**Chronic elevation of glucocorticoids late in life generates long lasting changes
in physiological state without a life history switch**

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Abstract

Chronic stressors have profound impacts on phenotypes and life history strategies on the short term, but delayed effects of stress experienced late in life remain poorly investigated in wild populations. Here, we used a combined laboratory and field experiment to test if chronic stress late in life has immediate and delayed effects on physiological and demographic traits in the common lizard, *Zootoca vivipara*. We increased plasma corticosterone levels in adults and yearlings during three weeks of the post-reproductive season. We quantified immediate responses in the laboratory, delayed intra-generational effects in field enclosures one month and one year later during the next reproductive season, and delayed inter-generational effects in the first generation of offspring. Our phenotypic assays included metabolism, immune capacities, lipid metabolism and oxidative stress. Relative to placebos, lizards treated with corticosterone had higher body condition and lower oxidative damages but an increased skin swelling response directly after the manipulation. Delayed responses in field enclosures were of three types. First, we found catch-up growth for body mass such the placebos had similar body conditions one month after the laboratory manipulation. Second, we found persistent differences in oxidative damages during one month but not one year later. Third, during the next reproductive season, corticosterone-treated females had higher levels of plasma triglycerides, whereas corticosterone-treated individuals had a higher skin swelling response. We found no delayed inter-generational effects on demographic traits of offspring. Our study demonstrates the potential for long-lasting physiological consequences of chronic corticosterone enhancement despite no changes in life history.

1. Introduction

Individuals face multiple stressors throughout their life, and behavioural and physiological strategies have therefore evolved to avoid the deleterious effects of these stressors. Among vertebrates, the adrenocortical response, characterized by a rapid rise of glucocorticoid secretion in response to a wide range of stressor stimuli, is one of the most conserved physiological mechanisms for that purpose (Wingfield, 2003). Glucocorticoid secretion after a stressful event induces a cascade of organismal responses that promote immediate survival by reallocating energy from storage to activity (Crespi et al., 2013; Sapolsky et al., 2000). Indeed, glucocorticoids are intimately associated to metabolic activities involved in energy balance (Sapolsky et al., 2000; Solomon et al., 2011), which in turn controls growth, reproduction, and survival (Artacho and Nespolo, 2009; Steyermark, 2002). In addition, glucocorticoids are powerful regulators of immune functions (Dhabhar, 2002; Dhabhar and McEwen, 1997). High chronic levels of plasma glucocorticoid can suppress immune functions (Sapolsky et al., 2000) and have also been associated to oxidative stress increase (Sahin and Gumuslu, 2007). Therefore, chronic stressful events can induce deleterious effects such as immune depression, lower survival or reproduction failures (Crespi et al., 2013).

The ecological relevance of these glucocorticoid effects has been well investigated in both captive and natural populations where energy expenditure, immunity and oxidative stress responses can be potentially important fitness related traits (Alonso-Alvarez et al., 2006). Physiological and demographic responses are generally measured during or right after the stressful event and can be referred to as “immediate” effects of chronic elevation of glucocorticoids. Yet, the life of individuals is made out of a series of critical events or life stages, and there is accumulating evidence that environmental variation at a given life stage can impact the following life stages through delayed, intra-generational effects as well as the following generations through inter-generational effects (Beckerman et al., 2002; Meylan et al. 2012). Thus,

prolonged stressful events, such as chronic food restriction, can have long lasting effects throughout the entire life of the individual and may shift its life history strategy. In particular, chronic elevation of glucocorticoids may have organizational effects and thus could influence ontogenic trajectories on the long-term (Schoech et al., 2011). For example, Spencer and collaborators (Spencer and Verhulst, 2007; Spencer et al., 2005) chronically supplemented nestlings of zebra finch (*Taeniopygia guttata*) with corticosterone (one of the two major glucocorticoid with cortisol in birds, amphibians, reptiles and mammals) and found immediate negative effects on growth as well as delayed effects on song characteristics in adults. Exposure to elevated glucocorticoids early in life can also disrupt HPA axis regulation (Kitaysky et al., 2005) and has profound, well-known long-term effects on the physiology, behaviour, and life history (Blas et al., 2007; Dufty et al., 2002; Matthews, 2002; Meylan and Clobert, 2005). These impacts of glucocorticoid can carry over across generations through maternal effects (Catalani et al., 2000; Khan et al., 2016).

Glucocorticoid secretion can induce phenotypic plasticity at adulthood as well (Dufty et al., 2002), but its long lasting, delayed effects have been little investigated so far in late life stages. Here, we used common lizards (*Zootoca vivipara*) as a model system to investigate for the first time the joint immediate and delayed physiological and demographic effects of a prolonged corticosterone increase late in life. Immediate effects of chronic corticosterone increase in adult common lizards include changes in resting and activity metabolism, immune capacities, oxidative stress, and behaviours such as food consumption, locomotor activity and basking (Cote et al., 2006; Meylan and Clobert, 2005; Meylan et al., 2010). Moreover, a chronic corticosterone manipulation increases the future survival of adult males but not females suggesting sex-specific long lasting, delayed effects (Cote et al., 2006). In this viviparous species, increased corticosterone secretion during gestation changes the morphology, behavioural activity and dispersal strategy of juveniles at birth (de Fraipont et al., 1999; Meylan et al., 2002; Meylan et al.,

2004), but can also influence their growth and survival later in life (Meylan and Clobert, 2005). However, we do not know if increased corticosterone secretion some months before the gestation period can cause maternal effects.

To quantify immediate and delayed intra-generational and inter-generational effects, we used a three-stage experimental design where we first exposed yearlings and adults to a chronic corticosterone exposure during the summer post-reproductive season in the laboratory. We next released them in outdoor populations until the next reproductive season, and then followed for an additional season their first generation of offspring. In yearlings and adults, we investigated four sets of physiological parameters (standard metabolic rate, immune capacities, oxidative stress and plasmatic triglyceride concentration) directly after the treatment, one month later in the field and one year later in the field to identify most relevant components of the adrenocortical response. Consequences on life history were examined by quantifying reproductive performances, growth, survival rate and the intergenerational effects on the first generation of offspring. At the end of the laboratory manipulation, we expected an increase of baseline corticosterone, as well as of triglyceride concentration (Karatsoreos et al., 2010; Peckett et al., 2011), oxidative stress (Cote et al., 2010b), and leanness (Cote et al., 2006; Cote et al., 2010a). In contrast, we predicted no change in immune response in accordance with previous work (Meylan et al., 2010) but sex-dependent changes of metabolic rates (Cote et al., 2010b; Meylan et al., 2010). Since previous works have shown sex-specific delayed effects on survival in adults (Cote et al., 2006), we expected different long-lasting responses between sexes. If chronic corticosterone exposure increases maintenance costs, we predicted a long-term decrease of immune capacities and an increase of oxidative stress, plasmatic triglyceride concentrations and baseline corticosterone (Cote et al., 2006; McEwen and Wingfield, 2003; Romero et al., 2009). Moreover, since the energetic costs of maintenance change between age classes, sex and reproductive cycles (Massot et al., 2011;

McEwen and Wingfield, 2003), we expected stronger delayed effects during the reproductive period, one year after the manipulation, than one month after the treatment because energy demands peak during the reproductive season in both sexes.

2. Materials and methods

2.1. Model species

The lizard *Zootoca vivipara* is a small ovoviparous (adult snout-vent length SVL: 53-77 mm) species inhabiting humid habitats across northern Eurasia. It is characterized by a 3 to 4 year life expectancy, continuous growth and plastic life history (Le Galliard et al., 2005; Mugabo et al., 2010). Natural populations are structured in three age classes: juveniles (newborns), yearlings (1 year old individuals) and adults (2 year old or more). In our study site, males start to emerge from hibernation around the beginning of March, followed shortly by juveniles and by females. Mating period starts upon emergence of females (Bauwens et al., 1989). From June to July, females lay an average clutch of five non-calcified eggs (range 1-13). Offspring hatch shortly after parturition and are immediately autonomous. Lizards enter in hibernation in October.

2.2. Capture and rearing condition

Lizards were captured between 18 and 26 May 2014 in outdoor enclosures (10 × 10 m) at the Centre de Recherche en Ecologie Expérimentale et Prédictive (Saint-Pierre-lès-Nemours, France, 48°17'N, 2°41' E). All animals were identified by their unique toe clip code and measured for body size (SVL, ± 0.5 mm) and body mass (± 1 mg). They were then maintained in individual terraria (25 × 15 × 16 cm) with a shelter, peat soil as substrate and opportunities for optimal thermoregulation. We used incandescent light bulbs (25 W) for 9 h/d to ensure a thermal gradient from room temperature at 17-23°C to 35-38°C below the bulb during daytime. Room temperature was maintained at 16°C from 21:00 to 7:30 (night time) and at 25°C during daytime. We provided lizards with water and food *ad libitum* and kept all

individuals under strictly identical conditions until gravid females gave birth (20 June to 14 July). Immediately after parturition, newborns were separated from their mother, marked by toe clipping and measured for their SVL and body mass, and immediately released in an outdoor enclosure.

2.3. Experimental design

We sampled 68 adult and 60 yearling females and 33 adult and 60 yearling males for this experiment. At day 1, a balanced number lizards from each age and sex class was assigned to a corticosterone (33 adult and 31 yearling females; 17 adult and 30 yearling males) or a placebo group (35 adult and 29 yearling females; 16 adult and 30 yearling males). We subsequently manipulated the circulating levels of corticosterone during 3 weeks using a non-invasive method designed by Meylan et al. (2003). This method increases the baseline corticosterone concentration within the natural range of variation without causing mortality or abortion in pregnant females (Meylan et al., 2003). We diluted corticosterone (Sigma-Aldrich, France, C2505-500mg 92%, $C_{21}H_{30}O_4$) in commercial sesame oil, which facilitates skin penetration of corticosterone, to reach a final concentration of 3 μ g corticosterone for 1 μ l of sesame oil. We applied 4.5 μ L of corticosterone mixture (corticosterone group) or pure sesame oil (placebo) each evening between 20:00 h and 21:00 local time during 21 days (a duration corresponding to 10% of the yearly activity period). At day 0, prior to the start of the experiment, there was no difference in SVL ($\chi^2 = 0.35$, $df = 1$, $P = 0.55$) and body mass ($\chi^2 = 0.14$, $df = 1$, $P = 0.70$) between treatment groups.

At the end of the laboratory experiment, all lizards were released in 10 outdoor enclosures from 20 July to 6 August. We used the standard set-up and maintenance procedures in each enclosure (Lecomte and Clobert, 1996). We visited all enclosures repeatedly between 2 to 10 September 2014 and recaptured all live animals. Captured lizards were then maintained during 4 days in the laboratory for subsequent measurements (see

below) and later released in their capture enclosure. A second visit after the wintering period was done between 18 May to 12 June 2015 and captured lizards were again maintained in the laboratory for similar measurements. Gravid females were maintained until the parturition to assess their reproductive traits. Immediately after parturition, newborns were separated from their mother, marked by toe clipping and measured for SVL and body mass. Each sibling group was released in one of 10 enclosures (N=342), and newborns were recaptured in May-June 2016 (N=70) to measure their size growth and annual survival. The experimental process is described in the figure 1

2.4. Life history data

We monitored yearlings and adults during one year to assess body size and condition, growth, survival and female reproductive performance one year after the manipulation. We recaptured 164 lizards from the two treatment groups in September 2014 and 107 individuals in May-June 2015. At each time step of the experimental design (pre-experimental, post-experimental, September 2014, and May-June 2015), we sampled blood and measured immuno-competence for all animals and whole-organism metabolism for a subset of lizards (see below). Body growth rates were calculated as the change in SVL between two captures divided by the time interval. We subtracted the number of inactive days spent in hibernation for the growth rate whenever needed. Hibernation is assumed to be from end of October to beginning of March. We estimated annual survival probabilities assuming that capture probability was very close to 1 (Le Galliard et al., 2005). To assess reproductive performance of females, we counted live new-borns, dead new-borns and aborted or unfertilized eggs of each clutch. Parturition date, total fecundity (live, dead and aborted newborns), fit fecundity (live newborns) and offspring characteristics (sex, SVL, body mass) were recorded.

2.5. Blood sample and plasma measurements

Blood samples (40-80 µl whole blood) were taken from the infra-orbital sinus using 2- 3 20µl microhematocrit tubes, within 3 min of handling to avoid the handling-induced increase in plasma corticosterone levels (Dauphin-Villemant and Xavier, 1987). Plasma was obtained by centrifugation at 5,000 G for 3 minutes of the blood samples and was stored at -20°C until assay. Because of the low available amount of plasma, the different dosages could not be made on the same samples. Then, the samples were distributed in different lots in order to be able to carry out all the expected dosages. For subsequent analyses, 3-6 individuals from each age class, sex, treatment and session (pre and post-experiment sessions, and the two delayed sessions) were used to measure corticosterone levels and 3-10 individuals were used to measure triglyceride, anti-oxidant barrier and oxidative damage (table 1).

Corticosterone level was measured with a competitive enzyme-immunoassay method using corticosterone EIA (IDS Corticosterone EIA kit, ref AC-14F1, IDS EURL Paris, France) after 1:10 dilution of all samples. This method quantifies total plasmatic corticosterone using a polyclonal corticosterone antibody and is based on a colorimetric assay of absorbance at 450 nm. Sensitivity is 0.55 ng/mL. Measurements were highly repeatable (intra-plate repeatability: $n = 63$, $F_{3,60} = 102.61$, $P < 0.001$, $r = 0.89$; inter-plate repeatability: $n = 63$, $F_{16,47} = 51.3$, $P < 0.001$, $r = 0.93$). Levels of circulating triglyceride were measured by colorimetric assays using 2.5 µl of plasma (Triglyceride Colorimetric Assay kit, ref. 10010303, Cayman Chemical, USA). This method quantifies the total triglyceride by using a chain of three enzymatic reactions ending up in hydrogen peroxide production. Sensitivity is 0.5 mg/dL. The measurement was highly repeatable (inter-plate repeatability: $n = 24$, $F_{4,20} = 795.48$, $P < 0.001$, $r = 0.99$, intra-plate repeatability : $n = 24$, $F_{2,22} = 728.06$, $P < 0.001$, $r = 0.98$).

To assess oxidative stress in lizards, we used two complementary colorimetric tests. First, we used d-ROMs test (MC003, Diacron International, Via Zircone 8, 58100 Grosseto (GR), Italy) to assess the concentration of reactive oxygen metabolites in 4 μ L plasma sample. Second, to assess the anti-oxidant barrier of plasma, we used the OXY-Absorbent test (MC435, Diacron International, Via Zircone 8, 58100 Grosseto (GR), Italy) after 1:100 dilution of 3 μ L of plasma. These two tests were also highly repeatable (d-ROMs test : inter-plate repeatability : $n = 16$, $F_{4,12} = 37.53$, $P < 0.001$, $r = 0.91$; intra-plate repeatability : $n = 16$, $F_{2,14} = 46.69$, $P < 0.001$, $r = 0.88$ and OXY test : inter-plate repeatability : $n = 18$, $F_{4,14} = 153.26$, $P < 0.001$, $r = 0.88$; intra-plate repeatability : $n = 18$, $F_{3,15} = 214.96$, $P < 0.001$, $r = 0.98$). Prior to the beginning of the manipulation, there was no difference between treatment groups for corticosterone (Likelihood-ratio tests, $\chi^2 = 0.99$, $df = 1$, $P = 0.32$), triglyceride concentration ($\chi^2 = 0.06$, $df = 1$, $P = 0.81$), oxidative damage ($\chi^2 = 0.09$, $df = 1$, $P = 0.76$) and antioxidant barrier capacity ($\chi^2 = 0.15$, $df = 1$, $P = 0.69$).

2.6. Evaluation of immune capacities

Immuno-competence was assessed by measuring skin swelling in response to an injection of a mitogen, the phytohaemagglutinin (PHA). This procedure triggers a local haemagglutination and leukocyte infiltration and involves both adaptive and innate immune components (Martin et al., 2006). This implies that primary responses measured in naive animals may differ from the secondary responses measured in animals already exposed to PHA earlier in their life. Previous study in this species have shown no correlation between the primary and the secondary swelling responses, but an increase in the swelling response after a first exposure to PHA (Mugabo et al., 2015). In order to avoid biases due to differences between first and subsequent injections, we thus first injected all animals with a solution of phosphate buffered saline (PBS) containing 2.5 mg/mL of PHA (PHA-M, Sigma-Aldrich; reference 9008-97-3) in the right posterior leg in order to elicit a primary response. To obtain our subsequent

measurements, we then administered a second subcutaneous injection of PHA solution in the right posterior leg and quantified the secondary skin-swelling response. Just before and 12h after the injection, we measured the thickness of the right posterior leg using a spessimeter (Mitutoyo, ID-C112, Kanagawa, Japan) with an accuracy of 0.01 mm. We spaced the two measurements by 12 hours because this coincides with the greatest swelling response (Mugabo et al., 2015). Swelling response was calculated as the difference in leg thickness between the post- and pre-injection measurements. At the start of the experiment, we did not find difference between treatment groups for PHA response ($\chi^2 = 0.72$, $df = 1$, $P = 0.394$).

2.7. Whole-organism metabolic expenditure

We defined at the start of the experiment a sub-sample of 60 males and 60 females from the two age classes to measure repeatedly their resting metabolic rate during the four measurement periods. All individuals were measured during the pre-experiment and post-experiment sessions and recaptured animals were measured during the sessions of September 2014 and May-June 2015. The resting metabolic rate, or standard metabolic rate (SMR) for ectotherms, is the minimum energetic cost of maintenance estimated in fasting individuals at rest and at a given temperature. Here, we measured the SMR after a fasting period of 3 days to ensure a post-absorptive state (Artacho et al., 2013). Before the measurement, lizards were moved in a dark room maintained at 20°C during 3-4 hours and were then placed in their metabolic chamber during 1 hour at 16°C before the beginning of the measurements. This temperature was chosen because it is a body temperature where lizards are inactive. All measurements were recorded at night between 08:00 pm and 08:00 am. The CO₂ production was sampled during 40 mins for each animal with a multiple-channel, flow-through respirometry system (Qubit Systems, Kingston, ON, Canada) compounded of a CO₂ analyzer (S157) connected to a respirometry software (QS Research). Incoming air flowed through columns of sodalime (Spherasorb, Wokingham, Berkshire, RG41 2RZ, UK) and drierite to

remove CO₂ and H₂O, respectively. After the absorbent columns, air was pushed through seven channels of a multiplexer at 150 mL.min⁻¹. Six channels were used for measuring lizards and one channel for the CO₂ reference. Outgoing air was pushed into the CO₂ analyzer at 50 mL.min⁻¹. Metabolic records of CO₂ were processed by a macro program C950 data acquisition software (Qubit systems) in order to transform the ppm measure of CO₂ production in mL per hour, which takes into account the flow rate..

2.8. Statistical analyses

Analyses were performed with R 3.1.0 (R Core Team 2014, [https:// www.r-project.org/](https://www.r-project.org/)) using the *lmer* statistical procedure available in the *lme4* package for linear mixed models (Bates et al., 2015) and *lm* procedure from *stats* package for linear models. Analyses of variance procedure were done with the *Anova* procedure from *Car* package (Fox and Weisberg 2011). All initial models included as fixed factors the effects of treatment group, age class, sex and their first-order interactions. Analyses of corticosterone and triglyceride concentrations, antioxidative-barrier and oxidative damage, swelling response, SMR (CO₂ production) and body mass from the post-experimental session were done by calculating the difference between the post-experimental (after 20 days of treatment application) and the initial measurement and using this as a response variable in a linear model. Data for corticosterone, triglyceride concentrations, antioxidative-barrier, oxidative damage, swelling response and SMR were log-transformed to fit the normality assumption. Because SMR may increase with body mass, we used the mass gain between pre and post experimental session as a covariate, and initial measurement of SVL was also used as covariate in other models.

During the September 2014 session, the choice of samples was made in such a way as to maximize repeated measurements per individual for each measured parameter through sessions and also to balance the numbers by treatment, sex and age class in order to test their effect. Then, we analysed the same traits with linear mixed models including population

identity as a random factor. Physiological traits were log-transformed to fit the normality assumption. To assess the change in body mass and SMR, we used the difference between the September and the post-experimental measurements since repeated measures on the same animals were obtained for these traits. In addition, we used SVL as covariate for all traits except body mass change and SMR change where we used change in SVL and body mass as covariates, respectively. The same procedures were used to analyze data collected during the May-June 2015 session. In addition, we analysed SVL growth rate from September 2014 to May-June 2015 with a linear mixed model including the effect of initial SVL to control for growth deceleration with increasing body size and the effect of the date of release to control for seasonal changes in growth rates.

Annual survival was similarly analysed with mixed-effect logistic regressions including a logit link and binomial error term. Initial SVL was used as covariate to control for size effect on survival. All adult and yearling females bred during the summer 2015. Parturition date and offspring characteristics (SVL and body condition) were analysed with linear mixed models, whereas total fecundity and fit fecundity were analysed with mixed effect log regressions including a log link and a Poisson error term. Body growth and annual survival of the first generation of offspring were analysed with the same procedures including the mother identity as an additive random effect.

In all cases, parameters of linear mixed models were estimated with the maximum likelihood procedure, whereas a Laplace approximation of the maximum likelihood was used in the case of logistic and log regressions. Fixed effects were tested with Wald χ^2 statistics for mixed models and Fisher test in the case of linear models. A minimum adequate model was obtained by a backward selection procedure where we removed non-significant terms one by one. Assumptions of normality and homoscedasticity were verified and over-dispersion of logistic and log regression were tested with a Pearson's chi squares. When a minimum

adequate model was found, we used the Tukey's procedure to conduct post hoc tests (pairwise comparisons) with the *lsmeans* package (Lenth, 2016). Results are presented as mean parameter estimates (β) $\pm$ standard error or [lower,upper] at 95% confidential interval unless otherwise stated.

3. Results

3.1. Immediate physiological effects

Relative to the placebo, the corticosterone treatment increased significantly plasma corticosterone concentration until the end of the laboratory manipulation (Table 2, Figure 2A) and the skin swelling response (Table 2, Figure 2C), but it did not influence circulating plasma triglyceride concentration (Table 2, Figure 2B), antioxidant barrier (Table 3, Figure 3A) and SMR ($F_{1,114} = 0.06$, $P = 0.81$). In addition, oxidative damages were more important in placebo than in corticosterone treated lizards (Table 3, Figure 3B). Corticosterone treatment significantly increased body mass change during the laboratory manipulation ($F_{1,207} = 11.94$, $P < 0.001$, Figure 4A) after controlling for effects of sex ($F_{1,207} = 7.3$, $P = 0.007$), age class ($F_{1,207} = 227.65$, $P < 0.001$), and initial body mass ($F_{1,207} = 358.74$, $P < 0.001$).

3.2. Delayed physiological and demographical effects

One month after manipulation, baseline corticosterone concentration returned to similar levels between placebo and treatment groups (Figure 2D), and baseline concentrations were higher in adults and in females after controlling for a negative effect of body size (Table 2). In sharp contrast, we found a delayed effect on triglyceride concentration and swelling response in May-June 2015 but not in September 2014. One year after manipulation, triglyceride concentration was significantly higher in females from the corticosterone group than the placebo (Tukey's contrast = 0.94 ± 0.04 , $df = 22.48$, $t = 2.36$, adjusted $P = 0.03$) but not for males (contrast = -0.53 ± 0.37 , $df = 19$, $t = -1.43$, adjusted $P = 0.17$). Skin swelling response of the corticosterone treated lizards was significantly higher than in placebos (see Table 2,

Figure 2I). At the same time, we found delayed effects on oxidative damage but not for anti-oxidative barrier (see Figure 3C-F and Table 3). Oxidative damage was significantly lower in corticosterone treated group than the placebo in September 2014 (Figure 3D). We did not observe delayed effect of corticosterone treatment on SMR (September 2014: $\chi^2 = 0.07$, $df = 1$, $P = 0.79$; May-June 2015: $\chi^2 = 0.39$, $df = 1$, $P = 0.53$).

During the interval time between release and September 2014, lizards from the corticosterone treatment gained less body mass than placebos ($\chi^2 = 9.67$, $df = 1$, $P = 0.002$, Figure 4) after controlling for a significant interaction between sex and age ($\chi^2 = 10.64$, $df = 1$, $P = 0.001$). There was no difference between treatment groups for size growth between release in 2014 and recapture in May-June 2015 (placebo: $0.016 \pm 0.0015 \text{ mm.days}^{-1}$, corticosterone: $0.015 \pm 0.002 \text{ mm.days}^{-1}$; $\chi^2 = 0.43$, $df = 1$, $P = 0.51$) after controlling for a negative effect of initial SVL ($\chi^2 = 200.5$, $df = 1$, $P < 0.001$) and a sex effect ($\chi^2 = 254.8$, $df = 1$, $P < 0.001$). We also found a marginal difference between treatment groups for mass change between September 2014 and May-June 2015 ($\chi^2 = 3.48$, $df = 1$, $P = 0.06$), with a lower mass gain in corticosterone treated group.

Estimated annual survival rate was not different between treatment groups (placebo = 0.40 [0.26, 0.56] and corticosterone = 0.52 [0.37, 0.67], $\chi^2 = 2.61$, $df = 1$, $P = 0.11$) and between sexes ($\chi^2 = 2.18$, $df = 1$, $P = 0.14$) but estimated survival rate of yearling lizards was higher than adults (adults = 0.35[0.22, 0.50]; yearlings = 0.58[0.43,0.71], $\chi^2 = 3.88$, $df = 1$, $P = 0.05$). Similarly, we found no delayed effects of corticosterone enhancement on female reproductive traits, including parturition date, number of live offspring and total fecundity (all $P > 0.1$).

3.3. Inter-generational effects

Juveniles born from corticosterone treated females did not differ from those of placebo females for body size (SVL: $\chi^2 = 0.54$, $df = 1$, $P = 0.46$) and body condition at birth ($\chi^2 = 0.8$,

df = 1, P = 0.37) after controlling for sex effects (SVL: $\chi^2 = 68.81$, df = 1, P < 0.001; body condition: $\chi^2 = 21.97$, df = 1, P < 0.001). Estimated annual survival rate of juveniles between 2015 and 2016 was not different between maternal treatment groups (placebo = 0.19 [0.13, 0.27], corticosterone = 0.24 [0.18, 0.32], $\chi^2 = 0.97$, df = 1, P = 0.32). The body size growth rate of juveniles born from corticosterone treated females did not differ from placebos ($\chi^2 = 0.68$, df = 1, P = 0.41) after controlling for the faster growth of females ($\chi^2 = 3.92$, df = 1, p = 0.05) and the decrease of growth rate with birth date ($\chi^2 = 8.77$, df = 1, P = 0.003).

4. Discussion

Our combined laboratory and field experiment evidenced immediate and delayed effects of chronic corticosterone elevation on physiological parameters related to lipid metabolism, immune capacities and oxidative stress, independently of age classes but with some differences between sexes. In addition, body mass increased more rapidly in the laboratory in corticosterone treated lizards, but we later observed compensatory responses in the field with stronger relative body mass change in placebo lizards. In contrast, standard metabolism, survival, reproduction of lizards and the demographic traits of their first generation of offspring were relatively insensitive to the corticosterone manipulation. Thus, experimental enhancement of corticosterone level, which was used to simulate a chronic stressful event late in life, was a significant factor of physiology and body mass dynamics, but not of the life history strategy.

4.1. Effects on immune and antioxidant capacities

As in previous studies using the same protocol (Cote et al., 2006; Cote et al., 2010b; Meylan et al., 2003), daily inputs of corticosterone for 21 days induced an increase of individual corticosterone level, which was about 3 times higher in the corticosterone group (167.34 ng.ml⁻¹ vs. 48.34 ng.ml⁻¹ in placebos). An inhibition of the swelling response by the corticosterone treatment was expected given the well-known immunosuppressive actions of

the chronic secretion of corticosterone (Berger et al., 2005; Dhabhar and McEwen, 1997; Martin et al., 2005; Sapolsky et al., 2000). Unexpectedly, our chronic corticosterone elevation was instead associated with a higher immune response and less oxidative damage. The increase of swelling response in corticosterone treated lizards did not last in the field one month after the end of the laboratory manipulation; yet, the swelling response measured during the next reproductive season was again higher in corticosterone-treated lizards than in placebos. We speculate that this increased swelling response during the reproduction following corticosterone increase may be adaptive. Indeed, it has been shown that a higher basal level of corticosterone in males resulted an increase in the number of mating attempts, which may expose individuals to more injury or pathogens, thus selecting for higher cellular defences (Gonzalez-Jimena and Fitze, 2012; Richard et al., 2012).

The observed decrease of plasmatic oxidative damage was even more surprising, especially since the treatment did not influence the antioxidant capacity. Chronic application of corticosterone in adult males was previously found to increase oxidative damage under some circumstances, as shown by higher levels of lipid peroxidation in muscular tissues, but it had complex and contrasted effects on antioxidant enzyme activities (Cote et al., 2010b; Meylan et al., 2010). During a chronic stress, oxidative damage is expected to increase because higher circulating levels of corticosterone should increase the basal metabolic rate, locomotor activity and foraging behaviour (Costantini et al., 2011). Aerobic metabolism produces reactive oxygen species (ROS), which in turn can result in oxidative damage if ROS generation exceeds antioxidant defences. In our study, the standard metabolic rate of lizards did not change between treatment groups, implying that the decrease of oxidative damage during the laboratory manipulation was not due to changes in basal metabolism. However, we recently demonstrated that the liver mitochondria from corticosterone-treated lizards produced less reactive oxygen species without change in the metabolic rate or oxygen consumption

(Voituron et al., 2017). The persistent difference between treatment groups in the field one month after the end of the laboratory manipulation, despite no differences in the immune response, corticosterone concentration or antioxidant barrier, suggests a short-lasting carry-over effect on blood oxidative damage.

4.2. Effects on body mass and metabolism

Although a positive effect of corticosterone enhancement on basal metabolic rate is generally expected, the experimental evidence is rather contradictory and we found no immediate and delayed effects on SMR. In birds, for example, corticosterone elevation has been associated with a reduction of nocturnal metabolism (Buttemer et al., 1991). In contrast, Wikelski et al. (1999) found no apparent relationship between resting metabolic rate and corticosterone levels in white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Moreover, an experimental study in two wild passerine bird species, house sparrows (*Passer domesticus*) and gray catbirds (*Dumetella carolinensis*), demonstrated that the relationship between metabolic rate and corticosterone concentration differed qualitatively between the species (Cohen et al., 2008). In the common lizard, the effect of the corticosterone on metabolism can be condition-dependent as well. Indeed, Meylan et al. (2010) found an increase of the SMR after 21 days of corticosterone application in pregnant females; whereas SMR decreased after 10 days of treatment in adult males (Cote et al. 2010a) and did not change in yearlings (Voituron et al., 2017).

Despite the lack of change in whole-organism metabolic expenditure, we observed a significantly higher mass gain in the corticosterone treated group during the laboratory manipulation followed by a higher mass gain from placebo lizards in the field suggesting a compensatory response. The laboratory result is in contradiction with previous studies (Cote et al., 2006; Cote et al., 2010a) that used other food items (*Pyrallis sp.* larva instead of *Acheta domestica*) provided in much smaller quantities (250 mg every 5 days instead of 300-400 mg

every 2 days), which are insufficient for sustained body growth in the laboratory. In contrast, our laboratory protocol brought food in excess and allowed for significant size growth like in the field. In the common lizard, a prolonged corticosterone elevation promotes behaviours associated with food intake including exploratory activity, foraging and basking (Cote et al. 2010a). Corticosterone can also induce lipid storage in non-fasted state by activating lipogenesis and adipogenesis (Peckett et al., 2011; Voituron et al., 2017). The body mass gain during the laboratory experiment could thus result from an increase of both food intake and lipid storage. Against the later hypothesis, we however found no difference between treatment groups for plasma triglyceride concentration in the laboratory and one month after the end of the laboratory manipulation.

In the field, the body mass gain in corticosterone treated group was lower in placebo group, and body mass between the two groups was not different one month after the end of the laboratory manipulation (post-hoc test, $t = -0.53$, $df = 148.9$, adjusted $P = 0.595$). This suggests that corticosterone treated lizards decreased their energy storage and/or their food intake in the field resulting in a lower body mass relative to placebos. In addition, females from corticosterone treated group had much higher triglyceride levels than placebo group during the next reproductive season. Difference between sexes could be explained by the higher baseline corticosterone levels in females and its action on lipolysis (Peckett et al., 2011) and also by the additional costs associated with gestation. Indeed higher triglyceride levels in females suggest a higher energetic requirement to maintain reproduction output, and so a delayed cost of the past chronic enhancement of corticosterone.

4.3. Demographic and intergenerational effects

Contrary to our predictions, the chronic corticosterone elevation in the laboratory had no intra-generational delayed effects on demographic traits, including body size growth, annual survival, and future reproduction, and no inter-generational effects on the demographic traits

of the first generation offspring. The absence of delayed, intra-generational effects is surprising because earlier studies using a grossly similar protocol (apart for feeding conditions) found higher annual survival rate of adult males following corticosterone enhancement (Cote et al., 2006). However, the present study included both adults and yearlings, and our sample size was too small to test for age- and sex-dependent effect of corticosterone enhancement on survival with sufficient statistical power.

Inter-generational effects were predicted on the basis of earlier findings where reproductive traits of females and the phenotype of their offspring changed following a chronic increase in the baseline corticosterone concentration during gestation (Meylan and Clobert, 2005). In viviparous lizards, corticosterone elevation during gestation can impair embryonic development (Cree et al., 2003), decrease juvenile locomotor performance (Meylan and Clobert, 2004), inhibit juvenile growth rate (Meylan and Clobert, 2005) and promote juvenile philopatry (de Fraipont et al., 2000). Contrary to our experiment, chronic exposure to the corticosterone was applied during the gestation, whereas we expected delayed maternal effects during the next reproductive season. Indeed, in rats, it has been demonstrated that stress effects can persist in second-generation rats bred from females whose own mothers had been stressed during pregnancy (Pollard, 1986). It is noteworthy that these effects persisted into adulthood. Daughters of physically stressed mothers are less fertile and less fecund than daughters of unstressed mothers (Clark and Galef, 1995). Nevertheless, the absence of inter-generational effects in our study can be explain by differences in term of timing of hormonal treatment. The corticosterone elevation took place 9 months before the next reproduction, too early to affect juvenile phenotype. Therefore, even if females still carry stress effects (higher triglyceride levels), corticosterone levels that can be considered as good candidate in shaping

an offspring's phenotype during development (Dufty et al., 2002; Meylan et al., 2012) do not differ anymore between experimental groups.

Author Contributions Statement

S. M., J.-F. L.G. and R.J. designed the study. All authors contributed to protocols. R. J. analysed the data and wrote a first draft of the manuscript. S. M. and J.-F. L.G. contributed to writing.

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Data Accessibility

Data supporting the results in a paper will be archived in the public archive Dryad upon acceptance of the paper.

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664 **Table 1**

665 Sample sizes for each age and sex per hormonal treatment during each session (pre-experiment and immediate post-experiment, and delayed
666 sessions (September 2014 and May-June 2014) for physiological measurements. We used exactly the same samples for pre-and immediate post-
667 experiment measurements, then, the sample sizes in May and June 2014 are presented only once in the first column. The two following samples
668 were dependant of the recapture collection.

	Sessions 1 (May 2014) & 2 (June 2014)								Session September 2014								Session May-June 2015							
	Control				Corticosterone-treated				Control				Corticosterone-treated				Control				Corticosterone-treated			
	Females		Males		Females		Males		Females		Males		Females		Males		Females		Males		Females		Males	
	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG	AD	YLG
CORTICOSTERONE	4	4	4	3	4	4	4	3	5	5	3	5	5	4	4	4	3	5	0*	5	4	4	3	3
TOTAL	15				15				18				17				13				14			
OXIDATIVE DAMAGE	6	9	6	9	6	7	6	7	6	10	4	9	6	10	4	10	3	4	3	5	4	4	3	4
TOTAL	30				26				29				30				15				15			
ANTI-OXIDANT BARRIER	6	8	6	7	6	7	6	7	6	10	4	9	6	10	4	9	3	4	3	5	4	4	4	4
TOTAL	27				26				29				29				15				16			
TRIGLYCERIDE	6	10	6	10	6	10	6	10	7	10	4	10	6	10	4	10	3	3	3	4	4	3	3	4
TOTAL	32				32				31				30				13				14			
PHA RESPONSE	35	29	16	30	33	31	17	30	16	20	16	22	16	26	16	24	10	14	6	15	13	19	9	17
TOTAL	110				111				74				82				45				58			
METABOLISM	15	15	15	15	15	15	14	15	11	11	10	11	10	13	9	12	10	14	6	14	10	12	9	13
TOTAL	60				60				43				44				44				44			

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674 Immediate and delayed (September 2014: one month later and May-June 2015: next year) effects of experimental treatment (corticosterone vs placebo) on
 675 plasma corticosterone (A) and triglyceride (B) concentration as well as the swelling response (C), a measure of the inflammatory immune response. Test
 676 statistics from a backward elimination procedures are provided and bolded values are significant (<0.05). Sample size (N) is underlined.

	Factor	Immediate effects			Delayed effects one month				Delayed effects next year			
		F	P	N	χ^2	df	P	N	χ^2	df	P	N
A. Corticosterone concentration (ng.ml⁻¹)	Treatments	F1,27 = 7.75	0.01		0.29	1	0.89		0.54	1	0.46	
	Age	F1,24 = 2.53	0.11		5.94	1	0.01		8.23	1	0.004	
	Sex	F1,25 = 1.4	0.25		18.92	1	<0.001		5.29	1	0.02	
	SVL	F1,26 = 0.54	0.47		7.63	1	0.006		4.67	1	0.03	
	Initial measurement	F1,27 = 17.71	<0.001									
	Age × Treatments	F1,21 = 2.50	0.13		0.03	1	0.87		0.65	1	0.42	
	Sex × Treatments	F1,21 = 0.59	0.45		0.67	1	0.41		0.02	1	0.88	
	Age × Sex	F1,21 = 0.01	0.91	<u>30</u>	0.26	1	0.61	<u>35</u>	0.5	1	0.48	<u>27</u>
B. Triglyceride concentration (mg.dl⁻¹)	Treatments	F1,32 = 1.72	0.2		2.45	1	0.11		0.62	1	0.43	
	Age	F1,31 = 0.2	0.66		4.79	1	0.03		3.41	1	0.06	
	Sex	F1,29 = 0.05	0.82		0.44	1	0.5		22.83	1	<0.001	
	SVL	F1,30 = 0	0.98		0.06	1	0.81		1.16	1	0.28	
	Initial measurement	F1,32 = 43.5	<0.001									
	Age × Treatments	F1,26 = 0.03	0.86		0.17	1	0.67		0.77	1	0.38	
	Sex × Treatments	F1,26 = 0.27	0.61		0.53	1	0.47		7.63	1	0.006	
	Age × Sex	F1,26 = 0.53	0.47	<u>34</u>	0.10	1	0.75	<u>61</u>	0.05	1	0.82	<u>27</u>
C. Skin swelling response (mm)	Treatments	F1,217 = 3.74	0.05		0.05	1	0.83		5.47	1	0.02	
	Age	F1,217 = 26.39	<0.001		0.01	1	0.91		1.85	1	0.17	
	Sex	F1,216 = 0.42	0.51		0.18	1	0.67		0.1	1	0.75	
	SVL	F1,214 = 2.66	0.10		15.42	1	<0.001		10.08	1	0.001	
	Initial measurement	F1,217 = 277.56	<0.001		0.87	1	0.35		0.1	1	0.75	
	Age × Treatments	F1,212 = 0.18	0.67		2.55	1	0.11		2.47	1	0.12	
	Sex × Treatments	F1,212 = 1.34	0.25		2.28	1	0.13		3.02	1	0.08	
	Age × Sex	F1,215 = 1.92	0.17	<u>218</u>	0.61	1	0.44	<u>161</u>	0	1	0.99	<u>103</u>

679 Immediate and delayed (September 2014 and May-June 2015) effects of experimental treatment (corticosterone *vs* placebo) on (A) oxidative damage and (B)
 680 anti-oxidant barrier. Test statistics from a backward elimination procedures are provided and bolded values are significant (<0.05). Sample size (N) is
 681 underlined.
 682

	Factor	Immediate effects			Delayed effects one month				Delayed effects next year			
		F	P	N	χ^2	df	P	N	χ^2	df	P	N
A. Oxidative damage (Conc_U)	Treatments	F1,53 = 6.19	0.02		3.92	1	0.05		0.01	1	0.93	
	Age	F1,51 = 2.38	0.13		1.46	1	0.23		0.27	1	0.60	
	Sex	F1,50 = 1.85	0.18		5.8	1	0.02		3.41	1	0.06	
	SVL	F1,52 = 2.03	0.16		6.19	1	0.01		2.08	1	0.15	
	Initial measurement	F1,53 = 16.75	<0.001									
	Age × Treatments	F1,47 = 2.09	0.15		1.73	1	0.19		0.16	1	0.69	
	Sex × Treatments	F1,47 = 0.51	0.36		0.09	1	0.76		0.94	1	0.33	
	Age × Sex	F1,47 = 0.85	0.43	<u>56</u>	1.28	1	0.3	<u>59</u>	1.78	1	0.18	<u>30</u>
B. Anti-oxidant barrier (µl HCLO.ml⁻¹)	Treatments	F1,50 = 2.24	0.14		0.16	1	0.69		0.40	1	0.52	
	Age	F1,47 = 0.04	0.84		0.81	1	0.37		0.78	1	0.67	
	Sex	F1,49 = 1.43	0.24		3.47	1	0.06		3.01	1	0.08	
	SVL	F1,48 = 0.7	0.41		0.51	1	0.48		0.18	1	0.67	
	Initial measurement	F1,50 = 230.04	<0.001									
	Age × Treatments	F1,45 = 1.16	0.29		3.03	1	0.08		0.73	1	0.39	
	Sex × Treatments	F1,45 = 0.08	0.78		1.1	1	0.29		0.08	1	0.77	
	Age × Sex	F1,45 = 0.67	0.42	<u>53</u>	1.59	1	0.11	<u>58</u>	0.12	1	0.73	<u>31</u>

Figure legends

Figure 1. Diagram representing the timing of the experiment over the 3 years. Lizards alternated laboratory and outdoor phases. After the captures in May and September, the lizards are kept in the laboratory for 2 days to acclimatize and then a series of physiological measurements (indicated “M”) was done: first a blood sample, the day after an injection of PHA to estimate the inflammatory response and the next day after 3 days of fasting the metabolism was measured overnight.

Figure 2. Immediate effects of corticosterone increase (corticosterone increase: red, placebo: blue) on baseline corticosterone ($\text{ng}\cdot\text{ml}^{-1}$), circulating triglycerides ($\text{mg}\cdot\text{dl}^{-1}$) and skin swelling response (mm) (A-C) as well as delayed effects one month later (September 2014, D-F) and the next year (May-June 2015, G-I). Panels A-C display the differences between post and pre-experimental values, while panels D-K represent individual scores. Values are presented as means $\pm$ 95% confidence intervals and dotplots of raw data per group.

Figure 3 Immediate effects of corticosterone increase (corticosterone increase: red, placebo: blue) on antioxidant barrier (Conc U) and oxidative damage ($\mu\text{l HClO}\cdot\text{ml}^{-1}$) (A-B) as well as delayed effects one month later (September 2014, C-D) and the next year (May-June 2015, E-F). Panels A and B represent differences between post and pre-experimental values in the laboratory, while panels C to F represent raw individual values. Values are presented as means $\pm$ 95% confidence intervals and dotplots of raw data per group. In panel B, raw data are residuals from a linear regression against body size.

Figure 4. Immediate (from pre- to post-experimental values in the laboratory, A) and delayed body mass change (one month after the manipulation, B) in lizards from corticosterone increase (red) and placebo (blue) groups. Values are presented as means $\pm$ 95% confidence intervals and dotplots of raw data per group