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RESEARCH ARTICLE

The persistent effects of corticosterone administration during lactation on the physiology of maternal and offspring mitochondria

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ABSTRACT

Reproduction and environmental stressors are generally thought to be associated with a cost to the individual experiencing them, but the physiological mechanisms mediating costs of reproduction and maternal effects remain poorly understood. Studies examining the effects of environmental stressors on a female's physiological state and body condition during reproduction, as well as the physiological condition of offspring, have yielded equivocal results. Mitochondrial physiology and oxidative stress have been implicated as important mediators of life-history trade-offs. The goal of this investigation was to uncover the physiological mechanisms responsible for the enhanced trade-off between self-maintenance and offspring investment when an animal is exposed to stressful conditions during reproduction. To that end, we manipulated circulating corticosterone (CORT) levels by orally supplementing lactating female mice with CORT and investigated mitochondrial physiology and oxidative stress of both the reproductive females and their young. We found that maternal CORT exposure resulted in lower litter mass at weaning, but mitochondrial performance and oxidative status of females were not impacted. We also found potential beneficial effects of maternal CORT on mitochondrial function (e.g. higher respiratory control ratio) and oxidative stress (e.g. lower reactive oxygen species production) of offspring in adulthood, suggesting that elevated maternal CORT may be a signal for early-life adversity and prepare the organism with a predictive, adaptive response to future stressors.

KEY WORDS: Stress, Glucocorticoids, Mitochondria, Oxidative stress, Maternal effects

INTRODUCTION

The proximate mechanisms underlying the costs of reproduction and maternal effects have garnered significant attention in the field of biology over the past few decades (Harshman and Zera, 2007; Speakman and Garratt, 2014; Stearns, 1992; Williams, 1966, 2018). However, these physiological mechanisms remain poorly understood (Veasey et al., 2001; Williams, 2012).

According to life-history theory, when the demands of reproduction or cost of self-maintenance are high, these two variables will trade-off (Reznick, 1985; Stearns, 1989) as organisms cannot simultaneously maximize energy allocation to multiple energetically demanding processes. A reduction in somatic maintenance could attenuate a female's lifespan and/or her capacity to allocate resources to future reproduction, whereas a reduction in resource allocation to her offspring can have lasting effects on offspring fitness (Williams, 1957, 1966). Some studies have found negative effects of environmental stressors on females' physiological state and body condition during reproduction (Salmon et al., 2001; Wiersma and Verhulst, 2005). Yet, several empirical studies have found that reproduction can benefit a female's body condition, which would benefit future reproduction and longevity and also play a role in the evolution of life-history patterns (Cardoso et al., 2002; Hood et al., 2019; Olijnyk and Nelson, 2013; Skibiel et al., 2013). These seemingly conflicting findings could potentially be because different animals employ different life-history strategies (Harrison et al., 2011; Zhang and Hood, 2016).

In animals with small body size, the energetic demands of lactation, growth and development are particularly pronounced (Oftedal, 1984). In house mice (*Mus musculus*), food intake increases nearly 3–5 times, and liver mass roughly doubles to support the demands of milk synthesis. Further, the body mass of pups increases around 10–15 times during the first 2 months of life (Hammond and Diamond, 1994; Hollister et al., 1987; Johnson et al., 2001). At the cellular level, these heightened energetic demands are supported by mitochondria, which are responsible for most of an animal's cellular energy (ATP) production. To support lactation, ATP production is amplified by increasing mitochondrial number via mitochondrial biogenesis and increasing mitochondrial efficiency by removing old or damaged mitochondria via autophagy (Benard et al., 2010; Youle and Van Der Bliek, 2012). Experimental evidence has shown reproducing house mice to have higher mitochondrial densities in the liver during lactation compared with non-reproductive controls (Pichaud et al., 2013), allowing them to meet the heightened energetic demands of lactation.

Additionally, mitochondrial respiration is linked to the production of reactive oxygen species (ROS) and the resulting oxidative damage to proteins, lipids and DNA, which may cause declines in mitochondrial function (Cui et al., 2012; Shigenaga et al., 1994). Oxidative stress has also been implicated as an important mediator of life-history trade-offs (Dowling and Simmons, 2009; Monaghan et al., 2009). Given the elevated energetic demand of reproduction and the central role of mitochondria in both energy production and oxidative stress, it is possible that females would struggle to maintain homeostasis and body condition when challenged with an environmental stressor during reproduction.

In such situations, females could elect to prioritize self-maintenance over investment in current offspring (Hegemann et al., 2013; Reznick, 1985; Stearns, 1989). Experimental studies in rats showed that reduced nutrient transfer to young during fetal development because of uteroplacental insufficiency not only

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retarded offspring growth but also diminished mitochondrial function in the pups' liver and skeletal muscle (Peterside et al., 2003; Selak et al., 2003). However, branding these observed effects as simply positive or negative ignores the fact that these adjustments may provide fitness benefits to both the mother and offspring in the short and long term (Hales and Barker, 2001; Love and Williams, 2008).

According to the match–mismatch hypothesis first proposed by Cushing (1969), maternal effects that provide information about environmental stress from mother to offspring can be adaptive when the offspring's environment mirrors that of its mother (Love and Williams, 2008; Sheriff and Love, 2013) and when a brief stressor reduces both behavioral and neuroendocrine responses to stressors encountered later in life. For instance, mothers that are reproducing in stressful environments (e.g. food scarcity) can modulate the development of their offspring so that the offspring will be prepared for survival in similarly stressful environments. This often results in traits such as small body size and insulin insensitivity (Hales and Barker, 2001), which are commonly viewed as negative effects on offspring. However, these seemingly negative traits can confer fitness benefits to both the mothers and offspring if the early-life and adult environment of the offspring match those of the mothers' developmental environment.

Studies investigating how environmental stress affects physiology often utilize administration of glucocorticoids. Glucocorticoids are produced by the adrenal glands as a part of the hypothalamic-pituitary–adrenal (HPA) axis, the system responsible for the endocrine response to stressors. Corticosterone (CORT) represents the main glucocorticoid in rodents and is secreted as the end-product of HPA activation (Nelson and Kriegsfeld, 2016). Because of the tight link between stress induction, HPA activation and CORT production, CORT is frequently used as an exogenous stressor in studies that manipulate stress physiology in animals (Catalani et al., 2000; Dantzer et al., 2020; Love and Williams, 2008). CORT is commonly linked to an individual's energetic status (Holberton et al., 1996; Kitaysky et al., 2006; Love et al., 2005), and the administration of CORT is known to influence the generation of oxidative stress (Costantini et al., 2011).

The objective of the present study was to evaluate the physiological mechanisms responsible for the enhanced trade-off between self-maintenance and offspring investment when an animal is exposed to stressful conditions during reproduction. First, we sought to investigate how CORT exposure during reproduction affects the mitochondrial physiology and oxidative stress of reproducing females. We manipulated circulating CORT levels by supplementing lactating female mice with CORT orally. We predicted that when their CORT levels are artificially elevated during reproduction, baseline mitochondrial physiology will be negatively impacted, and oxidative damage will be greater than that of unmanipulated reproducing females.

Given that stressful maternal conditions are also expected to impact offspring development, we then asked whether and how maternal CORT treatment influences mitochondrial physiology and oxidative stress of offspring. Species that give birth to altricial young, such as rodents, are anticipated to complete most neuroendocrine development in the postnatal period (Darlington et al., 1999; Matthews, 2002), and so lactation was targeted for our study's manipulation of maternal CORT. Lactation requires substantial physiological investment beyond pregnancy for the continual development of offspring postnatally (Power and Schulkin, 2013). We expected CORT transfer in milk to be the primary mechanism for these developmental effects, as maternal CORT treatment has been discovered in the stomach milk of neonatal rats and shown to increase serum levels of CORT in offspring post-treatment, before returning to baseline immediately post-weaning (Brummelte et al., 2010). We predicted that maternal stress, induced by an elevation in circulating CORT, will negatively influence mitochondrial physiology and induce higher oxidative damage in offspring. Alternatively, if elevated maternal CORT served as a signal for deteriorating environmental conditions (e.g. high predation risks, low food abundance, etc.), then offspring exposed to maternal stress could develop higher mitochondrial function and lower oxidative stress in anticipation of experiencing similarly poor environmental conditions.

MATERIALS AND METHODS

Animal housing

Adult female wild-derived house mice (*Mus musculus* Linnaeus 1758) were utilized in this investigation. Wild-derived mice were used because they have enhanced, and presumably more ecologically relevant, responsiveness to stressors compared with their laboratory counterparts (Abolins et al., 2017; Gaukler et al., 2015; Harper, 2008; Ruff et al., 2015). The founding population of mice was collected in Gainesville, FL, USA, by Wayne Potts, University of Utah (Meagher et al., 2000). We received mice from Potts in 2012 and 2015. We maintained genetic diversity by crossing each maternal lineage to a different paternal lineage each year. Just before the initiation of this study (2019), each lineage was crossed once with mice collected on the Farallon Islands, CA, USA (mice obtained from John Godwin, North Carolina State University). At the time of this study, our lineages were approximately 19 generations removed from collection in Gainesville. All husbandry and experimental procedures were approved by the Auburn University Institutional Animal Care and Use Committee (PRN 2018-3447, 2018-3432). All animals were kept in polypropylene rodent boxes and provided with rodent chow (Teklad Global Diet 2019, Envigo, Indianapolis, IN, USA) and water *ad libitum*. Animals were exposed to a natural light and dark cycle, as well as natural ambient temperatures and humidity.

Experimental design

Twenty-six adult virgin female mice were divided into experimental (CORT; n=14) and vehicle (n=12) control groups. Mice were age-matched across groups to diminish potential age effects between treatment groups. All female mice were paired with a male until visible signs of pregnancy (enlarged nipples and increased girth – usually around 14 days of pregnancy), at which time the male was removed. Because wild-derived mice commonly cannibalize their first litter (W.R.H., personal observation), all females were bred twice and successfully weaned their second litter. All experimental manipulations and data collection were based on the second litter. Litter size was adjusted to five pups on the day that females gave birth to their second litter in an attempt to standardize energy allocation to offspring. Twenty-one litters had greater than five pups at parturition, each of which were then culled to a litter size of five. The other seven litters had fewer than five pups either at parturition (n=2) or following events of pup cannibalism (n=5). Experimental females were exposed to CORT daily, from days 7 to 21 post-partum. CORT was provided to each female in peanut butter pellets (described below) an hour before dark. To acclimate the females to the method of CORT delivery, peanut butter pellets without CORT (vehicle pellets) were offered to mice daily from parturition to day 6 post-partum. Parturition was identified as day 1. On days 7–21, the experimental animals received a peanut butter pellet with CORT

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and non-experimental (control) animals continued to receive vehicle pellets. This time period was targeted for CORT treatment because it spans peak lactation (i.e. greatest milk production) in the house mouse and avoids the period in early lactation when females facing a stressor are more likely to cannibalize their young (Hood, 2012). CORT treatment was terminated as the intestinal tract of the young changed to prepare for the switch to adult food in the third week of life when milk yield drops (Henning and Sims, 1979; Knight et al., 1986). Pups were removed from the female's box when they were weaned at 28 days of age. Females were then returned to a non-reproductive state and killed by decapitation 10 days after weaning. This timing was selected to ensure that the changes in organ function supporting reproduction had returned to a condition optimized for self-maintenance, thus allowing us to quantify how elevated CORT during reproduction alters the baseline physiological condition of females, which could subsequently impact their longevity (Zhang and Hood, 2016). Age-matched dams were killed on different dates because reproduction occurred at different rates across first and second litters.

Offspring were evaluated for body mass and mitochondrial physiology at two different ages: juvenile (5.5 weeks of age) and adulthood (10 weeks of age). One pup of each sex, for a total of two pups per litter, was collected at each time point as maternal stress has been shown to have distinct sex effects in offspring (Liu et al., 2001; Slotten et al., 2006; Weintraub et al., 2010). There was one dam with only female pups, for which we were unable to collect any male progeny. Effects of indirect CORT exposure through the maternal milk supply were evaluated in juvenile offspring of both vehicle (n=12 females, 12 males) and CORT (n=13 males, 15 females) groups. Effects were also evaluated in adult offspring of both vehicle (n=11 males, 12 females) and CORT (n=12 males, 14 females) groups to evaluate the delayed onset or persistence of these effects. Body mass (g) was measured in offspring on days 2, 12 and 28 post-partum, as well as at the time of death in both sexes of both age groups. A summary of the experimental timeline is shown in Fig. S1.

Peanut butter pellet

The CORT-treated mice were given peanut butter pellets with corticosterone mixed in, while vehicle (control) mice were provided with peanut butter pellets made with peanut butter alone. We chose the CORT dosage to fall near levels that have been previously shown to influence energy metabolism in mitochondria of rodent livers (Jani et al., 1991). To make the CORT pellets, 11 mg of CORT (≥98% purity; Enzo, Farmingdale, NY, USA) was thoroughly mixed into 2.5 g of organic peanut butter (Simple Truth, The Kroger Co., Cincinnati, OH, USA). Hence, the concentration of CORT per pellet was calculated as 4.4 mg g^{-1}. To ensure that the peanut butter and CORT were not contaminated, they were weighed separately in sterile, disposable weigh boats and delivered with sterile, disposable spatulas. The peanut butter was warmed to 100°C in a water bath. This temperature limit was used to prevent CORT degradation (Wotiz et al., 1958). The vehicle batch of peanut butter pellets received no addition of CORT. This warm mixture was then dripped into a 4.5×4.5×5 mm, 15-well pellet mold (Ted Pella, Inc., Redding, CA, USA) and frozen at −80°C to form the frozen pellet. Pellets were then carefully removed from the mold and stored at −20°C until ready for consumption. Animals were fed pellets daily during treatment and 3 days prior to the beginning of treatment. Because of the 3 day training regimen, most dams ingested the peanut butter pellet immediately. In order to reduce exogenous sources of stress (i.e. presence of the experimenter), peanut butter ingestion was not monitored past the training regimen. Instead, CORT ingestion was confirmed *post hoc* via quantification of fecal CORT metabolites.

Fecal CORT metabolites

Glucocorticoids were quantified via metabolite concentration in feces of experimental (CORT-treated and vehicle) females at three time points: baseline (pre-mating and pre-treatment), once during CORT treatment (at 12 days post-parturition), and 1 week post-treatment (once offspring were weaned). We also evaluated fecal CORT metabolites in offspring upon reaching adulthood (10 weeks of age). Fecal CORT metabolites were not evaluated in juvenile offspring (5.5 weeks of age), because mothers commonly groom the feces of their young pups and increase coprophagy to offset the nutritional demands of lactation (Soave and Brand, 1991). For collection, mice were put into clean boxes and then fecal samples were collected from the boxes 24 h later. Female offspring were housed together by litter, so samples were difficult to differentiate between individuals. All fecal analyses included the dam as a random factor. Male offspring were housed individually to prevent fighting. The average mass of fecal samples collected was 0.28±0.07 g. Samples were stored at −20°C until analysis.

We measured CORT steroid hormone levels in fecal extracts using DetectX corticosterone enzyme-linked immunosorbent assay (ELISA; Arbor Assays, Ann Arbor, MI, USA). For extraction, fecal samples were dried in an oven at 60°C and homogenized by hand using a disposable pestle in 15 ml Falcon tubes after adding 1 ml of 80% ethanol for every 0.1 g of feces. Samples were then vortexed for 5 min and shaken vigorously by hand for 30 min, while being quickly vortexed every 5 min in between. Samples were then centrifuged at 2000 ***g*** for 10 min (Thermo IEC Centra-CL2). The supernatant was diluted down to 5% ethanol with assay buffer and loaded into 96-well clear microtiter plates coated with donkey anti-sheep IgG and read at an optical density of 450 nm with a Synergy H1 Hybrid plate reader (BioTek, Winooski, VT, USA).

To confirm that fecal CORT metabolites were correlated with circulating CORT, a separate validation study was conducted. Our results suggested that plasma and fecal CORT are moderately correlated with each other across all time points (Pearson correlation, r=0.55; Fig. S2). This finding is generally consistent with other studies that show that fecal and plasma measures of an animal's stress physiology are concordant (Dehnhard et al., 2003; Sheriff et al., 2010). Hence, the correlation between fecal CORT and plasma CORT in our validation study suggests that our treatment should have increased circulating levels of CORT in experimental females.

Mitochondrial isolation

Mitochondria were isolated from liver and skeletal muscle tissues of the CORT and control (vehicle) females, as well as their offspring, following previously outlined procedures (Hood et al., 2018; Hyatt et al., 2017; Zhang et al., 2018). Juvenile (5.5 weeks) and adult (10 weeks) offspring were both evaluated for mitochondrial measurements. Briefly, the largest lobe of the liver and skeletal muscle from the left hindlimb were dissected for live mitochondrial isolation. If the initial tissues were too small for proper mitochondrial isolation, tissue was taken from the next largest lobe of the liver or skeletal muscle from the right hindlimb. The initial tissues and next largest pieces were then combined for mitochondrial isolation. All remaining liver and hindlimb skeletal muscle tissue were flash frozen in liquid nitrogen and stored in −80°C for future analysis.

Skeletal muscle tissue was trimmed to remove fat and connective tissues, and quickly minced with scissors in cold isolation buffer 1

(for composition, see Hood et al., 2018; Hyatt et al., 2017; Zhang et al., 2018). The minced muscle was then homogenized for 5 s with a polytron Vitris homogenizer (Kinematica, Inc., Bohemia, NY, USA). Protease (trypsin) was added (5 mg g^{-1} wet muscle) and the sample was mixed continually for 7 min, at the end of which an equal volume of isolation buffer 1 was added to terminate the digestion. The homogenate was centrifuged (Heraeus Megafuge, Life Technologies Corporation, Grand Island, NY, USA) at 500 ***g*** for 10 min at 4°C. The resulting supernatant was passed through cheesecloth and centrifuged at 3500 ***g*** for 10 min at 4°C. The resulting supernatant was discarded, and the pellet was resuspended with isolation buffer 1 and centrifuged at 3500 ***g*** for 10 min. The supernatant was again discarded, and the mitochondrial pellet was resuspended in isolation buffer 2 (same formula as buffer 1, but without BSA) and centrifuged at 3500 ***g*** for 10 min. The final mitochondrial pellet was suspended in 250 µl of mannitol–sucrose solution.

Liver tissue was minced with scissors in cold liver isolation buffer and homogenized in a Potter-Elvhjem PTFE pestle and glass tube. The homogenate was centrifuged at 500 ***g*** for 10 min at 4°C. The supernatant was filtered through cheesecloth before another centrifugation round at 3500 ***g*** for 10 min. The resulting supernatant was discarded, and the mitochondrial pellet was washed with liver isolation buffer and centrifuged one last time at 3500 ***g*** for 10 min. The final mitochondrial pellet was then suspended in a mannitol–sucrose solution.

Mitochondrial hydrogen peroxide emission

Mitochondrial hydrogen peroxide (H_2O_2) production was assessed in isolated mitochondria using AmplexRed Hydrogen Peroxide/Peroxidase assay kits (ThermoFisher, Waltham, MA, USA). The assay was performed as per the manufacturer's instructions. Resorufin (oxidized AmplexRed) formation occurs with addition of H_2O_2, and the fluorescent signal emitted by resorufin indicates the level of H_2O_2 production. The Synergy H1 Hybrid plate reader excited at a wavelength of 544 nm and measured emission at 590 nm at 37°C in a 96-well black plate. Resulting values were normalized to mitochondrial protein concentration determined by the Bradford assay (Bradford, 1976).

Respiratory control ratio

Mitochondrial isolates were suspended in a respirometer at 37°C (Oxytherm, Hansatech Instruments, King's Lynn, UK). Complex I-driven respiration was measured using 2 mmol l^{-1} pyruvate, 2 mmol l^{-1} malate and 10 mmol l^{-1} glutamate as a substrate, while complex II-driven respiration was measured using 5 mmol l^{-1} succinate. For complex II-driven respiration, 5 µmol rotenone was added to stop electron backflow to complex I prior to the addition of succinate. ADP was added after all substrates were introduced for both complex I and complex II respiration.

The respiratory control ratio (RCR), a measure of mitochondrial respiratory capacity, was evaluated for each tissue type as the ratio of maximum (state 3) to basal (state 4) respiration of isolated mitochondria. State 3 represents the maximal respiration rate initiated by addition of 0.25 mmol l^{-1} ADP substrate, while state 4 or basal respiration is measured when the phosphorylation of ADP is completed. Respiration rates were normalized to mitochondrial protein concentration using the Bradford assay.

Mitochondrial density

Citrate synthase (CS) activity of liver and skeletal muscle tissues was measured as a proxy for mitochondrial volume density (Larsen et al., 2012; Spinazzi et al., 2012), using tissue homogenate from liver and skeletal muscle. Homogenization included a 1:10 (w/v) ratio of 5 mmol l^{-1} Tris-HCl, 5 mmol EDTA and protease inhibitor cocktail (VWR, Radnor, PA, USA) and centrifugation at 1500 ***g*** for 10 min in 4°C. The supernatant was collected, and the protein content was quantified using the Bradford assay. CS activity reactions were quantified as the increase in absorbance from 5,5′-dithiobis-2-nitrobenzoic acid reduction over a minute, reading values from the plate every 10 s.

Oxidative damage markers

4-Hydroxynonenal (4HNE) and protein carbonyls are two common markers for lipid damage and protein damage, respectively, in studies of animal oxidative stress (Casagrande and Hau, 2018; Jenni-Eiermann et al., 2014; Peterside et al., 2003). Therefore, for our study, markers of oxidative damage were quantified via western blot, including adducts of lipid peroxidation (4HNE, Abcam, Cambridge, MA, USA) and carbonyls of protein oxidation (OxyBlot, EMD Millipore, Billerica, MA, USA). Sample preparation for western blot analysis involved homogenization of liver and skeletal muscle tissues. Whole-tissue homogenates of each tissue were prepared with a 1:10 w/v ratio of 5.5 mmol l^{-1} Tris-HCL (pH 7.5), 5 mmol l^{-1} EDTA (pH 8.0) and protease inhibitor cocktail (VWR) before centrifugation at 1500 ***g*** for 10 min in 4°C. Following protein content determination by the Bradford assay, homogenates were further diluted to a concentration of 2 µg $µl^{-1}$. Proteins from tissue homogenates were separated by electrophoresis with the use of 4–15% Criterion TGX precast gels (Bio-Rad, Hercules, CA, USA). Proteins were transferred to polyvinylidene difluoride (PVDF) membranes following separation and washed using Tris-buffered saline solution with 0.1% Tween-20 (TBS-T; 3 times, 5 min). Non-specific site binding was blocked in gels measuring 4HNE with the use of TBS-T and 5% non-fat milk. Gels evaluating protein carbonyls using OxyBlot were blocked in 1% bovine serum albumin (BSA) with TBS-T solution. Membranes were incubated with primary antibodies for either 4HNE (1:2000 dilution, ab46545, Abcam) or protein carbonyls (1:2000 dilution, OxyBlot s7150, EMD Millipore) and then secondary antibodies (1:2000 dilution, anti-rabbit IgG, #7074, Cell Signaling, Danvers, MA, USA) after washing with TBS-T (3 times, 5 min). Amersham ECL prime western blotting detection reagent was used to illuminate membranes and detect labeled proteins (GE Healthcare, Chalfont St Giles, Bucks, UK). Western blot membranes were revealed with a ChemiDoc-It imaging system (Ultra-Violet Products Ltd, Cambridge, UK) and VisionWorks software (Analytik Jena AG, Jena, Germany), but were analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA). All western blot membranes were analyzed separately by age class because they were randomized in this manner upon sample loading. Because of variability between membranes, we were unable to compare samples across membranes.

Statistical analyses

Grubbs' test was used to identify and remove outliers in our dataset prior to subsequent analyses. General linear models were used to evaluate the impact of CORT treatment on mitochondrial performance and oxidative damage variables in reproductive females. Specifically, all physiological metrics and measures of mitochondrial respiration were included in the models as dependent variables, with group as a main effect and date of death and body mass as covariates. Tukey's HSD was used to evaluate pairwise comparisons following a significant mixed model. Females were

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age matched prior to the investigation and age did not differ between treatment groups at the termination of the study (t_{24}=−0.82, P=0.42), averaging 341.8 days for all groups (vehicle control: 332±15.4 days, CORT: 350±14.3 days). Date of death was included as a covariate to control for any seasonal effects in our model because, by chance, the vehicle group bred more quickly than the CORT group (t_{24}=−3.34, P=0.003). The average date of death for females from the vehicle group (24 April ±6.19 days) was approximately a month earlier than for CORT females (23 May ±5.74 days). Body mass was also included as a covariate in all models because previous research has shown mass dependency in measured mitochondrial variables (Mélanie et al., 2019; Park et al., 2020; Roussel et al., 2015). Cannibalism was observed in 3 litters in the CORT treatment group and 2 litters in the vehicle group.

Linear mixed effects models were used to evaluate the impact of maternal CORT treatment on fecal CORT levels, body mass, mitochondrial performance and oxidative damage in offspring. Juvenile (5.5 weeks) and adult (10 weeks) offspring were both evaluated for mitochondrial measurements. Offspring mass was analyzed with group (i.e. CORT versus vehicle) and age class (i.e. juvenile versus adult) as fixed effects, maternal body mass as covariate, and maternal ID as a random factor. Fecal CORT levels were analyzed with group as a fixed effect, and maternal ID as a random factor. Time point was not included as part of the model in the offspring fecal CORT analysis because fecal samples were only collected from offspring at 10 weeks of age. Physiological metrics, and measures of mitochondrial respiration were analyzed with group and age as fixed effects, body mass and date of death as covariates, and maternal ID as a random factor. Maternal age was not included as a covariate because there was no significant difference in age of mothers between treatment groups (t_{24}=−0.82, P=0.42), which averaged 341.8 days for both (vehicle: 332±15.4 days, CORT: 350±14.3 days). Offspring age, however, was included as a covariate to separate emerging and persisting effects of indirect CORT exposure on offspring physiological development. Finally, date of death was included as a covariate in all models to control for seasonal differences between treatment groups. The analysis of fecal CORT metabolites in adult offspring also included body mass as a co-variate because body mass has been previously shown to be impacted by CORT treatment (Singleton and Garland, 2019).

Additionally, detailed statistical output showing all variables and statistical models is presented in Tables S1–S4. Specifically, Tables S1 and S2 show all statistical output pertaining to post-reproductive females (i.e. dams), where Table S2 includes measurements of their mitochondrial physiology. Tables S3 and S4 show all statistical output pertaining to offspring, where Table S4 includes all measurements of mitochondrial physiology in offspring.

RESULTS

Fecal CORT levels, body mass and litter mass of mothers

Reproductive females were evaluated for mitochondrial physiology and oxidative damage effects of CORT administration 10 days after weaning their second litter. There was a group×time interaction for fecal CORT metabolites ($F_{2,45}$=12.48, P<0.0001; Table S1; Fig. 1). Fecal CORT metabolites at peak lactation (day 12 post-partum) were higher in CORT-treated females than in those in the vehicle group ($t_{67.8}$=5.99, P<0.0001; Fig. 1), and at the other two time points (Fig. 1). There was no difference in fecal CORT metabolites between vehicle females at all time points (P>0.05; Fig. 1). There was no effect of CORT treatment on body mass of reproductive females at the time of death (10 days after weaning; t_{24}=−1.43, P=0.168; Table S1) and throughout the study. CORT treatment resulted in lower litter mass at weaning or 28 days post-partum (t_{16}=3.68, P=0.003; Table S1; Fig. 2).

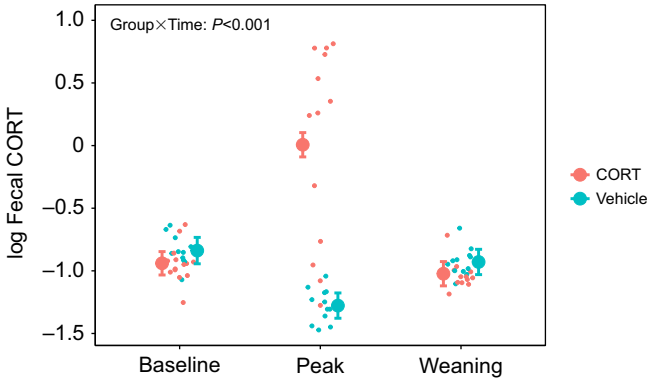

Fig. 1. Effect of maternal corticosterone (CORT) treatment on fecal CORT levels. Fecal CORT metabolites (μg g^{-1} fecal solid) were measured across baseline (n=12 vehicle control, n=14 CORT, before pairing with male), peak lactation (n=12 vehicle; n=14 CORT, day 12 post-partum) and weaning (n=12 vehicle, n=13 CORT, day 28 post-partum) in CORT- and vehicle-treated reproducing mice. Data shown are least-squares means±s.e.m., as well as individual data points. Data are plotted with log-transformed values as the transformed values were used in our analysis.

Mitochondrial physiology and oxidative stress of mothers

At the time of death (10 days after weaning), mitochondrial H_2O_2 emission (ROS) in skeletal muscle tissue was significantly lower in CORT-treated females than in those in the vehicle group (t_{24}=2.36, P=0.028; Table S2; Fig. 3). There was no influence of CORT treatment on mitochondrial H_2O_2 emission (ROS) in liver tissue (P>0.05; Table S2). There was no effect of CORT treatment on any of the mitochondrial respiratory indices (complex I or II: state 3, state 4, RCR), mitochondrial density (CS activity) or oxidative damage markers (4HNE, protein carbonyls) in either liver or skeletal muscle tissue (P>0.05 in all cases; Table S2).

Fecal CORT levels and body mass of offspring

Juvenile (5.5 weeks old) and adult (10 weeks old) offspring were also evaluated for effects of postnatal maternal CORT on fecal CORT levels and body mass, as well as effects on mitochondrial physiology and oxidative damage. There was no effect of maternal CORT treatment on offspring body mass at these two time points

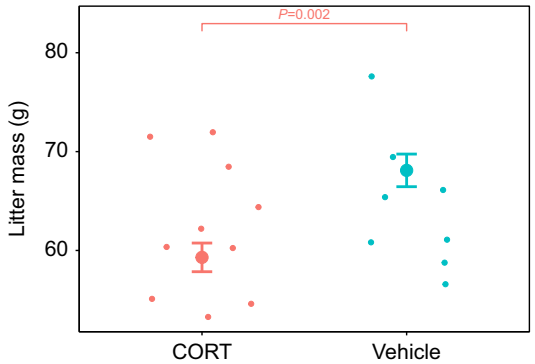

Fig. 2. Effect of maternal CORT treatment on litter mass. Cumulative litter mass of offspring at weaning (day 28 post-partum) of mothers from the CORT and vehicle groups. Data shown are least-squares means±s.e.m., as well as individual data points.

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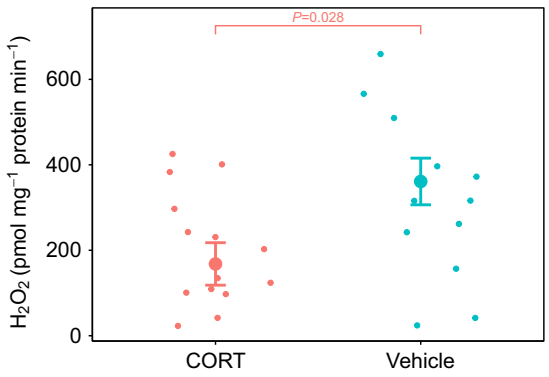

Fig. 3. Effect of maternal CORT treatment on mitochondrial respiration. Mitochondrial H_2O_2 emission in skeletal muscle tissue of reproducing female mice from the CORT and vehicle groups. Data shown are least-squares means±s.e.m., as well as individual data points.

($F_{1,24}$=1.55, P=0.23; Table S3). Feces were only collected from adult (10 weeks of age) offspring. Maternal CORT treatment did not affect fecal CORT metabolite levels of adult offspring ($F_{1,22}$=2.10, P=0.16; Table S3).

Mitochondrial physiology and oxidative stress of offspring

There was a significant group×age interaction for complex I-driven state 4 respiration in the skeletal muscle of offspring ($F_{1,67}$=16.56, P=0.0001; Table S4; Fig. 4A). Specifically, adult offspring from vehicle-treated mothers had higher state 4 respiration than adult and juvenile offspring from CORT-treated mothers ($t_{37.5}$=−2.67, P=0.05), as well as juvenile offspring from vehicle-treated mothers ($t_{84.8}$=4.15, P=0.005). Similarly, RCR showed a significant group×age interaction in skeletal muscle mitochondria using complex II substrates ($F_{1,65}$=9.25, P=0.009; Fig. 4C). Adult offspring from CORT-treated mothers had significantly higher complex II-driven RCR than adult offspring from vehicle-treated mothers ($t_{40.5}$=2.13, P=0.039), but similar RCR to juvenile offspring from both CORT- (t_{80}=−0.37, P=0.98) and vehicle-treated mothers (t_{30}=0.90, P=0.80). There was also a significant group×age interaction for complex II-driven state 3 respiration ($F_{1,67}$=4.27, P=0.04). However, despite observing a trend for lower state 3 respiration in offspring from CORT-treated mothers, *post hoc* comparisons after Tukey adjustments revealed no significant differences between offspring from different treatment and age groups (P>0.15 in all cases). Likewise, there was a significant group×age interaction for state 4 respiration using complex II substrates in skeletal muscle ($F_{1,67}$=18.58, P=0.0001; Table S4; Fig. 4B). Adult offspring from CORT-treated mothers had lower complex II-driven state 4 respiration than adult offspring from vehicle-treated mothers ($t_{41.3}$=−2.88, P=0.03), but similar state 4 respiration to juvenile offspring from both treatment groups (P>0.80 in both cases). We did not find any significant main effects or interactions for liver mitochondrial respiratory indices (complex I or II: state 3, state 4, RCR) (P>0.05 in all cases).

We did not find any significant main effects or interactions for liver ROS emissions (P>0.30 in all cases). In skeletal muscle tissue, however, there was a significant group×age interaction for ROS emission of offspring ($F_{1,69}$=15.2, P=0.0003; Table S2; Fig. 4D). Overall, skeletal muscle ROS emission increased significantly with age in offspring of vehicle-treated mothers (t_{90}=2.70, P=0.04). In the adult age class, offspring of CORT-treated mothers had significantly lower ROS emissions than those from vehicle-treated mothers ($t_{41.2}$=−3.23, P=0.013). There were no effects of maternal CORT on offspring mitochondrial density in liver or skeletal muscle tissues (CS activity; P>0.05 in all cases; Table S4).

Compared with adult offspring from vehicle-treated mothers, adult offspring from CORT-treated mothers had significantly lower levels of protein oxidation (protein carbonyls) in skeletal muscle tissue ($F_{1,23}$=4.64, P=0.04; Table S4; Fig. 5A). Similar trends, albeit not significant, were found for markers of lipid peroxidation (4HNE adducts) in the liver tissue of the adult offspring of CORT-treated mothers ($F_{1,23}$=3.26, P=0.08; Table S4; Fig. 5B). There was no influence of maternal CORT treatment on protein carbonyls of liver tissue in juvenile and adult offspring (P>0.05 in both cases; Table S4). There was also no influence of maternal CORT treatment on 4HNE adducts in the skeletal muscle tissues of both adult and juvenile offspring (P>0.05 in both cases; Table S4). Protein carbonyls of juveniles also did not differ with maternal CORT treatment in skeletal muscle tissue (P>0.05; Table S4).

DISCUSSION

We investigated the physiological mechanisms responsible for enhanced trade-offs between self-maintenance and reproductive effort by studying the impact of a 2 week artificial elevation in CORT during lactation on mitochondrial physiology and oxidative stress of reproducing female mice and their young. Further, we evaluated the impact of maternal CORT exposure on offspring just after weaning (juvenile) and as they reached reproductive maturity (adult). Despite elevated fecal (and presumably circulating) CORT levels, CORT treatment had no effect mitochondrial physiology of mothers, apart from lower ROS emission in skeletal muscle tissue. However, we found that maternal CORT exposure resulted in lower litter mass at weaning and had lasting impacts on mitochondrial respiration, ROS emissions and oxidative damage of offspring.

Our results suggest that reproductive females are well equipped to minimize their own cost of elevated CORT during reproduction. As stress during reproduction has the potential to shorten maternal lifespan (Kirkwood et al., 2000), the lasting effects of maternal CORT on respiratory performance of mitochondria, ROS production and oxidative damage markers were evaluated 17 days after CORT treatment ended to establish a baseline. We found lower ROS emissions in the skeletal muscle of mothers exposed to CORT. It is likely that a reduction of mitochondrial ROS post-CORT exposure was used as a compensation mechanism to protect mitochondria in this tissue against damage induced by CORT treatment (Brand, 2000). Skeletal muscle tissue is particularly susceptible to oxidative damage because cells are post-mitotic, meaning they have a slow turnover rate and are no longer able to proliferate (Crane et al., 2013). This potentially explains the significantly lower ROS production with maternal CORT treatment, as this tissue may be prioritized for protection from oxidative damage associated with treatment. Indeed, there were no measurable, persistent effects of CORT treatment on oxidative damage markers in maternal tissues. Although it is known from past studies that glucocorticoid exposure can result in oxidative damage (Costantini et al., 2011), there is also strong evidence that mice are able to recover from an oxidative challenge (Zhang et al., 2018).

While it is possible that treatment levels of CORT were insufficient for revealing persistent effects on baseline mitochondrial physiology in reproductive females, prior research has shown muscle mitochondrial metabolism to be dynamic in response to both the level and duration of exogenous glucocorticoid exposure (Duclos et al., 2001). Similarly, in this study, it seems that maternal mitochondria were able to recover from any changes experienced following elevation of CORT during reproduction.

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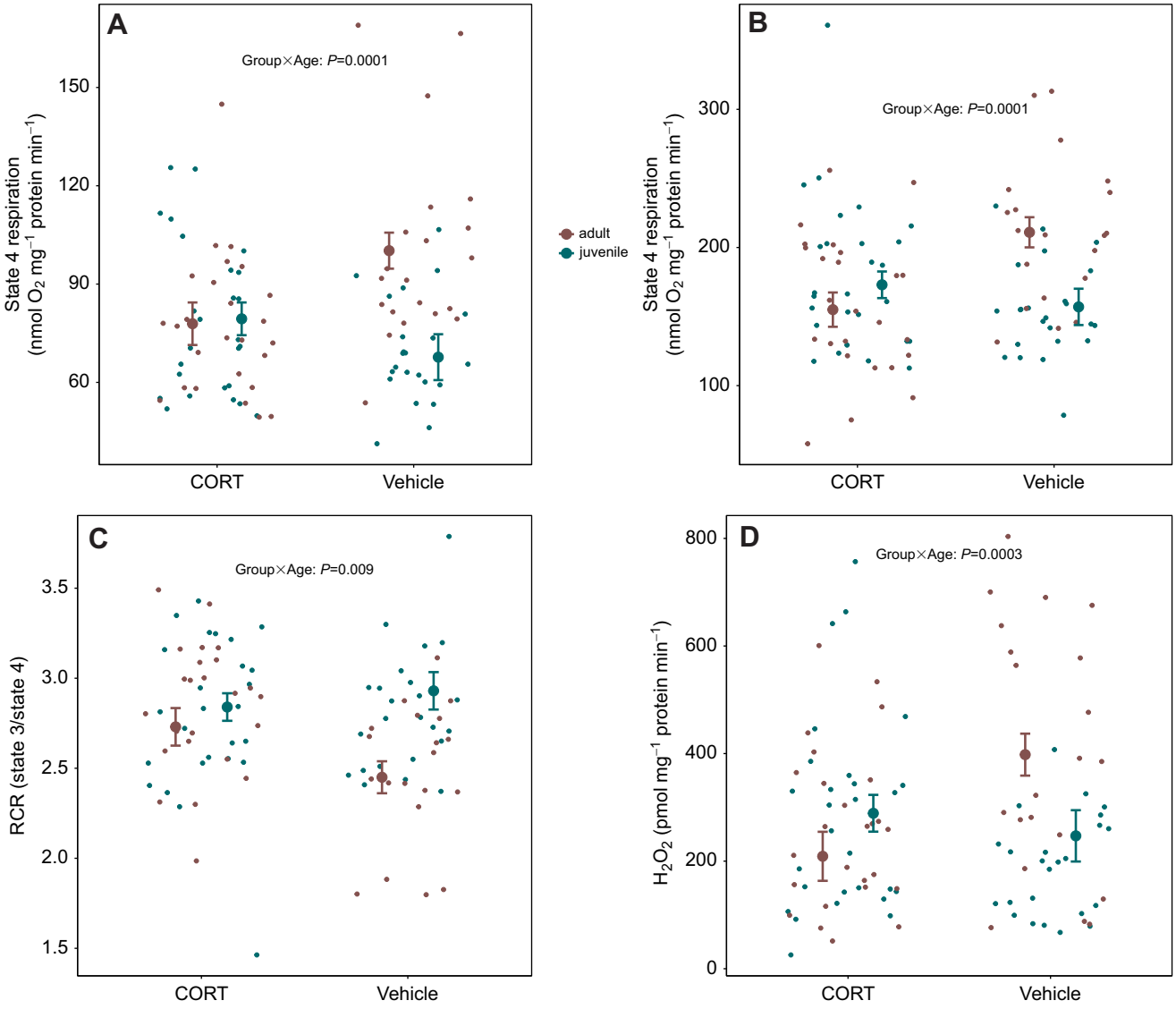

Fig. 4. Effect of maternal CORT treatment on skeletal muscle mitochondria of juvenile and adult offspring. (A) State 4 respiration using complex I substrates. (B) State 4 respiration using complex II substrates. (C) Respiratory control ratio (RCR) using complex II substrates. (D) Mitochondrial H_2O_2 emission. Data shown are least-squares means±s.e.m., as well as individual data points.

Stress exposure is often associated with a decrease in body mass (Iio et al., 2011; Jeong et al., 2013), but as animals in our study were exposed to CORT via their food source, and as the CORT dosage in our study is considered moderate, we did not necessarily expect a decrease in body size. Indeed, we found no influence of exogenous CORT on reproductive females' body mass at the time of death (10 days post-weaning). This result suggests minimal negative effects of CORT treatment on resource allocation to self-maintenance during reproduction. While the effect of treatment had no impact on maternal mass, it appears that mothers may have transferred less milk energy to their offspring, as indicated by a lower litter mass at weaning. After peak lactation, pups begin supplementing their dietary intake with adult food, so it remains possible that this effect was in part or entirely based on differences in rodent chow consumption by pups. While we expected that maternally ingested CORT would result in increased circulating CORT in the nursing offspring (Brummelte et al., 2010), it should be noted that we did not explicitly measure circulating CORT in the offspring. Thus, it is also possible that the effects may be due to differences in maternal care, as chronic social stress has been shown to reduce the period of nursing leading to reduced growth in offspring (Nephew and Bridges, 2010).

Several effects of maternal CORT on mitochondrial function were revealed in adult offspring. In skeletal muscle tissue, we found significant interactions between maternal CORT treatment and offspring age class in state 4 respiration using both complex I and II substrates, as well as RCR using complex II substrates. Specifically, adult offspring from CORT-treated mothers had lower state 4 respiration than adult offspring from vehicle-treated mothers. State 4 respiration is often used as a proxy for proton conductance across the mitochondrial inner membrane (Nicholls and Ferguson, 2002). In this study, lower state 4 respiration was observed in respiration rate driven by both complex I and complex II substrates, indicating lower proton conductance in CORT-treated adult offspring. Paired with increased RCR using complex II substrates, the relative capacity for fatty acid metabolism in skeletal muscle could be maintained during adulthood in the young of mothers exposed to CORT. Energy efficiency of animals whose mother experienced

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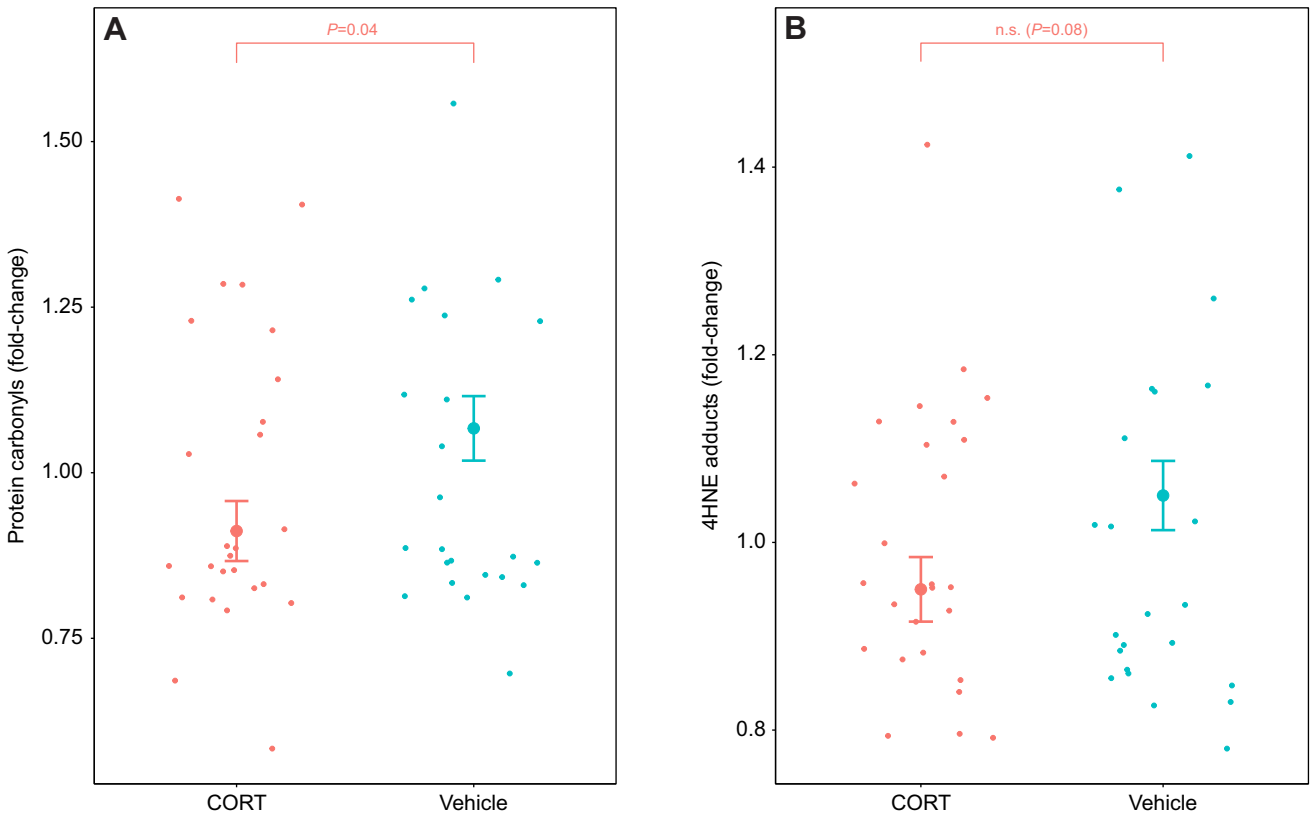

Fig. 5. Effect of maternal CORT treatment on oxidative damage in adult offspring. (A) Protein carbonyls in skeletal muscle. (B) 4HNE in liver. Data shown are least-squares means±s.e.m., as well as individual data points.

stress is expected to increase with RCR as it reflects the electron transport system's capacity to synthesize ATP and decrease ROS production, which could even slow aging processes (Brand, 2000; Brand and Nicholls, 2011; Speakman et al., 2004).

ROS emissions in skeletal muscle were also lower in the adult offspring of CORT-treated mothers. Mitochondrial condition may be improved with significantly lower ROS emissions from skeletal muscle tissue (Nicholls and Ferguson, 2002). This could be a protective mechanism from oxidative stress, supported by differences in protein oxidative damage in the skeletal muscle of adult offspring of CORT-treated mothers. Skeletal muscle ROS emissions were shown to increase with age in offspring of vehicle-treated mothers, presumably as a result of the effects of hormones associated with growth and development (e.g. growth hormone, thyroid hormones, sex steroids, etc.) (Darbandi et al., 2018; Harper and Seifert, 2008; Rosa et al., 2008). However, this is not the case in adult offspring of CORT-treated mothers as they maintained lower ROS emission levels than adult offspring of vehicle-treated females. This finding is consistent with previous research in our lab which revealed that increased ROS emissions following an oxidative event quickly increased ROS and oxidative damage markers, followed by a drop below control levels several days post-exposure (Zhang et al., 2018). These results are reflective of mitohormetic patterns, where a stressor initially causes damage that ultimately leads to improved baseline mitochondrial function (Zhang and Hood, 2016; Hood et al., 2018).

At least under the conditions describe herein, it appears that maternal CORT elevation during lactation has a greater impact on the young than on the mother who was directly exposed to the hormone. While the young of CORT-treated mothers were smaller at weaning, there was no influence of maternal CORT treatment on body mass of juvenile or adult offspring at the time of death. This result suggests catch-up growth patterns, which have been shown to reduce lifespan (Metcalfe and Monaghan, 2001). While the negative effects of catch-up growth are often attributed to oxidative damage (Alonso-Alvarez et al., 2007; De Block and Stoks, 2008; Tarry-Adkins et al., 2016), an accumulation of oxidative damage was not described herein. Our results are supported by research in wild red squirrels (*Tamias hudsonicus*) showing a comparable elevation of maternal glucocorticoids during lactation will lead to the production of offspring with lower birth weight and higher growth rate, without inducing long-term oxidative costs that could influence survival of young (Dantzer et al., 2020). Similar responses to early-life CORT exposure have been observed in avian studies, including slower growth rates in CORT-treated nestlings without any significant changes in oxidative state (Casagrande et al., 2020). However, contrary to findings by Casagrande et al. (2020), our findings showed that offspring from CORT-treated mothers had lower mitochondrial state 4 respiration than offspring from vehicle-treated mothers. This discrepancy could be due to species differences (house mouse versus great tit), target tissues (skeletal muscle versus erythrocytes) and/or experimental design (CORT administered to mothers versus offspring themselves). It should also be noted that in our study, the differences observed in offspring physiology could be due to differences in maternal care and behavior, as well as milk production and composition in CORT- versus vehicle-treated mothers.

Overall, our results of lower birth weight and higher mitochondrial performance in offspring exposed to maternal CORT provide support for the match-mismatch hypothesis (Cushing, 1969), which suggests that early-life adversity may prepare the organism with a predictive, adaptive response for future stressors (Gluckman et al., 2007; Love

and Williams, 2008; Sheriff and Love, 2013). This has been demonstrated in post-natal stress studies, where HPA axis reactivity is often diminished with exposure to early-life stress (Macrì et al., 2011). A higher threshold for tolerance to CORT exposure would allow these individuals to prioritize somatic maintenance towards growth and maturation, potentially explaining the benefits seen in mitochondrial respiration, ROS emissions and subsequent oxidative damage. Future studies should investigate whether exposure to elevated maternal glucocorticoid dampens HPA axis reactivity of offspring or influences maternal care, as well as whether the increased mitochondrial performance observed in the current study augments physical performance measures, such as endurance capacity or burst speed of offspring exposed to elevated maternal CORT.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: K.N.Y., V.A.A., W.R.H.; Methodology: K.N.Y., V.A.A., K.Y.Y., A.N.K., W.R.H.; Validation: K.N.Y., V.A.A.; Formal analysis: K.N.Y., V.A.A., K.Y.Y., W.R.H.; Investigation: K.N.Y., V.A.A., A.S.W., K.Y.Y., S.Z., W.R.H.; Resources: W.R.H.; Data curation: K.N.Y., V.A.A., W.R.H.; Writing - original draft: K.N.Y., V.A.A., W.R.H.; Writing - review & editing: K.N.Y., V.A.A., A.S.W., K.Y.Y., A.N.K., W.R.H.; Visualization: K.N.Y., V.A.A.; Supervision: W.R.H.; Project administration: K.N.Y., V.A.A., A.S.W., K.Y.Y., S.Z., W.R.H.; Funding acquisition: W.R.H.

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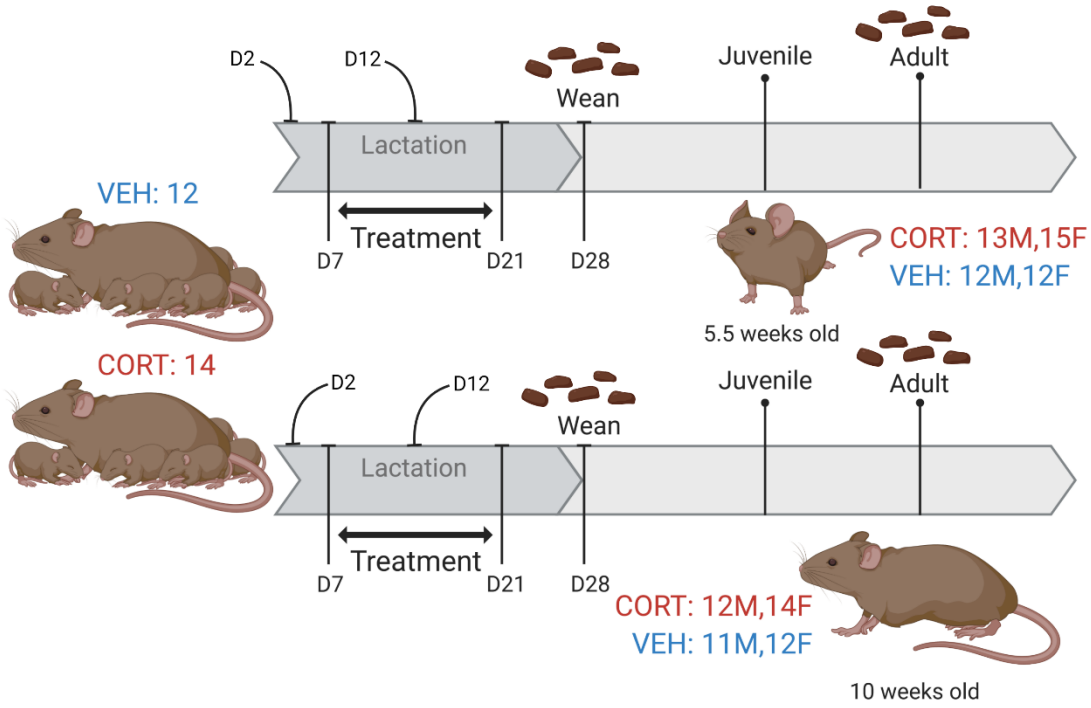

Fig. S1. Experimental timeline of vehicle (VEH: 12) and corticosterone-treated (CORT: 14) reproducing dams, as well as their Juvenile (CORT: 13M, 15F; VEH: 12M, 12F) and Adult (CORT: 12M, 14F; VEH: 11M, 12F) offspring. Units are indicated as days post parturition (D#). Litter mass was taken at day 2 post-partum (D2), day 12 post-partum (D12) and weaning (D28). Body mass of dams were measured at sacrifice (10 days post-weaning). Juvenile (5.5 weeks) and Adult (10 weeks) offspring mass were also measured at sacrifice. Fecal samples were collected on the day of sacrifice from dams at weaning (D28) and Adult offspring (10 weeks).

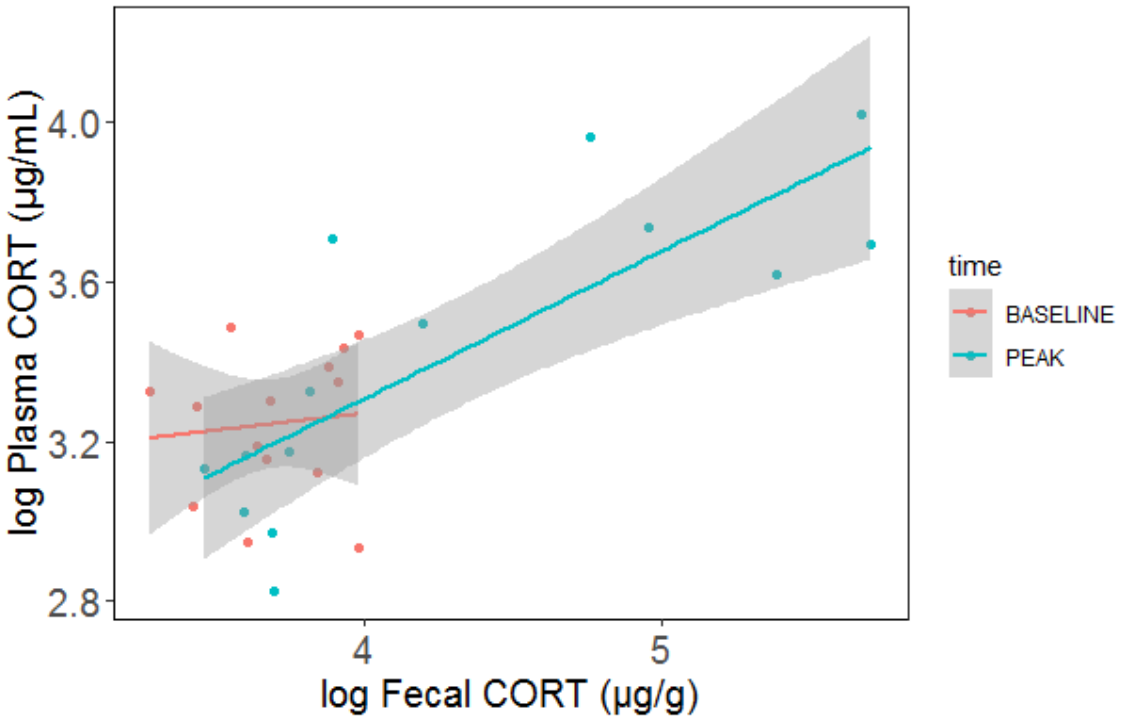

Fig. S2. To confirm that fecal CORT metabolites were correlated with circulating CORT, a separate validation study, 14 female mice were evenly divided into VEH (n=7) and CORT (n=7) groups. Experimental females were bred and given the exact same CORT treatment described above starting on day 7 post-partum. A blood sample of approximately 70-100 µl was collected from each mouse on day 12 post-partum from the facial vein (Hoff, 2000). Once blood was obtained, each female was immediately returned to her pups in a clean box. Feces were then collected from the box the next morning, approximately 12 hours later. The CORT content of these blood and fecal samples were analyzed as described above, except plasma was extracted from blood samples through a single centrifugation event. Dissociation reagent (5 µl) from the ELISA kit was vortexed gently with plasma (5 µl) and incubated at room temperature for 5 minutes. Samples were then diluted 1:100 with 490 µl of assay buffer before plate loading. Our results showed a positive correlation between corticosterone (CORT) metabolites (pg/g) found in both plasma and fecal samples of reproducing female mice (Pearson correlation, $r=0.55$, $p<0.001$), when baseline and peak lactation stages were combined. When stages were assessed separately, there was a positive correlation between corticosterone (CORT) metabolites (pg/g) found in both plasma and fecal samples during peak lactation (Pearson correlation, $r=0.80$, $p<0.001$) but not during baseline (Pearson correlation, $r=0.10$, $p>0.05$).

Table S1. General linear model comparisons of body mass, fecal corticosterone, and litter mass (D2, D12, D28 post-partum) in corticosterone-treated (CORT) or vehicle (VEH) dams. Data shown are least-squared means ± s.e. and units are indicated as days post parturition (D#). Fecal corticosterone metabolites (µg/gm fecal solid) across baseline (before pairing with male), peak lactation (D12 post-partum), and weaning (D28 post-partum) timepoints. Death date and mass were included as covariates and interactions between groups were explored. The p-values are given for all comparisons. Significant findings are reported in bold.

	VEH	**CORT**		**Statistics**			
	$\bar{x}$**+se**	$\bar{x}$**+se**	**num DF**	**den DF**	**Main effect/Int-eraction**	**F-value**	**p-value**
Body mass (g)	20.5±0.61	21.7±0.56	1	24	Group	-1.43	0.17
Fecal CORT metabolites (µg/g)	B: 0.155±0.32 P: 0.0514±0.31 W: 0.118±0.31	B: 0.122±0.29 P: 2.62±0.30 W: 0.104±0.30	2	45	**Group xTime**	12.48	**<0.0001**
D2 Litter mass (g)	8.11±0.47	7.82±0.43	1	24	Group	0.42	0.68
D12 Litter mass (g)	29.9±1.8	27.7±1.6	1	24	**Group**	0.83	0.42
D28 Litter mass (g)*	68.1±1.7	59.3±1.5	1	16	**Group**	3.68	**0.003**

For each model: parent = random factor; B = baseline; P = peak; W = weanling
*Dams with final litter size < 5 pups removed

Table S2. General linear model comparisons of mitochondrial physiology and oxidative damage of liver and skeletal muscle in corticosterone-treated (CORT) or vehicle (VEH) dams. Data shown are least-squared means ± s.e. Maternal death date and mass were included as covariates and interactions between groups were explored. The p-values are given for all comparisons and significant findings are reported in bold.

	VEH	CORT	Statistics				
	$\bar{x}$+se	$\bar{x}$+se	num DF	den DF	Main effect/Int-eraction	F-value	p-value
LIVER:							
Citrate synthase (nM/min/mg protein)	1.95±0.13	1.77±0.12	1	24	Group	0.95	0.35
H_2O_2 emission (pmols/mg protein/min)	34.5±4.1	34.9±3.8	1	24	Group	-0.065	0.95
Complex I substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	124±8.8	137±7.6	1	23	Group	-1.03	0.31
State 4 respiration (nmole O_2/mg/ protein/min)	25.7±2.0	29.4±1.8	1	24	Group	-1.24	0.23
Respiratory control ratio (state 3/state 4)	5.23±0.36	4.86±0.33	1	24	Group	0.69	0.50
Complex II substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	226±11.6	224±10.5	1	24	Group	0.79	0.38
State 4 respiration (nmole O_2/mg/ protein/min)	50.5±3.3	55.2±3.0	1	24	Group	-0.94	0.36
Respiratory control ratio (state 3/state 4)	4.53±0.13	4.18±0.24	1	24	Group	0.87	0.39
Oxidative damage markers							
4HNE adducts (fold-change)	0.993±0.035	0.996±0.032	1	24	Group	-0.059	0.95

Protein carbonyls (fold-change)	0.970±0.063	0.923±0.057	1	24	Group	0.49	0.63
SKELETAL MUSCLE:							
Citrate synthase (nM/min/mg protein)	1.61±0.18	1.70±0.16	1	24	Group	-0.35	0.73
H_2O_2 emission (pmols/mg protein/min)	361±54.6	168±49.7	1	24	**Group**	2.36	**0.03**
Complex I substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	351±31.5	271±28.7	1	24	Group	1.70	0.10
State 4 respiration (nmole O_2/mg/ protein/min)	81.8±8.1	65.9±7.4	1	24	Group	1.26	0.22
Respiratory control ratio (state 3/state 4)	4.45±0.48	4.39±0.44	1	24	Group	0.08	0.94
Complex II substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	449±39.9	383±36.3	1	24	Group	1.11	0.28
State 4 respiration (nmole O_2/mg/ protein/min)	167±17.1	148±15.5	1	24	Group	0.72	0.48
Respiratory control ratio (state 3/state 4)	2.70±0.13	2.67±0.12	1	24	Group	0.17	0.87
Oxidative damage markers							
4HNE adducts (fold-change)	0.984±0.032	0.995±0.029	1	24	Group	-0.26	0.83
Protein carbonyls (fold-change)	0.995±0.063	1.078±0.060	1	23	Group	-0.85	0.41

For each model: parent = random factor

Table S3. General linear mixed effect model comparisons of body mass and fecal corticosterone in the offspring of corticosterone-treated (CORT) or vehicle (VEH) mice. Offspring were evaluated at Juvenile (5.5 weeks) and Adult (10 weeks) timepoints. Body mass of offspring was measured at sacrifice. Fecal corticosterone metabolites (µg/gm fecal solid) were evaluated in Adult offspring only. Maternal death date and mass were included as covariates and interactions between groups were explored. The p-values are given for all comparisons and significant findings are reported in bold.

	VEH	**CORT**		**Statistics**			
	$\bar{x}$+se	**$\bar{x}$+se**	**num DF**	**den DF**	**Main effect/Int-eraction**	**F-value**	**p-value**
Body mass (g)	J: 16.2±0.57 A: 18.3±0.60	J: 15.6±0.52 A: 16.8±0.56	1	24	Group	1.55	0.23
Fecal CORT metabolites (µg/g)*	0.096±0.0101	0.075±0.0089	1	22	Group	2.10	0.16

For each model: parent = random factor; J = juvenile; A = adult;
*Adults only

Table S4. General linear model comparisons of mitochondrial physiology and oxidative damage of liver and skeletal muscle in the offspring of corticosterone-treated (CORT) or vehicle (VEH) dams. Data shown are least-squared means ± s.e. Maternal death date and mass were included as covariates and interactions between groups were explored. The p-values are given for all comparisons and significant findings are reported in bold.

	VEH	CORT	Statistics				
	$\bar{x}$+se	$\bar{x}$+se	num DF	den DF	Main effect/Int-eraction	F-value	p-value
LIVER:							
Citrate synthase (nM/min/mg protein)	1.03±0.046	0.97±0.043	1	24	Group	0.73	0.40
H_2O_2 emission (pmols/mg protein/min)	36.4±2.9	37.2±2.7	1	24	Group	0.033	0.86
Complex I substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	142±8.0	150±7.4	1	24	Group	0.48	0.50
State 4 respiration (nmole O_2/mg/ protein/min)	26.9±1.1	26.8±0.97	1	24	Group	0.0090	0.93
Respiratory control ratio (state 3/state 4)	5.28±0.31	5.74±0.28	1	24	Group	1.043	0.32
Complex II substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	224±8.4	226±7.8	1	24	Group	0.0097	0.92
State 4 respiration (nmole O_2/mg/ protein/min)	52.9±2.3	53.5±2.2	1	24	Group	0.030	0.86
Respiratory control ratio (state 3/state 4)	4.30±0.17	4.32±0.16	1	24	Group	0.0038	0.95
Oxidative damage markers							
Juvenile:							
4HNE adducts (fold-change)	1.05±0.034	0.97±0.032	1	22	Group	2.48	0.13

Protein carbonyls (fold-change)	1.04±0.056	0.95±0.051	1	23	Group	1.23	0.28
Adult:							
4HNE adducts (fold-change)	1.047±0.036	0.95±0.033	1	23	Group	3.26	0.08
Protein carbonyls (fold-change)	1.04±0.044	0.94±0.041	1	23	Group	2.41	0.13
SKELETAL MUSCLE:							
Citrate synthase (nM/min/mg protein)	1.38±0.14	1.51±0.13	1	24	Group	0.42	0.52
H_2O_2 emission (pmols/mg protein/min)	J: 247±47.7 A: 398±39.0	J: 289±34.2 A: 209±45.6	1	70	**Group ×Age**	14.39	**0.0003**
Complex I substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	382±16.8	377±15.2	1	24	Group	0.056	0.81
State 4 respiration (nmole O_2/mg/ protein/min)	J: 67.7±7.0 A: 100.2±5.5	J: 79.4±5.0 A: 77.9±6.5	1	67	**Group ×Age**	16.56	**0.0001**
Respiratory control ratio (state 3/state 4)	4.81±0.30	4.96±0.28	1	24	Group	0.12	0.73
Complex II substrates							
State 3 respiration (nmole O_2/mg/ protein/min)	J: 452±29.4 A: 507±25.1	J: 486±21.7 A: 440±28.5	1	67	**Group ×Age**	6.18	**0.02**
State 4 respiration (nmole O_2/mg/ protein/min)	J: 157±13.2 A: 211±10.9	J: 173±9.6 A: 155±12.4	1	67	**Group ×Age**	18.58	**0.0001**
Respiratory control ratio (state 3/state 4)	J: 2.93±0.10 A: 2.45±0.089	J: 2.84±0.077 A: 2.73±0.10	1	66	**Group ×Age**	6.10	**0.02**
Oxidative damage markers							
Juvenile:							
4HNE adducts (fold-change)	1.00±0.062	0.98±0.057	1	22	Group	0.039	0.85

Protein carbonyls (fold-change)	1.01±0.046	0.973±0.042	1	21	Group	0.39	0.54
Adult:							
4HNE adducts (fold-change)	1.03±0.032	0.995±0.029	1	23	Group	0.624	0.44
Protein carbonyls (fold-change)	1.07±0.049	0.91±0.045	1	23	**Group**	4.64	**0.04**

For each model: parent = random factor; J = juvenile; A = adult