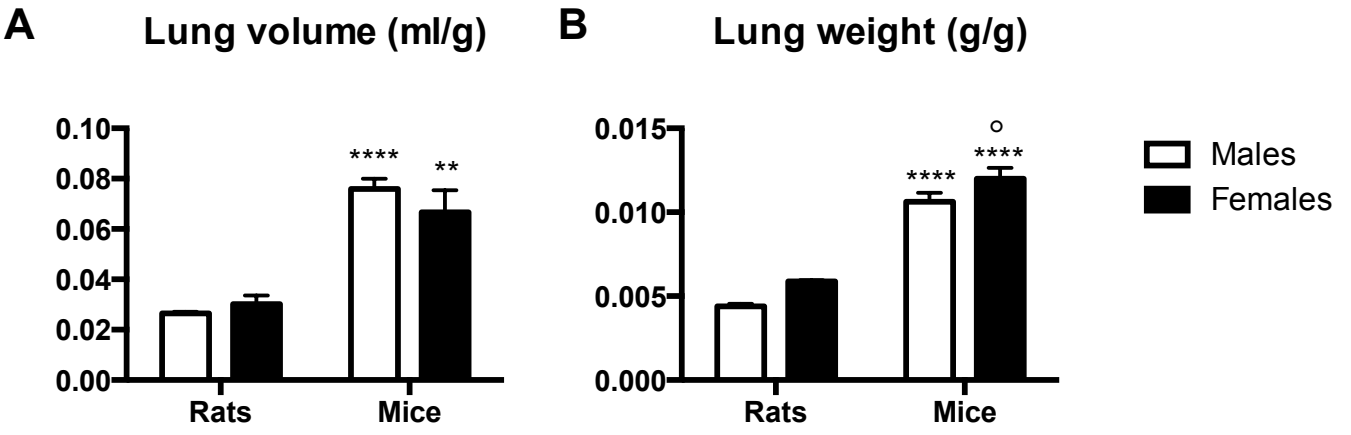

A
Lung volume (ml/g)
0.10
0.08
0.06
0.04
0.02
0.00
****
**
Rats
Mice
B
Lung weight (g/g)
0.015
0.010
0.005
0.000
****
°
****
Rats
Mice
Males
Females

Figure 4

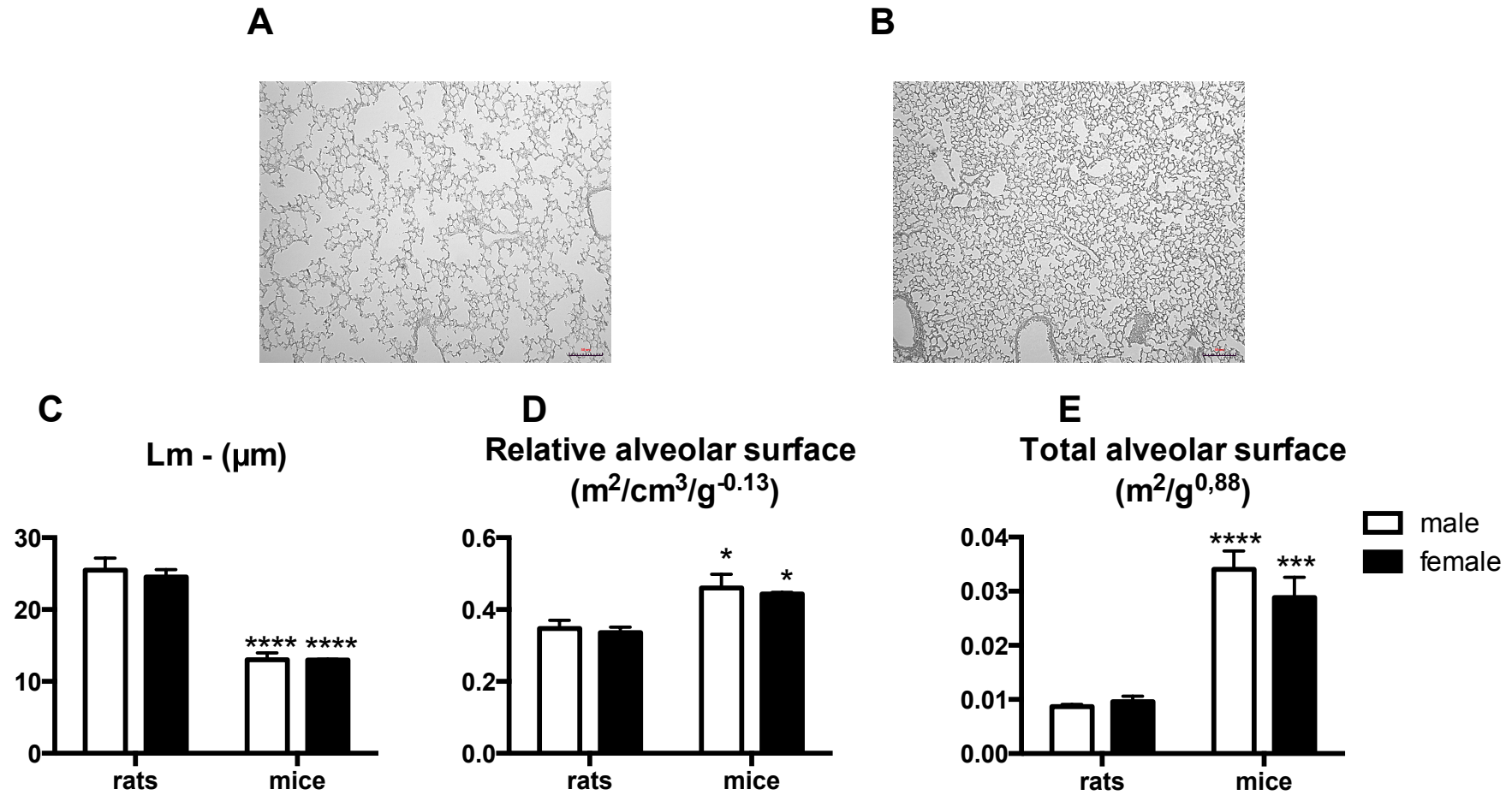

A
B
C
Lm - (µm)
30
20
10
0
****
****
rats
mice
D
Relative alveolar surface
(m2/cm3/g-0.13)
0.6
0.4
0.2
0.0
*
*
rats
mice
E
Total alveolar surface
(m2/g0,88)
0.04
0.03
0.02
0.01
0.00
****
***
rats
mice
male
female

Figure 5

Total alveolar surface / O_2 consumption ($m^2/ml.min^{-1}$)

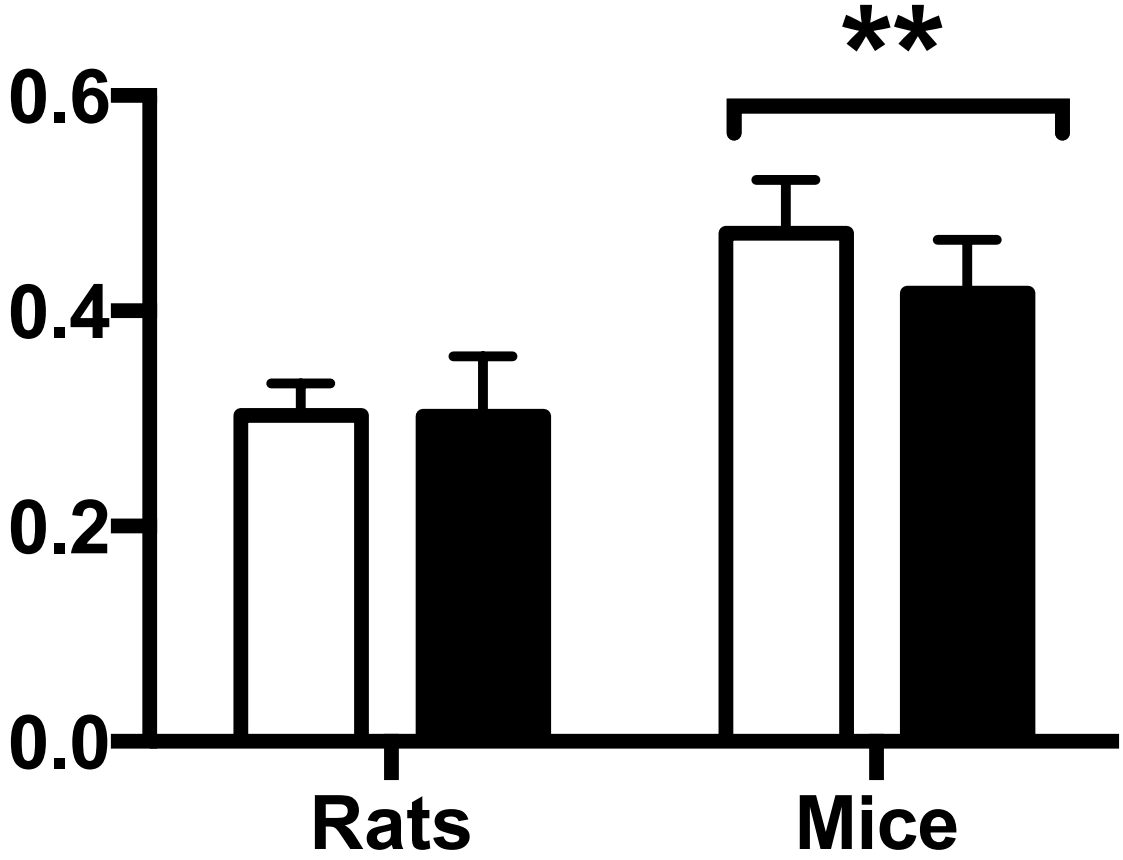
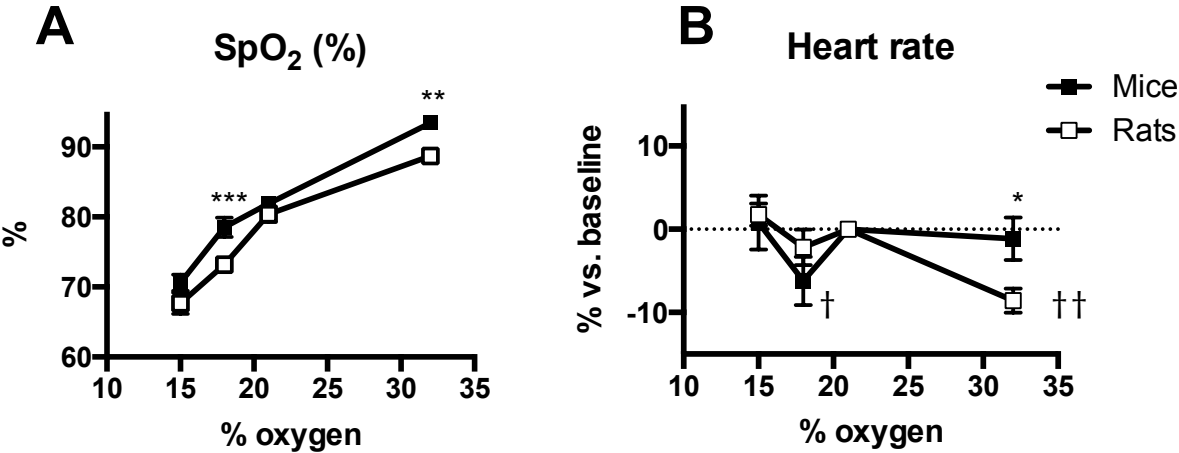


Figure 6



* mice vs rats

° vs. 21% O2

Figure 7

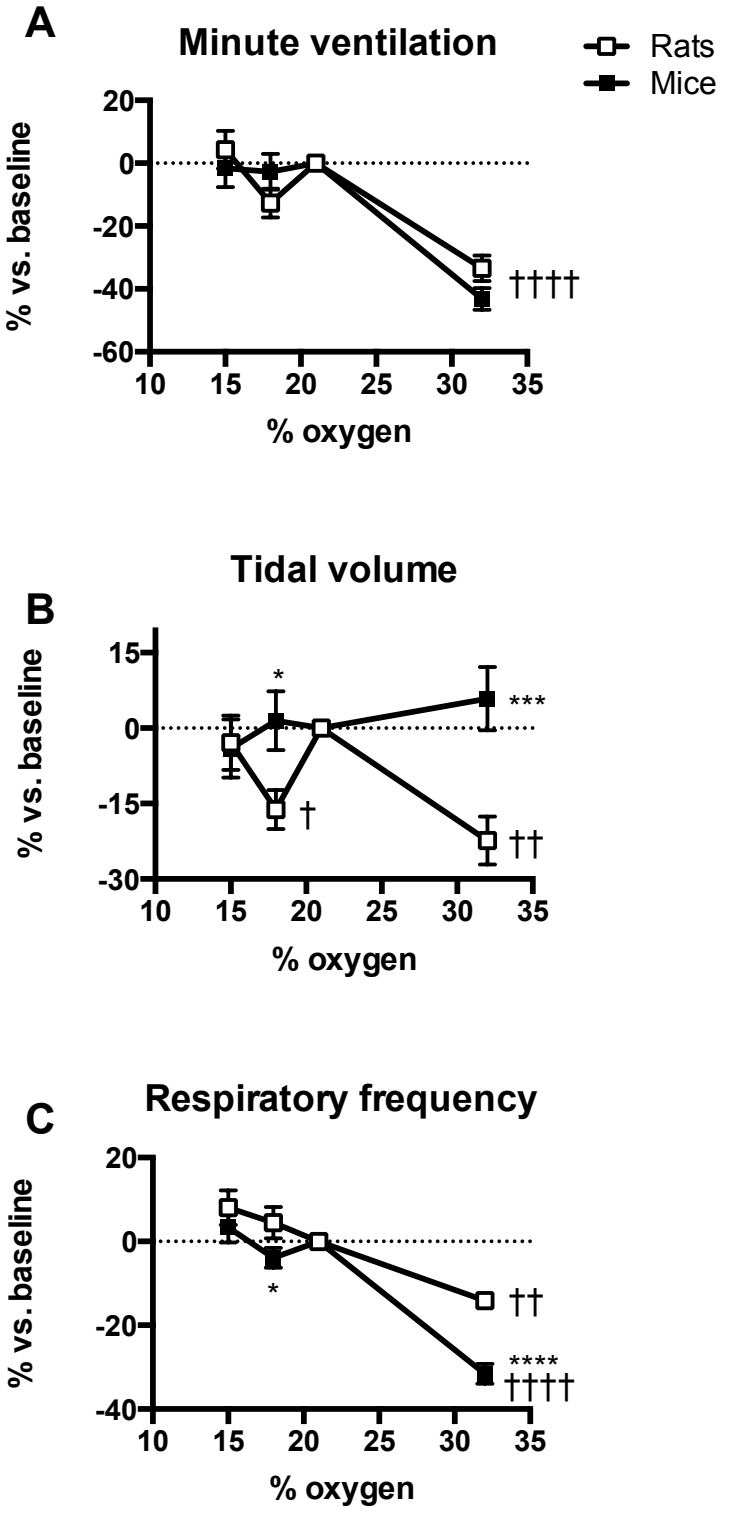

A
Minute ventilation
Rats
Mice
% vs. baseline
20
0
-20
-40
-60
10
15
20
25
30
35
% oxygen
††††
B
Tidal volume
% vs. baseline
15
0
-15
-30
*
***
†
††
% oxygen
C
Respiratory frequency
% vs. baseline
20
0
-20
-40
*
††
****
††††
% oxygen

Figure 8

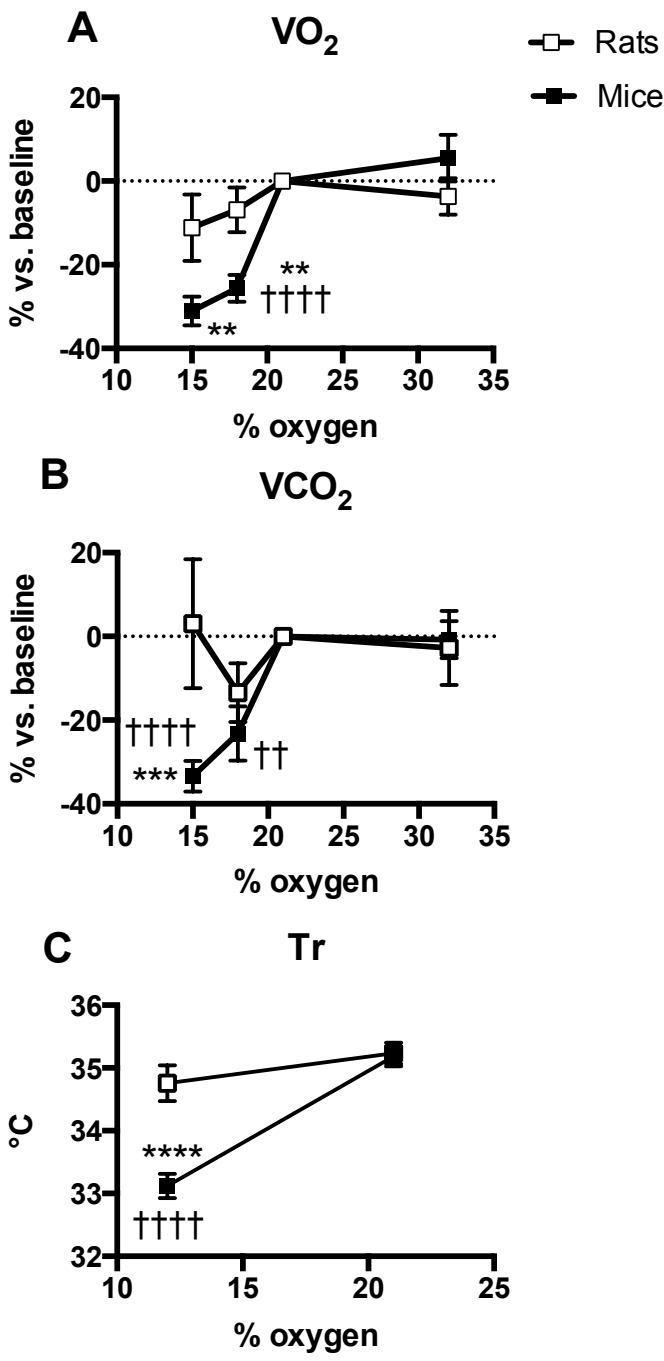

A
VO2
Rats
Mice
20
0
-20
-40
% vs. baseline
**
††††
**
10
15
20
25
30
35
% oxygen
B
VCO2
20
0
-20
-40
% vs. baseline
††††
***
††
10
15
20
25
30
35
% oxygen
C
Tr
36
35
34
33
32
°C
****
††††
10
15
20
25
% oxygen

Figure 9

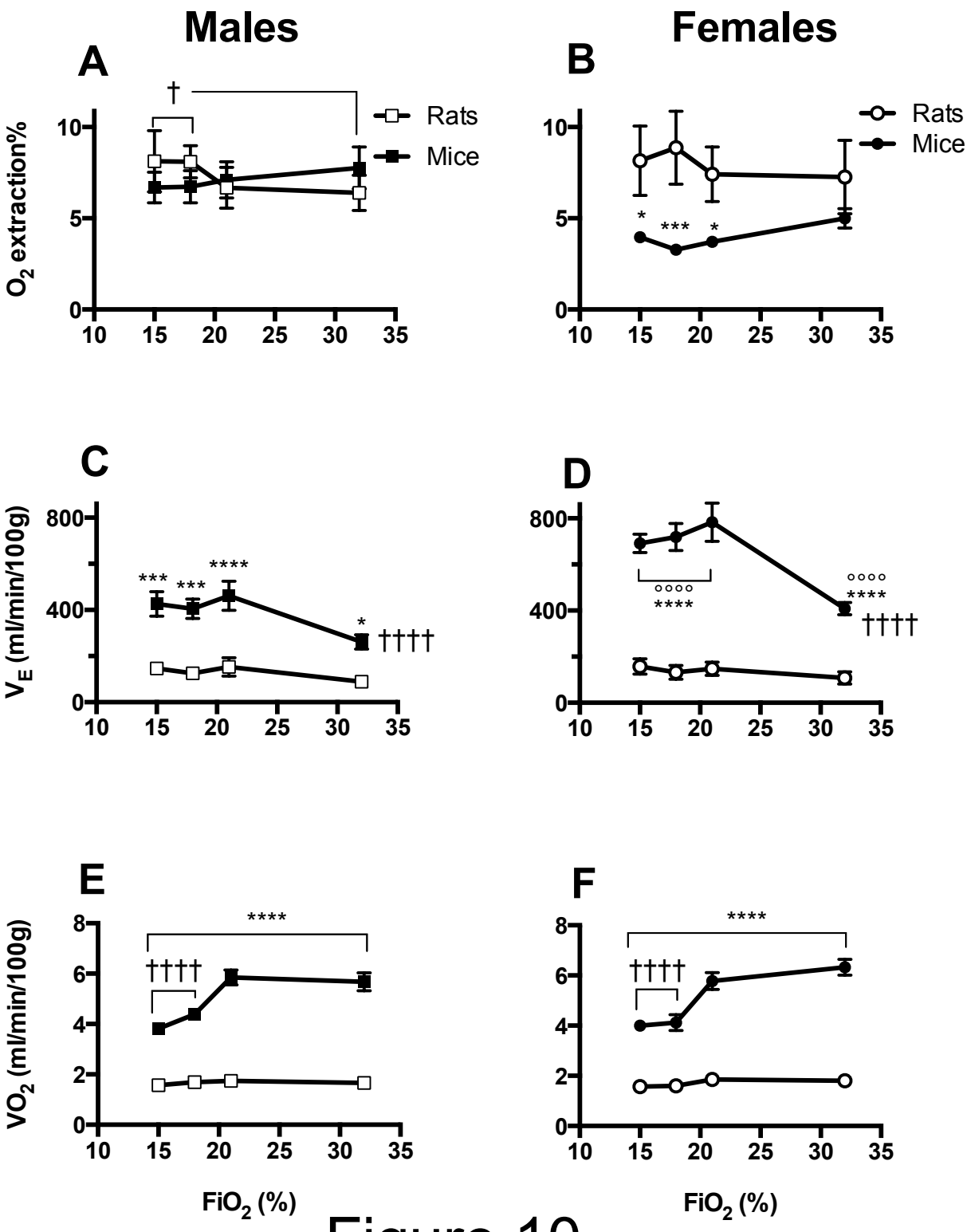

Males
Females
A
B
C
D
E
F
Rats
Mice
O_2 extraction%
V_E (ml/min/100g)
VO_2 (ml/min/100g)
FiO_2 (%)
FiO_2 (%)
†
*
***
****
°°°°
††††

Figure 10