



**HAL**  
open science

## Mitochondrial oxidative phosphorylation response overrides glucocorticoid-induced stress in a reptile

Yann Voituron, Damien Roussel, Jean-François Le Galliard, Andréaz Dupoué,  
Caroline Romestaing, Sandrine Meylan

► **To cite this version:**

Yann Voituron, Damien Roussel, Jean-François Le Galliard, Andréaz Dupoué, Caroline Romestaing, et al.. Mitochondrial oxidative phosphorylation response overrides glucocorticoid-induced stress in a reptile. *Journal of Comparative Physiology B*, 2022, 192 (6), pp.765-774. 10.1007/s00360-022-01454-5 . hal-03764878

**HAL Id: hal-03764878**

<https://cnrs.hal.science/hal-03764878v1>

Submitted on 30 Aug 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Mitochondrial oxidative phosphorylation resilience overrides glucocorticoid-induced**  
2 **stress in a reptile**

3  
4  
5  
6 4 Yann Voituron<sup>1\*</sup>, Damien Roussel<sup>1</sup>, Jean-François Le Galliard<sup>2,3</sup>, Andréaz Dupoué<sup>2</sup>, Caroline  
7  
8 Romestaing<sup>1</sup> and Sandrine Meylan<sup>2</sup>  
9

10  
11 6  
12  
13 7 1. Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés (U.M.R. CNRS 5023),  
14  
15 8 Université Claude Bernard Lyon1, Université de Lyon; Bd du 11 novembre 1918, Bât. Darwin  
16  
17 C; 69622 VILLEURBANNE Cedex, France  
18  
19 9

20  
21 10 2. Sorbonne Université, CNRS, IRD, INRA, Institut d'écologie et des sciences de  
22  
23 11 l'environnement (IEES), Paris, France  
24  
25

26 12 3. Centre de recherche en écologie expérimentale et prédictive (CEREEP-Ecotron  
27  
28 13 IleDeFrance), Département de biologie, Ecole normale supérieure, CNRS, PSL University,  
29  
30 14 77140 Saint-Pierre-lès-Nemours, France  
31  
32

33 15  
34  
35  
36 16  
37  
38 17 \* To whom correspondence should be addressed: Tel: +33 4 72 43 15 20; Fax: +33 4 72 43 11  
39  
40 18 71; E-mail: [yann.voituron@univ-lyon1.fr](mailto:yann.voituron@univ-lyon1.fr)  
41  
42

43 19  
44  
45 20 **Running title:** Mitochondrial resilience under chronic stress in a reptile  
46  
47  
48 21  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

22 **Abstract**

23 Stress hormones and their impacts on whole-organism metabolic rates are usually considered  
24 as appropriate proxies for animal energy budget that is the basement of numerous concepts and  
25 models aiming at predicting individual and population responses to environmental stress.  
26 However, the kinetics of energy re-allocation under stress make the link between metabolism  
27 and corticosterone complex and still unclear. Using ectopic application of corticosterone for 3  
28 days, 11 days and 21 days, we simulated acute, mild-time and chronic stress in a lizard (*Zootoca*  
29 *vivipara*). We then investigated whole-organism metabolism, muscle cellular O<sub>2</sub> consumption  
30 and liver mitochondrial oxidative phosphorylation processes (O<sub>2</sub> consumption and ATP  
31 production) and ROS production. The data showed that (i) while muscle is only slightly  
32 impacted, stress regulates the mitochondrial functionality in a time-dependent manner with  
33 contrasted pictures between acute, mild-time and chronic stress that is poorly described by the  
34 whole-organism metabolism and (ii) an unexpected “pathway” to obtain mitochondrial  
35 resilience. While acute stress is characterized by a decreased ATP synthesis rate and high H<sub>2</sub>O<sub>2</sub>  
36 release with no change in the rate of oxygen consumption, the mild-time stress reduced all three  
37 fluxes. Application of corticosterone for 21 days induced a return to initial oxidative  
38 phosphorylation capacities with lower H<sub>2</sub>O<sub>2</sub> production allowing a better capacity to respond to  
39 the energetic demands imposed by the environment. So, the global mitochondrial functioning  
40 has to be considered in order to better understand the proximal causes of resource allocation  
41 under stress periods.

42  
43 **Keywords:** Corticosterone, Lizard, Metabolism, Mitochondrial efficiency, ROS and ATP  
44 production, Oxygen consumption

## 47 **Introduction**

48 Glucocorticoids (GCs) secreted by the hypothalamus–pituitary–adrenal axis are crucial  
49 hormones orchestrating the tradeoff between survival and different physiological processes  
50 when vertebrates are facing stressors. However, physiological consequences are known to be  
51 different in accordance to short-term versus long-term elevations in GCs production. While  
52 many of the acute effects of GCs consist of mobilizing energy, chronically elevated circulating  
53 GCs enhance energy storage (Harris 2015, Sapolsky et al. 2000). Similarly, acute GC elevations  
54 can activate the adaptive immune system, whereas chronic GC elevations are linked to  
55 suppressed cell-mediated leucocyte trafficking (Sorrells et al. 2009; Dhabhar and Mc Ewen  
56 1997). Such biphasic responses in which acute and chronic stress or corticosteroid exposure  
57 induce contrasting and sometimes opposite effects have not yet been fully elucidated. While  
58 some studies stipulate potential differential actions of different nuclear receptors of GCs (e.g.,  
59 Joels and de Kloet 1992), others link it to larger magnitude changes in gene expression under  
60 acute stress rather than chronic stress (e.g., Wang et al. 2004).

61 Other studies demonstrated rapid, non-genomic effects of GCs (de Kloet, 2013) explained at  
62 least partly by the GCs translocation to the mitochondria that provide ATP through the Krebs  
63 tricarboxylic-acid cycle and oxidative phosphorylation (Du, 2009, Lee et al. 2013). Indeed, *in*  
64 *vitro* (with cortical neuronal cultures) and *in vivo* (with brains from male rats) experiments have  
65 demonstrated that mitochondrial oxidation, membrane potential, mitochondrial complex I  
66 activity and mitochondrial encoded genes (i.e. NADH dehydrogenase 1, 3, 6 and ATP synthase  
67 6) are modulated by corticosterone in a dose and time-dependent manner (Du et al. 2009; Hunter  
68 et al. 2016). In parallel, a reduction of the activity of specific mitochondrial electron transport  
69 chain complexes together with an increased mitochondrial Reactive Oxygen Species (ROS)  
70 production has been shown under chronic glucocorticoid treatment (Manoli et al. 2007), which  
71 could impact the oxidative balance (Costantini 2011). Altogether, these physiological studies

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
72 thus demonstrated (i) a time adjustments of energetic resources in response to stress and (ii) a  
73 direct linkage between GC secretion in response to stressor and mitochondrial physiology.  
74 These observations led to the emergent concept of “mitochondrial allostatic load” that is the  
75 mitochondrial analog of allostatic load in the broader context of stress biology (McEwen and  
76 Wingfield 2003). The mitochondrial allostatic load can be defined as the “deleterious structural  
77 and functional changes that mitochondria undergo” in response to elevated glucocorticoid  
78 secretion under a chronic stress situation (Picard et al. 2014, 2018). The mitochondria are the  
79 major ATP cell generators (Lehninger et al. 1993) sustaining physiological processes  
80 underlying both maintenance and physiological responses to environmental variations.  
81 Mitochondrial energy transduction system also continually produces ROS which can be  
82 involved in cell signaling and/or physiopathology (Starkov, 2008). Unfortunately, at that time,  
83 no direct assessments of mitochondrial functionality parameters like ATP and ROS under  
84 chronic GCs production were available. Yet such data are crucial to understand functional  
85 consequences of previously described responses to GC secretion, such as alteration of gene  
86 expression and/or epigenetic modifications (Hunter 2016), increased oxidized mtDNA and  
87 mitochondrial fission (Picard et al. 2014). A better knowledge of the temporal dynamics of  
88 mitochondrial responses to GC secretion will undoubtedly have biomedical applications but  
89 will also provide new visions in evolutionary biology (Eisner et al. 2018).

45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
90 The objective of this study was to explore the time-dependent metabolic responses at  
91 the whole-body and mitochondrial levels in lizards (*Zootoca vivipara*) exposed to 3 days, 11  
92 days and 21 days of corticosterone treatment. This protocol provides the temporal dynamic of  
93 the mitochondrial responses to GC secretion and answers the following three questions. First  
94 question: do short-term *versus* long-term elevations in GCs production induce similar  
95 physiological responses in ectotherms than in endotherms? Indeed, the commonly accepted  
96 conclusions on time effects on GCs impacts are strongly biased toward endotherms (mammals

1 97 and birds), neglecting the vast majority of ectothermic metazoans. Recent experimental  
2 98 approaches suggest different mitochondrial responses under stress in some ectotherms (e.g.,  
3  
4 99 Cote et al. 2010; Voituron et al. 2017). Comparative analyses demonstrate that evolution shapes  
5  
6  
7 100 GCs variations which leads to a different impact between ectotherms and endotherms (Jessop  
8  
9 101 et al. 2013; Vitousek et al. 2019). Second question: are ATP and ROS productions equivalently  
10  
11 102 impacted by GCs? Third question: given that the liver and muscle contribute about 50% of body  
12  
13  
14 103 O<sub>2</sub> consumption at rest and more during activity (Rolfe and Brown, 1997), does whole-  
15  
16 104 organism metabolism correctly reflect energetic adjustments at a cellular level of organization?  
17  
18  
19  
20 105

## 21 22 106 **Materials and methods**

### 23 24 25 107 *Capture and rearing condition*

26  
27 108 The common lizard (*Zootoca vivipara*) is a small lacertid species (adult's snout-vent length  
28  
29 109 SVL ranging from 50–70 mm) widely distributed across Eurasia. In spring 2016, 49 sub-adult  
30  
31 110 males (1-year-old) were captured by hand in outdoor enclosures (10 × 10 m) at CEREEP  
32  
33 111 (Centre de Recherche en Ecologie Expérimentale et Prédictive; Saint-Pierre-lès-Nemours,  
34  
35 112 France, 48°17'N, 2°41'E) field station from May 16th to May 25th. Animals were measured  
36  
37 113 for body size (SVL, ± 0.5 mm) and body mass (± 1 mg). All animals were maintained in  
38  
39 114 individual terraria (25 × 15 × 16 cm) with a shelter, peat soil as substrate and opportunities for  
40  
41 115 optimal thermoregulation. We used incandescent light bulbs (25 W) for 8 hours per day from  
42  
43 116 09:00 to 17:00 local time to ensure a thermal gradient ranging from 17-23°C to 35-38°C. We  
44  
45 117 provided lizards with water *ad libitum* and, every other day, with 300 ± 20 mg of food (*Acheta*  
46  
47 118 *domestica*).  
48  
49  
50  
51

### 52 119 *Experimental design and whole-organism assays*

53  
54  
55 120 The study was performed between June 12th and July 12th 2016. At the start of the experiment,  
56  
57 121 animals were distributed in six groups corresponding to two experimental treatments  
58  
59  
60  
61  
62  
63  
64  
65

122 (corticosterone enhancement [CORT] and placebos [CONT]) and three treatment exposure  
123 groups (3 days [3D], 11 days [11D] and 21 days [21D] of exposure to experimental treatments  
124 until the functional analyses of mitochondria). Similar sample sizes (N=8) were used in each  
125 group except in the CORT-21D group (N=9). We repeatedly measured the body mass and  
126 whole-organism metabolic expenditure 3 days before the start of the experiments, and the day  
127 before the cellular and mitochondrial measurements meaning days 2, 10 and 20 (in 3D, 11D  
128 and 21D groups respectively) after the start of the experiment. Before each whole-organism  
129 assay, the animals were left without food for 3 days to reach the post-absorptive state. After the  
130 final assay, the animals were fed and treated with corticosterone according to their treatment  
131 group. Then, the animals were euthanized to perform functional analyses of mitochondria (see  
132 below and Figure 1).

133 Whole-organism metabolic expenditure at night was quantified with closed respirometry  
134 techniques as previously described (Foucart et al., 2014). We measured oxygen consumption  
135 and carbon production overnight (approximately 20:00–08:00h) in a dark climatic chamber  
136 (AQUALYTIC® TC 135S, Dortmund, Germany). Trials were carried out at  $25\pm 1^\circ\text{C}$ , which  
137 correspond to body temperature at which functional analyses of mitochondria were performed  
138 *in vitro* (see below). The lizards were placed individually into glass jars (ca. 1,000 ml) within  
139 the chamber and allowed to acclimatize for 1h. A baseline air sample (two 140 ml syringes)  
140 was collected at the onset of the trial, and the glass vial was then carefully sealed. The trial  
141 duration was set to achieve adequate oxygen suppression based on previous studies and  
142 preliminary trials (mean=11.8 h, range=11.2-12.1h). A final sample of air was collected with  
143 two 140 ml syringes connected to a stopcock. Oxygen and CO<sub>2</sub> concentrations (% of total  
144 volume) of the air samples were determined using fuel-cell O<sub>2</sub> and infrared CO<sub>2</sub> sensors  
145 (FOXBOX, Sable Systems, Las Vegas, NV, USA) at room temperature with a constant flow (60  
146 ml.min<sup>-1</sup>) after water absorption through a column of Drierite. Oxygen consumption (VO<sub>2</sub>, in

147 ml min<sup>-1</sup>) and CO<sub>2</sub> production (VCO<sub>2</sub>) were calculated as: (final % – initial %) × exact chamber  
148 volume (ml)/trial duration (min). Body mass was recorded to the nearest mg before each trial.  
149 Respiratory quotient (RQ) was calculated like VCO<sub>2</sub>/VO<sub>2</sub> and ranged from 0.60 to 0.82 [mean  
150 = 0.72, 95% CI= 0.71-0.73]. We found no treatment effect on RQ (ANOVA, F<sub>1,47</sub>=0.65, P =  
151 0.42), but parallel, temporal changes in RQ for both control and treated lizards (F<sub>3,96</sub>=36.59, P  
152 < 0.0001). Since the theoretical RQ value for lipid substrates is 0.71, we therefore assumed that  
153 most lizards used stored lipids as the main energy source and used energy equivalents for lipids  
154 (19.8 J.mL<sup>-1</sup>) to convert VO<sub>2</sub> into standard metabolic rates (SMR) values (in J.min<sup>-1</sup>). These  
155 metabolic measurements proved to be extremely reliable and repeatable compared to our earlier  
156 studies (Foucart et al., 2014).

#### 157 ***Hormonal manipulation and plasma corticosterone assays***

158 Circulating levels of corticosterone were enhanced using a non-invasive method designed by  
159 Meylan et al. (2003). We diluted corticosterone (Sigma-Aldrich, France, C2505-500mg 92%,  
160 C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>) in commercial sesame oil according to 3µg of corticosterone per 1µL of sesame oil.  
161 Each evening between 20:00 h and 21:00 local time (when lizards are mostly inactive), 4.5 µL  
162 of corticosterone mixture (CORT) or pure sesame oil (CONT, a placebo) were topically applied  
163 on the backs of the lizards. To check effects on plasma levels of corticosterone, blood was  
164 sampled from the infraorbital sinus of the lizards 7 days before the start of the experiment and  
165 at the end of the treatment exposure using 2–3 20 µL microhematocrit tubes. In order to  
166 standardize the measurements, all samples were collected between 15:00 h and 16:00 local time  
167 within 3 min of removal of an animal from its home cage to avoid the handling-induced increase  
168 in plasma corticosterone levels (Dauphin-Villemant, 1987). Plasma was obtained by  
169 centrifugation at c.a. 5,000 g for 5 minutes of the blood samples and was stored at -40°C for  
170 subsequent measurements of plasma levels of corticosterone. Corticosterone level was  
171 measured with a competitive enzyme-immunoassay method using corticosterone EIA (IDS



172 Corticosterone EIA kit, ref AC-14F1, IDS EURL Paris, France) after 1:10 dilution of all  
173 samples following previously published guidelines (Mugabo, 2017).

#### 174 ***Respiratory capacities of muscular permeabilized fibres***

175 At the end of the experiment, animals were euthanized by decapitation. Skeletal muscles were  
176 rapidly and entirely dissected, weighed and then placed in an ice-cold isolation buffer (BIOPS  
177 containing 2.77 mM Ca-EGTA, 7.23 mM, 20 mM imidazole, 20 mM taurine, 50 mM K-MES,  
178 0.5 mM DTT, 6.56 mM MgCl<sub>2</sub>, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.2). Skeletal  
179 muscle fibres were permeabilized in BIOPS solution supplemented with saponin (50 µg ml<sup>-1</sup>)  
180 according to a standard protocol (Pesta and Gnaiger, 2012). Permeabilized fibres were weighed  
181 and their respiration were monitored with a high-resolution respirometer (Oxygraph-2k,  
182 Oroboros Instruments; Austria) in a hyper-oxygenated respiratory buffer maintained at 25 °C  
183 (110 mM sucrose, 0.5 mM EGTA, 3 mM MgCl<sub>2</sub>, 60 mM K-lactobionate, 20 mM taurine, 10  
184 mM KH<sub>2</sub>PO<sub>4</sub>, 1 g l<sup>-1</sup> fatty acid-free bovine serum albumin, 20 mM Hepes, pH 7.1). A mixture  
185 of respiratory substrates (5 mM pyruvate, 2.5 mM malate and 5 mM succinate) was added to  
186 obtain the basal respiration (state 2). Cellular ATP synthesis was initiated by the addition of 1  
187 mM ADP. The maximal fully uncoupled respiration (state 3<sub>unc</sub>) was initiated by addition of 2  
188 µM carbonyl cyanide p-tri-fluoro-methoxy-phenyl-hydrazone (FCCP), in presence of  
189 oligomycin (2 µg ml<sup>-1</sup>), an inhibitor of ATP synthase.

#### 190 ***Extraction of liver and functional analyses of mitochondria***

191 After weighing the liver, the mitochondria were isolated in an ice-cold isolation buffer (250  
192 mM sucrose, 1 mM EGTA, 20 mM Tris-HCl, pH 7.4 at 4°C) as previously described (Voituron  
193 et al., 2017). Briefly, the isolation procedure involved Potter-Elvehjem homogenization (three  
194 passages) and differential centrifugations (all steps at 4°C), with the liver mitochondria being  
195 pelleted at 9,000×g (10 min). The protein concentration of mitochondrial suspension was  
196 spectrophotometrically determined at 540 nm by a Biuret method with bovine serum albumin

197 as a standard. Because lizard mitochondrial preparations contained a dark pigment that  
198 absorbed at 540 nm, the absorbance of the same volume of mitochondria in a solution  
199 containing 0.06% Na deoxycholate, 0.6% K-Na-tartrate and 3% NaOH was subtracted.

200 Rates of oxygen consumption and ATP synthesis were measured in a closed glass cell fitted  
201 with a Clark oxygen electrode (Rank Brothers Ltd, UK) at 25°C in a respiratory buffer  
202 containing 120 mM KCl, 5 mM KH<sub>2</sub>PO<sub>4</sub>, 1 mM EGTA, 2 mM MgCl<sub>2</sub>, 0.3 % fatty acid-free  
203 bovine serum albumin, 1.6 U/ml hexokinase, 20 mM glucose, 3 mM Hepes (pH 7.4). Liver  
204 mitochondria (0.5-1.5 mg/mL) were energized with a mixture of substrates (5 mM pyruvate,  
205 2.5 mM malate and 5 mM succinate). Mitochondrial ATP synthesis was initiated by the addition  
206 of 500 μM ADP. After recording the phosphorylating respiration rate for 3 min, four 100 μL  
207 samples were withdrawn from the suspension every 1 min and were quenched in 100 μL ice-  
208 cold perchloric acid solution consisting of 10% HClO<sub>4</sub> and 25 mM EDTA. After centrifugation  
209 (15,000×g, 5 min) and neutralization of the resulting supernatant with a KOH solution (2 M  
210 KOH, 0.3 M MOPS), ATP production was determined from the slope of the linear accumulation  
211 of glucose-6-phosphate content of samples (Voituron et al., 2017). To make sure that the rates  
212 we measured were specific of the mitochondrial ATP synthase activity and not associated with  
213 contaminated ATPase activity such as adenylate kinase, we determined oxygen consumption  
214 and ATP synthesis rates in the presence of oligomycin (2 μg/mL). These values were taken into  
215 account to calculate the rate of mitochondrial ATP synthesis that is associated with  
216 mitochondrial ATP synthase activity and oxygen consumption.

### 217 **Mitochondrial reactive oxygen species production**

218 The rate of H<sub>2</sub>O<sub>2</sub> released by isolated liver mitochondria was measured at 25°C in a respiratory  
219 buffer supplemented with 5 U/mL horseradish peroxidase and 1 μM Amplex red fluorescent  
220 dye using a fluorescence spectrophotometer (SFM-25, Kontron Instrument) at excitation and  
221 emission wavelengths of 560 nm and 584 nm, respectively. The fluorescent signal was

222 calibrated using a standard curve obtained after successive addition of H<sub>2</sub>O<sub>2</sub> (up to 35 pmoles).  
1  
2 223 Amounts of H<sub>2</sub>O<sub>2</sub> release were corrected from background rate of product formation in the  
3  
4 224 absence of exogenous substrate as described previously (Voituron et al., 2017). Free radical  
5  
6  
7 225 electron leak (ROS/O ratio) was calculated as the fraction (%) of basal non-phosphorylating  
8  
9  
10 226 oxygen consumption that is reduced into H<sub>2</sub>O<sub>2</sub> at the respiratory chain instead of H<sub>2</sub>O at the  
11  
12 227 cytochrome-c oxidase. Similarly, the oxidative cost of mitochondrial ATP synthesis was  
13  
14 228 calculated from the ratio of H<sub>2</sub>O<sub>2</sub> generation under phosphorylating state divided by the  
15  
16  
17 229 corresponding rate of ATP synthesis (ROS/ATP ratio).  
18

### 19 230 **Statistical analyses**

20  
21  
22 231 All statistical analyses were performed with linear models in R 3.3.2 (R Development Core  
23  
24 232 Team, 2015). We first checked the effects of treatment groups on change in plasma  
25  
26  
27 233 corticosterone levels from before the end of the experiment with a linear model fitted with the  
28  
29 234 *lm* package (Venables and Ripley, 2002). We included initial plasma corticosterone level and  
30  
31 235 body mass as covariates in this model. Next, repeated measurements (body mass and SMR)  
32  
33  
34 236 were analyzed with mixed-effects linear models in the *lme* package. Dependence among  
35  
36 237 repeated measurements were accounted using random intercept model where the intercept of  
37  
38  
39 238 the model is allowed to vary randomly among individuals. A compound symmetry covariance  
40  
41 239 structure that includes within-subject correlated errors was used and the random term was  
42  
43  
44 240 always kept in the models. This model assumes that individuals react in the same way to  
45  
46 241 treatments and provides information about intra-class correlations and therefore consistency of  
47  
48  
49 242 inter-individual differences. In addition, all models included fixed effects of experimental  
50  
51 243 treatments, treatment exposure groups (categorical variable) and their interactions. Since SMR  
52  
53 244 increases exponentially with body mass, we log transformed SMR prior to the analyses and  
54  
55  
56 245 included log-transformed body mass as a covariate in the analyses. The normality and  
57  
58 246 homogeneity of variance of residuals was systematically checked in the full models and was  
59  
60  
61  
62  
63  
64  
65

247 found to be satisfactory. In a third set of analyses, we compared final measurements of liver  
248 mass and mitochondria functioning across treatment groups with a linear model including fixed  
249 effects of experimental treatments, treatment exposure groups and their interactions. In all  
250 models, the significance of fixed effects was tested with type III F statistics using the *Anova*  
251 procedure in the *car* package. We removed non-significant variables ( $\alpha=0.05$ ) one by one using  
252 a backward elimination procedure. Whenever significant differences were found among  
253 treatment groups, we used Tukey's procedure to conduct post hoc tests (pairwise comparisons  
254 between the experimental groups) with the *lsmeans* package (Lenth, 2016). Results are  
255 presented as mean  $\pm$  standard error unless otherwise stated.

## 257 **Results**

### 258 **Corticosterone supplementation increases body and liver masses**

259 Before the experiment, animals had similar SVL, body mass and body condition, SMR and  
260 initial plasma corticosterone levels among treatment groups (ANOVA, all  $P > 0.66$ ). Analysis  
261 of intra-individual change in plasma corticosterone levels confirmed that treated groups  
262 exhibited higher concentration over the entire experiment (treatment:  $F_{2,44}=71.6$ ,  $P < 0.0001$ ;  
263 exposure group:  $F_{2,44}=6.49$ ,  $P = 0.003$ ). On average, plasma corticosterone levels were 154.8  
264  $\text{ng.mL}^{-1}$  ( $\pm 18.3$ ) higher in treated than in control lizards, similar to previous effects reported  
265 with the same protocol (Voituron et al. 2017). In addition, treated lizards exhibited an increase  
266 of body mass over time (+2% at 3D, +5% at 11D and +17% at 21D), whereas the body mass of  
267 control lizards did not change significantly over time (treatment  $\times$  exposure group:  $F_{3,94}=18.13$ ,  
268  $P < 0.0001$ ) (see Table 1). Total liver mass (treatment  $\times$  exposure group:  $F_{2,43}=8.24$ ,  $P = 0.0009$ )  
269 and relative liver mass (g of liver per 100 g body mass; liver mass controlled for variation in  
270 body mass; treatment  $\times$  exposure group:  $F_{2,42}=8.33$ ,  $P = 0.0009$ ) differed also between

271 treatments after 11 days of exposure only. There was a significant increase of liver mass at D11  
272 and D21 in treated lizards relative to controls (Table 1).

### 273 **Corticosterone supplementation and whole-organism oxygen consumption**

274 Overall, SMR remained relatively similar between treatments apart for a slight, close to  
275 significant decrease in SMR from treated lizards relative to controls at D21 (Table 1; treatment  
276  $\times$  exposure group:  $F_{3,92}=2.69$ ,  $P = 0.05$ ; Student's  $t$  test at D21:  $P = 0.07$ ). However, when  
277 controlling for the positive relationship between individual body mass and individual SMR  
278 (log-log slope= $0.67 \pm 0.13$ ;  $F_{1,90}=25.78$ ,  $P < 0.0001$ ), the best fit model indicated that mass-  
279 corrected SMR differences over time between treatments ranged from a small positive  
280 difference for CORT at D3, followed by a return to normal at D11, and a significant decrease  
281 in mass-corrected SMR from treated lizards at D21 (treatment  $\times$  exposure group:  $F_{3,90}=5.01$ ,  
282  $P=0.003$ ). These results can be interpreted as evidence that corticosterone treated lizards  
283 maintained relatively similar whole-organism SMR despite their increase in body mass during  
284 the 21-day long exposure period, thus had lower mass-corrected SMR through time.

### 285 **Corticosterone supplementation and liver mitochondrial functions**

286 The treatment affected mitochondrial activities with contrasted effects between the rates of ATP  
287 synthesis and oxygen consumption over the course of the study (Table 1 and Figure 2, see Table  
288 S1 for statistical details). The rate of ATP synthesis was significantly lower in the corticosterone  
289 treatment irrespective of treatment exposure. In contrast, both basal and phosphorylating rates  
290 of oxygen consumption did not change after 3 days of treatment, then were significantly  
291 decreased in 11-day treated lizards compared with control animals, and eventually returned to  
292 same levels than in controls at day 21 (Table 1). Consequently, mean values of the mitochondrial  
293 coupling efficiency (ATP/O ratio) were significantly decreased after 3 days of corticosterone  
294 treatment, but were not significantly different from control values after 11 days and 21 days of  
295 treatment (treatment  $\times$  exposure group:  $F_{2,41}=4.14$ ,  $P = 0.02$ , see Figure 2).

296 The rates of mitochondrial reactive oxygen species production under both basal non-  
297 phosphorylating and active phosphorylating states were not significantly altered after 3 days of  
298 glucocorticoid treatment but exhibited between 40% to 50% decrease in treated lizards relative  
299 to controls after 11 and 21 days (Table 1, see Table S1 for statistical details). Overall, the  
300 electron leak defined by the ROS/O ratio was not significantly changed by the glucocorticoid  
301 treatment neither in basal non-phosphorylating state (treatment  $\times$  exposure group:  $F_{2,41}=2.09$ ,  $P$   
302 = 0.14; treatment:  $F_{1,43}=0.13$ ,  $P = 0.82$ ; exposure group:  $F_{2,44}=10.21$ ,  $P = 0.0002$ ) nor in active  
303 phosphorylating state (treatment  $\times$  exposure group:  $F_{2,41}=1.81$ ,  $P = 0.18$ ; treatment:  $F_{1,43}=0.89$ ,  
304  $P = 0.35$ ; exposure group:  $F_{2,44}=2.26$ ,  $P = 0.12$ ). In contrast, the oxidative cost of ATP synthesis  
305 (ROS/ATP ratio) was marginally affected by hormonal treatment, being higher in the 3-day  
306 treated group than in the control group, but returning to the level of control groups after 11 days  
307 and 21 days of treatment (treatment  $\times$  exposure group:  $F_{2,41}=3.02$ ,  $P = 0.06$ ; see Figure 3).

### 308 **Corticosterone supplementation and muscle mitochondria functioning**

309 Whatever the state of activation (basal non-phosphorylating, phosphorylating and maximal),  
310 cellular oxygen consumption of lizard muscle was not different between control and treated  
311 individuals neither at 3 days, 11 days nor at 21 days of treatment (Table 1 and Table S1).

## 313 **Discussion**

314 This study aimed at unraveling the time-dependent mechanisms linking corticosterone and  
315 metabolism at different levels of organization (whole-organism, cellular and mitochondrial) in  
316 an ectothermic organism. The data presented here provide first clear evidence of GC-dependent  
317 regulation of the liver mitochondrial functionality in a time-dependent manner leading to a  
318 “functional resilience” after 21 days of chronic corticosterone increase. Indeed, short-term GC  
319 supplementation induced a significant decrease of mitochondrial ATP synthesis without change  
320 of oxygen consumption and  $H_2O_2$  release, thus causing a lower efficiency (ATP/O) and a higher

1 321 oxidative cost of an ATP molecule production (ROS/ATP). In contrast, longer-term GC  
2 322 supplementation induced a significant decrease of mitochondrial H<sub>2</sub>O<sub>2</sub> release with  
3  
4 323 mitochondrial oxidative phosphorylation characteristics to returning to the same level than  
5  
6  
7 324 controls. In an important synthesis, Mc Ewen and Wingfiels (2003) defined the allostatic state  
8  
9 325 as the “*altered and sustained activity levels of the primary mediators, e.g., glucocorticosteroids,*  
10  
11 326 *that integrate physiology and associated behaviors in response to changing environments and*  
12  
13  
14 327 *challenges such as social interactions, weather, disease, predators, pollution, etc*”. The present  
15  
16 328 data show that liver mitochondria decrease first their efficiency and then their fluxes (both in  
17  
18  
19 329 terms of O<sub>2</sub> and ATP) but restore their functional capacity in response to the energetic  
20  
21  
22 330 dysfunction imposed by the endocrine variations. In addition, the data also suggest a tissue  
23  
24 331 dependent response, with corticosterone treatment affecting functioning of mitochondria in the  
25  
26 332 liver but not that of skeletal muscles. Interestingly, changes in metabolism of mitochondria *in*  
27  
28  
29 333 *vitro* were not reflected by variation in the whole-organism metabolism *in vivo*.

30  
31 334         The present data expand results of a previous study on this species (Voituron et al. 2017)  
32  
33 335 providing the time dynamics of functional regulation of the mitochondrial oxygen consumption,  
34  
35  
36 336 ATP and H<sub>2</sub>O<sub>2</sub> productions under GC secretion. In response to the glucocorticoid  
37  
38  
39 337 supplementation, the liver mitochondria first lowered its ATP synthesis rate without changing  
40  
41 338 its oxygen consumption (see Figure 2). Even if mechanisms underpinning this lower efficiency,  
42  
43  
44 339 the cellular ATP/AMP ratio being modified, a cascade of genes upregulation required for  
45  
46 340 mitochondrial respiratory chain expression and function is triggered (Teperino et al. 2010;  
47  
48  
49 341 Mouchiroud et al. 2014). At 11 days of treatment, the mitochondrial pattern is characterized by  
50  
51 342 low values of all fluxes reflecting a diminished activity of the respiratory chain. Interestingly,  
52  
53 343 this mitochondrial hypometabolism restored both the efficiency (ATP/O) and oxidative cost of  
54  
55  
56 344 an ATP molecule production (ROS/ATP). Altogether, these elements are in line with scenarios  
57  
58 345 of downregulation of the respiratory chain subunits (Pandya et al., 2004) and of the activity of  
59  
60  
61  
62  
63  
64  
65

346 substrate oxidation reported after glucocorticoids treatment in rodents (Roussel et al., 2004;  
1  
2 347 Arvier et al., 2007). The nearly total recovering of both oxygen and ATP after 21 days of  
3  
4 348 treatment associated with lower ROS release might be ascribed to *de novo* mitogenesis  
5  
6  
7 349 (Jornayvaz and Shulman 2010). Indeed, glucocorticoids can induce higher expression of key  
8  
9  
10 350 nuclear genes that are required to produce new mitochondria (Psarra and Sekeris 2011). Such a  
11  
12 351 functional resilience in terms of ATP production associated with lower H<sub>2</sub>O<sub>2</sub> production  
13  
14 352 corroborates previous results on male common lizards that showed lower superoxide dismutase  
15  
16  
17 353 activity in corticosterone-treated individuals after 21 days (Cote et al. 2010). These results are  
18  
19 354 however not congruent with data on endotherms in which chronically elevated GC levels  
20  
21  
22 355 accelerate aging and reduce lifespan (Schoenle et al. 2018), thus imposing fitness costs  
23  
24 356 potentially through oxidative balance (Costantini 2011).

25  
26 357 All these mitochondrial adjustments occurred without modification of whole-organism  
27  
28  
29 358 metabolism (present study; Voituron et al. 2017). Even if only two tissues were tested in the  
30  
31  
32 359 study, the data thus strongly suggest that whole-organism oxygen consumption cannot be used  
33  
34 360 as an accurate proxy for neither ATP production nor ROS release by tissue-specific  
35  
36 361 mitochondria (Salin et al. 2015). However, the GC effect on whole-organism metabolism, an  
37  
38  
39 362 important parameter of the total energy budget, still remains an open question since it has been  
40  
41 363 demonstrated that chronic increase of corticosterone slightly increases metabolic rates at rest in  
42  
43  
44 364 pregnant female common lizards (Meylan et al. 2010) when other lizard species reduce their  
45  
46 365 total oxygen consumption rate when exposed to an increase in corticosterone (Miles et al. 2007;  
47  
48  
49 366 Durant et al. 2008). Nevertheless, the functional mitochondrial resilience observed *in vivo*  
50  
51 367 might constitute a proximal explanation of the increase in survival of male common lizards with  
52  
53 368 corticosterone enhancement (Meylan and Clobert 2005; Cote et al. 2006).

54  
55  
56 369 Even if oxygen consumption between tissues has been assessed at different levels of  
57  
58 370 organization, the data suggests that muscles and liver showed differential response patterns.  
59  
60  
61  
62  
63  
64  
65



1 371 This statement would be wrong only if mitochondrial content in muscles strongly increase  
2 372 during acute and mild-time stress that has never been reported and not congruent with  
3  
4 373 mitogenesis dynamic (Jornayvaz and Shulman 2010). The data available on mammals are  
5  
6  
7 374 puzzling with no muscular effect in rodents under dexamethasone (Dumas et al. 2003, Roussel  
8  
9 375 et al. 2004) or muscle wasting mediated, in part, by GR-dependent transactivation of genes that  
10  
11 376 drive myocyte atrophy (Patel et al. 2014). In addition, the muscular impact of GC is dose-  
12  
13  
14 377 dependent with moderate or transient exposure to GCs enhancing muscle performance (Caruso  
15  
16 378 et al. 2014). Even if muscles of common lizards do not exhibit a mitochondrial response,  
17  
18  
19 379 muscles could be involved as amino-acids source for liver neoglucogenesis under  
20  
21  
22 380 corticosterone.

23  
24 381

## 25 26 382 **Conclusions and perspectives**

27  
28  
29 383 This study demonstrates that liver mitochondrial energetics of ectotherms is directly influenced  
30  
31 384 in a time-dependent manner by exposure to higher plasma concentrations of circulating GCs.  
32  
33  
34 385 After chronic exposure to GCs, the liver mitochondria reached a functional resilience with a  
35  
36 386 complex interplay between O<sub>2</sub> consumption, ATP production and H<sub>2</sub>O<sub>2</sub> release. The coupling  
37  
38  
39 387 between these three mitochondrial processes is thus plastic and differentially regulated leading  
40  
41 388 to a critical period when stress is acute (low ATP production with high H<sub>2</sub>O<sub>2</sub> release), an  
42  
43  
44 389 intermediate period when efficiency returns to the initial levels but with low respiratory chain  
45  
46 390 activity, and finally a complete “recovery” to the initial fluxes and ratios. This time-dependent  
47  
48  
49 391 relationship between GC and mitochondria in ectotherms needs to be taken into account to  
50  
51 392 better understand mechanisms that ensure and drive the flow of energy during  
52  
53 393 physiopathological responses (e.g., Rohleder 2012) but also towards adaptive processes.  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

394

1

2

## 3 395 **Acknowledgements**

4

5 396 We are thankful to field assistants and technical staff for their support, especially Beatriz

6

7 397 Decencière, Simon Agostini, Victorien, Virginie and Amandine. We thank Olivier Lourdais for

8

9 398 renting the respirometry equipment.

10

11

12

13 399

14

## 15 400 **Declarations**

16

17

## 18 401 **Funding**

19

20 402 This study was funded by the Centre National de la Recherche Scientifique (CNRS), the Agence

21

22 403 Nationale de la Recherche (ANR-13-JSV7-0011-01 to S.M.) and the Région Île-de-France

23

24 404 R2DS program (grant 2013-08 to S.M., J.F.L.G. and R.J.). This work has also benefited from

25

26 405 technical and human resources provided by CEREEP-Ecotron IleDeFrance (CNRS/ENS UMS

27

28 406 3194), which is supported by Regional Council of Ile-de-France under the DIM Program R2DS

29

30 407 bearing the reference I-05-098/R and the program " Investissements d'Avenir" launched by the

31

32 408 French government and implemented by ANR with the reference ANR-11-INBS-0001 AnaEE

33

34 409 France.

35

## 36 410 **Conflict of interest/Competing interests**

37

38 411 There is no conflict of interest to declare

39

## 40 412 **Availability of data and material**

41

42 413 The datasets generated during the current study are available from the corresponding author on

43

44 414 reasonable request.

45

## 46 415 **Author contributions**

47

48 416 Conceptualization: Y.V., S.M., D.R., J.F.L.G., C.R.; Methodology: D.R., Y.V., S.M., C.R.,

49

50 417 J.F.L.G.; Validation: D.R., Y.V.; Formal analysis: D.R., J.F.L.G., A.D., C.R., Y.V.; Investigation:

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

1  
2 418 D.R., J.F.L.G., S.M., A.D., C.R., Y.V.; Writing - original draft: Y.V., D.R.; Writing - review &  
3 editing: Y.V., S.M., D.R., J.F.L.G., A.D.

4  
5 420 **Ethics approval**

6  
7 421 The present investigation was carried out according to the ethical principles of the Préfecture  
8  
9 422 de Seine-et-Marne under agreement A77-341-1.

10  
11  
12 423  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

424 **Bibliography**

- 1  
2  
3 425 Arvier M, Lagoutte L, Johnson G, Dumas JF, Sion B, Grizard G, Malthiery Y, Simard G, Ritz  
4  
5 426 P (2007) Adenine nucleotide translocator promotes oxidative phosphorylation and mild  
6  
7 427 uncoupling in mitochondria after dexamethasone treatment. *Am J Physiol Endocrinol*  
8  
9  
10 428 *Metab* 293: E1320-1324.
- 11  
12 429 Casuso RA, Melskens L, Bruhn T, Secher NH, Nordsborg NB (2014) Glucocorticoids  
13  
14  
15 430 improve high-intensity exercise performance in humans. *Eur J Appl Physiol* 114: 419–  
16  
17 431 424.
- 18  
19 432 Costantini D, Marasco V, Møller AP (2011) A meta-analysis of glucocorticoids as modulators  
20  
21  
22 433 of oxidative stress in vertebrates. *J Comp Physiol B* 181:447-56. doi: 10.1007/s00360-  
23  
24 434 011-0566-2.
- 25  
26  
27 435 Cote J, Clobert J, Meylan, S, Fitze PS (2006) Experimental enhancement of corticosterone  
28  
29 436 levels positively affects subsequent male survival. *Horm Behav* 49: 320-327.  
30  
31  
32 437 doi:10.1016/j.yhbeh.2005.08.004.
- 33  
34 438 Cote J, Meylan S, Clobert, J, Voituron Y (2010) Carotenoid-based coloration, oxidative stress  
35  
36  
37 439 and corticosterone in common lizards. *J Exp Biol* 213, 2116-2124.  
38  
39 440 doi:10.1242/jeb.040220.
- 40  
41  
42 441 de Kloet ER (2013) Functional profile of the binary brain corticosteroid receptor system:  
43  
44 442 mediating, multitasking, coordinating, integrating. *Eur J Pharmacol* 719:53–62.
- 45  
46 443 Dhabhar FS, McEwen BS (1997) Acute stress enhances while chronic stress suppresses cell-  
47  
48  
49 444 mediated immunity in vivo: A potential role for leukocyte trafficking. *Brain Behav*  
50  
51 445 *Immun* 65:360-368.
- 52  
53  
54 446 Du J, Wang Y, Hunter R, Wei Y, Blumenthal R, Falke C, Khairova R, Zhou R, Yuan P,  
55  
56 447 Machado-Vieira R, McEwen BS, Manji HK (2009) Dynamic regulation of  
57  
58  
59 448 mitochondrial function by glucocorticoids. *Proc Natl Acad Sci USA* 106: 3543-3548.

449 Dumas JF, Simard G, Roussel D, Douay O, Foussard F, Malthiery Y, Ritz P (2003)  
1  
2 450 Mitochondrial energy metabolism in a model of undernutrition induced by  
3  
4 451 dexamethasone. *Brit J Nutri* 90: 969-977.  
5  
6  
7 452 Durant SE, Romero LM, Talent LG, Hopkins, WA (2008) Effect of exogenous corticosterone  
8  
9 453 on respiration in a reptile. *Gen Comp Endo* 156:126-133.  
10  
11 454 doi:10.1016/j.ygcen.2007.12.004  
12  
13  
14 455 Eisner V, Picard M, Hajnóczky G (2018) Mitochondrial dynamics in adaptive and  
15  
16 456 maladaptive cellular stress responses. *Nat Cell Biol* 20:755–765.  
17  
18 457 doi.org/10.1038/s41556-018-0133-0  
19  
20  
21 458 Foucart T, Lourdais O, DeNardo DF, Heulin B (2014) Influence of reproductive mode on  
22  
23 459 metabolic costs of reproduction: insight from the bimodal lizard *Zootoca vivipara*. *J Exp*  
24  
25 460 *Biol* 217:4049-56. 2014. doi: 10.1242/jeb.104315  
26  
27  
28  
29 461 Harris RBS (2015) Chronic and acute effects of stress on energy balance: are there  
30  
31 462 appropriate animal models? *Am J Physiol – Regul Integ Comp Physiol* 308: R250-  
32  
33 463 R265.  
34  
35  
36 464 Hunter RG, Seligsohn M, Rubin TG, Griffiths BB, Ozdemir Y, Pfaff DW, Datson NA, McEwen  
37  
38 465 BS (2016) Stress and corticosteroids regulate rat hippocampal mitochondrial DNA gene  
39  
40 466 expression via the glucocorticoid receptor. *Proc Natl Acad Sci USA*. 113: 9099-9104. doi:  
41  
42 467 10.1073/pnas.1602185113.  
43  
44  
45  
46 468 Jani MS, Telang SD, Katyare SS (1991) Effect of corticosterone treatment on energy  
47  
48 469 metabolism in rat liver mitochondria. *J Steroid Biochem Mol Biol* 38:587-591.  
49  
50  
51 470 Jessop TS, Woodford R, Symonds MRE (2013) Macrostress: do large-scale ecological  
52  
53 471 patterns exist in the glucocorticoid stress response of vertebrates? *Funct Ecol* 27:120–  
54  
55 472 130. 2013doi: 10.1111/j.1365-2435.2012.02057.x  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1 473 Joels M, De Kloet ER (1992) Coordinative mineralocorticoid and glucocorticoid receptor-  
2 474 mediated control of responses to serotonin in rat hippocampus. *Neuroendocrinology*  
3  
4 475 55:344–350.  
5  
6  
7 476 Jornayvaz FR, Shulman GI (2010) Regulation of mitochondrial biogenesis. *Essays Biochem*  
8  
9 477 47:69-84. doi: 10.1042/bse0470069  
10  
11  
12 478 Lee SR, Kim HK, Song IS, Youm J, Dizon LA, Jeong SH, Ko TH, Heo HJ, Ko KS, Rhee BD,  
13  
14 479 Kim N, Han J (2013) Glucocorticoids and their receptors: insights into specific roles in  
15  
16 480 mitochondria. *Prog Biophys Mol Biol* 112:44-54.  
17  
18  
19 481 Lehninger AL, Nelson DL, Cox MM (1993) *Principles of Biochemistry*, 2nd edn. New York,  
20  
21 482 USA: Worth Publishers.  
22  
23  
24 483 Lenth RV (2016) Least-Squares Means: The R Package lsmeans. *J Stat Soft* 69:1-33. doi:  
25  
26 484 10.18637/jss.v069.i01  
27  
28  
29 485 McEwen BS, Wingfield JC (2003) The concept of allostasis in biology and biomedicine.  
30  
31 486 *Horm Behav* 43: 2–15.  
32  
33  
34 487 Manoli I, Alesci S, Blackman MR, Su YA, Rennert OM, Chrousos GP (2007) Mitochondria as  
35  
36 488 key components of the stress response. *Trends Endo Metab* 18:190-198.  
37  
38 489 doi:10.1016/j.tem.2007.04.004  
39  
40  
41 490 Meylan S, Clobert J (2005) Is corticosterone-mediated phenotype development adaptive?  
42  
43 491 Maternal corticosterone treatment enhances survival in male lizards. *Horm Behav*  
44  
45 492 48:44-52. doi:10.1016/j.yhbeh.2004.11.022.  
46  
47  
48 493 Meylan S, Dufty AM, Clobert J (2003) The effect of transdermal corticosterone application on  
49  
50 494 plasma corticosterone levels in pregnant *Lacerta vivipara*. *Comp Biochem Physiol A*  
51  
52 495 13:497-503.  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

496 Meylan S, Haussy C, Voituron Y (2010) Physiological actions of corticosterone and its  
1  
2 497 modulation by an immune challenge in reptiles. *Gen Comp Endo* 169:158-166.  
3  
4 498 doi:10.1016/j.ygcen.2010.08.002.  
5  
6  
7 499 Miles DB, Calsbeek R, Sinervo B (2007) Corticosterone, locomotor performance, and  
8  
9 500 metabolism in side-blotched lizards (*Uta stansburiana*). *Horm Behav* 51: 548-554.  
10  
11 501 doi:10.1016/j.yhbeh.2007.02.005.  
12  
13  
14 502 Mouchiroud L, Eichner LJ, Shaw RJ, Auwerx J (2014) Transcriptional Coregulators: Fine-  
15  
16 503 Tuning Metabolism. *Cell Metab* 20:26-40.  
17  
18 504 doi:<https://doi.org/10.1016/j.cmet.2014.03.027>  
19  
20  
21 505 Mugabo M, Le Galliard JF, Perret S, Decencière B, Haussy C, Meylan S (2017) Sex-  
22  
23 506 specific density- dependent secretion of glucocorticoids in lizards: insights from  
24  
25 507 laboratory and field experiments. *Oikos* 126:1051-1061.  
26  
27 508 <https://doi.org/10.1111/oik.03701>  
28  
29  
30  
31 509 Pandya JD, Agarwal NA, Katyare SS (2004) Effect of dexamethasone treatment on oxidative  
32  
33 510 energy metabolism in rat liver mitochondria during postnatal developmental periods.  
34  
35 511 *Drug Chem Toxicol* 27:389-403.  
36  
37  
38 512 Patel, R., Williams-Dautovich, J. and Cummins, C. L. (2014). New molecular mediators of  
39  
40 513 glucocorticoid receptor activity in metabolic tissues. *Mol Endocrinol* 28, 999–1011.  
41  
42  
43 514 Pesta D, Gnaiger E (2012) High-resolution respirometry: OXPHOS protocols for human cells  
44  
45 515 and permeabilized fibers from small biopsies of human muscle. *Methods Mol Biol*  
46  
47 516 810:25-58. doi: 10.1007/978-1-61779-382-0\_3.  
48  
49  
50  
51 517 Picard M, Juster RP, McEwen BS (2014) Mitochondrial allostatic load puts the 'gluc' back in  
52  
53 518 glucocorticoids. *Nat Rev Endocrinol* 10: 03-310.  
54  
55  
56 519 Picard M, McEwen BS, Epel ES, Sandi C (2018) An energetic view of stress: Focus on  
57  
58 520 mitochondria. *Front Neuroendocrinol* 49:72–85.  
59  
60  
61  
62  
63  
64  
65

- 521 Picard M, McEwen BS (2018) Psychological Stress and Mitochondria: A Systematic Rev  
1  
2 522 Psychosom Med 80:141-153. doi: 10.1097/PSY.0000000000000545.  
3  
4 523 Psarra AM, Sekeris CE (2011) Glucocorticoids induce mitochondrial gene transcription in  
5  
6  
7 524 HepG2 cells: role of the mitochondrial glucocorticoid receptor. Bioch Biophys Acta  
8  
9  
10 525 1813:1814-1821. doi:10.1016/j.bbamcr.2011.05.014.  
11  
12 526 Rohleder N (2012) Acute and chronic stress induced changes in sensitivity of peripheral  
13  
14 527 inflammatory pathways to the signals of multiple stress systems.  
15  
16 528 Psychoneuroendocrinol 37:307-316.  
17  
18  
19 529 Rolfe DFS, Brown GC (1997) Cellular energy utilization and molecular origin of standard  
20  
21  
22 530 metabolic rate in mammals. Physiol Rev 77:731-58. doi:10.1152/physrev.1997.77.3.731  
23  
24 531 Roussel, D., Dumas, J. F., Simard, G., Malthiery, Y. and Ritz, P. (2004). Kinetics and control  
25  
26 532 of oxidative phosphorylation in rat liver mitochondria after dexamethasone treatment.  
27  
28  
29 533 Biochem. J. 382, 491-499.  
30  
31 534 Salin K, Luquet E, Rey B, Roussel D, Voituron Y (2012) Alteration of mitochondrial  
32  
33  
34 535 efficiency affects oxidative balance, development and growth in frog (*Rana temporaria*)  
35  
36 536 tadpoles. J Exp Biol 215:863-869.  
37  
38  
39 537 Salin K, Auer SK, Rey B, Selman C, Metcalfe NB (2015) Variation in the link between  
40  
41 538 oxygen consumption and ATP production, and its relevance for animal performance.  
42  
43 539 Proc Biol Sci 282:20151028. doi:10.1098/rspb.2015.1028.  
44  
45  
46 540 Sapolsky RM, Romero LM, Munck AU (2000) How do glucocorticoids influence stress  
47  
48 541 responses? Integrating permissive, suppressive, stimulatory, and preparative actions.  
49  
50  
51 542 Endo Rev 1:55–89. doi:10.1210/er.21.1.55  
52  
53 543 Schoenle LA, Zimmer C, Vitousek MN (2018) Understanding context dependence in  
54  
55 544 glucocorticoid-fitness relationships: the role of the nature of the challenge, the intensity  
56  
57  
58 545 and frequency of stressors, and life history. Int Comp Biol 58:777–789.  
59  
60  
61  
62  
63  
64  
65



- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
- 546 Sorrells SF, Caso JR, Munhoz CD, Sapolsky RM (2009) The stressed CNS: when  
547 glucocorticoids aggravate inflammation. *Neuron* 64:33-39. doi:  
548 10.1016/j.neuron.2009.09.032.
- 549 Starkov AA (2008) The role of mitochondria in reactive oxygen species metabolism and  
550 signaling. *Ann N Y Acad Sci* 1147:37-52. doi: 10.1196/annals.1427.015.
- 551 Strack AM, Bradbury MJ, Dallman MF (1995) Corticosterone decreases nonshivering  
552 thermogenesis and increases lipid storage in brown adipose tissue. *Am J Physiol*  
553 268:R183-191.
- 554 Team RDC (2008) R: A language and environment for statistical computing. R Foundation for  
555 Statistical Computing, Vienna, Austria.
- 556 Teperino R, Schoonjans K, Auwerx J (2010) Histone methyl transferases and demethylases;  
557 can they link metabolism and transcription? *Cell Metab* 12:321-327. doi:  
558 10.1016/j.cmet.2010.09.004.
- 559 Venables WN, Ripley BD (2002) *Modern Applied Statistics with S*, Fourth edition. Springer,  
560 New York. ISBN 0-387-95457-0
- 561 Vitousek MN, Johnson MA, Downs CJ, Miller ET, Martin LB, Francis CD, Donald JW,  
562 Fuxjager MJ, Goymann W, Hau M, Husak JF, Kircher BK, Knapp R, Schoenle LA,  
563 Williams TD (2019) Macroevolutionary Patterning in Glucocorticoids Suggests  
564 Different Selective Pressures Shape Baseline and Stress-Induced Levels. *Am Nat*  
565 193:866-880. doi: 10.1086/703112.
- 566 Voituron Y, Josserand R, Le Galliard JF, Haussy C, Roussel D, Romestaing C, Meylan S  
567 (2017) Chronic stress, energy transduction and free radical production in a reptile.  
568 *Oecologia* 185:195-203.
- 569 Wang XL, Herzog B, Waltner-Law M, Hall RK, Shiota M, Granner, DK (2004) The  
570 synergistic effect of dexamethasone and all-trans-retinoic acid on hepatic

571 phosphoenolpyruvate carboxykinase gene expression involves the coactivator p300. J

1

2 572

3

Biol Chem 279:34191–34200.

4

5 573

6

7 574

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

575 **Figure 1:** Experimental design

576

577 **Figure 2:** Effect of 3 days, 11 days and 21 days of exogenous treatment with corticosterone

578 (CORT: 3µg of corticosterone per µL of sesame oil) on liver metabolism mitochondria from

579 *Zootoca vivipara*. (A) Mitochondrial oxygen consumption and ATP production; the values are

580 represented as % of control values ( $\pm$ SEM represented by the horizontal gray-shaded

581 rectangle). (B) Mitochondrial efficiency (ATP/O) in function of treatment time. Circles

582 represent individuals' data and squares represent means for each group. \* and † Significantly

583 different from the corresponding control.

584

585 **Figure 3:** Effect of exogenous treatment of corticosterone (CORT: 3µg of corticosterone per

586 µL of sesame oil; control: only sesame oil (white circles and square)) during 3 days (black

587 circles and square), 11 days (black circles and square) and 21 days (black circles and square)

588 in the oxidative cost of an ATP production. Circles represent individuals' data and squares

589 represent means for each group. Values are means  $\pm$  s.e.m. for 8-9 animals.

590

591 **Table 1.** Effects of exogenous treatment of corticosterone (CORT: 3µg of corticosterone per µL

592 of sesame oil; CONT: only sesame oil) during 3 days, 11 days and 21 days in *Zootoca vivipara*.

593 The metabolic traits studied include whole-organism standard metabolic rates (SMR), muscle

594 fibers and mitochondrial liver oxygen consumptions, liver mitochondrial ATP and ROS

595 production. Data are reported as mean  $\pm$  SE ( $n = 8-9$  per group). Significant difference among

596 groups from Tukey post-hoc tests of the best model (see main text) are specified with different

597 letters.

598

Figure 1

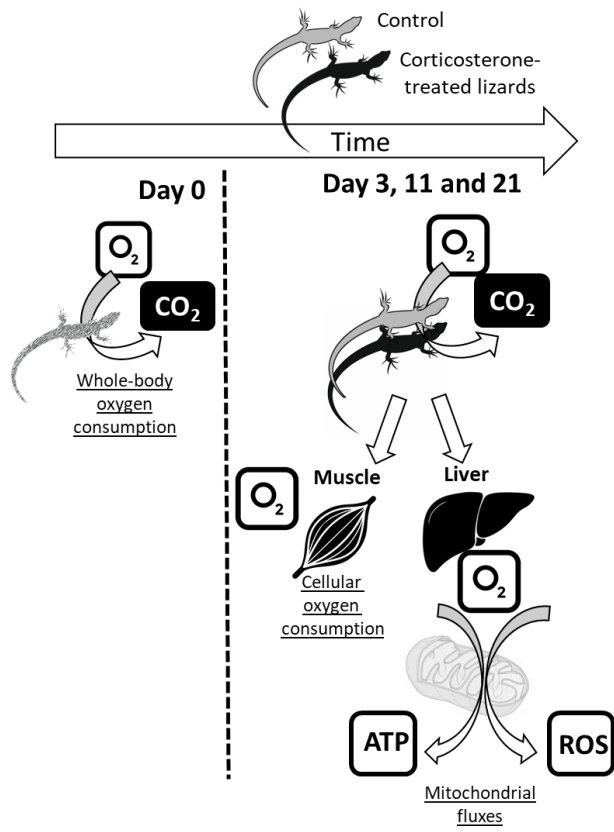


Figure 2

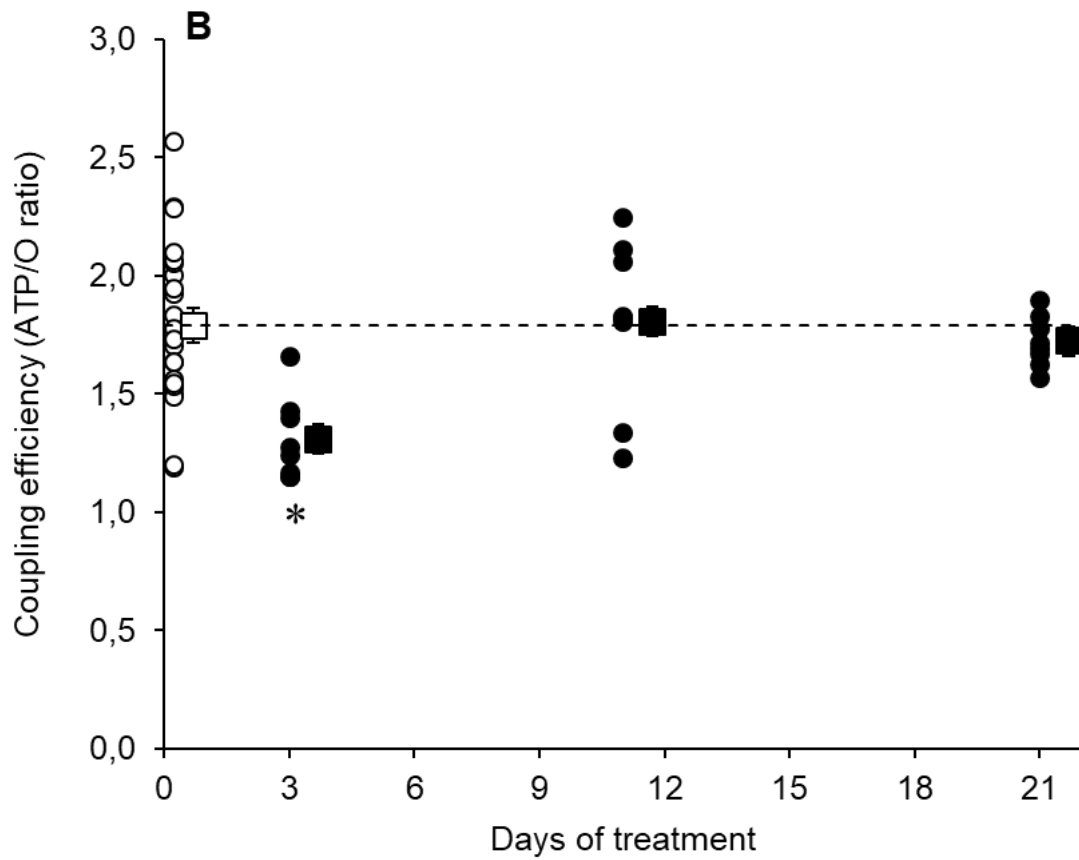
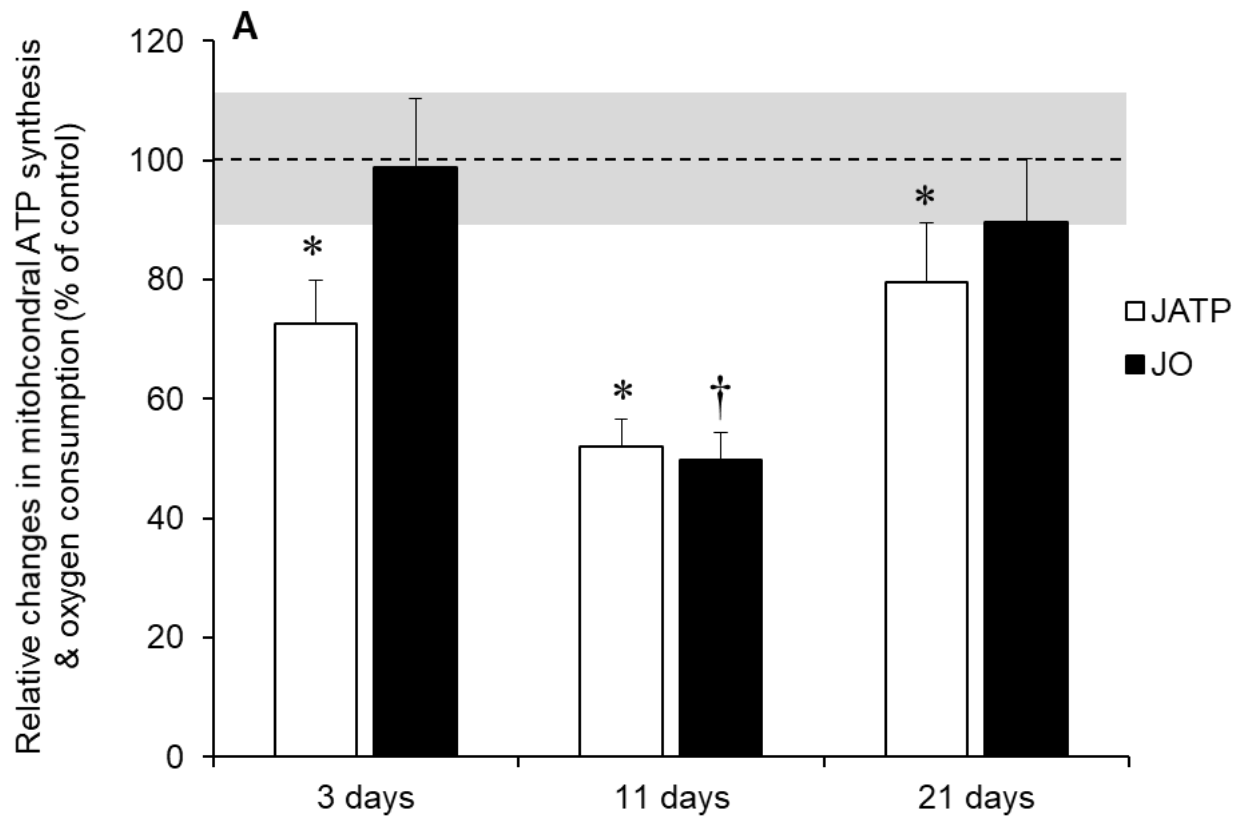
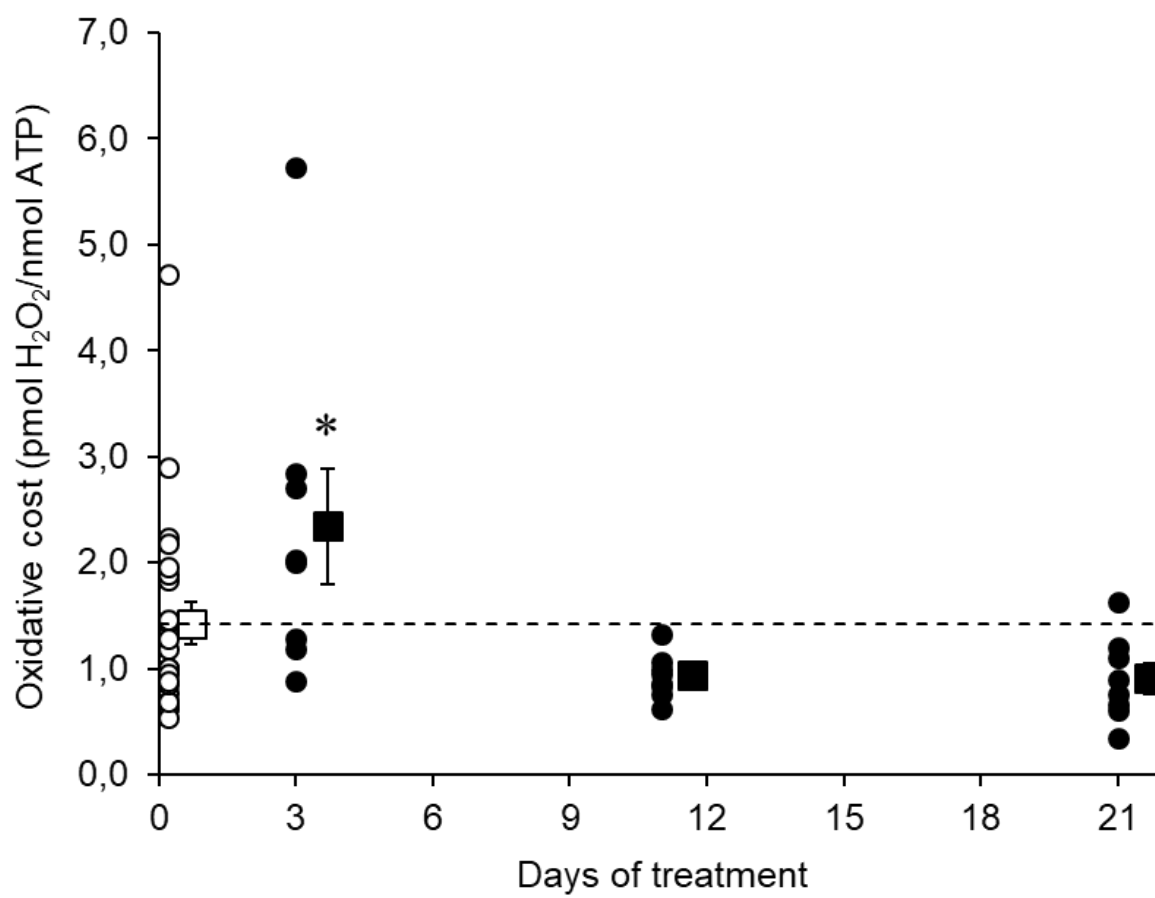


Figure 3



1 **Table 1.** Effects of exogenous treatment of corticosterone (CORT: 4.5 $\mu$ g of corticosterone per  $\mu$ L of sesame oil; CONT: only sesame oil) during 3 days, 11 days  
 2 and 21 days in *Zootoca vivipara*. The metabolic traits studied include whole-organism standard metabolic rates (SMR), muscle fibers and mitochondrial liver  
 3 oxygen consumptions, liver mitochondrial ATP and ROS production. Data are reported as mean  $\pm$  SE ( $n = 8-9$  per group). Significant difference among groups  
 4 from Tukey post-hoc tests of the best model (see main text) are specified with different letters.

5

<b>Morphological and Metabolic traits</b>	<b>Treatment</b>	<b>Day 0</b>	<b>Day 3</b>	<b>Day 11</b>	<b>Day 21</b>
<b>Body and liver mass</b>					
Body mass (g)	CONT	2.00 $\pm$ 0.03 <sup>a</sup>	2.01 $\pm$ 0.04 <sup>a</sup>	1.95 $\pm$ 0.04 <sup>a</sup>	2.03 $\pm$ 0.04 <sup>a</sup>
	CORT	2.00 $\pm$ 0.04 <sup>a</sup>	2.02 $\pm$ 0.04 <sup>a</sup>	2.06 $\pm$ 0.04 <sup>a</sup>	2.26 $\pm$ 0.06 <sup>b</sup>
Liver mass (mg)	CONT		66.13 $\pm$ 3.04 <sup>a</sup>	56.12 $\pm$ 2.18 <sup>b</sup>	57.12 $\pm$ 1.68 <sup>b</sup>
	CORT		60.25 $\pm$ 2.40 <sup>a</sup>	69.12 $\pm$ 4.34 <sup>b</sup>	71.78 $\pm$ 2.44 <sup>b</sup>
Relative liver mass (% of BW)	CONT		3.07 $\pm$ 0.15 <sup>a</sup>	2.64 $\pm$ 0.10 <sup>b</sup>	2.71 $\pm$ 0.09 <sup>b</sup>
	CORT		2.88 $\pm$ 0.12 <sup>a</sup>	3.30 $\pm$ 0.19 <sup>b</sup>	3.46 $\pm$ 0.09 <sup>b</sup>
<b>SMR (J.min<sup>-1</sup>)</b>					
Whole-organism SMR	CONT	6.70 $\pm$ 0.19 <sup>a</sup>	7.00 $\pm$ 0.16 <sup>a</sup>	7.30 $\pm$ 0.22 <sup>b</sup>	7.26 $\pm$ 0.32 <sup>ab</sup>
	CORT	6.69 $\pm$ 0.17 <sup>a</sup>	7.29 $\pm$ 0.18 <sup>ab</sup>	7.58 $\pm$ 0.22 <sup>b</sup>	6.96 $\pm$ 0.20 <sup>a</sup>
<b>Muscle fibers oxygen consumption (pmol O<sub>2</sub>.s<sup>-1</sup>.mg<sup>-1</sup> fresh mass)</b>					
Maximal respiration	CONT		19.92 $\pm$ 1.59 <sup>a</sup>	19.01 $\pm$ 0.70 <sup>a</sup>	20.53 $\pm$ 0.85 <sup>a</sup>
	CORT		19.26 $\pm$ 1.75 <sup>a</sup>	20.97 $\pm$ 0.93 <sup>a</sup>	20.54 $\pm$ 1.54 <sup>a</sup>

Phosphorylating respiration	CONT		15.24 ± 1.07 <sup>a</sup>	14.18 ± 0.71 <sup>a</sup>	15.55 ± 0.57 <sup>a</sup>
	CORT		15.18 ± 1.37 <sup>a</sup>	15.0 ± 0.85 <sup>a</sup>	16.29 ± 1.41 <sup>a</sup>
Basal respiration	CONT		4.80 ± 0.25 <sup>a</sup>	4.96 ± 0.20 <sup>a</sup>	5.23 ± 0.09 <sup>b</sup>
	CORT		5.05 ± 0.34 <sup>a</sup>	4.99 ± 0.32 <sup>a</sup>	6.11 ± 0.34 <sup>b</sup>
<b>Liver mitochondrial oxygen consumption and ATP fluxes (nmol ATP/min.mg protein or nmol O.min<sup>-1</sup>.mg protein<sup>-1</sup>)</b>					
ATP synthesis	CONT		37.21 ± 4.39 <sup>a</sup>	42.06 ± 6.08 <sup>a</sup>	44.43 ± 7.52 <sup>a</sup>
	CORT		27.0 ± 2.72 <sup>b</sup>	21.87 ± 1.94 <sup>b</sup>	35.09 ± 4.43 <sup>b</sup>
Phosphorylating respiration	CONT		21.35 ± 2.35 <sup>ab</sup>	24.94 ± 3.39 <sup>a</sup>	22.34 ± 2.90 <sup>ab</sup>
	CORT		21.10 ± 2.44 <sup>ab</sup>	12.40 ± 1.18 <sup>b</sup>	20.32 ± 2.40 <sup>ab</sup>
Basal respiration	CONT		3.85 ± 0.44 <sup>a</sup>	3.93 ± 0.39 <sup>a</sup>	2.33 ± 0.41 <sup>b</sup>
	CORT		3.92 ± 0.45 <sup>a</sup>	2.12 ± 0.19 <sup>b</sup>	1.99 ± 0.22 <sup>b</sup>
<b>Liver mitochondrial ROS production (pmol H<sub>2</sub>O<sub>2</sub>/min.mg protein)</b>					
Basal state	CONT		167.69 ± 22.4 <sup>a</sup>	129.86 ± 13.05 <sup>a</sup>	164.86 ± 27.92 <sup>ab</sup>
	CORT		181.80 ± 30.6 <sup>a</sup>	63.92 ± 8.85 <sup>b</sup>	97.57 ± 7.35 <sup>b</sup>
Phosphorylating state	CONT		50.84 ± 6.83 <sup>ab</sup>	42.87 ± 4.58 <sup>ab</sup>	57.24 ± 10.72 <sup>a</sup>
	CORT		60.26 ± 11.5 <sup>a</sup>	20.64 ± 3.13 <sup>b</sup>	29.97 ± 4.14 <sup>b</sup>