Maternal glucocorticoids promote offspring growth without inducing oxidative stress or shortening telomeres in wild red squirrels

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Abstract

Elevations in glucocorticoid levels (GCs) in breeding females may induce adaptive shifts in offspring life histories. Offspring produced by mothers with elevated GCs may be better prepared to face harsh environments where a faster pace of life is beneficial. We examined how experimentally elevated GCs in pregnant or lactating North American red squirrels (*Tamiasciurus hudsonicus*) affected offspring growth in body mass, structural (skeletal) size, oxidative stress levels (balance of two antioxidants and one measure of oxidative protein damage) in three different tissues (blood, heart, liver), and liver telomere lengths. We predicted that offspring from mothers treated with GCs would grow faster but would also have higher levels of oxidative stress and shorter telomeres, which may predict reduced longevity. Offspring from mothers treated with GCs during pregnancy grew (in body mass) 17.0% faster than those from controls, whereas offspring from mothers treated with GCs during lactation grew 34.8% slower than those from controls. Treating mothers with GCs during pregnancy or lactation did not alter the oxidative stress levels or telomere lengths of their offspring. Fast-growing offspring from any of the treatment groups did not have higher oxidative stress levels or shorter telomere lengths, indicating that offspring that grew faster early in life (~1 to 25 d of age) did not exhibit oxidative costs after this period of growth, when these measures of oxidative stress and telomere lengths were obtained (~70 d of age). Our results indicate that elevations in maternal GCs may induce plasticity in offspring growth without long-term oxidative costs to the offspring that might result in a shortened lifespan.

Keywords: Developmental plasticity, Life history, Maternal effects, Stress

42 **Introduction**

43 Parents can have long-lasting impacts on their offspring across a diversity of taxa. These
44 parental or maternal effects have drawn substantial interest because they suggest that parental
45 characteristics or the parental environment itself could induce adaptive shifts in offspring traits
46 that prepare them for specific environments (i.e., adaptive transgenerational phenotypic
47 plasticity: 1-4). Furthermore, changes in maternal hormone levels, especially glucocorticoids
48 (GCs), are widely suspected to act as a mediator of transgenerational phenotypic plasticity in
49 vertebrates (6-7)

50 GCs are metabolic hormones released by the hypothalamic-pituitary-adrenal (HPA) axis
51 (8), in response to a variety of ecologically salient cues. In mammals, studies in laboratory
52 animals (9, 10) and in humans (11, 12) show that elevated maternal GCs can generate stable
53 individual differences in offspring physiology and behaviour through the transfer of maternally-
54 derived GCs to offspring across the placenta (10, 12), in milk (13, 14), or through changes in
55 maternal behaviour (15-17). Maternal GCs could also act as an internal cue for offspring to
56 modify their own development (18). Regardless of the pathway, there is much evidence that
57 changes in maternal GCs mediate parental effects whose influence may even persist across
58 generations via epigenetic mechanisms (19).

59 Some of these changes in offspring characteristics that are caused by elevations in
60 maternal GCs are suspected to reflect adaptive plasticity in offspring life history traits, such as
61 modifying the trade-off between early life growth and lifespan (20-21). Elevated maternal GCs
62 may induce a "faster" life history strategy whereby offspring produced by mothers with elevated
63 GCs grow or develop faster (4, 22). Such adjustments in the "pace of life" may be adaptive, as
64 fast postnatal growth or a quicker developmental time may be beneficial when the risk of

65 extrinsic mortality is heightened (23-24), which may coincide with elevated maternal GCs.
66 However, this increased investment in postnatal growth is expected to carry costs for offspring
67 longevity whereby fast-growing individuals exhibit a shortened lifespan (25-28).
68 Identifying if maternal stress induces a trade-off between growth and lifespan in wild
69 animals is needed, as any costs of increased exposure to maternal GCs may be masked by benign
70 environmental conditions in the laboratory. Accurately documenting lifespan in wild animals
71 remains challenging in many species, and the stochastic nature of mortality in wild animals may
72 obscure any mechanistic costs of increased exposure to maternal GCs for offspring.
73 Consequently, one way to examine if elevated maternal GCs induce a trade-off between growth
74 and longevity in offspring is to examine how it affects the possible underlying mechanisms of
75 reduced longevity or physiological correlates that may predict a shortened lifespan. The free-
76 radical theory of aging (29) provides one framework to examine the mechanisms by which
77 maternal GCs may induce this trade-off between offspring growth and longevity. Reactive
78 oxygen species (ROS) produced during aerobic respiration can have damaging effects on cells
79 (29-30). ROS production may be elevated by increased aerobic respiration due to enhanced
80 investment in growth or reproduction (28, 31-33) or by increased GCs (34-37). Antioxidants
81 produced by individuals (enzymatic antioxidants such as superoxide dismutase) as well as those
82 antioxidants from the external environment (non-enzymatic antioxidants in the diet) can lessen
83 the impact of ROS production (38). An important type of oxidative damage occurs to the
84 protective ends of chromosomes, called telomeres. Telomeres are the repetitive DNA sequences
85 that occur at the ends of eukaryote chromosomes whose length is shortened during each cell
86 division (39-40), and may also be reduced by the increased production of ROS (28, 41-43). Cells
87 with a reduced telomere length become senescent and stop dividing unless the enzyme

88 telomerase, or another elongation process, is produced to elongate the telomeres (44-45).
89 Telomere length or rate of loss has been found to be predictive (but perhaps not causal: 46) of the
90 mortality risk of individuals (47-51), though the strength of this relationship may vary among
91 taxa and in relation to other life history traits. Furthermore, avian or mammalian species with
92 longer lifespans have been shown to exhibit slower age-specific rates of telomere loss (52-55).
93 Elevated exposure to maternal GCs may, therefore, induce a life history trade-off
94 between offspring growth and longevity, because offspring may experience elevated oxidative
95 damage, decreased antioxidant levels, and/or shortened telomeres, either due to oxidative stress
96 or increased cell division associated with elevated growth (20, 28, 56-58). Previous studies
97 across taxa show that elevated exposure to maternal GCs can shorten telomere lengths in
98 offspring (37, 59-61) or increase their rate of attrition as they age (57), which could cause or be
99 associated with a shortened lifespan. For example, experimental studies in captive and wild birds
100 show that offspring that had exogenous GCs added to their eggs or were administered GCs
101 during chick growth had a heightened physiological stress response, higher levels of oxidative
102 stress, and shorter telomeres early in life (37, 61). Despite much interest in this topic, few studies
103 in wild animals have examined if experimental elevations in the GCs of breeding females impact
104 the oxidative state of offspring or explicitly tested the prediction that elevations in the GCs of
105 breeding females increase early life growth. Additionally, few studies have tested whether
106 elevated GCs in breeding females or fast early life growth comes at some cost by promoting
107 oxidative stress and shortening telomeres in offspring.
108 We tested the hypothesis that elevations in maternal GCs would promote a faster life
109 history strategy in offspring of wild North American red squirrels (*Tamiasciurus hudsonicus*).
110 We treated females with GCs using a protocol that allowed us to increase circulating GCs within

111 a physiologically-relevant range (62). We treated females with GCs either during pregnancy or
112 lactation to assess if the timing of exposure to maternal GCs influenced their effects on offspring.
113 Other than for offspring growth in body mass, we did not have strong *a priori* expectations of
114 how the timing of elevated maternal GCs would differentially impact offspring because elevated
115 maternal GCs during pregnancy or lactation can impact offspring through the same pathways:
116 direct transfer of maternal GCs to offspring across the placenta or through milk
117 ("programming"), altering maternal behaviour, or affecting offspring behaviour (see references
118 above). However, based upon our previous study (4), we predicted that offspring produced by
119 mothers treated with GCs during pregnancy would grow faster in body mass. We did not have an
120 *a priori* expectation for how treating mothers with GCs during lactation would impact offspring
121 growth in body mass, though results from a previous study suggested that it should reduce
122 growth (17).

123 We measured offspring postnatal growth in body mass prior to weaning (~1 to 25 d of
124 age) and subsequently obtained measures of oxidative stress when pups were weaned (~70 d of
125 age). In three tissues (liver, heart muscle, and blood) collected from weaned offspring, we
126 measured one enzymatic antioxidant (superoxide dismutase), one type of non-enzymatic
127 antioxidant (total antioxidant capacity), and one type of oxidative damage (protein damage
128 measured via protein carbonyls). We used multiple tissues because other studies have
129 highlighted how experimental manipulations can have tissue-specific effects (63). To assess the
130 cumulative impact of elevated maternal GCs on the oxidative state of offspring and how
131 offspring growth impacted telomere lengths, we also measured telomere lengths in DNA from
132 the liver. Although we only measured telomere lengths in one tissue, previous studies indicate
133 that telomere lengths measured in one somatic tissue are strongly correlated with those in others

134 (64). Because our estimate of offspring growth from ~1 to 25 d of age was temporally separated
135 from when we obtained our measures of oxidative stress and telomere lengths (when offspring
136 were ~70 d), we were able to assess whether there were persistent or cumulative oxidative costs
137 to fast growth.

138 We predicted that offspring from mothers treated with GCs during pregnancy would
139 grow quicker in body mass after birth, but would experience more oxidative stress (manifested as
140 a reduction in antioxidants and an increase in oxidative damage) and decreased telomere length,
141 which would be a result of increased oxidative stress or increased cell division associated with
142 faster growth. Because we have previously found that female red squirrels can ameliorate the
143 trade-off between offspring number and growth (4, 65), we examined if elevated maternal GCs
144 altered the trade-off between litter size and offspring growth or structural (skeletal) size. Because
145 early life exposure to GCs may modify the direction and strength of the association between two
146 variables (66-67), we also examined if increases in maternal GCs affected the expected negative
147 relationship between offspring growth and oxidative stress state (33) by assessing the statistical
148 interaction between offspring growth and maternal treatment.

149

150 **Materials and Methods**

151 *Study area & measuring offspring growth*

152 We conducted this study as a part of a long-term study of red squirrels in the Yukon,
153 Canada that takes place on the traditional territory of the Champagne and Aishihik First Nations.
154 Squirrels in our study population were all marked individually with unique ear tags and
155 combinations of coloured wire threaded through the ear tags (68). Females in our study
156 population usually produce one litter in the spring, and rarely produce more than one litter of

157 offspring to weaning per year (69). Females were captured and handled every ~3 to 21 d to
158 assess reproductive status through abdominal palpation and nipple condition. Pups were retrieved
159 from the nest two times. The first nest entry occurred immediately after parturition and the
160 second nest entry occurred when pups were approximately 25 d of age. At both nest entries, pups
161 were briefly removed from their nest, sexed, and weighed to the nearest 0.01 g using a portable
162 balance. At the first nest entry, we marked them uniquely by obtaining a small ear biopsy (for
163 later paternity analyses) and then we permanently marked pups at the second nest entry with
164 unique metal ear tags. Because offspring growth in body mass during this period of time is
165 approximately linear (70), we estimated offspring growth as the change in body mass from the
166 first to second nest entry divided by the total number of days elapsed between the two measures
167 of body mass. Red squirrels usually first emerge from their nest around 30-35 d old and are
168 usually weaned around 70 d old, such that this period of growth from ~1-25 d of age represents a
169 period in which offspring are only consuming milk from their mother. At the second nest entry,
170 we measured zygomatic arch width and right hind foot length to the nearest 1 mm using digital
171 callipers or a ruler, respectively. Because we only obtained these morphological measures at the
172 second nest entry (when pups were ~25 d old), we were not able to measure the change in
173 offspring structural size as we did for growth.

174 *Maternal treatments*

175 We used four separate treatment groups to assess the effects of elevated maternal GCs on
176 offspring over four different years (2012, 2015-2017), although in 2012 we only collected
177 growth in body mass data. Individual females were treated with GCs either during pregnancy or
178 lactation ("Pregnancy GCs" and "Lactation GCs"), whereas other females were treated as
179 controls during pregnancy or lactation ("Pregnancy Controls" or "Lactation Controls"). We

180 increased maternal GCs either during pregnancy or lactation using an established experimental
181 protocol (4, 62). Briefly, we treated females in the Pregnancy GCs (n = 45 total litters from 44
182 unique females) and Lactation GCs (n = 18 litters from 17 unique females) treatment groups with
183 exogenous cortisol (hydrocortisone, Sigma H4001) dissolved in peanut butter and wheat germ
184 mixture (8 g of peanut butter, 2 g of wheat germ). Females in the Pregnancy Control (n = 32 total
185 litters from 32 unique females) or Lactation Control (n = 17 litters from 16 unique females)
186 treatment groups were fed the same amount of peanut butter and wheat germ mixture but lacking
187 the hydrocortisone. GC treatments were prepared by dissolving hydrocortisone in 1 mL of 100%
188 ethanol and then 5 mL of 100% peanut oil before allowing the emulsion to sit overnight so that
189 the ethanol could evaporate. The following morning, the hydrocortisone emulsion was
190 thoroughly mixed with the appropriate amount of peanut butter and wheat germ, weighed out
191 into individual dosages (~10 g each), placed into an individual container, and then frozen at -20
192 °C until provisioning to the treated squirrels.
193 Each day during the treatment period, we placed individual dosages into a bucket that
194 was hung ~7-10 m off the ground on the centre of the squirrel's territory. Squirrels defend these
195 buckets from all other conspecifics and heterospecifics (62), so we can, therefore, be confident
196 that the squirrels that were given these treatments were consuming them. The dosage of
197 hydrocortisone varied among some of the treatment groups but we have previously shown that
198 either 3, 6, 8, or 12 mg of hydrocortisone per day significantly elevates baseline plasma cortisol
199 and faecal glucocorticoid metabolite levels but within a physiologically-relevant range (4, 62).
200 Females in the Pregnancy GCs treatment group were provisioned either with 3 mg (n = 4 litters),
201 6 mg (n = 6 litters), 8 mg (n = 26 litters), or 12 mg (9 litters) of hydrocortisone whereas females
202 in the Lactation GCs treatment groups were provisioned either with 8 mg per day (n = 10 litters)

203 or 12 mg per day (n = 8 litters). Although the dosage administered to females treated during
204 pregnancy or lactation varied, we grouped those administered GCs in the same treatment group
205 regardless of dosage (i.e., Pregnancy GCs contained females administered 3-12 mg of
206 hydrocortisone per day, Lactation GCs contained females administered 8 or 12 mg of
207 hydrocortisone per day). We did this for three reasons. First, we have previously shown that
208 females provisioned with 6, 8, or 12 mg of hydrocortisone per day did not differ in their faecal
209 glucocorticoid metabolite levels (FGM), although there were non-significant trends where
210 squirrels fed higher dosages of hydrocortisone had higher FGM (62). Second, in preliminary
211 analyses of the data presented here and in a previous study (4), we found that the effects of
212 different GC dosages (3, 6, 8, and 12 mg per day) that were provided to pregnant females on
213 offspring growth were in the same direction (increased postnatal growth). For females treated
214 with Lactation GCs (8 or 12 mg per day), we also found that the effects on offspring growth
215 were in the same direction (decreased postnatal growth). Finally, our sample sizes in some of the
216 treatment groups (3 and 6 mg per day in the Pregnancy GCs treatment group) were too small to
217 assess whether there were statistical differences among the different dosage groups
218 We aimed to treat females in the pregnancy treatments from approximately 20 d after
219 conception until 5 d after birth (or 20-40 d post-conception as red squirrels on average have a
220 ~35 d gestation period), whereas we actually treated females in the pregnancy GCs treatment
221 group from 24.1 ± 0.9 d (mean ± SE) to 38.9 ± 0.6 d post-conception (mean ± SE treatment
222 duration: 14.9 d ± 0.8 d) and females in the pregnancy control treatment group from 20.2 ± 0.9 d
223 to 38.4 ± 0.5 d (treatment duration: 18.2 ± 0.8 d). We aimed to treat females in the lactation
224 treatments from approximately 5 d after parturition until 15 d post-parturition, whereas we
225 actually treated females in the Lactation GCs treatment group from 5.4 ± 0.5 d (mean ± SE) to

226 14.5 ± 0.6 d post-conception (mean ± SE treatment duration: 9.1 d ± 0.1 d) and females in the
227 Lactation Control treatment group from 4.9 ± 0.4 d to 14.1 ± 0.5 d (treatment duration: 9.2 ± 0.5
228 d). Given a ~35 d gestation period and a ~70 d lactation period in this population, our pregnancy
229 treatments corresponded to treating females during the last trimester of gestation and into the
230 first few days of lactation whereas our lactation treatments corresponded to early lactation before
231 offspring begin to feed independently (they typically leave the nest on their own for the first time
232 at ~35 d: Boutin et al., unpub. data). Note that this means that the lactation treatments occurred
233 during a time when the offspring would not be able to consume any of the treatments themselves
234 so any effects on offspring were likely due to the maternal phenotype.

235 Some females in the Pregnancy GCs (n = 3 litters) or Pregnancy Control (n = 2 litters)
236 treatment groups aborted their litters prior to the first nest entry. Some females lost their litters
237 (likely due to nest predation: 91) after the first nest entry but before the second nest entry when
238 we could obtain the second measure of pup body mass to estimate their growth and the only
239 measures of offspring morphology, thereby reducing our sample sizes to estimate the treatment
240 effects on offspring growth and size for Pregnancy GCs (n = 23 litters from 23 unique females),
241 Pregnancy Control (n = 20 litters from 20 unique females), Lactation GCs (n = 16 litters from 15
242 unique females), and Lactation Control (n = 14 litters from 14 unique females). Below we show
243 that there was no evidence that the treatments had differential effects on litter survival from the
244 first to second nest entry (see Results).

245 *Tissue sample collection*

246 Pups are weaned when they are approximately ~70 d of age and generally stay on their
247 natal territory until dispersal soon after (71). When juvenile squirrels were ~70 d of age, they
248 were euthanized and tissues (liver and cardiac muscle) were immediately removed, rinsed with

249 PBS buffer, snap frozen on dry ice, and then stored in liquid nitrogen or in a -80 °C freezer until
250 analysis. Trunk blood was collected through decapitation and then centrifuged at 10,000 *g* for 10
251 min at room temperature to separate plasma and red blood cells.

252 *Haematocrit*

253 We measured packed red blood cell volume (haematocrit) as a measure of body condition
254 as some (though not all) previous studies have found that higher haematocrit levels correspond to
255 better body condition or improved reproductive performance, at least in some studies of wild
256 birds (72, 89, 90). Before pups were euthanized, we collected a blood sample from the hind nail
257 into a heparinized capillary tube. Haematocrit was quantified using a micro-capillary reader after
258 centrifuging blood samples at 10,000 *g* for 10 min at room temperature.

259 *Protein carbonyls*

260 We measured oxidative damage to proteins (73) using the protein carbonyl colorimetric
261 kit by Cayman Chemical (Ann Arbor, USA). Briefly, ~200 mg of cardiac muscle or liver were
262 homogenized in ~1000 µL of 50mM MES buffer containing 1mM EDTA using a sonicator, and
263 then centrifuged at 10,000 *g* for 15 min at 4 °C. The protein concentration of tissue homogenate
264 supernatant and plasma samples was measured prior to the assay using a Biotek Take3 protocol
265 (Biotek, Vermont, USA) and samples were diluted in PBS buffer to give a protein range between
266 1-10 mg/ml, as recommended by the manufacturer. The average intra-assay CV for samples for
267 plasma, heart, or liver were 1.2%, 2.8%, and 1.6%, respectively. Inter-assay CVs for a red
268 squirrel pooled sample run on repeat assays for plasma (n = 7 assays), heart (n = 6), or liver (n =
269 7) were 3.5%, 9.0%, and 8.3%, respectively. We also ran a positive control (oxidized bovine
270 serum albumin) in two different assays and the inter-assay CV was 3.1%.

271 *Superoxide Dismutase*

272 We obtained one measure of the levels of enzymatic antioxidants (73) by quantifying
273 levels of superoxide dismutase (SOD) using the SOD kit from Cayman Chemical. SOD was
274 expressed as units/mg/ml protein (quantified using a Biotek Take3 protocol). Red blood cells
275 were lysed as per the manufacturer's protocol. The average intra-assay CV for samples for
276 RBCs, heart, or liver were 2.3%, 4.7%, and 3.1%, respectively. Inter-assay CVs for a red squirrel
277 pooled sample run on repeat assays for RBCs (n = 10 assays), heart (n = 2), or liver (n = 4) were
278 16.9%, 4.6%, and 6.5%, respectively.

279 *Total Antioxidant Capacity*

280 We obtained one measure of the levels of non-enzymatic antioxidants (73) by quantifying
281 total antioxidant capacity (TAC) using the TAC kit from Cayman Chemical. Plasma was diluted
282 in assay buffer and assayed according to the manufacturer's protocol. Liver and cardiac muscle
283 (~47 mg) were separately homogenized in 250 µL PBS using a sonicator and the supernatant was
284 diluted in assay buffer and used in the assay. The average intra-assay CVs for samples for
285 plasma, heart, or liver were 4.7%, 3.2%, and 4.7%, respectively. The inter-assay CVs for
286 standards run on all the plates for plasma (n = 5 assays) was 15.8% whereas the inter-assay CV
287 for a red squirrel pooled sample run on repeat for heart (n = 8) or liver (n = 2) were 15.4% and
288 5.4%, respectively.

289 *Telomeres*

290 Liver telomere lengths were measured using the telomere restriction fragment (TRF)
291 assay following established methods (74). Briefly, 2 to 10 g slices of liver tissue were
292 homogenized in cell lysis solution and proteinase K (Qiagen, Germantown, USA). DNA was
293 extracted from the liver homogenates and resuspended in buffer. The resuspended DNA was
294 restriction digested with 15 U of HinfI, 75U of HaeIII and 40U of RsaI (New England BioLabs,

295 Ipswich, USA) at 37 °C. DNA was then separated using pulsed field electrophoresis at 14˚C for
296 19 hours followed by in-gel hybridization overnight at 37˚C with a radioactively labeled
297 telomere-specific oligo (CCCTAA)4. Hybridized gels were placed on a phosphorscreen
298 (Amersham Biosciences, Buckinghamshire, UK), which was scanned on a Typhoon Imager
299 (Amersham Biosciences). Densitometry in ImageJ (v. 1.51s) was used to determine the position
300 and the strength of the radioactive signal in each of the lanes compared with the molecular
301 marker (Quick-Load1 kb DNA Extend DNA Ladder; New England BioLabs) to calculate
302 telomere lengths for each sample. Inter-gel variation was accounted for by calculating the mean
303 TRF length of standard samples run on each gel.

304 *Statistical analyses*

305 We assessed the effects of maternal treatments on litter survival from the first to second
306 nest entry (proportion of pups present at both first and second nest entries) and litter size and
307 litter sex ratio (proportion of litter composed of males) as recorded at the first and second nest
308 entry using generalized linear mixed-effects models (GLMMs: litter survival and litter sex ratio,
309 using binomial errors) or a linear mixed-effect model (LMM: litter size). Each of these separate
310 models contained maternal treatment, birth date, and year as fixed effects. Models for lactation
311 and pregnancy treatments were run separately. There was one litter from a Pregnancy Control
312 treatment female where her litter size at the second nest entry was greater than the at the first,
313 likely because we missed a pup in the nest at the first nest entry, and we therefore excluded this
314 litter from our analyses of the effects of the treatments on litter survival, litter size, and litter sex
315 ratio. We confirmed that none of the GLMMs were overdispersed as all dispersion parameters
316 were <1 (0.48 to 0.72).

317 We assessed the effects of maternal treatments on offspring growth in body mass and a
318 single measure of size using separate LMMs for pregnancy and lactation treatments. Each of the
319 four LMMs (one model for growth and one model for size for pregnancy treatments; one model
320 for growth and one model for size for lactation treatments) included a fixed effect for maternal
321 treatment and covariates (sex, year [categorical variable], birth date, litter size) that could impact
322 offspring growth or size. We included a two-way interaction term between treatment and litter
323 size to identify if elevations in maternal GCs altered the trade-off between litter size and
324 offspring growth, as shown previously for offspring growth (4). We included a two-way
325 interaction between treatment and sex to assess if the treatments had sex-specific effects on
326 growth and size, as documented in other species (75). We used a principal component analysis
327 (PCA) using a covariance matrix in the R package ade4 (version 1.7-13, 76) to generate a
328 composite score of offspring size. The first principal component axis (PC1, hereafter "size")
329 explained 69.8% of the variation in offspring size as measured by zygomatic arch width and hind
330 foot length. Both zygomatic arch width (0.71) and hind foot length (0.71) loaded positively on
331 PC1, indicating that larger PC1 scores corresponded to offspring with longer hind feet and wider
332 crania.
333 Oxidative stress reflects an imbalance between antioxidants and the production of ROS
334 that can damage proteins, lipids, or DNA (73). Consequently, the effects of our treatments on
335 measures of antioxidants should not be viewed in absence of their effects on our measures of
336 oxidative damage (77). We used a PCA to create a composite variable that reflected the oxidative
337 state of an offspring. The PCA was composed of the two antioxidants (SOD, TAC) and one
338 measure of oxidative damage (PCC). We conducted a separate PCA (using a correlation matrix)
339 for each tissue type using the package ade4. For some individuals, we were missing measures of

340 TAC (heart: n = 3; plasma: n = 2) or PCC (heart: n = 2; plasma: n = 4) so we substituted average
341 values for the PCA.

342 Low scores for Blood PC2, Heart PC2, or Liver PC1 corresponded to squirrels that were
343 exhibiting oxidative stress as they represented lower levels of the two antioxidants (SOD, TAC)
344 for blood and liver tissue or just one antioxidant (SOD) for heart tissue and, for heart tissue,
345 higher levels of protein damage (PCC, Table S1). We used these composite variables describing
346 oxidative state of each tissue, haematocrit, or telomere length as the response variables in
347 separate LMMs. Each of these LMMs contained a fixed effect for maternal treatment and
348 offspring sex, year [categorical variable], birth date, and litter size. Because offspring growth
349 may impact oxidative stress levels or telomere lengths (28), we included a two-way interaction
350 term between treatment and offspring growth to examine if mothers with elevated maternal GCs
351 exhibited an altered relationship between growth and the response variable (66-67). Due to
352 smaller sample sizes for these variables, we did not include an interaction between sex and
353 treatment. In the model to assess treatment effects on telomere lengths, we also included a fixed
354 effect for the oxidative stress levels in the liver (Liver PC1).

355 All analyses were conducted in R (version 3.5.2, 78) using lme4 (version 1.1-18-1, 79)
356 and *P*-values for fixed effects were estimated using *lmerTest* (version 3.0-1, 80). Continuous
357 predictor variables were standardized (mean of 0, SD of 1) with birth date, litter size, and growth
358 being standardized within each grid-year combination. Assumptions of homoscedasticity,
359 normality of residuals for our LMMs, and a lack of high leverage observations were confirmed
360 using diagnostic plots (81). As we only had one estimate of growth, size, oxidative stress, or
361 telomere lengths per individual pup, we did not include random intercept terms for individual
362 identity in any of the models described above. Whenever we had repeated observations of the

same litter (e.g., multiple estimates of offspring growth from different pups within the same litter), we included a random intercept term for litter identity. However, we did not include a random intercept in some models (shown in electronic supplementary materials) due to model convergence issues that were likely the result of having only a few observations of multiple pups being measured within one litter or for having a relatively small number of litters. As we had few observations of the same females across years for most of our response variables (sample sizes shown above), we did not include a random intercept for maternal identity as this was usually redundant with litter identity. Random effects were not included in our models of the effects of the treatments on litter characteristics (survival, size, sex ratio) as we only had a few females observed across multiple years. We estimated variance inflation factors (VIFs) from our models to assess multicollinearity among the predictor variables (81) and VIFs indicated that multicollinearity was not an issue in these models (all VIF < 3.31 except if included in an interaction or a multi-level categorical variable).

Results

Effects of Treatments on Litter Survival, Litter Size, & Litter Sex Ratio

There was no evidence that treating mothers with GCs during pregnancy or lactation caused litter failure or altered litter size or litter sex ratio compared to the controls. For those mothers producing offspring until at least the first nest entry (occurring soon after pups were born), the proportion of the litter that survived from the first to the second nest entry did not differ between mothers treated with GCs during pregnancy (n = 42 litters, 51.8 ± 9% of total pups survived from the first to second nest entry) and the pregnancy (time-matched) controls (n = 29 litters, 62 ± 9%, $z = -1.0$, $P = 0.32$), nor between mothers treated with GCs during lactation

386 (n = 18 litters, 79.6 ± 8% pups survived) and the lactation (time-matched) controls (n = 17 litters,
387 72.6 ± 9.5% pups survived, z = -0.62, P = 0.53).
388 Litter size did not differ between mothers treated with GCs during pregnancy or the
389 controls at the first nest entry (Pregnancy GCs: n = 42 litters, 3.05 ± 0.14 pups, range = 1-5 pups;
390 Pregnancy Controls: n = 31 litters, 2.83 ± 0.17 pups, range = 1-5 pups, effect of treatment, t_{52} =
391 0.18, P = 0.85) nor at the second nest entry (Pregnancy GCs: n = 23 litters, 2.87 ± 0.22 pups,
392 range = 1-5 pups; Pregnancy Controls: n = 20 litters, 2.70 ± 0.16 pups, range = 1-4 pups, effect
393 of treatment, t_{37} = 0.23, P = 0.82). Litter size also did not differ between mothers treated with
394 GCs during lactation or the controls at the first nest entry (Lactation GCs: n = 18 litters, 2.67 ±
395 0.2 pups, range = 1-4 pups; Lactation Controls: n = 17 litters, 2.94 ± 0.13 pups, range = 2-4 pups,
396 effect of treatment, t_{30} = -1.29, P = 0.21), nor at the second nest entry (Lactation GCs: n = 16
397 litters, 2.25 ± 0.2 pups, range = 1-3 pups; Lactation Controls: n = 14 litters, 2.57 ± 0.2 pups,
398 range = 1-4 pup, effect of treatment, t_{25} = -1.45, P = 0.16).
399 The litter sex ratio (proportion of males) at the first nest entry did not differ between
400 mothers treated with GCs during pregnancy or the controls (Pregnancy GCs: n = 42 litters, 53 ±
401 4% males; Pregnancy Controls: n = 29 litters, 40.9 ± 5% males, effect of treatment: z = 0.62, P =
402 0.53), nor at the second nest entry (Pregnancy GCs: n = 23 litters, 59.3 ± 7% males; Pregnancy
403 Controls: n = 19 litters, 46.5 ± 7% males, effect of treatment: z = 0.34, P = 0.73). Similarly, the
404 litter sex ratio at the first nest entry did not differ between mothers treated with GCs during
405 lactation or the controls (Lactation GCs: n = 18 litters, 52.8 ± 7.2% males; Lactation Controls: n
406 = 17 litters, 53.4 ± 7.9% males, effect of treatment: z = 0.10, P = 0.92) nor at the second nest

407 entry (Lactation GCs: n = 16 litters, 58.3 ± 8.7% males; Lactation Controls: n = 14 litters, 64.9 ±
408 9.3% males, effect of treatment: z = -0.26, P = 0.79).

409 *Effects of treating pregnant females with GCs on offspring growth, oxidative stress, and telomere*
410 *lengths*

411 Offspring from mothers treated with GCs during pregnancy grew 17.0% faster ($t_{42.3}$ =
412 3.17, P = 0.003, Table S2A, Fig. 1A) but were not larger in structural size (Table S2B, Fig. 1C),
413 and did not differ in body condition (as reflected in their haematocrit levels: Table S3A) than
414 those produced by control mothers. There was no indication that the treatments had sex-specific
415 effects on offspring growth or size (Table S2). There was also no indication of a trade-off
416 between litter size and offspring growth or size during the years that we studied (Table S2) nor
417 was there any evidence that treating mothers with GCs during pregnancy altered the relationship
418 between litter size and growth rate, as indicated by the lack of significant interactions between
419 treatment and litter size for offspring growth and size (Table S2). Because the treatments had no
420 significant effects on litter size or litter sex ratio (see above), the effects of the treatments on
421 offspring growth were not simply due to a reduction in litter size.

422 Offspring from mothers treated with GCs during pregnancy did not have higher oxidative
423 stress levels in the blood, liver, or heart (Table S4, Fig. 2) and they also did not have shorter
424 telomere lengths (Table S5, Fig. 3). Offspring from mothers treated with GCs during pregnancy
425 or provided with supplemental food during pregnancy (controls) that grew faster did not have
426 higher oxidative stress levels in blood, heart, or liver nor did they have shorter telomere lengths
427 (Tables S4-S5). There was no indication that growth or its interaction with maternal treatment
428 impacted oxidative stress levels (Table S4) or liver telomere lengths (Table S5) in offspring from
429 females treated during pregnancy.

430 *Effects of treating lactating females with GCs on offspring growth, oxidative stress, and telomere*
431 *lengths*

432 Offspring from mothers treated with GCs during lactation grew 34.8% slower, but did not
433 grow significantly slower ($t_{26.3} = -1.71$, $P = 0.098$, Table S6A, Fig. 1B), were not significantly
434 smaller in structural size (Table S6B, Fig. 1D), and did not differ in body condition (as reflected
435 in haematocrit levels: Table S3B) from those of pups from control mothers. There was no
436 indication that the treatments had sex-specific effects on offspring growth or size, as reflected in
437 the lack of significant sex x treatment interactions (Table S6). There was also no indication of a
438 trade-off between litter size and offspring growth or size (Table S6) and no evidence that treating
439 females with GCs during lactation altered the relationship between litter size and growth rate, as
440 indicated by the lack of significant interactions between treatment and litter size for offspring
441 growth and size (Table S6). Because the treatments had no significant effects on litter size or
442 litter sex ratio (see above), the effects of the treatments on offspring growth were not simply due
443 to an increase in litter size.

444 Offspring from mothers treated with GCs during lactation did not have higher oxidative
445 stress levels than those from control mothers in any of the three tissues (blood, heart, liver: Table
446 S7, Fig. 2B, 2D, 2F) and they also did not have shorter telomere lengths (Table S5, Fig. 3B).
447 Offspring from mothers treated with GCs during lactation or provided with supplemental food
448 during lactation (controls) that grew faster did not have higher oxidative stress levels in blood,
449 heart, or liver nor did they have shorter telomere lengths (Table S7). There was also no
450 indication that growth or its interaction with maternal treatment impacted oxidative stress levels
451 (Table S7) or liver telomere lengths (Table S5) in offspring from females treated during
452 lactation.

453

454 **Discussion**

455 Mothers treated with GCs during pregnancy produced faster growing offspring whereas
456 mothers treated with GCs during lactation tended to produce slower growing offspring. There
457 were no treatment effects on offspring structural size, indicating that while offspring from
458 mothers treated with GCs gained mass at a different rate than the controls, the treatments did not
459 influence skeletal size. However, we only obtained one measure of structural size when offspring
460 were ~25 d of age, and therefore did not quantify any treatment effects in the rate of change in
461 structural size as we did for body mass. Our results differ from a recent literature analysis across
462 mammals showing that offspring from mothers experiencing late gestational stress grew more
463 slowly before weaning (21). One explanation for the difference between this previous literature
464 analysis and our results is that elevated GCs simply modulated the trade-off between offspring
465 quantity and quality (e.g., producing small litters of fast-growing offspring) or ameliorated the
466 trade-off between offspring quantity and quality (e.g., lessening the effect of increased litter size
467 on the growth rate of each individual offspring: 82). However, this is unlikely because we found
468 no treatment effects on litter size nor on the trade-off between litter size and growth rate. Thus,
469 somehow females treated with GCs during pregnancy produced fast growing offspring without
470 merely reducing their litter sizes, though it is notable that in the years in which we conducted this
471 study, we also did not document a trade-off between litter size and offspring growth in any of the
472 treated or control females, as we have found previously (4). GCs are metabolic hormones that
473 have well-known effects on partitioning resources dedicated to survival vs. reproduction within-
474 individuals (92). Here, it is possible that GCs also play a role in the partitioning of resources

475 between mothers and offspring where they increase investment in offspring (increasing growth
476 rates without a reduction in litter size) perhaps at the expense of maternal energy stores.
477 We did not find support for the hypothesis that elevated maternal GCs during pregnancy
478 or lactation or increased offspring growth elevated oxidative stress levels or shortened telomere
479 lengths in offspring, as other studies have predicted (20, 28, 57). This is surprising and requires
480 explanation. First, we measured oxidative stress levels in offspring when they were weaned (~70
481 d of age) whereas we treated their mothers with GCs either during pregnancy or early lactation.
482 Thus, it is possible that the offspring in our study experienced elevated oxidative stress levels but
483 these effects had disappeared by weaning. A second possibility is an artefact associated with
484 selective disappearance of poor-quality individuals from those mothers treated with GCs during
485 pregnancy, such as slow growing individuals with short telomeres dying before we could obtain
486 our measures of oxidative stress and telomere lengths. This is unlikely as we observed no
487 treatment effects on litter size or the reduction in litter size from the first to second nest entry.
488 Finally, the fact that mothers treated with GCs did not produce offspring with elevated oxidative
489 stress levels or shorter telomeres may have been because of the effects of maternally-derived
490 GCs on offspring telomerase levels, an enzyme that is capable of rebuilding telomeres or
491 buffering them from attrition (83). We did not measure telomerase levels, but a previous study
492 showed that long-term exposure of laboratory rats to unpredictable stressors increased the
493 production of telomerase (84). Thus, a testable prediction for future studies is that treating
494 mothers with GCs promotes increases in telomerase or enzymatic antioxidant production that has
495 protective effects on offspring. This would be consistent with predictions from the oxidative
496 shielding hypothesis (32) that proposes that females may reduce their own levels of oxidative

damage (perhaps by upregulation of enzymatic antioxidants) to mitigate their detrimental influence on offspring.

Our results indicate that elevated GCs can impact maternal investment in the current litter. Females experiencing elevated GCs during pregnancy increased their investment in the current litter whereas females experiencing elevated GCs during lactation tended to reduce their investment in the current litter, the latter possibly being because mothers spent less time interacting or nursing their offspring, as in a previous study in laboratory rats (12). Life history theory predicts that such changes in maternal investment in offspring could alter the survival or future reproduction of mothers (82) or increase their oxidative stress levels (32, 85). However, our previous work in red squirrels shows that their food caching nature results in unexpected patterns with respect to the potential costs of reproductive investment. Red squirrels can elevate reproductive output (i.e., producing a second litter or larger litters) in anticipation of increased future food abundance (69), or produce faster growing offspring when the fitness payoffs warrant increased investment in the current litter (4). They seem to be able to do this without additional access to food except the food that they already have stored from the previous autumn (4, 69, 86). Thus far, survival costs for this increased reproductive investment in the current litter exhibited by female red squirrels seem to be small or absent. Female red squirrels with increased reproductive effort do expend more energy (31, 86) and experience increased oxidative protein damage (31) but we have not yet documented substantive survival costs for females that increase their reproductive output (86, 87). We have not yet quantified any oxidative or survival costs to mothers who were treated with GCs during pregnancy and who on average produced faster growing offspring. Unless females upregulate the production of protective enzymatic antioxidants or telomerase (32), it seems likely that females with elevated GCs would experience

520 increased oxidative damage due to their elevated reproductive investment, or because of the
521 elevated levels of GCs that they experience. For example, previous studies in red squirrels (31)
522 and other species (32) highlight that increased reproductive investment or increased exposure to
523 GCs (34-37) may elevate oxidative damage in breeding females with elevated GCs during
524 reproduction.

525 Although it is not known whether increased antioxidants, reduced oxidative damage, or
526 elongated telomeres actually cause an increase in longevity (46), our results suggest that fast
527 growing offspring or those from mothers treated with GCs during pregnancy or lactation would
528 not experience a reduction in lifespan. Our results and our previous study in red squirrels (4)
529 show that maternal GCs during pregnancy or lactation can induce plasticity in offspring growth
530 and that this plasticity should be adaptive for the fluctuating environments experienced by
531 Yukon red squirrels (4). Specifically, when population density is elevated, females have elevated
532 GCs and fast offspring growth increases offspring survival (4). We have shown here that females
533 with elevated GCs during pregnancy produce faster growing offspring whereas females with
534 elevated GCs during lactation produce slower growing offspring. This suggests that plasticity in
535 GC levels during female reproduction (e.g., females have elevated GCs during pregnancy but
536 exert strong negative feedback on further GC production during lactation) would maximize
537 female reproductive success when density is high. However, we did not find support for the
538 hypothesis that elevated maternal GCs induce a faster pace of life where offspring grow faster
539 and are more competitive early in life but this comes at some oxidative cost that may predict a
540 shortened lifespan. Future studies should assess oxidative stress using an even broader array of
541 measures than the few measures we used here, and will of course need to assess if elevated
542 maternal GCs actually impact offspring lifespan.

Data Accessibility: All data files will be made available on FigShare.

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Figure 1. Effects of treating pregnant or lactating female red squirrels with glucocorticoids (GCs) on offspring postnatal growth and structural size. A) Offspring from females treated with GCs during pregnancy grew significantly faster than those from controls. **B)** Offspring from females treated with GCs during lactation grew significantly slower than those from controls. **C & D)** There were no treatment effects on the structural size of offspring. Offspring growth was measured as the change in body mass from ~1 to ~25 d of age. Offspring size is a composite variable where high scores of PC1 correspond to offspring with larger zygomatic arch widths and longer hind foot lengths when pups were ~25 d of age. Results in Tables S2 & S6. Upper and lower hinges correspond to the first and third quartiles while white diamonds indicate means and horizontal line indicate medians.

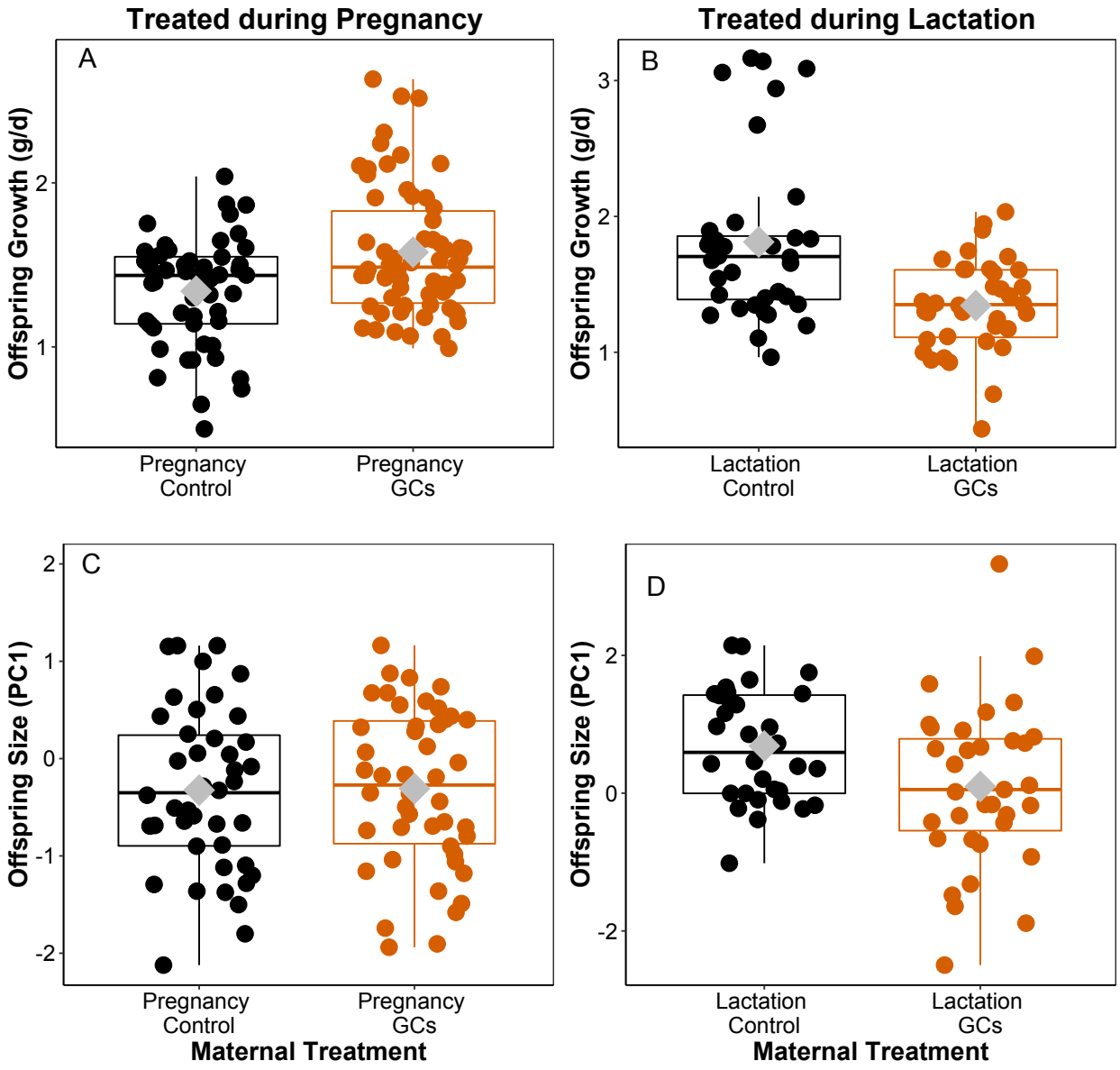
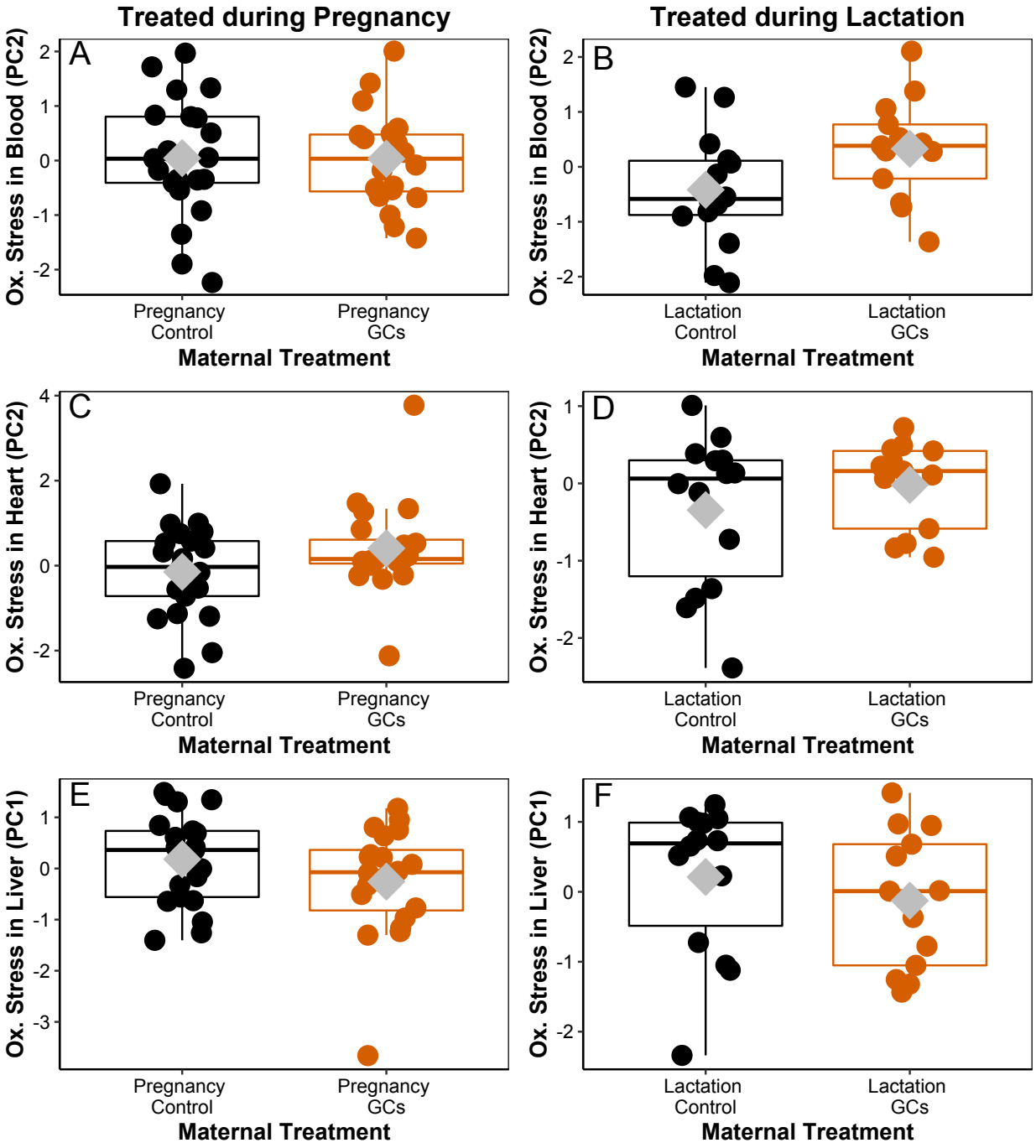
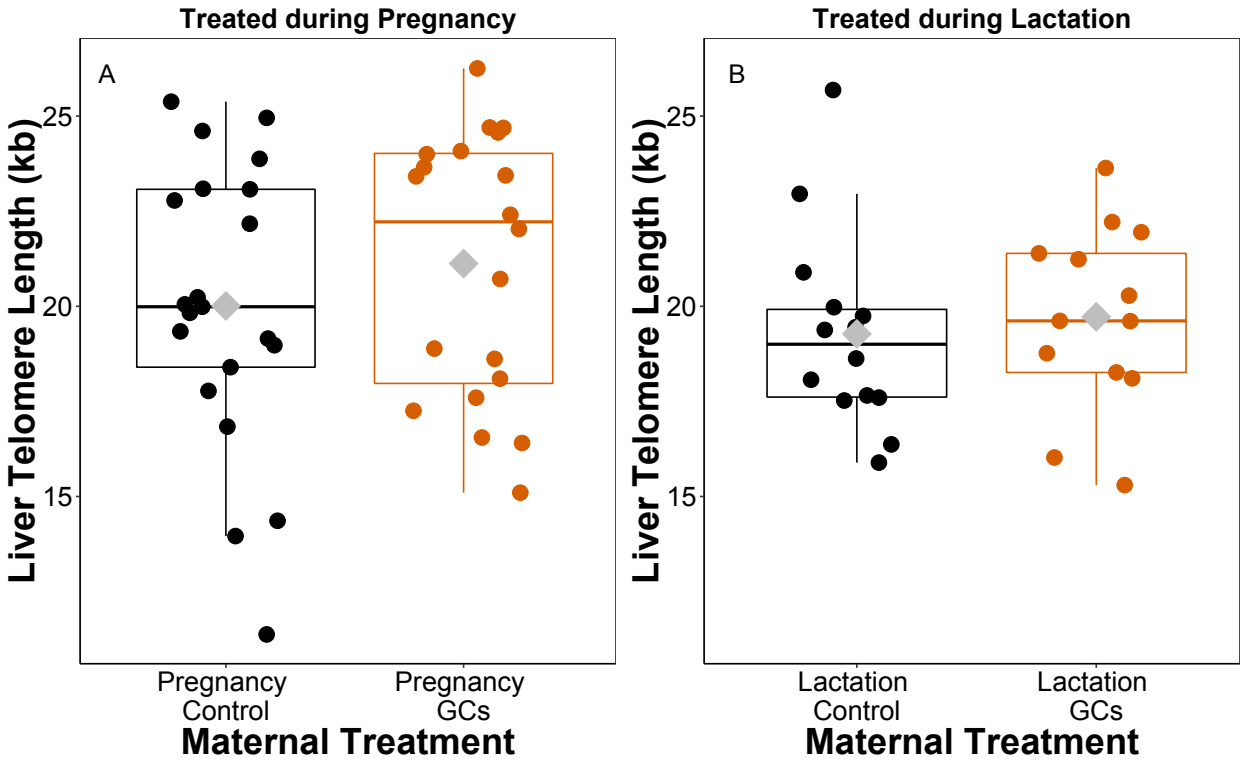


Figure 2. Effects of treating pregnant or lactating female red squirrels with glucocorticoids (GCs) on oxidative stress levels in blood, liver, and heart tissue from weaned juvenile red squirrels. There were no significant treatment effects on oxidative stress levels in the blood (**A, B**), heart (**C, D**), or liver (**E, F**) for offspring produced by mothers treated with GCs during pregnancy or lactation. Values on y-axes reflect a composite variable generated by separate principal component analyses for blood, heart, and liver tissue where high scores correspond to low levels of one or two of the antioxidants (TAC, SOD) and, for heart, higher levels of protein damage (PCC; see Table S1). Results in Tables S4 & S7. Note differences between y-axes among the panels.



801 **Figure 3. Effects of treating pregnant or lactating female red squirrels with glucocorticoids**
802 **(GCs) on mean liver telomere lengths in weaned offspring. A & B)** There were no significant
803 treatment effects on liver telomere lengths. Results shown in Table S5.
804



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Electronic Supplementary Material

Maternal glucocorticoids promote offspring growth without inducing oxidative stress or shortening telomeres in wild red squirrels

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9 **Supplementary Results**

10

Table S1. Results from principal component analyses to derive axes of variation of oxidative stress state in weaned pups. For each principal component shown, high values correspond to lower oxidative stress levels as they reflect samples with low levels of two (Blood PC2, Liver PC1) or one (Heart PC2) antioxidants (total antioxidant capacity, superoxide dismutase) and for heart tissue, higher levels of oxidative protein damage (protein carbonyls).

Measurement	**Tissue**		
	Blood PC2	**Heart PC2**	**Liver PC1**
Total Antioxidant Capacity	-0.57	-0.0007	-0.70
Superoxide Dismutase	-0.82	-0.71	-0.70
Protein Carbonyls	0.03	0.71	0.09
Prop. Variance Explained	34.3%	34.3%	38.5%

Table S2. Effects of treating female red squirrels with glucocorticoids (GCs) during pregnancy on offspring postnatal growth (A) and structural size (B). Offspring growth is the linear change in body mass from ~1 d to ~25 d of age. Offspring size is a composite variable where high scores of PC1 correspond to offspring (one estimate of size obtained at ~25 d of age) with larger zygomatic arch widths and longer hind foot lengths. Models contained random intercept term for litter identity (growth model: $\sigma^2 = 0.064$; size model: $\sigma^2 = 0.29$).

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Growth	**Intercept (2012, Control, Female)**	**1.87**	**0.12**	**15.6**	**35.6**	**<0.0001**
	Year (2015)	-0.15	0.2	-0.78	34.3	0.44
	Year (2016)	**-0.64**	**0.13**	**-4.92**	**34.2**	**<0.0001**
	Year (2017)	**-0.78**	**0.12**	**-6.6**	**34.4**	**<0.0001**
	Sex (Male)	**0.073**	**0.03**	**2.07**	**73.03**	**0.04**
	Birth date	0.014	0.05	0.28	34.5	0.78
	Litter size	0.15	0.07	2.04	35.9	0.048
	Treatment (GCs)	**0.28**	**0.09**	**3.17**	**42.3**	**0.003**
	Treatment (GCs) x Sex (Male)	-0.08	0.05	-1.61	74.1	0.11
	Treatment (GCs) x Litter size	-0.17	0.1	-1.77	35.9	0.08

Results based upon 114 offspring from 43 litters across 4 years

(B)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Size (PC1)	Intercept (2015, Control, Female)	0.004	0.40	0.01	31.2	0.99
	Year (2016)	**-0.98**	**0.41**	**-2.37**	**29.0**	**0.024**
	Year (2017)	-0.04	0.40	-0.10	29.4	0.92
	Sex (Male)	0.26	0.15	1.66	59.5	0.10
	Birth date	0.12	0.12	1.02	25.9	0.31
	Litter size	-0.05	0.23	-0.24	35.3	0.81
	Treatment (GCs)	0.06	0.25	0.23	45.6	0.82
	Treatment (GCs) x Sex (Male)	-0.09	0.22	-0.41	60.3	0.68
	Treatment (GCs) x Litter size	-0.12	0.28	-0.44	33.5	0.66

Results based upon 88 offspring from 34 litters across 3 years

Table S3. Effects of treating female red squirrels with GCs during (A) pregnancy or (B) lactation on offspring haematocrit levels (packed red blood cell volume) collected from weaned offspring. Results for the pregnancy model contained random intercept term for litter identity (pregnancy: $\sigma^2 = 18.5$) whereas results for the lactation model are from a general linear model.

(A)

Variable	b	SE	*t*	df	*P*-value
Intercept (2015, Control, Female)	**47.3**	**2.9**	**16.4**	**17.7**	**<0.0001**
Year (2016)	-5.86	3.3	-1.78	16.7	0.093
Year (2017)	-5.70	3.1	-1.82	16.8	0.086
Sex (Male)	1.86	1.1	1.71	17.0	0.10
Birth date	-0.61	1.5	-0.39	18.0	0.69
Growth	-1.25	1.4	-0.89	29.8	0.38
Litter size	-0.84	1.3	-0.69	18.1	0.51
Treatment (GCs)	-0.12	2.1	-0.06	18.7	0.95
Treatment (GCs) x Growth	1.99	2.4	0.84	29.3	0.41

Results based upon 39 offspring from 25 litters across 3 years

(B)

Variable	b	SE	t	df	P-value
Intercept (2015, Control, Female)	**44.7**	**2.6**	**16.9**	**15**	**<0.0001**
Year (2016)	**-8.75**	**4.3**	**-2.03**	**15**	**0.06**
Year (2017)	-3.18	2.2	-1.43	15	0.17
Sex (Male)	**4.65**	**2.1**	**2.18**	**15**	**0.045**
Birth date	**1.86**	**1.6**	**1.12**	**15**	**0.25**
Growth	-2.23	1.9	-1.19	15	0.25
Litter size	0.69	1.9	0.35	15	0.73
Treatment (GCs)	0.82	2.7	0.30	15	0.76
Treatment (GCs) x Growth	2.80	2.4	1.18	15	0.26

Results based upon 24 offspring from 16 litters across 3 years

Table S4. Effects of treating pregnant red squirrels with GCs on oxidative stress levels in (A) blood, (B) heart, and (C) liver tissue from weaned offspring. High PC scores correspond to low levels of antioxidants and, for heart, higher levels of protein damage (see Table 1). Models for blood and liver tissues contained random intercept term for litter ID (blood: σ^2 = 0.36; liver: σ^2 = 0.06).

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Blood PC2	Intercept (2015, Control, Female)	-0.08	0.5	-0.17	12.0	0.87
	Year (2016)	-0.14	0.6	-0.24	12.4	0.82
	Year (2017)	-0.29	0.5	-0.54	12.3	0.59
	Sex (Male)	0.59	0.3	2.06	22.9	0.051
	Birth date	-0.57	0.3	-2.05	16.7	0.057
	Growth	-0.09	0.3	-0.32	22.7	0.75
	Litter size	-0.37	0.2	-1.63	17.7	0.12
	Treatment (GCs)	0.05	0.4	0.13	15.1	0.89
	Treatment (GCs) x Growth	-0.61	0.5	-1.24	20.4	0.23

(B)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Heart PC2	**Intercept (2015, Control, Female)**	**-0.95**	**0.4**	**-2.16**	**32**	**0.038**
	Year (2016)	**1.29**	**0.5**	**2.48**	**32**	**0.019**
	Year (2017)	0.98	0.5	1.99	32	0.055
	Sex (Male)	-0.08	0.3	-0.24	32	0.81
	Birth date	0.52	0.3	1.97	32	0.057
	Growth	0.006	0.3	0.02	32	0.98
	Litter size	**0.49**	**0.2**	**2.23**	**32**	**0.033**
	Treatment (GCs)	0.31	0.3	0.86	32	0.39
	Treatment (GCs) x Growth	0.35	0.5	0.74	32	0.46

(C)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Liver PC1	**Intercept (2015, Control, Female)**	**-0.93**	**0.3**	**-2.78**	**12.5**	**0.016**
	Year (2016)	0.58	0.4	1.47	13.0	0.16
	Year (2017)	**1.75**	**0.4**	**4.65**	**12.6**	**0.0005**
	Sex (Male)	-0.001	0.2	-0.006	28.6	0.99
	Birth date	0.11	0.2	0.55	17.7	0.59
	Growth	-0.11	0.2	-0.51	19.5	0.61
	Litter size	0.05	0.2	0.29	20.1	0.78
	Treatment (GCs)	-0.37	0.3	-1.38	15.4	0.19
	Treatment (GCs) x Growth	-0.31	0.3	-0.89	18.3	0.38

Results from 41 offspring from 26 litters over 3 years.

Table S5. Effects of treating female red squirrels with GCs during (A) pregnancy or (B) lactation on liver telomere lengths (kb) of weaned offspring. Telomeres measured in DNA from liver tissue using the TRF method. Models contained random intercept term for litter identity (pregnancy: $\sigma^2 = 1.03$; lactation: $\sigma^2 = 6.5$).

(A)

Variable	b	SE	*t*	df	*P*-value
Intercept (2015, Control, Female)	**19.93**	**1.2**	**16.01**	**16.0**	**<0.0001**
Year (2016)	**4.37**	**1.4**	**3.20**	**14.0**	**0.006**
Year (2017)	-2.37	1.6	-1.48	20.4	0.15
Sex (Male)	0.40	0.8	0.52	26.9	0.60
Birth date	0.80	0.6	1.23	18.2	0.23
Growth	-0.45	0.7	-0.63	20.3	0.54
Litter size	-0.24	0.5	-0.45	19.9	0.66
Liver PC1	0.20	0.6	0.34	30.9	0.73
Treatment (GCs)	0.62	0.9	0.67	16.7	0.51
Treatment (GCs) x Growth	1.27	1.2	1.1	19.2	0.29

Results based upon 41 offspring from 26 litters across 3 years

(B)

Variable	b	SE	t	df	*P*-value
Intercept (2015, Control, Female)	**18.8**	**1.7**	**11.01**	**14.9**	**<0.0001**
Year (2016)	**2.14**	**3.6**	**0.59**	**10.3**	**0.56**
Year (2017)	0.44	2.4	0.18	15.6	0.86
Sex (Male)	0.22	0.9	0.24	10.5	0.81
Birth date	-0.04	1.1	-0.04	10.9	0.97
Growth	-0.03	0.8	-0.04	16.9	0.96
Litter size	0.66	1.5	0.43	11.0	0.68
Liver PC1	-0.49	0.8	-0.63	15.3	0.54
Treatment (GCs)	0.39	1.5	0.25	10.4	0.81
Treatment (GCs) x Growth	1.09	1.1	0.95	17.0	0.35

Results based upon 27 offspring from 18 litters across 3 years

Table S6. Effects of treating female red squirrels with GCs during lactation on offspring postnatal growth (A) and structural (skeletal) size (B). Offspring growth is the linear change in body mass from ~1 d to ~25 d of age. Offspring size is a composite variable where high scores of PC1 correspond to offspring (~25 d of age) with larger zygomatic arch widths and longer hind foot lengths. Models contained random intercept term for litter identity (growth model: σ^2 = 0.24; size model: σ^2 = 0.69).

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Growth	**Intercept (2015, Control, Female)**	**1.86**	**0.21**	**8.9**	**25.7**	**<0.0001**
	Year (2016)	-0.45	0.44	-1.03	22.4	0.31
	Year (2017)	-0.26	0.2	-1.29	22.6	0.21
	Sex (Male)	-0.005	0.06	-0.09	40.7	0.93
	Birth date	**0.19**	**0.11**	**1.69**	**22.7**	**0.10**
	Litter size	0.09	0.21	0.41	22.9	0.69
	Treatment (GCs)	**-0.37**	**0.21**	**-1.71**	**26.3**	**0.098**
	Treatment (GCs) x Sex (Male)	0.09	0.08	1.05	40.9	0.30
	Treatment (GCs) x Litter size	-0.01	0.24	-0.06	23.1	0.95

Results based upon 72 offspring from 30 litters across 3 years

(B)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Size (PC1)	Intercept (2015, Control, Female)	0.17	0.43	0.40	43.7	0.69
	Year (2016)	-0.7	0.71	-0.98	19.9	0.34
	Year (2017)	**0.74**	**0.34**	**2.18**	**22.6**	**0.04**
	Sex (Male)	0.19	0.3	0.63	43.1	0.53
	Birth date	**0.41**	**0.18**	**2.22**	**21.8**	**0.037**
	Litter size	-0.22	0.37	-0.60	25.9	0.55
	Treatment (GCs)	-0.67	0.43	-1.55	43.4	0.13
	Treatment (GCs) x Sex (Male)	0.04	0.37	0.10	42.5	0.92
	Treatment (GCs) x Litter size	0.60	0.42	1.44	26.5	0.16

Results based upon 67 offspring from 30 litters across 3 years

Table S7. Effects of treating lactating red squirrels with GCs on oxidative stress levels in (A) blood, (B) heart, and (C) liver tissue from weaned offspring. High PC scores correspond to low levels of antioxidants and, in heart tissue, higher levels of protein damage (Table 1). The model for liver contained a random intercept term for litter identity (liver: $\sigma^2 = 0.24$).

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Blood PC2	Intercept (2015, Control, Female)	0.41	0.5	0.80	18	0.43
	Year (2016)	-0.13	0.9	-0.15	18	0.88
	Year (2017)	-0.82	0.5	-1.67	18	0.11
	Sex (Male)	-0.51	0.5	-1.11	18	0.28
	Birth date	-0.19	0.3	-0.61	18	0.55
	Growth	0.36	0.3	1.18	18	0.25
	Litter size	-0.42	0.4	-0.97	18	0.35
	Treatment (GCs)	0.47	0.5	1.02	18	0.32
	Treatment (GCs) x Growth	-0.20	0.4	-0.49	18	0.63

(B)

Offspring trait	Variable	b	SE	t	df	*P*-value
Heart PC2	Intercept (2015, Control, Female)	-1.33	0.4	-3.37	18	0.0034
	Year (2016)	0.57	0.7	0.83	18	0.42
	Year (2017)	1.0	0.4	2.64	18	0.016
	Sex (Male)	0.33	0.3	0.93	18	0.36
	Birth date	0.21	0.2	0.89	18	0.38
	Growth	-0.04	0.2	-0.18	18	0.86
	Litter size	0.27	0.3	0.82	18	0.42
	Treatment (GCs)	0.68	0.3	1.92	18	0.07
	Treatment (GCs) x Growth	-0.24	0.3	-0.75	18	0.46

(C)

Offspring trait	Variable	b	SE	t	df	*P*-value
Liver PC1	**Intercept (2015, Control, Female)**	**-1.06**	**0.4**	**-2.78**	**13.2**	**0.015**
	Year (2016)	1.48	0.77	1.92	6.8	0.098
	Year (2017)	**2.21**	**0.4**	**5.68**	**9.2**	**0.0003**
	Sex (Male)	-0.03	0.3	-0.1	16.0	0.92
	Birth date	-0.31	0.2	-1.24	8.8	0.24
	Growth	-0.13	0.2	-0.59	16.8	0.56
	Litter size	-0.52	0.3	-1.49	8.6	0.17
	Treatment (GCs)	-0.17	0.4	-0.46	8.9	0.66
	Treatment (GCs) x Growth	-0.04	0.3	-0.12	13.9	0.90

Results from 27 offspring from 18 litters over 3 years.