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Hypoxia during embryonic development increases energy metabolism in normoxic juvenile chicks

Lara do Amaral-Silva [a,b,1], Carolina da S. Scarpellini [a,b,1], Paula Andrea Toro-Velasquez [a], Marcia H.M.R. Fernandes [c], Luciane H. Gargaglioni [a,b], Kênia C. Bícego [a,b,*]

[a] Department of Animal Morphology and Physiology, College of Agricultural and Veterinarian Sciences, São Paulo State University, Jaboticabal, São Paulo 14884-900, Brazil
[b] National Institute of Science and Technology – Comparative Physiology (INCT-Fisiologia Comparada), Brazil
[c] Department of Animal Science, College of Agricultural and Veterinarian Sciences, São Paulo State University, Jaboticabal, São Paulo 14884-900, Brazil

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ABSTRACT

Environmental changes during perinatal development can affect the postnatal life. In this sense, chicken embryos that experience low levels of O_2 over a specific phase of incubation can have their tissue growth reduced and the ventilatory response to hypoxia blunted, at least until hatching. Additionally, exposure to low level of O_2 after birth reduces the thermogenesis as well. In the present study, we tested the hypothesis that hypoxia over the third week of incubation affects the thermoregulation of juvenile chicks at an age when thermogenesis is already expected to be well-developed. To this end, we measured body temperature (Tb) and oxygen consumption ($\dot{V}O_2$) under acute hypoxia or different ambient temperatures (Ta) of 1 and 10 day-old chicks that have been exposed to 21% O2 for entire incubation (Nx) or to 15% O_2 in the last week of incubation (Hx). We also assessed the thermal preference under normoxia or acute hypoxia of the older chicks from both incubation groups in a thermocline. Hypoxia over incubation reduced growth but did not affect the cold-induced thermogenesis in hatchlings. Regarding the juvenile Hx, present data indicate a catch up growth with higher resting $\dot{V}O_2$, a thermal preference for warmer Tas and a possible higher thermal conductance. In conclusion, our results show that hypoxia over the third week of incubation can affect the thermoregulation at least until 10 days after hatch in chickens.

1. Introduction

Hypoxia induces decrease in metabolic rate ($\dot{V}O_2$) and body temperature (Tb) and increase in ventilation, which seems to minimize the imbalance between oxygen supply and demand. These responses are observed in newborns and adults of several species (Gautier, 1996; Bicego et al., 2007; Mortola, 2009; Mortola and Maskrey, 2011) whose metabolic suppression is accompanied by increase in autonomic (Tattersall and Milsom, 2003) and behavioral (Mortola and Feher, 1998; Bicego et al., 2007; Mortola and Maskrey, 2011) heat loss responses.

During prenatal stages, the hypoxic metabolic drop is observed mainly as depression of tissue growth because at this phase growth is the most energy-demanding function, which can lead to immaturity of organs/systems in hatchlings (Mortola and Cooney, 2008; Mortola, 2009; Mortola and Awam, 2010). The drop in growth is also observed during non-hypoxic reduction of metabolic rate such as cold exposure over incubation (Mortola and Toro-Velasquez, 2013).

In contrast, after birth thermogenesis becomes the principal source of energy expenditure and its inhibition is the main factor involved in hypoxia-induced metabolic depression (Mortola and Maskrey, 2011). The opposite response (increase in energy expenditure), however, is observed during cold exposure to avoid hypothermia (cf. Bicego et al., 2007). These facts indicate that, although these two stressors (hypoxia and cold) have similar effects on metabolic rate during prenatal stages, in post-natal life their effects are opposite because of the establishment of endothermy (Szdzuy et al., 2008). In this context, it is interesting to note that hypoxia, but not cold exposure, during incubation induces reduced ventilatory response to low oxygen in chicken hatchlings (Mortola and Toro-Velasquez, 2013), indicating a specificity of the hypoxic stimulus to the chemosensory development.

All the studies mentioned above addressed morphophysiogical responses during pre-hatching phases or in the first day after hatching. It is possible that exposure to low levels of O2 over embryogenesis can cause not only short, but also prolonged morphophysiological

Abbreviations: BM, body mass; H0, hatchlings (between 12 and 20 hs); H10, 10 day-old chicks; Hx, hypoxic incubation (last week, 15% O2); Nx, normoxic incubation (21% O2); Ta, ambient temperature; Tb, body temperature; $\dot{V}O_2$, metabolic rate assessed as the rate of oxygen consumed by the animal.

* Corresponding author at: Via de acesso Paulo Donato Castellane s/n, 14884-900, Departamento de Morfologia e Fisiologia Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, SP, Brazil.
E-mail addresses: keniacb@yahoo.com.br, keniacb@fcav.unesp.br (K.C. Bícego).
[1] These authors contributed equally to this work.

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alterations. The interest in postnatal consequences caused by environmental changes throughout perinatal development is because such alterations during critical phases can induce responses of genes that lead to different phenotypes later in life (Okubo and Mortola, 1989; Snyder et al., 1984). In the case of hypoxia, the phase of embryonic development that the animal is exposed to, is crucial to determine possible phenotypic changes (Chan and Burggren, 2005).

Because in birds the last week of incubation is a critical phase for some organs/system and thermogenesis maturation (Chan and Burggren, 2005; Ferner and Mortola, 2009; Szdzuy et al., 2008), we hypothesize that low levels of O2 over the third week of incubation would affect thermoregulation of chicks at the second week of life, i.e., when they are already expected to have a well-developed thermogenesis (Tzschentke and Nichelmann, 1999). To test this hypothesis, we measured Tb and oxygen consumption (index of thermogenesis) under acute hypoxia or different ambient temperatures (Ta) of 1 and 10 day-old chicks that have been exposed to 15% O2 in the last week of incubation. We also measured the thermal preference in normoxia or acute hypoxia of the older chicks from both incubation groups.

2. Methods

2.1. Animals

Freshly laid fertilized eggs of lineage Cobb were obtained from a local supplier (Globoaves, Itirapina, SP, Brazil). All the eggs were weighed at day 0, incubated at temperature of 37.5 °C and 60% of relative humidity and rotated every 2 h. All of those parameters were controlled by sensors inside the incubators. One group of eggs remained under normoxia for the entire incubation (21% O2; Nx), whereas another group was transferred into a hypoxic incubator (15% O2; Hx) between day 12 and day 18 of incubation. The desired level of hypoxia (15% O2) was obtained by pushing into the incubator a small stream of N2 (0.2–0.4 mL/min; White Martins, Osasco, SP, Brazil) controlled by a flowmeter and the O2 concentration was monitored continuously by an O2 analyzer (Sensepoint XCD, Honeywell, USA). The incubator was equipped with three thermometers disposed in strategic points and one of them was very close to the nearest egg to the N2 leaking to make sure that the introduction of compressed gas would not alter the temperature of the incubator. On the 19th day all eggs were transferred to a normoxic hatcher (37.5 °C). After hatchlings were dry, they were transferred to chambers (Premium Ecológica, Belo Horizonte, Brazil) with controlled temperature (33–31 °C until day 5 and to 29 °C until day 10), light:dark cycle 14 h:10 h and water and food ad libitum. The experiments were conducted with the same chicks at day 0 (H0; between 12 and 20 hs) and ten (H10) after hatching. The experimental protocols were in agreement with the guidelines of the National Council of Control in Animal Experimentation (CONCEA-MCT-Brazil) and approved by the local Animal Care and Use Committee (CEUA - # 024166/13).

2.2. Oxygen consumption

$\dot{V}0_2$ was measured using an open-flow respirometry method (Szdzuy et al., 2008). Chicks were placed individually in a respirometer (total volume: 540 mL for H0; 1000 mL for H10) inside a temperature controlled chamber (FANEM, Sao Paulo, SP, Brazil). The ambient temperature (Ta) and gas concentrations inside the respirometer varied according to the protocol and age (see "*Protocols*" item for details). The incurrent air was pulled (pull mode; MFS, Sable Systems, Las Vegas, NV, USA) into the respirometer at a rate of 800 mL/min (H0) or 1500 mL/min (H10) and the gases concentrations were monitored intermittently. Outflow air passed through a drying column (Drierite, Sigma Aldrich, St. Louis, MO, USA), was subsampled (180 mL/min; SS4, Sable Systems, Las Vegas, NV, USA) and finally pulled through calibrated analyzers for O2 (PA-10; Sable Systems, Las Vegas, NV, USA) and CO2 (CA-10; Sable Systems, Las Vegas, NV, USA) concentrations recording. The $\dot{V}0_2$ (mL O2/kg·min^{-1} STPD) was calculated using the following equation (Depocas and Hart, 1957; Lighton, 2008, Eq. 11.7):

$$\dot{V}_{O_2} = \frac{FR_e[(F_iO_2 - F_eO_2 -) - F_iO_2(F_eO_2 - F_iO_2 -)]}{1 - F_iO_2}$$

where:

FRe excurrent flow rate;
FiO2 incurrent fractional concentration of oxygen (from baseline);
FeO2 excurrent fractional concentration of oxygen;
FiCO2 incurrent fractional concentration of carbon dioxide (from baseline);
FeCO2 excurrent fractional concentration of carbon dioxide.

2.2.1. Body temperature (Tb) measurement

Colonic temperature was measured by inserting a thin temperature sensor at 3 cm through the animal's cloaca. The sensor was connected to an analog thermometer (Yellow Spring Instrument, Yellow Spring, Ohio, USA).

2.3. Thermal preference

The thermal gradient chamber used in the present study is the same described in Vizin et al. (2015) but slightly modified to be cooled down to 15 °C at one end of it and warmed up to 40 °C at the other end. For each experiment, a linear thermal gradient was considered acceptable if R2 ≥ 0.96. Pictures of chicks's position in the lanes were taken every minute by using a webcam positioned at the top of the apparatus. The temperatures selected by the animals were calculated based on the equation of the linear regression and the position of the chicks obtained from the pictures. The thermal gradient chamber has been built with eight thermometers even distributed just under the grid floor where the temperatures are slightly colder than the air temperature at mid chick height. Thus, a linear regression was calculated between the temperatures obtained under and above the grid for all thermometers and then used to correct the Ta recorded by the thermometers below the grid.

2.4. Protocols

2.4.1. Effect of hypoxic incubation on resting metabolic rate and Tb of normoxic chicks at days 0 (H0) and 10 (H10) after hatching

After having their Tb measured, animals of both ages (H0 and H10) and both incubation groups (Nx and Hx) were individually placed in the respirometer under normoxia (20.95% O2; at Ta of ~34 °C or 30 °C for H0 and H10, respectively) for approximately 30 min for habituation and then the $\dot{V}0_2$ was determined. Following these resting measurements, animals were divided in two groups and were exposed to either hypoxia or cold (see below).

2.4.2. Effect of hypoxic incubation on metabolic rate and Tb of chicks exposed to an acute hypoxic event at days 0 (H0) and 10 (H10) after hatching

After the normoxic resting measurements, incurrent gas was switched to a hypoxic gas mixture of 15% O2 (15% O2 and N2 balance, White Martins, Osasco, SP, Brazil) and gases concentrations inside the respirometer were measured after 13 min, for 2 min. Lastly, incurrent gas was once again switched to 10% O2 (10% O2 and N2 balance, White Martins, Osasco, SP, Brazil) and gas measurements were repeated after 13 min, for 2 min. As mentioned, Tb was measured before they were placed into the respirometer (initial Tb) and right after they were removed from there (after the 10% hypoxia exposure; final Tb).