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

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18 **Key words:** stress, maternal effect, oxidative stress, immune-competence, metabolism,

19 triglycerides

20

21 **Abstract**

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

42

## 43        **1. Introduction**

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

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

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

81       Glucocorticoid secretion can induce phenotypic plasticity at adulthood as well (Dufty et al.,  
82 2002), but its long lasting, delayed effects have been little investigated so far in late life stages.  
83 Here, we used common lizards (*Zootoca vivipara*) as a model system to investigate for the first  
84 time the joint immediate and delayed physiological and demographic effects of a prolonged  
85 corticosterone increase late in life. Immediate effects of chronic corticosterone increase in adult  
86 common lizards include changes in resting and activity metabolism, immune capacities, oxidative  
87 stress, and behaviours such as food consumption, locomotor activity and basking (Cote et al.,  
88 2006; Meylan and Clobert, 2005; Meylan et al., 2010). Moreover, a chronic corticosterone  
89 manipulation increases the future survival of adult males but not females suggesting sex-specific  
90 long lasting, delayed effects (Cote et al., 2006). In this viviparous species, increased  
91 corticosterone secretion during gestation changes the morphology, behavioural activity and  
92 dispersal strategy of juveniles at birth (de Fraipont et al., 1999; Meylan et al., 2002; Meylan et al.,

93 2004), but can also influence their growth and survival later in life (Meylan and Clobert, 2005).  
94 However, we do not know if increased corticosterone secretion some months before the gestation  
95 period can cause maternal effects.

96 To quantify immediate and delayed intra-generational and inter-generational effects,  
97 we used a three-stage experimental design where we first exposed yearlings and adults to a  
98 chronic corticosterone exposure during the summer post-reproductive season in the  
99 laboratory. We next released them in outdoor populations until the next reproductive season,  
100 and then followed for an additional season their first generation of offspring. In yearlings and  
101 adults, we investigated four sets of physiological parameters (standard metabolic rate,  
102 immune capacities, oxidative stress and plasmatic triglyceride concentration) directly after the  
103 treatment, one month later in the field and one year later in the field to identify most relevant  
104 components of the adrenocortical response. Consequences on life history were examined by  
105 quantifying reproductive performances, growth, survival rate and the intergenerational effects  
106 on the first generation of offspring. At the end of the laboratory manipulation, we expected an  
107 increase of baseline corticosterone, as well as of triglyceride concentration (Karatsoreos et al.,  
108 2010; Peckett et al., 2011), oxidative stress (Cote et al., 2010b), and leanness (Cote et al.,  
109 2006; Cote et al., 2010a). In contrast, we predicted no change in immune response in  
110 accordance with previous work (Meylan et al., 2010) but sex-dependent changes of metabolic  
111 rates (Cote et al., 2010b; Meylan et al., 2010). Since previous works have shown sex-specific  
112 delayed effects on survival in adults (Cote et al., 2006), we expected different long-lasting  
113 responses between sexes. If chronic corticosterone exposure increases maintenance costs, we  
114 predicted a long-term decrease of immune capacities and an increase of oxidative stress,  
115 plasmatic triglyceride concentrations and baseline corticosterone (Cote et al., 2006; McEwen  
116 and Wingfield, 2003; Romero et al., 2009). Moreover, since the energetic costs of  
117 maintenance change between age classes, sex and reproductive cycles (Massot et al., 2011;

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

## 121 **2. Materials and methods**

### 122 **2.1. Model species**

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

### 133 **2.2. Capture and rearing condition**

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

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

### 147 **2.3. Experimental design**

148 We sampled 68 adult and 60 yearling females and 33 adult and 60 yearling males for this  
149 experiment. At day 1, a balanced number lizards from each age and sex class was assigned to  
150 a corticosterone (33 adult and 31 yearling females; 17 adult and 30 yearling males) or a  
151 placebo group (35 adult and 29 yearling females; 16 adult and 30 yearling males). We  
152 subsequently manipulated the circulating levels of corticosterone during 3 weeks using a non-  
153 invasive method designed by Meylan et al. (2003). This method increases the baseline  
154 corticosterone concentration within the natural range of variation without causing mortality or  
155 abortion in pregnant females (Meylan et al., 2003). We diluted corticosterone (Sigma-Aldrich,  
156 France, C2505-500mg 92%, C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>) in commercial sesame oil, which facilitates skin  
157 penetration of corticosterone, to reach a final concentration of 3µg corticosterone for 1µl of  
158 sesame oil. We applied 4.5 µL of corticosterone mixture (corticosterone group) or pure  
159 sesame oil (placebo) each evening between 20:00 h and 21:00 local time during 21 days (a  
160 duration corresponding to 10% of the yearly activity period). At day 0, prior to the start of the  
161 experiment, there was no difference in SVL ( $\chi^2 = 0.35$ ,  $df = 1$ ,  $P = 0.55$ ) and body mass ( $\chi^2 =$   
162  $0.14$ ,  $df = 1$ ,  $P = 0.70$ ) between treatment groups.

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



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

#### 176 **2.4. Life history data**

177 We monitored yearlings and adults during one year to assess body size and condition, growth,  
178 survival and female reproductive performance one year after the manipulation. We recaptured  
179 164 lizards from the two treatment groups in September 2014 and 107 individuals in May-  
180 June 2015. At each time step of the experimental design (pre-experimental, post-  
181 experimental, September 2014, and May-June 2015), we sampled blood and measured  
182 immuno-competence for all animals and whole-organism metabolism for a subset of lizards  
183 (see below). Body growth rates were calculated as the change in SVL between two captures  
184 divided by the time interval. We subtracted the number of inactive days spent in hibernation  
185 for the growth rate whenever needed. Hibernation is assumed to be from end of October to  
186 beginning of March. We estimated annual survival probabilities assuming that capture  
187 probability was very close to 1 (Le Galliard et al., 2005). To assess reproductive performance  
188 of females, we counted live new-borns, dead new-borns and aborted or unfertilized eggs of  
189 each clutch. Parturition date, total fecundity (live, dead and aborted newborns), fit fecundity  
190 (live newborns) and offspring characteristics (sex, SVL, body mass) were recorded.

191 **2.5. Blood sample and plasma measurements**

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

202 Corticosterone level was measured with a competitive enzyme-immunoassay method  
203 using corticosterone EIA (IDS Corticosterone EIA kit, ref AC-14F1, IDS EURL Paris,  
204 France) after 1:10 dilution of all samples. This method quantifies total plasmatic  
205 corticosterone using a polyclonal corticosterone antibody and is based on a colorimetric assay  
206 of absorbance at 450 nm. Sensitivity is 0.55 ng/mL. Measurements were highly repeatable  
207 (intra-plate repeatability:  $n = 63$ ,  $F_{3,60} = 102.61$ ,  $P < 0.001$ ,  $r = 0.89$ ; inter-plate repeatability:  $n$   
208  $= 63$ ,  $F_{16,47} = 51.3$ ,  $P < 0.001$ ,  $r = 0.93$ ). Levels of circulating triglyceride were measured by  
209 colorimetric assays using 2.5 µl of plasma (Triglyceride Colorimetric Assay kit, ref.  
210 10010303, Cayman Chemical, USA). This method quantifies the total triglyceride by using a  
211 chain of three enzymatic reactions ending up in hydrogen peroxide production. Sensitivity is  
212 0.5 mg/dL The measurement was highly repeatable (inter-plate repeatability:  $n = 24$ ,  $F_{4,20} =$   
213  $795.48$ ,  $P < 0.001$ ,  $r = 0.99$ , intra-plate repeatability :  $n = 24$ ,  $F_{2,22} = 728.06$ ,  $P < 0.001$ ,  $r =$   
214  $0.98$ ).

215 To assess oxidative stress in lizards, we used two complementary colorimetric tests.  
216 First, we used d-ROMs test (MC003, Diacron International, Via Zircone 8, 58100 Grosseto  
217 (GR), Italy) to assess the concentration of reactive oxygen metabolites in 4  $\mu$ L plasma sample.  
218 Second, to assess the anti-oxidant barrier of plasma, we used the OXY-Absorbent test  
219 (MC435, Diacron International, Via Zircone 8, 58100 Grosseto (GR), Italy) after 1:100  
220 dilution of 3 $\mu$ L of plasma. These two tests were also highly repeatable (d-ROMs test : inter-  
221 plate repeatability :  $n = 16$ ,  $F_{4,12} = 37.53$ ,  $P < 0.001$ ,  $r = 0.91$ ; intra-plate repeatability :  $n = 16$ ,  
222  $F_{2,14} = 46.69$ ,  $P < 0.001$ ,  $r = 0.88$  and OXY test : inter-plate repeatability :  $n = 18$ ,  $F_{4,14} =$   
223  $153.26$ ,  $P < 0.001$ ,  $r = 0.88$ ; intra-plate repeatability :  $n = 18$ ,  $F_{3,15} = 214.96$ ,  $P < 0.001$ ,  $r =$   
224  $0.98$ ). Prior to the beginning of the manipulation, there was no difference between treatment  
225 groups for corticosterone (Likelihood-ratio tests,  $\chi^2 = 0.99$ ,  $df = 1$ ,  $P = 0.32$ ), triglyceride  
226 concentration ( $\chi^2 = 0.06$ ,  $df = 1$ ,  $P = 0.81$ ), oxidative damage ( $\chi^2 = 0.09$ ,  $df = 1$ ,  $P = 0.76$ ) and  
227 antioxidant barrier capacity ( $\chi^2 = 0.15$ ,  $df = 1$ ,  $P = 0.69$ ).

## 228 **2.6. Evaluation of immune capacities**

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

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

## 248 **2.7. Whole-organism metabolic expenditure**

249 We defined at the start of the experiment a sub-sample of 60 males and 60 females from the  
250 two age classes to measure repeatedly their resting metabolic rate during the four  
251 measurement periods. All individuals were measured during the pre-experiment and post-  
252 experiment sessions and recaptured animals were measured during the sessions of September  
253 2014 and May-June 2015. The resting metabolic rate, or standard metabolic rate (SMR) for  
254 ectotherms, is the minimum energetic cost of maintenance estimated in fasting individuals at  
255 rest and at a given temperature. Here, we measured the SMR after a fasting period of 3 days  
256 to ensure a post-absorptive state (Artacho et al., 2013). Before the measurement, lizards were  
257 moved in a dark room maintained at 20°C during 3-4 hours and were then placed in their  
258 metabolic chamber during 1 hour at 16°C before the beginning of the measurements. This  
259 temperature was chosen because it is a body temperature where lizards are inactive. All  
260 measurements were recorded at night between 08:00 pm and 08:00 am. The CO<sub>2</sub> production  
261 was sampled during 40 mins for each animal with a multiple-channel, flow-through  
262 respirometry system (Qubit Systems, Kingston, ON, Canada) compounded of a CO<sub>2</sub> analyzer  
263 (S157) connected to a respirometry software (QS Research). Incoming air flowed through  
264 columns of sodalime (Spherasorb, Wokingham, Berkshire, RG41 2RZ, UK) and drierite to

265 remove CO<sub>2</sub> and H<sub>2</sub>O, respectively. After the absorbent columns, air was pushed through  
266 seven channels of a multiplexer at 150 mL.min<sup>-1</sup>. Six channels were used for measuring  
267 lizards and one channel for the CO<sub>2</sub> reference. Outgoing air was pushed into the CO<sub>2</sub> analyzer  
268 at 50 mL.min<sup>-1</sup>. Metabolic records of CO<sub>2</sub> were processed by a macro program C950 data  
269 acquisition software (Qubit systems) in order to transform the ppm measure of CO<sub>2</sub>  
270 production in mL per hour, which takes into account the flow rate..

## 271 **2.8. Statistical analyses**

272 Analyses were performed with R 3.1.0 (R Core Team 2014, [https:// www.r-project.org/](https://www.r-project.org/)) using  
273 the *lmer* statistical procedure available in the *lme4* package for linear mixed models (Bates et  
274 al., 2015) and *lm* procedure from *stats* package for linear models. Analyses of variance  
275 procedure were done with the *Anova* procedure from *Car* package (Fox and Weisberg 2011)  
276 All initial models included as fixed factors the effects of treatment group, age class, sex and  
277 their first-order interactions. Analyses of corticosterone and triglyceride concentrations,  
278 antioxidative-barrier and oxidative damage, swelling response, SMR (CO<sub>2</sub> production) and  
279 body mass from the post-experimental session were done by calculating the difference  
280 between the post-experimental (after 20 days of treatment application) and the initial  
281 measurement and using this as a response variable in a linear model. Data for corticosterone,  
282 triglyceride concentrations, antioxidative-barrier, oxidative damage, swelling response and  
283 SMR were log-transformed to fit the normality assumption. Because SMR may increase with  
284 body mass, we used the mass gain between pre and post experimental session as a covariate,  
285 and initial measurement of SVL was also used as covariate in other models.

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

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

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

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

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

### 319 **3. Results**

#### 320 **3.1. Immediate physiological effects**

321 Relative to the placebo, the corticosterone treatment increased significantly plasma  
322 corticosterone concentration until the end of the laboratory manipulation (Table 2, Figure 2A)  
323 and the skin swelling response (Table 2, Figure 2C), but it did not influence circulating  
324 plasma triglyceride concentration (Table 2, Figure 2B), antioxidant barrier (Table 3, Figure  
325 3A) and SMR ( $F_{1,114} = 0.06$ ,  $P = 0.81$ ). In addition, oxidative damages were more important in  
326 placebo than in corticosterone treated lizards (Table 3, Figure 3B). Corticosterone treatment  
327 significantly increased body mass change during the laboratory manipulation ( $F_{1,207} = 11.94$ ,  
328  $P < 0.001$ , Figure 4A) after controlling for effects of sex ( $F_{1,207} = 7.3$ ,  $P = 0.007$ ), age class  
329 ( $F_{1,207} = 227.65$ ,  $P < 0.001$ ), and initial body mass ( $F_{1,207} = 358.74$ ,  $P < 0.001$ ).

#### 330 **3.2. Delayed physiological and demographical effects**

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

340 Figure 2I). At the same time, we found delayed effects on oxidative damage but not for anti-  
341 oxidative barrier (see Figure 3C-F and Table 3). Oxidative damage was significantly lower in  
342 corticosterone treated group than the placebo in September 2014 (Figure 3D). We did not  
343 observe delayed effect of corticosterone treatment on SMR (September 2014:  $\chi^2 = 0.07$ ,  $df =$   
344  $1$ ,  $P = 0.79$ ; May-June 2015:  $\chi^2 = 0.39$ ,  $df = 1$ ,  $P = 0.53$ ).

345 During the interval time between release and September 2014, lizards from the  
346 corticosterone treatment gained less body mass than placebos ( $\chi^2 = 9.67$ ,  $df = 1$ ,  $P = 0.002$ ,  
347 Figure 4) after controlling for a significant interaction between sex and age ( $\chi^2 = 10.64$ ,  $df =$   
348  $1$ ,  $P = 0.001$ ). There was no difference between treatment groups for size growth between  
349 release in 2014 and recapture in May-June 2015 (placebo:  $0.016 \pm 0.0015$  mm.days<sup>-1</sup>,  
350 corticosterone:  $0.015 \pm 0.002$  mm.days<sup>-1</sup>;  $\chi^2 = 0.43$ ,  $df = 1$ ,  $P = 0.51$ ) after controlling for a  
351 negative effect of initial SVL ( $\chi^2 = 200.5$ ,  $df = 1$ ,  $P < 0.001$ ) and a sex effect ( $\chi^2 = 254.8$ ,  $df =$   
352  $1$ ,  $P < 0.001$ ). We also found a marginal difference between treatment groups for mass change  
353 between September 2014 and May-June 2015 ( $\chi^2 = 3.48$ ,  $df = 1$ ,  $P = 0.06$ ), with a lower mass  
354 gain in corticosterone treated group.

355 Estimated annual survival rate was not different between treatment groups (placebo =  
356  $0.40$  [ $0.26, 0.56$ ] and corticosterone =  $0.52$  [ $0.37, 0.67$ ],  $\chi^2 = 2.61$ ,  $df = 1$ ,  $P = 0.11$ ) and  
357 between sexes ( $\chi^2 = 2.18$ ,  $df = 1$ ,  $P = 0.14$ ) but estimated survival rate of yearling lizards was  
358 higher than adults (adults =  $0.35$  [ $0.22, 0.50$ ]; yearlings =  $0.58$  [ $0.43, 0.71$ ],  $\chi^2 = 3.88$ ,  $df = 1$ ,  $P$   
359  $= 0.05$ ). Similarly, we found no delayed effects of corticosterone enhancement on female  
360 reproductive traits, including parturition date, number of live offspring and total fecundity (all  
361  $P > 0.1$ ).

### 362 **3.3. Inter-generational effects**

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



365 df = 1, P = 0.37) after controlling for sex effects (SVL:  $\chi^2 = 68.81$ , df = 1, P < 0.001; body  
366 condition:  $\chi^2 = 21.97$ , df = 1, P < 0.001). Estimated annual survival rate of juveniles between  
367 2015 and 2016 was not different between maternal treatment groups (placebo = 0.19 [0.13,  
368 0.27], corticosterone = 0.24 [0.18, 0.32],  $\chi^2 = 0.97$ , df = 1, P = 0.32). The body size growth  
369 rate of juveniles born from corticosterone treated females did not differ from placebos ( $\chi^2 =$   
370 0.68, df = 1, P = 0.41) after controlling for the faster growth of females ( $\chi^2 = 3.92$ , df = 1, p =  
371 0.05) and the decrease of growth rate with birth date ( $\chi^2 = 8.77$ , df = 1, P = 0.003).

## 372 **4. Discussion**

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

### 384 **4.1. Effects on immune and antioxidant capacities**

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

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

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

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

#### 419 **4.2. Effects on body mass and metabolism**

420 Although a positive effect of corticosterone enhancement on basal metabolic rate is generally  
421 expected, the experimental evidence is rather contradictory and we found no immediate and  
422 delayed effects on SMR. In birds, for example, corticosterone elevation has been associated  
423 with a reduction of nocturnal metabolism (Buttemer et al., 1991). In contrast, Wikelski et al.  
424 (1999) found no apparent relationship between resting metabolic rate and corticosterone  
425 levels in white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Moreover, an  
426 experimental study in two wild passerine bird species, house sparrows (*Passer domesticus*)  
427 and gray catbirds (*Dumetella carolinensis*), demonstrated that the relationship between  
428 metabolic rate and corticosterone concentration differed qualitatively between the species  
429 (Cohen et al., 2008). In the common lizard, the effect of the corticosterone on metabolism can  
430 be condition-dependent as well. Indeed, Meylan et al. (2010) found an increase of the SMR  
431 after 21 days of corticosterone application in pregnant females; whereas SMR decreased after  
432 10 days of treatment in adult males (Cote et al. 2010a) and did not change in yearlings  
433 (Voituron et al., 2017).

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

440 every 2 days), which are insufficient for sustained body growth in the laboratory. In contrast,  
441 our laboratory protocol brought food in excess and allowed for significant size growth like in  
442 the field. In the common lizard, a prolonged corticosterone elevation promotes behaviours  
443 associated with food intake including exploratory activity, foraging and basking (Cote et al.  
444 2010a). Corticosterone can also induce lipid storage in non-fasted state by activating  
445 lipogenesis and adipogenesis (Peckett et al., 2011; Voituron et al., 2017). The body mass gain  
446 during the laboratory experiment could thus result from an increase of both food intake and  
447 lipid storage. Against the later hypothesis, we however found no difference between treatment  
448 groups for plasma triglyceride concentration in the laboratory and one month after the end of  
449 the laboratory manipulation.

450 In the field, the body mass gain in corticosterone treated group was lower in placebo  
451 group, and body mass between the two groups was not different one month after the end of  
452 the laboratory manipulation (post-hoc test,  $t = -0.53$ ,  $df = 148.9$ , adjusted  $P = 0.595$ ). This  
453 suggests that corticosterone treated lizards decreased their energy storage and/or their food  
454 intake in the field resulting in a lower body mass relative to placebos. In addition, females  
455 from corticosterone treated group had much higher triglyceride levels than placebo group  
456 during the next reproductive season. Difference between sexes could be explained by the  
457 higher baseline corticosterone levels in females and its action on lipolysis (Peckett et al.,  
458 2011) and also by the additional costs associated with gestation. Indeed higher triglyceride  
459 levels in females suggest a higher energetic requirement to maintain reproduction output, and  
460 so a delayed cost of the past chronic enhancement of corticosterone.

#### 461 **4.3. Demographic and intergenerational effects**

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

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

471

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

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

#### 491 **Author Contributions Statement**

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

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500 were done under the agreement with the Regional ethics committee in animal experiment N°3  
501 of the Région Île-de-France. The authors declare that they have no conflict of interest.

#### 502 **Data Accessibility**

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

505

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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.  
 669

	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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672

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	$\chi^2$	df	P	N	$\chi^2$	df	P	N
<b>A. Corticosterone concentration (ng.ml<sup>-1</sup>)</b>	Treatments	<b>F1,27 = 7.75</b>	<b>0.01</b>		0.29	1	0.89		0.54	1	0.46	
	Age	F1,24 = 2.53	0.11		<b>5.94</b>	<b>1</b>	<b>0.01</b>		<b>8.23</b>	<b>1</b>	<b>0.004</b>	
	Sex	F1,25 = 1.4	0.25		<b>18.92</b>	<b>1</b>	<b>&lt;0.001</b>		<b>5.29</b>	<b>1</b>	<b>0.02</b>	
	SVL	F1,26 = 0.54	0.47		<b>7.63</b>	<b>1</b>	<b>0.006</b>		<b>4.67</b>	<b>1</b>	<b>0.03</b>	
	Initial measurement	<b>F1,27 = 17.71</b>	<b>&lt;0.001</b>									
	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>B. Triglyceride concentration (mg.dl<sup>-1</sup>)</b>	Treatments	F1,32 = 1.72	0.2		2.45	1	0.11		0.62	1	0.43	
	Age	F1,31 = 0.2	0.66		<b>4.79</b>	<b>1</b>	<b>0.03</b>		3.41	1	0.06	
	Sex	F1,29 = 0.05	0.82		0.44	1	0.5		<b>22.83</b>	<b>1</b>	<b>&lt;0.001</b>	
	SVL	F1,30 = 0	0.98		0.06	1	0.81		1.16	1	0.28	
	Initial measurement	<b>F1,32 = 43.5</b>	<b>&lt;0.001</b>									
	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		<b>7.63</b>	<b>1</b>	<b>0.006</b>	
	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>
<b>C. Skin swelling response (mm)</b>	Treatments	<b>F1,217 = 3.74</b>	<b>0.05</b>		0.05	1	0.83		<b>5.47</b>	<b>1</b>	<b>0.02</b>	
	Age	<b>F1,217 = 26.39</b>	<b>&lt;0.001</b>		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		<b>15.42</b>	<b>1</b>	<b>&lt;0.001</b>		<b>10.08</b>	<b>1</b>	<b>0.001</b>	
	Initial measurement	<b>F1,217 = 277.56</b>	<b>&lt;0.001</b>		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	$\chi^2$	df	P	N	$\chi^2$	df	P	N
<b>A. Oxidative damage (Conc_U)</b>	Treatments	<b>F1,53 = 6.19</b>	<b>0.02</b>		<b>3.92</b>	<b>1</b>	<b>0.05</b>		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		<b>5.8</b>	<b>1</b>	<b>0.02</b>		3.41	1	0.06	
	SVL	F1,52 = 2.03	0.16		<b>6.19</b>	<b>1</b>	<b>0.01</b>		2.08	1	0.15	
	Initial measurement	<b>F1,53 = 16.75</b>	<b>&lt;0.001</b>									
	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>B. Anti-oxidant barrier (<math>\mu\text{l HClO.ml}^{-1}</math>)</b>	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	<b>F1,50 = 230.04</b>	<b>&lt;0.001</b>									
	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>



683 **Figure legends**

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

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

697  
698 **Figure 3** Immediate effects of corticosterone increase (corticosterone increase: red, placebo:  
699 blue) on antioxidant barrier (Conc U) and oxidative damage ( $\mu\text{l HClO}\cdot\text{ml}^{-1}$ ) (A-B) as well as  
700 delayed effects one month later (September 2014, C-D) and the next year (May-June 2015, E-  
701 F). Panels A and B represent differences between post and pre-experimental values in the  
702 laboratory, while panels C to F represent raw individual values. Values are presented as  
703 means  $\pm$  95% confidence intervals and dotplots of raw data per group. In panel B, raw data  
704 are residuals from a linear regression against body size.

705  
706 **Figure 4.** Immediate (from pre- to post-experimental values in the laboratory, A) and delayed  
707 body mass change (one month after the manipulation, B) in lizards from corticosterone  
708 increase (red) and placebo (blue) groups. Values are presented as means  $\pm$  95% confidence  
709 intervals and dotplots of raw data per group