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

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Running title: Mitochondrial resilience under chronic stress in a reptile

Abstract

Stress hormones and their impacts on whole-organism metabolic rates are usually considered as appropriate proxies for animal energy budget that is the basement of numerous concepts and models aiming at predicting individual and population responses to environmental stress. However, the kinetics of energy re-allocation under stress make the link between metabolism and corticosterone complex and still unclear. Using ectopic application of corticosterone for 3 days, 11 days and 21 days, we simulated acute, mild-time and chronic stress in a lizard (*Zootoca vivipara*). We then investigated whole-organism metabolism, muscle cellular O_2 consumption and liver mitochondrial oxidative phosphorylation processes (O_2 consumption and ATP production) and ROS production. The data showed that (i) while muscle is only slightly impacted, stress regulates the mitochondrial functionality in a time-dependent manner with contrasted pictures between acute, mild-time and chronic stress that is poorly described by the whole-organism metabolism and (ii) an unexpected "pathway" to obtain mitochondrial resilience. While acute stress is characterized by a decreased ATP synthesis rate and high H_2O_2 release with no change in the rate of oxygen consumption, the mild-time stress reduced all three fluxes. Application of corticosterone for 21 days induced a return to initial oxidative phosphorylation capacities with lower H_2O_2 production allowing a better capacity to respond to the energetic demands imposed by the environment. So, the global mitochondrial functioning has to be considered in order to better understand the proximal causes of resource allocation under stress periods.

Keywords: Corticosterone, Lizard, Metabolism, Mitochondrial efficiency, ROS and ATP production, Oxygen consumption

Introduction

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

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

thus demonstrated (i) a time adjustments of energetic resources in response to stress and (ii) a direct linkage between GC secretion in response to stressor and mitochondrial physiology.

These observations led to the emergent concept of "mitochondrial allostatic load" that is the mitochondrial analog of allostatic load in the broader context of stress biology (McEwen and Wingfield 2003). The mitochondrial allostatic load can be defined as the "deleterious structural and functional changes that mitochondria undergo" in response to elevated glucocorticoid secretion under a chronic stress situation (Picard et al. 2014, 2018). The mitochondria are the major ATP cell generators (Lehninger et al. 1993) sustaining physiological processes underlying both maintenance and physiological responses to environmental variations. Mitochondrial energy transduction system also continually produces ROS which can be involved in cell signaling and/or physiopathology (Starkov, 2008). Unfortunately, at that time, no direct assessments of mitochondrial functionality parameters like ATP and ROS under chronic GCs production were available. Yet such data are crucial to understand functional consequences of previously described responses to GC secretion, such as alteration of gene expression and/or epigenetic modifications (Hunter 2016), increased oxidized mtDNA and mitochondrial fission (Picard et al. 2014). A better knowledge of the temporal dynamics of mitochondrial responses to GC secretion will undoubtedly have biomedical applications but will also provide new visions in evolutionary biology (Eisner et al. 2018).

The objective of this study was to explore the time-dependent metabolic responses at the whole-body and mitochondrial levels in lizards (*Zootoca vivipara*) exposed to 3 days, 11 days and 21 days of corticosterone treatment. This protocol provides the temporal dynamic of the mitochondrial responses to GC secretion and answers the following three questions. First question: do short-term *versus* long-term elevations in GCs production induce similar physiological responses in ectotherms than in endotherms? Indeed, the commonly accepted conclusions on time effects on GCs impacts are strongly biased toward endotherms (mammals

and birds), neglecting the vast majority of ectothermic metazoans. Recent experimental approaches suggest different mitochondrial responses under stress in some ectotherms (e.g., Cote et al. 2010; Voituron et al. 2017). Comparative analyses demonstrate that evolution shapes GCs variations which leads to a different impact between ectotherms and endotherms (Jessop et al. 2013; Vitousek et al. 2019). Second question: are ATP and ROS productions equivalently impacted by GCs? Third question: given that the liver and muscle contribute about 50% of body O_2 consumption at rest and more during activity (Rolfe and Brown, 1997), does whole-organism metabolism correctly reflect energetic adjustments at a cellular level of organization?

Materials and methods

Capture and rearing condition

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

Experimental design and whole-organism assays

The study was performed between June 12th and July 12th 2016. At the start of the experiment, animals were distributed in six groups corresponding to two experimental treatments

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

Whole-organism metabolic expenditure at night was quantified with closed respirometry techniques as previously described (Foucart et al., 2014). We measured oxygen consumption and carbon production overnight (approximately 20:00–08:00h) in a dark climatic chamber (AQUALYTIC® TC 135S, Dortmund, Germany). Trials were carried out at 25±1°C, which correspond to body temperature at which functional analyses of mitochondria were performed *in vitro* (see below). The lizards were placed individually into glass jars (ca. 1,000 ml) within the chamber and allowed to acclimatize for 1h. A baseline air sample (two 140 ml syringes) was collected at the onset of the trial, and the glass vial was then carefully sealed. The trial duration was set to achieve adequate oxygen suppression based on previous studies and preliminary trials (mean=11.8 h, range=11.2-12.1h). A final sample of air was collected with two 140 ml syringes connected to a stopcock. Oxygen and CO_2 concentrations (% of total volume) of the air samples were determined using fuel-cell O_2 and infrared CO_2 sensors (FOXBOX, Sable Systems, Las Vegas, NV, USA) at room temperature with a constant flow (60 $ml.min^{-1}$) after water absorption through a column of Drierite. Oxygen consumption (VO_2, in

ml min^{-1}) and CO_2 production (VCO_2) were calculated as: (final % – initial %) × exact chamber volume (ml)/trial duration (min). Body mass was recorded to the nearest mg before each trial. Respiratory quotient (RQ) was calculated like VCO_2/VO_2 and ranged from 0.60 to 0.82 [mean = 0.72, 95% CI= 0.71-0.73]. We found no treatment effect on RQ (ANOVA, $F_{1,47}$=0.65, P = 0.42), but parallel, temporal changes in RQ for both control and treated lizards ($F_{3,96}$=36.59, P < 0.0001). Since the theoretical RQ value for lipid substrates is 0.71, we therefore assumed that most lizards used stored lipids as the main energy source and used energy equivalents for lipids (19.8 $J.mL^{-1}$) to convert VO_2 into standard metabolic rates (SMR) values (in $J.min^{-1}$). These metabolic measurements proved to be extremely reliable and repeatable compared to our earlier studies (Foucart et al., 2014).

Hormonal manipulation and plasma corticosterone assays

Circulating levels of corticosterone were enhanced using a non-invasive method designed by Meylan et *al.* (2003). We diluted corticosterone (Sigma-Aldrich, France, C2505-500mg 92%, $C_{21}H_{30}O_4$) in commercial sesame oil according to 3µg of corticosterone per 1µL of sesame oil. Each evening between 20:00 h and 21:00 local time (when lizards are mostly inactive), 4.5 µL of corticosterone mixture (CORT) or pure sesame oil (CONT, a placebo) were topically applied on the backs of the lizards. To check effects on plasma levels of corticosterone, blood was sampled from the infraorbital sinus of the lizards 7 days before the start of the experiment and at the end of the treatment exposure using 2–3 20 µL microhematocrit tubes. In order to standardize the measurements, all samples were collected between 15:00 h and 16:00 local time within 3 min of removal of an animal from its home cage to avoid the handling-induced increase in plasma corticosterone levels (Dauphin-Villemant, 1987). Plasma was obtained by centrifugation at c.a. 5,000 *g* for 5 minutes of the blood samples and was stored at -40°C for subsequent measurements of plasma levels of corticosterone. Corticosterone level was measured with a competitive enzyme-immunoassay method using corticosterone EIA (IDS

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

Respiratory capacities of muscular permeabilized fibres

At the end of the experiment, animals were euthanized by decapitation. Skeletal muscles were rapidly and entirely dissected, weighed and then placed in an ice-cold isolation buffer (BIOPS containing 2.77 mM Ca-EGTA, 7.23 mM, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM $MgCl_2$, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.2). Skeletal muscle fibres were permeabilized in BIOPS solution supplemented with saponin (50 µg ml^{-1}) according to a standard protocol (Pesta and Gnaiger, 2012). Permeabilized fibres were weighed and their respiration were monitored with a high-resolution respirometer (Oxygraph-2k, Oroboros Instruments; Austria) in a hyper-oxygenated respiratory buffer maintained at 25 °C (110 mM sucrose, 0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM K-lactobionate, 20 mM taurine, 10 mM KH_2PO_4, 1 g l^{-1} fatty acid-free bovine serum albumin, 20 mM Hepes, pH 7.1). A mixture of respiratory substrates (5 mM pyruvate, 2.5 mM malate and 5 mM succinate) was added to obtain the basal respiration (state 2). Cellular ATP synthesis was initiated by the addition of 1 mM ADP. The maximal fully uncoupled respiration (state 3_{unc}) was initiated by addition of 2 µM carbonyl cyanide p-tri-fluoro-methoxy-phenyl-hydrazone (FCCP), in presence of oligomycin (2 µg ml^{-1}), an inhibitor of ATP synthase.

Extraction of liver and functional analyses of mitochondria

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

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

Rates of oxygen consumption and ATP synthesis were measured in a closed glass cell fitted with a Clark oxygen electrode (Rank Brothers Ltd, UK) at 25°C in a respiratory buffer containing 120 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 2 mM $MgCl_2$, 0.3 % fatty acid-free bovine serum albumin, 1.6 U/ml hexokinase, 20 mM glucose, 3 mM Hepes (pH 7.4). Liver mitochondria (0.5-1.5 mg/mL) were energized with a mixture of substrates (5 mM pyruvate, 2.5 mM malate and 5 mM succinate). Mitochondrial ATP synthesis was initiated by the addition of 500 µM ADP. After recording the phosphorylating respiration rate for 3 min, four 100 µL samples were withdrawn from the suspension every 1 min and were quenched in 100 µL ice-cold perchloric acid solution consisting of 10% $HClO_4$ and 25 mM EDTA. After centrifugation (15,000×*g*, 5 min) and neutralization of the resulting supernatant with a KOH solution (2 M KOH, 0.3 M MOPS), ATP production was determined from the slope of the linear accumulation of glucose-6-phosphate content of samples (Voituron et al., 2017). To make sure that the rates we measured were specific of the mitochondrial ATP synthase activity and not associated with contaminated ATPase activity such as adenylate kinase, we determined oxygen consumption and ATP synthesis rates in the presence of oligomycin (2 µg/mL). These values were taken into account to calculate the rate of mitochondrial ATP synthesis that is associated with mitochondrial ATP synthase activity and oxygen consumption.

Mitochondrial reactive oxygen species production

The rate of H_2O_2 released by isolated liver mitochondria was measured at 25°C in a respiratory buffer supplemented with 5 U/mL horseradish peroxidase and 1 µM Amplex red fluorescent dye using a fluorescence spectrophotometer (SFM-25, Kontron Instrument) at excitation and emission wavelengths of 560 nm and 584 nm, respectively. The fluorescent signal was

calibrated using a standard curve obtained after successive addition of H_2O_2 (up to 35 pmoles). Amounts of H_2O_2 release were corrected from background rate of product formation in the absence of exogenous substrate as described previously (Voituron et al., 2017). Free radical electron leak (ROS/O ratio) was calculated as the fraction (%) of basal non-phosphorylating oxygen consumption that is reduced into H_2O_2 at the respiratory chain instead of H_2O at the cytochrome-c oxidase. Similarly, the oxidative cost of mitochondrial ATP synthesis was calculated from the ratio of H_2O_2 generation under phosphorylating state divided by the corresponding rate of ATP synthesis (ROS/ATP ratio).

Statistical analyses

All statistical analyses were performed with linear models in R 3.3.2 (R Development Core Team, 2015). We first checked the effects of treatment groups on change in plasma corticosterone levels from before the end of the experiment with a linear model fitted with the *lm* package (Venables and Ripley, 2002). We included initial plasma corticosterone level and body mass as covariates in this model. Next, repeated measurements (body mass and SMR) were analyzed with mixed-effects linear models in the *lme* package. Dependence among repeated measurements were accounted using random intercept model where the intercept of the model is allowed to vary randomly among individuals. A compound symmetry covariance structure that includes within-subject correlated errors was used and the random term was always kept in the models. This model assumes that individuals react in the same way to treatments and provides information about intra-class correlations and therefore consistency of inter-individual differences. In addition, all models included fixed effects of experimental treatments, treatment exposure groups (categorical variable) and their interactions. Since SMR increases exponentially with body mass, we log transformed SMR prior to the analyses and included log-transformed body mass as a covariate in the analyses. The normality and homogeneity of variance of residuals was systematically checked in the full models and was

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

Results

Corticosterone supplementation increases body and liver masses

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

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

Corticosterone supplementation and whole-organism oxygen consumption

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

Corticosterone supplementation and liver mitochondrial functions

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

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

Corticosterone supplementation and muscle mitochondria functioning

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

Discussion

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

oxidative cost of an ATP molecule production (ROS/ATP). In contrast, longer-term GC supplementation induced a significant decrease of mitochondrial H_2O_2 release with mitochondrial oxidative phosphorylation characteristics to returning to the same level than controls. In an important synthesis, Mc Ewen and Wingfiels (2003) defined the allostatic state as the "*altered and sustained activity levels of the primary mediators, e.g., glucocorticosteroids, that integrate physiology and associated behaviors in response to changing environments and challenges such as social interactions, weather, disease, predators, pollution, etc*". The present data show that liver mitochondria decrease first their efficiency and then their fluxes (both in terms of O_2 and ATP) but restore their functional capacity in response to the energetic dysfunction imposed by the endocrine variations. In addition, the data also suggest a tissue dependent response, with corticosterone treatment affecting functioning of mitochondria in the liver but not that of skeletal muscles. Interestingly, changes in metabolism of mitochondria *in vitro* were not reflected by variation in the whole-organism metabolism *in vivo*.

The present data expand results of a previous study on this species (Voituron et al. 2017) providing the time dynamics of functional regulation of the mitochondrial oxygen consumption, ATP and H_2O_2 productions under GC secretion. In response to the glucocorticoid supplementation, the liver mitochondria first lowered its ATP synthesis rate without changing its oxygen consumption (see Figure 2). Even if mechanisms underpinning this lower efficiency, the cellular ATP/AMP ratio being modified, a cascade of genes upregulation required for mitochondrial respiratory chain expression and function is triggered (Teperino et al. 2010; Mouchiroud et al. 2014). At 11 days of treatment, the mitochondrial pattern is characterized by low values of all fluxes reflecting a diminished activity of the respiratory chain. Interestingly, this mitochondrial hypometabolism restored both the efficiency (ATP/O) and oxidative cost of an ATP molecule production (ROS/ATP). Altogether, these elements are in line with scenarios of downregulation of the respiratory chain subunits (Pandya et al., 2004) and of the activity of

substrate oxidation reported after glucorticoids treatment in rodents (Roussel et al., 2004; Arvier et al., 2007). The nearly total recovering of both oxygen and ATP after 21 days of treatment associated with lower ROS release might be ascribed to *de novo* mitogenesis (Jornayvaz and Shulman 2010). Indeed, glucocorticoids can induce higher expression of key nuclear genes that are required to produce new mitochondria (Psarra and Sekeris 2011). Such a functional resilience in terms of ATP production associated with lower H_2O_2 production corroborates previous results on male common lizards that showed lower superoxide dismutase activity in corticosterone-treated individuals after 21 days (Cote et al. 2010). These results are however not congruent with data on endotherms in which chronically elevated GC levels accelerate aging and reduce lifespan (Schoenle et al. 2018), thus imposing fitness costs potentially through oxidative balance (Costantini 2011).

All these mitochondrial adjustments occurred without modification of whole-organism metabolism (present study; Voituron et al. 2017). Even if only two tissues were tested in the study, the data thus strongly suggest that whole-organism oxygen consumption cannot be used as an accurate proxy for neither ATP production nor ROS release by tissue-specific mitochondria (Salin et al. 2015). However, the GC effect on whole-organism metabolism, an important parameter of the total energy budget, still remains an open question since it has been demonstrated that chronic increase of corticosterone slightly increases metabolic rates at rest in pregnant female common lizards (Meylan et al. 2010) when other lizard species reduce their total oxygen consumption rate when exposed to an increase in corticosterone (Miles et al. 2007; Durant et al. 2008). Nevertheless, the functional mitochondrial resilience observed *in vivo* might constitute a proximal explanation of the increase in survival of male common lizards with corticosterone enhancement (Meylan and Clobert 2005; Cote et al. 2006).

Even if oxygen consumption between tissues has been assessed at different levels of organization, the data suggests that muscles and liver showed differential response patterns.

This statement would be wrong only if mitochondrial content in muscles strongly increase during acute and mild-time stress that has never been reported and not congruent with mitogenesis dynamic (Jornayvaz and Shulman 2010). The data available on mammals are puzzling with no muscular effect in rodents under dexamethasone (Dumas et al. 2003, Roussel et al. 2004) or muscle wasting mediated, in part, by GR-dependent transactivation of genes that drive myocyte atrophy (Patel et al. 2014). In addition, the muscular impact of GC is dose-dependent with moderate or transient exposure to GCs enhancing muscle performance (Caruso et al. 2014). Even if muscles of common lizards do not exhibit a mitochondrial response, muscles could be involved as amino-acids source for liver neoglucogenesis under corticosterone.

Conclusions and perspectives

This study demonstrates that liver mitochondrial energetics of ectotherms is directly influenced in a time-dependent manner by exposure to higher plasma concentrations of circulating GCs. After chronic exposure to GCs, the liver mitochondria reached a functional resilience with a complex interplay between O_2 consumption, ATP production and H_2O_2 release. The coupling between these three mitochondrial processes is thus plastic and differentially regulated leading to a critical period when stress is acute (low ATP production with high H_2O_2 release), an intermediate period when efficiency returns to the initial levels but with low respiratory chain activity, and finally a complete "recovery" to the initial fluxes and ratios. This time-dependent relationship between GC and mitochondria in ectotherms needs to be taken into account to better understand mechanisms that ensure and drive the flow of energy during physiopathological responses (e.g., Rohleder 2012) but also towards adaptive processes.

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Declarations

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Conflict of interest/Competing interests

There is no conflict of interest to declare

Availability of data and material

The datasets generated during the current study are available from the corresponding author on reasonable request.

Author contributions

Conceptualization: Y.V., S.M., D.R., J.F.L.G., C.R.; Methodology: D.R., Y.V., S.M., C.R., J.F.L.G.; Validation: D.R., Y.V.; Formal analysis: D.R., J.F.L.G., A.D., C.R., Y.V.; Investigation:

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

Ethics approval

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

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Figure 1: Experimental design

Figure 2: Effect of 3 days, 11 days and 21 days of exogenous treatment with corticosterone (CORT: 3µg of corticosterone per µL of sesame oil) on liver metabolism mitochondria from *Zootoca vivipara*. (A) Mitochondrial oxygen consumption and ATP production; the values are represented as % of control values (±SEM represented by the horizontal gray-shaded rectangle). (B) Mitochondrial efficiency (ATP/O) in function of treatment time. Circles represent individuals' data and squares represent means for each group. * and † Significantly different from the corresponding control.

Figure 3: Effect of exogenous treatment of corticosterone (CORT: 3µg of corticosterone per µL of sesame oil; control: only sesame oil (white circles and square)) during 3 days (black circles and square), 11 days (black circles and square) and 21 days (black circles and square) in the oxidative cost of an ATP production. Circles represent individuals' data and squares represent means for each group. Values are means ± s.e.m. for 8-9 animals.

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

Figure 1

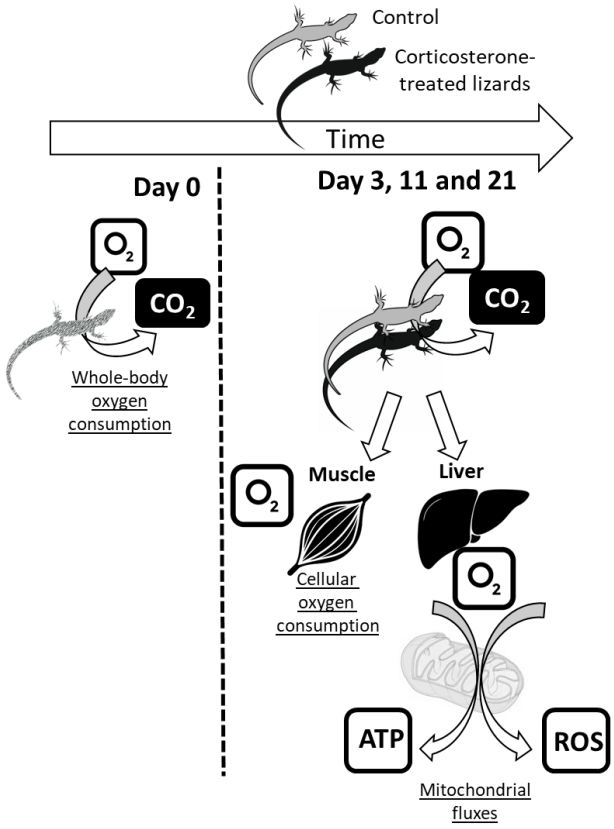
Control
Corticosterone-treated lizards
Time
Day 0
Day 3, 11 and 21
O_2
CO_2
Whole-body oxygen consumption
Muscle
Liver
Cellular oxygen consumption
ATP
ROS
Mitochondrial fluxes

Figure 2

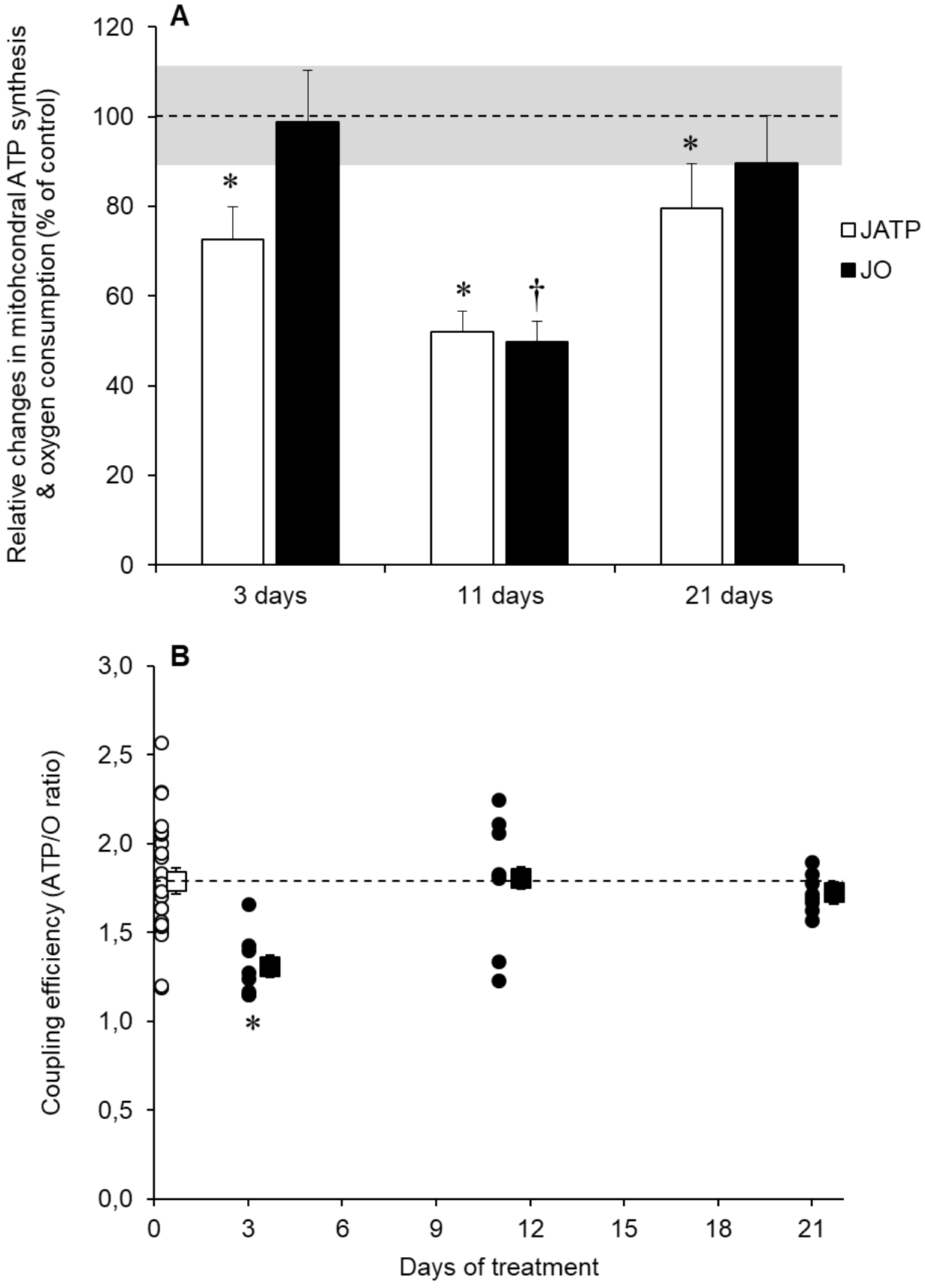
A
Relative changes in mitohcondral ATP synthesis & oxygen consumption (% of control)
120
100
80
60
40
20
0
*
*
†
*
JATP
JO
3 days
11 days
21 days
B
Coupling efficiency (ATP/O ratio)
3,0
2,5
2,0
1,5
1,0
0,5
0,0
*
0
3
6
9
12
15
18
21
Days of treatment

Figure 3

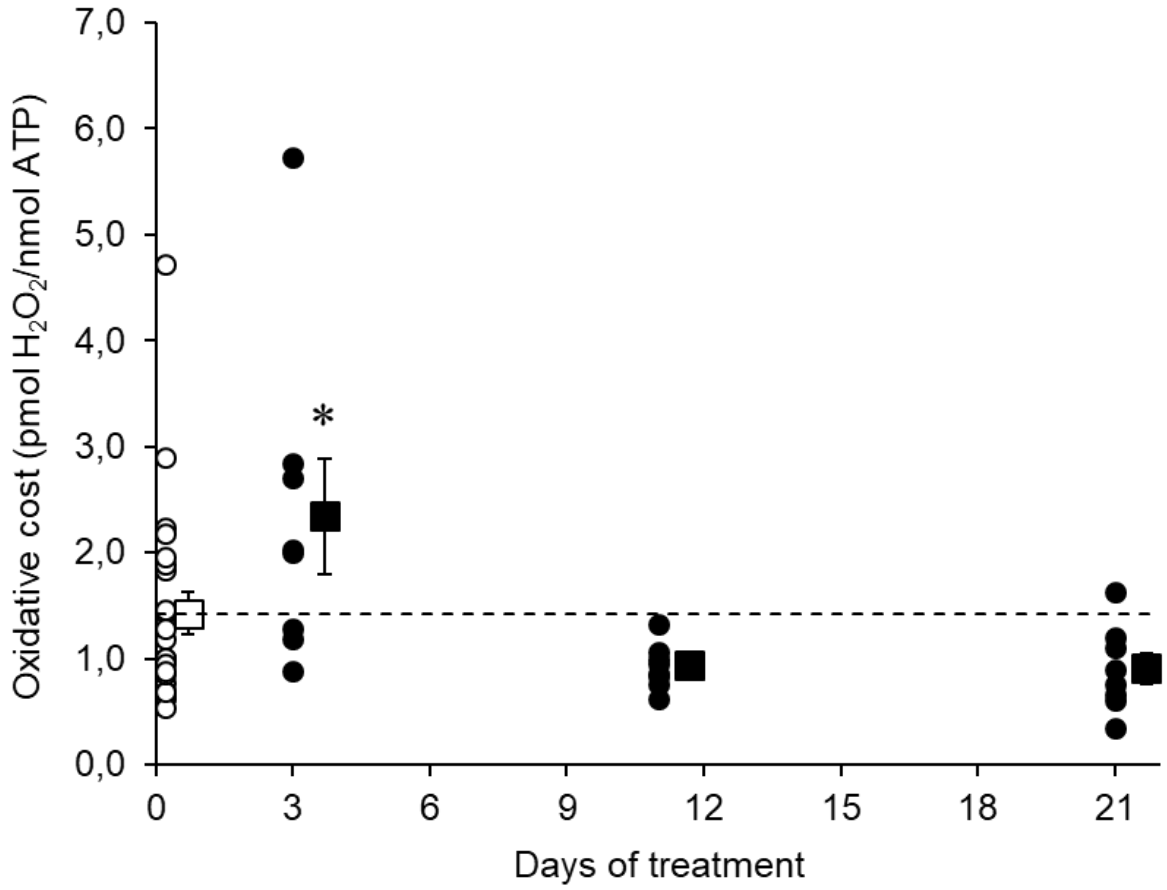
7,0
6,0
5,0
4,0
3,0
2,0
1,0
0,0
Oxidative cost (pmol H_2O_2/nmol ATP)
*
0
3
6
9
12
15
18
21
Days of treatment

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

Morphological and Metabolic traits	**Treatment**	**Day 0**	**Day 3**	**Day 11**	**Day 21**
Body and liver mass					
Body mass (g)	CONT	2.00 ± 0.03 [a]	2.01 ± 0.04 [a]	1.95 ± 0.04 [a]	2.03± 0.04 [a]
	CORT	2.00 ± 0.04 [a]	2.02 ± 0.04 [a]	2.06 ± 0.04 [a]	2.26 ± 0.06 [b]
Liver mass (mg)	CONT		66.13 ± 3.04 [a]	56.12 ± 2.18 [b]	57.12 ± 1.68 [b]
	CORT		60.25 ± 2.40 [a]	69.12 ± 4.34 [b]	71.78 ± 2.44 [b]
Relative liver mass (% of BW)	CONT		3.07 ± 0.15 [a]	2.64 ± 0.10 [b]	2.71 ± 0.09 [b]
	CORT		2.88 ± 0.12 [a]	3.30 ± 0.19 [b]	3.46 ± 0.09 [b]
SMR ($J.min^{-1}$)					
Whole-organism SMR	CONT	6.70 ± 0.19[a]	7.00 ± 0.16[a]	7.30 ± 0.22[b]	7.26 ± 0.32[ab]
	CORT	6.69 ± 0.17[a]	7.29 ± 0.18[ab]	7.58 ± 0.22[b]	6.96 ± 0.20[a]
Muscle fibers oxygen consumption (pmol $O_2.s^{-1}.mg^{-1}$ fresh mass)					
Maximal respiration	CONT		19.92 ± 1.59[a]	19.01 ± 0.70[a]	20.53 ± 0.85[a]
	CORT		19.26 ± 1.75[a]	20.97 ± 0.93[a]	20.54 ± 1.54[a]

Phosphorylating respiration	CONT CORT		15.24 ± 1.07[a] 15.18 ± 1.37[a]	14.18 ± 0.71[a] 15.0 ± 0.85[a]	15.55 ± 0.57[a] 16.29 ± 1.41[a]
Basal respiration	CONT CORT		4.80 ± 0.25[a] 5.05 ± 0.34[a]	4.96 ± 0.20[a] 4.99 ± 0.32[a]	5.23 ± 0.09[b] 6.11 ± 0.34[b]
Liver mitochondrial oxygen consumption and ATP fluxes (nmol ATP/min.mg protein or nmol O.min^{-1}.mg protein^{-1})					
ATP synthesis	CONT CORT		37.21 ± 4.39[a] 27.0 ± 2.72[b]	42.06 ± 6.08[a] 21.87 ± 1.94[b]	44.43 ± 7.52[a] 35.09 ± 4.43[b]
Phosphorylating respiration	CONT CORT		21.35 ± 2.35[ab] 21.10 ± 2.44[ab]	24.94 ± 3.39[a] 12.40 ± 1.18[b]	22.34 ± 2.90[ab] 20.32 ± 2.40[ab]
Basal respiration	CONT CORT		3.85 ± 0.44[a] 3.92 ± 0.45[a]	3.93 ± 0.39[a] 2.12 ± 0.19[b]	2.33 ± 0.41[b] 1.99 ± 0.22[b]
Liver mitochondrial ROS production (pmol H_2O_2/min.mg protein)					
Basal state	CONT CORT		167.69 ± 22.4[a] 181.80 ± 30.6[a]	129.86 ± 13.05[a] 63.92 ± 8.85[b]	164.86 ± 27.92[ab] 97.57 ± 7.35[b]
Phosphorylating state	CONT CORT		50.84 ± 6.83[ab] 60.26 ± 11.5[a]	42.87 ± 4.58[ab] 20.64 ± 3.13[b]	57.24 ± 10.72[a] 29.97 ± 4.14[b]