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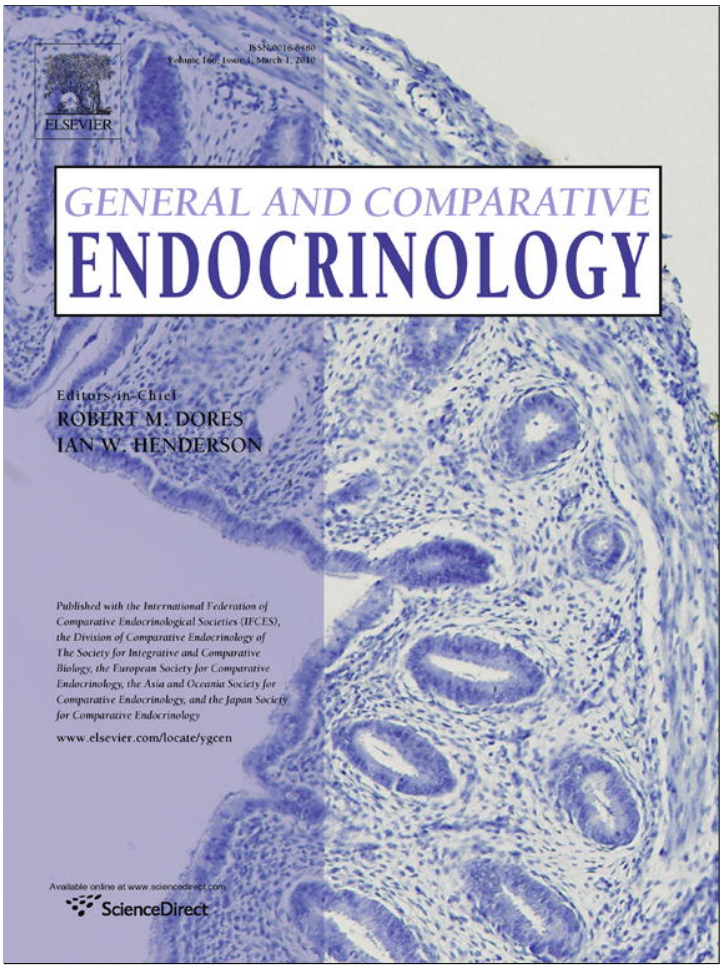

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Food deprivation modifies corticosterone-dependent behavioural shifts in the common lizard

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ABSTRACT

Stressful events typically induce glucocorticoid production that suppresses unnecessary physiological and behavioural functions. The glucocorticoid production also temporally activates alternative behavioural and physiological pathways. These responses are generally adaptive changes to avoid the negative effects of stressors. However, under low food availability, these behavioural and physiological modifications might lead to energetic costs. We therefore predict that these responses should not be activated when there are energetic constraints (e.g., low food availability). We experimentally tested whether food deprivation modifies corticosterone-induced behavioural and physiological responses in captive male common lizards. We measured corticosterone-induced responses in terms of body mass, metabolic rate, activity level and basking behaviour. We found that corticosterone-induced various behavioural and physiological responses which were dependent on food availability. Well-fed lizards treated with corticosterone were active earlier, and increased their basking behaviour. These behavioural modifications did not occur in food-deprived lizards. This inactivation of stress-related behavioural changes probably allows the lizard to save energy.

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1. Introduction

In many species, individuals adjust their phenotype in response to environmental changes (West-Eberhard, 1989). These phenotypic adjustments necessary to face a stressful event often require energetic expenditure that exceeds the energy necessary to maintain all current functions (McEwen and Wingfield, 2003; Romero, 2004; Wingfield, 2003). In many cases, responses to environmental perturbations involve the production of glucocorticoids that often mediate changes in physiological pathways and behavioural expression that minimize energy expenditure (i.e., emergency life history stages; Romero, 2004; Wingfield, 2003). For example, increased glucocorticoid levels can suppress reproductive behaviour (Moore and Jessop, 2003; Silverin, 1998), social activity (DeNardo and Licht, 1993) and partial regulation of the immune system (Berger et al., 2005; Morici et al., 1997), or can increase activity and foraging (Breuner et al., 1998; Cote et al., 2006; Gleeson et al., 1993; Tataranni et al., 1996). These stress responses constitute a set of adaptive changes that should promote survival (Breuner et al., 2008; Cote et al., 2006; Romero, 2004; Wingfield, 2003).

Corticosterone concentrations rise in response to both short-term (e.g., social interactions and food availability; Creel, 2001; Greenberg et al., 1984; Knapp and Moore, 1995; Wingfield and Ramenofsky, 1999) and long-term stressors (e.g., social status; Creel, 2001; Fox et al., 1997; Sapolsky, 1988). While short-term stressors are believed to induce different physiological responses than long-term stressors, the behavioural stress responses are often similar. For example, chronically increased plasma levels of corticosterone also reduce or suppress aggressive and reproductive behaviours (de Fraipont et al., 2000; DeNardo and Licht, 1993; Tokarz, 1987) as well as activity (DeNardo and Sinervo, 1994) in lizards. However, a temporary rise in glucocorticoid level increases energetic expenditure in a lizard species (DuRant et al., 2008). Moreover, a sustained elevation of glucocorticoid production in response to long-lasting stressors requires more energy than a temporary one (Pravosudov et al., 2001; Romero, 2004) and may then have negative consequences, such as catabolization of fast-twitch muscle fibres leading to loss of muscle function, reduced immunocompetence or neural degeneration (Berger et al., 2005; McEwen et al., 1997; Morici et al., 1997; Sapolsky et al., 2000). In many cases, individuals experiencing chronic stress may compensate

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for the energetic costs by enhancing, for example, food intake (Cote et al., 2006; Kitaysky et al., 2003; Tataranni et al., 1996).

Under some environmental circumstances, however, energetic requirements may exceed available environmental resources. From an evolutionary perspective, stress-induced behavioural and physiological responses should then be modulated according to energetic constraints encountered in the environment or to intrinsic factors such as the ability to mobilize energy from fat reserves. More precisely, in conditions in which resource availability/energetic reserves does not compensate for the energetic requirements of the stress response, the behavioural and physiological modifications may not be activated (Landys et al., 2006). For example, Astheimer et al. (1992) showed that birds modulate their activity and their metabolic rate according to their level of satiety to prevent unnecessary energetic expenditure. Some other studies also showed that corticosterone-induced modifications strongly depend on body condition (Angelier et al., 2007; Loiseau et al., 2008), suggesting the effect of energetic conditions on stress responses. Although previous studies have predicted adaptive responses to long-lasting stress (Cabezas et al., 2007; Cote et al., 2006), the consequences of reduced food availability on the development of behavioural responses to chronic corticosterone elevations still remain unclear. Increasing activity in a food-deprived environment is inefficient and may lead to undesirable increases in energy expenditure; thus, we predict that chronically stressed animals should not exhibit this inefficient stress response in a food-deprived environment.

Here, we experimentally tested whether food deprivation modifies the corticosterone-induced behavioural responses in the common lizard (*Lacerta vivipara*). In this species, a chronic elevation of corticosterone increases energetic expenditure, food consumption, activity and basking behaviour (Cote et al., 2006; de Fraipont et al., 2000). Furthermore, corticosterone has direct and indirect positive effects on male survival via its effects on behaviour and physiology (Cote et al., 2006). Moreover, in this species, food availability is known to affect several important life history traits, for example offspring dispersal (Massot and Clobert, 1995). Thus, we expect that underfed and well-fed lizards will respond to corticosterone treatment in different ways. Based on previous studies we predict that well-fed lizards chronically treated with corticosterone should have greater mass loss and higher metabolic rate, should start their activity earlier and spend a greater proportion of time active compared to control lizards. However, we expect these responses to be reduced or suppressed in food-restricted lizards.

2. Methods

2.1. Species, study-site and housing conditions

The common lizard (*L. vivipara* Jacquin, 1787) is a small lacertid (adult snout–vent length: males 40–60 mm, females 45–75 mm) living in humid habitats in Eurasia. The diet consists of small insects, spiders and earthworms (Avery, 1962).

The experimental design was applied to two separate groups of adult males (>2 years old) in which different variables were measured (because data collection continued on one group for an additional analysis to be reported elsewhere, Fig. 1). These two groups have been collected at the same time (in June 2006) over 4 days from three populations in close proximity (less than 1 km a part) on the Mont Lozère (France, 44°27′N, 3°44′E, average elevation of 1400 m) in Southern France. Group 1 consisted of 36 individuals (9 in each treatment combination) and were used to measure plasma corticosterone levels on day 11. Group 2 consisted of 84 individuals (21 in each treatment combination) and was used to measure body mass on days 1 and 11, standard metabolic rate on day 11 (on a subset of 40 individuals) and behaviour on day 11. Individuals were allocated to hormonal and feeding treatments (described thereafter) in a full crossed design. Half of the males were randomly chosen and treated with corticosterone, while the remaining males were treated with a control treatment. Within each hormonal treatment (corticosterone and control), half of the males were randomly chosen to receive a high feeding treatment, while the remaining males received a low feeding treatment. Treatment groups did not differ in body size (snout–vent length: SVL), body condition or date of capture ($p > 0.20$ for all the simple treatments effects and the interaction) and the same number of lizards from each population were allocated to each of the treatment groups. Prior to the experiment, lizards were individually housed in captivity in plastic terrariums (25 × 15.5 × 15 cm; Le Galliard et al., 2003) containing 3-cm deep litter. In one corner of the terrarium a bulb provided heat for thermoregulation from 09:00 to 12:00 h and from 14:00 to 17:00 h. The bulb created a temperature gradient in the terrarium from room temperature (19–24 °C night–day) to 35–37 °C (below the bulb), encompassing the thermal needs range of this species (Van Damme et al., 1986). An opaque egg carton was added, allowing lizards to hide. Lizards were able to behave normally and behaviour associated with escaping (e.g., scratching on the walls) was rarely observed. Experimental methods adhered to the National Institute of Health Guide for Care and Use of Laboratory Animals. The experiment described below was started 4 days after the capture of the last lizard.

2.2. Experimental treatments

2.2.1. Experimental corticosterone application

The males of each group were allocated to either a corticosterone or a control treatment. The corticosterone treatment consisted of a daily application of 4.5 μl of sesame oil mixed with corticosterone (3 μg of corticosterone/μl oil). Control lizards were treated with 4.5 μl of only sesame oil (for more details, see Cote et al., 2006; Meylan et al., 2003). The treatment was applied on the dorsal surface of the lizard each evening for 10 days (starting on day 1, Fig. 1).

This non-invasive method of corticosterone treatment is similar to that described by Knapp and Moore (1995). It leads to a 5- to 10-fold increase of the basal blood corticosterone levels (equivalent to an absolute increase of about 100 ng/ml) compared to natural populations (Meylan et al., 2003; Cote et al., 2006). Average basal plasma corticosterone levels of individuals that were housed for 1 day in the laboratory day were 21.64 ng/ml for females (max. 101.97 ng/ml, Meylan et al., 2003) and 77.03 ng/ml for males (max. 181 ng/ml, Cote et al., 2006). Our manipulation therefore corresponds to the upper level of corticosterone that naturally occurs in response to acute stress in reptiles (DuRant et al., 2008; Tyrrell and Cree, 1998; see Section 4 for more details).

2.2.2. Feeding treatment

Within each of the hormone treatments (corticosterone and control), half of the individuals were randomly allocated to well-fed and food-restricted treatments. The feeding treatment levels were based on three previous studies of the same species (Cote et al., 2006; Le Galliard et al., 2004; Massot and Clobert, 1995). At the start of the experiment (day 1, Fig. 1), each lizard of the well-fed group was offered one *Pyralis farinalis* larva, whereas lizards of the food-restricted group received no larva to create a diet restriction. Five days later (day 6), all lizards were offered one larva. Only larvae of similar body mass were used to feed the lizards (254 mg ± 12.64 SE). In nature, this species feeds on small insects, spiders and earthworms (Avery, 1962). Our feeding regime is less diverse than in nature, but (1) larvae are part of the feeding regimes of this species and (2) a single specific prey allowed us to precisely control the feeding

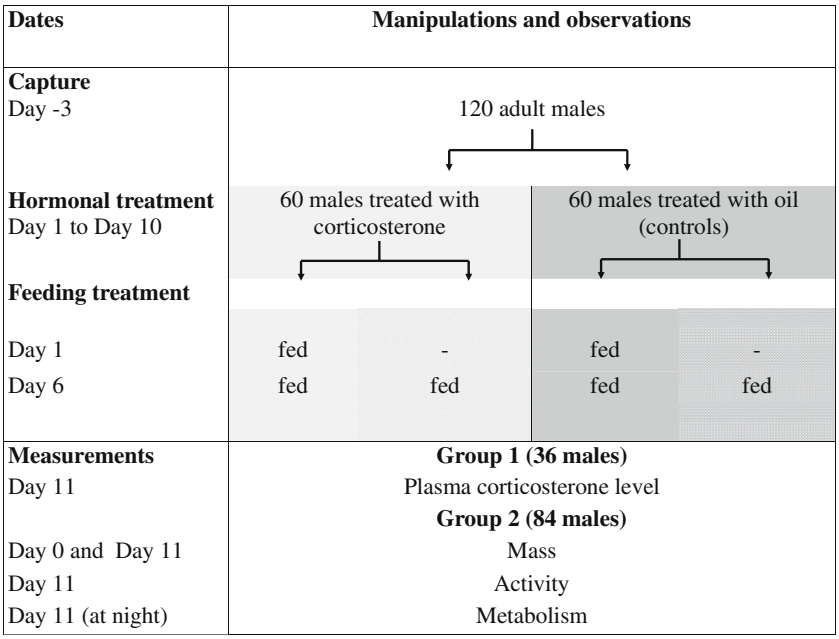

Fig. 1. Experimental design. Corticosterone and first-feeding treatment started 4 days after capture. At the beginning of the experiment (day 0), only lizards in the well-fed group were fed. Five days later, all lizards were fed. After 10 days of corticosterone treatment, we measured plasma corticosterone levels on 36 males (group 1; 9 from each treatment combination) and activity, body mass and standard metabolic rate on 84 males (group 2; 21 from each treatment combination).

treatments. Because, we did not have any data on previously acquired food items by the experimental animals, we randomized the distribution of lizards into the feeding treatment according to, e.g., the capture date. However, a pilot experiment (Massot, unpublished) aiming to study the intestinal fauna of this species (45 individuals) measured that the intestinal transit was not taking more than 3 days, a period which correspond to the time the lizards were kept unfed before to start the experiment. Previous studies have also showed that one larva every 5 days fulfils the energy demands of this species (Massot and Clobert, 1995) and that individuals temporarily fed with this diet presented similar survival or reproduction characteristics into their natural habitats to those not manipulated (Clobert et al., 2000). Living larvae were presented to the lizards between 11:30 and 12:30 h and were usually immediately attacked and eaten and, in all cases, eaten by the evening. Terraria were covered with fine mesh to avoid animals gaining additional prey accidentally. Water was available *ad libitum*.

2.3. Physiological and behavioural measurements

2.3.1. Body mass and standard metabolic rate

The body mass of each lizard (group 2) was measured to within 2 mg, both on the day before the first feeding treatment (day 0) and on the 11th day of the experiment (Fig. 1).

On day 11 (at night), we measured the standard metabolic rate (SMR) for 40 males from group 2, i.e., 10 lizards from each treatment group, randomly chosen. On this sub-sample, we checked that treatment groups did not differ in body size, initial body condition or date of capture ($p > 0.30$ for all the simple treatments effects and the interaction). The SMR was measured as the minimum rate of energy expenditure measured under post-absorptive conditions in the inactive phase of the daily cycle (Stevenson et al., 1957) and it is measured at a given temperature within the animal's range of activity (Lewis and Gatten, 1985). The estimation of SMR relies on the measurement of the volume of oxygen consumed per unit of time (ml O_2 g^{-1} h^{-1}). Our methodology assumes that energy production related to the consumption of a given volume of oxygen is roughly constant, a condition currently accepted (Schmidt-Nielsen, 1997).

Oxygen consumption was measured using an open air flow respirometer from Sable Systems (Las Vegas, Nevada, USA) comprising: a two-channel pump PP-2, two mass flow controller electronics units MFC-2, an eight-channel multiplexer TR-RM8, an FC-10a Oxygen analyzer and the sub-sampler/pump/mass flow meter unit TR-SS3. Animals were placed individually in one of the three 200 ml darkened chambers, with an airflow of 30 ml/min. Oxygen consumption was measured every 2.5 min over a 30-min period at 25 ± 0.5 °C. Oxygen consumption was calculated as the difference between the oxygen consumption in the ambient air and that at the exit of the chamber. All lizards have been deprived of food for 3 days before the measurements were taken. We modelled the mass-specific SMR by using the residuals of the regression of SMR with body mass as variable.

2.3.2. Behavioural measurements

Activity of each lizard (group 2) was measured on day 11 of the experiment. First, time of emergence from the nocturnal shelter (under the egg carton or in the ground) was measured by a naïve observer every 15 min between 09:00 and 12:00 h, and was expressed as the number of minutes after 09:00 that took a lizard to leave their nocturnal refuge (Cote et al., 2006). Second, activity was measured by the same naïve observer every 15 min between 16:00 and 17:00 h (five observations): (1) if the lizard was active or not (at least partially visible or not) and (2) if the lizard was basking or not (Lecomte, 1993; Lecomte et al., 1994). If the lizard was basking, the observer also distinguish between two basking behaviour: (a) full-basking below the light on the shelter (upright head position and increased respiration, see Carpenter and Ferguson, 1977; Huey, 1982, for a precise description) and b) half-basking below the light (the head under the light and the rest of the body hidden). We distinguished half-basking behaviour from basking, as the behaviours may differ in thermoregulatory efficiency or predation risk (in the natural situation). Half-basking behaviour may also reflect the trade-off between the need for basking and the risks of being exposed.

2.3.3. Plasma corticosterone levels

Blood samples (on average 40 μl), made on males from group 1, were taken from the post-orbital sinus using two to three 50 μl microhematocrit tubes to measure plasma corticosterone levels. All samples were taken in less than 3 min. Samples were taken between 16:00 and 17:30 h to reduce variability in corticosterone levels due to diurnal rhythms in its secretion. Corticosterone levels were determined using the enzyme-immunoassay procedure (IDS, Inc., USA Octeia Corticosterone Kit, ref. AC14F-1, lot 55642). In brief, this kit is a competitive enzyme-immunoassay utilizing a polyclonal corticosterone antibody coated on the inner surface of polystyrene microtiter wells. Calibrators, controls and samples were incubated with enzyme (peroxidase)-labelled corticosterone in the antibody-coated wells overnight at 28 °C. The wells were washed and colour was developed using a chromogenic substrate (tetramethylbenzidine). The absorbance of the stopped reaction mixtures were read in a microplate reader, with colour intensity developed being inversely proportional to the concentration of corticosterone of the samples. The kit's sensitivity is 0.55 ng/ml, plasma samples ranged from 23 to 363 ng/ml (CV intra assay = 4.9, CV inter assay = 7.8; correlation between two repeats for four individuals, $n = 4$; $F_{1,2} = 26.71$, $p = 0.036$, $r = 0.93$).

2.4. Statistics

The time of emergence, the change of body mass during the experiment (body mass after the end of the treatment minus body mass before the beginning of the treatment), plasma corticosterone levels and the SMR were analyzed using general linear model (Proc GLM in SAS v8.02). We analyzed the change in body mass with body length as an independent variable in a general linear model (Darlington and Smulders, 2001; Garcia-Berthou, 2001). The variables describing the activity between 16:00 and 17:00 h were analyzed using logistic regressions with a logit and a binomial error distribution (GENMOD procedure in SAS v8.02). The time spent active (number of times a lizard was visible) was the response term (i.e., events) and the number of observations (i.e., five observations) was the binomial denominator (i.e., trials). We applied the same procedure for the active time spent basking. The number of times a lizard was basking was the response term and the number of times a lizard was active was the denominator. Finally, we also analyzed the basking time a lizard spent in half-basking behaviour. The number of times a lizard was half-basking was the response term and the number of times a lizard was basking was the denominator. The assumptions in all presented models were verified on the residuals and were fulfilled. For logistic regressions, we checked models for overdispersion and scaled the deviance when necessary. Full models contained the treatments, body length, the population of origin and all interactions. All models were simplified using backward elimination of the non-significant interactions and factors (if not part of significant interactions). The significance level was set at $p = 0.05$. The population of origin and its interactions with the treatment were never significant (all $p > 0.50$), and thus we have not presented these effects. Differences in sample sizes reflect a missing value due to insufficient blood volume for plasma corticosterone levels and a missing value for SMR.

3. Results

3.1. Body mass change and standard metabolic rate

Lizards weighed around 2.5 g ± 0.06 SE at the start of the treatment, but body mass decreased on average during the experiment (−0.26 g ± 0.02 SE), with body mass change being negatively related to the body length ($F_{1,76} = 21.50$, $p < 0.0001$). In other words, large lizards lost less mass than small ones. Corticosterone had no main effect on body mass change (modelled with body length as an independent variable; $F_{1,76} = 1.38$, $p = 0.24$), but the interaction between corticosterone and feeding treatments was significant ($F_{1,76} = 4.20$, $p = 0.04$, Fig. 2A). Separate analyses of feeding treatments showed that in the well-fed group, body mass decreased in corticosterone-treated males ($F_{1,36} = 4.97$, $p = 0.03$), whereas corticosterone treatment had no significant effect on male body mass in the food-restricted group ($F_{1,36} = 0.44$, $p = 0.51$).

Corticosterone treatment significantly decreased the standard metabolic rate ($F_{1,33} = 14.61$, $p = 0.04$, Fig. 2B) but the interaction between corticosterone and feeding treatments was not significant ($F_{1,33} = 5.34$, $p = 0.20$). Feeding treatments did not affect the standard metabolic rate ($F_{1,33} = 2.89$, $p = 0.35$).

3.2. Behavioural measurements

Corticosterone interacted with the feeding treatment to modify the emergence time (Table 1). Corticosterone treatment significantly decreased the emergence time in the well-fed group, but this effect was not significant in the food-restricted group (Table 2 and Fig. 3A). Time of emergence also depended on population of origin (Table 1) which might reflect, e.g., a maintained effect of environmental conditions on activity pattern.

The two treatments did not significantly affect the proportion of times a lizard was active (Table 1 and Fig. 3B), but corticosterone interacted with the feeding treatment to modify the proportion of active time a lizard was basking (Table 1). Corticosterone treatment significantly increased the basking time in the well-fed group, but this effect tended to be opposite in the food-restricted group (Table 2 and Fig. 3C). The proportion of basking time spent in half-basking behaviour significantly depended on corticosterone treatment, but not on feeding treatments (Table 1). Corticosterone-treated lizards were half-basking significantly more than control lizards (Fig. 3D). However, separate analyses revealed that the effect of corticosterone treatment is only significant for well-fed lizards (well-fed group: $\chi_1^2 = 5.80$, $p = 0.02$; food-restricted group: $\chi_1^2 = 0.36$, $p = 0.55$).

3.3. Plasma corticosterone levels

Corticosterone-treated males had higher plasma corticosterone levels than control males while food restriction did not significantly affect plasma corticosterone levels (corticosterone treatment: $F_{1,31} = 4.93$, $p = 0.03$; feeding treatment: $F_{1,31} = 3.17$, $p = 0.08$; feeding treatment × corticosterone treatment: $F_{1,31} = 0.13$, $p = 0.71$; Fig. 4).

4. Discussion

Chronic elevation of corticosterone levels induced various behavioural stress responses according to the feeding treatment. Well-fed corticosterone-treated lizards were active earlier, and increased their basking behaviour, in particular half-basking behaviour, in comparison with control well-fed lizards. Well-fed lizards treated with corticosterone lost more mass than well-fed lizards treated with a control treatment. These observations were consistent with those reported in a former study for the same species (Cote et al., 2006) and might reflect the increased energetic expenditure necessary to develop a behavioural response to the stressor. If enough food is available, corticosterone appears to enhance activity, potentially counteracting the negative effects induced by the stressor, as predicted previously (Astheimer et al., 1992; Romero, 2004; Wingfield, 2003; Wingfield and Ramenofsky, 1999). Although basking in the open is more efficient, this behaviour may be risky for lizards as they

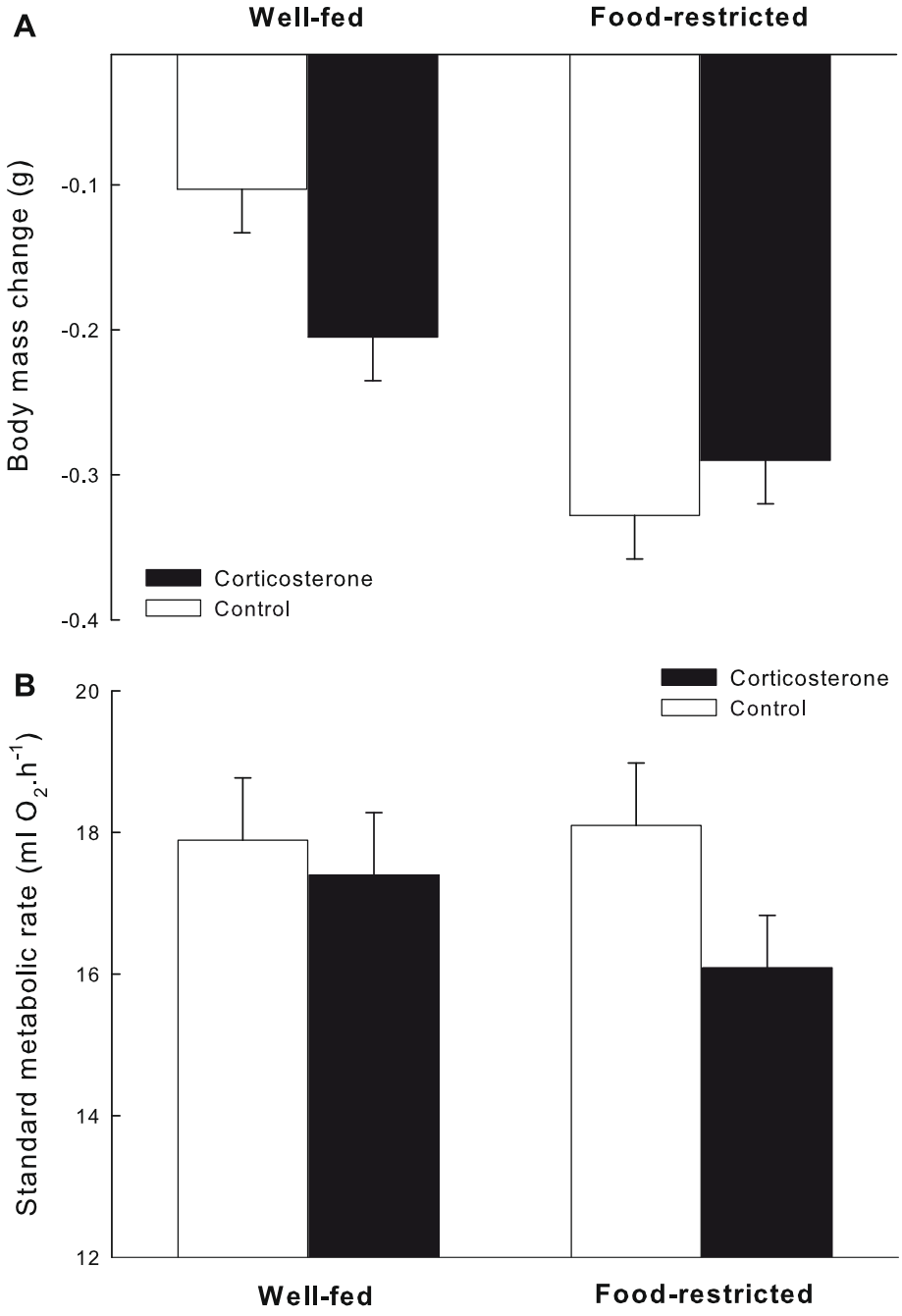

Fig. 2. Physiological changes of male lizards in relation to corticosterone and feeding treatments. Mean values (±SE) per treatment groups shown. (A) Mean (±SE) body mass change (body mass at the end of the laboratory experiment – body mass at the start of the experiment). (B) Standard metabolic rates of male lizards.

Table 1
Effect of corticosterone treatments, feeding treatments and population of origin on the time of emergence, the proportion of time spent active, the proportion of active time spent basking and the proportion of basking time spent in half-basking behaviour.

	Corticosterone	Feeding treatment	Corticosterone * feeding treatment	Population of origin
Time of emergence	$F_{1,78} = 4.63$, $p = 0.03$	$F_{1,78} = 0.35$, $p = 0.55$	$F_{1,78} = 5.53$, $p = 0.02$	$F_{2,78} = 5.20$, $p = 0.04$
Proportion of time spent active	$\chi_1^2 = 2.88$, $p = 0.09$	$\chi_1^2 = 0.39$, $p = 0.53$	$\chi_1^2 = 0.004$, $p = 0.95$	$\chi_2^2 = 0.09$, $p = 0.95$
Proportion of active time spent basking	$\chi_1^2 = 1.12$, $p = 0.29$	$\chi_1^2 = 0.26$, $p = 0.61$	$\chi_1^2 = 13.17$, $p = 0.0003$	$\chi_2^2 = 3.46$, $p = 0.18$
Proportion of basking time spent half-basking	$\chi_1^2 = 4.87$, $p = 0.03$	$\chi_1^2 = 4.82$, $p = 0.03$	$\chi_1^2 = 2.64$, $p = 0.10$	$\chi_2^2 = 4.46$, $p = 0.11$

face greater exposure to predators (Christian and Tracy, 1981; Clobert et al., 2000). Thus, half-basking is thought to be a strategy optimizing the trade-off between predation risks and basking efficiency. This possibly corresponds to a strategy for decreasing risk by increasing thermoregulation in stressful conditions. Our results suggest that, in an energetically unconstrained environment, even long-lasting high corticosterone levels induce an adaptive response to stressors.

Table 2
Effect of corticosterone treatments on the time of emergence from overnight stay and on the proportion of active time a lizard spent basking for each feeding treatment.

	Dependent variable	Corticosterone
Well-fed treatment	Emergence	$F_{1,40} = 10.42$, $p = 0.003$
	Active time spent basking	$\chi_1^2 = 10.60$, $p = 0.001$
Food-restricted treatment	Emergence	$F_{1,40} = 0.03$, $p = 0.87$
	Active time spent basking	$\chi_1^2 = 3.5$, $p = 0.06$

4.1. Energy-saving strategy under food restriction

Food deprivation has strongly affected lizards' physiology. Food-restricted lizards lost 12% of their mass during the experiment, whereas well-fed lizards lost only 3–6% (depending on corticosterone treatment). In captivity, body mass usually decreases in the first 2–3 weeks (personal observation). The extra body mass loss due to the corticosterone involves a reduction of individual energetic reserves. In energetically unconstrained environments, this reduction should act as a signal for initiating increased foraging activity. In an energetically constrained environment, however, a low energy reserve should on the contrary stop lizards from developing increased activity in response to stressors. Indeed, high corticosterone plasma levels did not increase activity or alter basking behaviour in food-restricted lizards. Thus, food-restricted lizards possibly adopt an energy-saving strategy, in which the increased energy pathway requiring a change of behaviour to enhance food consumption is shut down in an environment which

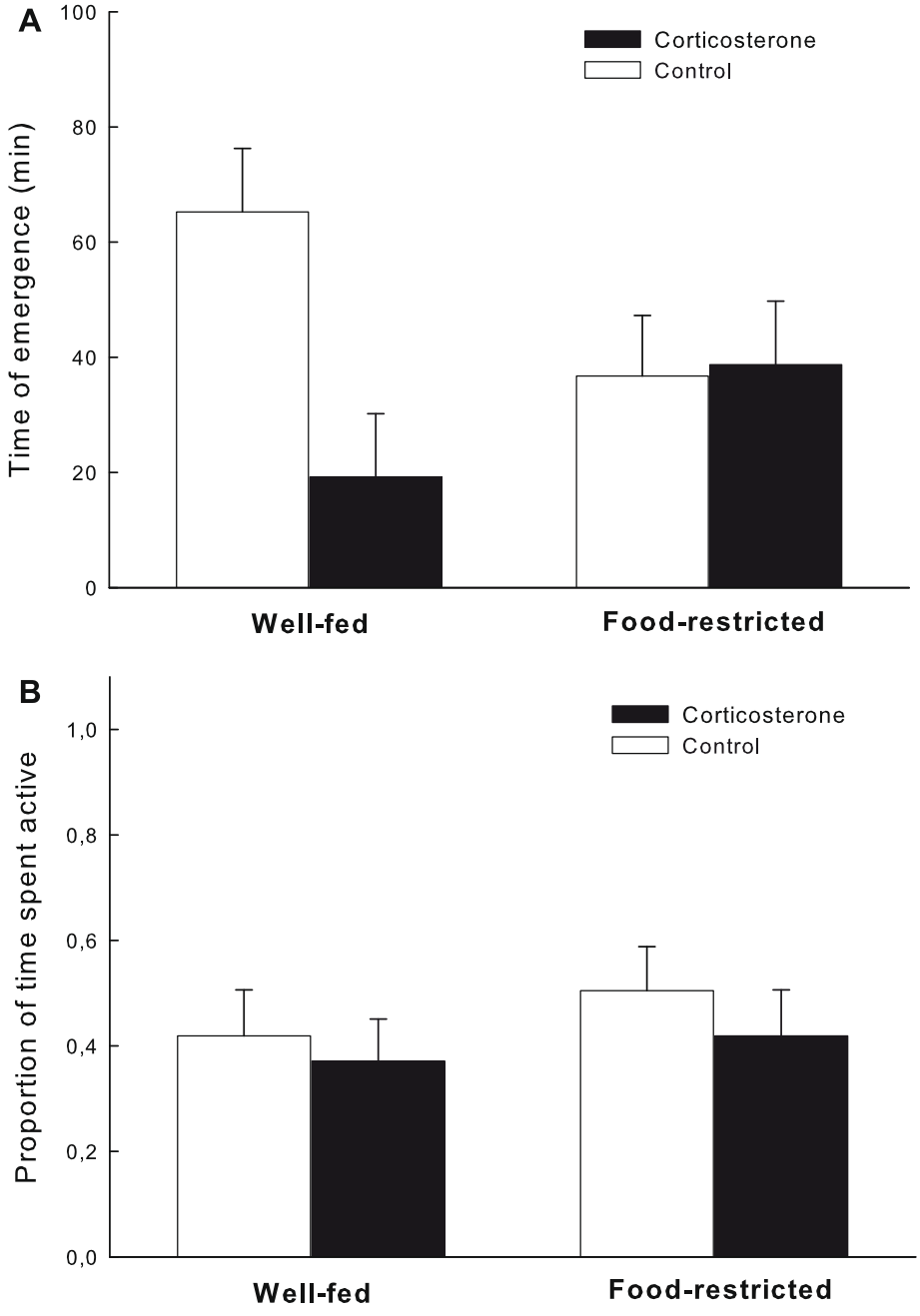

Fig. 3. Emergence from over night stay and activity between 16:00 and 17:00 h of male lizards in relation to corticosterone and feeding treatments. Mean values (±SE) per treatment groups shown. (A) Mean (±SE) time of emergence. (B) Mean (±SE) proportion of time spent active. (C) Mean (±SE) proportion of active time spent basking. (D) Mean (±SE) proportion of basking time spent in half-basking behaviour.

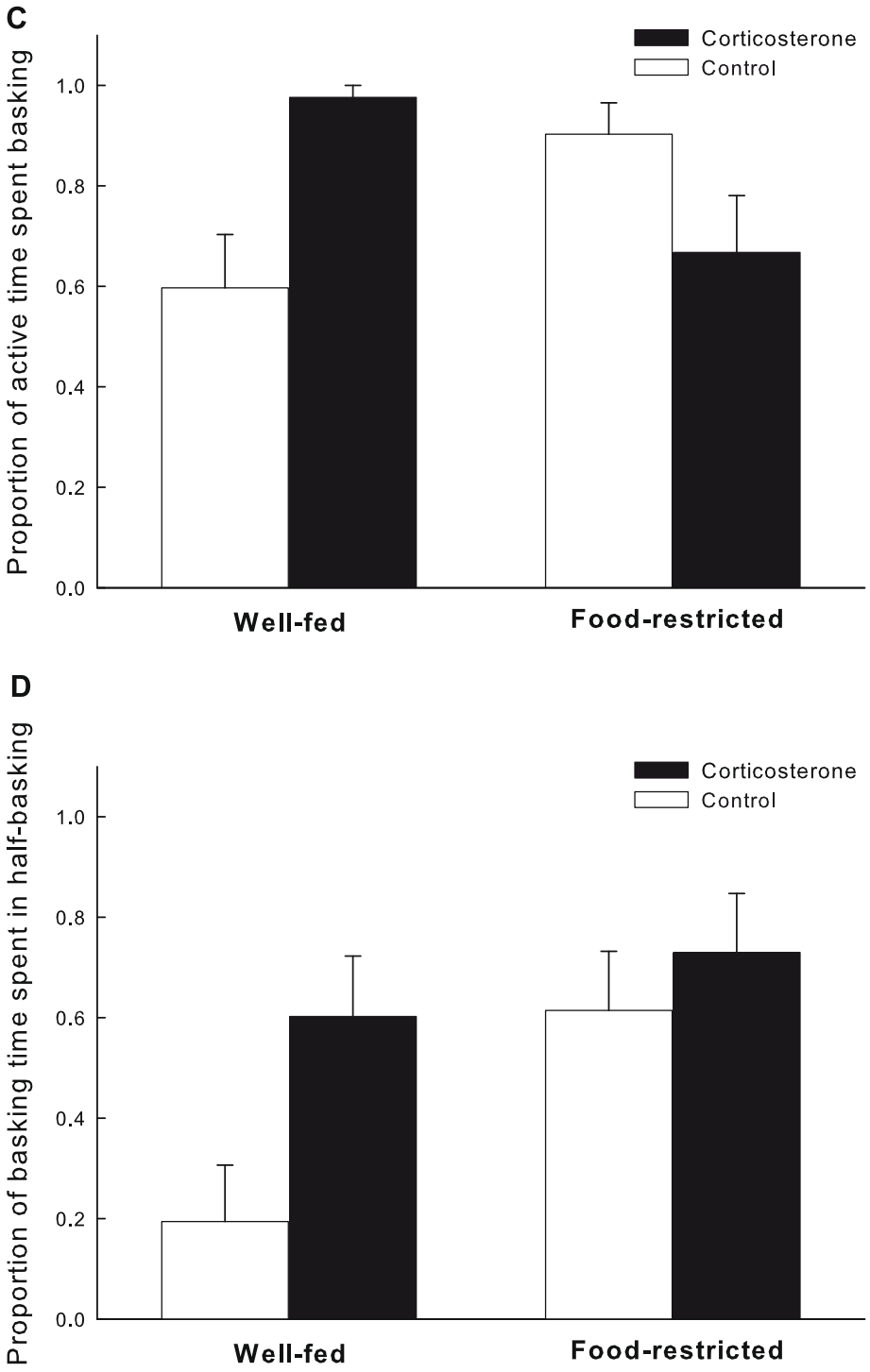

Fig. 3 (*continued*)

is energetically constrained, i.e., one in which foraging is likely to be unsuccessful. As expected in this case, food-restricted corticosterone-treated lizards had the same body mass loss as food-restricted control lizards. Corticosterone-induced energetic consumption appears to be compensated for by decreasing other energy demanding processes (such as an activity) in order to avoid losing excess weight in an environment not offering the possibility of increasing food intake. The decreased standard metabolic rate is also consistent with energy saving in a constrained environment (Astheimer et al., 1992; Hiebert et al., 2000). Decreasing the standard metabolic rate is one way for saving energy from non-vital expenditure. Even if a direct relationship between SMR and energy saving is missing, our results accords with studies on other species (Hiebert et al., 2000; Miles et al., 2007). Especially, Hiebert et al. (2000) showed that corticosterone treatment in hummingbirds increases nocturnal torpor to preserve energy during the night. In the side-blotched lizard *Uta stansburiana*, chronically elevated levels of corticosterone significantly reduced resting metabolic rate and oxygen consumption during the night (Miles et al., 2007). Miles et al. (2007) suggested that the corticosterone-induced reduction in resting oxygen consumption might help to reduce the daily energy requirements during periods of inactivity and minimize

Fig. 4. Plasma corticosterone levels of male lizards from group 1 in relation to corticosterone and feeding treatments. Mean values (±SE) per treatment groups shown.

energetic expenditure which in turn increases survival during stressful episodes. However, the link between corticosterone and metabolism is still unclear as highlighted by the increases in respiration subsequent to corticosterone treatment in western fence lizards (*Sceloporus occidentalis*; DuRant et al., 2008). In our study, whatever the food environment, lizards treated with corticosterone maintain low overnight metabolic expenditure. In a food-deprivation context, a reduction in energy requirement appears to be adaptive, and could be an example of a strategy where individuals wait for better environmental conditions. In an environment where less food is available, energy obtained from environmental resources cannot compensate for the energetic requirements of the behavioural stress response. Therefore, the cheapest strategy is to save energy until the stressor disappears or the conditions improve. From a functional perspective, our results might be explained by the "corticosterone insensitivity hypothesis" (Breuner et al., 2003). Some previous studies indeed showed that, in harsh conditions, the nervous system is "desensitized" at high corticosterone level. Breuner et al. (2003) hypotheses that the decreased sensitivity to circulating glucocorticoids may result from a reduction in neural glucocorticoid receptors (GR-like receptors), leading to reduced behavioural and physiological responses when environmental conditions are harsh. Future studies would require measures of corticosterone globulin levels or corticosterone-receptor density.

4.2. Acclimation or a context-dependant chronic stress response

Stress responses mediated by corticosterone are context-dependent. Evidence from several vertebrate classes suggests that corticosterone secretion in response to a stressor depends on physiological and environmental factors, including body condition, sex, reproductive status, social status, season and other environmental conditions (Landys et al., 2006; Romero, 2004; Wingfield, 2003; Wingfield and Ramenofsky, 1999). Recently, Lendvai et al. (2007) reported that adult house sparrows with experimentally enhanced brood sizes responded less strongly to a stressor than adults with experimentally reduced brood sizes and some other studies showed that stress response of an individual depend on its body condition (Angelier et al., 2007; Loiseau et al., 2008). Lendvai et al. (2007) concluded that individuals might actively modulate their stress response according to the costs and benefits of their current condition. More generally, individuals allocate energy between maintaining body functions and responding to stressors. If energy resources were low, individuals would allocate more energy to maintaining body functions than to developing a stress response.

Moreover, a chronically stressed animal may be less sensitive (acclimated) to additional stressors, as explained by Romero (2004) in his review about acclimation and facilitation processes. Indeed, the stress response of rats after several weeks of handling was smaller than their initial response (Dobrakova et al., 1993). Rats acclimatized to handling, stopped considering handling to be noxious and reduced the glucocorticoid response. Similar acclimatization has also been found in the common lizard (Dauphin-Villemant and Xavier, 1987). In our study, animals in low nutritive conditions may have acclimatized to stress. Indeed, we observed a complete reduction of corticosterone-induced behavioural and physiological changes (i.e., activity and body mass loss) in food-restricted lizards. However, the corticosterone treatment increased plasma corticosterone levels whatever the feeding treatment suggesting that the acclimatization is unlikely. Even if food restriction did not significantly affect significantly plasma corticosterone levels, it might be due to a low sample size. In particular, food-restricted control lizards had similar plasma corticosterone levels than well-fed corticosterone-treated lizards. Food restriction might create a chronically stressful situation (Kitaysky et al., 1999) leading to the reduction of corticosterone-induced behavioural and physiological changes. The decrease of food-restricted lizards stress response might thus reflect acclimation or a condition-dependent chronic stress response. Further studies are needed to understand the proximate origin of the decrease of food-restricted lizards stress response.

4.3. Validation of the corticosterone treatment

In our study, we detected higher corticosterone levels to those reported in other studies. Meylan et al. (2003) reported basal

corticosterone levels averaging 21 ng ml^{-1}, but these were using only pregnant females. The basal levels reported in this study for control well-fed males were much higher. However, Cote et al. (2006) reported that basal corticosterone levels averaged 77 ng ml^{-1} for males and, using a large dataset of free-living males, we can show that basal corticosterone levels averaged 54 ng ml^{-1} (Cote, unpublished data). We believe that the sex/status (pregnant) of the lizard is responsible for the observed difference in basal corticosterone levels between studies. Moreover, plasma corticosterone levels of control males and of non-manipulated free-living lizards were similar. It suggests that corticosterone levels were not affected by the manipulation when we applied the treatment, a result similar to the one obtained early no on the same species (Dauphin-Villemant and Xavier, 1987).

In our study, corticosterone treatment has been applied once a day for 10 following days. According to Meylan et al. (2003), this treatment induces repeated elevation of plasma corticosterone levels, simulating the effect of chronic stressors that may occur when stressors exist over a long period. Plasma corticosterone levels after treatment had increased 1.6-fold in corticosterone-treated males. Although these values were significantly elevated, the mean plasma corticosterone level in corticosterone-treated males was still within the range of plasma corticosterone levels detected in control well-fed lizards (from 20.05 to 143.24 ng.ml^{-1}) and only two corticosterone-treated lizards had a plasma corticosterone level out this range. The induced increase was large but it was still within the natural range of plasma corticosterone levels for this species. However, further investigations should study the dose-dependent effect of our treatment as well as the variation in plasma corticosterone level throughout life stages in males and females.

5. Conclusion

Our findings demonstrate that the stress response mediated by corticosterone is dependent on the food environment. Previous results have shown that chronic increased corticosterone levels increase energetic expenditure, food consumption and activity levels. Furthermore, chronically increased corticosterone promote increased survival in males (Cote et al., 2006; Meylan and Clobert, 2005). As also suggested by Breuner et al. (2008), we believe that chronic elevation of corticosterone may lead to an adaptive response to stressors if food is not limited. We furthermore suggested that the chronic elevation of corticosterone as a response to a long-lasting stressor may be an adaptive mechanism inducing behavioural and physiological changes similar to those involved in the acute stress response. Acute and chronic stress might reflect two ends of a continuum where the duration of the stress will shape the costs endured by the behavioural and physiological responses to a stressor. Further studies are required to understand the precise mechanisms underlying these context-dependent modifications and their fitness consequences, as well as the relationship between acute and chronic stress.

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References

Angelier, F., Clément-Chastel, C., Gabrielsen, G.W., Chastel, O., 2007. Corticosterone and time-activity budget: an experiment with Black-legged kittiwakes. Horm. Behav. 52, 482–491.

Astheimer, L.B., Buttemer, W.A., Wingfield, J.C., 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scand. 23, 355–365.

Avery, R.A., 1962. Notes on the biology of *Lacerta vivipara* L.. Br. J. Herpetol. 3, 165–170.

Berger, S., Martin II, L.B., Wikelski, M., Romero, L.M., Kalko, E.K.V., Vitousek, M.N., Rödl, T., 2005. Corticosterone suppresses immune activity in territorial Galapagos marine iguanas during reproduction. Horm. Behav. 47, 419–429.

Breuner, C.W., Greenberg, A.L., Wingfield, J.C., 1998. Noninvasive corticosterone treatment rapidly increases activity in Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Gen. Comp. Endocrinol. 111, 386–394.

Breuner, C.W., Orchinik, M., Hahn, T.P., Meddle, S.L., Moore, I.T., Owen-Ashley, N.T., Sperry, T.S., Wingfield, J.C., 2003. Differential mechanisms for regulation of the stress response across latitudinal gradients. Am. J. Physiol. Regul. Integr. Comp. Physiol. 285, R594–R600.

Breuner, C.W., Patterson, S.H., Hahn, T.P., 2008. In search of relationships between the acute adrenocortical response and fitness. Gen. Comp. Endocrinol. 157, 288–295.

Cabezas, S., Blas, J., Marchant, T.A., Moreno, S., 2007. Physiological stress levels predict survival probabilities in wild rabbits. Horm. Behav. 51, 313–320.

Carpenter, C.C., Ferguson, G.W., 1977. Variation and evolution of stereotyped behavior in reptiles. In: Tinkle, C.G.D.W. (Ed.), Biology of the Reptilia: Ecology and Behavior. Academic Press, New York, pp. 335–554.

Christian, K.A., Tracy, C.R., 1981. The effects of the thermal environment on the ability of hatchling Galapagos land iguanas to avoid predation during dispersal. Oecologia 49, 218–223.

Clobert, J., Oppliger, A., Sorci, G., Ernande, B., Swallow, J.G., Garland, T.J., 2000. Trade-offs in phenotypic traits: endurance at birth, growth, survival, predation and susceptibility to parasitism in a lizard, *Lacerta vivipara*. Funct. Ecol. 14, 675–684.

Cote, J., Clobert, J., Meylan, S., Fitze, P.S., 2006. Experimental enhancement of corticosterone levels positively affects subsequent male survival. Horm. Behav. 49, 320–327.

Creel, S., 2001. Social dominance and stress hormones. Trends Ecol. Evol. 16, 491–497.

Darlington, R.B., Smulders, T.V., 2001. Problems with residual analysis. Anim. Behav. 62, 599–602.

Dauphin-Villemant, C., Xavier, F., 1987. Nychthemeral variations of plasma corticosteroids in captive female *Lacerta vivipara* Jacquin: influence of stress and reproductive state. Gen. Comp. Endocrinol. 67, 292–302.

de Fraipont, M., Clobert, J., John-Adler, H., Meylan, S., 2000. Increased pre-natal maternal corticosterone promotes philopatry of offspring in common lizards *Lacerta vivipara*. J. Anim. Ecol. 69, 404–413.

DeNardo, D.F., Licht, P., 1993. Effects of corticosterone on social behavior of male lizards. Horm. Behav. 27, 184–199.

DeNardo, D.F., Sinervo, B., 1994. Effects of corticosterone on activity and home-range size of free-ranging male lizards. Horm. Behav. 28, 53–65.

Dobrakova, M., Kvetnansky, R., Oprsalova, Z., Jezova, D., 1993. Specificity of the effect of repeated handling on sympathetic adrenomedullary and pituitary–adrenocortical activity in rats. Psychoneuroendocrinology 18, 163–174.

DuRant, S.E., Romero, L.M., Talent, L.G., Hopkins, W.A., 2008. Effect of exogenous corticosterone on respiration in a reptile. Gen. Comp. Endocrinol. 156, 126–133.

Fox, H.E., White, S.A., Kao, M.H.F., Fernald, R.D., 1997. Stress and dominance in a social fish. J. Neurosci. 17, 6463–6469.

Garcia-Berthou, E., 2001. On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. J. Anim. Ecol. 70, 708–711.

Gleeson, T.T., Dalessio, P.M., Carr, J.A., Wickler, S.J., Mazzeo, R.S., 1993. Plasma catecholamine and corticosterone and their in vitro effects on lizard skeletal muscle lactate metabolism. Am. J. Physiol. 265, R632–R639.

Greenberg, N., Chen, T., Crews, D., 1984. Social status, gonadal state, and the adrenal stress response in the lizard, *Anolis carolinensis*. Horm. Behav. 18, 1–11.

Hiebert, S.M., Salvante, K.G., Ramenofsky, M., Wingfield, J.C., 2000. Corticosterone and nocturnal torpor in the rufous hummingbird (*Selasphorus rufus*). Gen. Comp. Endocrinol. 120, 220–234.

Huey, R.B., 1982. Phylogenetic and ontogenetic determinants of sprint performance in some diurnal Kalahari lizards. Koedoe 25, 43–48.

Kitaysky, A.S., Piatt, J.F., Wingfield, J.C., Romano, M., 1999. The adrenocortical stress–response of Black-legged Kittiwake chicks in relation to dietary restrictions. J. Comp. Physiol. B 169, 303–310.

Kitaysky, A.S., Kitaiskaia, E.V., Piatt, J.F., Wingfield, J.C., 2003. Benefits and costs of increased levels of corticosterone in seabird chicks. Horm. Behav. 43, 140–149.

Knapp, R., Moore, M.C., 1995. Hormonal responses to aggression vary in different types of agonistic encounters in male tree lizards, *Urosaurus ornatus*. Horm. Behav. 29, 85–105.

Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen. Comp. Endocrinol. 148, 132–149.

Le Galliard, J.F., Ferrière, R., Clobert, J., 2003. Mother–offspring interactions affect natal dispersal in a lizard. Proc. R. Soc. B: Biol. Sci. 270, 1163–1169.
Le Galliard, J.F., Clobert, J., Ferrière, R., 2004. Physical performance and Darwinian fitness in lizards. Nature 432, 502–504.
Lecomte, J., 1993. Comportements du lézard vivipare. Université Paris Sud, Paris.
Lecomte, J., Clobert, J., Massot, M., Barbault, R., 1994. Spatial and behavioural consequences of a density manipulation in the common lizard. Ecoscience 1, 300–310.
Lendvai, A.Z., Giraudeau, M., Chastel, O., 2007. Reproduction and modulation of the stress response: an experimental test in the house sparrow. Proc. R. Soc. B: Biol. Sci. 274, 391–397.
Lewis, L.Y., Gatten, R.E.J., 1985. Aerobic metabolism of American alligators, Alligator mississippiensis, under standard conditions and during voluntary activity. Comp. Biochem. Physiol. A 80, 441–447.
Loiseau, C., Fellous, S., Haussy, C., Chastel, O., Sorci, G., 2008. Condition-dependent effects of corticosterone on a carotenoid-based begging signal in house sparrows. Horm. Behav. 53, 266–273.
Massot, M., Clobert, J., 1995. Influence of maternal food availability on offspring dispersal. Behav. Ecol. Sociobiol. 37, 413–418.
McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15.
McEwen, B.S., Biron, C.A., Brunson, K.W., Bulloch, K., Chambers, W.H., Dhabhar, F.S., Goldfarb, R.H., Kitson, R.P., Miller, A.H., Spencer, R.L., Weiss, J.M., 1997. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. Brain Res. Rev. 23, 79–133.
Meylan, S., Clobert, J., 2005. Is corticosterone mediated phenotype development adaptive? Maternal corticosterone treatment enhances survival in male lizards. Horm. Behav. 48, 44–52.
Meylan, S., Dufty, A.M., Clobert, J., 2003. The effect of transdermal corticosterone application on plasma corticosterone levels in pregnant *Lacerta vivipara*. Comp. Biochem. Physiol. A 134, 497–503.
Miles, D.B., Calsbeek, R., Sinervo, B., 2007. Corticosterone, locomotor performance, and metabolism in side-blotched lizards (*Uta stansburiana*). Horm. Behav. 51, 548–554.
Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation an amphibians and reptiles. Horm. Behav. 43, 39–47.
Morici, L.A., Elsey, R.M., Lance, V.A., 1997. Effect of long-term corticosterone implants on growth and immune function in juvenile alligators, *Alligator mississippiensis*. J. Exp. Zool. 279, 156–162.
Pravosudov, V.V., Kitaysky, A.S., Wingfield, J.C., Clayton, N.S., 2001. Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). Gen. Comp. Endocrinol. 123, 324–331.
Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19, 249–255.
Sapolsky, R.M., 1988. Individual differences and the stress response: studies of a wild primate. Adv. Exp. Med. Biol. 245, 399–411.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.
Schmidt-Nielsen, K., 1997. Animal Physiology: Adaptation and Environment. Cambridge University Press, Cambridge.
Silverin, B., 1998. Stress responses in birds. Poult. Avian Biol. Rev. 9, 153–168.
Stevenson, O.R., Coulson, R.A., Hernandez, T., 1957. Effects of hormones on carbohydrate metabolism in the alligator. Am. J. Physiol. 191, 95–102.
Tataranni, P.A., Larson, D.E., Snitker, S., Young, J.B., Flatt, J.P., Ravussin, E., 1996. Effects of glucocorticoids on energy metabolism and food intake in humans. Am. J. Physiol. 271, E317–E325.
Tokarz, R.R., 1987. Effects of corticosterone treatment on male aggressive behavior in a lizard (*Anolis sagrei*). Horm. Behav. 21, 358–370.
Tyrrell, C., Cree, A., 1998. Relationships between corticosterone concentration and season, time of day and confinement in a wild reptile (Tuatara, *Sphenodon punctatus*). Gen. Comp. Endocrinol. 110, 97–108.
Van Damme, R., Bauwens, D., Verheyen, R.F., 1986. Selected body temperatures in the lizard *Lacerta vivipara*: variation within and between populations. J. Therm. Biol. 11, 219–222.
West-Eberhard, M.J., 1989. Phenotypic plasticity and the origin of diversity. Annu. Rev. Ecol. Evol. Syst. 20, 249–278.
Wingfield, J.C., 2003. Control of behavioural strategies for capricious environments. Anim. Behav. 66, 807–816.
Wingfield, J.C., Ramenofsky, M., 1999. Hormones and the behavioral ecology of stress. In: Balm, P.H.M. (Ed.), Stress Physiology in Animals. Sheffield Academic Press, pp. 1–51.