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Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpaAdjustments of the oxygen diffusing capacity to energetic demands during the development of the quail (*Coturnix coturnix japonica*)M. Canals^{a,b,*}, B.B. Martinez^c, D. Figueroa^c, P. Sabat^{a,d}^a Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile^b Departamento de Medicina, Facultad de Medicina, Universidad de Chile, Chile^c Departamento de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Chile^d Center for Advanced Studies in Ecology and Biodiversity, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile

ARTICLE INFO

Article history:

Received 20 December 2010

Received in revised form 29 March 2011

Accepted 31 March 2011

Available online 6 April 2011

Keywords:

Quail

Symmorphosis

Oxygen diffusion capacity

Oxygen consumption

ABSTRACT

One of the hypotheses that attempt to explain physiological limitations of energy budgets is the symmorphosis hypothesis, which proposes that if matching structures to functional needs were combined with the strict economy of energy and materials, the result would be an optimal organ design for the specific function it serves. Evidence in favor of symmorphosis in adults is as abundant as evidence against it, but the plasticity of some morphological traits may be dependent on the ontogenetic stage at which acclimation acts. Thus, here we studied the adjustment of structure and function in lungs at different stages of development in the quail *Coturnix coturnix japonica* under two thermal regimes. Our main results show that i) resting metabolic rate, maximum thermogenic oxygen consumption and oxygen diffusion capacity did not exhibit developmental plasticity for two thermal environments; and ii) oxygen diffusion capacity fully adjusted to resting metabolic rate and maximum oxygen consumption during development. *C. coturnix* has a low safety factor close to 1 which is consistent with the symmorphosis hypothesis.

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1. Introduction

Regarding only physiological constraints, there are three principal hypotheses that attempt to explain the physiological limitations of energy budgets in animals: i) shared central machinery limits the sustained metabolic rate ("central limitation hypothesis"); ii) energy consuming machinery limits the metabolic rate ("peripheral limitation hypothesis"); and iii) the capacity of the central machinery closely matches that of the peripheral tissues and energy requirements (Bacigalupe and Bozinovic, 2002). The central limitation hypothesis implies that metabolic limits are independent of the manner in which energy is expended and peripheral organs always possess an excess capacity. The peripheral limitation hypothesis proposes that central organs have an excess capacity. The third hypothesis proposes that organisms do not have excess capacities, but rather the capacity of central organs to supply energy matches the expenditure capacity of peripheral organs, just as proposed by the symmorphosis hypothesis (Taylor and Weibel, 1981). Symmorphosis is defined as "a state of structural design commensurate with functional needs resulting from regulated morphogenesis, whereby the formation of structural elements is regulated to satisfy but not exceed

the requirements of the functional system" (Taylor and Weibel, 1981). Thus the symmorphosis hypothesis proposes that if matching structures to functional needs were combined with the strict economy of energy and materials, the result would be an optimal organ design for the specific function it serves (Weibel et al., 1991, 1992). This hypothesis contains three predictions: i) organism design will be optimized; ii) if the design is optimized in the sense of material economy, then structure is the key factor in determining maximum organ capacities, and iii) adjustments to maximum capacities require morphogenetic processes (Jones, 1998; Weibel, 1998; Canals, 2002). Although symmorphosis was first proposed for the oxygen pathway in mammals, it has been established as a general hypothesis of economic design (Weibel, 1998).

The oxygen pathway is considered a good model to test these hypotheses for two main reasons. First, it involves a series of linked structures in which the effect of the structural parameters on functional capacity can be defined. For example, $\dot{V}_{O_2} = \Delta PO_2 \cdot D_{LO_2}$ (Bohr's equation), where $\dot{V}_{O_2}$ is the oxygen consumption, ΔPO_2 is the alveolus-capillary pressure gradient, and D_{LO_2} is the total oxygen diffusion capacity (air-erythrocyte) or oxygen conductance. The latter depends directly upon the alveolar and capillary surface, hematocrit and capillary volume, and inversely upon the thickness of the air-capillary barrier (Weibel et al., 1991). The second reason is that the overall function has a measurable upper limit, maximum oxygen consumption $\dot{V}_{O_2 \max}$ (Weibel, 1998). The symmorphosis hypothesis has generated great debate that to date remains unresolved. Evidence

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in favor of symmorphosis is as abundant as evidence against it (Bacigalupe and Bozinovic, 2002; Canals, 2002). Regarding the oxygen pathway, Weibel et al. (1992) found that mitochondrial volume, cardiac output and capillary volume varied with body mass with the same allometric exponent as maximum oxygen consumption, suggesting that mitochondria achieve the same rate of oxidative phosphorylation in all mammals, supporting the symmorphosis hypothesis (Weibel et al., 1991, 1992). However, the same authors reported that lung oxygen diffusion capacity did not adjust to functional needs, with the result that species of large body size have an excess capacity relative to that of small species (Weibel, 1998). Jones (1998) also reported an excess oxygen diffusion capacity in well-trained equines. Furthermore, from an evolutionary point of view, several objections have been proposed to symmorphosis (Garland, 1998; Bacigalupe and Bozinovic, 2002).

The authors who proposed the symmorphosis hypothesis were surprised when they observed that the diffusing capacity of the lung was only partly adjusted to maximum oxygen capacity both in adaptive and allometric variation (Taylor and Weibel, 1981; Weibel et al., 1991, 1992), so species of great body size had excess diffusing capacity, whereas smaller species did not. For example, while the safety factors (i.e. the ratio between maximal capacities/maximal requirements) of small animals are small, they can reach values as high as 6 in some artiodactyls of large size (Diamond, 1998; Canals et al., 2010). Alexander (1998) proposed that the presence of safety factors contradicts the material economy proposed in the hypothesis of symmorphosis, but is consistent with optimization of organs and organisms when the role of natural selection is incorporated. Also it has been proposed that the high safety factor found in adults of the leaf-eared mouse (*Phyllotis darwini*) may be the result of symmorphosis operating during development (Canals et al., 2010).

Although several studies have provided evidence in favor of symmorphosis (Taylor et al., 1996; Weibel et al., 1996; Chappel et al., 1999; Hammond et al., 2000; Seymour et al., 2004, 2008; Runciman et al., 2005) and optimization (Canals et al., 2002a, 2002b, 2004, 2005, 2008) in adults of mammals and birds, there are few studies of the structure–function adjustment during development. For example, in mammals, results during the ontogeny of *P. darwini* supported the symmorphosis hypothesis (Canals et al., 2009a; 2009b; 2010), similar to the studies of Seymour et al. (2004, 2005) and Runciman et al. (2005) in birds. The latter authors found that altricial pelicans and precocial turkeys have a parallel allometry between $\dot{V}_{O2\ max}$ and D_{LO2} , consistent with the concept of symmorphosis during development. Also, some authors have reported the existence of phenotypic plasticity of morphology and physiology during ontogeny but absence of response when animals are adults, i.e., exhibiting an inflexible norm of reaction (Toloza and Diamond, 1990; Biviano et al., 1993; Bozinovic, 1993; Zhao et al., 1996). Thus the presence of plasticity of some morphological traits may be dependent on the ontogenetic stage at which acclimation acts (Sabat and Bozinovic, 2000), whereas for others it is not (McKechnie et al., 2007). Thus, we expect that birds acclimated to environments of different energetic requirements adjust their morphological capacities to the maximal functional needs during the development. Thus, the aim of this work is to study the adjustment of structure and function in lungs at different stages of development in the quail *Coturnix coturnix japonica* under two thermal regimes which result in different energetic requirements.

2. Materials and methods

2.1. Animal model, sample size and experimental groups

Twenty seven individuals of *C. coturnix japonica* were randomly selected from multiple litters that were produced in a commercial hatchery in Santiago, Chile. The individuals were taken on the day of their hatching (that we call day 1). The oxygen consumption of three

individuals was studied on the same day, after maintenance in individual cages in a thermoregulated chamber at 30 °C for 12 h. The remaining 24 individuals were randomly separated into 2 groups of 12 individuals and exposed to 2 thermal regimes with water and food *ad lib* and a L:D 12:12 photoperiod in thermo-regulated rooms. The first group was kept at a constant temperature of 30 ± 1 °C (low energetic requirement group: LR); the second group was exposed initially to 30 °C for two days and thereafter the temperature was reduced by 2 °C every two days until it reached 15 ± 1 °C at day 15 (high energetic requirement group: HR). This protocol of gradual decrease in temperature was developed to avoid mortality of chicks, which are very sensitive to temperature changes. Three individuals of each group were studied at 10, 16, 24 and 45 ± 1 day. For each individual we first determined the resting (RMR) and maximum ($\dot{V}_{O2\ max}$) metabolic rates, then individuals were sacrificed to study lung morphology. The different thermoregulatory energy requirements of each thermal environment can be estimated through the sum of products between temperature and exposure time (Table 1).

2.2. Resting metabolic rate

All measures of oxygen consumption ($\dot{V}_{O2}$) were performed in an open-flow respirometer system (Sable Systems). Individual determinations of resting metabolic rate were performed in a 1 L steel chamber at 30 ± 0.5 °C. The metabolic chamber received dry air at a rate of 500 mL/min from a flow controller (Sierra Instruments), to ensure a correct air mix. CO₂ was removed and the air was dried before entering and after leaving the chamber. Oxygen was monitored every 5 s using an Oxygen Analyzer 1FC-1B (Sable Systems). Oxygen consumption was estimated using the equation of Whithers (1977). All measurements were made during the resting phase of the species. Body temperature was measured at the end of each measurement period with an intra-rectal Cu-constantan thermocouple (± 0.1 °C).

2.3. Maximum thermogenic oxygen consumption

Beginning at 1 day older, $\dot{V}_{O2\ max}$ was determined in an atmosphere of He–O₂ (80–20%) (Rosenmann and Morrison, 1974) in an open-flow respirometer system. The metabolic chamber received dried air at 1000 mL min^{−1} from a mass flow controller and through Bev-A-Line tubing (Thermoplastic Processes Inc.) and the chamber temperature was constantly monitored (5.0 ± 0.5 °C). This flow kept the partial oxygen pressure above 150 Torr, which is considerably above the level of hypoxia. The gas mixture was passed through CO₂ and H₂O absorbent (Baralyme and Drierite) at the entrance and exit of the chamber. To be sure that individuals reached $\dot{V}_{O2\ max}$, measurements were stopped when the reduction of $\dot{V}_{O2}$ was evident; hypothermia was checked with a Cole-Parmer copper–constantan thermocouple after each measurement.

2.4. Pulmonary structure

Beginning at day 10, individuals were euthanized by means of CO₂ exposure, complying with the current laws of Chile and the standards of the ethical committee of the Facultad de Ciencias, Universidad de

Table 1

Thermal exposure of the two experimental groups (HR and LR), corresponding to the area below the time–temperature curve.

	LR	HR
1 day	30 °C day	
10 days	300 °C day	260 °C day
16 days	480 °C day	366 °C day
24 days	720 °C day	486 °C day
45 days (adults)	1350 °C day	801 °C day

Chile, where the experiments were performed. A small tracheotomy was then performed and the lungs instilled with 2.5% glutaraldehyde in a 0.01 mol L⁻¹ phosphate buffer solution (pH = 7.4, 350 mOsm) using a plastic catheter with the reservoir located 20 cm above the level of the sternum. The trachea was tied off with surgical silk to maintain the intrapulmonary fixative volume. After 24 h, the lungs were removed by thoracotomy and their volumes (V_L) were estimated by means of a water displacement method (Scherle, 1970). After this, tissues (right and left lungs) were immersed in the same fixative at 4 °C for a minimum of 2 h and processed for routine light microscopy (LM) and transmission electron microscopy (TEM). Briefly, two slices of 1–2 mm thickness (one for LM and one for TEM) were obtained from each of three zones (upper, middle and basal) in each lung. The pieces were washed with buffer and post-fixed with 1% osmium tetroxide for 1 h at 4 °C. For light microscopy slices were dehydrated in ascending series of ethanol and infiltrated and embedded in epoxy resin constructing cubes of 2–3 mm³, obtaining semi-thin randomly oriented sections of 1 µm. Tissue samples were stained with 1% toluidine blue and viewed with an optical microscope. For TEM, the pieces were stained with 1% osmium tetroxide, *en bloc* stained with 2% uranyl acetate, and dehydrated in ascending series of alcohol. Ultrathin sections of 60–90 nm of thickness were made, contrasted with Pb-citrate, mounted on copper mesh grids and viewed in a JOEL/JEM 100SX transmission electron microscope. Sections were photographed and digitalized, and twelve semi-thin and twelve ultra-thin sections per individual were analyzed using Scion Image Software.

The respiratory surface density (RS_d) was estimated by means of the line-intersection stereological method (Weibel, 1970/71) in the semi-thin sections with light microscopy at 10× magnification: $RS_d = \frac{2I}{1/2 \cdot P_T \cdot Z}$, where I is the number of intersections between line probes of length Z with the respiratory surface and P_T is the number of testing points. The number of line segments was $\frac{1}{2}$ the number of points.

The harmonic mean thickness of the air–blood barrier (τ_h) was estimated by a stereological method in a square lattice grid as

$$\text{suggested by Weibel (1970/71) and Maina (2002): } \frac{1}{\tau_h} = \frac{3}{2} \cdot \frac{\sum_{j=1}^m f_j \cdot \frac{1}{l_j}}{\sum_{j=1}^m f_j},$$

where l_j is the mid-value of the intercept length of linear probes (in

the direction of the line), f_j the frequency of class j and m is the number of classes.

The thickness of the air–blood barrier, the density of the respiratory surface and the lung volume allowed the estimation of the oxygen diffusion capacity. The parenchymal lung volume was estimated as $V_p = 0.46 \cdot V_L$ (Maina, 2002). From these structural measurements the morphometric oxygen diffusion capacity (tissue) was estimated using:

$$D_{tO_2} = \kappa \frac{RS_d \cdot V_p}{\tau_h}, \text{ where } D_{tO_2} \text{ is the oxygen diffusion capacity of the air–blood barrier (tissue) and } \kappa \text{ is Krogh's diffusion coefficient } \kappa = 4.1 \cdot 10^{-10} \text{ cm}^2 \text{ s}^{-1} \text{ mbar}^{-1} = 4.1 \cdot 10^{-12} \text{ cm}^2 \cdot \text{s}^{-1} \cdot \text{Pa}^{-1} \text{ (Gehr et al., 1981). } RS_d \text{ is the respiratory surface density, } V_p \text{ is the parenchymal lung volume and } \tau_h \text{ is the harmonic mean thickness of the blood–gas barrier.}$$

2.5. Data analyses

Comparisons of metabolic and structural variables between HR and LR groups were performed with two way ANOVAs and two way ANCOVAs with body mass as the co-variable in all variables in which an effect of the body mass was detected, considering the age and the thermal environment as main effect. As animals were sacrificed after metabolic measurement to analyze structural variables of the lung, the data matrix consisted of three individuals analyzed at day one and three individuals for each age and acclimation temperature from day 10 onward. The comparison was performed from day 10 onward because at the hatching day individuals had not experienced the experimental conditions.

Based on that total oxygen diffusion capacity (air–erythrocyte: D_{tO_2}) is about 1/10 times D_{tO_2} (Canals et al., 2010), we estimated the gradients of partial pressure between air and blood (ΔPO_2) necessary to sustain the maximum metabolism of each animal from $\Delta PO_2 = \dot{V}O_{2 \max} / D_{tO_2}$. And then, since in birds the usual values of ΔPO_2 are around 7.5 kPa (60 mm Hg), the ratio between this value and that necessary to satisfy maximum oxygen consumption was used as a broad estimate of the safety factor associated with oxygen diffusion (i.e. $S_f = 7.5 / \Delta PO_2$). Thus, while values >1 suggest excess of morphological capacity, values = 1 or <1 indicates a full adjust between morphological capacity and maximal function (Canals et al., 2010). Simultaneously these ratios are indexes of the degree of fit between the oxygen requirements and the morphometric oxygen diffusion capacity during development. To compare estimated safety factors we

Table 2

Variation of body mass (m_b), lung volume (V_L), resting metabolic rate (RMR), maximum metabolic rate ($\dot{V}O_{2 \max}$), harmonic mean of thickness of the air–blood barrier (τ_h), respiratory surface density (RS_d), respiratory area (RA), oxygen diffusion capacity (tissue) (D_{tO_2}) and mass-specific oxygen diffusion capacity (tissue) (D_{tO_2}/g) in *Coturnix coturnix japonica* at different ages of development and for two thermal regimes (BR and HR).

BR				
Age	9	16	24	45
m_b (g)	11.93 ± 2.55	37.5 ± 5.37	74.53 ± 16.00	125.17 ± 7.31
V_L (cm ³)	0.15 ± 0.03	0.40 ± 0.15	1.00 ± 0.22	1.14 ± 0.14
RMR (mLO ₂ /gh)	3.61 ± 0.25	3.16 ± 0.11	2.07 ± 0.31	1.79 ± 0.31
$\dot{V}O_{2 \max}$ (mLO ₂ /gh)	7.87 ± 0.94	9.22 ± 1.31	7.54 ± 1.09	6.26 ± 0.64
τ_h (µm)	0.37 ± 0.06	0.45 ± 0.10	0.40 ± 0.03	0.43 ± 0.07
RS_d (cm ⁻¹)	1242.7 ± 184.5	1517.3 ± 262.2	1329.2 ± 81.2	965.4 ± 135.7
RA (cm ²)	233.5 ± 0.3	938.5 ± 180.6	1562.5 ± 214.9	1862.0 ± 271.3
D_{tO_2} (× 10 ⁻⁵ mLO ₂ /sPa)	2.59 ± 5.92	8.99 ± 3.62	15.91 ± 2.73	18.14 ± 3.97
D_{tO_2}/g (× 10 ⁻⁶ mLO ₂ /sPag)	2.51 ± 9.5	2.39 ± 8.25	2.14 ± 0.15	1.44 ± 0.25
AR				
Age	10	16	23	46
m_b (g)	9.34 ± 0.79	26.24 ± 1.17	56.28 ± 9.23	129.5 ± 12.52
V_L (cm ³)	0.11 ± 0.02	0.24 ± 0.09	0.87 ± 0.28	1.26 ± 0.10
RMR (mLO ₂ /gh)	2.87 ± 0.23	3.22 ± 0.19	2.61 ± 0.09	2.29 ± 0.59
$\dot{V}O_{2 \max}$ (mLO ₂ /gh)	7.67 ± 0.45	9.91 ± 0.87	7.84 ± 1.27	6.80 ± 0.64
τ_h (µm)	0.40 ± 0.09	0.45 ± 0.04	0.43 ± 0.03	0.57 ± 0.18
RS_d (cm ⁻¹)	1409.0 ± 88.6	1321.2 ± 112.1	1430.1 ± 196.9	1090.2 ± 176.9
RA (cm ²)	237.9 ± 88.7	588.6 ± 73.8	1318.7 ± 374.9	2152.9 ± 257.2
D_{tO_2} (× 10 ⁻⁵ mLO ₂ /sPa)	2.69 ± 0.70	5.41 ± 1.15	12.67 ± 4.26	16.24 ± 4.6
D_{tO_2}/g (× 10 ⁻⁶ mLO ₂ /sPag)	2.74 ± 0.79	2.05 ± 0.35	2.20 ± 0.40	1.25 ± 0.28

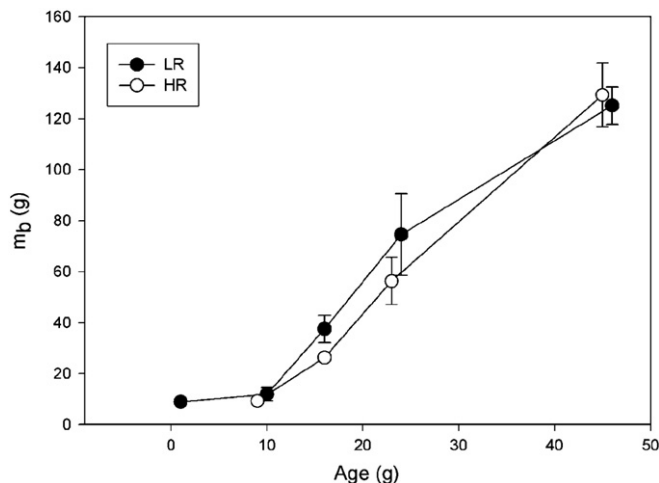


Fig. 1. Variation in body mass (m_b) with age (days) in *Coturnix coturnix japonica* under two thermal regimes (HR and LR).

used ANOVA. Power law regression analyses between metabolic and structural variables with body mass were performed to determine allometric relationships among these variables. As the metabolic developmental do not initially follow an allometric relationship (see Burggren, 1989) in this case the regressions were calculated from day 10 onward.

3. Results

Values of all variables for LR and HR groups for all developmental ages are shown in Table 2.

3.1. Body mass (m_b) and lung volume (V_L)

Body mass increased with age in both groups ($F_{3,16}=211.2$, $p<0.0001$), the body mass was slightly less in the LR group ($F_{1,16}=4.0$, $p=0.06$) at 14 and 21 days, but mass was similar at adulthood (Fig. 1). The V_L increased with age ($F_{3,16}=63.1$, $p<0.0001$), but without differences between the LR and HR groups ($F_{1,16}=0.75$, $p=0.39$) (Fig. 2). Eliminating the body mass effect with ANCOVA we found similar results with age ($F_{3,15}=6.78$, $p=0.004$), and experimental groups ($F_{1,15}=0.001$, $p=0.97$).

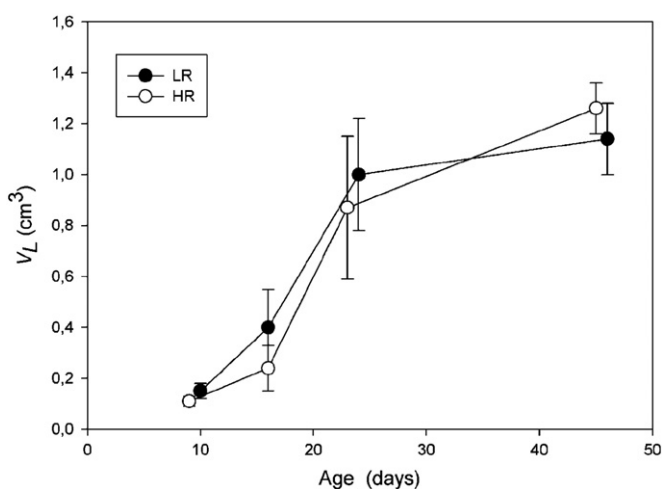


Fig. 2. Variation in lung volume (V_L) with age in *Coturnix coturnix japonica* under two thermal regimes (HR and LR).

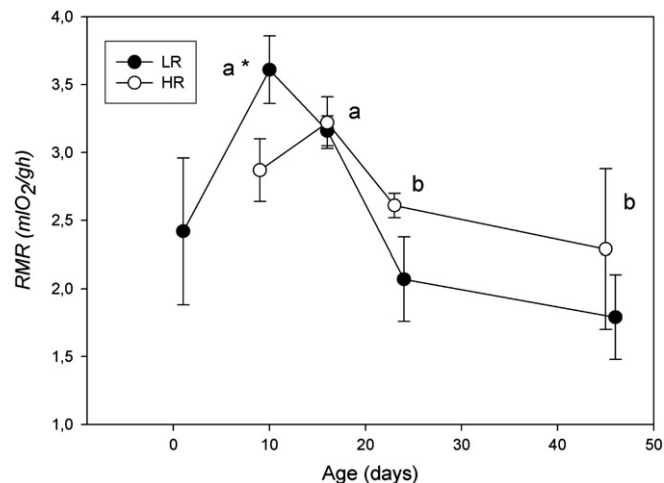


Fig. 3. Variation of the mass-specific resting metabolic rate (RMR) (mL O₂/gh) with age in *Coturnix coturnix japonica* under two thermal regimes (HR and LR). Different letters indicates differences in RMR among ages and asterisk indicates RMR differences between HR and LR groups using Newman–Keuls multiple comparisons.

3.2. Resting and maximum metabolic rate

Mass-specific RMR reached a maximum value at 10 days, gradually decreasing until adulthood ($F_{3,15}=3.50$, $p=0.042$), in a similar way in the LR and HR groups ($F_{1,15}=0.02$, $p=0.89$), however an interaction between the age and acclimation temperature was detected ($F_{3,15}=6.76$, $p=0.004$) (Fig. 3). Newman–Keuls multiple comparisons showed that the only difference between LR and HR groups was established at 7 days old. Similar results were obtained for mass-specific $\dot{V}O_{2\max}$ ($F_{3,15}=13.1$, $p=0.0001$ for age effect and $F_{1,15}=1.05$, $p=0.32$ for temperature effect) but in this case no interaction was found ($F_{3,15}=1.88$, $p=0.18$) (Fig. 4).

3.3. Lung structure

The thickness of the air–blood barrier remained constant with age ($F_{3,15}=0.79$, $p=0.22$) and no difference between HR and LR groups was found ($F_{1,15}=1.85$, $p=0.19$) (Fig. 5). The respiratory surface density decreased slowly with age ($F_{3,16}=7.8$, $p=0.002$) but we did

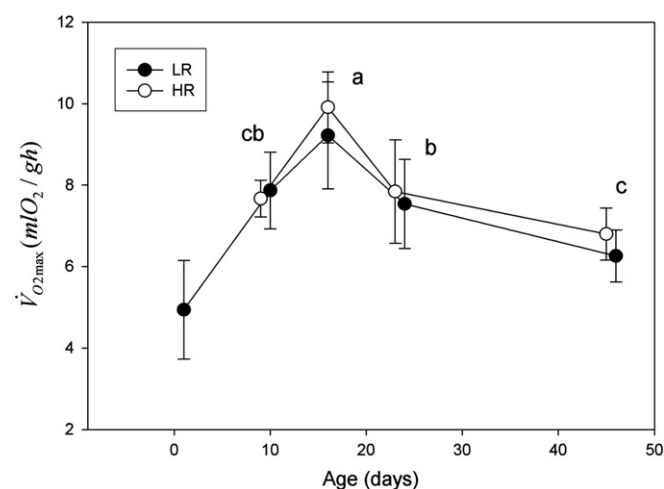


Fig. 4. Variation of the mass-specific maximum metabolic rate ($\dot{V}O_{2\max}$) (mL O₂/gh) with age in *Coturnix coturnix japonica* under two thermal regimes (HR and LR). Different letters indicate differences in RMR among ages using Newman–Keuls multiple comparisons.

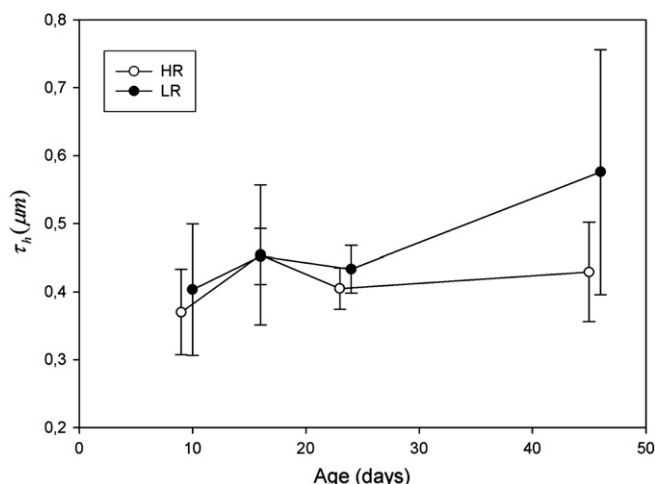


Fig. 5. Variation of the thickness of the air-blood barrier (τ_h) (μm) with age in *Coturnix coturnix japonica* under two thermal regimes (HR and LR).

not find differences between the HR and LR groups ($F_{1,16}=0.81$, $p=0.38$) (Fig. 6).

3.4. Oxygen diffusion capacity-tissue (D_tO_2)

The oxygen diffusion capacity increased with age ($F_{3,13}=7.25$, $p=0.001$) showing a similar pattern in the HR and LR groups ($F_{1,13}=0.11$, $p=0.75$) (Fig. 7) while the mass-specific oxygen diffusion capacity decreased ($F_{3,14}=5.66$, $p=0.009$) without differences between the LR and HR groups ($F_{1,14}=0.08$, $p=0.78$).

3.5. Estimations of S_f

The average safety factors were lower than the unity: $S_f=0.77 \pm 0.22$ ($F_{1,14}=16.5$, $p=0.002$). We did not find differences in S_f with age ($F_{3,14}=1.14$, $p=0.38$) or between the LR and HR groups ($F_{1,14}=0.31$, $p=0.58$). A $\Delta P_{O_2}=1.169 \pm 0.379$ kPa is required only to cross the tissue barrier satisfying $\dot{V}_{O_2 \max}$ (Table 3).

3.6. Allometric relationships between metabolic and structural variables with body mass

Maximum metabolic rate and the lung volume fit to power laws with exponents close to unity, whilst RMR had lower allometric

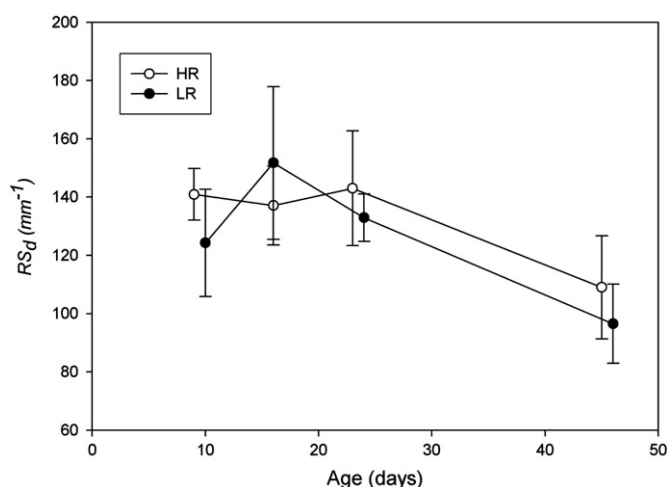


Fig. 6. Variation of respiratory surface density (RS_d) (μm) with age in *Coturnix coturnix japonica* under two thermal regimes (HR and LR).

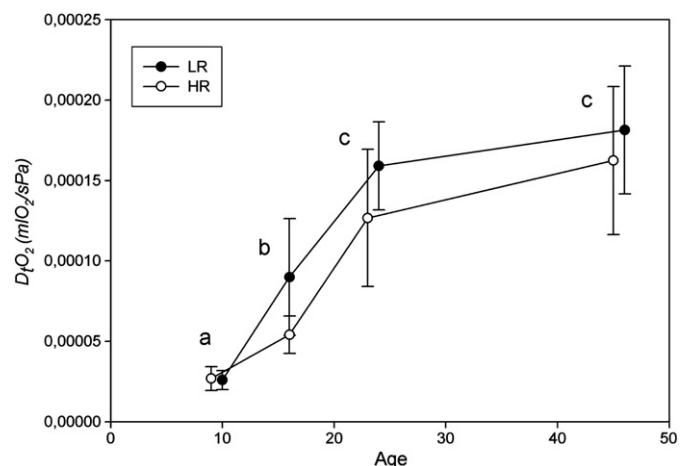


Fig. 7. Variation of the oxygen diffusion capacity (D_tO_2) (mLO_2/sPa) with age in *Coturnix coturnix japonica* under two thermal regimes (HR and LR). Different letters indicate differences in RMR among ages using Newman-Keuls multiple comparisons.

exponents. The thickness of the air-blood barrier and the respiratory surface density were independent of body mass, with exponents near 0. The oxygen diffusion coefficient (tissue) showed exponents near 0.8, but the ratios of RMR and $\dot{V}_{O_2 \max}$ with D_tO_2 respectively were independent of body mass, with exponents close to zero (Table 4).

4. Discussion

We found developmental changes in $\dot{V}_{O_2}$ in *C. coturnix japonica* during development, but the only difference between the two experimental conditions LR and HR was established at 10 days old. Although the temperature differences between experimental groups were established gradually, avoiding death during development, these differences were important because the body temperature of this species is 42.1°C and complete thermoregulatory capacity is reached at age 13 days (Spiers et al., 1977). Furthermore, both thermal regimes are below the thermoneutral zone of this species and other quails (Freeman, 1967; McNabb and McNabb, 1977; Blem, 1978; Pis and Lusnia, 2005). Actually, the lower critical temperatures vary according to age and body mass in this species and they have been estimated at 35°C in individuals younger than 1 week, 31°C at two weeks, 23°C at three weeks, 21°C at 4 weeks and 19°C at age six weeks (Freeman, 1967). Thus, while for the group of low energy requirements (LR) the ambient temperature was slightly lower than the critical temperature only until the first week, in the HR group the temperature was well below the lower critical temperature during the entire experimental period.

Table 3

Maximum metabolic rate to oxygen diffusion capacity ratios $\dot{V}_{O_2 \max} / D_tO_2$ with age and safety factor (S_f) in *Coturnix coturnix japonica* in two thermal regimes.

Age	$\dot{V}_{O_2 \max} / D_tO_2$ (kPa)	
	BR	AR
10 $\pm$ 1 day	0.95 $\pm$ 0.50	0.83 $\pm$ 0.29
16 $\pm$ 1 day	1.17 $\pm$ 0.43	1.38 $\pm$ 0.33
24 $\pm$ 1 day	0.97 $\pm$ 0.12	1.03 $\pm$ 0.36
45 $\pm$ 1 day	1.23 $\pm$ 0.24	1.60 $\pm$ 0.57
Age	S_f	
	BR	AR
10 $\pm$ 1 day	0.92 $\pm$ 0.48	0.95 $\pm$ 0.32
16 $\pm$ 1 day	0.83 $\pm$ 0.21	0.63 $\pm$ 0.14
24 $\pm$ 1 day	0.78 $\pm$ 0.09	0.79 $\pm$ 0.25
45 $\pm$ 1 day	0.69 $\pm$ 0.01	0.59 $\pm$ 0.06

Table 4

Allometric exponents (with body mass) for lung volume (V_L), resting metabolic rate (RMR), maximum metabolic rate ($\dot{V}_{O_2 \max}$), harmonic mean of thickness of the air–blood barrier (τ_h), respiratory surface density (RS_d), oxygen diffusion capacity (tissue) (D_tO_2), RMR/ D_tO_2 ratio and $\dot{V}_{O_2 \max} / D_tO_2$ ratio during development in *Coturnix coturnix japonica* at two thermal regimes (LR and HR). (*) indicates whether the exponent was different from 0 in the F-test in the power law regression. The R^2 coefficient is indicated between parentheses.

	LR	HR
V_L^*	1.03 ± 0.07 (0.94)	1.07 ± 0.06 (0.94)
RMR*	0.69 ± 0.05 (0.98)	0.90 ± 0.04 (0.99)
$\dot{V}_{O_2 \max}^*$	0.90 ± 0.05 (0.98)	0.97 ± 0.06 (0.98)
τ_h	0.004 ± 0.05 (0.001)	0.11 ± 0.06 (0.15)
RS_d	-0.104 ± 0.07 (0.11)	-0.08 ± 0.04 (0.08)
$D_tO_2^*$	0.80 ± 0.09 (0.87)	0.74 ± 0.09 (0.87)
$\dot{V}_{O_2 \max} / D_tO_2$	0.10 ± 0.10 (0.09)	0.16 ± 0.11 (0.09)
RMR/ D_tO_2	-0.11 ± 0.11 (0.09)	0.14 ± 0.09 (0.08)

Despite the different temperature requirements in both conditions, RMR only was different between HR and LR groups at 10 days old, but in general similar to the values obtained by other authors throughout development in this species (Freeman, 1967; McNabb and McNabb, 1977). The developmental patterns of mass-specific BMR and $\dot{V}_{O_2 \max}$ were similar to that described for metabolism and heart rate in some birds and other vertebrates (Burggren, 1989) with a sharp increase in early development up until day 10 concomitant with an increase in body mass.

There were no differences in $\dot{V}_{O_2 \max}$ between groups or in the structural measures of lung capacity or oxygen diffusion. That is, this species showed no developmental plasticity in metabolic parameters and lung structure by being exposed to these temperature variations except in RMR at 10 days old. One explanation for this result could be that quail chick does not attain full endothermy until day 13, and presumably the ectothermic nature of the chicks, at least early after hatching, will prevent RMR from increasing in response to lowered ambient temperature. The lack of differences in the development of lung morphology between the experimental groups could be explained by the possibility that these structures were rigid (at least with the thermal ranges used in this study). In a similar way, for example, in *P. darwini*, a small altricial rodent, no plasticity of lung morphology was found as a function of thermal requirements, although this species exhibits a marked difference in thermoregulatory capacity (Canals et al., 2009a,b).

The thickness of the air–blood barrier showed no changes in the different stages of development. Allometric exponents were not different from 0 for the HR and LR groups. This result is similar to those found for the Australian brush turkey and Australian pelicans (Seymour et al., 2008). In individuals of different developmental stages of *C. coturnix* thickness varied between 0.37 and 0.57 μm , a value similar to that found in *Meleagris gallopavo* (Seymour et al., 2008) and near other Galliformes that have high values ranging between 0.25 and 0.39 μm (Maina, 2002). These values contrast for example with the thickness of the thin air–blood barrier of the Columbines, which is about 0.2 μm (Maina, 2002; Figueroa et al., 2007; Canals et al., 2007), with an upper limit of 0.302 μm for *Columbina picui* (Alfaro et al., 2010). The high value of *C. coturnix* is probably associated with the cursorial life habit of Galliformes, which in fact is shared with other birds such as the Tinamiformes, whose air–blood barrier is as thick as 0.469 μm in the Chilean tinamou *Notoprocta perdicaria* (Figueroa et al., 2007).

We also found no significant change in respiratory surface density, which had negative allometric exponents but not different from 0. The respiratory surface density was low but close to that reported for other Galliformes which vary between 135 and 192 mm^{-1} (Maina, 2002), which places *C. coturnix* in the lower range of this group, although Duncker (1974) reported higher values for this species.

The allometric exponent for $\dot{V}_{O_2 \max}$ was in the range to the value 1.07 ± 0.16 reported for *M. gallopavo* (Table 3). But, the allometric exponent for RMR was lower to that reported for this species: 0.99 ± 0.22 (Seymour et al., 2008). Also, the allometric exponent for D_tO_2 in *C. coturnix* was somewhat lower than the value reported for *M. gallopavo*: 1.23 ± 0.12 , but slightly larger than the value reported for adult birds 0.71 (Maina, 2002). Despite the small differences between the metabolic exponents and the D_tO_2 exponent, the allometric exponent between RMR/ D_tO_2 and $\dot{V}_{O_2 \max} / D_tO_2$ ratios and body mass did not differ from 0 and our safety factors did not change with age during development. This shows a good adjustment between metabolic variables and oxygen diffusion capacity, remaining constant for all ages and body mass during development, which is consistent with the symmorphosis hypothesis. These ratios measure the adjustment between function and morphology at the level of the respiratory system, but also represent the differential needed to overcome the barrier to meet and RMR and $\dot{V}_{O_2 \max}$ respectively. This oxygen tension difference averaged 0.31 kPa and 0.916 kPa for RMR and $\dot{V}_{O_2 \max}$. Considering that the ratio of the total capacity of oxygen diffusion (D_tO_2) (including plasma and red blood cells in addition to the tissue barrier) and D_tO_2 in birds is $D_tO_2/D_tO_2 \approx 1/10$ (Canals et al., 2010) (actually $D_tO_2/D_tO_2 = 0.091$ with a confidence interval of 0.066–0.116; based on data of Maina, 2002), the baseline ΔPO_2 requirements range between 2.7 and 4.7 kPa. However, to meet the maximum requirements a ΔPO_2 between 7.89 kPa and 13.8 kPa would be necessary. Our safety factors were lower than the unity, which can be explained by the assumptions of our estimation. For example a value of 7.5 kPa (=60 Torr) was used as a “typical” ΔPO_2 . For example, sea-level chicken (*Gallus gallus*) had an air cell pressure value of $O_2 = 102.3 \pm 2.7$ Torr (Monge & León-Velarde, 1994) and atrial and arterial blood samples of PO_2 had values over the range of 40 to 50 Torr (Nightingale and Richardi 1977). These values suggest ΔPO_2 between 60 and 65 Torr in this species, indicating that small intra-specific and inter-specific variations can exist in ΔPO_2 . Despite these variations our safety factors close to 1 suggest that *C. coturnix* have a morphological capacity (D_tO_2) probably too close to the maximum needs along all the developmental period, just as predicted by symmorphosis hypothesis.

Acknowledgments

We thank Lafayette Eaton for his useful comments on the manuscript. Funding by FONDECYT 1040649 to MCL.

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