HEART FUNCTION, CIRCULATION AND RESPIRATION IN EMBRYO AND HATCHING

Early Development of Thermoregulatory Competence in Chickens: Responses of Heart Rate and Oxygen Uptake to Altered Ambient Temperatures


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ABSTRACT

Avian embryos need a heat supply from the external environment to develop, because heat loss from an egg overwhelms heat production of an embryo and accordingly they cannot maintain body temperature constant during incubation. In the domestic fowl, a minute homeothermic competence appears during the end of incubation with subsequent large augmentation soon after hatching. However, hatchling's metabolic rate still produces less heat than that required to offset heat loss. This stage prior to reaching "full-blown" homeothermy is designated as "power-limited". In this power-limited stage of thermoregulation, development of thermoregulatory competence in response to altered ambient temperature \( T_a \) involves the rapid maturation of heart rate (HR) regulation. The first part of this report reviews briefly: (1) early development of homeothermic thermal and metabolic responses to altered \( T_a \) in chick embryos and hatchlings, and (2) HR responses to altered \( T_a \) in newly hatched chicks during the power-limited stage of thermoregulation, which depend on the state of development of thermoregulatory competence. The last part concerns our recent preliminary experiment and hypothesis with regard to the early development of thermoregulatory competence in hatchlings developing in the power-limited stage. The preliminary experiment shows possible influence of brooding temperatures (i.e. preferred brooding \( T_a \) of 35°C and low brooding \( T_a \) of 24–27°C) to the development of thermoregulatory competence in terms of HR responses to altered \( T_a \). We hypothesise that homeothermic-metabolic response to altered \( T_a \) develops in parallel with the homeothermic HR response and additionally the brooding temperature will influence the maturation of homeothermic-metabolic response so that hatchlings maintained at low \( T_a \) will develop the homeothermic-metabolic response earlier than those at the preferred brooding \( T_a \). The result shows that homeothermic-metabolic response obtained in the white leghorn chickens does not develop in parallel with the homeothermic HR response obtained previously in the broiler chickens. In addition, maturation of homeothermic-metabolic response is observed on just hatched chicks maintained at both the preferred brooding \( T_a \) and low \( T_a \). The failure of proving the hypothesis suggests other possibilities of which investigation will improve our knowledge of thermoregulation in avian embryos and hatchlings.

Keywords: thermoregulatory competence, heart rate response, metabolic response, embryo, hatchling, altered ambient temperature, preferred brooding temperature, cooling exposure, warming exposure, chick, duck, emu

1. INTRODUCTION

Avian embryos developing inside an eggshell need a heat supply from the environments, i.e. parent birds or an incubator. When they are exposed to low ambient temperature \( T_a \), the embryos cannot maintain body temperature \( T_b \) because of their small heat production compared with a possible large decrease in \( T_a \) caused...
by isolation of heat supply and so avian embryos are effectively poikilotherms. In the domestic fowl (Gallus gallus), thermal conductance of the egg is estimated to be about 70–75 mW°C⁻¹ during incubation, while embryos generate heat at most of about 130–140 mW prior to pipping and about 160 mW during external pipping (EP) at an incubation temperature (T_a) of 38°C (Tazawa et al., 1988a, 1988b, 2001). Accordingly, these metabolic heats raise the egg temperature about 2°C or a bit more above T_a during the last stages of incubation (Tazawa and Rahn, 1987). However, if the eggs are exposed to an environment 10°C cooler than T_a, the rate of heat loss during cooling exceeds the embryos’ maximum rate of heat production by about 5–6-fold. In fact, if the embryos are exposed to air 10°C cooler than the egg’s centre they have to generate heat at a rate of about 800 mW to keep the egg temperature steady (Turner, 1986). Eventually, chick embryos cannot maintain constant body temperature when they are exposed to low T_a and thus they are poikilothermic. However, the transition from poikilothermy to homeothermy begins to occur during the late stages of incubation. When chicken eggs were cooled gradually for a prolonged period to low T_a to make the imbalance between heat loss from the eggs and heat production of the embryos as small as possible, 18-day-old pre-pipped embryos showed a weak metabolic response to cooling with a subsequent augmented response in externally pipped embryos (gradual cooling test, Tazawa et al., 1988b). When the drop of T_a was small, e.g. 6°C, a feeble compensatory metabolic response was also confirmed in the late pre-pipped embryos. Namely, during prolonged cooling with the small drop in T_a, they had a temperature coefficient (Q10) of oxygen uptake (M_o2) lower than 2 (the Arrhenius value; Tazawa et al., 1989; Kuroda et al., 1990). A transient homeothermic-metabolic response to cooling greater than 3°C was also demonstrated in late pre-pipped and pipped chick embryos (Freeman, 1964).

While the thermoregulatory competence begins to emerge during the last stages of embryonic development, in chickens, it is rapidly augmented soon after hatching (Romijn and Lokhorst, 1955; Freeman, 1964, 1967, 1971; Wekstein and Zolman, 1967; Tazawa and Rahn, 1987), or after a predicted time of hatching irrespective of emergence from the eggshell (Tazawa and Rahn, 1987; Tazawa et al., 1988b). However, the hatchling’s metabolic rate still produces less heat than that required to offset heat loss. In models of the development of thermoregulation, illustrating the transition from poikilothermy to homeothermy in precocial and altricial birds (Tazawa et al., 1988b; Whittow and Tazawa, 1991), this stage prior to reaching “full-blown” homeothermy where hatchlings are capable of fully defending their body temperature against cooling has been designated as “power limited”. In this stage, the hatchling’s capacity to generate heat is limited. In chickens, development of thermoregulatory competence during the power-limited stage still progresses after hatching (Tazawa and Rahn, 1987). Previously, it was shown that the development of thermoregulatory competence in response to altered T_a during the first week of post-hatch involved the rapid maturation of heart rate (HR) regulation. HR responses to altered T_a in newly hatched chicks during the power-limited stage of thermoregulation depended on the state of development of thermoregulatory competence (Khandoker et al., 2004). Another preliminary experiment indicated that brooding temperature of hatchlings influenced the development of thermoregulatory competence in reference to HR responses to altered T_a. Based on our previous experience with the thermoregulatory responses of HR, we hypothesise that metabolic responses to altered T_a in chick hatchlings during the power-limited stage also depend on the state of development of thermoregulatory competence and brooding temperature. The present report reviews briefly the HR responses to altered T_a in chick hatchlings and the possible influences of brooding temperature on the HR responses. In addition to the review, we examine whether or not metabolic responses to altered T_a also depend on the state of development of thermoregulatory competence and brooding temperature.

2. DEVELOPMENT OF THERMOREGULATORY COMPETENCE IN EMBRYOS AND HATCHLINGS

Many studies on the thermogenesis in chick embryos and hatchlings show that during embryonic development chickens are substantially poikilothermic and after hatching rapidly develop the competence to maintain body temperature (Romijn and Lokhorst, 1955; Freeman, 1964, 1967, 1971; Wekstein and Zolman, 1967; Tazawa and Rahn, 1987). Development of thermoregulatory competence can be shown by determining egg temperature (T_egg) or body temperature (T_b) of animals that are exposed to low T_a.
The equilibrium temperature of eggs and hatchlings at $T_a$ of 38°C and quasi-equilibrium temperature at $T_a$ of 28°C in chickens are shown in Figure 1 (Tazawa and Rahn, 1987). A quasi-equilibrium state of embryo’s temperature is defined as a change in $T_{egg}$ of less than 0.1°C·h$^{-1}$ and has been reached 5-hour after exposure to low $T_a$. The equilibrium temperature of the embryos increases steadily to reach a value of about 2°C above $T_a$ of 38°C just before hatching due to increasing metabolism during development while maintaining a constant thermal conductance of the egg. After hatching, $T_b$ increases further to reach a plateau about 3°C above $T_a$ of 38°C on day 3-4. After exposure to $T_a$ of 28°C for the 5-hour period, $T_{egg}$ reaches the quasi-equilibrium temperature that also gradually increases with embryonic development to reach a value of about 1°C above $T_a$ just before pipping and hatching. In consequence, the large, prolonged drop in $T_a$ swamps the nascent thermoregulatory response of $T_{egg}$ to $T_a$. However, upon hatching, the quasi-equilibrium temperature 5-hour after exposure to the lower $T_a$ is remarkably higher than 28°C and reaches levels approaching 38°C by day 3. This augmented $T_b$ confirms the previous studies on the rapid development of thermoregulatory competence soon after hatching (Romijn and Lokhorst, 1955; Freeman, 1964, 1967, 1971; Wekstein and Zolman, 1967). Additionally, the closed circles in the initial temperature and open circles in the final temperature show the equilibrium temperature and quasi-equilibrium temperature of a single embryo that fails to hatch at predicted time of hatching. Although it stays within the eggshell on day 22 (equivalent to day 0 post-hatch) and day 23 (day 1 post-hatch), the quasi-equilibrium temperature is remarkably above that at the EP period. This result indicates that emergence from the eggshell at a time of predicted hatching is not necessary for an increase in metabolic rate in response to cooling.

The ratio of oxygen uptake ($M_o_2$) after 5 hours at a $T_a$ of 24°C to that at 37°C in chick embryos and hatchlings is shown in Figure 2 (Tazawa and Rahn, 1987). The ratio is remarkably constant at about 40% throughout the last half of incubation until hatching. The $Q_{10}$ of $M_o_2$ is about 2, indicating a simple physiochemical process without an attempt of thermoregulation by the embryos. However, immediately upon hatching, the ratio increases to 70-95%, supporting rapid development of thermoregulatory competence. The two ringed circles in Figure 2 show two eggs that pipped, but the embryos failed to emerge from the eggshell on day 22 of incubation as in the case of the embryo shown in Figure 1. Their $M_o_2$ is appreciably higher than that during the previous day, indicating again that emergence from the eggshell is not necessary for augmented metabolic response to

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**Fig. 1** The temperature of eggs and hatchlings before and after 5-hour exposure to ambient temperature of 28°C. The shaded area corresponds to the equilibrium temperature of the eggs and hatchlings at 38°C before exposure to 28°C, and final temperature corresponds to the temperature of eggs and hatchlings that reaches after 5-hour exposure to 28°C; that is, quasi-equilibrium temperature. Solid lines connect the results from the same individuals. Reproduced with permission from Tazawa and Rahn (1987).

**Fig. 2** The ratio of oxygen uptake at quasi-equilibrium temperature of 24°C after 5-hour to that at equilibrium temperature of 37°C in chick embryos and hatchlings. The shaded area corresponds to pipping period and symbols I and E indicate internally pipped and externally pipped embryos. Reproduced with permission from Tazawa and Rahn (1987).
cooling and rapid development of thermoregulatory competence occurs in unhatched embryos that reach a maturity to hatch. The similar thermoregulatory response of an unhatched embryo is shown by changes in its HR in response to cooling (Tazawa et al., 2001).

3. HEART RATE RESPONSES TO ALTERED $T_a$ IN NEWLY HATCHED CHICKS

While the thermoregulatory competence improves immediately upon hatching, it involves the rapid maturation of HR regulation in birds (Tazawa et al., 2002; Tamura et al., 2003; Khandoker et al., 2004). The HR response to altered $T_a$ in newly hatched chicks depends on the state of development of thermoregulation. The maturation of the thermoregulatory HR response to altered $T_a$ has been investigated on the broiler hatchlings in Muroran, Japan (Khandoker et al., 2004). Figure 3 shows representative responses of instantaneous heart rate (IHR) and skin temperature ($T_s$) to altered $T_a$ in newly hatched chicks. The hatchlings were kept in a brooding cage placed under an electric lamp maintaining an environmental temperature at about 24–27°C until the experiment. After setting non-invasively three flexible ECG electrodes and thermistor probe on the skin of the chest wall and abdomen, the chicks were placed in a programmable incubator maintained initially at 25°C. The chicks were kept at the temperature of 24–27°C, so the measurement of IHR and $T_s$ was began soon after placing them in the incubator. After 1-hour measurement at 25°C, $T_a$ was changed to 35°C for the next hour and then returned to 25°C for an hour. On day 0, i.e. the period of 24 hours after hatching, measurements were made during the first 8-hours after hatching (group 0–7 hours), the second 8-hours (group 8–15 hours), and the following two 4-hour periods (group 16–19 hours and group 20–23 hours).

A chick in group 0–7 hours (Figure 3, panel A) shows a flat and narrow HR baseline with spontaneous HR decelerations during the first 1-hour exposure to 25°C. Upon exposure to increased $T_a$ (35°C), HR baseline increases in an exponential fashion in parallel with increase in $T_s$, and it decreases as in $T_a$ decreases during the final cooling period. Accordingly, this chick responds to the warming and cooling bout with a thermoconformity pattern of HR baseline. As the age of hatchlings advances, $T_a$ at $T_s$ of 25°C increases and the elevation in $T_s$ during warming at 35°C becomes small, indicating development of thermoregulatory competence. A chick in group 16–19 hours (Figure 3, panel B), of which thermoregulatory competence is more developed, responds to warming with wide range HR baseline that remains at a similar level to the
preceeding 1-hour period. In addition, HR baseline increases transiently against cooling during the last 1-hour period. The HR baseline of a 1-day-old chick (Figure 3, panel C) decreases in response to warming and increases against cooling. The thermoregulatory patterns of HR response during warming and cooling bout are more evident in a 7-day-old chick of which \( T_s \) changes minutely (Figure 3, panel D). These are the representative patterns of the responses of HR and \( T_s \) to warming and cooling bouts according to chick age, which suggests that the developmental stage (age) has a significant effect on the HR response to altered \( T_a \) and thermoregulatory competence.

The results of the responses of \( T_s \) and HR to warming and cooling bouts measured for 114 hatchlings ranging in age from day 0 to day 7 are summarised in Figure 4 (Khandoker et al., 2004). For \( T_s \) and HR responses, the mean value of the last 10-minute period of individual 1-hour exposure periods was obtained and all the mean values for a given developmental stage were averaged. As the chicks age and develop, the \( T_s \) increases when exposed to the warm \( T_a \) (35°C) and the magnitude in the change of \( T_s \) between warming and cooling exposures decreases, indicating that thermoregulatory competence is progressing with age. Meanwhile, HR increases on warming and decreases on cooling in the two youngest groups (0–7 hours and 8–15 hours on day 0), showing the thermoconformity pattern in HR response. During the last 8-hour period of day 0, the thermoconformity response of HR to warming turns to be insignificant change and the insignificant increase of \( T_s \) and HR during cooling turns to be significant. Therefore, HR increases significantly against the decrease in \( T_s \), showing a thermoregulatory response.

The development of thermoregulatory competence differs among the birds. The difference is evident between the altricial and precocial birds (cf. Whittow and Tazawa, 1991). Among the precocial species, it is shown that early thermoregulatory competence progresses in aquatic domestic ducks (\textit{Anas platyrhynchos} var. \textit{domestica}) more than in terrestrial chickens (Kuroda et al., 1990). The size of body also influences the development of thermoregulatory competence. In general, the larger the hatchling is, the smaller the increase in mass-specific heat production required to maintain body temperature during cooling exposure. The development of thermoregulatory competence involves the maturation of HR regulation, and so HR responses to altered \( T_s \) should be different between precocial species. Measurements of HR responses to cooling in large ratite emu hatchlings show the thermoregulatory response on the day of hatching and suggest that ability of thermo-
regulation progresses considerably before hatching compared with chickens (Tamura et al., 2003).

Accordingly, we provisionally examined ducks and emus for HR responses to altered $T_a$ (unpublished data). In the duck, IHR baseline tends to decrease with cooling when the plumage is still wet soon after hatching, but a few hours later when the plumage dries HR baseline increases in response to mild cooling. During the external pipping period, some duck embryos respond to mild cooling with an increase in HR baseline, while other embryos decrease HR baseline with or without initial increase. The early thermoregulatory response of HR seems to be more advanced in ducks than in chickens. In the emu a marked increase in HR baseline in response to mild cooling is observed in externally pipped embryos. Even internally pipped (IP) embryos tend to increase HR baseline during an early period of mild cooling. This thermoregulatory HR response in ratite IP embryos corresponds to that in chick hatchlings on the day of hatching (day 0) and that in duck embryos during the external pipping period.

4. POSSIBLE INFLUENCE OF BROODING TEMPERATURE TO THERMOREGULATORY RESPONSES OF HEART RATE

When the changes in $T_a$ induce no response of carbonic acid discharged by respiratory exchange in animals, this condition is termed the “neutral condition” (Pembrey, 1895; Freeman, 1964) and the zone of thermal neutrality for newly hatched chicks is narrow around 35°C (Freeman, 1963). The thermoneutral temperature is now defined as the temperature that produces the lowest heat production of the animals and a preferred ambient temperature for hatchlings is situated near the thermoneutral temperature (Nichelmann and Tzschentke, 2002). Meanwhile, for studies on ontogeny of thermoregulation, hatchlings are kept at $T_a$ of 25°C with the possibility of moving to a warmer place (Nickelmann and Tzschentke, 2002). In our previous experiment investigating into the maturation of thermoregulatory HR response to altered $T_a$, the hatchlings were kept at $T_a$ of 24–27°C. The preferred brooding $T_a$ is much higher (circa 35°C), so a low brooding $T_a$ may induce a thermal stress to the hatchlings. Accordingly, it is feasible that the low brooding $T_a$ prior to experiments influences the development of thermoregulatory HR responses to altered $T_a$.

In a preliminary experiment, influence of brooding temperature on HR responses to altered $T_a$ was briefly investigated on broiler chicks in Muroran. Chick hatchlings were divided into two groups soon after hatching. Hatchlings in one group were kept in the incubator maintained at 38°C (group A) and those in group B were moved into a cage placed in a laboratory room and warmed by an electric lamp to 24–27°C. Individual chicks were tagged to identify the time of hatching. ECG electrodes and a thermistor probe were attached on a hatchling in the same manner as in the previous experiment (Khandoker et al., 2004). The hatching was put in the programmable incubator of which temperature was set at 35°C. After an hour, the measurements of IHR and $T_a$ were made for the first hour at 35°C, then a 2-hour period at 25°C, a third hour at 35°C and finally an hour at 25°C. Two hatchlings from groups A and B were selected so that ages were similar and eight hatchlings were examined for the HR responses to the series of cooling, warming and cooling bouts.

Figures 5 and 6 present the responses of IHR and $T_a$ in four hatchlings kept at 38°C and 24–27°C environments prior to the experiment, respectively. The ages of hatchlings were 22-hour (panel A in both Figures 5 and 6), 30 and 32-hour (panel B), 38 and 39-hour (panel C) and 47 and 40-hour (panel D). The solid lines indicate changes in $T_a$ in response to altered $T_a$. The mean of $T_a$ at 35°C during the first 1-hour exposure was 38.7°C (SD = 0.7) and 38.6°C (SD = 0.3) for chicks of groups A and B, respectively. In both groups, $T_a$ during the 2-hour cooling period decreased in an exponential fashion to the values of final 10-minute period that tended to increase with ages. The mean of $T_a$ during the final 10-minute period at 25°C was –7.5°C (SD = 1.0) and –5.3°C (SD = 2.8) lower than $T_a$ at 35°C for chicks of groups A and B, respectively. Although statistical analysis was not carried out due to the small number of experimental animals, these values, as well as patterns of $T_a$ changes during the first 2-hour cooling, indicate that thermoregulatory competence improved in advanced hatchlings of both groups, but the largest improvement occurred in group B. Meanwhile, HR baseline tended to increase upon cooling that began at the first hour of recording, but decreased with the lapse of time towards the end of the 2-hour cooling period, particularly in group A (Figure 5). The subsequent
warming at $35^\circ C$ was accompanied by increases in HR baseline even in advanced chicks in group A (e.g. 38-hour and 47-hour chicks), showing a thermoconformity response of HR to warming. After 1-hour of warming, HR baseline transiently increased upon cooling with a subsequent gradual decrease as during the first cooling. As a result, although the chicks maintained at $38^\circ C$ responded to cooling with transient thermoregulatory increases in HR baseline at the beginning of cooling, their HR changes during warming were a thermoconformity response (Figure 5).

On the other hand, advanced chicks maintained at 24–27°C changed HR baseline with a thermoregulatory pattern in response to altered $T_a$ (Figure 6). A 22-hour chick (panel A) showed the thermoconformity pattern for the HR baseline. A 32-hour chick (Figure 6, panel B) increased HR baseline transiently in response to cooling which began at 1-hour and 4-hour of the recording and decreased HR baseline transiently during warming with subsequent transient increases and again decreased towards the end of warming. While a 39-hour old chick (Figure 6, panel C) maintained almost constant HR baseline in response to warming, a 40-hour old chick (Figure 6, panel D) down-regulated HR baseline during warming, showing a thermoregulatory response. Although the experimental data were obtained from only four hatchlings in both groups and lacked statistical analysis, Figures 5 and 6 indicate possible influence of brooding temperature on the development of thermoregulatory competence and the HR regulation in relation to the development of thermoregulatory competence in chick hatchlings.

5. METABOLIC RESPONSES TO ALTERED $T_a$ IN CHICK HATCHLINGS

5.1. Hypothesis

Based on the previous study on the homeothermic response of HR to altered $T_a$ (Khandoker et al., 2004) and the preliminary experiment on the possible influence of brooding temperature on the development of thermoregulatory competence in relation to HR responses, we hypothesised that homeothermic-metabolic response to altered $T_a$ develops in parallel with the homeothermic response of HR in chick hatchlings. In addition, the brooding temperature will influence the maturation of homeothermic-metabolic response so that hatchlings maintained at low $T_a$ will develop the homeothermic-metabolic response earlier than those at the preferred brooding $T_a$ ($35^\circ C$).

5.2. Materials and methods

White Leghorn chicken eggs were brought from Texas A & M University to the University of North Texas and were incubated for 20 days in a Lyon forced-draught incubator maintained at a temperature of $37.5^\circ C$ and a relative humidity of about 60%. Eggs were turned automatically every 4h. On day 20, all eggs were

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**Fig. 5** Responses of skin temperature ($T_s$) and instantaneous heart rate (IHR) to changes in ambient temperature ($T_a$) during 5-hour period in four newly hatched chicks that were kept in $38^\circ C$ environment after hatching. $T_s$ and $T_a$ are shown by solid and dotted lines, respectively. The mean values of $T_s$ and IHR during the last 10-minute period in the first $35^\circ C$ environment, the next $25^\circ C$ environment and the second $35^\circ C$ environment are $38.2^\circ C$, $29.2^\circ C$ and $37.2^\circ C$ and $319$ bpm, $200$ bpm and $311$ bpm for the 22-hour chick, $39.5^\circ C$, $32.5^\circ C$ and $38.7^\circ C$ and $313$ bpm, $271$ bpm and $312$ bpm for the 30-hour chick, and $39.2^\circ C$, $33.1^\circ C$ and $39.0^\circ C$ and $362$ bpm, $303$ bpm and $355$ bpm for the 47-hour chick, respectively.
transferred alternately to an incubator maintained at 35°C (group A) or a brooding cage placed under an electric lamp maintaining temperature at 24–27°C (group B). Water and food were given in the incubator and the brooding cage. The day of hatching was designated as day 0.

Metabolic rate was determined as oxygen uptake \( (\dot{M}_{\text{O}_2}) \) by a hatchling using flow through respirometry previously described for chicken eggs (Dzialowski et al., 2002). Hatchlings were placed in individual PVC respirometers (with an approximate volume of one litre) and submerged into a water bath. A gas mixture (20.94% O2/N2) was pumped from a cylinder through the individual respirometers. The flow rate of the gas mixture ventilating the respirometers was regulated with fine needle valves and was measured at the inflow side of the respirometers for the individual chicks. The gas mixture passed through copper coils submerged in the water bath prior to entering the respirometers to allow it to equilibrate the temperature of the water bath. The outflow gas from the respirometers passed through drierite and soda lime to remove water vapour and CO2 respectively before the percentage of O2 was measured using a Sable Systems O2 analyser and recorded with a data acquisition system (Sable Systems). \( \dot{M}_{\text{O}_2} \) was calculated using the equation of Hill (1972) and expressed as mlO2 h\(^{-1}\) g\(^{-1}\) at standard temperature, pressure and dry (STPD).

The body temperature \( (T_b) \) of individual chicks and \( T_a \) inside the respirometers were measured to 0.1°C by fine gauge thermocouples. For cloacal temperature, the thermocouple wire was wound through three small holes in the centre of a small plastic disk about 1 cm in diameter so that 1–1.5 cm length of wire came out of the plastic disk. The end of wire was inserted into the cloaca and the plastic disk was glued to the feathers with superglue. After the experiment, the plastic disk was removed from the feathers by melting superglue with acetone.

The responses of \( \dot{M}_{\text{O}_2} \) and \( T_b \) to a cooling and warming bout were measured on 0, 1, 2 and 3 day-old hatchlings in both groups. The single measurement for a hatchling was made over a 4-hour period. The hatchling of known age had the thermocouple placed in the cloaca and was placed in the respirometer. A lid of the respirometer had a small hole through which two thermocouple wires were passed in advance. One thermocouple measured \( T_a \) in the respirometer and another had a female connector to connect a male connector from the thermocouple in the cloaca. After

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**Fig. 6** Responses of skin temperature \( (T_s) \) and instantaneous heart rate (IHR) to changes in ambient temperature \( (T_a) \) during 5-hour period in four newly hatched chicks that were kept in 24–27°C environment after hatching. \( T_s \) and \( T_a \) are shown by solid and dotted lines, respectively. The mean values of \( T_s \) and IHR during the last 10-minute period in the first 35°C environment, the next 25°C environment and the second 35°C environment are 38.1°C, 29.0°C and 36.8°C and 313 bpm, 206 bpm and 315 bpm for the 22-hour chick, 38.9°C, 32.1°C and 38.8°C and 287 bpm, 279 bpm and 254 bpm for the 32-hour chick, 38.4°C, 36.3°C and 38.6°C and 365 bpm, 415 bpm and 413 bpm for the 39-hour chick, and 38.8°C, 35.5°C and 38.2°C and 361 bpm, 364 bpm and 305 bpm for the 40-hour chick, respectively.
the lid was closed hermetically, the hole was also hermetically sealed with clay. Initially, the respirometer with the hatchling was submerged in a water bath maintained at about 35–36°C for 2-hour period. Then, the water in the bath was replaced at once with cool water (circa 24–25°C) that was maintained in it for the next hour. Lastly, the water was warmed back to 35–36°C for the final hour by replacing the bath at once with warm water. During these cooling and warming bouts, the averaged values of $M_{o2}$, $T_b$ and $T_a$ during the last 10-minute period prior to water change were determined and used as the value of individual parameters at the altered $T_a$. After the experiment, the hatchling was taken out of the respirometer and the thermocouple was removed from the cloaca. The hatchling was weighed to 0.1 g and returned to the original incubator or cage. The data are shown as means and standard deviations.

The effects of the thermal environment and age on $M_{o2}$ and $T_b$ were tested with multivariate repeated-measures ANOVA. To examine the significance of differences between group means after an ANOVA was significant, Tukey’s post hoc test was made.

### Table 1 Mean masses with SD of chicks in groups A and B

<table>
<thead>
<tr>
<th>Chick age</th>
<th>Group A</th>
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<th>Group B</th>
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<tr>
<td></td>
<td>chick mass (g)</td>
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<td>chick mass (g)</td>
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<tr>
<td>0–11 hours</td>
<td>39.6 ± 3.7</td>
<td>41.4 ± 4.7</td>
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<tr>
<td>12–23 hours</td>
<td>36.4 ± 4.2</td>
<td>37.2 ± 4.2</td>
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<tr>
<td>Day 1</td>
<td>35.7 ± 3.6</td>
<td>34.6 ± 2.8</td>
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<tr>
<td>Day 2</td>
<td>40.4 ± 3.6</td>
<td>38.9 ± 5.0</td>
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<tr>
<td>Day 3</td>
<td>41.5 ± 7.7</td>
<td>35.7 ± 7.6</td>
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Patterns of $T_a$ during cooling were not significantly different between the two brooding temperature groups. Meanwhile, developmental changes in $T_b$ at both $T_a$’s of 35°C and 24–27°C occurred on day 2, i.e. $T_{8s}$ on days 2 and 3 were significantly higher than those on days 0 and 1. The results of statistical analysis on $M_{o2}$ were the same as those for $T_b$. The developmental patterns of $M_{o2}$ at the preferred $T_a$ and low $T_a$ were not significantly different between groups A and B with significant increases in $M_{o2}$ on day 2.

### 5.4. Discussion

#### 5.4.1. Lack of transitional responses of $M_{o2}$ to altered $T_a$ during the day of hatching

In contrast to the hypothesis based on the previous study on HR responses to altered $T_a$ in chick hatchlings, responses of $M_{o2}$ to altered $T_a$ did not show various patterns on day 0 in either group A or B. $M_{o2}$ was up-regulated during cooling and down-regulated during warming, showing the thermoregulatory response even in the newly hatched (0–11 hour) chicks from both groups.

$M_{o2}$ is a function of HR and O$_2$ pulse which is determined by a product of the stroke volume and arterio-venous O$_2$ content difference and so we hypothesised that $M_{o2}$ would respond to altered $T_a$ as did HR on the assumption that the O$_2$ pulse during warming and cooling bouts would not change significantly so as not to influence $M_{o2}$. However, we failed to obtain the metabolic responses to altered $T_a$ that would change in parallel with HR responses on the day of hatching. HR responses to altered $T_a$ on day 0 changed from the thermoconformity pattern to the thermoregulatory pattern (Khandoker et al., 2004), while metabolic responses to altered $T_a$ are thermoregulatory pattern throughout all the experimental days. If both are the case, the increase in $M_{o2}$ in response to cooling during the first half of day 0 is accompanied with the decrease in HR and instead a
large increase in O₂ pulse. During the last half of day 1 and afterwards, the increase in $M_{O_2}$ in response to cooling is accompanied by an increase in HR and accordingly a small increase or decrease in O₂ pulse. Thus, the O₂ pulse is predicted to have large changes in response to warming and cooling bouts during the first half of day 0 with subsequent small change at advanced ages. However, it is questionable that the stroke volume and/or the arterio-venous O₂ content difference of just hatched chicks can change largely in response to altered $T_a$.

Another possibility to explain the inconsistency between the metabolic responses and HR responses is due to the different breeds used in the two experiments, i.e. white leghorn eggs in Texas and broiler eggs in Muroran. Investigations into the development of tonic vagal control of HR in chick embryos have produced controversial results. While cholinergic chronotropic control began to occur around the end of the second week of incubation in broiler chickens (Höchel et al., 1998; Chiba et al., 2003), white leghorns lacked this control during the entire period of embryonic development (Crossley and Altirias, 2000). Bantam chick embryos also showed obvious cholinergic tone and it was suggested that different breeds might develop different levels of cholinergic chronotropic control at different times (Crossley et al., 2003). The early development of thermoregulatory competence may also differ between different breeds of chickens.

5.4.2. No significant effects of brooding $T_a$ on development of homeothermic metabolic response

Metabolic responses to altered $T_a$ were not significantly different between both groups of hatchlings maintained at a high temperature (35°C) and at a low temperature (24–27°C). The thermoneutral temperature (35°C) may be a preferred $T_a$ for the newly hatched chicks in terms of the changes in HR fluctuation. In the low temperature environment (25°C), HR baseline of newly hatched chicks is narrow, indicating lack of HR fluctuation. The HR baseline becomes wide when hatchlings are exposed to warm temperature of 35°C and also in advanced hatchlings which are provided with more developed competence of thermoregulation even in the low temperature environment. Thus, it is assumed that HR fluctuations occur in chick hatchlings that live in thermal environment favourable to their thermoregulatory competence. A low $T_a$ in contrast to the preferred $T_a$ results in thermal stress depressing HR fluctuations. It seems that the thermal stress, i.e. exposure to low $T_a$ or cold conditioning, does not necessarily induce adverse effects on physiological functions of hatchlings. In
broiler chickens, short repetitive cold exposure at an early age (cold conditioning) improves their thermotolerance in later life (Shinder et al., 2002). We hypothesised that a mild thermal stress would improve development of thermoregulatory competence in newly hatched chicks. The present results fail to prove the hypothesis, but at least indicates that the thermal stress does not induce adversely development of homeothermic metabolic response in newly hatched chicks. Another mild thermal stress, e.g. exposure to $T_a$ less than 10°C below the preferred $T_a$, may prove the hypothesis. The influence of brooding temperature on HR responses to altered $T_a$ remains to be investigated again in broiler chickens and because of the possibility of different results due to different breeds, it should also be investigated in white leghorn chickens.

REFERENCES


