Comparative Biochemistry and Physiology, Part A 212 (2017) 81–87

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Aerobic scope in chicken embryos

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ARTICLE INFO

Keywords:
Gas exchange
Growth
Hyperthermia
Oxygen limitation
Prenatal metabolism

ABSTRACT

We investigated the aerobic scope of chicken embryos, that is, the margin of increase of oxygen consumption ($\dot{V}_{O_2}$) above its normal value. $\dot{V}_{O_2}$ was measured by an open-flow methodology at embryonic ages E3, E7, E11, E15, E19 and at E20 at the internal (IP) and external pipping (EP) phases, at the normal incubation temperature (Ta = 38 °C), in hypothermia (Ta = 30 °C) and in hyperthermia (Ta = 41 and 44 °C). In the cold, Q_{10} averaged ~2 at all ages, except in IP and EP when lower values (~1.5) indicated some degree of thermogenesis. In hyperthermia (38–44 °C) Q_{10} was between 1 and 1.4. Hyperthermia had no significant effects on $\dot{V}_{O_2}$ whether the results combined all ages or considered individual age groups, except in IP (in which $\dot{V}_{O_2}$ increased 8% with 44 °C) and EP embryos (+13%). After opening the air cell, which exposed the embryo to a higher O_2 pressure, hyperthermic $\dot{V}_{O_2}$ was significantly higher than in normothermia in E19 (+13%), IP (+22%) and EP embryos (+22%). We conclude that in chicken embryos throughout most of incubation neither heat nor oxygen availability limits the normal (normoxic-normothermic) values of $\dot{V}_{O_2}$. Only close to hatching O_2-diffusion represents a limiting factor to the embryo's $\dot{V}_{O_2}$. Hence, embryos differ from postnatal animals for a nearly absent aerobic scope, presumably because their major sources of energy expenditure (growth and tissue maintenance) are constantly maximized.

1. Introduction

In animals with no thermal control changes in ambient temperature (Ta) cause parallel changes in body temperature (Tb); these, then, are responsible for changes in oxygen consumption ($\dot{V}_{O_2}$) according to the Q10 (Arrhenius) factor. The Q10 term is a quantitative approximation of the general concept that reactions rates depend on the absolute temperature; it expresses the change in velocity of metabolic processes for a 10 °C change in Ta, as

$$Q10 = (M1/M2)^{[10/(T1-T2)]} \quad (1)$$

where M1 and M2 are the speed of metabolic reactions at the corresponding temperatures T1 and T2. Around physiologically relevant temperatures, the rate of common chemical reactions increases by a factor of ~2 for every 10-degree Celsius change in temperature. At the organismal level, therefore, in absence of Tb-regulation a 10 °C change in Ta approximately doubles $\dot{V}_{O_2}$; that is, metabolic Q10 = 2 (Bennett, 1984; Elias et al., 2014). Q10 values for $\dot{V}_{O_2}$ < 2 indicate some effectiveness in the control of Tb. In the cold, the stability of Tb despite the drop of Ta implies the presence of effective thermogenesis, in which case, metabolic Q10 < 1. In the heat, the stability of Tb in the face of a rise in Ta (i.e., metabolic Q10 = 1) indicates an efficient control of heat loss.

Avian embryos have almost no thermogenic capacity, which, in fact, initiates only close to hatching. Indeed, measurements in various species including the chicken embryos have shown that both Tb and $\dot{V}_{O_2}$ drop as Ta decreases, with Q10 ~2 (Romanoff, 1972; Nair et al., 1983; Williams and Ricklefs, 1984; Tazawa and Rahn, 1987; Tazawa et al., 1989a; Feast et al., 1998; Nichelmann et al., 1998; Whittow and Tazawa, 1991; Mortola and Labbè, 2005; Mortola, 2006).

By comparison to the response to cold, the embryo's metabolic response to heat has received less attention, although prenatal hyperthermia has biological interest and carries clinical implications. During pregnancy, fever or other conditions of a modest rise in Tb can have serious consequences on fetal development (Edwards, 1967, Smith et al., 1978; Edwards et al., 2003; Power and Blood, 2011). Mammalian experimental models to the study of prenatal hyperthermia have been used, but the uterine, maternal and placental responses to changes in Ta complicate the interpretation of the effects of hyperthermia on the fetus; furthermore, maternal stress during pregnancy by itself impacts on fetal development (Fernandez-Cano, 1958; Hensleigh and Johnson, 1971; Saetta et al., 1988; Tazumi et al., 2005). Avian embryos are free from some of the confounding factors typical of mammalian preparations; hence, they have been the experimental models for many studies, mostly concerned with small increments in incubation temperature on embryonic growth. The increase in Ta (by 1–2 °C) at various times in

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http://dx.doi.org/10.1016/j.cbpa.2017.07.017
Received 26 April 2017; Received in revised form 26 July 2017; Accepted 27 July 2017
Available online 31 July 2017

incubation have produced mixed results, with accelerations of embryonic growth (Hammond et al., 2007; Collins et al., 2013), decreases (Yalçin and Siegel, 2003; Yalçin et al., 2008; Piestun et al., 2009) or no significant changes (Iqbal et al., 1990; Moraes et al., 2004; Yahav et al., 2004; Yalçin et al., 2005; Ipek et al., 2014; Krischek et al., 2016). The scatter of results probably originated from the transversal protocols on separate groups of embryos, compounded by the large normal variability in embryo's development (Mortola and Al Awam, 2010).

Only a handful of studies have considered the effects of hyperthermia on embryo's $\dot{V}_{O_2}$. In chicken embryos incubated at 39.5 °C for several days $\dot{V}_{O_2}$ was slightly higher, similar or lower than in controls (37.8 °C) depending on the embryo's age (Piestun et al., 2009). Higher temperatures have not been tested, possibly because incompatible with survival when protracted for a long fraction of incubation (Romanoff, 1972). A brief (3 h) exposure to 39 °C of chicken and duck embryos in the second half of incubation raised $\dot{V}_{O_2}$ by < 5% (Janke et al., 2002); higher temperatures were not tested. In another study, a progressive increase of egg temperature (5 °C in 2 h) in chicken embryos just before or during the internal pipping stage had, respectively, no effect or a 13% increase in $\dot{V}_{O_2}$ (Bícego and Mortola, submitted for publication). Younger ages have never been tested. Hence, we do not have enough information on the embryonic Q10 during hyperthermia, and whether it varies throughout incubation.

Based on the information currently available, several possibilities can be anticipated. First, a rise in Ta may increase $\dot{V}_{O_2}$ with a $Q_{10} = 2$. This result would imply that the amount of heat available to the normothermic embryo limits its $\dot{V}_{O_2}$ ("heat-limitation"). A second possibility is that the rise in $\dot{V}_{O_2}$ with Ta occurs only in the presence of additional oxygen. This possibility ("O_2-limitation") stems from previous observations in normothermic embryos close to hatching, in which exposure to hyperoxia increased $\dot{V}_{O_2}$ (Høiby et al., 1983; Burton and Tullett, 1985; Tazawa et al., 1992a; Dzialowski et al., 2007). With respect to oxygenation in hyperthermia the only previous study was on turtle eggs; it showed no effect of hyperthermia on embryo's body mass or hatching success either in normoxia or hyperoxia (Liang et al., 2015). A third hypothetical result should contemplate the eventuality that $\dot{V}_{O_2}$ remains unaltered after lifting both the O_2- and the heat-limitation. Such absence of aerobic scope would imply that in normothermia the embryo's metabolic processes are working at maximal power with no room for further acceleration of the cellular biochemical functions ("power-limitation").

The purpose of this study was to quantify the potential limitations imposed by the two essential substrates (heat and oxygen) on the aerobic scope of chicken embryos at various ages of incubation. To this end, first, we have measured the Q10 of the $\dot{V}_{O_2}$ response to hypothermia; then, we have compared to it the data of acute hyperthermia before and after opening the eggshell region above the air cell, an intervention that lifts the barrier to O_2 diffusion (Nakazawa and Tazawa, 1988). The results have revealed that of the three possibilities listed above the third one occurs most frequently throughout incubation; that is, the embryo's metabolic activity constantly operates close to its maximal value.

2. Methods

Freshly laid chicken (*Gallus gallus domesticus*, layer line) eggs were purchased from a local supplier. They were weighed individually and at midday (embryonic day 0, E0, out of 20.5 days of total incubation) were placed in a still air incubator (Hova-Bator model 1602, Savannah, GA, USA) set at 38 °C temperature, 60% relative humidity and automatic 90° egg rotation four times per day. Measurements were conducted at E3, E7, E11, E15, E19 and at E20 on embryos that had entered the IP (internal pipping) or the external pipping (EP) phase of the hatching process. The number of embryos studied at each age varied between 16 and 27. The IP phase is the period of time between the piercing of the chorioallantoic membrane (CAM) of the air cell and the breaking of the eggshell; in chickens, it usually occurs at E20, lasts several hours and is accompanied by the initiation of pulmonary ventilation. The star fracture of the eggshell initiates the EP phase, characterized by a rapid increase in $\dot{V}_{O_2}$ (Szdzuy et al., 2008). The IP staging was identified by transillumination and verified at the end of the experiment after opening of the eggshell.

On the day of the experiment the egg was placed in a 120-ml plastic container that acted as a completely sealed respirometer. The respirometer was submerged into a circulating water bath that provided the desired ambient temperature (Ta), measured by two tungsten-constantan thermocouples placed at middle egg height. The respirometer had inflow-outflow leads for the passage of gas flow. The time required to detect a gas perturbation within the respirometer corresponded to the time needed by the analyzer to sense a rapidly injected bolus of CO_2; it was 44 s and adequate for the measurements performed, which occurred in steps of 40 min (*2.2*).

The relationship between Ta and the temperature inside the egg (Tegg) was obtained in a group of sterile eggs. To this end, an additional thermocouple was threaded through a small hole drilled at the blunted end of the egg and positioned approximately in the egg center. Then, Ta was changed either above or below normothermia following the same protocols of the experimental eggs (*2.2*); the resulting Ta-Tegg data points were plotted and the relationship best described by the exponential $Y = 14.208^{0.0252 \cdot X}$ ($r = 1$), Y and X being, respectively, Tegg and Ta. The Ta-Tegg difference was about 1 °C at the highest Ta (44.5 °C) and nil at the lowest (30 °C).

2.1. Oxygen consumption

Oxygen consumption ($\dot{V}_{O_2}$) was measured by an open flow methodology (Mortola and Labbè, 2005; Szdzuy et al., 2008). A negative pressure pump located downstream the circuitry maintained the flow through the respirometer (50 ml/min at E3 and E7, 100 ml/min at the older ages), under the control of a mass flow meter (Sable Systems International Fox, Henderson, NV). Calibrated gas analyzers (Sable Systems International Fox, Henderson, NV), arranged in series, recorded continuously the outflow O_2 and CO_2 concentrations after the gas had passed through a drying column (anhydrous calcium sulfate, Drierite®, Hammond, Xenia, OH); the inflow concentrations of the gases were monitored intermittently. The gas flow, O_2 and CO_2 concentrations were displayed continuously on a computer monitor. The gas fractional concentrations were mathematically corrected for the error introduced by a respiratory exchange ratio different from unity (Depocas and Hart, 1957; Mortola and Besterman, 2007); then, $\dot{V}_{O_2}$ corresponded to the product of flow rate and inflow-outflow O_2 concentration difference, calculated in μl/min at standard temperature, pressure and dry conditions.

2.2. Protocols

On the day of the experiment the egg was taken from the incubator and placed in the respirometer for at least 30 min before collection of the first set of measurements during normothermia. Two main protocols were followed. In one protocol, after the measurements in normothermia, the eggshell was opened to eliminate its diffusive resistance and raise O_2-availability (Nakazawa and Tazawa, 1988). To this end, the egg was taken from the respirometer and the opening of the eggshell (1 cm in diameter) was performed with micro scissors in the region immediately above the air cell, at the blunted end. The process took about 1 min. Then, the egg was repositioned and measurements taken once temperature equilibrium was re-established, in any case not before 30 min. Finally, the bath was set to produce a Ta inside the respirometer of ~30 °C over a period of 40 min, and maintained for additional 50 min for the measurement in hypothermia. Therefore all embryos measured in the cold had the eggshell open; the purpose of these measurements was to compute the Q10 over the hypothermic