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RESEARCH ARTICLE

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

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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 *Coturnix 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 4–5 h with a reduction of metabolism by >40% and another approached the 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 development in small precocial and not only altricial birds.

KEY WORDS: Endothermy, Metabolic rate, King quail, *Coturnix chinensis*, Heterothermy, Thermal energetics

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 are 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 nests 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 passerina*, *Spizella pusilla* and *Pooecetes gramineus*; Dawson and Evans, 1957, 1960) and storm petrel (*Oceanodroma furcata*; Boersma, 1986) to up to 3 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, a 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 10 days of age (Nichelmann and Tzschentke, 2002), whereas large species can already thermoregulate efficiently 1 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 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).

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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), 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), delay hatching until conditions are favourable for parents and offspring (Geiser and Brigham, 2012) and enhance 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 a 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: 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 the early stages of development, around the time they become endothermic.

In this study, we first aimed to determine at what age the king quail *Coturnix 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. Second, 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 is relevant when determining torpor, because the purpose of torpor is energy conservation and not T_b reduction. Moreover, entry into torpor usually requires a calm and undisturbed animal. Because of 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 surface temperature (T_s) would disturb the birds, and may interrupt torpor, whereas 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.

MATERIALS AND METHODS

Experimental animals

King quails, *Coturnix chinensis* (Linnaeus 1766), occur widely from India to Southeast Asia, Indonesia, New Guinea, and 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 on 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 12 h light:12 h dark with lights on from 06:00 h to 18:00 h. 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 to two brooders made of plastic cages (43.3×80×51 cm H×W×D), bedded with pine shavings 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 was 35°C below the heating lamp 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 09:30 h and 11:00 h and placed individually into a paper cup at T_a=22.0±0.1°C. Immediately upon removal from the brooder, 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 min intervals until the last measurement after 40 min (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 a 50 g common poorwill (Brigham, 1992) and less than 4°C in an 80 g owl (Smit and 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 does not exceed 1–2°C. Animals were weighed with an electronic balance at the end of the cooling experiment to the nearest 0.1 g. These measurements were conducted until 12 days of age, when the T_s change over 40 min 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.

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, thermoneutral conditions for adult king quail (28–35°C; Roberts and Baudinette, 1986), for at least 60 min 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 min (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 (FXO301-01R field oxygen analysis system version 1.01, Sable Systems International). 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.K. 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 respiratory quotient (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 was 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 min at T_a=22.0±0.1°C, we tested whether they could use torpor. Chicks were removed from the brooder between 16:00 h and 16:30 h and placed individually into the respirometry chamber overnight for approximately 15 h on a 12 h light:12 dark cycle, 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 $\dot{V}_{O_2}$, respectively, measured consecutively over a 36 min period (i.e. 4 consecutive values). 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 MR.

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

$$\mathrm{BMR} + C(T_{c,\mathrm{lower}} - T_a), \quad (1)$$

where C is the wet thermal conductance, calculated using the equation for the active phase C=0.994$M^{-0.509}$ (where M is mass in g; Schleucher and Withers, 2001) and $T_{c,lower}$ is the lower critical temperature: $T_{c,lower}$=T_b−4.24$M^{0.317}$ (Swanson and Weinacht, 1997). Individual basal metabolic rate (BMR, in ml g^{-1} h^{-1}; because the chicks were still growing) was calculated using the equation for non-passerines birds: logBMR=0.699×log$M$−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 T_s under the wing using an infrared thermometer in four individuals at 22:00 h 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 s of removing the chick from the respirometry chamber and subsequently returning it. 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:

$$M(t) = A\exp[-\exp\{-k(t-t_i)\}] \quad (2)$$

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' (https://CRAN.R-project.org/package=growthrates).

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 the general linear model (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 min) as the dependent variable, in relation to age and body mass. In addition, we divided the dataset into four different body mass groups (<5, 5–<8, 8–13 and >13 g) 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 the cooling experiment (at time zero and after 40 min) as the dependent variable in relation to time (start and end of the experiment). Finally, we calculated an index of homeothermy for nestlings following Ricklefs (1987) and Visser and Ricklefs (1993). This index measures the gradient between equilibrium T_b and the environmental temperature, and 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:

$$H = (T_{end} - T_a)/(T_{start} - T_a), \quad (3)$$

where T_{start} and T_{end} are T_s at time of extraction from brooder and after 40 min 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 min.

RMR in the TNZ

We used general linear modelling with individual as a random effect to examine the effect of body mass on RMR at TNZ (T_a=30°C) in the 3–12 day old chicks. RMR was calculated as the lowest mean rate of $\dot{V}_{O_2}$, measured consecutively over a 27 min period (i.e. 3 consecutive values). We analysed both total and mass-specific RMR.

Overnight MR 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 a 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). The R function *lme* in the R package 'nlme' (https://CRAN.R-project.org/package=nlme) was used to perform mixed-effect models with the function *visreg* in the 'visreg' package for visualization (https://CRAN.R-project.org/package=visreg).

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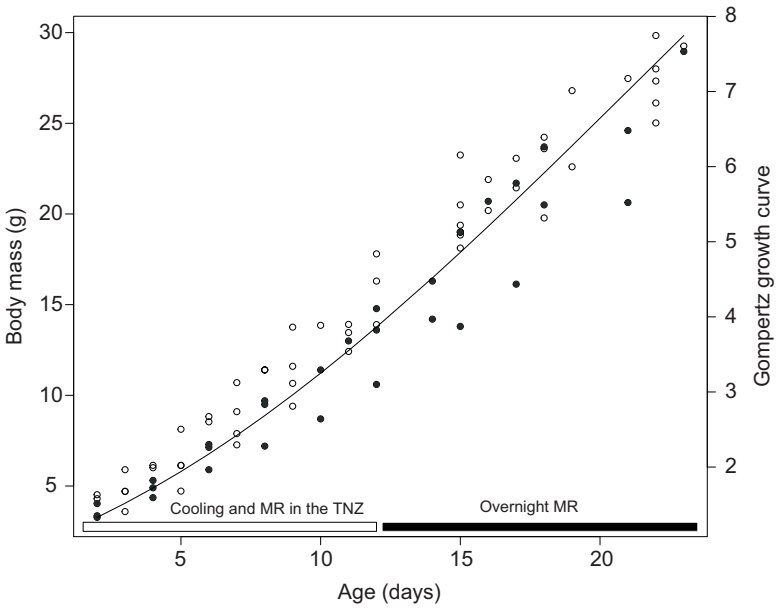

Fig. 1. Body mass in developing king quail and experimental time course. Data are *n*=83 measurements from *N*=9 individuals. Solid line is the Gompertz growth curve (right *x*-axis). MR, metabolic rate; TNZ, thermoneutral zone. Filled circles represent individuals that expressed torpor and had the slowest growth rate (Fig. 3 and Table 3).

All statistical analyses were conducted using R version 3.3.0 (http://www.R-project.org/).

Numeric values are presented as means±s.d for the number of individuals (*N*) measured *n*=number of measurements.

RESULTS

Body mass (*M*) and growth rate

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

T_s

The mean T_s of chicks measured immediately after removal from the brooder at thermoneutrality increased with age from 34.6±2.2°C at 2–5 days, to 36.9±0.7°C at 3–8 days, 38.6±0.7°C at 6–12 days and 40.1±0.4°C at 10–15 days (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 min 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 min (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 days old. 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 days 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

MR in the TNZ

At 3–12 days, 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 and 6.04 ml O_2 g^{-1} h^{-1} (with one value of 1.97 ml O_2 g^{-1} h^{-1} in one individual at 5.3 g). The mean total RMR 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 RMR=1.43+4M; t=8.94, P<0.001).

Determination of torpor expression

At 12–22 days, 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; Table S1). Generally, MR started to decrease soon after lights off (at 18:00 h), and increased again at approximately 02:00 h, 4 h before lights on (Fig. 3). Mass loss during the night ranged between 2.96 and 4.6 g, with an average mass loss of 3.84±0.48 g (Table S1), which to some extent was due to loss of faeces. The differential between both total and mass-specific MR_{min} and MR_{max} was significantly and negatively related to body mass (Fig. 4, Table 4), with this differential being smaller in heavier birds.

Table 1. Summary table of age, surface temperature (T_s) and the associated *t*-values for chicks in the four body mass groups

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

M, body mass; *N*, number of measurements; *n*, number of individuals; T_{start} and T_{end}, T_s measured with an infrared thermometer under the wings before and after (40 min) the cooling experiment, respectively. *t*-values are shown for the models testing the difference between T_{start} and T_{end}. Bold indicates significance.

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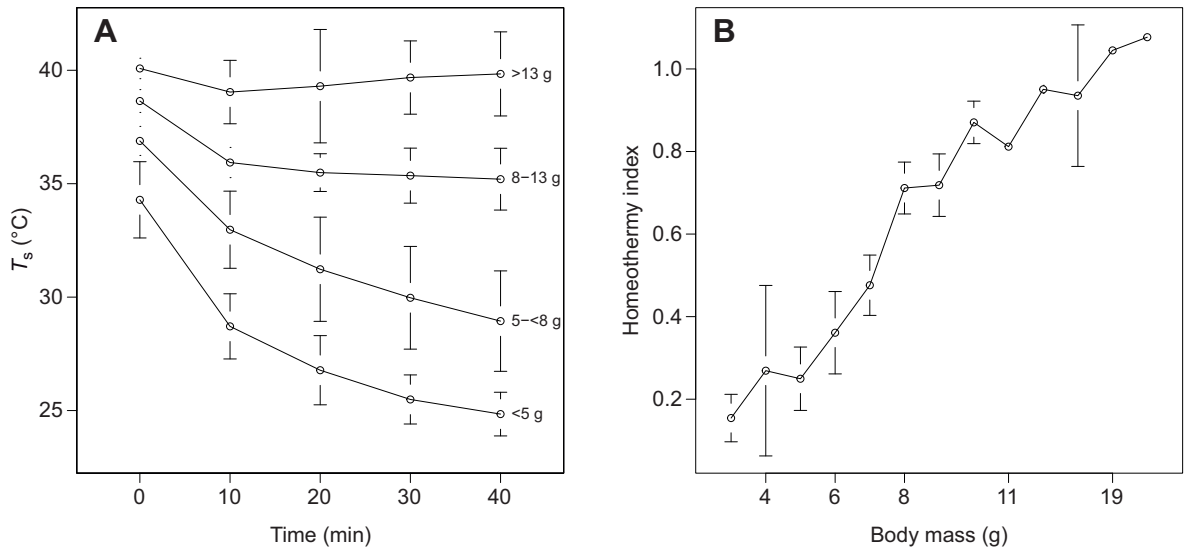

Fig. 2. Development of endothermy with body mass. (A) Change in surface temperature (T_s) of king quails in different body mass groups at an ambient temperature (T_a) of 22°C in a 40 min cooling experiment. Each data point represents the mean T_s with confidence intervals (measured with an infrared thermometer under the wing) of the indicated body mass group (see Table 1 for number of measurements and individuals for each group). (B) Homeothermy index (*H*) following Ricklefs (1987) and Visser and 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 min. Chick body mass was rounded to the nearest 1 g.

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 days, with 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 h, after which MR increased again to 4.46 ml O_2 g^{-1} h^{-1} at around 04:00 h. After this increase, MR plummeted to 1.15 ml O_2 g^{-1} h^{-1} and when the animal was removed at 07:30 h, 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 h (from 22:00 h until 03:00 h; Fig. 3A, Table 3). Similarly, chick 2 (body mass 16.1 g) exposed to T_a=18°C at 17 days steadily reduced MR from ∼5.5 ml O_2 g^{-1} h^{-1} after lights off. The T_s measured at 22:00 h was 33.8°C, 6.2°C below the starting T_s, with a MR at 3.39 ml O_2 g^{-1} h^{-1}. After a brief increase to

Table 2. Summary table of oxygen consumption as a measure of metabolic rate for each of the chicks in our study at 3-12 days

Chick	Age (days)	*M* (g)	Total RMR (ml O_2 h^{-1})	Mass-specific RMR (ml O_2 g^{-1} h^{-1})
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

Total resting metabolic rate (RMR) and mass-specific RMR were calculated as the lowest consecutive values over 27 min during a >60 min trial at an ambient temperature (T_a) of 30°C.

Table 3. Summary table of the overnight metabolic measurements for each chick in the study

Chick	Age (days)	*M* (g)	MR_{min} (ml O_2 g^{-1} h^{-1})	MR_{max} (ml O_2 g^{-1} h^{-1})	Predicted RMR (ml O_2 h^{-1})	MR reduction (%)
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

Grey shading indicates chicks that expressed torpor. MR_{min} and MR_{max}, minimum and maximum metabolic rate, calculated as the lowest and highest mean rate of oxygen consumption, respectively, measured consecutively over a 36 min period. Predicted RMR was calculated as $BMR+C(T_{c,lower}-T_a)$, where *C* is wet thermal conductance, calculated using the equation for the active phase $C=0.994M^{-0.509}$ (Schleucher and Withers, 2001); $T_{c,lower}$ is the lower critical temperature: $T_b-4.24M^{0.317}$ (where T_b is body temperature; Swanson and Weinacht, 1997); and BMR is basal metabolic rate, calculated using the equation for non-passerines birds: $\log BMR=0.699\times\log M-1.371$ (McKechnie et al., 2006). MR reduction was calculated as the percentage reduction in MR between MR_{min} and the predicted RMR.

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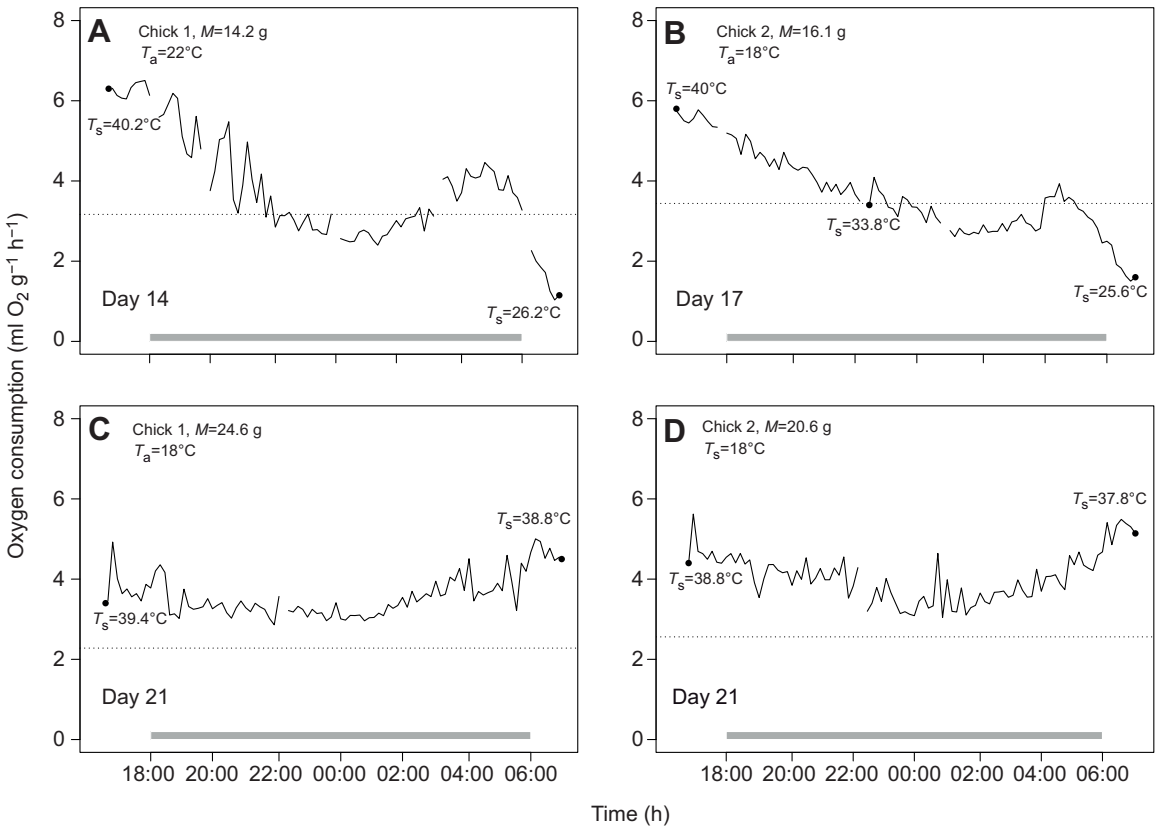

Fig. 3. Mass-specific oxygen consumption overnight. Data are for chicks 1 and 2 (chick number and body mass on the day of measurement are indicated in the upper left corner of each panel). Both chicks showed torpor in their first measurement night (A,B), but defended a high MR during the second night of measurement (C,D). Horizontal dotted lines depict the torpor threshold (75% of the calculated RMR; see Materials and Methods for detailed calculations). Horizontal grey bar indicates the rest phase (lights off). T_s (measured with an infrared thermometer under the wings) at the beginning and end of the trial is indicated at the start and end of the measurement line and at 22:00 h on the first night for chick 2.

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 01:30 h (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 04:00 h, but then again decreased until it reached 1.60 ml O_2 g^{-1} h^{-1} at 07:30 h, 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 h (from 00:00 h until 04:00 h; Fig. 3B, Table 3). Both chicks did not enter torpor on the second trial at T_a=18°C at 21 days (Fig. 3C,D). Chick 5 at T_a=22°C at 14 days reduced MR to values very close to the torpor threshold

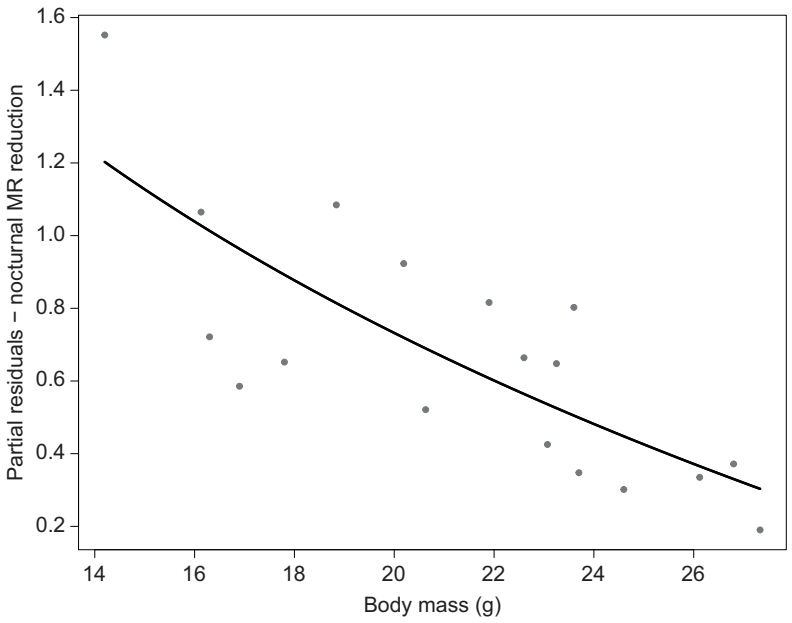

Fig. 4. Partial residual plot showing the relationship between MR reduction during the rest phase and body mass in king quail chicks (age 12–22 days). MR reduction was calculated as the difference between maximum and minimum metabolic rate, measured as oxygen consumption. Data are presented after accounting for age in a general linear mixed-effect model with individual as a random effect (n=18 measurements, N=9 individuals).

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Table 4. Model estimation for a general linear model describing the effect of body mass and age on the difference between MR_{max} and MR_{min} measured during a 15 h trial after chicks were able to defend their body temperature from day 12

	Variable	Estimate	*t*-value	d.f.	*P*-value
Total MR	*M*	−0.57	−3.74	8*	0.005
	Age	0.006	0.17	7	0.36
Mass-specific MR	*M*	−1.56	−4.10	7	0.004
	Age	0.04	1.96	7	0.09

Individual was a random effect. Results are shown for both total (ml O_2 h^{-1}) and mass-specific (ml O_2 g^{-1} h^{-1}) MR. *Model d.f. is higher because we report the values of the final model, excluding age.

(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 h while they were in the respirometry chamber on days 17, 18 and 19. The mean T_s of all chicks at the age of 12–22 days, before the respirometry measurements, was 40.2±0.6°C. The T_s of chicks 2, 4, 5 and 7 was 33.8, 35.4, 35.6 and 37.8°C, respectively, which was 6.2, 5.6, 5.4 and 2.8°C below their initial T_s, respectively. Except for chick 2 (details above), all the chicks rewarmed (to 38.8, 36.6 and 37.8°C for chicks 4, 5 and 7) when they were removed from the chambers at around 07:30 h.

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 days, and at 30% of adult body mass. The chicks reduced MR by up to 41% below the predicted RMR, almost twice below the torpor threshold of 25% below 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).

T_s and the development of endothermy

The 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 days. The latter is in line with the value of T_b=41.7°C reported in the 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 endothermy was positively correlated with growth rate (Dunn, 1975). Therefore, it is difficult to determine a specific age at which a species becomes fully endothermic because of individual variation in growth rates and probably also environmental variables (Beintema and 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 days, 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 days in the king quail. Spiers et al. (1974) report 13–15 days for the Japanese quail *Coturnix coturnix japonica*, and 14 days for the Bobwhite quail *Colinus virginianus* (Spiers et al., 1985). Although quails are rather similar with regard to the development of thermoregulatory efficiency, this varies between precocial avian species, and is 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).

MR

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 days, ranging between 4 and 7 ml O_2 g^{-1} h^{-1} (Pearson, 1994). Bernstein (1973) reported lower values of RMR in king quail chicks at a body mass of 3–9 g, but a RMR of 6.3 ml O_2 g^{-1} h^{-1} in a 6.8 g chick at T_a=25°C. Both 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 has also been reported in other bird species (domestic pigeon: Riddle et al., 1932; red-necked pheasant, domestic fowl and California quail: Koskimies 1962). The changes in RMR in the chicks aged 3–12 days 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 a 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 a body mass of almost 4 g, so the lower values are probably missing from our data. Our data of RMR at 3–12 days, therefore, probably depicted the peak values of RMR, between the phases of low RMR just after hatching and later at the decrease of metabolic requirement, and therefore were 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 days 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 chicks 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 be hypothermic (Geiser et al., 2014). Similar behaviour was observed in young crimson chat (*Epthianura 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 has been reported for 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 poikilothermic phase and a completely endothermic phase, in which the young enter into 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 thermoenergetics 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 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 appear to express torpor only after a 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 would 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 of T_s fluctuation for the three chicks that were measured at 22:00 h (and did not express torpor according to our MR definition), was 5.6, 5.4 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 and 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, 3.3 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. However, 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 is 29.2 and 31.8°C in chicks 2 and 1, respectively. These calculated values are 4.7 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 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 6 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 because of 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 a 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 the 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
The authors declare no competing or financial interests.

Author contributions
Conceptualization: Y.A., G.K., F.G.; Methodology: Y.A., G.K., C.W., F.G.; Software: G.K.; Formal analysis: Y.A.; Investigation: Y.A.; Writing - original draft: Y.A.; Writing - review & editing: G.K., C.W., F.G.; Supervision: F.G.; Project administration: Y.A.; Funding acquisition: Y.A.

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Data availability
The datasets generated during the current study are available from Dryad (Aharon-Rotman et al., 2020): dryad.brv15dv80

Supplementary information
Supplementary information available online at
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