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Physiology

Maternal corticosterone increases thermal sensitivity of heart rate in lizard embryos

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While it is well established that maternal stress hormones, such as corticosterone (CORT), can induce transgenerational phenotypic plasticity, few studies have addressed the influence of maternal CORT on pre-natal life stages. We tested the hypothesis that experimentally increased CORT levels of gravid female eastern fence lizards (*Sceloporus undulatus*) would alter within-egg embryonic phenotype, particularly heart rates. We found that embryos from CORT-treated mothers had heart rates that increased faster with increasing temperature, resulting in higher heart rates at developmentally relevant temperatures but similar heart rates at maintenance relevant temperatures, compared with embryos of control mothers. Thus, maternal CORT appears to alter the physiology of pre-natal offspring. This may speed development and decrease the amount of time spent in eggs, the most vulnerable stage of life.

1. Introduction

Environmental stressors such as predation, competition and temperature extremes can alter concentrations of glucocorticoid (stress) hormones such as corticosterone (CORT) [1,2]. Increases in maternal stress hormones during gravidity can alter the resulting offspring's phenotype via maternal stress effects [3,4]. Several studies have demonstrated effects of maternal CORT on post-natal offspring phenotype [3], but few have addressed effects on embryo phenotypes (see [5]) despite likely links. For example, elevating CORT of gravid female fence lizards, *Sceloporus undulatus*, resulted in increased CORT concentrations in the yolk of freshly laid eggs [4]. Given the effects of CORT *in utero* and on egg contents [4,6], it is likely that offspring phenotypic changes begin in the embryonic stages.

Since exposure to stressors increases an organism's heart rate [7], we hypothesize that elevated maternal CORT may increase embryonic heart rate, potentially via elevations in egg yolk CORT. To test this, we experimentally elevated CORT of gravid female eastern fence lizards to ecologically relevant levels. We then measured embryonic heart rate in the resulting eggs at metabolically (less than 26°C) and developmentally (greater than or equal to 26°C) relevant temperatures [8]. We predicted that embryos of CORT-treated mothers would have elevated heart rates and that such effects would be greater at higher temperatures.

2. Material and methods

(a) Animal collection and husbandry

We captured 18 gravid female eastern fence lizards from three sites in southern Alabama with similar habitat: Geneva State Forest, Conecuh National Forest and

Blakeley State Park. Females were returned to the laboratory and housed in pairs until laying. Each member of a pair was assigned to a different treatment and at a different stage of gravidity to ensure we could assign maternity to eggs [4]. Eggs were placed as clutch groups into plastic tubs containing moist vermiculite (approx. −200 kPa), sealed with Seran wrap and incubated at 31°C until hatching (approx. 45 days), per MacLeod *et al*. [9]. Tubs were rotated daily.

(b) Maternal corticosterone treatments

Females were assigned to one of two treatments and received either a daily dose of 0.2 µl g^{-1} lizard of a solution of 4 mg CORT (greater than or equal to 92%, Sigma C2505, Saint Louis, MO, USA) in 1 ml of sesame oil vehicle (approx. 0.8 µg CORT g^{-1} lizard; range = 0.74–0.87 µg CORT g^{-1}), or a vehicle-only control [4]. This was applied transdermally via pipette at 19.30, after lizards had ceased activity for the evening, eliminating the need for handling. The treatment was applied from capture until laying (mean ± s.e. = 34 ± 3 days).

(c) Egg heart rates

We measured heart rates of embryos using an infrared heart rate monitor (Buddy system Mk1; Avian Biotech, Tallahassee, FL, USA). We tested 28 eggs from eight CORT-treated females (1–9 eggs per female) and 34 eggs from 10 control females (1–7 eggs per female) at days 12–41 post-laying. Eggs were tested between 12.30 and 20.30 at temperatures of 20–35°C. This encompasses temperatures at which embryonic metabolic effort is directed primarily, but not solely, to maintenance (less than 26°C) or to growth and development (greater than or equal to 26°C), with more heart beats needed to complete embryogenesis at cooler temperatures [8]. Eggs were initially measured for heart rate at two temperatures ($n = 18$), but logistics constrained subsequent measurements to a single temperature ($n = 44$).

The heart rate monitor and eggs (within their incubation tub) were allowed 30 min to reach the testing temperature prior to measuring heart rates. An egg was placed on the monitor, and the external egg (and background monitor) temperature was measured using an infrared thermometer (Minitemp MT6; Raytek, Santa Cruz, CA, USA). Eggs were then weighed (nearest 0.01 g).

(d) Statistical analyses

We analysed effects of maternal CORT on embryo heart rate using three separate linear models, with embryonic heart rate (beats min^{-1}) as the dependent variable. Our first model included data across our entire temperature range (20–35°C). We then divided the dataset into eggs measured at primarily maintenance relevant (less than 26°C [8]; $n = 49$ measures of 42 eggs) and developmentally relevant (greater than or equal to 26°C [8]; $n = 31$ measures of 31 eggs) temperatures and analysed these groups separately. All models included maternal treatment (CORT or control), temperature, egg mass, time of day measurement was taken and days since laying as covariates, with an interaction term of treatment * temperature. For the first overall model, maternal identity (nested within treatment) and egg identity were included as random effects to account for repeated representation (multiple eggs per mother and multiple measurements of some eggs). For the two split-temperature models, maternal identity (nested within treatment) was included as a fixed effect (the number of levels within eggs of CORT-treated mothers tested at greater than or equal to 26°C was too low for a random effect to adequately estimate the distribution [10]). Egg identity was included as a random effect for only the model of eggs measured at less than 26°C as there were no eggs tested more than once at greater than or equal to 26°C.

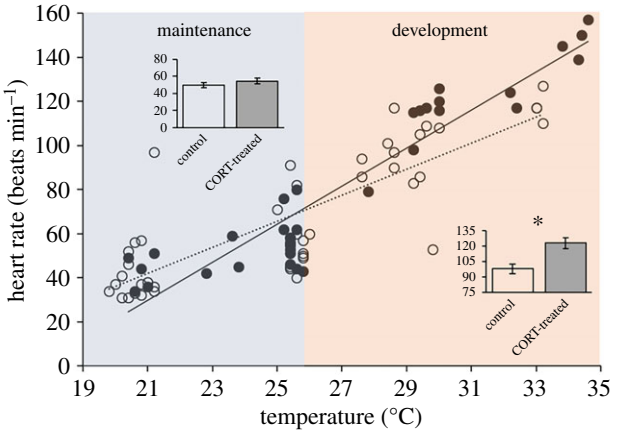

Figure 1. Main figure shows the relationship between embryo heart rate (beats min^{-1}) and temperature for eastern fence lizard (*S. undulatus*) eggs obtained from control (open circles, dotted line; $n = 34$) and CORT-treated (solid circles, solid line; $n = 28$) mothers. Lines are best fit trend lines. Shading represents temperatures at which heart beats contribute primarily to maintenance (less than 26°C, left panel) or development (greater than or equal to 26°C, right panel). The two outlying data points do not qualitatively change the statistical results. Insets show the mean (±1 s.e.) heart rate of embryos from control and CORT-treated mothers at maintenance relevant (upper left) and developmentally relevant (lower right) temperatures. *$p < 0.05$. (Online version in colour.)

Non-significant terms (egg mass, time, days since laying, treatment * temperature (only in the divided datasets); $p > 0.07$) were omitted from final models to preserve degrees of freedom. Retaining these factors did not qualitatively change the results. There are two anomalous data points (figure 1) from the control treatment. Omitting these did not qualitatively change the results, so we retained these in our final model for completeness of data. All analyses were conducted in JMP Pro 13 (SAS Institute Inc., Cary, NC, USA) with $\alpha = 0.05$. *$p < 0.001$.

3. Results

Embryonic heart rate was affected by an interaction between maternal treatment and temperature (treatment * temperature: $F_{1,38} = 5.42$, $p = 0.025$; treatment: $F_{1,10} = 0.04$, $p = 0.845$; temperature: $F_{1,38} = 185.46$, $p < 0.001$). Embryos from CORT-treated mothers exhibited greater increases in heart rates with increasing temperature than those from control mothers (figure 1).

Across maintenance relevant temperatures (less than 26°C), embryo heart rate increased with temperature (temperature: $F_{1,14} = 6.49$, $p = 0.023$) and was not affected by maternal treatment (treatment: $F_{1,32} = 0.31$, $p = 0.579$). Across developmentally relevant temperatures (greater than or equal to 26°C), embryo heart rate also increased with temperature (temperature: $F_{1,1} = 8.51$, $p = 0.009$), but embryos from CORT-treated mothers had higher heart rates than did embryos from control mothers (treatment: $F_{1,1} = 15.66$, $p < 0.001$; figure 1 insets).

4. Discussion

Embryos from CORT-treated mothers had more temperature-sensitive heart rates, and relatively higher heart rates at temperatures primarily associated with growth and development (greater than or equal to 26°C), than embryos from

control mothers (figure 1). There was no difference in heart rates at temperatures primarily associated with maintenance functions (less than 26°C; [8]). Since higher heart rate can result in faster development and shorter time to hatching [8], effects of maternal CORT on embryonic heart rate are likely to speed development of embryos and result in earlier hatching, at no cost to maintenance.

The effect of maternal CORT on offspring heart rate could be a physiological side effect of high maternal CORT, and the increased thermal sensitivity of embryos from CORT-treated mothers could incur important costs by decreasing their thermal tolerance range [11]. For example, rapid increases in heart rate at higher temperatures could result in offspring reaching or exceeding their physiological performance threshold. Greater metabolic rates at higher temperatures could create hypoxic conditions because of embryonic oxygen demand [12,13], increasing embryonic mortality. Embryos of control mothers, whose heart rate was less affected by temperature, may be better suited to warming events than those of mothers with elevated CORT. Consequently, offspring of stressed mothers may in fact be maladapted to future environmental change scenarios [14], such as global warming.

Despite potential costs, the effect of maternal CORT on offspring heart rate may be an adaptive response to particular environmental stressors experienced by wild lizards [14]. For example, earlier hatching of eggs, which is a vulnerable life stage, may reduce the chance of desiccation or overheating under drought conditions or help avoid oophagous predators (e.g. [15]). The predatory red imported fire ant, *Solenopsis invicta*, is abundant at our study sites [16]. Encounters with these venomous ants elevate CORT concentrations of lizards (including during gestation) [17]. Females with elevated CORT produce eggs with higher yolk CORT [4], and embryos with elevated heart rates (this study). Another study using a similar protocol found that offspring from CORT-treated mothers hatched earlier than those from control mothers ([4]; we could not measure incubation duration of measured eggs in this study owing to unexpected egg mortality). Since fire ants can depredate eggs but pose a lower threat to juveniles [18], it seems likely that offspring from CORT-elevated mothers that develop faster and hatch earlier would be less vulnerable to fire ant predation.

Our results demonstrate that maternal CORT can increase the thermal sensitivity of an important physiological trait, heart rate, in embryos. This maternal effect could speed embryo development and time to hatching, minimizing time spent in the vulnerable egg stage, but could make embryos more susceptible to negative effects of increased temperatures associated with global climate change. Further examination of the costs and/or benefits of maternal CORT on heart rate at the embryonic and subsequent life stages will be critical to understanding the potential adaptive significance of maternal CORT.

Ethics. This research was approved by the Institutional Animal Care and Use Committees at The Pennsylvania State University (no. 44595).

Data accessibility. Data have been uploaded to Penn State's data repository, ScholarSphere, https://scholarsphere.psu.edu/concern/generic_works/zcr56n3390.

Authors' contributions. D.A.S.O. and T.L. conceived and designed the project. D.A.S.O., J.J.H., D.C.E., K.J.M. and H.G. carried out the experiment. D.A.S.O. analysed the data and wrote the manuscript, with support from T.L. and M.J.S. All authors provided edits and approved the final manuscript, and all authors agree to be held accountable for the work therein.

Competing interests. We have no competing interests.

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