Comparative Biochemistry and Physiology, Part A xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*

Miriam Ben-Hamo [a,*], Berry Pinshow [a], Marshall D. McCue [a], Scott R. McWilliams [b], Ulf Bauchinger [a,b]

[a] *Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 84990 Midreshet Ben-Gurion, Israel*
[b] *Department Natural Resources Science, 105 Coastal Institute in Kingston, University of Rhode Island, Kingston, RI 02881, USA*

ARTICLE INFO

Article history:
Received 25 November 2009
Received in revised form 30 December 2009
Accepted 31 December 2009
Available online xxxx

Keywords:
Aves
Body mass
Body temperature
Energetics
Homeothermy
Metabolic rate
Temperature regulation

ABSTRACT

We tested three hypotheses regarding the cues that elicit facultative hypothermia in Japanese quail (*Coturnix japonica*): H_1) Ambient temperature (T_a), alone, influences the onset and depth of hypothermia; H_2) Fasting, alone, influences the onset and depth of hypothermia; H_3) T_a acts synergistically with fasting to shape the use of hypothermia. Eight quail were maintained within their thermoneutral zone (TNZ) at 32.6 ± 0.2 °C, and eight below their lower critical temperature (T_{lc}) at 12.7 ± 3.0 °C. All quail entered hypothermia upon food deprivation, even quail kept within their TNZ. Body temperature (T_b) decreased more (38.36 ± 0.53 °C *vs.* 39.57 ± 0.57 °C), body mass (m_b) loss was greater (21.0 ± 7.20 g *vs.*12.8 ± 2.62 g), and the energy saved by using hypothermia was greater (25.18–45.01% *vs.* 7.98–28.06%) in low the T_a treatment than in TNZ treatment. Interestingly, the depth of hypothermia was positively correlated with m_b loss in the low T_a treatment, but not in TNZ treatment. Our data support H_3, that both thermoregulatory costs and body energy reserves are proximate cues for entry into hypothermia in quail. This outcome is not surprising below the T_{lc}. However, the quail kept at their TNZ also responded to food deprivation by entering hypothermia with no apparent dependence on m_b loss. Therefore inputs, other than thermoregulatory costs and body condition, must serve as cues to enter hypothermia. Consequently, we address the role that tissue sparing may play in the physiological 'decision' to employ hypothermia.

1. Introduction

Facultative hypothermia, the down regulation of metabolic rates and body temperature (T_b) below normorthermic levels, is a widespread physiological mechanism used by birds to save energy (McKechnie and Lovegrove, 2002; Schleucher, 2004). Some birds use hypothermia on a regular basis, even daily, to reduce energy expenditure required for thermoregulation despite their nutritional status, while other species enter hypothermia only in response to food deprivation (Graf et al., 1989; Hohtola et al., 1991; Schleucher, 2001; McKechnie and Lovegrove, 2002). Although hypothermia has been shown to occur in many avian species (McKechnie and Lovegrove, 2002), the cues eliciting this phenomenon are not yet well understood.

Numerous researchers found that the decrease in T_b during hypothermia is dependent on ambient temperature (T_a) and therefore concluded that hypothermia is a mechanism for reducing energy expenditure necessary for thermoregulating in the cold (Haftorn, 1972; Chaplin, 1976; Reinertsen and Haftorn, 1983; Reinertsen and Haftorn, 1986; Merola-Zwartjes, 1998; Brigham et al., 2000; Merola-Zwartjes and Ligon, 2000; Dolby et al., 2004; Fletcher et al., 2004; Cooper and Gessaman, 2005). Their assumption was that at low T_a, a decrease in T_b will lower the T_b–T_a difference and decrease the rate of heat loss, thereby reducing the demand for metabolic heat production (Kleiber, 1975; McNab, 2002; Welton et al., 2002). However, in eight passerine species, no relationship between T_a and the depth of hypothermia (*i.e.*, the decrease in T_b during the rest-phase compared to nighttime normothermia) was found within a range of temperatures below their lower critical temperatures (T_{lc}) (Steen, 1958; Cooper and Gessaman, 2005). Furthermore, in 17 species of hummingbirds, in Japanese quail (*Coturnix japonica*) and in red-headed finches (*Amadina erythrocephala*), T_a was not found to trigger hypothermia (Kruger et al., 1982; Hohtola et al., 1991; McKechnie and Lovegrove, 2003). Thus, T_a alone may not fully explain the use of hypothermia.

Some cases of decreased T_b in birds have been linked to reduced food availability or depletion of body energy reserves. With respect to the latter, food-restricted rock pigeons (*Columba livia*) and Japanese quail used shallow nocturnal hypothermia (Graf et al., 1989; Hohtola et al., 1991), and it has been found that the depth of hypothermia is proportional to changes in body energy reserves (Reinertsen and Haftorn, 1983; Reinertsen and Haftorn, 1986; Cooper and Gessaman, 2005). For example, in Japanese quail, continuous fasting induced progressively deeper nocturnal hypothermia (Laurila and Hohtola, 2005). A decline in energy reserves is thus followed by enhanced

---

* Corresponding author. Tel.: +972 86596773; fax: +972 86596772.
E-mail addresses: miriammi@bgu.ac.il (M. Ben-Hamo), pinshow@bgu.ac.il (B. Pinshow), mmccue@bgu.ac.il (M.D. McCue), srmcwilliams@uri.edu (S.R. McWilliams), ulf@etal.uri.edu (U. Bauchinger).

1095-6433/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2009.12.020

energy conservation that presumably enables the birds to withstand periods of fasting (Castellini and Rea, 1992).

Given that the use of hypothermia in birds seems to be influenced by T_a as well as by nutritional status, it is likely that the 'trigger' to entering hypothermia is under the control of multiple physiological inputs (Schleucher, 2004) and may even differ among species. Consequently, we examined whether the use of hypothermia by Japanese quail is associated with thermoregulatory requirements, fasting-induced decreases in energy reserves, or a combination of both.

We manipulated T_a, thus changing the quails' thermoregulatory demands, and evaluated their energy reserves by monitoring body mass loss (Δm_b). We hypothesized (H_1) that T_a alone, triggers hypothermia and that quail acclimated to thermal conditions within their thermal neutral zone (TNZ) avoid facultative hypothermia even when they are deprived of food. An alternative hypothesis (H_2) is that fasting alone, triggers hypothermia and that Δm_b is correlated with depth of hypothermia independent of T_a. Lastly, we hypothesized (H_3) that T_a acts synergistically with fasting-induced decreases in body energy reserves to trigger the use of hypothermia. Consequently, we tested the following corresponding predictions: (1) When deprived of food, quail kept within their TNZ do not use hypothermia, while birds kept below their T_{lc} respond by decreasing T_b during their rest-phase. Substantiation of this prediction implies that thermoregulatory costs are the main trigger of hypothermia since quail kept within their TNZ do not use hypothermia. (2) When deprived of food, quail kept within their TNZ and birds kept below their T_{lc} all respond with similar reductions of T_b. Support for this prediction implies that endogenous energy reserves are the main cue for use of hypothermia because of the similar depth of hypothermia observed in birds kept at different T_as. (3) When deprived of food, quail kept within their TNZ and those kept below the T_{lc} decrease their T_bs to different extents, with the latter entering deeper hypothermia. Finally, if this prediction holds, we will conclude that both energy reserves and T_a influence the hypothermic response.

2. Materials and methods

2.1. Animals

Sixteen Japanese quail chicks, eight of each sex and of similar age were purchased from a commercial breeder (Joseph Yanai, Mata, Israel) and were maintained in outdoor aviaries (2.5×2.5×3 m) on the Sede Boqer Campus of Ben-Gurion University (30° 52′ N, 34° 46′ E) until the experiments began. Fully-grown quail (6 weeks old) were assigned to one of two experimental groups of about the same m_b (173.7 ± 12.44 g and 171.4 ± 7.14 g), each of four males and four females. During experiments the quail were housed individually in cages (40×30×30 cm), where tap water and feed consisting of grained corn, wheat, soy, plus a mixture of vitamins and minerals (Hemed Lachay, Hemed, Israel) were available *ad libitum*; during periods of food deprivation only tap water was supplied.

2.2. Experimental design

First, we measured the metabolic rates (MR) of nine birds using indirect calorimetry to quantify rates of oxygen consumption (VO_2) and rates of carbon dioxide production (VCO_2) over a range of T_as wide enough to allow determination of their TNZ. Next, two groups of eight quail each were acclimated for seven days to two T_as until they reached stable m_b. One group was kept in a temperature-controlled room, at a constant T_a of 32.6 ± 0.2 °C, within the birds' TNZ; the second group was kept in an air-conditioned animal room, where the T_a was always below the quails' T_{lc} (12.7 ± 3.0 °C). Photoperiods were identical in both treatment groups (following natural cycle). After seven days, four quail from each group were deprived of food for four days to induce hypothermia. Food was removed from the cages at sunset, and the following photophase and the subsequent scotophase were considered as the first day of food deprivation. We measured MR of four quail from each treatment group on the first and fourth days of food deprivation, and of two individuals that were fed *ad libitum* for comparison. Measurement of VO_2 and VCO_2 were made during their rest-phase (ρ-phase) to determine resting metabolic rate (RMR) and MR during hypothermia (HMR), while we took the minimum value overnight for each bird measured. We then repeated the procedure in the reverse order, *i.e.* the four birds in each group that were deprived of food were allowed to feed and those that were fed *ad libitum* were deprived of food. Quail were weighed to ± 0.1 g at sunrise each morning. Ambient temperature was continually monitored using temperature-sensitive data loggers (iButton, Maxim Integrated Products, Dallas Semiconductor), that were placed in shaded, well-ventilated areas adjacent to each quail cage.

2.3. Body temperature measurements

We implanted miniature data loggers (iBBat modified by Alpha Mach Inc., Mont-Saint-Hilaire, Canada) weighing 1.1 ± 0.1 g in the peritoneal cavities of the quail to record T_b. Birds were anesthetized with Isoflurane®, and the loggers, potted in paraffin wax, were inserted into the peritoneal cavity through a small incision in the abdominal wall. The incision was sutured, and four days were allowed for recovery in outside aviaries before experiments. We used the temperature correction factors supplied for each logger by the manufacturer to adjust the measured values. Although we began with eight birds in each treatment group, logger malfunctions occurred in six birds so we report results for five birds per treatment group.

2.4. Respirometry

We measured MR by indirect calorimetry with an eight-channel open-flow gas analysis system. VO_2 was calculated from measurements made while six quail rested in individual 1.9 L plastic chambers (Lock and Lock, Korea) with walls covered with black tape to insure that chamber temperature and T_a were equal (Porter, 1969). Each chamber was equipped with a hardware-cloth floor above a paraffin oil trap for excreta. Chamber temperatures were maintained by placing them together in a controlled temperature cabinet (Refritherm-5, Struers, Denmark). Each group of birds was measured at its maintenance T_a, namely 12 °C and 32 °C for quail kept below their T_{lc} and within their TNZ, respectively.

Air from outside the building was pumped through a purge gas generator (Pure Gas, Broomfield, CO, USA, model #PCDA-1-12-m-32-C) that removed CO_2 and water vapor to less than 1 ppm. The fractional concentrations of oxygen in the incurrent and excurrent gas streams (F_IO_2; F_EO_2) were monitored by a dual cell oxygen analyzer (AEI Technologies, Inc. Naperville, IL, USA, model S-3A) and the concentration of carbon dioxide (F_ECO_2) was measured with a CO_2 analyzer (AEI Technologies, Inc. Naperville, IL, USA, model CD-3A). The CO_2 analyzer was zeroed using CO_2-free air and spanned using a 10% CO_2 mixture (Scott Specialty Gases, Plumsteadville, PA, USA), and the differential O_2 analyzer was calibrated by flushing dry air through both cells at the same flow rates.

$\dot{V}O_2$ and $\dot{V}CO_2$ were calculated using well-known respirometry equations (Hill, 1972; Depocas and Hart, 1975; Withers, 1977), andVO_2 (mL O_2 h^{-1}) was converted to units of power (watts), taking the respiratory quotient (RQ) into account (Kleiber, 1975; Schmidt-Nielsen, 1997) and was expressed in mass-specific units (W/g).

2.5. Energetic benefit of hypothermia

We used McKechnie and Wolf's (A. E. McKechnie, personal communication) bioenergetic model to estimate the energetic benefits of hypothermia. This approach allowed us to quantitatively

Please cite this article as: Ben-Hamo, M., et al., Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*, Comp. Biochem. Physiol. A (2010), doi:10.1016/j.cbpa.2009.12.020

compare the energetic costs and benefits of hypothermia among the treatment groups, and to estimate energy expenditure overnight which was impossible to do directly because we used an eight-channel open-flow gas analysis system that sampled sequentially from the birds, and did not produce continuous measurements for each. The model estimates the energetic benefit of hypothermia by considering the difference between normothermic energy expenditure (E_{norm}) and the cumulative energy expenditure ($E_{hypothermia}$) during each of the three phases of hypothermia (i.e. entry, E_{entry}; maintenance, E_{maint}; and rewarming, E_{rewarm}) using the following equations:

$$E_{entry} = D_{entry}\left(HMR + \frac{RMR - HMR}{2}\right) \tag{1}$$

where D_{entry} is the time required to down-regulate T_b (h)

$$E_{maint} = D_{maint} HMR \tag{2}$$

where D_{maint} is the duration of the maintenance phase of the hypothermia bout (h) and,

$$E_{rewarm} = s(T_{norm} - T_{hypothermia})m_b + D_{rewarm}\left(HMR + \frac{RMR - HMR}{2}\right) \tag{3}$$

where

E is energy (J)
s is the average specific heat of animal tissues (3.43 J/g °C),
T_{norm} is normothermic rest-phase T_b (°C),
$T_{hypothermia}$ is T_b during hypothermia (°C), and
D_{rewarm} is the duration of the rewarming phase of the hypothermia bout (h).

Finally, the energetic benefit of hypothermia, as a %, was calculated by Eq. (4):

$$Energetic\ benefit = \left(1 - \frac{E_{hypothermia}}{E_{norm}}\right) \cdot 100. \tag{4}$$

Since RMR was measured on birds that were feeding *ad libitum*, it must be overestimated by value of the heat increment of feeding (HIF). Therefore, we also compared data from postabsorptive Japanese quail that were measured at the same T_as, but not acclimated to them, to our RMR data.

E_{norm} was estimated using both RMR of our fed birds and RMR of the postabsorptive birds.

2.6. Data analysis

The bounds of the TNZ, T_{lc} and the upper critical temperature (T_{uc}) were determined following Pinshow et al. (1976). In brief, a range of temperatures was chosen that was broad enough to clearly include the upper and lower critical temperatures. The data points were then divided into two groups, an upper temperature group and a lower temperature group, and the corresponding pair of linear regression lines and their pooled mean squares (PMS) was calculated. This process was repeated for all possible divisions of the data within the range of temperatures. The intersection of the two regression lines that had the smallest PMS was considered to be the critical temperature.

The duration of each of the three phases of hypothermia were determined by following T_b overnight, and assuming that it is positively correlated with changes in metabolic rate. Entry phase duration was determined as the time it took to down-regulate T_b, starting at sunset; maintenance phase duration was determined as the time until T_b stopped fluctuating and began increasing steadily; and the rewarming phase duration was determined as the time from which T_b increased steadily until sunrise.

We used two-way multiple analysis of covariance (MANCOVA) to investigate the relationship between T_a, T_b and feeding status. T_a and feeding status (either *ad libitum* or fasted) were the independent categorical variables and the maximum and minimum core T_bs for each individual in each treatment group were the continuous dependent variables. We controlled for the effect of m_b by using it as the covariate. In addition, we used repeated-measures analysis of covariance (RM-ANOVA) to test for differences in Δm_b and $E_{energetic\ benefit}$ between groups and over time. When the sphericity assumption of RM-ANOVA was violated we used the Greenhouse–Geisser (GG) adjustment to modify the degrees of freedom of both treatment and error. We then used Tukey's *post hoc* test to rank the differences between and among the treatment groups. Means are presented ±1 SD and $\alpha = 0.05$ was chosen as the highest acceptable level of significance.

3. Results

3.1. Thermoneutral zone of Japanese quail

Metabolic rates were measured at T_as from 16.0 °C to 39.5 °C (Fig. 1). Mean m_b ($\overline{m}_b$) of Japanese quail whose metabolic rates were measured, was 166.0 ± 8.87 g ($n = 9$). Their rest-phase T_{lc} and T_{uc} were determined to be 23.2 °C and 36.0 °C, respectively (Table 1), and the mean mass-specific BMR of the quail was 8.76 ± 0.80 mW/g.

3.2. Use of hypothermia at different ambient temperatures

Both T_a and food availability affected T_b of the quail (Two-way MANCOVA: T_a: $F_{(2,14)} = 24.98$, $p < 0.001$, food availability: $F_{(2,14)} = 21.23$, $p < 0.001$, see also Fig. 2). Body mass did not have a significant effect on hypothermia within treatment groups (Two-way MANCOVA — $\overline{m}_b$: $F_{(2,14)} = 1.99$, $p = 0.17$), but fasting caused hypothermia, independent of T_a (Two-way MANCOVA: $T_a \times$ Food availability: $F_{(2,14)} = 1.50$, $p = 0.25$).

We found that during the day, T_b of the birds was not affected by either T_a or fasting (Tukey's *post hoc* test). In contrast, T_b during scotophase at both T_as was significantly lower when food was unavailable (Tukey's *post hoc* test: Below T_{lc}, $T_{b\ (fasting)} = 38.36$ °C, $T_{b\ (food)} = 39.70$ °C, $p < 0.01$, TNZ: $T_{b\ (fasting)} = 39.57$ °C, $T_{b\ (food)} = 40.68$ °C, $p < 0.05$). Moreover, the minimum T_b during food deprivation was significantly lower

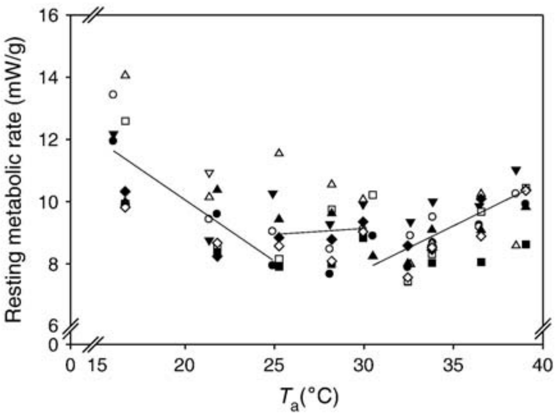

Fig. 1. Resting metabolic rate (RMR) *vs.* ambient temperature (T_a) in nine Japanese quail. Each point represents the mean metabolic rate of a single bird measured at a specific T_a and each bird has a different symbol. The statistical information we used for applying (Pinshow et al., 1976) method for estimating the bounds of the TNZ is to be found in Table 1 and the method in Section 2.6 of the text.

Please cite this article as: Ben-Hamo, M., et al., Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*, Comp. Biochem. Physiol. A (2010), doi:10.1016/j.cbpa.2009.12.020

Table 1
Equations of regression lines relating metabolic (MR) rate to ambient temperature (T_a) for Japanese quail. First column gives the range of the T_as used for the linear regression analysis. MR in mW/g and T_a in °C. N is the sample size, r^2 is the coefficient of determination, *F* is the statistics for linear term, *df* is the degrees of freedom and MS is the mean squared error.

Temp. (°C)	*N*	Regression equation	r^2	*F*	*df*	*p*-value	MS
16.00–24.90	184	$MR = -0.39T_a + 17.95$	0.24	57.90	1183	<0.001	2.663
25.25–29.50	81	$MR = 0.04T_a + 7.97$	0.003	0.24	180	NS	1.558
30.50–39.50	135	$MR = 0.28T_a - 0.67$	0.20	32.32	1134	<0.001	2.297

in quail kept below their T_{lc} than in quail kept within their TNZ (Tukey's *post hoc* test, $p<0.05$), indicating that the depth of hypothermia was significantly greater in quail kept below their T_{lc}.

3.3. Changes in hypothermia over time

Fed quail kept within their TNZ and below their T_{lc} had minimum T_bs that were independent of experimental day (Fig. 3). However, when they were deprived of food, minimum T_b of quail kept below their T_{lc} decreased significantly with each day of fasting (Fig. 3a). In contrast, minimum T_b in quail kept within their TNZ did not decrease with experimental day, even when deprived of food (Fig. 3b).

3.4. Body mass loss

Body mass loss differed between the two temperature treatments (RM-ANOVA: $F_{(1,14)} = 16.02$, $p<0.01$). Quail kept below their T_{lc} had greater Δm_b during the four days of fasting than quail kept within their TNZ (RM-ANOVA: $F_{(1,18)} = 7.86$, G–G adjusted $p<0.01$). Furthermore, the depth of hypothermia was positively correlated with Δm_b in quail kept below their T_{lc}, but not in those kept within their TNZ (Fig. 4).

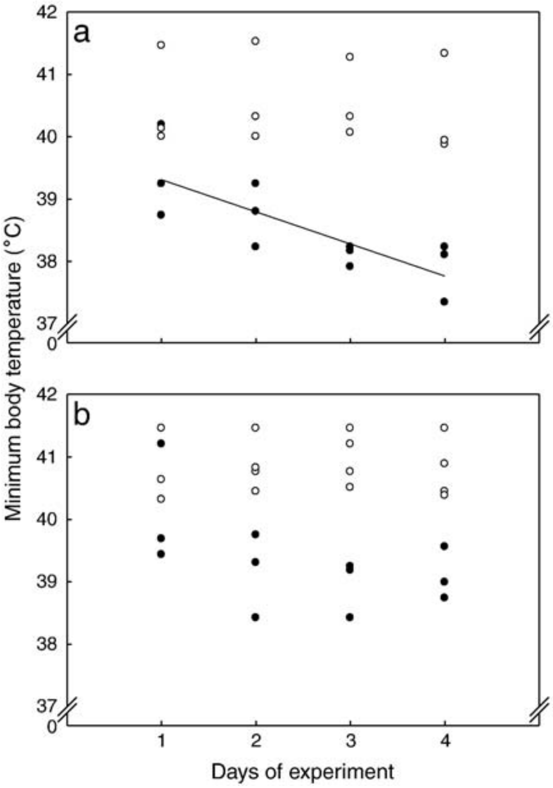

Fig. 3. The relationship between the minimum body temperature (T_b) and experimental day. Empty circles represent quail feeding *ad libitum* and filled ones quail deprived of food. a) Quail kept below their lower critical temperature (T_{lc}); Feeding *ad libitum*: $T_b = 40.65 - 0.05 \times \text{day}$, $p = 0.78$, $r^2 = 0.01$; Deprived of food: $T_b = 39.83 - 0.52 \times \text{day}$, $p<0.01$, $r^2 = 0.64$. b) Quail kept within their thermoneutral zone (TNZ); Feeding *ad libitum*: $T_b = 40.81 + 0.02 \times \text{day}$, $p = 0.84$, $r^2 = 0.003$; Deprived of food: $T_b = 40.47 - 0.24 \times \text{day}$, $p = 0.36$, $r^2 = 0.06$.

3.5. Energetic benefits of hypothermia

The duration of the entry phase was significantly higher in quail kept within their TNZ only on the first day of hypothermia (Table 2; Student's *t*-test: $t_{14} = 8.21$, $p<0.001$). The duration of the maintenance phase did not differ between the two temperature groups (Student's *t*-test: 1st day of fasting: $t_{14} = 1.58$, $p = 0.14$; 4th day of fasting: $t_{14} = 0.96$, $p = 0.35$), and the rewarming phase was significantly longer in quail kept below their T_{lc} only on the fourth day of hypothermia (Student's *t*-test: $t_{14} = 2.42$, $p<0.05$). Finally, the total duration of a hypothermic bout on the first day of hypothermia was significantly longer in quail kept within their TNZ (Student's *t*-test: $t_{14} = 2.63$, $p<0.05$).

Our calculations show that Japanese quail kept at different T_as derived significant energetic benefits from using hypothermia, both when we used RMR values of birds feeding *ad libitum* and RMR values of postabsorptive birds to correct for HIF (RM-ANOVA, $F_{(1,14)} = 53.40$,

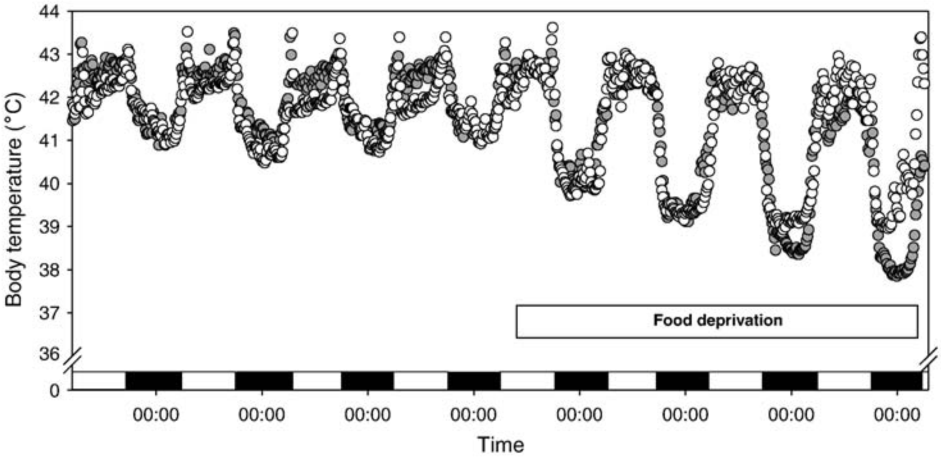

Fig. 2. Mean body temperature ($\overline{T}_b$) of Japanese quail offered food *ad libitum* for 4 d, followed by 4 d of food deprivation (indicated by the text box below the plot). White and black bars correspond to photophase and scotophase, respectively. Empty circles are $\overline{T}_b$ of five quail kept at a thermoneutral temperature (32.6 ± 0.2 °C); grey circles are $\overline{T}_b$ of five quail kept at an ambient temperature below their lower critical temperature (T_{lc}) (12.7 ± 3.0 °C).

Please cite this article as: Ben-Hamo, M., et al., Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*, Comp. Biochem. Physiol. A (2010), doi:10.1016/j.cbpa.2009.12.020

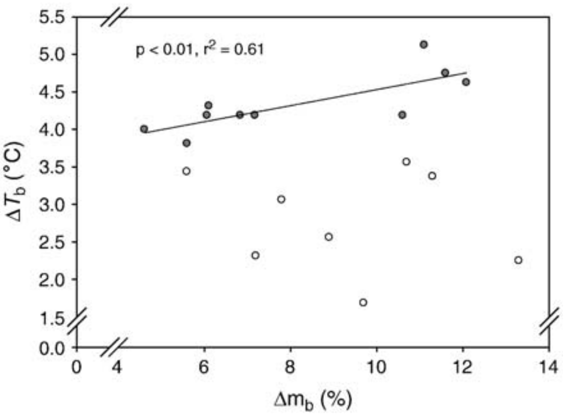

Fig. 4. The relationship between the decrease in body temperature (T_b) during hypothermia (ΔT_b) and body mass loss (% of initial). Empty circles are for quail kept within their thermoneutral zone and grey ones for quail kept below their lower critical temperature (T_{lc}). The p and r^2 values refer to the regression equation relating the decrease in T_b *vs.* % body mass loss (Δm_b) in quail kept below their T_{lc} ($\Delta T_b = 3.46 + 0.11 \times \Delta m_b$). The $n = 10$, since five quail from each treatment group were measured in first and fourth days of hypothermia.

$p<0.001$, corrected for HIF: $F_{(1,14)} = 30.51$, $p<0.001$, see also Fig. 5). Quail kept below their T_{lc} had greater energetic benefit on both the first and fourth days of hypothermia than those kept within their TNZ (Tukey's *post hoc* test: 1st day of fasting: below T_{lc}: 45.01 ± 4.54%, TNZ: 28.06 ± 3.33%, $p<0.001$; 4th day of fasting: below T_{lc}: 43.18 ± 2.80%, TNZ: 33.78 ± 5.30%, $p = 0.01$; corrected for HIF: 1st day of fasting: below T_{lc}: 25.18 ± 6.61%, TNZ: 7.98 ± 4.59%, $p<0.001$; 4th day of fasting: below T_{lc}: 27.05 ± 4.06%, TNZ: 16.21 ± 7.29%, $p<0.01$). Energetic benefit increased significantly with successive days of fasting in quail kept within their TNZ, but not in those kept below the T_{lc} (Tukey's *post hoc* test: Below T_{lc}: $p = 0.57$; TNZ: $p<0.01$; corrected for HIF: Below T_{lc}: $p = 0.78$; TNZ: $p<0.01$).

4. Discussion

Quail entered hypothermia upon food deprivation even when they were kept within their TNZ (Figs. 1 and 2). This response is further evidence that body energy reserves are a proximate cue for entry into hypothermia in birds (Hohtola et al., 1991; Phillips et al., 1991b; Reinertsen and Bech, 1994; Dolby et al., 2004; Laurila et al., 2005; Nord et al., 2009). However, although fasted quail used hypothermia when kept within their TNZ, there was a difference in the depth of hypothermia between groups with quail kept below T_{lc} entering deeper hypothermia. Moreover, the energetic benefit was greater in quail kept below their T_{lc} than in those kept within their TNZ (Fig. 5). Quail kept below their T_{lc} had two distinctive responses that were not found in those that were kept within their TNZ. First, hypothermia became deeper with sequential days of food deprivation (Fig. 3a). Second, the depth of hypothermia was positively correlated with Δm_b (Fig. 4).

4.1. The effect of thermoregulatory cost

We found that T_a affects the hypothermic response in Japanese quail at several levels. The depth of hypothermia was negatively correlated with T_a, from which we concluded that as thermoregulatory costs increase, quail respond by further reducing their nocturnal T_b when hypothermic. These results concur with those of previous studies that show a correlation between T_a and the depth of hypothermia (Haftorn, 1972; Chaplin, 1976; Reinertsen and Haftorn, 1983; Reinertsen, 1996; Brigham et al., 2000; Merola-Zwartjes and Ligon, 2000; Dolby et al., 2004; Cooper and Gessaman, 2005; but see: Steen, 1958; Haftorn, 1972; Chaplin, 1976; Reinertsen and Haftorn,

Table 2
Different measurements taken during the hypothermic response of Japanese quail. Values are given for the first and the fourth days of hypothermia when available. Data represented as mean ± SD. MR are given in kJ/day, all temperature data is given in °C, and duration values of the different phases of hypothermia are given in hours. The last row gives the p-values for the Student's t-test comparison of each two vertical values in the rows above. HMR is hypothermic metabolic rate, RMR is resting metabolic rate, $T_{hypotherm}$ is body temperature during nocturnal hypothermia, T_{norm} is body temperature during normorthermic night, D_{entry} is the duration of entry phase, D_{maint} is the duration of the maintenance phase, D_{rewarm} is the duration of the rewarming phase, and D_T is the total duration of hypothermia.

	HMR		RMR	$T_{hypotherm}$		T_{norm}	D_{entry}		D_{maint}		D_{rewarm}		D_T	
	1st day	4th day		1st day	4th day		1st day	4th day	1st day	4th day	1st day	4th day	1st day	4th day
Below T_{lc}	142.70 ± 17.66	107.48 ± 10.84	226.64 ± 39.00	39.39 ± 0.74	38.27 ± 0.95	40.53 ± 0.81	1.33 ± 0.37	2.67 ± 0.52	6.25 ± 0.75	5.92 ± 1.42	2.50 ± 0.75	3.42 ± 1.03	10.08 ± 0.72	12.00 ± 0.85
TNZ	99.41 ± 7.17	86.98 ± 10.38	148.01 ± 36.60	40.45 ± 1.03	39.69 ± 1.23	40.77 ± 0.49	3.50 ± 0.65	2.92 ± 0.87	5.25 ± 1.63	6.42 ± 0.37	2.67 ± 0.28	2.25 ± 0.90	11.42 ± 1.25	11.58 ± 0.37
p-value	<0.001	<0.01	=0.001	<0.05	<0.05	NS	<0.001	NS	NS	NS	NS	<0.05	<0.05	NS

Please cite this article as: Ben-Hamo, M., et al., Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*, Comp. Biochem. Physiol. A (2010), doi:10.1016/j.cbpa.2009.12.020

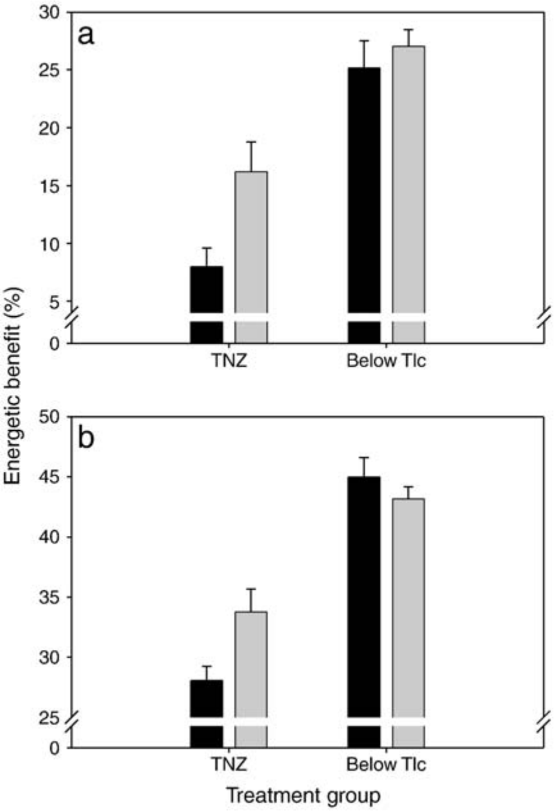

Fig. 5. Mean (±1 SD) energetic benefit (%) of using hypothermia in Japanese quail on the first and fourth day of fasting. a) Energetic benefit was calculated using postabsorptive quail to determine normorthermic energy expenditure (E_{norm}). b) Energetic benefit was calculated using quail feeding *ad libitum* to determine E_{norm}. Energetic benefit was calculated using Eq. (4) in the text. Black bars represent the first day of fasting and grey bars the fourth day. Each bar shows the mean of eight quail.

1983; Prothero and Jurgens, 1986; Hohtola et al., 1991; Reinertsen, 1996; Brigham et al., 2000; Merola-Zwartjes and Ligon, 2000; Dolby et al., 2004; Fletcher et al., 2004; Cooper and Gessaman, 2005). The equivocal relationship between T_a and hypothermia can be partly explained by our results. T_a did not trigger the occurrence of hypothermia, although it modulated its depth in birds that did enter hypothermia.

The energetic benefit was greater in quail maintained at the lower T_a (Fig. 5). Hence, we concluded that with increasing thermoregulatory costs, hypothermia increases the energetic benefit; T_b in quail kept below their T_{lc} continued to decrease with successive days of hypothermia, yet their energetic benefit remained constant (Figs. 3a and 5). This conclusion implies that higher thermoregulatory costs compel the quail into further reduce the difference between T_b and T_a in order to derive the equivalent energetic benefit to the first day of fasting. Surprisingly, we found that with a decrease of no more than 1 °C in T_b birds saved more than 25% of their energy expenditure during a normorthermic night. This value should be treated with caution as the birds that served for determining normorthermic rest-phase metabolism were feeding *ad libitum*, and thus their metabolic rates were probably increased due to HIF. When correcting for HIF, using values from postabsorptive birds, the energetic benefit declined to 7.98% for birds kept in their TNZ and 25.18% for birds kept below T_{lc}. Note that the postabsorptive birds were measured at the treatment temperatures overnight without being acclimated to them. Moreover, we consider a period of 24 h of fasting to be sufficient to elicit hypothermia. Therefore, we argue that it is important to present both estimates. In addition, our estimates are conservative because we used the minimum values for each night, and since birds s are known to have their peak of postprandial increase in metabolic rates about 2 h after feeding; thus we are reasonably confident that we have minimized the effects of specific dynamic action (McCue, 2006; Secor, 2009).

The small change in T_b, but the high energetic savings indicates that the fasting quail significantly reduced their thermal conductance. This is consistent with previous studies on fasting pigeons (*Columba livia*), king penguins (*Aptenodytes patagonicus*) and barn owls (*Tyto alba*) that all responded by reducing thermal conductance (Duchamp et al., 1989; Phillips et al., 1991a; Thouzeau et al., 1999a). The mechanism responsible for decreased conductance is unclear, but it can be achieved by changes in posture, degree of ptiloerection (Schmidt-Nielsen, 1997), decreased evaporative heat loss through respiratory adjustments (Aschoff, 1981), and peripheral vasoconstriction (Thouzeau et al., 1999a). Measurements of surface temperatures and respiratory variables may elucidate these mechanisms.

The rewarming phase was protracted in quail kept below their T_{lc} only on the fourth day of fasting (Table 2). The relatively long rewarming phase incurs a high energetic cost (Prothero and Jurgens, 1986), and its duration could explain why, even after reducing T_b further, the energetic benefit on the fourth day remained the same in quail kept below their T_{lc}. Interestingly, energy was also saved by using hypothermia within the TNZ, and this energetic benefit increased with successive days of food deprivation (Fig. 5), suggesting that the hypothermic response in Japanese quail is independent of thermoregulatory costs since it occurs within the birds' TNZ, at which thermoregulation costs are minimal.

4.2. The relationship between energy reserves and hypothermia

The depth of hypothermia was positively correlated with Δm_b during food deprivation in quail kept below their T_{lc}, but not in those kept within their TNZ (Fig. 4). Further Δm_b was negatively correlated with T_a, with quail kept below their T_{lc} losing more mass during the period of food deprivation. This result is consistent with other studies on birds (Torre-Bueno, 1978; Hohtola et al., 1991). In addition, we found that the depth of hypothermia was shallower in birds kept within their TNZ (Fig. 2), and therefore it is likely that the small differences observed in these birds together with the small sample size prevented us from detecting statistically significant relationships. There is conflicting evidence regarding the role of energy reserves in triggering hypothermia since in Puerto Rican todies (*Todus mexicanus*) and barnacle geese (*Branta leucopsis*) no relationship was found between m_b and the occurrence of hypothermia (Merola-Zwartjes and Ligon, 2000; Butler and Woakes, 2001). Nevertheless, the lack of such a relationship does not rule out the possibility that the use of hypothermia is independent of body condition for two reasons. First, we did not measure variables other than m_b that indicate physiological condition in birds (*e.g.*, fat reserves or hormone levels). Second, the quail only became hypothermic when deprived of food, which suggests a direct association between body energy reserves and the use of hypothermia.

The energetic benefit of hypothermia increased with successive days of hypothermia only in quail that were kept within their TNZ (Fig. 5). Furthermore, the decrease in T_b did not change significantly with the successive days of hypothermia in quail kept within their TNZ, but increased in quail kept below their T_{lc} (Fig. 3). These results suggest that the decline in body energy reserves over time accounts for the increase in the energetic benefit in quail within their TNZ since the T_a remained constant. Welton et al. (2002) presents a model that predicts a threshold level of body energy reserves below which using hypothermia becomes beneficial, and above which is more economical to rest at a normothermic T_b. This threshold decreases during the night, and the rate of the decrease follows the rate of decline in energy expenditure with decreasing T_b. In accordance with this model, our data further support the postulate that energy reserve levels are responsible for shaping the hypothermic response.

Please cite this article as: Ben-Hamo, M., et al., Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*, Comp. Biochem. Physiol. A (2010), doi:10.1016/j.cbpa.2009.12.020

4.3. Hypothermia may facilitate protein tissue maintenance

We have shown that within their TNZ the hypothermic response in Japanese quail is independent of body mass and thermoregulatory costs. This finding raises a question regarding the energetic benefit of decreasing T_b within the TNZ. According to Klaassen and Biebach (1994) 73% of the catabolized tissue that birds use to fuel during fasting is fat, but 4–10% of the total energy expenditure is generated through protein catabolism. Hypothermia may therefore permit further energy savings by reducing rates of protein turnover and ultimately rates of protein depletion. While *de novo* synthesis of protein diminishes during prolonged food deprivation, the rates of protein degradation remain constant (Swick and Benevenga, 1977), and may even increase (Robin et al., 1987; Henry et al., 1988; Cherel et al., 1992; Thouzeau et al., 1999b; McCue, 2007). Therefore, any reduction in T_b may function to slow down the overall metabolism including the process of protein degradation and may represent a mechanism for protein sparing. Such a mechanism could explain the hypothermic responses of quail kept within their TNZ.

Furthermore, the need to conserve protein may also explain the stronger hypothermic response in cold acclimated quail. House sparrows (*Passer domesticus*) exposed to T_a below their T_{lc} had higher red blood cell protein turnover rates (Carleton and Martinez del Rio, 2005) and Zebra finches (*Taeniopygia guttata*) showed higher tissue protein turnover rates (Bauchinger et al., in press) than birds maintained within their TNZ. Because carbon turnover is typically measured after fat extraction and glycogen stores are virtually not present in birds (apart from small amounts stored in the liver), carbon turnover represents a reliable measure of protein turnover (Carleton and Martinez del Rio, 2005; Bauchinger and McWilliams, 2009). Thus, a possible explanation for quail kept in their TNZ becoming hypothermic under ostensibly non-stressful conditions, independent of thermoregulatory costs and m_b, is to minimize maintenance costs and prolong survival when fasting.

5. Conclusions

The data support our hypothesis (H_3) that both thermoregulatory costs and body energy reserves serve as proximate cues for entry into hypothermia in quail. Not only did the quail respond to food deprivation by entering hypothermia, but their T_b also decreased when thermoregulatory costs were minimal. Because hypothermia was evident even within the TNZ with no apparent dependence on m_b loss, it is likely that stimuli besides T_a and energy reserves serve as cues to enter hypothermia. The continuous process of protein turnover may act as such a stimulus, either under maintenance conditions, or when food is not available. We therefore speculate that reducing T_b plays an important role in slowing down the process of protein degradation and thus be a mechanism to spare protein. In close association with protein turnover it is also possible, that the reduction in MR and concomitant decrease in T_b within the TNZ permits reduced maintenance costs. Thus, we conclude that Japanese quail modulate their hypothermic response patterns (*i.e.*, the drop in T_b and the length of each hypothermic bout) in response to different ecological conditions in a way that economizes energy expenditure.

Acknowledgments

This project was funded by US–Israel Binational Science Foundation Grant 2005119 to B.P. and S.R.M. M.D.M. and U.B. were funded by a Blaustein fellowship. We would like to thank Michał Wojciechowski, Justin Boyles and an anonymous reviewer for their insightful comments that helped improve our original manuscript. We would also like to thank Andrew McKechnie and Blair Wolf for allowing us to use their energetic model for hypothermia, and Ortal Mizrahy, Sarah Aamidor, Oren Amitai, and Sarah Hasslinger-Frey who helped care for the birds prior to and during the experiments. This study was done under authorization number BGU-R-08-2009 to BP of the Animal Care and Ethics Committee of Ben-Gurion University. This is paper #636 of the Mitrani Department of Desert Ecology.

References

Aschoff, J., 1981. Thermal conductance in mammals and birds — its dependence on body size and circadian phase. Comp. Biochem. Physiol. A 69, 611–619.

Bauchinger, U., Keil, J., McKinney, R., Starck, J.M., McWilliams, S.R., in press. Exposure to Cold But Not Exercise Increases Carbon Turnover Rates in Specific Tissues of a Passerine. J. Exp. Biol.

Bauchinger, U., McWilliams, S., 2009. Carbon Turnover in Tissues of a Passerine Bird: allometry, Isotopic Clocks, and Phenotypic Flexibility in Organ Size. Physiol. Biochem. Zool. 82, 787–797.

Brigham, R.M., Kortner, G., Maddocks, T.A., Geiser, F., 2000. Seasonal use of torpor by free-ranging Australian Owlet-Nightjars (*Aegotheles cristatus*). Physiol. Biochem. Zool. 73, 613–620.

Butler, P.J., Woakes, A.J., 2001. Seasonal hypothermia in a large migrating bird: Saving energy for fat deposition? J. Exp. Biol. 204, 1361–1367.

Carleton, S.A., Martinez del Rio, C., 2005. The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15} N incorporation in house sparrows (*Passer domesticus*). Oecologia 144, 226–232.

Castellini, M.A., Rea, L.D., 1992. The biochemistry of natural fasting at its limits. Experientia 48, 575–582.

Chaplin, S.B., 1976. Physiology of hypothermia in Black-capped chickadee, *Parus atricapillus*. J. Comp. Physiol. B 112, 335–344.

Cherel, Y., Robin, J.P., Heitz, A., Calgari, C., Maho, Y., 1992. Relationships between lipid availability and protein utilization during prolonged fasting. J. Comp. Physiol. B 162, 305–313.

Cooper, S.J., Gessaman, J.A., 2005. Nocturnal hypothermia in seasonally acclimatized mountain chickadees and juniper titmice. Condor 107, 151–155.

Depocas, F., Hart, J.S., 1975. Use of the Pauling oxygen analyzer for measurement of oxygen consumption of animals in open circuit and in short-lag, closed-circuit apparatus. J. Appl. Physiol. 10, 388.

Dolby, A.S., Temple, J.G., Williams, L.E., Dilger, E.K., Stechler, K.M., Davis, V.S., 2004. Facultative rest-phase hypothermia in free-ranging white-throated sparrows. Condor 106, 386–390.

Duchamp, C., Barre, H., Delage, D., Rouanet, J.L., Cohenadad, F., Minaire, Y., 1989. Nonshivering thermogenesis and adaptation to fasting in King penguin chicks. Am. J. Physiol. 257, R744–R751.

Fletcher, Q.E., Fisher, R.J., Willis, C.K.R., Brigham, R.M., 2004. Free-ranging common nighthawks use torpor. J. Therm. Biol. 29, 9–14.

Graf, R., Krishna, S., Heller, H.C., 1989. Regulated nocturnal hypothermia induced in pigeons by food deprivation. Am. J. Physiol. 256, R733–R738.

Haftorn, S., 1972. Hypothermia of tits in the Arctic winter. Ornis Scand. 3, 153–166.

Henry, C.J., Rivers, J.P., Payne, P.R., 1988. Protein and energy metabolism in starvation reconsidered. Eur. J. Clin. Nutr. 42, 543.

Hill, R.W., 1972. Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. J. Appl. Physiol. 33, 261–263.

Hohtola, E., Hissa, R., Pyornila, A., Rintamaki, H., Saarela, S., 1991. Nocturnal hypothermia in fasting Japanese quail: the effect of ambient temperature. Physiol. Behav. 49, 563–567.

Klaassen, M., Biebach, H., 1994. Energetics of fattening and starvation in the long distance migratory Garden warbler, *Sylvia borin*, during the migratory phase. J. Comp. Physiol. B 164, 362–371.

Kleiber, M., 1975. The Fire of Life: An Introduction to Animal Energetics. Publishing Co., Huntington, NY.

Kruger, K., Prinzinger, R., Schuchmann, K.L., 1982. Torpor and metabolism in hummingbirds. Comp. Biochem. Physiol. A 73, 679–689.

Laurila, M., Hohtola, E., 2005. The effect of ambient temperature and simulated predation risk on fasting-induced nocturnal hypothermia of pigeons in outdoor conditions. J. Therm. Biol. 30, 392–399.

Laurila, M., Pilto, T., Hohtola, E., 2005. Testing the flexibility of fasting-induced hypometabolism in birds: effect of photoperiod and repeated food deprivations. J. Therm. Biol. 30, 131–138.

McCue, M.D., 2006. Specific dynamic action: a century of investigation. Comp. Biochem. Physiol. A 144, 381–394.

McCue, M.D., 2007. Snakes survive starvation by employing supply-and demand-side economic strategies. Zoology 110, 318–327.

McKechnie, A.E., Lovegrove, B.G., 2002. Avian facultative hypothermic responses: a review. Condor 104, 705–724.

McKechnie, A.E., Lovegrove, B.G., 2003. Facultative hypothermic responses in an Afrotropical arid-zone passerine, the red-headed finch (*Amadina erythrocephala*). J. Comp. Physiol. B 173, 339–346.

McNab, B.K., 2002. The Physiological Ecology of Vertebrates: A View from Energetics. Cornell University Press, New York.

Merola-Zwartjes, M., 1998. Metabolic rate, temperature regulation, and the energetic implications of roost nests in the Bananaquit (*Coereba flaveola*). Auk 115, 780–786.

Merola-Zwartjes, M., Ligon, J.D., 2000. Ecological energetics of the Puerto Rican Tody: heterothermy, torpor, and intra-island variation. Ecology 81, 990–1003.

Nord, A., Nilsson, J.F., Sandell, M.I., Nilsson, J.A., 2009. Patterns and dynamics of rest-phase hypothermia in wild and captive blue tits during winter. J. Comp. Physiol. B 179, 737–745.

Please cite this article as: Ben-Hamo, M., et al., Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*, Comp. Biochem. Physiol. A (2010), doi:10.1016/j.cbpa.2009.12.020

Phillips, D.L., Rashotte, M.E., Henderson, R.P., 1991a. Energetic responses of pigeons during food-deprivation and restricted feeding. Physiol. Behav. 50, 195–203.

Phillips, D.L., Rashotte, M.E., Henderson, R.P., 1991b. Energetic responses of pigeons during food deprivation and restricted feeding. Physiol. Behav. 50, 195–203.

Pinshow, B., Fedak, M.A., Battles, D.R., Schmidt-Nielsen, K., 1976. Energy expenditure for thermoregulation and locomotion in emperor penguins. Am. J. Physiol. 231, 903–912.

Porter, W.P., 1969. Thermal radiation in metabolic chambers. Science 166, 115–117.

Prothero, J., Jurgens, K.D., 1986. An energetic model of daily torpor in endotherms. J. Theor. Biol. 121, 403–415.

Reinertsen, R.E., 1996. Physiological and ecological aspects of hypothermia. In: Carey, C. (Ed.), Avian Energ. Nutr. Ecol. Chapman and Hall, New-York, pp. 125–157.

Reinertsen, R.E., Bech, C., 1994. Hypothermia in pigeons; relating body temperature regulation to the gastrointestinal system. Naturwissenschaften 81, 133–136.

Reinertsen, R.E., Haftorn, S., 1983. Nocturnal hypothermia and metabolism in the willow tit *Parus montanus* at 63 degrees-N. J. Comp. Physiol. 151, 109–118.

Reinertsen, R.E., Haftorn, S., 1986. Different metabolic strategies of northern birds for nocturnal survival. J. Comp. Physiol. B 156, 655–663.

Robin, J.P., Cherel, Y., Girard, H., Géloen, A., Maho, Y., 1987. Uric acid and urea in relation to protein catabolism in long-term fasting geese. J. Comp. Physiol. B 157, 491–499.

Schleucher, E., 2001. Heterothermia in pigeons and doves reduces energetic costs. J. Therm. Biol. 26, 287–293.

Schleucher, E., 2004. Torpor in birds: taxonomy, energetics, and ecology. Physiol. Biochem. Zool. 77, 942–949.

Schmidt-Nielsen, K., 1997. Animal Physiology: Adaptation and Environment. Cambridge University Press.

Secor, S.M., 2009. Specific dynamic action: a review of the postprandial metabolic response. J. Comp. Physiol. B 179, 1–56.

Steen, J., 1958. Climatic adaptation in some small northern birds. Ecology 39, 625–629.

Swick, R.W., Benevenga, N.J., 1977. Labile protein reserves and protein turnover. J. Dairy Sci. 60, 505.

Thouzeau, C., Duchamp, C., Handrich, Y., 1999a. Energy metabolism and body temperature of barn owls fasting in the cold. Physiol. Biochem. Zool. 72, 170–178.

Thouzeau, C., Robin, J.P., Le Maho, Y., Handrich, Y., 1999b. Body reserve dynamics and energetics of barn owls during fasting in the cold. J. Comp. Physiol. B 169, 612–620.

Torre-Bueno, J.R., 1978. Evaporative cooling and water-balance during flight in birds. J. Exp. Biol. 75, 231–236.

Welton, N.J., Houston, A.I., Ekman, J., McNamara, J.M., 2002. A dynamic model of hypothermia as an adaptive response by small birds to winter conditions. Acta Biotheor. 50, 39–56.

Withers, P.C., 1977. Measurement of VO_2, VCO_2, and evaporative water loss with a flow-through mask. J. Appl. Physiol. 42, 120–123.

Please cite this article as: Ben-Hamo, M., et al., Fasting triggers hypothermia, and ambient temperature modulates its depth in Japanese quail *Coturnix japonica*, Comp. Biochem. Physiol. A (2010), doi:10.1016/j.cbpa.2009.12.020