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Effects of Prenatal Environment on Phenotype Are Revealed by Postnatal Challenges: Embryonic Hormone Exposure, Adrenocortical Function, and Food in Seabird Chicks

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ABSTRACT

The interaction between prenatal environments and postnatal environments is an important source of phenotypic variability. We examined the ability of prenatal steroid exposure and postnatal energy restriction to explain adrenocortical function and fledging age in captive seabird chicks. We proposed and tested two hypotheses: (1) the strength of prenatal effects is attenuated by challenging postnatal environments (postnatal override) and (2) the strength of prenatal effects increases with the severity of postnatal challenges (postnatal reveal). We reared common murre (*Uria aalge*) chicks and measured prenatal exposure to corticosterone (CORT) and testosterone (T) from allantoic waste. Adrenocortical function was assessed after 10 d of ad lib. feeding and then after 5 and 10 d on controlled diets. Postnatal override predicts that prenatal steroids will explain more phenotypic variation before implementation of energy restriction; postnatal reveal predicts that the contribution of prenatal steroids will increase with duration and severity of energy restriction. Energy restriction increased secretion of baseline CORT and the adrenocortical response to the standardized stressor of handling and restraint. The ability of prenatal steroids to explain baseline CORT increased with duration of energy restriction, and for day 20 free baseline CORT, there was a significant interaction between kilojoules per day and prenatal CORT levels; severity of restriction strengthened the relationship between prenatal hormone levels and postnatal hormone levels. Both maximum CORT at day 20 and fledging age were best explained by diet treatment and day 15 or day 20 baseline CORT, respectively. Overall, prenatal CORT increased fledging age and baseline secretion of CORT, while prenatal T decreased them. However, prenatal effects on adrenocortical function were apparent only under the energy restriction conditions. Thus, we found some support for the postnatal reveal hypothesis; our results suggest that some prenatal effects on phenotype may be more likely to manifest in challenging postnatal environments.

Keywords: corticosterone, testosterone, prenatal steroids, stress response, fledging, food restriction, murre.

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Introduction

It is a classic tenet of biology that phenotype reflects the interaction of genes and environment. In particular, aspects of the early environment, both prenatal and postnatal, can have long-term consequences. However, environmental contributions to phenotype reflect a combination of not only prenatal and postnatal factors but also their interactions. There is a growing literature demonstrating interactions between prenatal environments and postnatal environments, such that prenatal effects can have different consequences for phenotype, depending on the environment into which offspring emerge (Monaghan 2008; Sheriff and Love 2013). An important aspect of phenotype that is shaped by the interaction of pre- and postnatal factors is the ability of an organism to cope with developmental transitions and physiological challenges. This ability is mediated by adrenocortical function, the secretion of glucocorticoids from the adrenal glands in response to acute or prolonged stressful stimuli (Wingfield and Kitaysky 2002; Crespi et al. 2012). Variation in adrenocortical activity can have substantial implications for physiology and behavior and thereby potentially fitness (Breuner et al. 2008; Bonier et al. 2009). Here we examine contributions of pre- and postnatal environments to interindividual variation in adrenocortical function and developmental transitions of a long-lived vertebrate, the common murre (Aves, *Uria aalge*; Pontoppidan 1763).

Sources of Environmental Variability

In animals, much of the variation in the prenatal environment is generated by exposure to endogenously produced or maternally provided biomolecules, including antibodies, nutrients, and hormones (Mousseau and Fox 1998). Variation in prenatal hormone exposure can be particularly powerful at programming, or

permanently altering, multiple aspects of offspring phenotype (Fowden and Forhead 2009; Bertram and Hanson 2010). Embryonic exposure to androgens and glucocorticoids has received particular attention in vertebrates. Cross-generational transmission and embryonic production of both of these steroid hormones fluctuate in response to the internal and external environment experienced by mothers and embryos; they both affect traits relevant to fitness, such as growth, behavior, and endocrine activity, and, particularly relevant for this study, both have been shown to affect avian adrenocortical function (Sockman and Schwabl 2001; Groothuis et al. 2005; Henriksen et al. 2011; Schoech et al. 2011). The phenotypic impacts of prenatal experiences such as hormone exposure may be altered by challenging postnatal environments, which tend to force physiological trade-offs and alter resource allocation during development (Metcalfe and Monaghan 2001; Monaghan 2008).

Variation in the postnatal environment is more directly controlled by external biotic and abiotic factors, including climate, predation pressure, and food availability, though the influence of these factors may still be mediated by parents (Kitaysky et al. 2001*a*; Monaghan 2008; Love et al. 2012; Sheriff and Love 2013). Food availability is a particularly important aspect of the postnatal environment because food restriction forces changes in allocation of limited resources toward growth (Benowitz-Fredericks and Kitaysky 2006; Hou et al. 2011), alters adrenocortical activity (Kitaysky et al. 2001*a*, 2006), and can have other important effects on phenotype (Monaghan 2008).

Are Prenatal Effects Revealed or Overridden?

Understanding relationships between prenatal environments and postnatal environments is critical to interpreting manipulations of either environment and understanding the results in the context of selection. While many studies have focused on the fitness consequences of matches and mismatches between the maternal environment and the offspring's postnatal environment (Love and Williams 2008; Monaghan 2008; Sheriff and Love 2013), we are still missing a more basic framework for predicting the contexts in which variation in the prenatal environment will manifest at all in offspring phenotype. For example, a lack of consistent effects of experimental manipulations has generated questions about the importance of variation in maternal effects such as yolk steroid deposition, despite the probability that the consequences are context dependent (Benowitz-Fredericks et al. 2013). We propose two nonexclusive study hypotheses to describe the context-dependent manifestation of prenatal effects in different postnatal environments. Specifically, we propose that for phenotypic traits affected by both prenatal environments and postnatal environments, the postnatal environment may affect manifestation of the prenatal effects in one of two ways: (1) postnatal override, where the impact of prenatal effects on a trait is attenuated by challenging postnatal environments, or (2) postnatal reveal, where the impact of prenatal effects on a trait increases with the severity of postnatal challenges (fig. 1). Although these broad hypotheses about the potential interactions between prenatal environments and postnatal environments have not been explicitly articulated before, evidence exists for both scenarios (Mainwaring et al. 2010; Vergauwen et al. 2011; Kim et al. 2013). While the strength and direction of the relationship are likely to differ for different combinations of traits and aspects of pre- and postnatal environments, both hypotheses have implications for the interpretation of experimental results. For example, prenatal variables that act as potent modifiers of phenotype in lab conditions lacking behavioral or physiological challenges may often be irrelevant in free-living animals if they are usually combined with environmental constraints that obscure them (postnatal override). Similarly, prenatal variables identified as irrelevant in the lab may contribute significantly to phenotype in a resource-limited environment (postnatal reveal). Thus, the postnatal override/reveal framework is a useful tool for assessing the interactions between prenatal effects and postnatal environment.

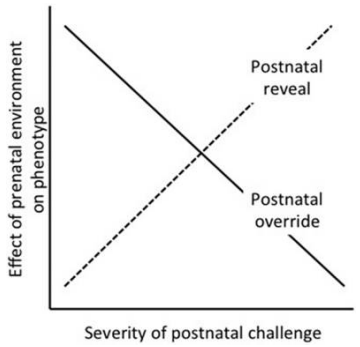

Figure 1. Schematic of two competing hypotheses about the potential effects of the postnatal environment on the manifestation of a prenatal effect. While some traits may be affected solely by either prenatal environments or postnatal environments, in cases where the two environments interact to affect a phenotypic trait, the postnatal override hypothesis (solid line) posits that the contribution of the prenatal environment to phenotype is reduced by challenging postnatal environments. In contrast, the postnatal reveal hypothesis (dashed line) posits that the impact of the prenatal environment is magnified by postnatal challenges.

Goals and Predictions

The goal of this study was to test the roles of postnatal override and postnatal reveal in the context of generating variation in adrenocortical function and initiation of a developmental transition (fledging) in captive chicks of a seabird, the common murre. Murres are colonial seabirds that lay a single egg and rear their single semiprecocial chick at the colony (Ainley et al. 2002). We measured allantoic corticosterone (CORT) and testosterone (T) at hatch as integrative measures of in ovo hormone excretion and thus indicators of the prenatal endocrine environment (Benowitz-Fredericks et al. 2005) and manipulated posthatch energy intake to generate variation in the postnatal environment. Availability of food is a highly variable aspect of the postnatal environment for seabird chicks, and it can shape multiple aspects of phenotype, including adrenocortical function (Kitaysky et al. 2001*a*) and fledging (reviewed in Crespi et al. 2012). The pheno-

typic traits we measured quantified components of adrenocortical function: total and free baseline CORT levels and total acute stress–induced CORT levels (Romero 2004), in addition to the age at which chicks chose to fledge from their nests. Fledging age for seabirds is variable and is a major life-history transition that not only can be affected by postnatal food availability but also may be mediated by adrenocortical function (Quillfeldt et al. 2007; Sprague and Breuner 2010; Riou et al. 2012).

In the context of our study, postnatal override predicts that the ability of prenatal steroids to explain variation in adrenocortical function will (*a*) decrease with duration of postnatal challenge (i.e., prenatal steroids will better explain interindividual variation in the adrenocortical function before implementation of restricted diets than after implementation of restricted diets) and (*b*) decrease with the strength of the postnatal challenge (i.e., at any single time point, relationships between prenatal steroids and phenotypic traits will be weaker in chicks with more restricted diets). Alternatively, postnatal reveal predicts the opposite: the ability of prenatal steroid levels to explain variation in phenotypic traits will increase with the duration and severity of postnatal nutritional stress.

Material and Methods

Egg and Chick Husbandry

Egg collection, incubation conditions, and chick husbandry and feeding have been previously described in detail (Benowitz-Fredericks and Kitaysky 2006). Briefly, in 2000, eggs of unknown lay dates were collected on the same day from incubating parents on a single ledge on Gull Island, a colony of free-living murres in the Gulf of Alaska, and transported to the University of Washington. Eggs were artificially incubated, and when chicks hatched (average duration of artificial incubation was 12 d; range = 9–14 d), they were housed in individual cages and hand-fed whole smelt (*Osmerus mordax*) and silverside (*Menidia menidia*) five times daily (with a daily vitamin supplement) for the duration of the study. Food was offered ad lib. for the first 10 d, and intake (kJ/d) was recorded; for the remainder of the study, each chick was placed on one of four controlled diets yielding experimental caloric intakes of 187 kJ/d ($n = 6$), 247 kJ/d ($n = 6$), 266 kJ/d ($n = 5$), or 353 kJ/d ($n = 5$; Benowitz-Fredericks and Kitaysky 2006). Chicks were assigned to treatment groups in order of hatching; thus, hatch dates were distributed evenly across treatment groups. Chicks were sexed after euthanasia by visual inspection of gonads.

Sample Collection

Collection of allantoic waste from eggs immediately after hatching has been previously described (Benowitz-Fredericks et al. 2005). Allantoic waste is the accumulated excreta from in ovo development that remains in egg shells after chicks have hatched and can provide an integrated measure of steroid metabolism during development (Benowitz-Fredericks et al. 2005). Each chick was bled for baseline CORT when it was 10, 15, and 20 d posthatch. On sampling days, postabsorptive chicks were taken quietly from their individual cages before feeding and were bled from the alar vein within 3 min of disturbance (thus preceding elevation of CORT in response to sampling-induced stress; Romero and Reed 2005). On day 20, chicks were subject to a standardized acute-stress series; they were bled for a baseline sample and then held in a cloth bag in between collection of 15-, 30-, and 60-min blood samples (maximum CORT being the highest concentration of CORT found in any of these samples). While it would have been valuable to assess maximum CORT before energy restriction as well as after implementation of controlled diets, the stress series was conducted only once because repetition of the acute-stress protocol results in a stressor that is no longer novel and can alter adrenocortical responsiveness (Lynn et al. 2013).

Fledging Behavior

Free-living common murre chicks fledge at night, at approximately 20 d of age (Cameron-McMillian et al. 2006), by leaping from their open cliff-face nests to the beach or water, often hundreds of feet below. They fledge before they are able to fly, and the exact age at fledging is variable, ranging from 16 to 30 d, with no difference in fledging age between the sexes (Nettleship and Birkhead 1985; Cameron-McMillian et al. 2006). Starting on day 20 posthatch, the evening after the acute-stress series samples were taken, we gave chicks the opportunity to fledge by leaving their cage open so that they could jump to the floor below (maximum height = 6 ft). A chick's cage was left open every night until the chick was found on the floor on two consecutive mornings, after which it was considered fledged and its cage was not left open again.

Sample Analysis

Allantoic waste was lyophilized, finely powdered and homogenized, and extracted and analyzed using radioimmunoassay as described earlier (Benowitz-Fredericks et al. 2005). Blood samples were centrifuged, and plasma was separated and frozen in two aliquots (one for CORT, one for CORT-binding globulin [CBG]; no CBG sample was taken on day 15) at −20°C until assays were run. For CORT, all samples were assayed in duplicate using the radioimmunoassay protocol described in Benowitz-Fredericks et al. (2008) and Kitaysky et al. (2003) with the Endocrine Sciences/Esoteric CORT antibody (B3-163; Calabasas Hills, CA; cross reactivity with other steroids less than 1%). Briefly, for each sample, 2,000 cpm of tritiated CORT was added to determine postextraction hormone recovery; final values were corrected for percent recovered. Steroids were extracted from 20 μL of plasma with dichloromethane, dried down under nitrogen, and reconstituted in phosphate-buffered saline. Samples were distributed across two assays, with sampling ages and treatments randomized across assays. Postextraction steroid recoveries ranged from 83% to 100% (average = 91%). Inter- and intraassay coefficients of variation were less than 2%. In order to calculate free baseline CORT (CBG was not measured for maximum CORT), binding capacity of plasma CBG was quantified for day 10 and day 20 samples as described in Shultz et al. (2008) and optimized for the common murre. Briefly, plasma was stripped of

endogenous CORT using charcoal and then incubated in triplicate with tritiated CORT; bound CORT was retained on a glass fiber filter that was counted in scintillation fluid. Optimal plasma dilution for murre chicks was 1:194, and incubation time was 3 h; the dissociation constant, K_d, was 2.98 ± 0.25. Free CORT was calculated as described by Barsano and Baumann (1989). Plasma T was not detectable (assay sensitivity at 1.95 pg/sample; antibody T-3003, Research Diagnostics), even in pooled samples of more than 100 μL of plasma.

Statistical Analysis

To assess the relative contributions of prenatal environment (as indicated by allantoic steroids) and postnatal environment (experimental food intake) on phenotype, we used an information-theoretic approach with the Akaike information criterion corrected for finite sample sizes (AICc; Burnham and Anderson 2002). We generated a candidate set of models based on a priori identified competing hypotheses with biological significance (table 1). Candidate model sets for baseline CORT levels had a similar structure. For maximum CORT at day 20, we added models including day 10, day 15, and day 20 baseline CORT because previous studies in seabirds have suggested that prior or recent adrenocortical activity may be the best predictor of maximum CORT secretion (Kitaysky et al. 2007; Benowitz-Fredericks et al. 2008). We included total baseline CORT because it has been clearly established that it changes in seabirds as a consequence of food availability (Kitaysky et al. 2007) and free baseline CORT because it not only fluctuates in response to environmental conditions such as food availability (Shultz and Kitaysky 2008) but also has been specifically linked to variation in seabird fledging age (Sprague and Breuner 2010).

For fledging age we used the same set of models as for baseline CORT but also included day 20 baseline CORT because there is evidence that fledging decisions may be regulated by adrenocortical function (reviewed in Crespi et al. 2012) and early food intake (day 0–10) because early developmental trajectories can also affect life-history transitions (Day and Rowe 2002). We did not include body mass in our analyses because changes in body

Table 1: Candidate set of models for effects on total and free baseline corticosterone (CORT) at chick ages 10 d (models 1–5, 7–10), 15 d, and 20 d (models 1–4, 6–10); for maximum CORT at day 20 (models 1–4, 6–13); and for fledging age (models 1–10, 13–15)

Model	Parameters	Biological significance
1	Intercept only	Null model (models not fitting data)
2	Sex	Expanded null model; only sex determines phenotype
3	Hatch date	Expanded null model; only hatch date determines phenotype
4	Allantoic CORT + allantoic T	Prenatal environment determines phenotype
5	Energy intake early[a]	Postnatal environment determines phenotype
6	Energy intake late[a]	Postnatal environment determines phenotype
7	Allantoic CORT + allantoic T + energy intake[b]	Pre- and postnatal environments determine phenotype additively
8	Allantoic CORT + energy intake[b] + allantoic CORT × energy intake[b]	Different postnatal environments lead to differential expression of prenatal effects (i.e., support for postnatal reveal or postnatal override); CORT is the most relevant prenatal hormone
9	Allantoic T + energy intake[b] + allantoic T × energy intake[b]	Different postnatal environments lead to differential expression of prenatal effects (i.e., support for postnatal reveal or postnatal override); T is the most relevant prenatal hormone
10	Allantoic CORT + allantoic T + energy intake[b] + allantoic CORT × energy intake[b] + allantoic T × energy intake[b]	Different postnatal environments lead to differential expression of prenatal effects (i.e., support for postnatal reveal or postnatal override); both CORT and T are relevant prenatal hormones
11	Total baseline CORT (day 10)	Early adrenocortical activity is the best predictor
12	Total baseline CORT (day 15)	Recent adrenocortical activity is the best predictor
13	Total baseline CORT (day 20)	Current adrenocortical activity (total CORT) is the best predictor
14	Free baseline CORT (day 20)	Current adrenocortical activity (free fraction of CORT) is the best predictor
15	Maximum CORT (day 20)	Current adrenocortical capacity is the best predictor

Note. T = testosterone.

[a]Energy intake early in the model selection for 10-d baseline CORT (i.e., before the postnatal challenge) and for 20-d maximum CORT was calculated as kilojoules per day consumed while fed ad lib.; energy intake late in the model selection for 15- and 20-d baseline CORT and for 20-d maximum CORT was calculated as kilojoules per day of the restricted diet treatments.

[2]Models 7–10 include energy intake early for 10-d baseline CORT and energy intake late for 15- and 20-d baseline CORT and 20-d maximum CORT.

mass in our study were driven by the daily energy intake (fig. 3 in Benowitz-Fredericks et al. 2005); thus, daily energy intake and body mass were highly autocorrelated. However, body mass at fledging varies dramatically across colonies and years for murres (e.g., Davoren and Montevecchi 2003) and is considered a less direct predictor of fitness in semiprecocial species such as murres, which fledge when they are many months from adult body size and mass (Ainley et al. 2002).

We proceeded with a model selection based on AICc values and multimodel averaging. We calculated relative variable importance and model-averaged parameter estimates from the set of top models selected by ΔAICc < 4, based on Burnham and Anderson (2001), which states that ΔAIC values >4 have considerably less support than those <4. We then examined support for the predictions of the postnatal override hypothesis and the postnatal reveal hypothesis via two lines of inquiry, both of which assessed the ability of prenatal environment (allantoic CORT and/or allantoic T) to explain phenotypic traits at day 10 (before experimental manipulation of the postnatal environment) and at day 15 and day 20 (after imposition of postnatal challenges).

First, we considered the length of postnatal challenges. The postnatal override hypothesis was supported if there was a decreased influence of prenatal environment on chick phenotype (free and total baseline CORT, total maximum CORT) with duration of the postnatal challenge, that is, if allantoic hormones explained chick baseline CORT better before implementation of controlled diets (age 10 d) than after either 5 or 10 d of controlled diets (age 15 or 20 d). In contrast, the postnatal reveal hypothesis was supported if there was an increased influence of prenatal environment (allantoic hormones) on chick phenotype with duration of controlled diets. The influence of prenatal environment was assessed by whether selected candidate models included allantoic hormones (i.e., models 4, 7–10; table 1) and by the relative importance of allantoic CORT and allantoic T. Second, we considered the influence of the magnitude of postnatal challenges. This was the only way to test our hypotheses for the measures taken at only one time point: maximum CORT and fledging age. If the interaction model (i.e., model 7) was selected (i.e., ΔAICc < 4), we examined the relationship between prenatal environment and chick phenotype at the different levels of postnatal challenge by regression analysis of the allantoic hormones and diet treatment. The postnatal override hypothesis was supported if the more challenging postnatal environments (more restricted diets) yielded an increased slope in the relationship between prenatal environment and chick phenotype (both after 5 and 10 d of challenge). In contrast, the postnatal reveal hypothesis was supported if the most challenging postnatal environment resulted in a decreased slope between prenatal environment and chick phenotype.

Finally, to determine the direction of relationships between prenatal or postnatal variables and phenotypic traits, we relied on estimates of slopes for energy intake and the effects of both allantoic steroids on measures of CORT and fledging behavior in the best-performing models (excluding those containing interactions between the explanatory variables, where slope estimates are confounded by the interaction). R was used for all statistical analyses with packages AICcmodavg and MuMIn (R Core Team 2012).

Results

Effects of Diets on Baseline CORT

Energy intake at all stages was selected into the set of top models for baseline CORT (table 2). Both total and free baseline CORT increased with the duration and severity of energy restriction (but decreased from day 10 to day 20 in the highest–energy intake group; 353 kJ/d; fig. 2).

Role of Allantoic Steroids in Baseline CORT

A consistent pattern emerged for measures of baseline CORT. Experimental energy intake always affected CORT; however, the ability of allantoic steroids to explain variation grew stronger after the transition from ad lib. (day 10) to controlled (days 15 and 20; tables 2, 3) energy intake.

Total Baseline CORT

At 10 d of age, selected top models for baseline total CORT included the null model (intercept only), expanded null models (hatch date, sex), and a simple postnatal environment model (ad lib. food intake; table 2). The strongest model including prenatal environment (e.g., allantoic steroids) was >5 AICc away from the best model and accounted for only 3% of the model weights (table 2). After 5 d of controlled energy intake, the set of top models included prenatal environment in an interaction model with postnatal environment, though a simple postnatal environment model (controlled energy intake) scored best (table 2). Although the interaction between prenatal environments and postnatal environments at day 15 reflected different slopes among treatments, those slopes were not consistently associated with the level of restriction of daily energy intake. At day 20, after 10 d of restricted intake, prenatal environment remained in the top models in an additive model with postnatal environment, while the simple postnatal environment model (controlled energy intake) remained the best model (table 2). Overall, relative importance of allantoic hormones increased through the postnatal challenge, from 0.04 (day 10), to 0.15 (day 15), to 0.20 (day 20) for allantoic CORT and from 0.04 (day 10), to 0.08 (day 15), to 0.19 (day 20) for allantoic T (table 3).

Free Baseline CORT

Free baseline CORT showed a stronger version of the same pattern. Although there was limited evidence (the null model was selected as the top model, ΔAICc $= 0$) for a role of prenatal environment on day 10 (ΔAICc $= 3.54$), at day 20 a pre/postnatal environment additive model scored best among the top models (ΔAICc $= 0$), and a pre/postenvironment

Table 2: Model selection results for effects of pre- and postnatal environment on baseline corticosterone (CORT; free and total), maximum stress-induced CORT, and fledging age

Response and model	df	Log likelihood	AICc	ΔAICc	AICc weights
Total baseline CORT (day 10):					
Intercept only	**2**	**−2.33**	**9.28**	**.00**	**.46**
Hatch date	**3**	**−1.68**	**10.70**	**1.42**	**.23**
Energy intake	**3**	**−2.23**	**11.79**	**2.51**	**.13**
Sex	**3**	**−2.25**	**11.83**	**2.55**	**.13**
Allantoic CORT + allantoic T	4	−2.28	14.92	5.63	.03
Allantoic T + energy intake + allantoic T × energy intake	5	−1.45	16.64	7.36	.01
Allantoic CORT + energy intake + allantoic CORT × energy intake	5	−1.86	17.47	8.19	.01
Allantoic CORT + allantoic T + energy intake	5	−2.18	18.12	8.84	.01
Allantoic CORT + allantoic T + energy intake + allantoic CORT × energy intake + allantoic T × energy intake	7	−1.31	24.62	15.34	.00
Total baseline CORT (day 15):					
Energy intake	**3**	**−3.52**	**14.38**	**.00**	**.72**
Allantoic CORT + energy intake + allantoic CORT × energy intake	**5**	**−2.24**	**18.24**	**3.86**	**.10**
Intercept only	2	−7.63	19.88	5.50	.05
Hatch date	3	−6.47	20.27	5.89	.04
Allantoic CORT + allantoic T + energy intake	5	−3.34	20.43	6.06	.03
Allantoic T + energy intake + allantoic T × energy intake	5	−3.35	20.44	6.06	.03
Sex	3	−7.09	21.52	7.14	.02
Allantoic CORT + allantoic T + energy intake + allantoic CORT × energy intake + allantoic T × energy intake	7	−1.42	24.84	10.46	.00
Allantoic CORT + allantoic T	4	−7.43	25.22	10.84	.00
Total baseline CORT (day 20):					
Energy intake	**3**	**1.33**	**4.68**	**.00**	**.74**
Allantoic CORT + allantoic T + energy intake	**5**	**2.79**	**8.17**	**3.49**	**.13**
Allantoic CORT + energy intake + allantoic CORT × energy intake	5	2.15	9.44	4.76	.07
Allantoic T + energy intake + allantoic T × energy intake	5	1.91	9.93	5.24	.05
Intercept only	2	−5.52	15.66	10.98	.00
Allantoic CORT + allantoic T + energy intake + allantoic CORT × energy intake + allantoic T × energy intake	7	2.96	16.07	11.39	.00
Sex	3	−5.12	17.57	12.89	.00
Hatch date	3	−5.50	18.33	13.65	.00
Allantoic CORT + allantoic T	4	−5.28	20.91	16.23	.00
Free baseline CORT (day 10):					
Intercept only	**2**	**71.82**	**−139.00**	**.00**	**.37**
Hatch date	**3**	**72.93**	**−138.52**	**.48**	**.29**
Sex	**3**	**72.07**	**−136.80**	**2.20**	**.12**
Energy intake	**3**	**72.02**	**−136.71**	**2.29**	**.12**
Allantoic CORT + allantoic T	**4**	**72.90**	**−135.46**	**3.54**	**.06**
Allantoic CORT + allantoic T + energy intake	5	72.96	−132.17	6.83	.01
Allantoic T + energy intake + allantoic T × energy intake	5	72.85	−131.95	7.05	.01
Allantoic CORT + energy intake + allantoic CORT × energy intake	5	72.65	−131.55	7.45	.01

Table 2 (*Continued*)

Response and model	df	Log likelihood	AICc	ΔAICc	AICc weights
Allantoic CORT + allantoic T + energy intake + allantoic CORT × energy intake + allantoic T × energy intake	7	73.33	−124.66	14.34	.00
Free baseline CORT (day 20):					
Allantoic CORT + allantoic T + energy intake	**5**	**75.45**	**−137.15**	**.00**	**.55**
Energy intake	**3**	**71.40**	**−135.47**	**1.68**	**.24**
Allantoic CORT + energy intake + allantoic CORT × energy intake	**5**	**74.26**	**−134.76**	**2.39**	**.17**
Allantoic T + energy intake + allantoic T × energy intake	5	72.14	−130.53	6.62	.02
Allantoic CORT + allantoic T + energy intake + allantoic CORT × energy intake + allantoic T × energy intake	7	76.15	−130.30	6.85	.02
Intercept only	2	65.06	−125.49	11.66	.00
Hatch date	3	65.59	−123.86	13.29	.00
Sex	3	65.46	−123.59	13.56	.00
Allantoic CORT + allantoic T	4	66.13	−121.92	15.23	.00
Maximum CORT (day 20):					
Energy intake	**3**	**5.50**	**−3.67**	**.00**	**.53**
Total baseline CORT (day 15)	**3**	**4.20**	**−1.07**	**2.59**	**.14**
Allantoic CORT + energy intake + allantoic CORT × energy intake	5	6.70	.35	4.02	.07
Intercept only	2	1.93	.76	4.43	.06
Total baseline CORT (day 20)	3	2.99	1.36	5.03	.04
Allantoic T + energy intake + allantoic T × energy intake	5	6.19	1.37	5.04	.04
Allantoic CORT + allantoic T + energy intake	5	6.13	1.48	5.15	.04
Total baseline CORT (day 10)	3	2.78	1.77	5.44	.03
Sex	3	2.03	3.27	6.93	.02
Hatch date	3	1.95	3.43	7.10	.02
Allantoic CORT + allantoic T	4	2.30	5.75	9.42	.00
Allantoic CORT + allantoic T + energy intake + allantoic CORT × energy intake + allantoic T × energy intake	7	6.84	8.31	11.98	.00
Fledging age:					
Energy intake late	**3**	**−41.89**	**91.11**	**.00**	**.43**
Allantoic T + energy intake + allantoic T × energy intake	**5**	**−40.01**	**93.76**	**2.66**	**.11**
Allantoic CORT + allantoic T + energy intake	**5**	**−40.04**	**93.83**	**2.72**	**.11**
Free baseline CORT (day 20)	**3**	**−43.35**	**94.04**	**2.93**	**.10**
Total baseline CORT (day 20)	**3**	**−43.54**	**94.41**	**3.30**	**.08**
Allantoic CORT + energy intake + allantoic CORT × energy intake	5	−40.79	95.33	4.22	.05
Allantoic CORT + allantoic T + energy intake + allantoic CORT × energy intake + allantoic T × energy intake	7	−36.96	95.92	4.81	.04
Intercept only	2	−46.13	96.88	5.77	.02
Maximum CORT (day 20)	3	−45.42	98.16	7.06	.01
Allantoic CORT + allantoic T	4	−44.27	98.90	7.79	.01
Energy intake early	3	−45.87	99.08	7.97	.01
Hatch date	3	−46.10	99.54	8.43	.01
Sex	3	−46.13	99.58	8.48	.01

Note. Models with ΔAICc < 4 are shown in bold. df = degree of freedom; AICc = Akaike information criterion corrected for finite sample sizes.

interaction model was also selected into the top models (ΔAICc = 2.39). Relative importance of allantoic hormones increased in a pattern that was similar to that for total baseline CORT (allantoic CORT: day 10 = 0.08, day 20 = 0.74; allantoic T: day 10 = 0.09, day 20 = 0.59; table 3). Further analysis of the interaction between prenatal environment and postnatal environment at 20 d revealed that the slope of the positive relationship between allantoic CORT and free base-

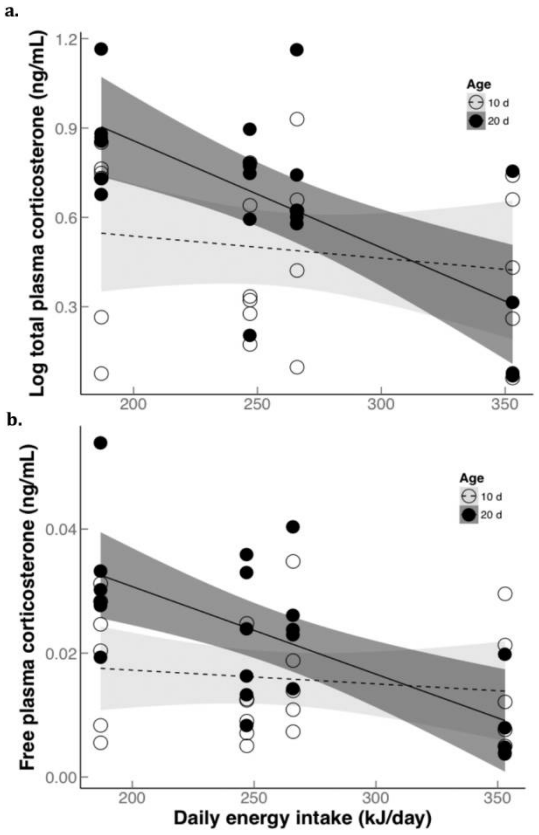

Figure 2. Total (*a*) and free (*b*) baseline corticosterone (CORT) in captive common murre chicks. Lines are regression lines, and shading indicates 95% confidence intervals. CORT was not different among treatment groups at day 10, before implementation of controlled diets. For illustrative purposes, treatments are indicated as chick daily energy intake. At day 20 posthatch, after 10 d of controlled diets, there was a negative relationship between energy intake and both total and free baseline CORT. Baseline CORT increased in the three most restricted groups (187, 247, and 266 kJ/d) but decreased in the least restricted group (353 kJ/d).

line CORT was significantly different from 0 ($P = 0.022$; adjusted $r^2 = 0.71$) only in the most restricted group (187 kJ/d; fig. 3).

Maximum CORT (Day 20)

Adrenocortical responsiveness to the acute stress of handling and restraint was tested once at day 20 posthatch; thus, it was not possible to assess whether the role of allantoic steroids changed after imposition of diet treatments. However, roles for allantoic steroids or an interaction between allantoic steroids and diet were not strongly supported. Instead, top models for maximum CORT included only energy intake and baseline CORT at 15 d (table 2).

Fledging Age

No chick opted to fledge before day 26 posthatch. Top models for fledging age included energy intake, a model combining additive effects of allantoic T and diet with the interaction between allantoic T and diet, additive effects of energy intake and allantoic steroids, and CORT at day 20 (free and total CORT were similarly weighted; table 2). However, further investigation of the interactions yielded no consistent effect of severity of energy restriction.

Directionality of Effects

Values of total and free baseline CORT, maximum CORT, and fledging age are summarized by treatment in table 5. Neither energy intake nor allantoic steroids showed any strong relationships with CORT before imposition of controlled diets on day 10 (table 2). After day 10, energy intake was negatively correlated with all CORT measures but positively associated with fledging age (table 4). Allantoic CORT and allantoic T had opposite relationships with baseline CORT at day 20 and fledging but had no effect on maximum CORT (table 4). High allantoic CORT was associated with higher baseline CORT and later fledging, and higher allantoic T was associated with lower plasma CORT and earlier fledging (table 4). A schematic summary of these results is shown in table 6.

Discussion

Summary

In this study we examined the relative contributions of pre- and postnatal environments to aspects of phenotype in seabird chicks. Specifically, we assessed phenotypic traits related to adrenocortical function (baseline and maximum CORT) and fledging—a postnatal behavior associated with a major developmental transition. In our sample of captive seabird chicks, these traits were strongly affected by postnatal food availability; a postnatal challenge in the form of energetic restriction increased baseline and maximum CORT secretion (table 4; fig. 2) and promoted chicks' readiness to fledge (table 4; fig. 4). Interestingly, when considering contributions of the prenatal environment, we found support for the postnatal reveal hypothesis (fig. 1): indicators of the prenatal environment (allantoic steroids) did not contribute significantly to explaining phenotype until after the imposition of a postnatal challenge (tables 2, 6), and there is evidence that the strength of their contribution increased with the severity of challenge (tables 2, 3; fig. 3). Our results emphasize the importance of interpreting studies of prenatal effects in light of the postnatal environment.

Adrenocortical Function

Baseline CORT. Changes in baseline CORT levels over the course of the experiment revealed a strong effect of postnatal energy intake (fig. 2) but also yielded two lines of evidence that effects of the prenatal environment on CORT production can be

Table 3: Model-averaged parameter estimates with confidence intervals (CIs; 2.5% and 97.5%) and relative importance of predictor variables

Response and parameter	Relative importance	Estimate	Lower CI	Upper CI
Total baseline CORT (day 10):				
Hatch date	.23	.0655	−.0592	.1902
Energy intake early	.16	.0249	−.1047	.1544
Sex	.13	.0455	−.2055	.2965
Allantoic T	.04	.0036	−.1411	.1482
Allantoic CORT	.04	−.0128	−.1548	.1292
Allantoic T × energy intake early	.01	.1039	−.0920	.2998
Allantoic CORT × energy intake early	.01	.0745	−.1302	.2791
Total baseline CORT (day 15):				
Energy intake	.89	−.1971	−.3366	−.0575
Allantoic CORT	.15	.0435	−.1596	.2465
Allantoic CORT × energy intake late	.11	.1417	−.0674	.3508
Allantoic T	.08	−.1107	−.2657	.0443
Allantoic T × energy intake late	.04	−.0297	−.1833	.1240
Hatch date	.04	−.0338	−.2674	.1999
Sex	.02	−.1495	−.4624	.1634
Total baseline CORT (day 20):				
Energy intake late	.99	−.2189	−.3314	−.1064
Allantoic CORT	.20	.0403	−.1141	.1947
Allantoic T	.19	−.0718	−.1907	.0472
Allantoic CORT × energy intake late	.07	−.0753	−.2462	.0956
Allantoic T × energy intake late	.06	−.0092	−.1919	.1735
Sex	.00	−.1178	−.4038	.1683
Hatch date	.00	−.0131	−.1614	.1352
Free baseline CORT (day 10):				
Hatch date	.29	.0029	−.0013	.0071
Energy intake early	.15	.0028	−.0058	.0114
Sex	.12	.0012	−.0033	.0056
Allantoic T	.09	−.0018	−.0066	.0030
Allantoic CORT	.08	−.0018	−.0064	.0029
Allantoic T × energy intake early	.01	.0016	−.0051	.0083
Allantoic CORT × energy intake early	.01	.0008	−.0061	.0077
Free baseline CORT (day 20):				
Energy intake late	1.00	.0040	−.0022	.0103
Allantoic CORT	.74	−.0043	−.0088	.0001
Allantoic T	.59	−.0090	−.0134	−.0046
Allantoic CORT × energy intake late	.19	−.0045	−.0110	.0020
Allantoic T × energy intake late	.04	.0010	−.0062	.0083
Hatch date	.00	−.0028	−.0087	.0031
Sex	.00	−.0048	−.0164	.0068
Maximum CORT (day 20):				
Energy intake late	.68	−.1200	−.2136	−.0264
Total baseline CORT (day 15)	.14	.0979	.0025	.1934
Allantoic CORT	.12	−.0548	−.1938	.0841
Allantoic T	.09	−.0867	−.2251	.0518
Allantoic CORT × energy intake late	.07	.0685	−.0324	.1693
Allantoic T × energy intake late	.04	−.0426	−.1415	.0564
Total baseline CORT (day 20)	.04	−.0241	−.1745	.1264
Total baseline CORT (day 10)	.03	.0618	−.0400	.1636
Sex	.02	−.0422	−.2489	.1644
Hatch date	.02	−.0093	−.1150	.0964

Table 3 (*Continued*)

Response and parameter	Relative importance	Estimate	Lower CI	Upper CI
Fledging age:				
Energy intake late	.75	1.1271	.2847	1.9695
Allantoic T	.27	−.7198	−1.5561	.1166
Allantoic CORT	.21	.1711	−1.0764	1.4186
Allantoic T × energy intake late	.15	.3859	−.8135	1.5853
Free baseline CORT (day 20)	.10	−.9511	−1.7800	−.1222
Allantoic CORT × energy intake late	.09	−.9229	−1.7587	−.0870
Total baseline CORT (day 20)	.08	.9644	−.3139	2.2428
Maximum CORT (day 20)	.01	−.5040	−1.4143	.4064
Energy intake early	.01	−.3027	−1.2322	.6268
Hatch date	.01	−.0923	−1.0315	.8469
Sex	.01	.0167	−1.8280	1.8614

Note. Relative variable importance was calculated as the sum of weights of Akaike information criterion corrected for finite sample sizes over all models containing the parameter of interest.

revealed by postnatal challenges. First, the ability of allantoic steroids to explain variation in baseline CORT grew stronger after the transition from ad lib. food intake to controlled diets (tables 2, 6); second, the effect of allantoic steroids on baseline free CORT was stronger in the most energetically restricted treatment group (fig. 3). Despite the small sample size, these results suggest that some effects of the prenatal environment would not be apparent in all postnatal environments and, in the case of baseline CORT, some prenatal influences on phenotype may manifest only in response to challenges.

Glucocorticoids are generally involved in regulating behavioral and physiological traits associated with maintaining energy balance (Kitaysky et al. 2001*b*, 2003; Lynn et al. 2003; Loiseau et al. 2008; Schultner et al. 2013*a*, 2013*b*) and have specifically been shown to respond to short-terms changes in food availability in adult murres (Barrett et al. 2015). Thus, it is not surprising that the imposition of energetic restriction increased adrenocortical activity in chicks. Though experimental diets were well within the range of food intake reported for free-living common murre chicks (Benowitz-Fredericks and Kitaysky 2006), the transition from ad lib. food intake to controlled diets induced an increase in baseline total CORT after 5 d (tables 2, 3). After 10 d, both total and free baseline CORT were elevated in all groups except for the least restricted (fig. 2), suggesting that despite the lack of a true ad lib. control group, 353 kJ/d did not compromise energy balance of 20-d-old chicks sufficiently to induce an adrenocortical response. The lack of change in hypothalamo-pituitary adrenal (HPA) function in this group (table 5) provided useful confirmation that changes in the more restricted groups were due to treatment and not age.

Responsiveness to Acute Stressors. We found no strong evidence that maximum CORT at day 20 was affected by the prenatal environment. Maximum CORT in response to a standardized stressor (a key component of the acute adrenocortical response, or glucocorticoid reactivity) has been generally interpreted as a reflection of an animal's ability to shift resource allocation toward self-maintenance (Landys et al. 2006; Breuner et al. 2008). Studies in seabirds have provided compelling evidence that, at least in some species, maximum CORT reflects recent (on the scale of days to weeks) stress, specifically as induced by aspects of the environment that reflect food intake (Kitaysky et al. 2010). These findings in seabirds corroborate biomedical studies using rats that demonstrate that recent stress facilitates upregulated glucocorticoid responsiveness (Dallman et al. 1992; Bhatnagar and Vining 2003). Because we measured maximum CORT at only day 20 and cannot compare to levels before the imposition of restricted diets, we cannot rule out the possibility that the prenatal environment might affect maximum CORT in the absence of postnatal challenges. However, based on the lack of explanatory power for either allantoic steroids themselves or their interaction with energy intake (which we would expect to find—with stronger relationships in more restricted groups—if severity of restriction overrode prenatal effects on maximum CORT), the effect of energy restriction appears to override any potential prenatal effects and seems to be the strongest predictor of adrenal response to acute stress of capture and handling.

Fledging

In free-living murres, there is an extended period of parental care postfledging, and the act of fledging is triggered by an interaction between parents and chicks, with adults in the nest or from the water below vocally encouraging chicks to jump (Ainley et al. 2002). Thus, our measure of fledging age reflects only the chicks' contribution to this transition. No chick fledged before day 26; however, we did not provide opportunity to fledge until day 20, whereas with the encouragement of their parents, free-living chicks fledge as early as day 16. Thus, we cannot exclude the possibility that chicks that would have fledged early were thwarted, and the fledging ages we observed reflect a revised fledging age for some chicks. However,

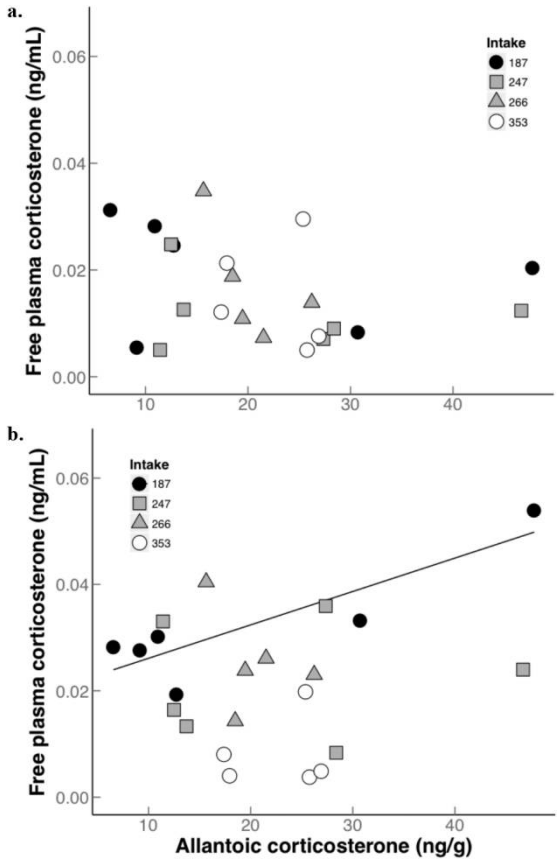

Figure 3. Allantoic corticosterone (CORT) and free baseline CORT before and after implementation of restricted diets. *a*, There were no significant relationships between allantoic CORT and free baseline CORT at day 10 posthatch. *b*, At day 20 posthatch, after 10 d of controlled diets, a treatment by allantoic CORT interaction (table 2) was evident—only the most energy-restricted chicks showed a significant positive relationship between allantoic CORT levels and free baseline CORT levels (indicated by solid line).

given that average fledging age in the wild ranges from 20 to 24 d (Uttley et al. 1994; Cameron-McMillian et al. 2006) and chicks in this study jumped multiple nights in a row after their first jump, it seems plausible that our data reflect first fledging attempts.

We found that an assortment of pre- and postnatal factors contributed to explaining fledging age, with higher energy intake and allantoic CORT associated with later fledging and higher baseline plasma CORT and exposure to higher embryonic T associated with earlier fledging (table 4). Because fledging is a single event, the presence and nature of interactions between energy intake and allantoic steroids are the only vehicles with which to assess our hypotheses. While an interaction between allantoic T and intake is present among the top models (table 2), it does not yield clear support for either hypothesis because there is no evidence that the strength of the relationship is related linearly to severity of restriction.

Although there are no studies directly investigating the role of prenatal steroid exposure on fledging age in seabirds, many studies have found that a combination of energy intake and baseline CORT affects fledging age of seabird chicks. For example, in thin-billed prions and storm petrels, CORT levels increase before fledging, while Laysan albatross chicks with higher free CORT fledge sooner and parental feeding rates often decrease as fledging approaches (Quillfeldt et al. 2007; Kozlowski et al. 2010; Sprague and Breuner 2010). Thus, it appears that the presence of sufficient food and low CORT in the least restricted group contributed to an extended nestling stage (fig. 4; table 4).

Allantoic Steroids as Indicators of the Prenatal Environment

Relatively little research has been conducted to elucidate factors determining the steroid content of allantoic waste from bird eggs. Levels of allantoic steroids reflect the interaction between genotype (in this case, both maternal and chick) and environment. Though we are not able to control for the genetic contribution to allantoic steroid levels in this study, they are likely to reflect variation in the prenatal environment generated by a combination of maternal steroid deposition into eggs and endogenous production by embryos (Benowitz-Fredericks et al. 2005). Because CORT is not highly heritable in birds (though maximum CORT is substantially more heritable than baseline; Jenkins et al. 2014; Homberger et al. 2015), variation in allantoic CORT levels likely reflects variation in the prenatal environment, both from maternal CORT deposition into eggs and from embryonic adrenals, which are active at a baseline level in ovo and capable of responding to prenatal stressors by elevating CORT (Wise and Frye 1973). In contrast, though there is active embryonic gonadal steroidogenesis (Bruggeman et al. 2002), there is little information about how the prenatal environment, including yolk T levels, might affect variation in embryonic androgen production. Though we use levels of allantoic steroids as proxy for an unspecified aspect of prenatal experience in this study and cannot distinguish maternal from embryonic contributions, some of our findings are consistent with other studies, suggesting that our measures of steroids in allantoic waste reflect developmental hormone exposure in relevant ways. For instance, the association between high allantoic CORT and high free baseline CORT at day 20 in the most restricted chicks is similar to studies showing that elevation of maternal CORT enhances HPA activity in quail chicks (Hayward and Wingfield 2004) and that elevated yolk CORT generated sustained CORT elevation in response to an acute stressor in chickens (Haussmann et al. 2012). However, it is in contrast to the marked suppression of HPA function by exogenously elevated yolk CORT in starlings (Love and Williams 2008). The differences in consequences of prenatal CORT exposure among studies may reflect differences in life-history strategies among avian species (Love and Williams 2008) or may reflect differences in postnatal environments, as described here. Elevated yolk androgens can alter avian adrenal function, but the direction of the effect appears to be mixed. For example, elevated yolk androgens increased circulating CORT in kestrel

Table 4: Slopes for parameters in top models from model selection (table 2) for baseline corticosterone (CORT; total and free), maximum stress-induced CORT (total), and fledging age following imposition of controlled diets (at day 10 posthatch)

Trait and model rank	Parameter	β	SE	Lower CL	Upper CL
Total baseline CORT (day 15):					
1	Energy intake	−.5679	.1841	−.9518	−.1839
Total baseline CORT (day 20):					
1	Energy intake	−.5378	.1885	−.9311	−.1446
2	Energy intake	−.6035	.1768	−.9750	−.2321
2	Allantoic CORT	.3809	.1898	−.0179	.7797
2	Allantoic T	−.3484	.1900	−.7475	.0507
Free baseline CORT (day 20):					
1	Energy intake	−.7293	.1489	−1.0422	−.4164
1	Allantoic CORT	.4033	.1599	.0674	.7392
1	Allantoic T	−.3451	.1600	−.6813	−.0089
2	Energy intake	−.6620	.1676	−1.0116	−.3124
Maximum CORT (day 20):					
1	Energy intake	−.5120	.1921	−.9126	−.1113
2	Total baseline CORT (day 15)	.5143	.1918	.1143	.9143
Fledging age:					
1	Energy intake	.5655	.1844	.1807	.9502
3	Energy intake	.5264	.1811	.1459	.9069
3	Allantoic CORT	.0720	.1945	−.3365	.4806
3	Allantoic T	−.3466	.1946	−.7555	.0622
4	Free baseline CORT (day 20)	−.4719	.1971	−.8831	−.0606
5	Total baseline CORT (day 20)	−.3523	.2093	−.7888	.0843

Note. Standard errors (SEs) and confidence limits (CLs; 5%–95%) are also shown.

chicks (Sockman and Schwabl 2001) but appeared to decrease fecal CORT in response to isolation in quail chicks (Daisley et al. 2005).

Adaptive Maternal Effects?

We did not have a strong basis on which to base a priori predictions about the ability of postnatal food availability to differentially override or reveal the effects of allantoic CORT versus T. Thus, interpretations of our data in the context of fitness can be made only speculatively. If, however, we assume that elevated allantoic steroids reflect differences in maternal steroid allocation to eggs and if the relationships between allantoic steroids and phenotype are causal, we can speculate that maternally deposited yolk CORT in murres may serve to facilitate acquisition of food from parents by increasing chick adrenal activity. Free-living adult common murres elevate plasma CORT in response to reduced food availability (Kitaysky et al. 2007; Barrett et al. 2015) and therefore may also elevate yolk CORT in low-food environments (Hayward and Wingfield 2004). In the wild, parents provision chicks that have experimentally elevated CORT at higher rates than control chicks (A. Kitaysky, unpublished data). Similarly, in another seabird species, black-legged kittiwakes (*Rissa tridactyla*), experimentally elevated CORT in chicks promoted begging behavior, and parents responded by increasing feeding (Kitaysky et al. 2001*b*). Thus, our results suggest the possibility that developmental exposure to elevated CORT may not alter adrenocortical function when food is not limited (postnatal override; no relationship between allantoic CORT and plasma CORT before restriction) but may make energy-limited chicks better able to respond to food shortages by elevating CORT to secure resources from their parents and by remaining in the nest longer (postnatal reveal; table 4). Similarly, if allantoic T were to directly reflect yolk T, our data suggest that the deposition of more T into eggs, potentially a reflection of maternal effects aimed at synchrony of fledging as an antipredatory tactic in colonial-breeding common murres (Benowitz-Fredericks et al. 2005, 2006), may dampen adrenal responsiveness of chicks to food shortages and reduce their time in the nest (table 4). Both causes of variation in allantoic steroids and fitness implications of variation in HPA function remain speculative, and CORT data from adult murres at the same colony from which these eggs were collected suggest that it was a relatively moderate year in terms of food availability (Kitaysky et al. 2007). However, similar to the scenarios described above (particularly

Table 5: Plasma corticosterone (CORT) levels, fledging age and mass, and sex by treatment

Food intake (after day 10; kJ/d)	Baseline CORT (ng/mL), total/free			Maximum CORT (ng/mL), total	Fledging age (d)	Fledging mass (g)	Sex ratio (M:F)
	Day 10	Day 15	Day 20	Day 20			
187	4.5 ± 2.4/.020 ± .011	**7.8 ± 3.4**	**7.8 ± 3.5/.032 ± .012**	**22.9 ± 5.9**	**27.0 ± 1.55**	**150 ± 10**	3:2
247	3.8 ± 2.4/.015 ± .010	**3.6 ± 2.5**	**6.4 ± 3.9/.025 ± .009**	**17.3 ± 8.6**	**26.3 ± .52**	**196 ± 15**	4:2
266	3.0 ± 1.8/.011 ± .007	**2.7 ± 2.3**	**5.2 ± 2.2/.022 ± .011**	**11.3 ± 4.0**	**26.6 ± .89**	**192 ± 10**	2:3
353	3.6 ± 1.9/.016 ± .009	**1.9 ± .8**	**2.7 ± 2.0/.010 ± .006**	**11.7 ± 4.3**	**30.0 ± 2.45**	**252 ± 12**	3:2

Note. Values shown in bold indicate measurements taken after the imposition of controlled diets. All values shown as average ± SD.

Table 6: Summary of the effects of daily energy intake and allantoic steroid concentrations on characteristics of HPA function and fledging age in common murre chicks growing under ad lib. (day 0–10) versus controlled (day 10–20) feeding conditions

	Predictor variable (effect)		
Trait	Energy intake	Allantoic CORT	Allantoic T
Total baseline CORT (ad lib./controlled)	○/−	○/+	○/−
Free baseline CORT (ad lib./controlled)	○/−	○/+	○/−
Maximum CORT (controlled)	−	○	○
Fledging age (controlled)	+	+	−

Note. Directionality is based on slope estimates from top models as indicated in table 4. Open circle = no effect; plus sign = positive; negative sign = negative. CORT = corticosterone; T = testosterone.

with CORT), future studies could integrate postnatal override/reveal with the maternal/offspring match-mismatch hypothesis (Love and Williams 2008; Sheriff and Love 2013). In this context, maternal effects manifest and are valuable when prenatal and postnatal conditions align but may be costly and therefore overridden by the postnatal environment when they do not.

Other Tests of Postnatal Override and Postnatal Reveal

Several studies have manipulated both prenatal environments and postnatal environments in birds. Love and Williams (2008) and Marasco et al. (2012) assessed pre- and postnatal contributions to adrenocortical function by experimentally manipulating both prenatal stress and postnatal stress for birds and assessing adrenocortical function. Interestingly, Love and Williams (2008) essentially found no interaction between prenatal stressors and postnatal stressors, with a dose of prenatal CORT (designed to bring the average level of CORT in eggs from 15.5 to 28.3 ng/g) completely overriding postnatal experience in starlings (*Sturnus vulgaris*), while Marasco et al. (2012) found strong evidence of postnatal override in determining interactions between the prenatal environments and postnatal environments in Japanese quail. At 22 d posthatch, there was no effect of prenatal treatment with 8.5 ng of CORT (1.8 times the standard deviation), but chicks treated with CORT postnatally showed similar patterns of adrenocortical activity regardless of prenatal treatment. At day 64 posthatch, an effect of prenatal environment appeared—prenatally treated chicks showed enhanced adrenocortical function but only if they were not also treated postnatally (Marasco et al. 2012). Another experiment that manipulated both prenatal environments and postnatal environments in partridges found that for the same aspect of the environment (predictability of food), postnatal treatments overrode prenatal (parental) treatments for some phenotypic traits (oxidative stress, immunity) but prenatal treatment overrode postnatal treatment for others (adrenocortical function; Homberger et al. 2013). Thus, it is probable that the interactions among prenatal environment, postnatal environment, and ontogeny will vary both among species and with different combinations of environmental factors and phenotypic traits.

Conclusion

Recently, Killen et al. (2013) described the inconsistent relationships between physiology and behavior both across and within taxa and proposed that the inconsistency is due to the ability of environmental stressors to either amplify or attenuate the strength of the relationship between these traits. Similarly, the postnatal environment may attenuate or amplify phenotypic manifestations of the prenatal environment, potentially generating a variety of seemingly contradictory responses to the same prenatal manipulations. We found that in the common murre baseline adrenocortical function and the decision of chicks to fledge are governed primarily by their daily energy intake but that the prenatal environment can contribute to variation in these aspects of phenotype in certain postnatal contexts. Specifically, we found that some effects of the prenatal environment are not always apparent but can be revealed by postnatal challenges (table 6). Although this study focused on prenatal steroids, food, and HPA function in

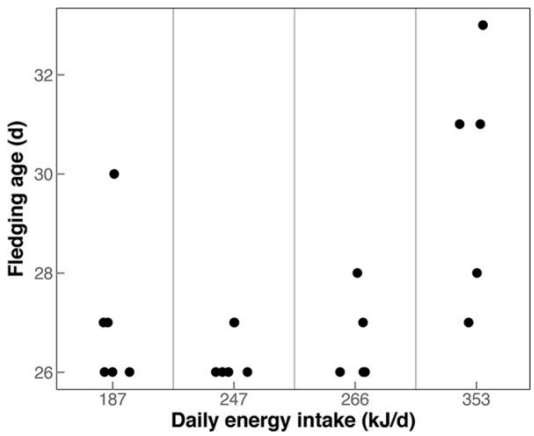

Figure 4. Fledging age of captive common murre chicks was best explained by daily energy intake. Chicks consuming more energy fledged at an older age than those consuming less.

murres, postnatal override and postnatal reveal can provide a general framework for investigating the interactions between the prenatal environment and the postnatal environment. The degree to which relationships can be generalized or predicted across combinations of pre- and postnatal environments, phenotypic traits, and study organisms remains to be determined. However, our ability to anticipate fitness consequences of variation in pre- or postnatal environments is contingent on understanding the ability of the postnatal environment to alter the manifestation of prenatal effects.

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