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Metabolic response to cooling temperatures in chicken embryos and hatchlings after cold incubation

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Abstract

We asked to what extent cold exposure during embryonic growth, and the accompanying hypometabolism, may interfere with the normal development of thermogenesis. White Leghorn chicken eggs were incubated in control conditions (38 °C) or at 36 or 35 °C. Embryos incubated at a lower temperature (34 °C) failed to hatch. The cold-incubated embryos had lower oxygen consumption ($\dot{V}_{O_2}$) and body weight (W) throughout incubation, and hatching was delayed by about, respectively, 1 and 2 days. The W–$\dot{V}_{O_2}$ relationship of the cold-incubated embryos was as in controls, indicating that cold-induced hypometabolism was at the expense of the growth, not the maintenance, component of $\dot{V}_{O_2}$. At embryonic day E11, the metabolic response to changes in ambient temperature (T) over the 30–39 °C range was typically poikilothermic, with Q_{10}=1.8–1.9, and similar among all sets of embryos. Toward the end of incubation (E20), the thermogenic responses of the cold-incubated embryos were significantly lower than in controls. This difference occurred also in the few-hour old hatchlings (H1), even though, at this time, W was similar among groups. Exposure to cold during only the last 3 days of incubation (from E18 to H1), i.e. during the developmental onset of the endothermic mechanisms, did not lower the thermogenic capacity of the hatchlings. In conclusion, sustained cold-induced hypometabolism throughout incubation blunted the rate of embryonic growth and the development of thermogenesis. This latter phenomenon could be an example of epigenetic regulation, i.e. of environmental factors exerting a long-term effect on gene expression.

Keywords: Embryonic development; Epigenetic adaptation; Hatching; Hypometabolism; Hypothermia; Thermoregulation; Development

1. Introduction

In adult mammals and birds, cold exposure stimulates metabolic rate, as part of the endothermic defence of body temperature. A sustained exposure to cold improves the ability to withstand further cold challenges, through an enhancement of the thermogenic capacity (Arieli et al., 1979; Gordon, 1990). Also in neonatal animals cold exposure during the first postnatal weeks stimulates brown fat, non-shivering thermogenesis, and the biomolecular machinery involved in heat production (Skála and Hahn, 1974; Bertin and Portet, 1981; Cannon and Nedergaard, 1983; Bertin et al., 1993; Sant'Anna and Mortola, 2003).

Contrary to the postnatal situation, little attention has been given to the implications that a prenatal cold-induced hypometabolic condition may have on the establishment of the thermogenic capacity. For a large fraction of embryonic development birds and mammals have minimal endothermic capabilities, and a drop in temperature lowers their metabolic rate (Whittow and Tazawa, 1991; Power et al., 2004). In rats, sustained cold during pregnancy reduced fetal growth, resulting in offsprings of smaller size and with lower oxygen consumption ($\dot{V}_{O_2}$) (Saetta et al., 1988). In mammals, however, the maternal protection of the fetal temperature and the fact that maternal thermal stress has an impact on fetal development (Fernandez-Cano, 1958; Hensleigh and Johnson, 1971; Tazumi et al., 2005) complicate the interpretation of the effects of cold-induced hypometabolism on fetal outcome. Experiments in avian preparations circumvent the issue of the maternal interference in the embryo's response to cold. In a recent study (Black and Burggren, 2004a,b), chicken eggs were incubated at 38 °C or at 35 °C; toward the end of incubation, those incubated in the cold were less capable of maintaining $\dot{V}_{O_2}$ during cooling than the embryos incubated at 38 °C. This difference, although small, remained

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significant after taking into account the differences in developmental staging between the two groups. On the other hand, studies performed on the hatchlings of turkeys, chickens and Muscovy ducks, incubated at low temperatures during the last few days of incubation, indicated an increase in heat production compared to controls (Minne and Decuypere, 1984; Tzschentke and Nichelmann, 1999; Tzschentke et al., 2001). One interpretation of these discordant results is that cold incubation may have qualitatively different effects on heat production between the late embryo and the hatchling. Perhaps, this difference could be due to the fact that hatching, by lifting the O_2-limitation on $\dot{V}_{O_2}$ during the terminal phases of embryonic development, causes a drastic and rapid change in heat production (Tazawa et al., 1988; Whittow and Tazawa, 1991; Nichelmann and Tzschentke, 2003; Black and Burggren, 2004a,b; Mortola and Labbè, 2005). Alternatively, cold exposures could have different effects depending on whether they occur throughout the whole incubation or only toward the end of it, this latter being the time when the thermogenic capacity begins to form. Hence, the primary goal of the current study was to assess the effects of cold exposure throughout incubation on the thermogenic response of both the late embryos and the hatchlings, to evaluate the possibility of a qualitative difference between these two developmental stages. The effects of cold exposure limited to the last few days of incubation have also been considered. A secondary aim was to examine the body weight–oxygen consumption (W–$\dot{V}_{O_2}$) relationship during embryonic development under various degrees of cold exposure. In fact, the drop in body weight (W) in embryos reared at 35 °C was proportional to their decrease in $\dot{V}_{O_2}$ (Black and Burggren, 2004a,b). Should the W–$\dot{V}_{O_2}$ relationship of the cold-incubated embryos be similar to controls, it would imply that, of the total embryonic energy budget, the cold-induced hypometabolism has curtailed solely the component related to body growth.

2. Methods

Freshly laid fertilized eggs of White Leghorn chickens (*Gallus gallus*) were obtained from a local supplier. Incubation started around midday (day 0). The eggs were weighed and placed in incubators (Hova-Bator, Savannah, GA, USA) pre-set either at the temperature (T) of 38 °C (Control group), or at lower T, i.e. 36, 35 or 34 °C. The incubators provided a 45° egg rotation four times a day. In all incubators, the relative humidity was 60%. A T-data logger and a hygrometer were placed inside the incubators; the former collected the T value every 10-min interval, while humidity was read daily.

A first set of eggs was used to measure body growth and metabolic rate from embryonic day 8 to day 20, at 3-day intervals, and to consider the degree of hatchability under the low T conditions. The metabolic responses to changes in T were measured in embryos at the same chronological age (E11, E20), and in the hatchlings on the day of hatching (H1).

2.1. Gaseous metabolism

Metabolic rate was measured by indirect calorimetry (oxygen consumption, $\dot{V}_{O_2}$, and carbon dioxide production, $\dot{V}_{CO_2}$), with an open-flow methodology (Frappell et al., 1992) adapted to the chicken embryo (Menna and Mortola, 2002, 2003; Mortola and Labbè, 2005). Measurements were performed on embryos (grouped in sets of two) and on hatchlings (singly) in a respirometer, which consisted of a 300-mL plastic container maintained at the desired T by a water bath. A steady gas flow of 100 mL/min was continuously delivered through the respirometer, and controlled by a precision flowmeter. The inflow and outflow O_2 and CO_2 concentrations were monitored by gas analysers (OM-11, Beckman and CD-3A, Applied Electrochemistry) arranged in series, after the gas passed through a drying column. The output of the analysers was displayed on a computer monitor during on-line acquisition. $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were computed from the flow rate and the inflow–outflow concentration difference over a period of 5 min at the end of each condition. The values, calculated at standard temperature, pressure, and dry conditions are presented in mL/min (1 mL O_2 STPD = 0.0446 mmol O_2).

2.2. Protocols

Eggs were weighed at the beginning of the incubation and on the day of the measurements. The average daily water loss (delta H_2O) was computed from the difference between the initial and final egg weight, divided by the number of days of incubation. The difference in partial pressure of water $P(H_2O)$ between the egg and the incubator was calculated from the corresponding T and relative humidity. From these data, the water conductance of each egg $G(H_2O)$ was equal to delta H_2O/delta $P(H_2O)$, mL day^{-1} Torr^{-1}. At the end of the experiment, the embryo was killed by exposure to CO_2 and cold, the egg was opened, the embryo examined to exclude gross anatomical abnormalities, and body weight (W) and head weight were measured on a digital scale.

For each group of embryos, the metabolic response to changes in T was studied on day 11 (E11) and day 20 (E20) embryos, and in hatchlings within the first 24 h after hatching (H1). Embryos were studied in sets of two, while hatchlings were studied individually. Each group, at any given age, had $N = 10$, i.e., 10 sets of two embryos each, or 10 hatchlings. The metabolic response to T consisted of measurements performed at $T = 39$–36–33–30 °C, either in ascending or descending sequence, alternating the order among experiments. Each T was maintained for 1 h, and data refer to the last 5 min of each exposure.

An additional group of eggs was incubated in control conditions (38 °C) until E18, when it was switched to 35 °C and left at that T until hatching. Then, the metabolic response to changes in T was studied in the hatchlings with the same protocol described above.

2.3. Normalisation and statistics

The data of $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were normalised by embryo's W. All group data are presented as means ± 1 S.E.M. Linear regression through the data points was employed to compute the Q_{10} values of E11 and E20, according to the van't Hoff