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Effects of a short period of elevated circulating corticosterone on postnatal growth in free-living Eurasian kestrels *Falco tinnunculus*

Claudia Müller[1,2,*], Susanne Jenni-Eiermann[1] and Lukas Jenni[1]

[1]Swiss Ornithological Institute, Luzernerstrasse 6, CH-6204 Sempach, Switzerland and [2]Zoological Museum of the University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich

*Author for correspondence (e-mail: claudia.mueller@vogelwarte.ch)

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SUMMARY

Environmental conditions affect growth and development and, through developmental plasticity, create phenotypic variation. In suboptimal conditions current survival is traded-off against development. Corticosterone, the main glucocorticoid in birds, may be involved in the reallocation of energy from growth to maintenance, but its effect on growth has rarely been investigated in altricial birds under natural conditions in the wild. In free-living Eurasian kestrel *Falco tinnunculus* nestlings, we artificially elevated corticosterone to stress-induced levels over 2–3 days in the middle of the nestling stage by implanting biodegradable implants, controlling the treatment with a placebo group. We measured the length of primary feather 8, hand length, tarsus length, body mass and subcutaneous fat stores from day 10 to 25. During corticosterone elevation, primary growth of cort-nestlings was significantly reduced to 71% of placebo-nestlings, hand and tarsus growth were significantly reduced to 14% and 26% of placebo-nestlings, respectively, and body mass increase stopped, while subcutaneous fat-store growth was not affected. Over the following 5 days, primary growth was still significantly suppressed to 84% of placebo-nestlings, while hand, tarsus and body mass growth were back to normal. During the subsequent 4 days, cort-nestlings partly compensated for the lag in body mass by significantly accelerating the body mass increase compared with placebo-nestlings. Before fledging, primary length was 10% shorter, hand and tarsus 5% and 4% shorter and body mass 8.5% lower in cort-nestlings than in placebo-nestlings, while fat score did not differ significantly between the two groups. Thus, we have shown that in free-living, altricial nestlings a few days of elevated plasma corticosterone levels alone, without food restriction, suppressed growth and this could only partly be compensated for afterwards. Feather, bone and body mass growth were reduced to different degrees, indicating that corticosterone had a differential effect on different structures. This demonstrates that corticosterone is probably involved in the control of developmental plasticity.

Key words: bone growth, corticosterone implants, feather growth, morphology, stress effects.

INTRODUCTION

Growth and development of animals generally are influenced by environmental conditions, which, in addition to genetic variation, create variation in phenotype, upon which selection acts (e.g. Stearns and Hoekstra, 2005). Energetic restrictions or other environmental perturbations during growth and development may provoke a trade-off between current survival and development. Many effects of environmental factors on growth and development are irreversible and thus often have consequences for life (Lindström, 1999).

Glucocorticoids may play an important role in this trade-off between maintenance and development. Across all vertebrate taxa, the activation of the hypothalamo-pituitary-adrenal (HPA) axis, leading to a rise in glucocorticoids, helps an animal to redirect the available energy and behaviour from normal activities into a survival mode, to cope with the critical situation (e.g. Wingfield et al., 1998). Elevated glucocorticoid levels inhibit anabolic processes including growth, suppress parts of the immune system and influence appetite (e.g. Bray, 1993; Sapolsky et al., 2000; Lin et al., 2006). Thus environmental factors may affect growth and development directly (e.g. limited nutrients) and indirectly through glucocorticoids which may have suppressing effects on growth and development, such as on body size, body condition, immune system and cognitive functions (e.g. Davison et al., 1983; Saino et al., 2003; Kitaysky et al., 2003; Hull et al., 2007). Therefore, glucocorticoids, as mediators of environmental conditions and through their indirect effects, may be an important additional factor causing developmental plasticity and thus, through long-term or life-long effects, shape phenotype (Dufty et al., 2002).

The most common environmental factor affecting growth and development is nutritional restriction (through food shortage, competition with siblings, parasites), but disease, heat, cold and water shortage can also have an effect. Most studies have used food restriction to investigate developmental plasticity as a response to environmental conditions, and thereby have studied the combined effects of both the nutrient restriction *per se* and elevated glucocorticoids. Most studies available on the effects of glucocorticoids during growth and development are from precocial species such as quail and chicken under lab conditions (e.g. Davison et al., 1983; Donker and Beuving, 1989; Hayashi et al., 1994). Studies on the effect of glucocorticoids during growth in altricial chicks and under natural conditions are few and focus mainly on behavioural aspects (Kitaysky et al., 2001b; Loiseau et al., 2008; Wada and Breuner, 2008). Precocial chicks forage for themselves and glucocorticoids may have effects similar to those in adults, i.e. enhance food searching behaviour (Astheimer et al., 1992; Sapolsky et al., 2000). Altricial nestlings, however, are completely dependent on their parents for food, but

also have a HPA axis responsive to stressors (e.g. Love et al., 2003). The function of increased glucocorticoids in altricial nestlings may be (a) to improve energy intake by increased begging and by becoming more aggressive towards their siblings (Kitaysky et al., 2003) and (b) to re-allocate the available energy to the most important processes (e.g. Sapolsky et al., 2000; Hochberg, 2002).

The aim of this study was to investigate the effects of a temporary increase of circulating corticosterone (the glucocorticoid in birds) on growth in an altricial bird species, the Eurasian kestrel *Falco tinnunculus*, in natural conditions in the wild. Because we artificially elevated circulating corticosterone for a few days with implants, we could investigate the effects of corticosterone without the confounding effects of food restriction. In most food restriction studies, glucocorticoid levels have not been measured and, thus, it was not known what the direct effects of food restriction and the indirect effects of elevated glucocorticoid levels were, while in some food restriction studies glucocorticoids were measured but the effects on structural growth were not documented (Kitaysky et al., 1999; Kitaysky et al., 2001a; Pravosudov and Kitaysky, 2006; Strochlic and Romero, 2008).

Contrary to most studies investigating the effects of a long stress period on postnatal development, we were interested in the effects of a short period (2–3 days) of clearly elevated baseline corticosterone levels. We used different growth and body condition measures in order to investigate whether growth of skeletal elements, feathers, body mass and subcutaneous body fat stores was affected differently by elevated corticosterone levels. Food restriction studies demonstrated a hierarchy in resource allocation favouring important structures, such as the nervous system and skeletal structures, at the expense of muscles, the digestive system and fat stores (Oyan and Anker-Nilssen, 1996; Schew and Ricklefs, 1998; Moe et al., 2004). However, there are hardly any studies investigating the effects of elevated corticosterone on various structures (including body size) and tissues in birds; exceptions investigating internal organs include the precocial chicken and quail (e.g. Lin et al., 2006; Hull et al., 2007).

We investigated the effects of elevated circulating corticosterone in the middle of the nestling stage and were therefore able to test for compensatory growth after the corticosterone levels returned back to normal. Food restriction studies have shown that delays in growth may be partly or fully compensated for through a prolongation or acceleration of growth (e.g. Emlen et al., 1991; Negro et al., 1994; Bize et al., 2003; Bize et al., 2006) possibly with associated costs (Metcalfe and Monaghan, 2001). Because this study was done in the wild, we examined whether compensatory growth occurred under natural, rather than *ad libitum*, food conditions. Thus our results are directly relevant to natural populations.

In the kestrel, males are slightly smaller than females and nestlings hatch partially asynchronously. Therefore we also investigated whether elevated corticosterone levels affected growth of the smaller sex or smaller chicks differently from growth of the larger ones.

MATERIALS AND METHODS

Study species and study site

The Eurasian Kestrel (*Falco tinnunculus* Linnaeus 1758) is a small raptor species with reversed size dimorphism. Females incubate the 4–6 eggs for 29 days starting after the third egg; the three oldest nestlings within a brood therefore have the same age. Tarsus growth is completed at about day 20, maximum body mass is reached on about day 25. Fledging occurs on day 32–39. The field work was carried out in north-western Switzerland in an area of 100 km^2 (7°50′E/47°25′N), where Eurasian kestrels breed in nest boxes mounted on agricultural buildings in open rural landscapes.

Experimental corticosterone elevation

The experiment was carried out with 109 nestlings of 13 broods in 2004 and 17 broods in 2005. There was no difference between the years in all parameters; therefore the year was not included in the analysis.

During both breeding seasons, nest boxes were checked weekly from April onwards and before hatching (from May to June) at 4 day intervals to determine hatching date. At the age of 13 days, two randomly selected nestlings out of the four oldest within a brood were implanted with a biodegradable corticosterone implant (Innovative Research of America, Sarasota, FL, USA; 10 mg corticosterone, 7 day release) and called cort-nestlings, and the other two were implanted with a placebo pellet (placebo-nestlings). To monitor the effect of the implant on circulating corticosterone levels, we took baseline blood samples at the age of 10, 13, 16 and 21 days in all nestlings. In a subgroup of the nestlings we took an additional sample at day 14 or 15. Within 3 min of taking nestlings out of the nest box, the alar vein was punctured and about 80 µl blood was sampled with heparinized capillary tubes. Corticosterone levels did not rise significantly within 3 min as a response to capture (F=3.29, d.f.=1, P=0.071). Within 30 min, the blood was centrifuged and the plasma stored in liquid nitrogen in the field and at –20°C once in the laboratory. All methods described in this study were approved by the Cantonal committee for animal research (animal experiment permit no. 274 from the Cantonal Veterinarian Office of Baselland).

Growth and body condition measurements

Nestlings were measured at the age of 10, 13, 16, 21 and 25 days (age of the oldest nestlings of the brood). We refrained from visiting the nests after day 25 to avoid premature fledging. The length of the wing and of primary 8 (second longest primary) were measured to the nearest 0.5 mm. An estimate of the skeletal hand length was obtained by subtracting the length of primary 8 from the wing length. Tarsus length was measured to the nearest 0.1 mm with digital calipers. Body mass was determined with a spring balance to the nearest gram. As in passerines (Kaiser, 1993), we assessed the subcutaneous fat stores at the furcula by assigning a fat score ranging from 0 to 4 (0: no visible fat; 1: 1 mm stripe of fat at the bottom of the furcular pit; 2: fat stripes 2–3 mm wide; 3: furcular pit nearly covered with fat (about 75%), 4: furcular pit completely filled with fat). Growth rates were calculated on the basis of the number of hours between measurements and expressed as growth rates per 24 h.

From nest controls at hatching and wing length at day 10, we determined the age difference between the oldest nestlings and their siblings. Between the treated nestlings within a brood, this age difference ranged from 0 to 3 days (0 days: 56 nestlings; 1 day: 43 nestlings; 2 days: 12 nestlings; 3 days: 1 nestling). For 18% of the sampling days, we were unable to measure the nestlings on the intended day for logistical reasons and did it 1 day earlier or later. Because there was no significant effect of bringing forward or delaying measurements on growth parameters, we omitted it from the analysis.

At the age of 10 and 13 days, 3 and 0 days before the treatment, there were no significant differences between the future corticosterone and placebo groups in any of the parameters measured (data not shown, but see day 10 and 13 values in Fig. 1).

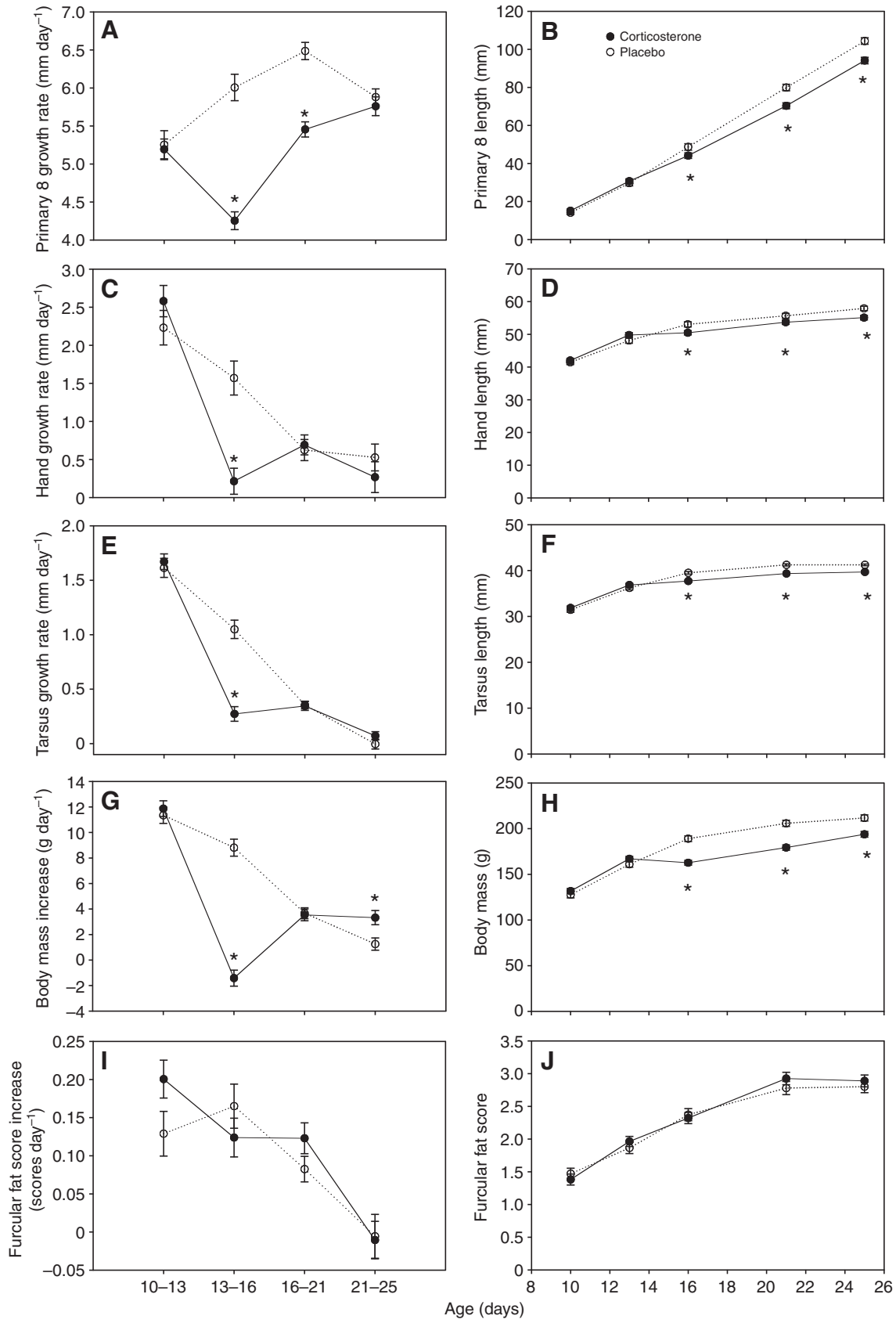

Fig. 1. Mean daily growth rates (±s.e.m.; left column) and mean absolute values (±s.e.m.; right column) of the length of primary 8, hand length, tarsus length, body mass and furcular fat score in kestrel nestlings from age 10 to 25 days. Corticosterone pellets were implanted on day 13 and circulating corticosterone was elevated from day 13 to 16. Asterisks indicate significant differences between cort- and placebo-nestlings (*post-hoc* mixed models, treatment: $P<0.001$).

Hormone assay

Plasma corticosterone concentration was determined using an enzyme immunoassay. Corticosterone in 5 µl plasma and 195 µl water was extracted with 4 ml dichloromethane, re-dissolved in phosphate buffer and given in triplicate in the enzyme immunoassay. The dilution of the corticosterone antibody (Chemicon, Temecula, CA, USA; cross-reactivity: 11-dehydrocorticosterone 0.35%, progesterone 0.004%, 18-OH-DOC 0.01%, cortisol 0.12%, 18-OH-B 0.02% and aldosterone 0.06%) was 1:8000. Horseradish peroxidase (1:400,000) linked to corticosterone served as the enzyme label and ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)] as the substrate. The concentration of corticosterone in plasma samples was calculated by using a standard curve run in duplicate on each plate. Plasma pools from chickens with two different corticosterone concentrations were included as internal controls on each plate. If the concentration was below the detection threshold, the determination was repeated with 10 µl plasma. If the concentration was still below the detection threshold, the value of the lowest detectable concentration (1 ng ml^{-1}) was assigned. Intra-assay variation ranged from 4.5 to 10.8% and inter-assay variation from 9.6 to 17.6%, depending on the concentration of the internal control and the year of determination.

Sex determination

Nestlings were sexed with molecular methods by fragment analysis on CHD1W/CHD1Z (Fridolfsson and Ellegren, 1999) using blood cells of blood samples extracted with the QIAamp DNA extraction kit (Qiagen, Hombrechtikon, Switzerland) in 2004 and after Kawasaki (Kawasaki, 1990) in 2005. Samples from 2004 were analysed at the Swiss Federal Institute for Forest, Snow and Landscape Research in Birmensdorf, Switzerland and those from 2005 at the Agroscope Research Station ACW in Wädenswil, Switzerland.

Statistical analysis

Growth rates were analysed with a mixed model for repeated measurements in Genstat 9.1 (Payne, 2003; Thompson and Welham, 2003). In the fixed model, the effect of the corticosterone treatment on primary, hand and tarsus growth rate and body mass and furcular fat store increase was examined taking into account age (in days), time of day, sex, age difference to the oldest nestling within the brood (in days), brood size, the absolute measure of the parameter of this individual at day 10 and hatching date (Julian date). After the main parameters, biologically relevant interactions between age, sex, age difference within the brood and treatment were tested. The design of the random model was brood×age. The nestling variance component was very small and therefore omitted from the random model.

To assess the effect of corticosterone treatment on growth rate, body size and condition before and during the experiment and just before fledging, the growth rates and absolute morphological measurements (primary 8, hand and tarsus length, body mass, fat score) at day 10, 13, 16, 21 and 25 were analysed separately in *post-hoc* mixed models with time of day, sex, the age difference to the oldest nestling within the brood, brood size, hatching date, treatment and relevant two-way interactions in the fixed model and brood as random model. Because individuals were measured repeatedly, significance levels were adjusted according to Bonferroni (Sokal and Rohlf, 2000). All model residuals were normally distributed.

RESULTS

The biodegradable corticosterone implants elevated circulating baseline corticosterone levels from 5.5±0.40 ng ml^{-1} (N=88, no difference between the future placebo and future corticosterone groups) on day 13 before implantation to 45.0±4.7 ng ml^{-1} (range 29.7–63.8 ng ml^{-1}, N=7) the following day and 25.2±2.96 ng ml^{-1} (range 17–30 ng ml^{-1}, N=4) on day 15. On day 16, the levels in the corticosterone-implanted nestlings (11.8±1.26 ng ml^{-1}) were only slightly elevated compared with those in the placebo group, whose corticosterone levels averaged 6.65±1.55 ng ml^{-1} from day 14 to 16. Thus, the corticosterone implants clearly elevated circulating corticosterone levels during the middle of the nestling stage for 2–3 days (see also Müller et al., 2009).

Corticosterone treatment occurred when the growth rate of primary 8 was high and temporarily reduced it (interaction age×treatment highly significant; Table 1; Fig. 1A). Primary growth rate in cort-nestlings was significantly reduced (for statistics see Fig. 1) to 71% of the placebo-nestlings during the period of elevated circulating corticosterone (from nestling day 13 to 16; Fig. 1A). During the 5 days after treatment (day 16–21), primary growth rate was still significantly reduced to 84% of the placebo group and recovered only in the following period from day 21 to 25, when it corresponded to the placebo group again. At day 25, cort-nestlings had a significantly shorter primary (10.3 mm or 10%; Table 2; Fig. 1B) than the placebo-nestlings. At this age, primary 8 had reached about 53% of its final length.

For both hand and tarsus, corticosterone treatment occurred during the final growth phase as shown by the strongly decreasing growth rates in placebo birds from day 10 to day 21 (Fig. 1C,E; age significant, Table 1). Corticosterone treatment temporarily reduced hand and tarsus growth significantly (interaction age×treatment significant; Fig. 1C,E). During the period of elevated circulating corticosterone (days 13–16), hand growth rate of the cort-nestlings was only 14% of the rate of the placebo group (Fig. 1C) and tarsus growth rate only 26% (Fig. 1E). After the period of high corticosterone levels (days 16–21), hand and tarsus growth rates of the cort-nestlings had recovered and were indistinguishable from those of the placebo group. At day 25, when hand growth is almost completed, cort-nestlings had a 2.8 mm or 5% shorter hand length (Table 2; Fig. 1D). In contrast to the hand, cort-nestlings still grew their tarsi from day 21 to 25 and compensated for the reduction to a certain degree, while tarsus growth was already completed in the placebo group (Fig. 1E,F). On day 25, cort-nestlings had a 1.5 mm or 4% shorter tarsus than the placebo group (Table 2; Fig. 1F).

Corticosterone treatment strongly affected body mass growth (Table 1; Fig. 1G). Body mass growth rate of the cort-nestlings was negative during the period of elevated circulating corticosterone while the placebo-nestlings gained about 9 g day^{-1}. During the 5 days after the treatment, from day 16 to 21, the two groups had similar growth rates. From day 21 to 25, the body mass retardation of cort-nestlings was partly compensated for by growing at 265% compared with the placebo group (difference in growth rate significant). On day 25, cort-nestlings had a significantly lower body mass (by 18 g or 8.5%) than the placebo group (Table 2; Fig. 1H). At this age, adult body mass has been reached or surpassed by normally developing nestlings.

Corticosterone treatment had no overall significant effect on furcular fat score increase and placebo- and cort-nestlings had about the same furcular fat score on day 25 (Fig. 1I,J), but the growth curves of the two groups had slightly different shapes (interaction age×treatment significant; Fig. 1I; Table 1). This may be partly due to the fact that placebo-nestlings by chance had more subcutaneous fat reserves on day 10 than future cort-nestlings and therefore fat was accumulated at a slightly lower rate until day 13 than in cort-nestlings.

Table 1. Effect of corticosterone treatment and other parameters on primary, hand and tarsus growth rates and body mass and fat score increase of Eurasian kestrel nestlings

		Primary growth rate		Hand growth rate		Tarsus growth rate		Body mass increase		Fat score increase	
Explanatory variable	d.f.	Wald	χ^2-*P*	Wald	χ^2-*P*	Wald	χ^2-*P*	Wald	χ^2-*P*	Wald	χ^2-*P*
Age of oldest nestling in brood (days)	**3**	**41.09**	**<0.001**	**90.78**	**<0.001**	**620.90**	**<0.001**	**156.59**	**<0.001**	**36.46**	**< 0.001**
Time of day (h)	**1**	2.36	0.124	2.37	0.123	3.46	0.063	**14.43**	**<0.001**	0.14	0.706
Sex	**1**	**3.86**	**0.049**	3.02	0.082	2.85	0.091	**17.27**	**<0.001**	0.30	0.581
Age difference to oldest nestling (days)	**1**	2.36	0.124	**6.81**	**0.009**	**16.58**	**<0.001**	**18.86**	**<0.001**	0.01	0.906
Brood size	1	0.37	0.543	0.00	0.982	0.45	0.501	1.44	0.231	0.06	0.803
Measure at day 10 (mm or g)	**1**	**13.18**	**<0.001**	**15.06**	**<0.001**	**66.42**	**<0.001**	**11.61**	**<0.001**	**19.70**	**< 0.001**
Hatching date (day)	1	0.12	0.728	0.01	0.935	0.61	0.436	1.58	0.209	0.76	0.384
Treatment group	**1**	**102.82**	**<0.001**	**6.13**	**0.013**	**16.97**	**<0.001**	**49.44**	**<0.001**	0.58	0.447
Age × sex	**3**	3.92	0.271	5.99	0.112	**11.04**	**0.012**	3.09	0.377	0.64	0.888
Age × age difference to oldest nestling	**3**	2.60	0.457	1.23	0.745	**10.70**	**0.013**	**10.66**	**0.014**	0.80	0.850
Age × treatment group	**3**	**85.00**	**<0.001**	**34.62**	**<0.001**	**105.46**	**<0.001**	**360.78**	**<0.001**	**8.99**	**0.029**
Sex × treatment group	1	0.97	0.324	0.46	0.498	0.26	0.609	0.01	0.923	0.01	0.917
Age difference to oldest nestling × treatment group	1	1.14	0.287	1.28	0.258	0.01	0.928	0.30	0.581	0.06	0.800
Age × sex × treatment group	3	0.75	0.860	5.71	0.126	2.50	0.476	6.38	0.094	0.78	0.854
Age × age difference to oldest nestling × treatment group	3	3.27	0.352	7.57	0.056	2.37	0.499	2.14	0.544	7.05	0.070

Data were collected from 109 nestlings in 30 broods. They were measured at the age of 10, 13, 16, 21 and 25 days resulting in 428 measurements. Growth rates per nestling were analysed using a repeated measures mixed model. Significant differences are in bold.

Despite some significant variation in growth rate with sex, developmental stage and within-brood age differences, corticosterone treatment did not affect growth of the sexes and asynchronously hatched or less developed nestlings differentially in any of the measurements taken (Table 1).

DISCUSSION

Our study revealed pronounced effects of a short-term corticosterone treatment on various growth parameters in altricial wild kestrel chicks under natural conditions. These effects were only partly compensated for after corticosterone levels returned to normal after 3 days. By administering corticosterone *via* implants, we evaluated the effects of increased corticosterone levels on postnatal growth without the confounding effects of a reduced availability of growth substrates caused by food restriction, the most common stressor used in lab studies. In altricial nestlings, glucocorticoids may also be released as a response to stressors not involving food reduction, such as disease, disturbance by predators and humans, interactions with siblings, etc. The biodegradable corticosterone pellets elevated circulating corticosterone to moderate or high levels for 3 days during the middle of the nestling stage. This is a short period of stress compared with other studies administering corticosterone (e.g. Lin et al., 2006; Hull et al., 2007) or restricting food over weeks in chicks (e.g. Oyan and Anker-Nilssen, 1996; Benowitz-Fredericks et al., 2006). The plasma levels of corticosterone induced by the implants were within the range occurring naturally in the smallest kestrel chicks during nutritional stress (25–100 ng ml^{-1}) and in kestrel chicks after 20 min of handling (10–50 ng ml^{-1}; C.M., unpublished data).

Immediate effects of corticosterone on growth

During the period of elevated circulating corticosterone (days 13–16 of age), corticosterone treatment affected all growth

Table 2. Effect of corticosterone treatment and other parameters on primary, hand and tarsus length, body mass and fat score at day 25 (before fledging) of Eurasian kestrel nestlings

		Primary length		Hand length		Tarsus length		Body mass		Fat score	
Explanatory variable	d.f.	Wald	χ^2-*P*	Wald	χ^2-*P*	Wald	χ^2-*P*	Wald	χ^2-*P*	Wald	χ^2-*P*
Time of day (h)	**1**	0.36	0.549	1.84	0.175	**6.96**	**0.008**	1.77	0.184	**4.60**	**0.032**
Sex	**1**	**4.22**	**0.040**	**8.04**	**0.005**	0.07	0.784	**36.77**	**<0.001**	0.30	0.585
Age difference to oldest nestling (days)	**1**	**35.26**	**<0.001**	0.01	0.930	**4.82**	**0.028**	0.02	0.882	3.48	0.062
Brood size	1	2.33	0.127	0.23	0.635	2.65	0.105	0.41	0.524	0.16	0.693
Hatching date (day)	**1**	0.00	0.997	0.31	0.579	1.86	0.103	**4.45**	**0.035**	1.17	0.279
Treatment group	**1**	**98.90**	**<0.001**	**21.89**	**<0.001**	**39.78**	**<0.001**	**37.94**	**<0.001**	0.33	0.568
Sex × treatment group	1	1.62	0.203	1.05	0.305	1.02	0.312	0.03	0.859	0.01	0.939
Age difference to oldest nestling × hatching date	**1**	**4.06**	**0.044**	0.77	0.379	0.13	0.716	2.63	0.105	0.30	0.586
Age difference to oldest nestling × treatment group	1	1.13	0.289	1.17	0.280	0.03	0.857	0.39	0.531	0.48	0.488

Data were collected from 109 nestlings in 30 broods. Significant differences are in bold.

parameters investigated, but to very different degrees (reduction to 71% in feather growth rate, to 14% in hand growth rate, to 26% in tarsus growth rate, to 0% in body mass increase and no effect on subcutaneous fat stores). Feather growth was only reduced to 71%, although wing feather growth was at its maximum, and is a very costly and inefficient process with only a 20% energetic efficiency of feather synthesis in the kestrel (Dietz et al., 1992). Feather growth reduction (to 84%) also extended into the days when circulating corticosterone had returned to normal levels, as observed in moulting starlings (Romero et al., 2005). Growth of the skeletal elements of all extremities was strongly reduced during the period of elevated corticosterone levels. Contrary to feather growth, growth of the hand and tarsus recovered completely to the (then low) rate of the control group directly after the period with elevated corticosterone levels.

That body mass growth was completely stopped and body mass even reduced under corticosterone treatment agrees with studies in precocial bird species, such as chicken and quail (Davison et al., 1983; Buyse et al., 1987; Donker and Beuving, 1989; Siegel et al., 1989; Bray, 1993; Hayashi et al., 1994; Post et al., 2003; Dong et al., 2007; Hull et al., 2007). Body mass growth recovered after the treatment period. Because no birds were killed, we cannot explore which parts of the body were most affected by corticosterone treatment, except for growth of feathers and skeletal extremities (see above) and fat stores. Corticosterone treatment did not reduce peripheral body fat stores, demonstrating that nestlings were not in a normal fasting state, such as under food restriction.

Corticosterone treatment thus had a differential effect on the growth of different body parts and organs. This hierarchy in growth allocation under elevated circulating corticosterone agrees partly with the hierarchy found in food restriction experiments, indicating a regulating role of corticosterone during food restriction. Structural growth is protected at the expense of more flexible body tissues such as muscles (Oyan and Anker-Nilssen, 1996; Moe et al., 2004; Benowitz-Fredericks et al., 2006). Feather growth is even more strongly supported than bone growth, similar to zebra finch nestlings raised with low-quality food (Boag, 1987). An exception are fat stores which are depleted first under food restriction, but kept under corticosterone treatment; this may be due to the well-known fattening effect of chronic corticosterone administration (e.g. Davison et al., 1983; Buyse et al., 1987). Flight feathers are crucial for flight performance and were in their main growth phase during elevated corticosterone levels; presumably, therefore, their growth was most buffered. Additionally, hand and tarsus were in their final growth phase during the treatment and had almost reached their definite length. Thus corticosterone could only have a small effect on their final size. Corticosterone treatment in another developmental stage may possibly show a different growth allocation.

There may be two mechanisms by which corticosterone reduces feather growth. The first is by interference with the growth hormone–IGF-1 axis (Hochberg, 2002), the primary control of postnatal growth (McNabb et al., 1998). The suppressed growth rate of feathers (consisting of up to 95% protein) can be explained by the inhibition of protein synthesis with high corticosterone levels (Sapolsky et al., 2000). The depressed feather growth rate in the days when circulating corticosterone levels had returned to the level of the control group could be the result of the continued presence of products induced by corticosterone affecting gene transcription (Sapolsky et al., 2000). Glucocorticoids impair bone growth (1) indirectly with catabolic effects on bone and cartilage protein, interfering with the growth hormone–IGF-1 axis and by disturbing normal calcium balance, and (2) directly, by impeding anabolic processes at the growth plate and the adjacent tissues of the bones (Hochberg, 2002). As a second mechanism, corticosterone treatment may also have affected growth by reducing appetite (Sapolsky et al., 2000) and possibly by a reducing digestive efficiency or increasing maintenance energy expenditure as observed in precocial chicks (Dong et al., 2007). However, the evidence of the effects of corticosterone on food intake is controversial. Other studies observed increased or no change in food intake in quail and chicken (Bray 1993, Buyse et al., 1987; Simon, 1984; Davison et al., 1983) and increased (Hayashi et al., 1994) or decreased food conversion (Siegel et al., 1989). Whether kestrel nestlings could increase food intake by increased begging and aggressiveness against siblings (Kitaysky et al., 2003) remains to be shown. Food intake of cort-nestlings during the 2 days after implantation was not measurably reduced compared with placebo-nestlings (video observations of feeding rates, C.M., unpublished data). This would indicate that elevated corticosterone increased energy expenditure which contributed to the loss of body mass (DuRant et al., 2008).

Compensatory growth

The compensatory growth pattern varied widely between body structures. Accelerated growth occurred only in body mass and to a slight extent in tarsus, while growth of hand and feather just resumed the growth rate of the corresponding age (i.e. the growth rate of the placebo-nestlings). A prolonged growth period probably occurred in body mass and feather length. Primary feather length, measured at day 25, had only reached about 53% of its final value; the primaries continue to grow after fledging. Therefore, we were unable to assess whether the cort-nestlings prolonged or accelerated primary growth after day 25 when their primary length was 10% shorter than in placebo-nestlings. Growth of the two skeletal structures was terminated by day 25. Maturation of the tarsus and the hand bone seemed not to be slowed down, which prevents prolonged or accelerated growth to reach a normal tarsus and hand length.

Cort-nestlings did not accelerate body mass growth rate during the period immediately following elevated corticosterone levels, but did so later before fledging. On day 25, their body mass was only 9% lower than in placebo-nestlings, compared with a body mass that was 14% lower on days 16 and 21, indicating a prolongation of the body mass growth phase. It is possible that the cort-nestlings further compensated for their lag in body mass until fledging by prolonging growth or by not reducing body mass just before fledging, as normally developed placebo-nestlings do, similar to food-stressed altricial song sparrows *Melospiza melodia* (Searcy et al., 2004). As the corticosterone-treated nestlings fledged about 2 days later than their placebo siblings (C.M., unpublished data), it is likely that they prolonged the build-up of body mass by 2 days and reached a similar fledging mass to their placebo siblings.

To our knowledge, this is the first study that has examined the development of body mass well after artificially elevated corticosterone levels returned to normal. All other studies known to us stopped at the end of corticosterone administration without monitoring potential compensatory growth. Studies investigating a natural or experimental food restriction in altricial nestlings, which lead to a similar lag in body mass to our corticosterone treatment, found either a similar restoration of the growth rate to the normal rates of the control group with prolonged growth (Schew, 1995) or accelerated growth (Negro et al., 1994; Bize et al., 2006), so that the reduction was at least partially compensated for. The timing and extent of the compensatory growth seems to depend on the severity, the developmental phase and the duration of the nutritional restriction.

Long-term effects of corticosterone on morphology and body condition

Only 2 days of elevated corticosterone levels resulted in a life-long impact on morphology. Bone growth is completed before fledging and at this point tarsus remained 4% shorter and hand skeleton 5% shorter than controls [as in three altricial food-restricted species (Boag, 1987; Schew, 1995; Searcy et al., 2004)], and this was irreversible. The consequences of a shorter leg and wing length in the kestrel are unknown. Concerning sexual selection, female kestrels select males with shorter tarsi and do not discriminate short-winged males (Hakkarainen et al., 1996), so the slightly shorter cort-nestling males are probably not at a disadvantage. However, this might be different in female kestrels, and in species without reversed sexual size dimorphism, where smaller birds often have a reduced fitness (Richner, 1989).

If wing feathers of cort-nestlings do not fully recover, wing length and wing area would be somewhat smaller, possibly negatively affecting flight performance and hunting capabilities and presumably survival. In male kestrels, however, a smaller wing does not have to be a disadvantage. Short-winged male kestrels are somewhat better hunters than longer-winged ones (Hakkarainen et al., 1996). If fledglings survive the first year, they have the opportunity to replace shorter primaries with longer ones during moult the following summer. This might be a good strategy, because accelerated feather growth is associated with a lower feather quality (Dawson et al., 2000) and general costs of compensatory growth (Metcalfe and Monaghan, 2001).

Cort-nestlings fledged with similar fat stores to placebo nestlings. However, if the reduction in body mass of cort-nestlings was not compensated for until fledging, cort-nestlings would have fledged at a lower body mass. Several studies have shown that body mass at fledging is a good predictor of survival (Lindén et al., 1992; Naef-Daenzer et al., 2001). In kestrels, fledglings are still fed by their parents up to 4 weeks after fledging and it is possible that the corticosterone-treated nestlings could catch up then.

No differential effect of corticosterone on the sexes and ages

In all morphological parameters investigated corticosterone treatment did not differentially affect the sexes and younger nestlings within the brood. We did not find indications that later born, smaller nestlings are affected more strongly than older, larger ones. Hence, we have no evidence that elevated corticosterone levels amplify size-dependent mortality resulting from asynchronous hatching. However, size differences between sexes and ages were not large in our kestrels.

Conclusions

This study demonstrated in altricial and free-living nestlings that elevated plasma levels of corticosterone alone, without food restriction, suppress growth and, thus, that the action of corticosterone alone is involved in the control of developmental plasticity. It follows that environmental stressors without energetic restrictions (e.g. human disturbance, disease) may have a growth-suppressing effect and consequently the potential to shape phenotype. A relatively short disturbance of 2–3 days resulting in high corticosterone levels can have far-reaching consequences on morphology and fitness. In the context of conservation biology, it will be important to investigate the effect of repeated high corticosterone levels, as may occur as a response to repeated human disturbance, on growth and development.

With the corticosterone administered in this study, effective for only a few days, we provoked a reduced primary feather and tarsus length before fledging occurred, corresponding to that of siblings born 3 days later. However, feather, bone and body mass growth were reduced to different degrees. This indicates that corticosterone has not an overall suppressing effect on growth but a differential effect favouring presumably the most sensitive tissues of the actual developmental phase. Such a differential effect was also observed in nutritional restriction experiments. Because food shortage usually results in elevated corticosterone levels, this points to a steering role of corticosterone on growth allocation during nutritional restriction and other disturbances.

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