Do small precocial birds enter torpor to conserve energy during development?

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Keywords: Precocial, metabolic rate, King quail *Cortunix chinensis*, heterothermy, thermal energetics

Summary statement: Torpor as a strategy to conserve energy have not been investigated in precocial developing chicks. We show that such strategy may be more common than previously thought.

Abstract

Precocial birds hatch feathered and mobile, but when they become fully endothermic soon after hatching, their heat loss is high and they may become energy-depleted. These chicks could benefit from using energy-conserving torpor, which is characterised by controlled reductions of metabolism and body temperature (T_b). We investigated at what age the precocial king quail *Cortunix chinensis* can defend a high T_b under a mild thermal challenge and whether they can express torpor soon after achieving endothermy to overcome energetic and thermal challenges. Measurements of surface temperature (T_s) using an infrared thermometer showed that king quail chicks are partially endothermic at 2-10 days, but can defend high T_b at a body mass of ~13 g. Two chicks expressed shallow nocturnal torpor at 14 and 17 days for 4 to 5 hours with a reduction of metabolism by > 40% and one approached torpor threshold. Although chicks were able to rewarm endogenously from the first torpor bout, metabolism and T_s decreased again by the end of the night, but they rewarmed passively when removed from the chamber. The total metabolic rate increased with body mass. All chicks measured showed a greater reduction of nocturnal metabolism than previously reported in quails. Our data show that shallow torpor can be expressed during the early postnatal phase of quails, when thermoregulatory efficiency is still developing, but heat loss is high. We suggest that torpor may be a common strategy for overcoming challenging conditions during the development in small precocial and not only altricial birds.

Introduction

The majority of mammals and birds are homeothermic endotherms as adults, and rely on endogenous heat production to keep a constant, high body temperature (T_b; Yahav, 2015). However, at birth or hatching, most endotherms are only partially endothermic (Dawson and Evans, 1960) and unable to produce sufficient heat to maintain a high and constant T_b when exposed to temperatures below the thermal neutral zone (TNZ). Nevertheless, within thermoneutrality the heat produced by resting metabolic rate (RMR) is enough to maintain a normothermic T_b often even in the early stages of development (Price and Dzialowski, 2018). During exposure to cold, developing endotherms depend on parental heat production or heat conservation from insulated nest for maintenance of a high T_b (Ricklefs, 1984).

Endothermy is energetically demanding (Visser and Ricklefs, 1993) and in birds, it requires the maturation of the skeletal muscles for heat production (Sirsat et al., 2016; Hohtola and Visser, 1998). The age at which endothermy is established differs substantially within, and among species, often reflecting the duration of the nestling period (Dunn, 1975; Ricklefs, 1974) and brood size (Andreasson et al., 2016). Most birds are altricial, featherless and immobile at hatching, and are unable to physiologically thermoregulate via internal heat production; their T_b and metabolic rate (MR) are a direct function of ambient temperature (T_a; Price and Dzialowski, 2018; Dawson and Evans, 1960). The age at which altricial species develop a strong endothermic metabolic response during cold exposure varies from several days in sparrows (*Spizella passerine*, *Spizella pusilla* and *Pooecetes gramineus*; Dawson and Evans, 1960, 1957) and storm petrel *Oceanodroma furcata* (Boersma, 1986), up to three weeks in large species, such as American white Pelicans *Pelecanus erythrorhynchos* (Abraham and Evans, 1999) and Double-crested Cormorants *Phalacrocorax auritus* (Dunn, 1976).

At the opposite end of the spectrum, few species are precocial, fully feathered and mobile, and develop thermoregulation during the early postnatal phase (Nichelmann and Tzschentke, 2002). Many precocial species are able to maintain, rather than increase, metabolism under cold exposure at the time of hatching (Sirsat et al., 2016; Dzialowski et al., 2007), but can form an endothermic metabolic response soon after hatching (Brown and Prior, 1999; Tamura et al., 2003). Most small precocial birds are completely endothermic only from about ten days of age (Nichelmann and Tzschentke, 2002), whereas large species thermoregulate efficiently already one day after hatching (Brown and Prior, 1999; Tamura et al., 2003).

Once the young endotherms become thermally independent and can increase MR to defend their T_b at moderate T_a, they still may face excessive heat loss in the absence of parental heat transfer during brooding. This heat loss, mainly an issue for small species, must be compensated for via endogenous heat production, which can result in substantial loss of energy reserves. To overcome these energetic and thermal challenges, some developing altricial birds and mammals use torpor, which is characterised by controlled reductions of MR and T_b (Boersma, 1986; Renninger et al., 2020; Eichhorn et al., 2011). While there is more than one definition of torpor (Schleucher, 2004), a common definition is a reduction by >25% of resting metabolic rate (RMR) at the same T_a (Hudson and Scott, 1979), and/or a reduction by >5°C below the normothermic T_b at rest (Schleucher, 2004; Ruf and Geiser, 2015). To be able to use and benefit from torpor during development, young endotherms must not only establish the capability of active thermoregulation for maintenance of a high and stable T_b in the cold, but also be able to actively rewarm and increase MR from torpor at low T_b at the end of the torpor bout (Geiser et al., 2014; Wacker et al., 2017).

While torpor can be an effective survival strategy, the reduced T_b and depressed physiological functions can have negative implications for young animals. These include increased predation risk for the inexperienced chicks (Eichhorn et al., 2011; Wheelwright and Boersma, 1979; Andreasson et al., 2019), a possible metabolic imbalance (Jensen and Bech 1992), and delayed prenatal and juvenile development (Boersma and Wheelwright, 1979; McAllan and Geiser, 2014). However, in many cases, the slow rate of development does not affect the chances of survival in offspring (McAllan and Geiser, 2014; Prinzinger and Siedle, 1988; Racey and Swift, 1981), or the mass gain of juveniles (Giroud et al., 2014), and therefore the ability to enter torpor during development is likely to increase fitness. Moreover, torpor has many other selective advantages beyond efficient energy conservation to survive the energetically challenging period of development (Boersma, 1986; Bae et al., 2003; Geiser et al., 2006; Giroud et al., 2014; Wacker et al., 2017; Geiser et al., 2019). For example, torpor has been shown to aid survival of bad weather and natural disasters (Nowack et al., 2017), delayed hatching until conditions are favourable for parents and offspring (Geiser and Brigham, 2012) and enhanced fat accumulation when food is scarce (Giroud et al., 2014).

Although a potentially crucial survival strategy for many endotherms, data on torpor during early stages of development are scarce and limited to only few altricial mammalian and avian species (e.g. Nagel, 1977; Eichhorn et al., 2011; Giroud et al., 2014). Currently data on torpor

in precocial species are lacking entirely although the energetic and thermal demands during development are similarly excessive especially in small species. One of these, the precocial Japanese quail (*Coturnix japonica*) has been investigated as an adult with regard to thermal energetics. These adult birds (body mass ~ 150 g) entered shallow nocturnal torpor after food deprivation and reduced their T_b by 5 °C (Hohtola et al., 1991). Although at hatching these quails weigh as little as 3.5 g, it has not been investigated whether torpor is used as a strategy to enhance survival during early stages of development, around the time they become endothermic.

In this study, we first aimed to determine at what age the king quail *Cortunix chinensis* (adult body mass ~50 g), a close relative to the Japanese quail, develops competent endothermic thermoregulation, and is able to defend a high T_b when thermally challenged. Secondly, we aimed to test the hypothesis that precocial king quail can use torpor soon after achieving endothermy. To quantify this, we measured MR as the rate of oxygen consumption. Metabolic reduction is regarded as a more reliable indicator of torpor than reduction in T_b (Hiebert, 1993; McKechnie and Lovegrove, 2002; Willis, 2007) and relevant when determining torpor, because the purpose of torpor is energy conservation and not T_b reduction. Moreover, entry into torpor usually requires calm and undisturbed animal. Due to their small size, implanting temperature sensitive devices in the king quail chicks was not possible, and the use of external devices to measure T_b or T_s would disturb the birds, and may interrupt torpor, while MR measurements can be conducted non-invasively while the bird is resting. Measurements were conducted overnight when torpor is more likely to be used by diurnal birds.

Methods

Experimental animals

King quails occur widely from India to Southeast Asia, Indonesia, New Guinea, the northern and eastern coast of Australia and are also a popular aviary bird. For our study, fertile quail eggs were obtained from commercial breeders in the Northern Tablelands in northern New South Wales. Eggs were incubated (LUMIA 8 incubator in heat- resistant ABS, Borroto, Buttapietra (Verona, Italy)) at 37.7°C, and humidity was 50%. The photoperiod was LD 12:12 with lights on from 06:00 to 18:00. Nine chicks hatched after 18 days of incubation and were kept in a temperature-controlled room at T_a 22.0 ± 0.1 °C. Chicks were randomly

assigned in two brooders made of plastic cages (43.3cm (H) x 80cm (W) x 51cm (D)), bedded with pine shaving and heated with a ceramic lamp suspended above the middle of the cage to create a thermal gradient. Chicks were identified by a marker on the back of their head. Four chicks were housed in one cage, and five in the other. The T_a in the cage below the heating lamp was 35°C, and 25 ± 1°C at the edges of the cage, furthest from the lamp. Chicks could therefore move around in the thermal gradient to behaviourally regulate their T_b. Commercial game birds starter (28% protein, 3% fat) and fresh water were provided *ad libitum*, except during cooling and respirometry measurements.

Cooling measurements

From the time when the chicks were 2 days old, 2-4 individuals were removed from the brooder between 9:30-11:00 am and placed individually into a paper cup at T_a 22.0 ± 0.1°C. Immediately upon removal from the brooder, skin temperature (T_s) was measured under the wing to the nearest 0.1 °C (T_{start}) using an infrared thermometer (Digitech, QM-7218), and then at 10 minute intervals until the last measurement after 40 minutes (T_{end}). Previous studies found the difference between T_s and cloacal T_b to be ~0.4°C in an 8-g bat (Bondarenco et al., 2014), less than 2°C in the 50-g common poorwill (Brigham, 1992) and less than 4°C in 80-g owl (Smit & McKechnie, 2010) and nightjar (McKechnie et al., 2007). It is therefore likely that in the small king quail chicks, the difference between T_b and T_s will not exceed 1-2°C. Animals were weighed with an electronic balance at the end of the cooling experiment to the nearest 0.1g. These measurements were conducted until 12 days of age, when the T_s change over 40 minutes was no longer significant. Each chick was measured between 3 and 4 times during the cooling experiment, and always had at least 1 day for recovery between measurements.

Resting metabolic rate (RMR)

Oxygen consumption measurements using open-flow respirometry of chicks in the TNZ were conducted from 3 to 12 days of age. Two animals were measured concurrently and were placed individually into 500 ml metabolic chambers in a temperature-controlled cabinet at T_a 30 ± 1.1°C, thermo-neutral conditions for adult king quail (28-35°C; Roberts and Baudinette, 1986), for at least 60 minutes to determine RMR. The T_a was measured to the nearest 0.1 °C in the respirometry chambers using calibrated thermocouples. Dried outside air was pumped through these chambers at a rate of approximately 300 ml min^{-1}. By employing two-way

solenoid valves, reference outside air and the air from the metabolic chambers was measured sequentially every 9 minutes, 3 min for each channel. Air exiting the chambers was again dried and flow rate was measured with a mass flow meter (Omega FMA-5606); the oxygen content in a 100 ml min^{-1} subsample was then determined with an O_2 analyser (FOX Field oxygen analysis system Version 1.01, Sable System, FXO301-01R). Outputs from the flow meter and the thermocouples from each respirometry chamber were digitized via a 14 bit A/D converter (Data Taker DT100), whereas the O_2 analyser was interfaced with the PC directly via a serial port. Temperature control within the climatic chamber, channel switching, calculations and data storage were performed with a custom program written by G. Körtner in Visual Basic 6 (Microsoft Inc.). Oxygen consumption was calculated based on flow rate and the O_2 differential between reference and chamber air using Eqn. 3a from Withers (1977) assuming a RQ of 0.85. This RQ would result in a maximum error of 3% if the RQ was actually 0.7 or 1 (Withers, 1977). Prior to measurements, the span of the O_2 analyser was set against outside air and the mass flow meter were calibrated with a custom-made bubble meter (Levy 1964).

Determination of torpor expression

Once the chicks were 12 days of age, and able to maintain a constant T_s over 40 minutes at T_a 22.0 ± 0.1°C, we tested whether they could use torpor. Chicks were removed from the brooder between 4:00 and 4:30pm and placed individually into the respirometry chamber overnight for approximately 15 hours at LD 12:12, without food and water. We then measured MR as the rate of oxygen consumption using the respirometry equipment described above. In the first two nights of metabolic measurements the chicks were measured at T_a 22.0 ± 0.3°C and from the third night onwards, we decreased the T_a to 18.0 ± 0.3°C, both well below the TNZ (Roberts and Baudinette, 1986).

The minimum and maximum metabolic rate (MR_{min} and MR_{max}) were calculated as the lowest and the highest mean rate of VO_2, measured consecutively over a 36- minute period (i.e. 4 consecutive values), respectively. Chicks were weighed to the nearest 0.1 g before and after each respirometry measurement. We assumed a linear mass loss to calculate the mass-specific metabolic rate.

To determine torpor occurrence in king quail, we calculated the individual expected RMR as:

$BMR+C\,(Tl_c-T_a)$,

where *C* is the wet thermal conductance, calculated using the equation for the active phase $C=0.994\ M^{-0.509}$ (Schleucher and Withers, 2001) and Tl_c is the lower critical temperature: $Tl_c = T_b-4.24\ M^{0.317}$ (Swanson and Weinacht, 1997). Individual basal metabolic rate ("BMR", because the chicks were still growing) was calculated using the equation for non-passerines birds: logBMR (ml g^{-1} h^{-1}) = 0.699 x logBM(g)-1.371 (McKechnie et al., 2006). A chick was deemed to be torpid if MR during the rest phase fell >25% below the expected RMR (Hudson and Scott, 1979).

We measured surface temperature (T_s) under the wing using an IR thermometer in four individuals at 10 pm during the metabolic measurements when the chicks were 17, 18, and 19 days old. These T_s measurements were completed within no more than 30 seconds of removing the chick from the respirometry chamber and returning it back. We defined torpor as a reduction of T_s by >5°C below the resting T_s (Schleucher, 2004; Ruf and Geiser, 2015).

All experiments were approved by the University of New England Animal Ethics Committee (Authority No. AEC19-079), and were conducted in conformity with the NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Statistical analysis

Growth rate

A Gompertz growth curve (Tsoularis and Wallace, 2002) was fitted through the individual body masses with the non-linear model, according to the formula:

$$M(t)=A\ \exp[-\exp\{-k(t-t_i)\}s]$$

Where M(t) is body mass (g) at age t (days), A is asymptotic body mass (g), k is Gompertz growth constant (days-1), and t_i is the age at the inflection point (days). We used the function *grow_gompertz* from the R package "growth rates".

Changes in T_s

We applied a general additive model (GAM) to describe temporal changes in T_s because the increase in T_s with age is not linear. The GAM approach is an extension of GLM and is well suited for non-linear trends. The model included log of body mass and age as the response variables, with T_s as the dependent variable.

Cooling experiment

We fitted a general linear mixed effect model with individual as a random effect to analyse the change in T_s over time with age and body mass**.** We set the difference between T_s at the start of the experiment (T_{start}, at time "zero") and T_s at the end of the experiment (T_{end}, after 40 minutes) as the dependent variable, in relation to age and body mass. In addition, we divided the dataset into four different body mass groups (<5g, 5-8g, 8-13g, >13g) and fitted a general linear mixed effect model with individual as a random effect in each body mass group to determine the body mass at which chicks develop endothermy. We set T_s before and after cooling experiment (at time "zero" and after 40 minutes) as the dependant variable in relation to time (start and end of experiment). Finally, we calculated an index of homeothermy for nestlings following Ricklefs (1987) and Visser & Ricklefs (1993). This index measures the gradient between equilibrium body temperature and the environmental temperature, thus indicates the degree of homeothermy. We calculated the index (*H*) by dividing the final temperature difference between T_s and T_a by the initial difference:

$$H = (T_{end}-T_a) / (T_{start}-T_a)$$

where T_{start} and T_{end} are T_s at time of extraction from brooder and after 40 minutes of cooling at T_a 22°C, respectively. When H=1, a chick defends its initial T_s and when H=0, T_s drops to T_a within 40 minutes.

Resting metabolic rate in the TNZ

We used general linear modelling with individual as random effect to examine the effect of body mass on RMR at TNZ (T_a 30°C) in the 3-12 days old chicks. RMR was calculated as the lowest mean rate of VO2, measured consecutively over a 27- minute period (i.e. 3 consecutive values). We analysed both total and mass-specific RMR.

Overnight metabolic rate below the TNZ

We analysed the effect of age and body mass on the difference between MR during the active and rest phases (MR_{max}-MR_{min}) under mild cold-exposure (T_a 18 or 22°C). For this purpose, we fitted a general linear mixed effect model with individual as random effect.

Variables were excluded from models using ANOVA Type III, based on a threshold significance level set to p=0.05. We confirmed the use of random effect in the model by comparing the AIC of the best model with and without random effect using the REML

method (Zuur et al., 2009). R-function *lme* in R- package "nlme" was used to perform mixed effect models with the function *visreg* in the "visreg" package for visualization. All statistical analyses were conducted using R version 3.3.0 (R Development Core Team, 2019).

Numeric values are presented as mean ± s.d for the number of individuals (N) measured.

Results

Body mass and growth rates

Mean chick body mass was 3.9 ± 0.5 g 24-hours after hatching (n=5, N=5), 8.7 ± 1.5 g at 7 d (n=4, N=4), and 19 ± 2.6 g at 15 d (n=8, N=8). By the end of the experiment, at 22 d, the mean body mass was 27.8 ± 1.7 g (n=7, N=7; Fig. 1). Adult feathers were visible at 5 d.

Surface temperature

The mean T_s of chicks measured immediately after removal from the brooder at thermo-neutrality increased with age from 34.6± 2.2 °C at 2-5 d, 36.9 ± 0.7 °C at 3-8 d, 38.6 ± 0.7 °C at 6-12 d, and 40.1 ± 0.4 °C at 10-15 d (Table 1). The increase was significantly and non-linearly correlated with age ($F = 30.12$, $p<0.001$); body mass was not significant, probably because the importance of age was greater, and was removed from the model.

Changes in T_s during cooling in young chicks

The change in T_s over the 40 minutes of the cooling experiment at T_a 22°C was correlated with body mass ($t = -9.28$, $p < 0.001$). In chicks with a body mass < 5 g, mean T_s decreased from T_{start} (34.6 ± 2.2°C) to 24.8 ± 1.3°C after 40 minutes (T_{end}), and these means differed significantly ($t = 9.26$, $p<0.001$). The chicks were already able to visibly shiver when they were 2 d. Chicks with a body mass between 5 and 13 g had slower rates of cooling than the previous group (<5 g). Consequently, T_{end} was 28.9 ± 2.4 °C at 5-8 g, whereas at 8-13 g T_{end} was rather high at 35.2 ± 1.8 °C, but still significantly lower than T_{start}. Chicks weighing > 13 g, at 10-15 d were able to defend their T_b, and the mean T_{start} was indistinguishable from T_{end} (39.8 ± 1.5°C, *p = 0.74*, Table 1 and Fig. 2).

Metabolic trials

Metabolic rate in the TNZ

At 3-12 d, all chicks showed a substantial drop of MR at night with a minimum soon after midnight. Mass-specific mean RMR of all chicks was 4.2 ± 0.99 ml O_2 g^{-1} h^{-1} at T_a 30°C,

ranging between 3.14-6.04 ml O_2 g^{-1} h^{-1} (and one value of 1.97 ml O_2 g^{-1} h^{-1} in one individual at 5.3 g). The mean total MR was 38.43 ±15.64 ml O_2 h^{-1} (Table 2). Mass-specific RMR was independent of body mass. Total RMR (ml O_2 h^{-1}) significantly increased with body mass (Total MR (ml O_2 h^{-1})= 1.43 + 4 (body mass); t=8.94, $p < 0.001$).

Determination of torpor expression

At 12-22 d, the mean decrease of rest phase MR (RMR_{min}) for all chicks was 39.2% of the active phase MR (MR_{max}) at T_a 18-22°C. The average nightly reduction in mass-specific MR was 2.1± 0.62 ml O_2 g^{-1} h^{-1} or 43.81 ± 9.55 ml O_2 h^{-1} in total MR (Table 3 and A1). Generally, MR started to decrease soon after lights off (at 6pm), and increased again at approximately 2 am; four hours before lights on (Fig. 3). Mass loss during the night ranged between 2.96-4.6 g, with an average mass loss of 3.84± 0.48 g (Table A1), which to some extent due to loss of faeces. The differential between both total and mass-specific MR_{min} and MR_{max} were significantly and negatively related to body mass (Fig. 4, Table 4), with this differential being smaller in heavier birds.

Two of the chicks expressed shallow nocturnal torpor and one additional chick approached the RMR torpor threshold. Chick #1 exposed to T_a 22°C at 14 d, and a body mass of 14.2 g, steadily reduced MR from 6.31 ml O_2 g^{-1} h^{-1} after being placed into the respirometer in the afternoon to a minimum of 2.39 ml O_2 g^{-1} h^{-1} (60% of predicted RMR and slightly below the predicted "BMR" of 2.6 ml O_2 g^{-1} h^{-1}) at around 01:00 hours, after which MR increased again to 4.46 ml O_2 g^{-1} h^{-1} at around 04:00 hours. After this increase, MR plummeted to 1.15 ml O_2 g^{-1} h^{-1} and when the animal was removed at 07:30h, its T_s was 26.2 °C and the chick was returned to the brooder under the heat lamp. The MR of chick #1 remained below the torpor threshold for 5 hours (from 22:00h until 03:00h; Fig. 3A, Table 3). Similarly, chick #2 (body mass 16.1 g) exposed to T_a 18°C at 17 d steadily reduced MR from ~5.5 ml O_2 g^{-1} h^{-1} after lights off. The T_s measured at 22:00h was 33.8 °C, 6.2 °C below the starting T_s and MR at 3.39 ml O_2 g^{-1} h^{-1}. After a brief increase to 4.09 ml O_2 g^{-1} h^{-1}, MR continued to decline until it reached 2.72 ml O_2 g^{-1} h^{-1} at approximately 01:30h (59% of the predicted RMR, but 35% above the "BMR"). MR then increased briefly to 3.93 ml O_2 g^{-1} h^{-1} at around 04:00h, but then again decreased until MR reached 1.60 ml O_2 g^{-1} h^{-1} at 07:30 when the animal was removed from the chamber, and its T_s was 25.6 °C. The MR of chick #2 remained below the torpor threshold for 4 hours (from 00:00h until 04:00h; Fig. 3B, Table 3). Both chicks did not enter torpor on the second trial at T_a 18°C at 21 days (Fig. 3C and 3D). Chick #5 at T_a 22°C at 14

days reduced MR to values very close to the torpor threshold (2.77 ml O_2 g^{-1} h^{-1}, 77% of the predicted RMR), but not during the second trial at T_a 18°C (figure not shown).

We measured T_s in four chicks at 22:00 while chicks were in the respirometry chamber on days 17, 18 and 19. The T_s of all chicks at the age of 12-22d before the respirometry measurements was 40.2 ± 0.6 °C. The T_s of chicks # 2, 4, 5 and 7 was 33.8°C, 35.4 °C, 35.6 °C and 37.8 °C, respectively. The T_s values were 6.2 °C, 5.6 °C, 5.4 °C and 2.8 °C below their initial T_s respectively. Except chick #2 (details above), all three rewarmed to 38.8 °C, 36.6 °C and 37.8 °C when they were removed from the chambers at around 7:30am.

Discussion

We provide the first evidence of shallow nocturnal torpor expression in a developing precocial endotherm. King quail chicks develop endothermy and are able to defend their T_b at between 12 and 17 d, and at 30% of adult body mass. The chicks reduced MR by up to 41% below the predicted RMR, well below the torpor threshold of <75% of RMR. Two chicks remained in shallow torpor for nearly half the night, and all chicks displayed a day-night oscillation in MR, with an average reduction of metabolism and T_s greater than previously reported for the heavier adult Japanese quail (Hohtola et al., 1991).

Surface temperature and the development of endothermy

The surface temperature (T_s) of king quail chicks in our study increased from 32.6 °C at hatching to a maximum of ~41°C at approximately 15 d. The latter is in line with the value of T_b 41.7°C reported in TNZ by Roberts and Baudinette (1986) for the same species. This increase in T_b with development is well documented, and initially when chicks are small, it allows them to reduce energy expended for thermoregulation, by lowering T_b and reducing the differential between T_a and T_b (Hissa et al., 1983; Tzschentke and Nichelmann, 1999; Pis, 2003; Freeman, 1964). While most precocial birds are completely endothermic at 10 days of age (Nichelmann and Tzschentke, 2002), the age of onset of endothermic positively correlated to growth rate (Dunn, 1975). Therefore, it is difficult to determine a specific age at which a species becomes fully endothermic due to individual variation in growth rates and probably also environmental variables (Beintema & Visser, 1989). However, the results of our study indicate that the crucial period for the onset of endothermy in king quail chicks is between 11 and 15 d, or when body mass reaches ~13 g. This age range for this species is in agreement with Pearson (1994), however Pis and Luśnia (2005) reported the onset of endothermy between 16 and 19 d in the king quail. Spiers et al. (1974) report 13-15 d for the

Japanese quail *Corturnix corturnix japonica*, and 14 d for the Bobwhite quail *Colinus virginianus* (Spiers et al., 1985). Although quails are rather similar with regard to development of thermoregulatory efficiency, this varies between precocial avian species, especially affected by size. In large ostrich and emu chicks, for example, homeothermy is well-developed, and they are able to maintain a constant T_b soon after hatching (Tamura et al., 2003; Brown and Prior, 1999).

Metabolic rate

The RMR of king quail chicks under thermoneutral conditions at a body mass of 3.6-14.8 g in our study (Table 2) is similar to reports on the same species at 6-10 d, ranging between 4-7 ml O_2 g^{-1} h^{-1} (Pearson, 1994). Bernstein (1973) reported lower values of RMR in king quail chicks at body masses of 3-9 g, but RMR of 6.3 ml O_2 g^{-1} h^{-1} in a 6.8 g chick at T_a 25°C. The latter studies reported an increase in RMR from the minimum value at hatching to a maximum shortly after hatching, indicating an increase in the capacity for heat production with growth, followed by a decrease, reflecting a decrease in the metabolic requirements for heat production (Bernstein, 1973; Pearson, 1994). The same polynomial relationship between body mass and oxygen consumption was also reported in other bird species (Domestic pigeon, Riddle et al. (1932); Red-necked pheasants, Domestic fowl and California quail; Koskimies (1962)). The changes in RMR in the chicks aged 3-12 d in our study was highly variable and independent of body mass. Bernstein (1973) reported lowest values of MR in the first days after hatching, at body mass < 4 g, maximum RMR at about 4-12 g and a decrease starting at about 15 g (Fig. 3 in Bernstein (1973)). Our MR trial period was shorter than in the other king quail studies, and started only on day 3, at body mass of almost 4 g, so likely the lower values are missing from our data. Our data of RMR on 3-12 d, therefore, probably depicted the peak values of RMR, between the phases of low RMR just after hatching and later at the decreased of metabolic requirement, and therefore was not explained by body mass.

Torpor

The fast development of thermoregulation in the precocial king quail is followed by the ability to express shallow torpor at a young age under mild thermal conditions. The king quail chicks in our study expressed torpor between the ages of 14 and 17 d for a brief window during their development, when their ability to defend high T_b was rather limited. Torpor was characterised by a substantial, but reversible reduction in MR around and soon after

midnight. Although they were able to rewarm from the first torpor bout via an increase in MR independently of T_a (a controlled response, hence defined as torpor), their MR and T_s decreased again by the end of the night, but they rewarmed passively only after extraction from the chamber. They were therefore deemed to become hypothermic (Geiser et al., 2014). Similar behaviour was observed in young Crimson chat *Ephthianura tricolor* and captive young Banded whiteface *Aphelocephala nigricincta* by Ives (1973), that spent the night appearing to be in a state of torpor. When they were handled in the morning, they were still inert, and passively rewarmed gradually with increasing morning warmth (Ives, 1973). More anecdotal evidence on passive arousal from torpor in birds have been reported on White-backed swallow *Cheramoeca leucosternum* (Serventy, 1970) and Welcome swallow *Hirundo neoxena* (Dove, 1923). Marsupial dunnarts *Sminthopsis crassicaudata* also had an intermittent phase between a poikilotermic phase and a completely endothermic phase, in which the young enter to what appears to be torpor, but could only rewarm passively when basking under a heat lamp (Wacker et al., 2017).

Our data suggest that torpor can be expressed when quail chicks are at their early postnatal phase, where thermoregulatory efficiency is still developing, but heat loss is high (Nichelmann and Tzschentke, 2002). Indeed, the chicks expressing torpor also had the slowest growth rate (Fig. 1), supporting the occurrence of torpor during this time only in those chicks that developed competent endothermy later than the others. The flexible thermo-energetics obtained at this point was apparently used as a survival strategy in response to the mild thermal challenge. However, the rapid fall of MR after the increase during rewarming from torpor before the end of the dark phase implies an exhaustion of their energy reserves as they appeared unable to maintain a high T_b at T_a lower than thermoneutrality. When the chicks were more mature (on the second night of measurements on day 21), they no longer expressed torpor, but maintained a constant, high MR throughout the night. This sequence of expressing torpor immediately when the animal is able to control T_b, varies among species. Some mammalian and avian species may express torpor at early stages of development and decrease torpor frequency with age (Renninger et al., 2020; Geiser et al., 2019; Geiser, 1988; Prinzinger and Siedle, 1988), whereas some placental mammals appears to express torpor only after transient homeothermic phase, where T_b is high and constant following the poikilothermic phase (Bae et al., 2003; Geiser and Kenagy, 1990).

Those chicks that did not express torpor, according to our definition, still reduced metabolism and T_s during the rest phase, but not to the extent that we can define it as torpor. We did not measure T_s during the night in all birds to avoid interference with the MR measurements and risk that this interference will prevent torpor, but also because we were more interested in the energy currency, rather than the reduction of their T_b. However, we did measure T_s in four birds and can also calculate the expected T_s given the MR measurements. The amplitude in T_s fluctuation for the chicks that were measured at 22:00 (and did not express torpor according to our MR definition), was 5.6 °C, 5.4 °C and 2.8 °C. This fluctuation is much larger than the 1 °C difference in T_b between the active and rest phases that has been reported in adult Japanese quails under normothermic conditions (Hohtola et al., 1991). Prinzinger et al. (1991) suggested a normal range of T_b fluctuation (day/night) of 2.48-1.25 °C for birds weighing between 10-100,000 g, decreasing with increasing body mass. While a reduction of T_b may provide a potential definition of torpor accompanying the reduction in metabolic rate, it is first necessary to determine the rest phase values as a standard reference to be able to define it (Schleucher, 2004). Considering a rest phase T_b of 38.9 °C in birds from the order Galliformes (Prinzinger et al., 1991), the T_b reductions in the three chicks that did not express torpor would only be 3.5 °C, 3.3 °C and 1.1 °C, all smaller than the 5 °C T_b reduction torpor threshold (Ruf and Geiser, 2015) and 5.1°C in the chick that did express torpor, before MR reached the lowest value. Moreover, if we calculate the T_s for the minimum MR for the chicks that expressed torpor using thermal conductance (Schleucher and Withers, 2001), the T_s calculated are 29.2 °C and 31.8 °C in chicks #2 and #1, respectively. These calculated values are 4.7 °C and 2.1° C below the torpor threshold of 33.9°C, and 7-10°C below the resting phase T_b.

Torpor may be used at times of an energetic emergency, such as starvation, and not as a developmental strategy to save energy while encountering a minor thermal challenge. Fork-tailed storm petrel, Wilson' storm petrels *Oceanites oceanicus* and house martin *Delichon u. urbica* chicks, expressed torpor only after a period of food shortage or starvation (Boersma, 1986; Prinzinger and Siedle, 1988; Kuepper et al., 2018), while Antarctic petrel chicks *Thalassoica antarctica* maintained high T_b (>36 °C) throughout the day even if T_a decreased below -15° C (Bech et al., 1991). Juvenile garden dormice *Eliomys quercinus,* exposed to an intermittent starvation trial, expressed torpor at about six weeks of age, gained mass at the same rate as juveniles fed ad libitum, and reached similar pre-hibernation fattening (Giroud et al., 2014). The juveniles fed ad libitum at T_a 6° C, entered torpor only after gaining maximum

body mass at the age of about 12 weeks (Giroud et al., 2012; Giroud et al., 2014). It is therefore likely that due to the risk of slower growth rate as a result of reduced T_b (McAllan and Geiser, 2014; Racey and Swift, 1981), chicks do not use torpor as a regular developmental strategy to save energy, but rather as a strategy to overcome extreme challenges such as starvation. A greater thermal challenge, or prolonged starvation, as in Alpine swift *Apus melba* chicks (Bize et al., 2007), may possibly trigger chicks in older age groups to use torpor.

Our study shows that king quail chicks may express torpor soon after developing endothermy. Although they were able to increase metabolism and rewarm from the torpor bout at night, they were unable to maintain a high MR until the following morning, suggesting their energy reserves were depleted when exposed to a minor thermal challenge. Our data therefore suggest that this heterothermic step requires that king quail chicks have access to parental or other external heat source to rewarm and benefit from torpor to overcome energetically challenging periods. Our study is the first to show torpor in early stages of development in a precocial bird, and suggests that torpor may be a more common strategy during challenging conditions for developing birds, which deserves to be investigated further.

Competing interests

No competing interests declared

References

Abraham C.L, Evans RM .(1999). The development of endothermy in American white pelicans. *The Condor*. **101,** 832-841

Andreasson F, Nord A, Nilsson J-Å .(2016). Brood size constrains the development of endothermy in blue tits. *Journal of Experimental Biology*. **219,** 2212-2219

Andreasson F, Nord A, Nilsson J-Å .(2019). Age-dependent effects of predation risk on night-time hypothermia in two wintering passerine species. *Oecologia* **189,** 329-337

Bae H.H, Larkin J.E, Zucker I .(2003). Juvenile Siberian hamsters display torpor and modified locomotor activity and body temperature rhythms in response to reduced food availability. *Physiological and Biochemical Zoology*. **76**, 858-867

Bech C, Mehlum F, Haftorn S .(1991). Thermoregulatory abilities in chicks of the Antarctic Petrel *Thalassoica antarctica*. *Polar Biology*. **11**, 233-238

Beintema, A. and Visser, G. (1989). The effect of weather on time budgets and development of chicks of meadow birds. *Ardea.* **77,** 181-192.

Bernstein M.H (1973) Development of thermoregulation in painted quail, *Excalfactoria chinensis*. *Comparative Biochemistry and Physiology Part A: Physiology*. **44,** 355-366

Bize P, Klopfenstein A, Jeanneret C, Roulin A. (2007). Intra-individual variation in body temperature and pectoral muscle size in nestling Alpine swifts Apus melba in response to an episode of inclement weather. *Journal of Ornithology*. **148,** 387-393

Boersma P.D. (1986). Body temperature, torpor, and growth in chicks of fork-tailed storm-petrels *(Oceanodroma furcata)*. *Physiological Zoology*. **59**, 10-19

Boersma P.D, Wheelwright N.T. (1979). Egg neglect in the Procellariiformes: reproductive adaptations in the Fork-tailed Storm-Petrel. *The Condor*. **81**, 157-165

Bondarenco, A., Körtner, G. and Geiser, F. (2014). Hot bats: extreme thermal tolerance in a desert heat wave. *Naturwissenschaften*, **101,** 679-685.

Brigham, R. M. (1992). Daily torpor in a free-ranging goatsucker, the common poorwill (*Phalaenoptilus nuttallii*). *Physiological Zoology*. **65,** 457-472.

Brown C, Prior S. (1999). Development of body temperature regulation in ostrich chicks. *British Poultry Science.* **40,** 529-535

Dawson W.R, Evans F.C. (1957). Relation of growth and development to temperature regulation in nestling field and chipping sparrows. *Physiological Zoology*. **30**, 315-327

Dawson W.R, Evans F.C. (1960). Relation of growth and development to temperature regulation in nestling Vesper Sparrows. *The Condor*. **62**, 329-340

Dove S .(1923). Semi-Hibernation of Swallows. *Emu-Austral Ornithology*. **23**, 149

Dunn E.H. (1975). The timing of endothermy in the development of altrical birds. *The Condor*. **77**, 288-293

Dunn E.H. (1976). Development of endothermy and existence energy expenditure of nestling Double-crested Cormorants. *The Condor*. **78**, 350-356

Dzialowski E, Burggren W, Komoro T, Tazawa H. (2007). Development of endothermic metabolic response in embryos and hatchlings of the emu *(Dromaius novaehollandiae)*. *Respiratory physiology & neurobiology*. **155**, 286-292

Eichhorn G, Groscolas R, Le Glaunec G, Parisel C, Arnold L, Medina P, Handrich Y. (2011). Heterothermy in growing king penguins. *Nature communications*. **2**, 1-7

Freeman B. (1964). The emergence of the homeothermic-metabolic response in the fowl *(Gallus domesticus)*. *Comparative Biochemistry and Physiology*. **13**, 413-422

Geiser F. (1988). Daily torpor and thermoregulation in *Antechinus* (Marsupialia): influence of body mass, season, development, reproduction, and sex. *Oecologia* **77**, 395-399

Geiser, F., and R. M. Brigham. (2012). The other functions of torpor. In *Living in a seasonal world* (ed. T. Ruf, C. Bieber, W. Arnold, and E. Millesi), pp 109-121, Berlin: Springer.

Geiser F, Chi Q , Wang D, Wen J, Sukhchuluun G. (2019). Precocious torpor in an altricial mammal: the functional implications of heterothermy during development. *Frontiers in Physiology*. **10,** 469

Geiser F, Currie SE, O'Shea K.A, Hiebert S.M. (2014). Torpor and hypothermia: reversed hysteresis of metabolic rate and body temperature. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. **307**, R1324-R1329

Geiser F, Kenagy G. (1990). Development of thermoregulation and torpor in the golden-mantled ground squirrel *Spermophilus saturatus*. *Journal of Mammalogy*. **71,** 286-290

Geiser F, Westman W, McAllan B.M, Brigham R.M .(2006). Development of thermoregulation and torpor in a marsupial: energetic and evolutionary implications. *Journal of Comparative Physiology B*. **176**, 107-116

Giroud S, Turbill C, Ruf T. (2012). Torpor use and body mass gain during pre-hibernation in late-born juvenile garden dormice exposed to food shortage. In *Living in a Seasonal World* (ed. T. Ruf, C. Bieber, W. Arnold and E. Millesi), pp 481-491. Berlin: Springer

Giroud S, Zahn S, Criscuolo F, Chery I, Blanc S, Turbill C, Ruf T. (2014). Late-born intermittently fasted juvenile garden dormice use torpor to grow and fatten prior to hibernation: consequences for ageing processes. *Proceedings of the Royal Society B: Biological Sciences*. **281**, 20141131

Hiebert, S. (1993). Seasonal changes in body mass and use of torpor in a migratory hummingbird. *The Auk*. **110**, 787-797.

Hissa R, Saarela S, Rintamäki H, Linden H, Hohtola E. (1983). Energetics and development of temperature regulation in capercaillie *Tetrao urogallus*. *Physiological Zoology*. **56**, 142-151

Hohtola E, Hissa R, Pyörnilä A, Rintamäki H, Saarela S. (1991). Nocturnal hypothermia in fasting Japanese quail: the effect of ambient temperature. *Physiology & Behavior*. **49**, 563-567

Hohtola E, Visser H. (1998). Development of locomotion and endothermy in altricial and precocial birds. *Oxford Ornithology Series*. **8**, 57-173

Hudson J.W, Scott I.M. (1979). Daily torpor in the laboratory mouse, *Mus musculus* var. albino. *Physiological Zoology*. **52**, 205-218

Ives N. (1973). Overnight torpidity in Australian arid-country birds. *Emu-Austral Ornithology*. **73**

Jensen C, Bech C. (1992). Oxygen consumption and acid-base balance during shallow hypothermia in the pigeon. *Respiration Physiology*. **88**, 193-204

Koskimies J. (1962). Ontogeny of thermoregulation and energy metabolism in some gallinaceous birds. *Ricerche Zool Appl Caccia*. **4**, 149-160

Kuepper N.D, Marek C, Coria N, Libertelli M.M, Quillfeldt P. (2018). Facultative hypothermia as a survival strategy during snowstorm induced food shortages in Antarctic storm-petrel chicks. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **224**, 76-83

Levy A. (1964). The accuracy of the bubble meter method for gas flow measurements. *Journal of Scientific Instruments*. **41**, 449

McAllan B, Geiser F. (2014). Torpor during reproduction in mammals and birds: dealing with an energetic conundrum. *Integrative and Comparative Biology*. **54**, 516-532

McKechnie A.E, Ashdown R.A, Christian M.B, Brigham R.M. (2007) Torpor in an African caprimulgid, the freckled nightjar *Caprimulgus tristigma*. *Journal of Avian Biology*. **38**, 261-266

McKechnie A.E, Freckleton R.P, Jetz W. (2006). Phenotypic plasticity in the scaling of avian basal metabolic rate. *Proceedings of the Royal Society B: Biological Sciences* **273**, 931-93

McKechnie A.E, Lovegrove B.G. (2002). Avian facultative hypothermic responses: a review. *The Condor.* **104**, 705-724

Nagel A. (1977). Torpor in the European white-toothed shrews. *Experientia*. **33**, 1455-1456

Nichelmann M, Tzschentke B. (2002). Ontogeny of thermoregulation in precocial birds. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **131**, 751-763

Nowack J, Stawski C, Geiser F. (2017). More functions of torpor and their roles in a changing world. *Journal of Comparative Physiology B.* **187,** 889-897

Pearson J. (1994) Oxygen consumption rates of adults and chicks during brooding in king quail *(Coturnix chinensis)*. *Journal of Comparative Physiology B*. **164**, 415-424

Pis T. (2003). Energy metabolism and thermoregulation in hand-reared chukars *(Alectoris chukar)*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **136**, 757-770

Pis T, Luśnia D. (2005). Growth rate and thermoregulation in reared king quails *(Coturnix chinensis)*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **140**, 101-109

Price E.R, Dzialowski E.M. (2018). Development of endothermy in birds: patterns and mechanisms. *Journal of Comparative Physiology B.* **188**, 373-391

Prinzinger R, Preßmar A, Schleucher E. (1991). Body temperature in birds. *Comparative Biochemistry and Physiology Part A: Physiology*. **99**, 499-506

Prinzinger R, Siedle K. (1988). Ontogeny of metabolism, thermoregulation and torpor in the house martin *Delichon u. urbica* (L.) and its ecological significance. *Oecologia* **76**, 307-312

R: A language and environment for statistical computing (2019). R Foundation for Statistical Computing. Vienna, Austria. URL https://www.R-project.org/,

Racey P, Swift S.M. (1981). Variations in gestation length in a colony of pipistrelle bats (*Pipistrellus pipistrellus*) from year to year. *Reproduction*. **61**, 123-129

Renninger M, Sprau L, Geiser F. (2020). White mouse pups can use torpor for energy conservation. *Journal of Comparative Physiology B.* **190**, 253-259

Ricklefs R.E. (1974). The energetics of reproduction in birds. *Avian energetics.* **15**, 152-297

Ricklefs R.E. (1987). Characterizing the development of homeothermy by rate of body cooling. *Functional Ecology*. **1**, 151-157

Ricklefs R.E (1984) The optimization of growth rate in altricial birds. *Ecology*. **65**, 1602-1616

Riddle O, Nussmann T.C, Benedict F.G (1932) Metabolism during growth in a common pigeon. *American Journal of Physiology-Legacy Content.* **101**, 251-259

Roberts J, Baudinette R. (1986). Thermoregulation, oxygen-consumption and water turnover in stubble quail, *Coturnix pectoralis*, and king quail, *Coturnix chinensis*. *Australian Journal of Zoology*. **34**, 25-33

Ruf T, Geiser F. (2015). Daily torpor and hibernation in birds and mammals. *Biological Reviews*. **90**, 891-926

Schleucher E. (2004). Torpor in birds: taxonomy, energetics, and ecology. *Physiological and Biochemical Zoology*. **77**, 942-949

Schleucher E, Withers PC. (2001). Re-evaluation of the allometry of wet thermal conductance for birds. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **129**, 821-827

Serventy D.L. (1970). Torpidity in the white-backed swallow. *Emu-Austral Ornithology*. **70**, 27-28

Sirsat SK, Sirsat TS, Faber A, Duquaine A, Winnick S, Sotherland PR, Dzialowski EM. (2016). Development of endothermy and concomitant increases in cardiac and skeletal muscle mitochondrial respiration in the precocial Pekin duck (*Anas platyrhynchos domestica*). *Journal of Experimental Biology*. **219**, 1214-1223

Smit B, McKechnie A.E. (2010). Do owls use torpor? Winter thermoregulation in free-ranging pearl-spotted owlets and African scops-owls. *Physiological and Biochemical Zoology*. **83**, 149-156

Spiers D.E, Adams T, Ringer R.K. (1985). Homeothermic development in the bobwhite (*Colinus virginianus*). *Comparative Biochemistry and Physiology Part A: Physiology*. **81,** 921-927

Spiers D.E, McNabb R.A, McNabb F.A .(1974). The development of thermoregulatory ability, heat seeking activities, and thyroid function in hatchling Japanese quail (*Coturnix coturnix japonica*). *Journal of Comparative Physiology* **89**, 159-174

Swanson D.L, Weinacht D.P. (1997). Seasonal effects on metabolism and thermoregulation in northern bobwhite. *The Condor*. **99**, 478-489

Tamura A, Akiyama R, Chiba Y, Moriya K, Dzialowski E, Burggren W, Tazawa H. (2003). Heart rate responses to cooling in emu hatchlings. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. **134**, 829-838

Tsoularis A, Wallace J. (2002). Analysis of logistic growth models. *Mathematical Biosciences*. **179**, 21-55

Tzschentke B, Nichelmann M. (1999). Development of avian thermoregulatory system during the early postnatal period: development of the thermoregulatory set-point. *Ornis Fennica*. **76**, 189-198

Visser G.H, Ricklefs R.E. (1993). Development of temperature regulation in shorebirds. *Physiological Zoology*. **66**, 771-792

Wacker C.B, McAllan B.M, Körtner G, Geiser F. (2017). The role of basking in the development of endothermy and torpor in a marsupial. *Journal of Comparative Physiology B*. **187**, 1029-1038

Wheelwright N.T, Boersma P.D. (1979). Egg chilling and the thermal environment of the Fork-tailed Storm Petrel (*Oceanodroma furcata*) nest. *Physiological Zoology*. **52**, 231-239

Willis C.K. (2007). An energy-based body temperature threshold between torpor and normothermia for small mammals. *Physiological and Biochemical Zoology*. **80**, 643-651

Withers P.C. (1977). Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. *Journal of Applied Physiology*. **42**, 120-123

Yahav S. (2015). Regulation of body temperature: strategies and mechanisms. In *Sturkie's Avian Physiology*. Elsevier, pp 869-905

Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. (2009). *Mixed effects models and extensions in ecology with R*. Springer Science & Business Media

Tables

Table 1: Summary table of age, surface temperature measured with infrared thermometer under the wings before (T_{start}) and after (T_{end}) cooling experiment (40 minutes), and t-value for the models testing the difference between T_{start} and T_{end} in the four body mass (BM) groups.

BM group[1]	**Age range** (days)	**T_{start}** (°C)	**T_{end}** (°C)	**t-value (p-value)**
<5g (9, 7)	2-5	34.6±2.19	24.84±1.25	**9.26 (<0.001)**
5<g<8 (7, 7)	3-8	36.88±0.69	28.94±2.39	**9.39 (<0.001)**
8<g<13 (9, 8)	6-12	38.64±0.65	35.2±1.77	**5.98 (<0.005)**
>13g (6, 5)	10-15	40.08±0.36	39.84±1.49	0.35 (0.74)

[1]Number of measurements and individuals (n, N) are in brackets for each group.

Table 2: Summary table of oxygen consumption as a measure of metabolic rate (Total MR; ml O_2 h^{-1} and mass specific RMR; ml O_2 g^{-1} h^{-1}), calculated as the lowest consecutive values over 27 minutes during a 60 minutes trial at T_a 30°C for each of the chicks in our study at 3-12 days (d).

Chick	Age (d)	Body mass (g)	Total RMR	Mass specific RMR
7	3	3.59	11.66	3.25
6	3	4.7	20.63	4.39
4	4	6.01	31.98	5.32
5	4	5.31	10.27	1.97
8	5	8.13	40.03	4.92
9	5	6.14	37.09	6.04
3	6	8.83	30.19	3.42
2	6	5.9	26.22	4.44
7	7	7.27	27.22	3.74
6	7	9.1	40.29	4.43
1	8	9.7	40.2	4.14
4	8	11.4	47.64	4.18
8	9	13.76	53.03	3.85
9	9	10.66	59.2	5.55
6	11	13.91	58.41	4.20
7	11	12.42	64.46	5.19
5	12	14.78	46.44	3.14
1	12	13.6	46.72	3.44

Table 3: Summary table of the overnight metabolic measurements in each chick in the study. Chicks marked in grey expressed torpor.

Chick	Age (d)	Body mass (g)	MR_{min}[1]	MR_{max}[1]	Predicted RMR[2]	MR reduction[3] (%)
1	14	14.2	2.51	6.44	4.22	40.52
1	21	24.6	3.04	4.81	3.04	0.00
2	17	16.13	2.69	5.61	4.59	41.39
2	21	20.63	3.13	5.36	3.42	8.48
3	12	17.8	3.64	5.09	3.13	-16.29
3	17	23.07	2.78	4.41	3.46	19.65
4	12	16.3	4.11	6.53	3.58	-14.80
4	18	23.6	2.99	4.55	3.47	13.83
5	14	16.9	2.76	4.47	3.63	23.97
5	18	23.7	3.24	4.79	3.46	6.36
6	16	21.9	3.3	5.49	3.44	4.07
6	22	27.33	2.83	4.53	2.82	-0.35
7	15	18.84	3.69	6.33	4.15	11.08
7	19	22.6	3.05	5.33	3.52	13.35
8	15	23.25	3.19	4.91	3.43	7.00
8	19	26.8	3.72	5.42	3.09	-20.39
9	16	20.19	3.1	5.48	3.70	16.22
9	22	26.12	3.76	5.73	3.12	-20.51

[1]Minimum and maximum metabolic rate (MR_{min} and MR_{max}; ml O_2 g^{-1} h^{-1}) were calculated as the lowest, and highest mean rate of oxygen consumption, respectively, measured consecutively over a 36 minutes period

[2]predicted RMR was calculated as BMR+C (Tl_c-T_a), where C is the wet thermal conductance, calculated using the equation for the active phase C=0.994 $M^{-0.509}$ (Schleucher and Withers 2001) and Tl_c is the lower critical temperature: T_b-4.24 $M^{0.317}$ (Swanson and Weinacht 1997). BMR was calculated using the equation for non-passerines birds: logBMR=0.699 x logBM-1.371 (McKechnie et al. 2006).

[3]MR reduction is calculated as the percentage of reduction in MR between MR_{min} and the predicted RMR.

Table 4: Model estimation for general linear model with individual as random effect describing the effect of body mass (BM; in g) and age (d) on the difference between maximum metabolic rate (MR_{max}) and minimum metabolic rate (MR_{min}) measured during a 15 hours trial after chicks were able to defend their body temperature from day 12. Results are shown for both total (ml O_2 h^{-1}) and mass-specific (ml O_2 g^{-1} h^{-1}) MR.

	Variable	Estimate	t-value	df[1]	p-value
Total	BM	-0.57	-3.74	8*	*0.005*
	age	0.006	0.17	7	*0.36*
Mass -specific	BM	-1.56	-4.10	7	*0.004*
	age	0.04	1.96	7	*0.09*

[1]df= degrees of freedom.

*Model df is higher because we report the values of the final model, excluding age.

Figures

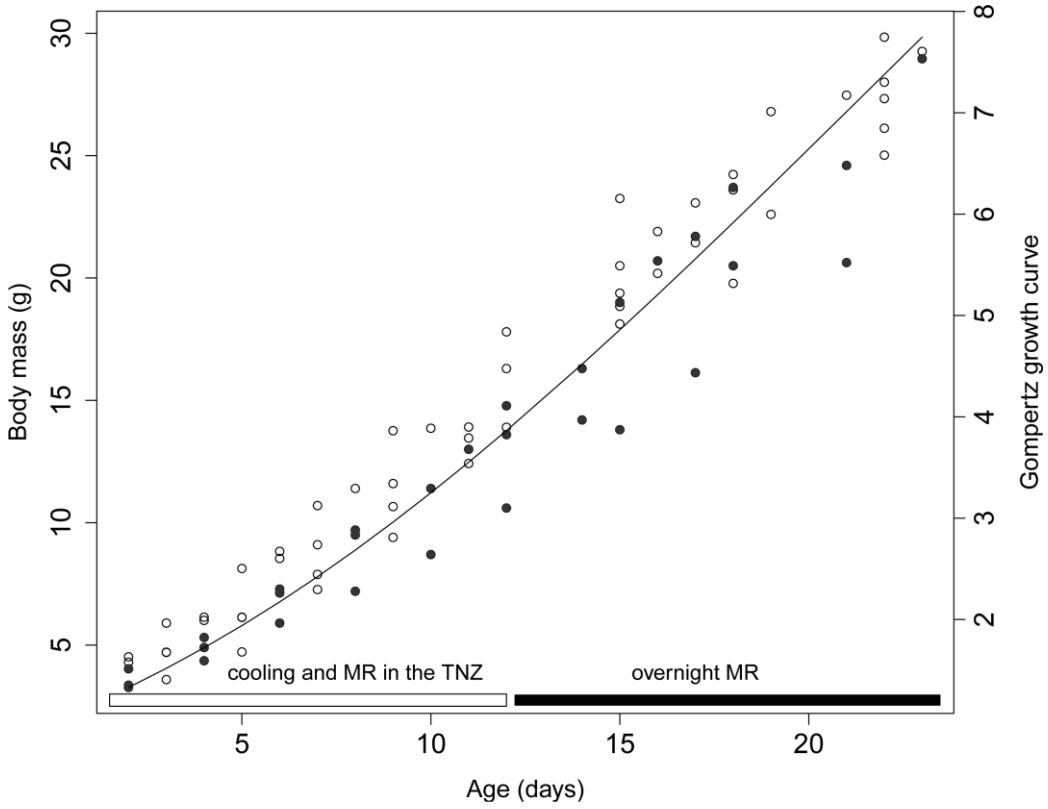

Fig. 1 Body mass in developing king quail and experiment time course (n=83, N=9). Solid line is the Gompertz growth curve (right x-axis). MR= metabolic rates measurements. Filled circles are individuals that expressed torpor and had the slowest growth rate (Fig. 3 and table 3).

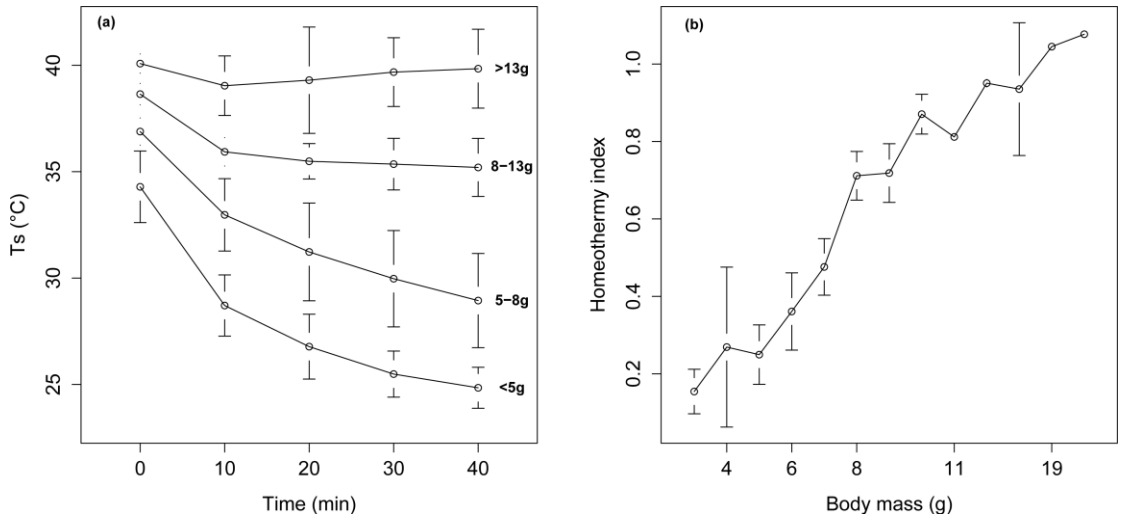

Fig. 2 (a) Changes in surface temperature (T_s) of king quails at different body mass groups at T_a 22° in a 40 minutes cooling experiment. Each line represent the mean T_s (°C) with confidence intervals, measured with infrared thermometer under the wing, of the body-mass group mentioned in the plot (see Table 1 for number of measurements and individuals for each group). (b) Homeothermy index (H) following Ricklefs (1987) and Visser & Ricklefs (1993), depicting stages of homeothermy for each body mass. *H*=1 in chicks that defend their initial T_s before cooling and *H*=0 when T_s drops to T_a within 40 minutes. Chicks body mass was round to the nearest 1 g.

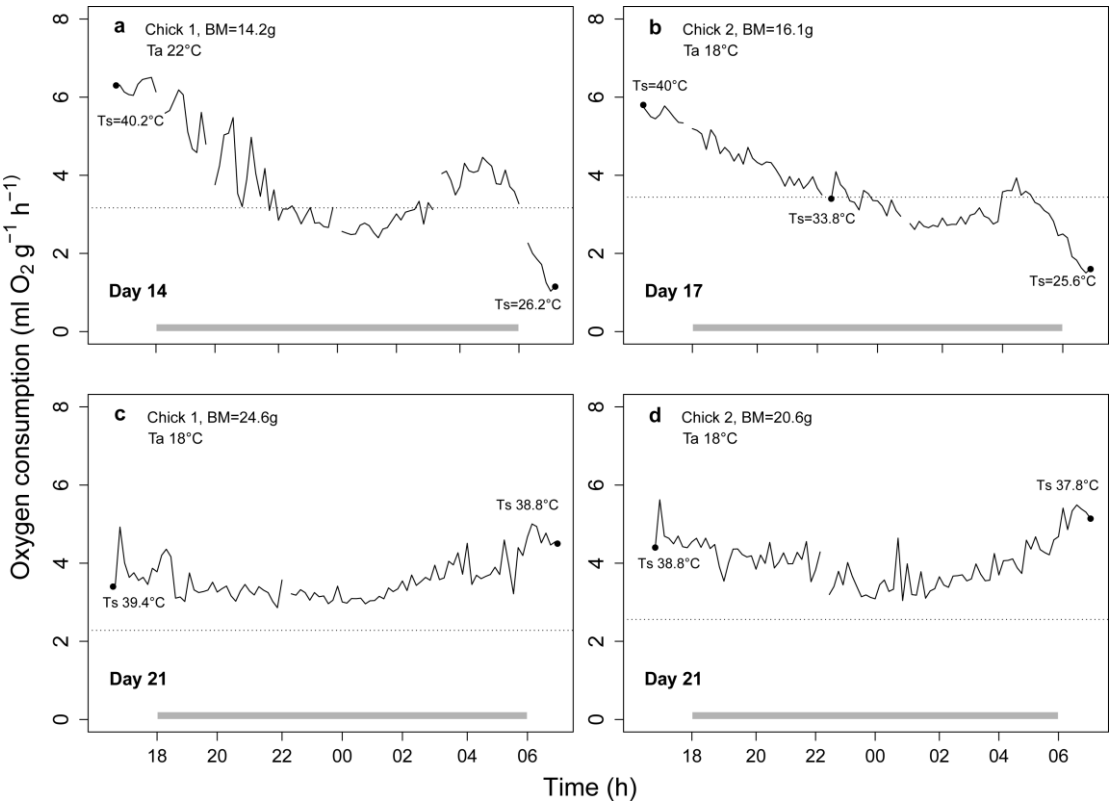

Fig. 3 Mass-specific oxygen consumption overnight for two chicks in our study (chick number and their body masses at day of measurement are depicted at the upper left corner of each panel). Both chicks (#1 and #2) show torpor in their first measurement night (panels **a** and **b**), but defended a constant MR during the second night of measurement (panels **c** and **d**). Horizontal dotted lines depict the torpor threshold (75% of the calculated RMR; see "Methods" section for detailed calculations). Horizontal grey bar depict the rest phase (lights off). Surface temperature (T_s) measured with infrared thermometer under the wings at the beginning and end of the trial are depicted at the start and end of measurements line and at 22:00h in the first night of chick #2.

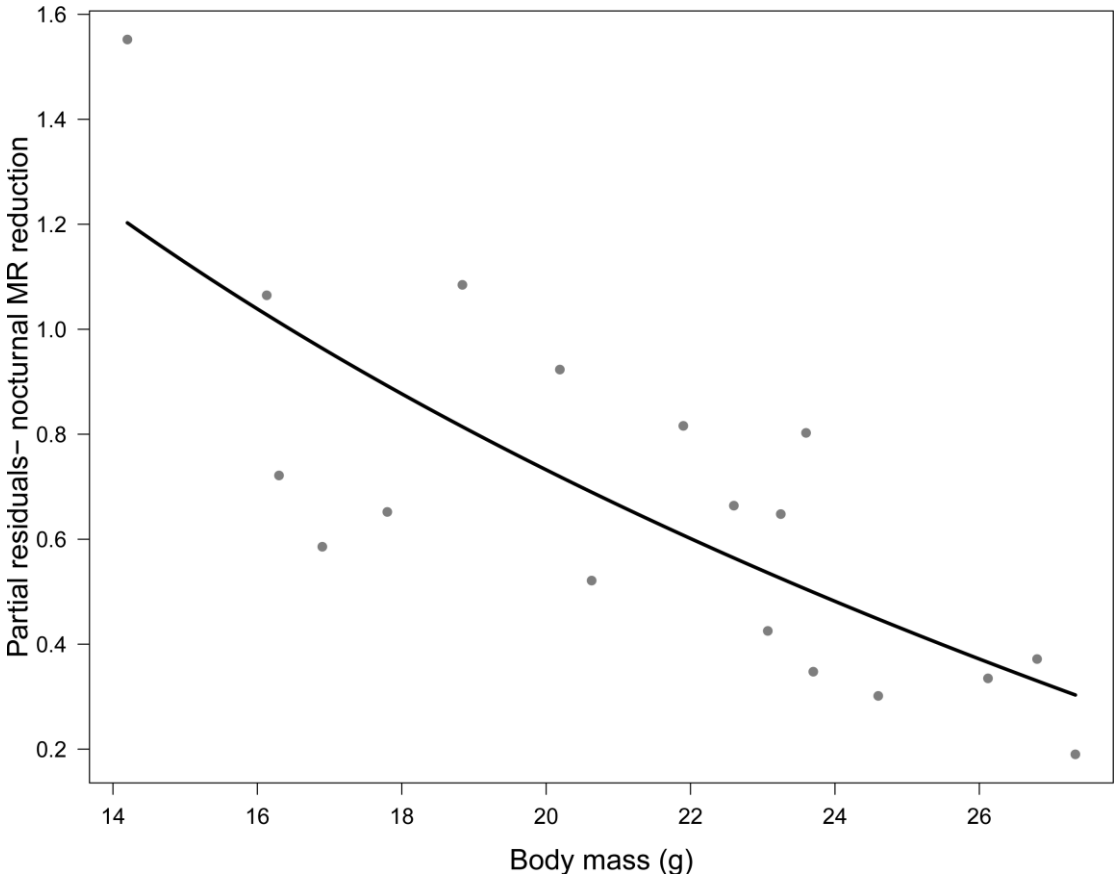

Fig. 4 Partial residual plot showing the relationship between metabolic reduction during the rest phase (the difference between maximum and minimum metabolic rate) measured as oxygen consumption (ml O_2 g^{-1} h^{-1}) and body mass (g) in king quail chicks (age 12-22 days), after accounting for age in a general linear mixed effect model with individual as a random effect (n=18, N=9).

Table S1: Summary table of the overnight metabolic measurements in each chick in the study (each chick was measured twice). Minimum and maximum metabolic rates are shown for both total (Total MR_{min} and Total MR_{max}; ml O_2 h^{-1}) and mass-specific (MR_{min} and MR_{max}; ml O_2 g^{-1} h^{-1}) units, and were calculated as the lowest, and highest mean rate of oxygen consumption, respectively, measured consecutively over a 36 minutes period.

Chick	Age (d)	Body mass (g)	Total MR_{min}	Total MR_{max}	MR_{min}	MR_{max}	Mass loss[1]	T_s start[2]	T_s end[2]
1	14	14.2	31.3	90.26	2.51	6.44	3.24	40.2	26.2
1	21	24.6	67.29	99.95	3.04	4.81	4.25	39.4	38.8
2	17	16.13	37.59	90.11	2.69	5.61	3.22	40	25.6
2	21	20.63	58.68	99.79	3.13	5.36	3.91	38.8	39
3	12	17.8	58.03	90.24	3.64	5.09	2.96	39.4	38
3	17	23.07	60.06	91.2	2.78	4.41	3.72	40.6	39.8
4	12	16.3	58.66	104.34	4.11	6.53	3.54	40	37.6
4	18	23.6	63.53	106.9	2.99	4.55	4.25	41	38.8
5	14	16.9	41.39	74.48	2.76	4.47	3.4	40.8	32.8
5	18	23.7	69.56	98.8	3.24	4.79	3.83	41	36.6
6	16	21.9	63.71	119.67	3.3	5.49	4.6	39.8	35.8
6	22	27.33	70.39	122.05	2.83	4.53	4.6	39.6	39.4
7	15	18.84	63.14	110.36	3.69	6.33	4.37	40.8	39.4
7	19	22.6	64.13	102.66	3.05	5.33	4.1	40.6	37.8
8	15	23.25	66.37	112.66	3.19	4.91	4.09	40.6	37.4
8	19	26.8	96.99	137.26	3.72	5.42	3.37	40.8	40
9	16	20.19	55.06	109.64	3.1	5.48	3.83	39.8	24.6
9	22	26.12	87.06	141.12	3.76	5.73	3.83	40.6	38.4

[1]Mass loss was calculated as the difference between mass measured before and after measurement night and was used to calculate mass-specific MR, assuming a linear mass loss during the night.

[2]T_s start and T_s end are surface temperatures before and after the nightly metabolic rates measurements.

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