Maternal stress promotes offspring growth without oxidative costs in wild red squirrels

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Abstract

Elevations in glucocorticoid levels (GCs) in breeding females (often called "maternal stress") 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% 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, and fast-growing offspring did not have higher oxidative stress levels or shorter telomere lengths. Our results indicate that elevations in maternal GCs may induce plasticity in offspring growth without oxidative costs to the offspring that might result in a shortened lifespan.

Introduction

Parents can have long-lasting impacts on their offspring across a diversity of taxa. These parental or maternal effects have drawn substantial interest because they suggest that parental characteristics or the parental environment itself could induce adaptive shifts in offspring traits

41 that prepare them for specific environments (i.e., adaptive transgenerational phenotypic
42 plasticity: 1-4). Furthermore, changes in maternal hormone levels, especially glucocorticoids
43 (GCs, are widely suspected to act as a mediator of transgenerational phenotypic plasticity in
44 vertebrates (6-7)

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

54 Some of these changes in offspring caused by elevations in maternal GCs are suspected
55 to reflect adaptive plasticity in offspring life history traits, such as modifying the trade-off
56 between early life growth and lifespan (20-21). Maternal stress may induce a "faster" life history
57 strategy whereby offspring produced by mothers with elevated GCs grow or develop faster (4,
58 22). Such adjustments in the "pace of life" may be adaptive, as fast postnatal growth or a quicker
59 developmental time may be beneficial when the risk of extrinsic mortality is heightened (23-24).
60 However, this increased investment in postnatal growth is expected to carry costs for offspring
61 longevity whereby fast-growing individuals exhibit a shortened lifespan (25-28).

62 Identifying if maternal stress induces a trade-off between growth and lifespan in wild
63 animals is needed, as any costs of maternal stress may be masked by benign environmental

64 conditions in the laboratory. Accurately documenting lifespan in wild animals remains
65 challenging in many species and the stochastic nature of mortality in wild animals may obscure
66 any mechanistic costs of maternal stress. Consequently, one way to examine if maternal stress
67 induces a trade-off between growth and longevity in wild animals is to examine how it affects
68 the possible underlying mechanisms of reduced longevity or physiological correlates that may
69 predict a shortened lifespan. The free-radical theory of aging (29) provides one framework to
70 examine the mechanisms by which maternal stress may induce this trade-off between offspring
71 growth and longevity. Reactive oxygen species (ROS) produced during aerobic respiration can
72 have damaging effects on cells (29-30). ROS production may be elevated by increased aerobic
73 respiration due to enhanced investment in growth or reproduction (28, 31-33) or by increased
74 GCs (34-37). Antioxidants produced by individuals (enzymatic antioxidants such as superoxide
75 dismutase) as well as those antioxidants from the external environment (non-enzymatic
76 antioxidants in the diet) can lessen the impact of ROS production (38). An important type of
77 oxidative damage occurs to the protective ends of chromosomes, called telomeres. Telomeres are
78 the repetitive DNA sequences that occur at the ends of eukaryote chromosomes whose length is
79 shortened during each cell division (39-40) and may also be reduced by the increased production
80 of ROS (28, 41-43). When telomeres reach a specific length, those cells become senescent and
81 stop dividing unless the enzyme telomerase, or another elongation process, is produced to
82 elongate the telomeres (44-45). Telomere length or rate of loss has been found to be predictive
83 (but perhaps not causal: 46) of the mortality risk of individuals (47-51), though the strength of
84 this relationship may vary among taxa and in relation to other life history traits. Furthermore,
85 avian or mammalian species with longer lifespans have been shown to exhibit slower age-
86 specific rates of telomere loss (52-55).

87 Maternal stress may, therefore, induce a life history trade-off between offspring growth
88 and longevity because offspring may experience elevated oxidative damage, decreased
89 antioxidant levels, and/or shortened telomeres either due to oxidative stress or increased cell
90 division associated with elevated growth (20, 28, 56-58). Previous studies across taxa show that
91 maternal stress can shorten telomere lengths in offspring (37, 59-61) or increase their rate of
92 attrition as they age (57), which could cause or be associated with a shortened lifespan. For
93 example, experimental studies in captive and wild birds show that offspring that had exogenous
94 GCs added to their eggs or GCs given during chick growth had a heightened physiological stress
95 response, higher levels of oxidative stress, and shorter telomeres early in life (37, 61). Despite
96 much interest in this topic, few studies in wild animals have examined if experimental elevations
97 in the GCs of breeding females impact the oxidative state of offspring or explicitly tested the
98 prediction that elevations in the GCs of breeding females increases early life growth.
99 Additionally, few studies have tested whether elevated GCs in breeding females or fast early life
100 growth comes at some cost by promoting oxidative stress and shortening telomeres in offspring.
101 We tested the hypothesis that elevations in maternal GCs would promote a faster life
102 history strategy in wild North American red squirrels (*Tamiasciurus hudsonicus*). We treated
103 females with GCs using a protocol that allowed us to increase circulating GCs within a
104 physiologically-relevant range (62). We treated females with GCs either during pregnancy or
105 lactation to assess if the timing of exposure to maternal GCs influenced their effects on offspring.
106 Other than for offspring growth in body mass, we did not have strong *a priori* expectations of
107 how the timing of maternal stress would differentially impact offspring because elevated
108 maternal GCs during pregnancy or lactation can impact offspring through the same pathways:
109 direct transfer of maternal GCs to offspring across the placenta or through milk

110 ("programming"), altering maternal behaviour, or affecting offspring behaviour (see references
111 above). However, based upon our previous study (4), we predicted that offspring produced by
112 mothers treated with GCs during pregnancy would grow faster in body mass. We did not have an
113 *a priori* expectation for how treating mothers with GCs during lactation would impact offspring
114 growth in body mass, though results from a previous study suggested that it should reduce
115 growth (17).

116 We measured offspring postnatal growth in body mass prior to weaning (~1 to 25 d of
117 age) and subsequently obtained measures of oxidative stress when pups were weaned (~70 d of
118 age). In three tissues (liver, heart muscle, and blood) collected from weaned offspring, we
119 measured one enzymatic antioxidant (superoxide dismutase), one type of non-enzymatic
120 antioxidant (total antioxidant capacity), and one type of oxidative damage (protein damage
121 measured via protein carbonyls). We used multiple tissues because other studies have
122 highlighted how experimental manipulations can have tissue-specific effects (63). To assess the
123 cumulative impact of elevated maternal GCs on the oxidative state of offspring and how
124 offspring growth impacted telomere lengths, we also measured telomere lengths in DNA from
125 the liver. Although we only measured telomere lengths in one tissue, previous studies indicate
126 that telomere lengths measured in one somatic tissue are strongly correlated with those in others
127 (64).

128 We predicted that offspring from mothers treated with GCs during pregnancy would
129 grow quicker in body mass after birth but would experience more oxidative stress (manifested as
130 a reduction in antioxidants and an increase in oxidative damage) and decreased telomere length,
131 which would be a result of increased oxidative stress or increased cell division associated with
132 faster growth. Because we have previously found that female red squirrels can ameliorate the

133 trade-off between offspring number and growth (4; 65), we examined if elevated maternal GCs
134 altered the trade-off between litter size and offspring growth or structural (skeletal) size. Because
135 early life exposure to GCs may modify the direction and strength of the association between two
136 variables (66-67), we also examined if increases in maternal GCs affected the expected negative
137 relationship between offspring growth and oxidative stress state (33) by assessing the statistical
138 interaction between offspring growth and maternal treatment.
139

140 **Materials and Methods**

141 *Study area & measuring offspring growth*

142 We conducted this study as a part of a long-term study of red squirrels in the Yukon,
143 Canada that takes place on the traditional territory of the Champagne and Aishihik First Nations.
144 Squirrels in our study population were all marked individually with unique ear tags and
145 combinations of coloured wire threaded through the ear tags (68). Females in our study
146 population usually produce one litter in the spring and rarely produce more than one litter of
147 offspring to weaning per year (69). Females were captured and handled every ~3 to 21 d to
148 assess reproductive status through abdominal palpation and nipple condition. Pups were accessed
149 from the nest two times. The first nest entry occurred immediately after parturition and the
150 second nest entry occurred when pups were approximately 25 d of age. At both nest entries, pups
151 were briefly removed from their nest, sexed, and weighed to the nearest 0.01 g using a portable
152 balance. At the first nest entry, we marked them uniquely by obtaining a small ear biopsy (for
153 later paternity analyses) and then we permanently marked pups at the second nest entry with
154 unique metal ear tags. Because offspring growth in body mass during this period of time is
155 approximately linear (70), we estimated offspring growth as the change in body mass from the

156 first to second nest entry divided by the total number of days elapsed between the two measures
157 of body mass. At the second nest entry, we measured zygomatic arch width and right hind foot
158 length to the nearest 1 mm using digital callipers or a ruler, respectively.

159 *Maternal treatments*

160 We used four separate treatment groups to assess the effects of elevated maternal GCs on
161 offspring over four different years (2012, 2015-2017), although in 2012 we only collected
162 growth in body mass data. Individual females were treated with GCs either during pregnancy or
163 lactation ("Pregnancy GCs" and "Lactation GCs"), whereas other females were treated as
164 controls during pregnancy or lactation ("Pregnancy Controls" or "Lactation Controls"). We
165 increased maternal GCs either during pregnancy or lactation using an established experimental
166 protocol (4, 62). Briefly, we treated females in the Pregnancy GCs (n = 42 litters) and Lactation
167 GCs (n = 18 litters) treatment groups with exogenous cortisol (hydrocortisone, Sigma H4001)
168 dissolved in peanut butter and wheat germ mixture (8 g of peanut butter, 2 g of wheat germ).
169 Females in the Pregnancy Control (n = 30 litters) or Lactation Control (n = 17 litters) treatment
170 groups were fed the same amount of peanut butter and wheat germ mixture but lacking the
171 cortisol. GC treatments were prepared by dissolving hydrocortisone in 1 mL of 100% ethanol
172 and then 5 mL of 100% peanut oil before allowing the emulsion to sit overnight so that the
173 ethanol could evaporate. The following morning, the hydrocortisone emulsion was thoroughly
174 mixed with the appropriate amount of peanut butter and wheat germ, weighed out into individual
175 dosages (~10 g each), placed into an individual container, and then frozen at -20 °C until
176 provisioning to the treated squirrels.

177 Each day during the treatment period, we placed individual dosages into a bucket that
178 was hung ~7-10 m off the ground on the centre of the squirrel's territory. Squirrels defend these

179 buckets from all other conspecifics and heterospecifics (62), so we can, therefore, be confident
180 that the squirrels that were given these treatments were consuming them. This procedure causes
181 long-term elevation of circulating cortisol levels and faecal glucocorticoid metabolite levels (62).
182 Additional details are provided in the electronic supplementary material (ESM).

183 *Tissue sample collection*

184 Pups are weaned when they are approximately ~70 d of age and generally stay on their
185 natal territory until dispersal soon after (71). When juvenile squirrels were ~70 d of age, they
186 were euthanized and tissues (liver and cardiac muscle) were immediately removed, rinsed with
187 PBS buffer, snap frozen on dry ice, and then stored in liquid nitrogen or in a -80 °C freezer until
188 analysis. Trunk blood was collected through decapitation and then centrifuged at 10,000 g for 10
189 min at room temperature to separate plasma and red blood cells.

190 *Haematocrit*

191 We measured packed red blood cell volume (haematocrit) as a measure of body condition
192 where higher levels correspond to better body condition (72). Before pups were euthanized, we
193 collected a blood sample from the hind claw into a heparinized capillary tube. Haematocrit was
194 quantified using a micro-capillary reader after centrifuging blood samples at 10,000 g for 10 min
195 at room temperature.

196 *Protein carbonyls*

197 We measured oxidative damage to proteins (73) using the protein carbonyl colorimetric
198 kit by Cayman Chemical (Ann Arbor, USA). Briefly, ~200 mgs of cardiac muscle or liver were
199 homogenized in ~1000 µL of 50mM MES buffer containing 1mM EDTA using a sonicator, and
200 then centrifuged at 10,000 g for 15 min at 4 °C. The protein concentration of tissue homogenate
201 supernatant and plasma samples was measured prior to the assay using a Biotek Take3 protocol

202 (Biotek, Vermont, USA) and samples were diluted in PBS buffer to give a protein range between
203 1-10 mg/ml, as recommended by the manufacturer. The average intra-assay CV for samples for
204 plasma, heart, or liver were 1.2%, 2.8%, and 1.6%, respectively. Inter-assay CVs for a red
205 squirrel pooled sample run on repeat assays for plasma (n = 7 assays), heart (n = 6), or liver (n =
206 7) were 3.5%, 9.0%, and 8.3%, respectively. We also ran a positive control (oxidized bovine
207 serum albumin) in two different assays and the inter-assay CV was 3.1%.

208 *Superoxide Dismutase*

209 We obtained one measure of the levels of enzymatic antioxidants (73) by quantifying
210 levels of superoxide dismutase (SOD) using the SOD kit from Cayman Chemical. SOD was
211 expressed as units/mg/ml protein (quantified using a Biotek Take3 protocol). Red blood cells
212 were lysed as per the manufacturer's protocol. The average intra-assay CV for samples for
213 RBCs, heart, or liver were 2.3%, 4.7%, and 3.1%, respectively. Inter-assay CVs for a red squirrel
214 pooled sample run on repeat assays for RBCs (n = 10 assays), heart (n = 2), or liver (n = 4) were
215 16.9%, 4.6%, and 6.5%, respectively.

216 *Total Antioxidant Capacity*

217 We obtained one measure of the levels of non-enzymatic antioxidants (73) by quantifying
218 total antioxidant capacity (TAC) using the TAC kit from Cayman Chemical. Plasma was diluted
219 in assay buffer and assayed according to the manufacturer's protocol. Liver and cardiac muscle
220 (~47 mg) were separately homogenized in 250 µL PBS using a sonicator and the supernatant was
221 diluted in assay buffer and used in the assay. The average intra-assay CVs for samples for
222 plasma, heart, or liver were 4.7%, 3.2%, and 4.7%, respectively. The inter-assay CVs for
223 standards run on all the plates for plasma (n = 5 assays) was 15.8% whereas the inter-assay CV

224 for a red squirrel pooled sample run on repeat for heart (n = 8) or liver (n = 2) were 15.4% and
225 5.4%, respectively.

226 *Telomeres*

227 Liver telomere lengths were measured using the telomere restriction fragment (TRF)
228 assay following established methods (74). Briefly, 2 to 10 g slices of liver tissue were
229 homogenized in cell lysis solution and proteinase K (Qiagen, Germantown, USA). DNA was
230 extracted from the liver homogenates and resuspended in buffer. The resuspended DNA was
231 restriction digested with 15 U of HinfI, 75U of HaeIII and 40U of RsaI (New England BioLabs,
232 Ipswich, USA) at 37˚C. DNA was then separated using pulsed field electrophoresis at 14˚C for
233 19 hours followed by in-gel hybridization overnight at 37˚C with a radioactively labeled
234 telomere-specific oligo (CCCTAA)4. Hybridized gels were placed on a phosphorscreen
235 (Amersham Biosciences, Buckinghamshire, UK), which was scanned on a Typhoon Imager
236 (Amersham Biosciences). Densitometry in ImageJ (v. 1.51s) was used to determine the position
237 and the strength of the radioactive signal in each of the lanes compared with the molecular
238 marker (Quick-Load1 kb DNA Extend DNA Ladder; New England BioLabs) to calculate
239 telomere lengths for each sample. Inter-gel variation was accounted for by calculating the mean
240 TRF length of standard samples run on each gel.

241 *Statistical analyses*

242 We assessed the effects of maternal treatments on offspring growth in body mass and a
243 single measure of size using separate linear mixed-effects models (LMMs) for pregnancy and
244 lactation treatments. Each of the four LMMs included a fixed effect for maternal treatment and
245 covariates (sex, year, birth date, litter size) that could impact offspring growth or size. We
246 included a two-way interaction term between treatment and litter size to identify if elevations in

247 maternal GCs altered the trade-off between litter size and offspring growth, as shown previously
248 for offspring growth (4). We included a two-way interaction between treatment and sex to assess
249 if the treatments had sex-specific effects on growth and size, as documented in other species
250 (75). We used a principal component analysis (PCA) using a covariance matrix in the R package
251 ade4 (version 1.7-13, 76) to generate a composite score of offspring size. The first principal
252 component axis (PC1, hereafter size) explained 69.8% of the variation in offspring size as
253 measured by zygomatic arch width and hind foot length. Both zygomatic arch width (0.71) and
254 hind foot length (0.71) loaded positively on PC1, indicating that larger PC1 scores corresponded
255 to offspring with longer hind feet and wider crania. Due to repeated observations on the same
256 litters, we included random intercept term of litter ID in these models.

257 Oxidative stress reflects an imbalance between antioxidants and the production of ROS
258 that can damage proteins, lipids, or DNA (73). Consequently, the effects of our treatments on
259 measures of antioxidants should not be viewed in absence of their effects on our measures of
260 oxidative damage (77). We used a PCA to create a composite variable that reflected the oxidative
261 state of an offspring. The PCA was composed of the two antioxidants (SOD, TAC) and one
262 measure of oxidative damage (PCC). We conducted a separate PCA (using a correlation matrix)
263 for each tissue type using the package ade4. For some individuals, we were missing measures of
264 TAC (heart: n = 3; plasma: n = 2) or PCC (heart: n = 2; plasma: n = 4) so we substituted average
265 values for the PCA.

266 Low scores for Blood PC2, Heart PC2, or Liver PC1 corresponded to squirrels that were
267 exhibiting oxidative stress as they represented lower levels of the two antioxidants (SOD, TAC)
268 for blood and liver tissue or just one antioxidant (SOD) for heart tissue and, for heart tissue,
269 higher levels of protein damage (PCC, Table S1). We used these composite variables describing

270 oxidative state of each tissue, haematocrit, or telomere length as the response variables in
271 separate LMMs. Each of these LMMs contained a fixed effect for maternal treatment and
272 offspring sex, year, birth date, and litter size. Because offspring growth may impact oxidative
273 stress levels or telomere lengths (28), we included a two-way interaction term between treatment
274 and offspring growth to examine if mothers with elevated maternal GCs exhibited an altered
275 relationship between growth and the response variable (66-67). Due to smaller sample sizes for
276 these variables, we did not include an interaction between sex and treatment. In the model to
277 assess treatment effects on telomere lengths, we also included a fixed effect for the oxidative
278 stress levels in the liver (Liver PC1). We included a random intercept term for litter ID for all of
279 these models except if the model indicated that the variance in the random effect was exactly 0.
280 All analyses were conducted in R (version 3.5.2, 78) using lme4 (version 1.1-18-1, 79)
281 and *P*-values were estimated using *lmerTest* (version 3.0-1, 80). Continuous predictor variables
282 were standardized (mean of 0, SD of 1) with birth date, litter size, and growth being standardized
283 within each grid-year combination. Assumptions of homoscedasticity, normality of residuals for
284 our LMMs, and a lack of high leverage observations were confirmed using diagnostic plots (81).
285 We estimated variance inflation factors (VIFs) from our models to assess multicollinearity
286 among the predictor variables (81) and VIFs indicated that multicollinearity was not an issue in
287 these models (all VIF < 3.31 except if included in an interaction or a multi-level categorical
288 variable).
289
290 **Results**
291 *Effects of treating pregnant females with GCs on offspring*

292 Offspring from mothers treated with GCs during pregnancy grew 17% faster ($t_{41.6} = 3.04$,
293 $P = 0.004$, Table S2A, Fig. 1A) but were not larger in structural size (Table S2B, Fig. 1C), and
294 did not differ in body condition (as reflected in their haematocrit levels: Table S3A) than those
295 produced by control mothers. There was no indication that the treatments had sex-specific effects
296 on offspring growth or size (Table S2). There was also no indication of a trade-off between litter
297 size and offspring growth or size during the years that we studied (Table S2) nor was there any
298 evidence that treating mothers with GCs during pregnancy altered the relationship between litter
299 size and growth rate, as indicated by the lack of significant interactions between treatment and
300 litter size for offspring growth and size (Table S2). Because the treatments had no significant
301 effects on litter size or litter sex ratio (ESM), the effects of the treatments on offspring growth
302 were not simply due to a reduction in litter size.

303 Despite growing faster, offspring from mothers treated with GCs during pregnancy did
304 not have higher oxidative stress levels in the blood, liver, or heart (Table S4, Fig. 2) and they
305 also did not have shorter telomere lengths (Table S5, Fig. 3). Offspring from mothers treated
306 with GCs during pregnancy that grew faster did not have higher oxidative stress levels in blood,
307 heart, or liver nor did they have shorter telomere lengths (Tables S4-S5). There was no indication
308 that growth or its interaction with maternal treatment impacted oxidative stress levels (Table S4)
309 or liver telomere lengths (Table S5) in offspring from females treated during pregnancy.

310 *Effects of treating lactating females with GCs on offspring*

311 Offspring from mothers treated with GCs during lactation grew 34.8% slower ($t_{26.4} = -$
312 2.14, $P = 0.04$, Table S6A, Fig. 1B), but were not significantly smaller in structural size (Table
313 S6B, Fig. 1D), and did not differ in body condition (as reflected in haematocrit levels: Table
314 S3B) from those of pups from control mothers. There was no indication that the treatments had

315 sex-specific effects on offspring growth or size, as reflected in the lack of significant sex x
316 treatment interactions (Table S6). There was also no indication of a trade-off between litter size
317 and offspring growth or size (Table S6) and no evidence that treating females with GCs during
318 lactation altered the relationship between litter size and growth rate, as indicated by the lack of
319 significant interactions between treatment and litter size for offspring growth and size (Table
320 S6). Because the treatments had no significant effects on litter size or litter sex ratio (ESM), the
321 effects of the treatments on offspring growth were not simply due to a reduction in litter size.
322 Offspring from mothers treated with GCs during lactation did not have higher oxidative
323 stress levels than those from control mothers in any of the three tissues (blood, heart, liver: Table
324 S7, Fig. 2B, 2D, 2F) and they also did not have shorter telomere lengths (Table S5, Fig. 3B).
325 Offspring from mothers treated during lactation that grew faster did not have higher oxidative
326 stress levels in blood, heart, or liver nor did they have shorter telomere lengths (Table S7). There
327 was also no indication that growth or its interaction with maternal treatment impacted oxidative
328 stress levels (Table S7) or liver telomere lengths (Table S5) in offspring from females treated
329 during lactation.
330
331 **Discussion**
332 Mothers treated with GCs during pregnancy produced faster growing offspring whereas
333 mothers treated with GCs during lactation produced slower growing offspring. There were no
334 treatment effects on offspring structural size, indicating that while offspring from mothers treated
335 with GCs gained mass at a different rate than the controls, the treatments did not influence
336 skeletal size. However, we only obtained one measure of structural size when offspring were ~25
337 d of age and therefore did not quantify any treatment effects in the rate of change in structural

338 size as we did for body mass. Our results differ from a recent literature analysis across mammals
339 showing that offspring from mothers experiencing late gestational stress grew more slowly
340 before weaning (21). One explanation is that elevated GCs simply modulated the trade-off
341 between offspring quantity and quality (e.g., producing small litters of fast-growing offspring) or
342 ameliorated the trade-off between offspring quantity and quality (e.g., lessening the effect of
343 increased litter size on the growth rate of each individual offspring: 82). This is unlikely because
344 we found no treatment effects on litter size (results in ESM) nor on the trade-off between litter
345 size and growth rate. Thus, somehow females treated with GCs during pregnancy produced fast
346 growing offspring without merely reducing their litter sizes, though it is notable that in the years
347 in which we conducted this study, we also did not document a trade-off between litter size and
348 offspring growth in any of the treated or control females.

349 We did not find support for the hypothesis that elevated maternal GCs during pregnancy
350 or lactation or increased offspring growth elevated oxidative stress levels or shortened telomere
351 lengths in offspring, as other studies have predicted (20, 28, 57). This is surprising and requires
352 explanation. First, we measured oxidative stress levels in offspring when they were weaned (~70
353 d of age) whereas we treated their mothers with GCs either during pregnancy or early lactation.
354 Thus, it is possible that the offspring in our study experienced elevated oxidative stress levels but
355 these effects had disappeared by weaning. A second possibility is an artefact associated with
356 selective disappearance of poor-quality individuals from those mothers treated with GCs during
357 pregnancy, such as slow growing individuals with short telomeres dying before we could obtain
358 our measures of oxidative stress and telomere lengths. This is unlikely as we observed no
359 treatment effects on litter size or the reduction in litter size from the first to second nest entry
360 (results shown in ESM). Finally, the fact that mothers treated with GCs did not produce offspring

361 with elevated oxidative stress levels or shorter telomeres may have been because of the effects of
362 maternally-derived GCs on offspring telomerase levels, an enzyme that is capable of rebuilding
363 telomeres or buffering them from attrition (83). We did not measure telomerase levels, but a
364 previous study showed that long-term exposure of laboratory rats to unpredictable stressors
365 increased the production of telomerase (84). We think that the most plausible explanation is that
366 treating mothers with GCs may have promoted increases in telomerase or enzymatic antioxidant
367 production that had protective effects on offspring. This would be consistent with predictions
368 from the oxidative shielding hypothesis (32) that proposes that females may reduce their own
369 levels of oxidative damage (perhaps by upregulation of enzymatic antioxidants) to mitigate their
370 detrimental influence on offspring.

371 Our results indicate that elevated GCs can impact maternal investment in the current
372 litter. Females experiencing elevated GCs during pregnancy increased their investment in the
373 current litter whereas females experiencing elevated GCs during lactation reduced their
374 investment in the current. Life history theory predicts that such changes in maternal investment
375 in offspring could alter the survival or future reproduction of mothers (82) or increase their
376 oxidative stress levels (32, 85). However, our previous work in red squirrels shows that their
377 food caching nature results in unexpected patterns with respect to the potential costs of
378 reproductive investment. Red squirrels can elevate reproductive output (i.e., producing a second
379 litter or larger litters) in anticipation of increased future food abundance (69) or produce faster
380 growing offspring when the fitness payoffs warrant increased investment in the current litter (4).
381 They seem to be able to do this without additional access to food except the food that they
382 already have stored from the previous autumn (4, 69, 86). Thus far, costs for this increased
383 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 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 increased oxidative damage due to their elevated reproductive investment or because of the elevated levels of GCs that they experience. For example, previous studies in red squirrels (31) and other species (32) highlight that increased reproductive investment or increased exposure to GCs (34-37) may elevate oxidative damage in breeding females with elevated GCs during reproduction.

Although it is not known whether increased antioxidants, reduced oxidative damage, or elongated telomeres actually cause an increase in longevity (46), our results suggest that fast growing offspring or those from mothers treated with GCs during pregnancy or lactation would not experience a reduction in lifespan. Our results and our previous study in red squirrels (4) show that maternal GCs during pregnancy or lactation can induce plasticity in offspring growth and that this plasticity should be adaptive for high density environments (4). However, we did not find support for the hypothesis that elevated maternal GCs induce a faster pace of life where offspring grow faster and are more competitive early in life but this comes at some oxidative cost that may predict a shortened lifespan. Future studies should assess oxidative stress using an even broader array of measures than the few measures we used here and will of course need to assess if elevated maternal GCs actually impact offspring lifespan.

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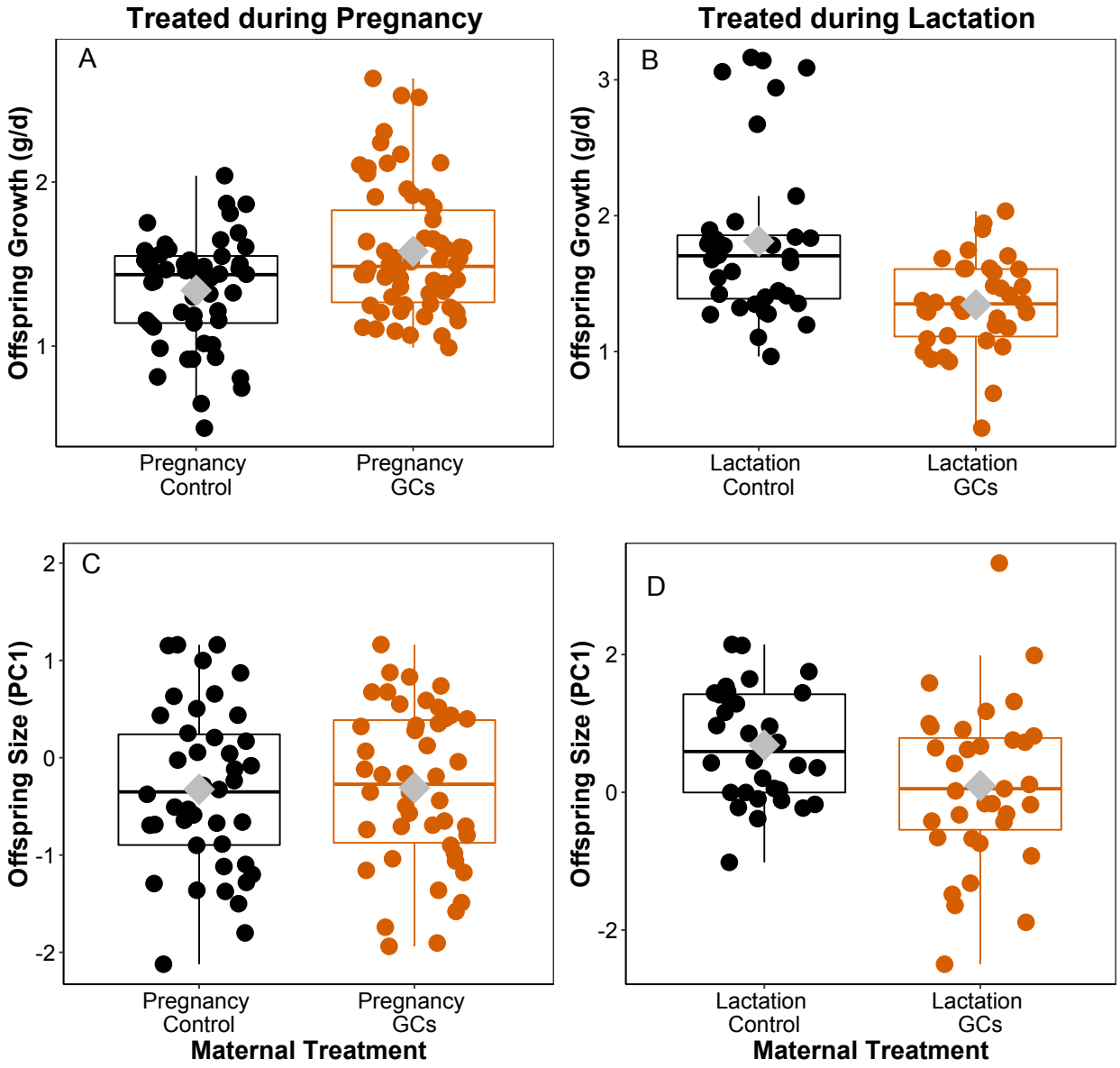
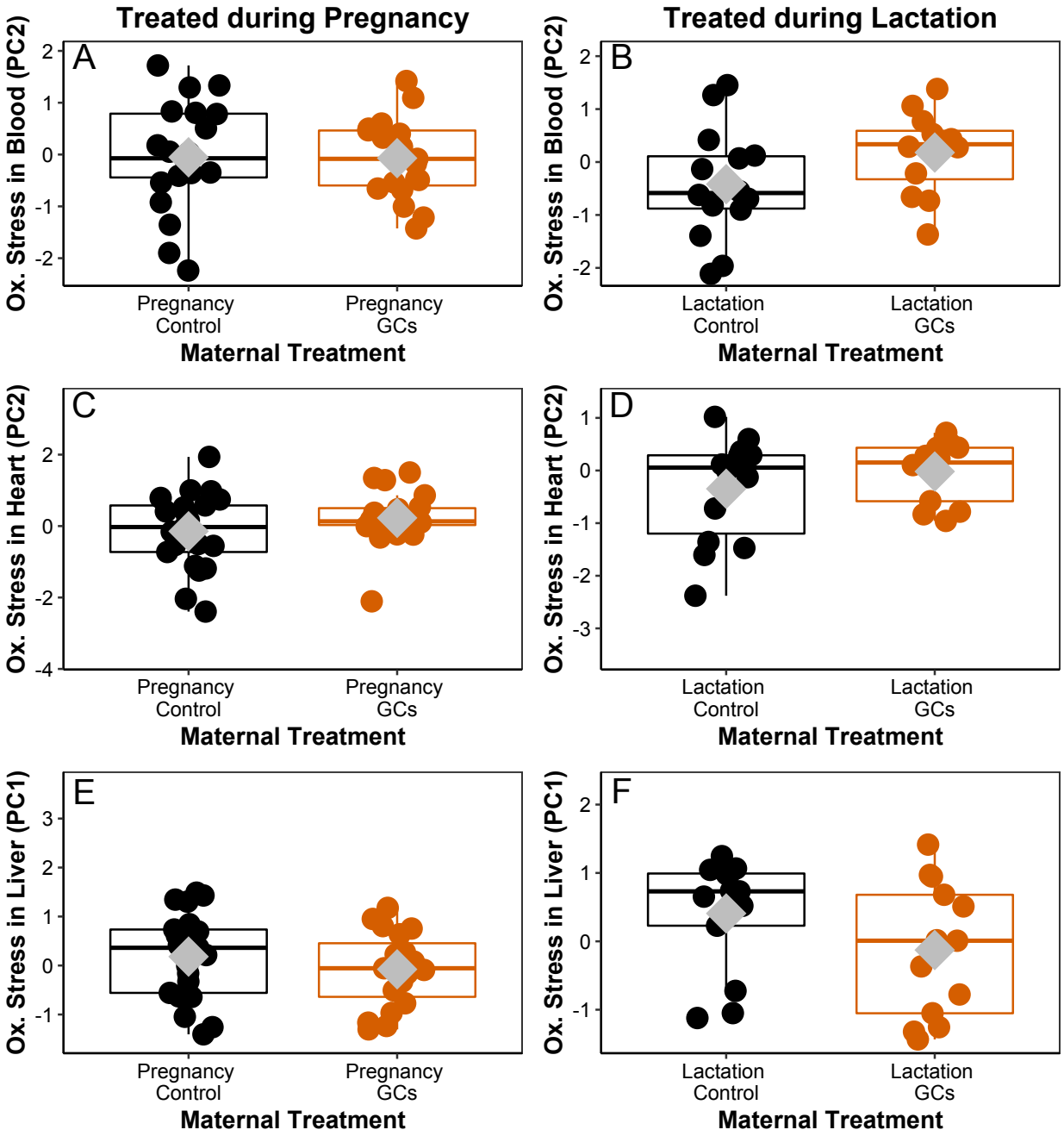
629

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 size is a composite variable where high scores of PC1 correspond to offspring with larger zygomatic arch widths and longer hind foot lengths. 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.

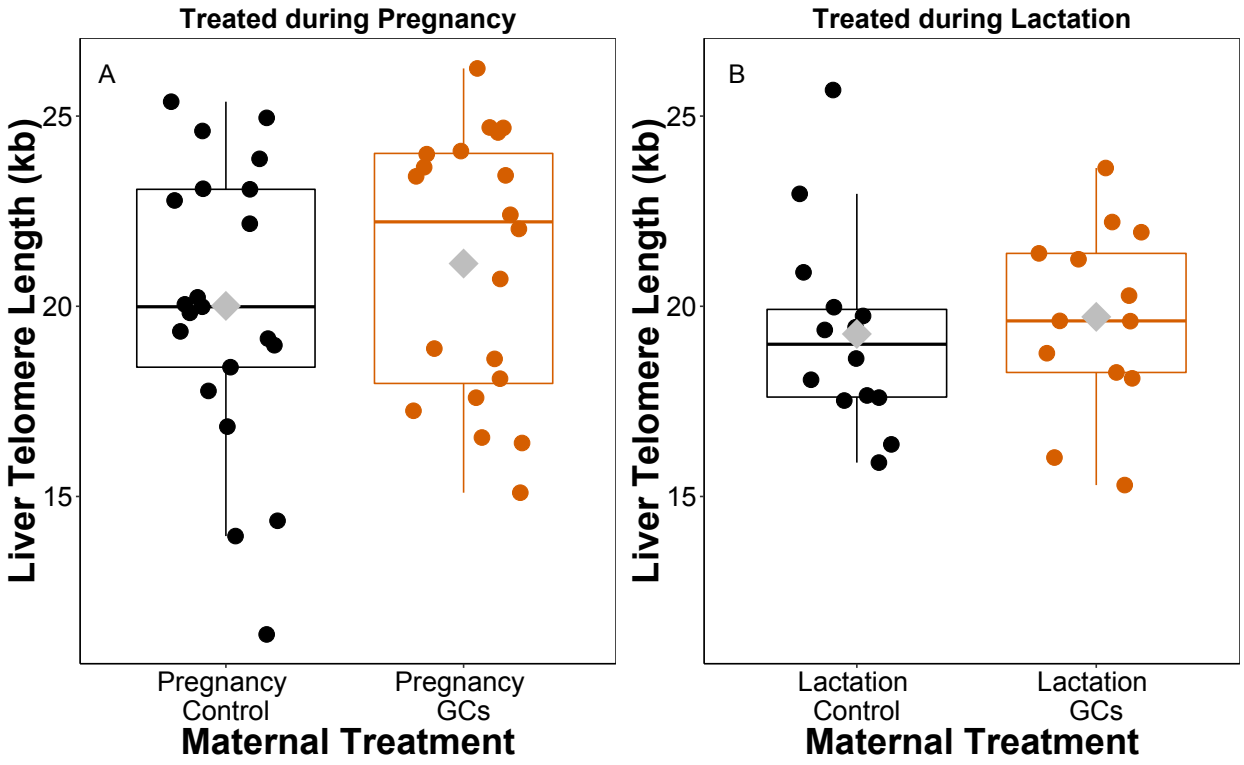


641 **Figure 2. Effects of treating pregnant or lactating female red squirrels with glucocorticoids**
642 **(GCs) on oxidative stress levels in blood, liver, and heart tissue from weaned juvenile red**
643 **squirrels.** There were no significant treatment effects on oxidative stress levels in the blood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



650

651 **Figure 3. Effects of treating pregnant or lactating female red squirrels with glucocorticoids**
652 **(GCs) on mean liver telomere lengths in weaned offspring. A & B)** There were no significant
653 treatment effects on liver telomere lengths. Results shown in Table S5.
654



655
656

657 *Electronic Supplementary Material*

658

659 **Maternal stress promotes offspring growth without oxidative costs in wild red squirrels**

660

661 Ben Dantzer[1,2], Freya van Kesteren[1], Sarah E. Westrick[1], Stan Boutin[3], Andrew G. McAdam[4],

662 Jeffrey E. Lane[5], Robert Gillespie[6], Ariana Majer[7], Mark Haussmann[7], Pat Monaghan[6]

663

664 **Supplementary Materials and Methods**

665 *Maternal treatments*

666 Dosage of hydrocortisone varied among some of the treatment groups but we have
667 previously shown that either 3, 6, 8, or 12 mg of hydrocortisone per day significantly elevates
668 plasma cortisol and faecal glucocorticoid metabolite levels within a biologically-relevant range
669 (Dantzer et al., 2013; van Kesteren et al., 2019). Females in the Pregnancy GCs treatment group
670 were provisioned either with 3 mg (4 litters), 6 mg (n = 6 litters), 8 mg (n = 25 litters), or 12 mg
671 (7 litters) of hydrocortisone whereas females in the Lactation GCs treatment groups were
672 provisioned either with 8 mg per day (10 litters) or 12 mg per day (8 litters). Although the
673 dosage administered to females varied, we grouped those administered GCs in the same
674 treatment group (e.g., Pregnancy GCs contained females administered 3-12 mg of
675 hydrocortisone) as in a previous one-year study (Dantzer et al., 2013) we found that the effects
676 GC dosages of 3, 6, and 12 mg per day on growth were in the same direction.

677 We aimed to treat females in the pregnancy treatments from approximately 20 d after
678 conception until 5 d after birth (or 20-40 d post-conception as red squirrels on average have a
679 ~35 d gestation period), whereas we actually treated females in the pregnancy GCs treatment
680 group from 24.1 ± 0.9 d (mean ± SE) to 38.9 ± 0.6 d post-conception (mean ± SE treatment
681 duration: 14.9 d ± 0.8 d) and females in the pregnancy control treatment group from 20.1 ± 0.9 d
682 to 38.2 ± 0.6 d (treatment duration: 18.1 ± 0.8 d). We aimed to treat females in the lactation
683 treatments from approximately 5 d after parturition until 15 d post-parturition, whereas we
684 actually treated females in the lactation GCs treatment group from 5.4 ± 0.5 d (mean ± SE) to
685 14.5 ± 0.6 d post-conception (mean ± SE treatment duration: 9.1 d ± 0.1 d) and females in the
686 lactation control treatment group from 4.9 ± 0.4 d to 14.1 ± 0.5 d (treatment duration: 9.2 ± 0.5

687 d). Given a ~35 d gestation period and a ~70 d lactation period in this population (Boutin et al.,
688 unpub. data), our pregnancy treatments corresponded to treating females during the last trimester
689 of gestation and into the first few days of lactation whereas our lactation treatments corresponded
690 to early lactation before offspring begin to feed independently (they typically leave the nest on
691 their own for the first time at ~35 d: Boutin et al., unpub. data). Note that this means that the
692 lactation treatments occurred during a time when the offspring would not be able to consume any
693 of the treatments themselves so any effects were due to the mother.

694 *Statistical Analyses*

695 We assessed the effects of maternal treatments on litter survival from the first to second
696 nest entry (proportion of pups present at both first and second nest entries) and litter size and
697 litter sex ratio (proportion of litter composed of males) as recorded at the first and second nest
698 entry using generalized linear mixed-effects models (GLMMs: litter survival and litter sex ratio,
699 using binomial errors) or a linear mixed-effect model (LMM: litter size). Each of these separate
700 models contained maternal treatment, birth date, and year as fixed effects. Models for lactation
701 and pregnancy treatments were run separately. There was one litter where litter size at the second
702 nest entry was greater than the at the first, likely because we missed a pup in the nest at the first
703 nest entry, and we excluded this litter from our analyses. We confirmed that none of the GLMMs
704 were overdispersed as all dispersion parameters were <1 (0.48 to 0.72).

705

706

707 **Supplementary Results**

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

709 There was no evidence that treating mothers with GCs during pregnancy or lactation
710 caused litter failure or altered litter size or litter sex ratio compared to the controls. For those
711 mothers producing offspring until at least the first nest entry (occurring soon after pups were
712 born), the proportion of the litter that survived from the first to the second nest entry did not
713 differ between mothers treated with GCs during pregnancy (n = 42 litters, 51.8 ± 9% of total
714 pups survived from the first to second nest entry) and the controls (n = 30 litters, 60 ± 8%, z = -
715 0.46, P = 0.65), nor between mothers treated with GCs during lactation (n = 18 litters, 79.6 ± 8%
716 pups survived) and the controls (n = 17 litters, 72.6 ± 9.5% pups survived, z = -0.67, P = 0.5).

717 Litter size did not differ between mothers treated with GCs during pregnancy or the
718 controls at the first nest entry (Pregnancy GCs: n = 42 litters, 3.05 ± 0.14 pups, range = 1-5 pups;
719 Pregnancy Controls: n = 31 litters, 2.84 ± 0.16 pups, range = 1-5 pups, effect of treatment, t_{67} =
720 0.58, P = 0.56) nor at the second nest entry (Pregnancy GCs: n = 23 litters, 2.87 ± 0.22 pups,
721 range = 1-5 pups; Pregnancy Controls: n = 21 litters, 2.71 ± 0.16 pups, range = 1-4 pups, effect
722 of treatment, t_{38} = 0.18, P = 0.85). Litter size also did not differ between mothers treated with
723 GCs during lactation or the controls at the first nest entry (Lactation GCs: n = 18 litters, 2.62 ±
724 0.2 pups, range = 1-4 pups; Lactation Controls: n = 17 litters, 2.93 ± 0.13 pups, range = 2-4 pups,
725 effect of treatment, t_{30} = -1.17, P = 0.25), nor at the second nest entry (Lactation GCs: n = 16
726 litters, 2.25 ± 0.2 pups, range = 1-3 pups; Lactation Controls: n = 14 litters, 2.57 ± 0.2 pups,
727 range = 1-4 pup, effect of treatment, t_{25} = -1.31, P = 0.2).

728 The litter sex ratio (proportion of males) at the first nest entry did not differ between
729 mothers treated with GCs during pregnancy or the controls (Pregnancy GCs: n = 42 litters, 53 ±

730 4% males; Pregnancy Controls: n = 30 litters, 41.8 ± 5% males, effect of treatment: $z = 0.49$, $P =$
731 0.63), nor at the second nest entry (Pregnancy GCs: n = 23 litters, 67.9 ± 12% males; Pregnancy
732 Controls: n = 20 litters, 47.5 ± 6% males, effect of treatment: $z = 0.32$, $P = 0.75$). Similarly, the
733 litter sex ratio at the first nest entry did not differ between mothers treated with GCs during
734 lactation or the controls (Lactation GCs: n = 18 litters, 43.5 ± 7.7% males; Lactation Controls: n
735 = 17 litters, 45.8 ± 8.7% males, effect of treatment: $z = 0.002$, $P = 0.99$) nor at the second nest
736 entry (Lactation GCs: n = 16 litters, 48.1 ± 8.8% males; Lactation Controls: n = 14 litters, 56.4 ±
737 10% males, effect of treatment: $z = -0.48$, $P = 0.63$).
738

739
740 **Table S1. Results from principal component analyses to derive axes of variation of**
741 **oxidative stress state in weaned pups.** For each principal component shown, high values
742 correspond to lower oxidative stress levels as they reflect samples with low levels of two (Blood
743 PC2, Liver PC1) or one (Heart PC2) antioxidants (total antioxidant capacity, superoxide
744 dismutase) and for heart tissue, higher levels of oxidative protein damage (protein carbonyls).
745

Measurement	**Tissue**		
	Blood PC2	**Heart PC2**	**Liver PC1**
Total Antioxidant Capacity	-0.57	-0.004	-0.70
Superoxide Dismutase	-0.82	-0.69	-0.70
Protein Carbonyls	0.03	0.71	0.09
Prop. Variance Explained	34.3%	34.3%	38.5%

746
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748

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: $\sigma^2 = 0.07$; size model: $\sigma^2 = 0.19$).

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Growth	**Intercept (2012, Control, Female)**	**1.86**	**0.12**	**15.2**	**35.6**	**<0.0001**
	Year (2015)	-0.17	0.2	-0.85	34.4	0.4
	Year (2016)	**-0.63**	**0.13**	**-4.74**	**34.2**	**<0.0001**
	Year (2017)	**-0.77**	**0.12**	**-6.4**	**34.4**	**<0.0001**
	Sex (Male)	**0.07**	**0.03**	**2.09**	**72.9**	**0.04**
	Birth date	-0.003	0.05	-0.06	34.5	0.95
	Litter size	0.12	0.07	1.66	35.6	0.11
	Treatment (GCs)	**0.27**	**0.09**	**3.04**	**41.6**	**0.004**
	Treatment (GCs) x Sex (Male)	-0.08	0.05	-1.64	74.1	0.1
	Treatment (GCs) x Litter size	-0.15	0.1	-1.47	35.8	0.15

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)	-0.04	0.4	-0.1	32.3	0.92
	Year (2016)	**-1.0**	**0.41**	**-2.42**	**29.1**	**0.022**
	Year (2017)	-0.02	0.41	-0.06	29.4	0.95
	Sex (Male)	0.26	0.15	1.68	59.6	0.1
	Birth date	0.16	0.11	1.43	26	0.16
	Litter size	0.02	0.21	0.09	33.2	0.93
	Treatment (GCs)	0.11	0.25	0.43	44.5	0.67
	Treatment (GCs) x Sex (Male)	-0.09	0.22	-0.42	60.8	0.67
	Treatment (GCs) x Litter size	-0.21	0.27	-0.76	32.2	0.45

Results based upon 88 offspring from 34 litters across 3 years

764 **Table S3. Effects of treating female red squirrels with GCs during (A) pregnancy or (B)**
765 **lactation on offspring haematocrit levels (packed red blood cell volume) collected from**
766 **weaned offspring**. Results for the pregnancy model contained random intercept term for litter
767 identity (pregnancy: $\sigma^2 = 19$) whereas results for the lactation model are from a general linear
768 model.
769

(A)

Variable	b	SE	*t*	df	*P*-value
Intercept (2015, Control, Female)	**47.4**	**2.9**	**16.1**	**18.2**	**<0.0001**
Year (2016)	-6.1	3.4	-1.82	17.4	0.085
Year (2017)	-5.8	3.1	-1.83	17	0.085
Sex (Male)	1.91	1.1	1.74	16.7	0.1
Birth date	-0.07	1.2	-0.06	18.9	0.95
Growth	-1.2	1.4	-0.84	29.7	0.41
Litter size	-0.58	1.2	-0.48	17.9	0.64
Treatment (GCs)	0.11	2.2	0.05	20.1	0.96
Treatment (GCs) x Growth	1.73	2.3	0.74	29.2	0.46

Results based upon 39 offspring from 25 litters across 3 years

(B)

Variable	b	SE	t	df	P-value
Intercept (2015, Control, Female)	**45.3**	**2.4**	**18.5**	**15**	**<0.0001**
Year (2016)	**-7.42**	**2.9**	**-2.6**	**15**	**0.02**
Year (2017)	-3.83	2.2	-1.74	15	0.1
Sex (Male)	**4.98**	**1.9**	**2.6**	**15**	**0.02**
Birth date	**3.94**	**1.7**	**2.35**	**15**	**0.033**
Growth	-1.62	1.7	-1.29	15	0.21
Litter size	3.1	1.9	1.65	15	0.12
Treatment (GCs)	2.12	2.4	0.89	15	0.39
Treatment (GCs) x Growth	2.72	2.1	1.27	15	0.22

770 Results based upon 24 offspring from 16 litters across 3 years
771
772

773 **Table S4. Effects of treating pregnant red squirrels with GCs on oxidative stress levels in**
774 **(A) blood, (B) heart, and (C) liver tissue from weaned offspring.** High PC scores correspond
775 to low levels of antioxidants and, for heart, higher levels of protein damage (see Table 1).
776 Models for blood and liver tissues contained random intercept term for litter ID (blood: $\sigma^2 = 0.3$;
777 liver: $\sigma^2 = 0.03$).
778

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Blood PC2	Intercept (2015, Control, Female)	0.09	0.47	0.19	12.3	0.85
	Year (2016)	-0.2	0.56	-0.36	12.8	0.72
	Year (2017)	-0.2	0.52	-0.38	12.2	0.71
	Sex (Male)	0.58	0.28	2.02	23.9	0.055
	Birth date	-0.39	0.2	-1.92	17.6	0.071
	Growth	0.02	0.29	0.08	21.5	0.93
	Litter size	-0.38	0.21	-1.85	16.9	0.08
	Treatment (GCs)	-0.19	0.38	-0.5	15.1	0.62
	Treatment (GCs) x Growth	-0.86	0.45	-1.9	19.06	0.071

(B)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Heart PC2	**Intercept (2015, Control, Female)**	**-1.1**	**0.46**	**-2.4**	**32**	**0.022**
	Year (2016)	**1.3**	**0.55**	**2.37**	**32**	**0.024**
	Year (2017)	0.94	0.5	1.86	32	0.072
	Sex (Male)	-0.08	0.35	-0.23	32	0.82
	Birth date	0.25	0.21	1.22	32	0.23
	Growth	-0.07	0.3	-0.23	32	0.81
	Litter size	0.4	0.21	1.88	32	0.07
	Treatment (GCs)	0.49	0.38	1.3	32	0.2
	Treatment (GCs) x Growth	0.45	0.47	0.98	32	0.34

(C)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Liver PC1	**Intercept (2015, Control, Female)**	**-0.91**	**0.33**	**-2.79**	**11.8**	**0.016**
	Year (2016)	0.44	0.4	1.15	12.2	0.27
	Year (2017)	**1.78**	**0.36**	**4.9**	**11.5**	**0.0004**
	Sex (Male)	0.01	0.24	0.08	29.3	0.94
	Birth date	0.08	0.15	0.53	17.5	0.6
	Growth	-0.04	0.2	-0.17	17.3	0.86
	Litter size	0.04	0.15	0.28	17.9	0.78
	Treatment (GCs)	-0.45	0.27	-1.66	13.8	0.12
	Treatment (GCs) x Growth	-0.47	0.32	-1.44	16.1	0.17

779 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: $\sigma^2 = 1.2$; lactation: $\sigma^2 = 6.6$).

(A)

Variable	b	SE	*t*	df	*P*-value
Intercept (2015, Control, Female)	**20.06**	**1.26**	**16.08**	**15.9**	**<0.0001**
Year (2016)	**4.06**	**1.4**	**2.92**	**13.4**	**0.012**
Year (2017)	-2.63	1.6	-1.61	20.7	0.12
Sex (Male)	0.38	0.77	0.49	26.5	0.62
Birth date	0.61	0.51	1.21	18.5	0.24
Growth	-0.3	0.72	-0.43	19.3	0.68
Litter size	-0.39	0.52	-0.75	18	0.46
Liver PC1	0.25	0.58	0.43	30.6	0.67
Treatment (GCs)	0.87	0.97	0.9	16.3	0.38
Treatment (GCs) x Growth	0.9	1.15	0.78	19.1	0.44

Results based upon 41 offspring from 26 litters across 3 years

(B)

Variable	b	SE	t	df	*P*-value
Intercept (2015, Control, Female)	**19.2**	**1.97**	**9.75**	**14.1**	**<0.0001**
Year (2016)	**2.1**	**2.7**	**0.79**	**10.6**	**0.45**
Year (2017)	0.13	2.6	0.05	15	0.96
Sex (Male)	0.22	0.92	0.24	10.2	0.82
Birth date	0.11	1.16	0.1	11.2	0.92
Growth	0.01	0.82	0.02	16.9	0.99
Litter size	0.68	1.56	0.44	10.9	0.67
Liver PC1	-0.44	0.79	-0.56	15.8	0.58
Treatment (GCs)	0.47	1.5	0.32	10.7	0.75
Treatment (GCs) x Growth	1.13	1.15	0.98	16.9	0.34

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 = 0.22; size model: σ^2 = 0.69).

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Growth	**Intercept (2015, Control, Female)**	**1.89**	**0.2**	**10.5**	**25.4**	**<0.0001**
	Year (2016)	-0.11	0.37	-0.3	22.2	0.77
	Year (2017)	-0.27	0.19	-1.42	22.6	0.17
	Sex (Male)	-0.006	0.06	-0.09	41.2	0.92
	Birth date	**0.23**	**0.1**	**2.37**	**22.5**	**0.027**
	Litter size	0.11	0.18	0.6	22.6	0.55
	Treatment (GCs)	**-0.4**	**0.19**	**-2.14**	**26.4**	**0.04**
	Treatment (GCs) x Sex (Male)	0.09	0.08	1.05	41.2	0.3
	Treatment (GCs) x Litter size	-0.006	0.21	-0.03	22.8	0.97

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)	0.07	0.35	0.2	41.6	0.84
	Year (2016)	0.04	0.6	0.07	18.2	0.94
	Year (2017)	**0.7**	**0.31**	**2.27**	**22.6**	**0.033**
	Sex (Male)	0.24	0.29	0.82	47.5	0.41
	Birth date	**0.46**	**0.15**	**2.98**	**21.5**	**0.007**
	Litter size	-0.02	0.29	-0.08	23.4	0.93
	Treatment (GCs)	-0.53	0.37	-1.44	41.9	0.16
	Treatment (GCs) x Sex (Male)	-0.04	0.37	-0.1	45.6	0.92
	Treatment (GCs) x Litter size	0.5	0.34	1.45	23.9	0.16

Results based upon 67 offspring from 30 litters across 3 years

806 **Table S7. Effects of treating lactating red squirrels with GCs on oxidative stress levels in**
807 **(A) blood, (B) heart, and (C) liver tissue from weaned offspring.** High PC scores correspond
808 to low levels of antioxidants and, in heart tissue, higher levels of protein damage (Table 1). The
809 model for liver contained a random intercept term for litter identity (liver: $\sigma^2 = 0.32$).
810

(A)

Offspring trait	Variable	b	SE	*t*	df	*P*-value
Blood PC2	Intercept (2015, Control, Female)	0.19	0.55	0.35	18	0.73
	Year (2016)	-0.25	0.7	-0.35	18	0.73
	Year (2017)	-0.75	0.55	-1.36	18	0.19
	Sex (Male)	-0.49	0.46	-1.06	18	0.3
	Birth date	-0.15	0.35	-0.42	18	0.68
	Growth	0.34	0.31	1.08	18	0.29
	Litter size	-0.37	0.45	-0.83	18	0.42
	Treatment (GCs)	0.53	0.44	1.2	18	0.24
	Treatment (GCs) x Growth	-0.22	0.42	-0.53	18	0.6

(B)

Offspring trait	Variable	b	SE	t	df	*P*-value
Heart PC2	Intercept (2015, Control, Female)	-1.2	0.43	-2.8	18	0.012
	Year (2016)	0.75	0.55	1.36	18	0.19
	Year (2017)	0.98	0.43	2.3	18	0.03
	Sex (Male)	0.33	0.36	0.91	18	0.37
	Birth date	0.2	0.27	0.75	18	0.46
	Growth	-0.02	0.24	-0.1	18	0.92
	Litter size	0.24	0.35	0.69	18	0.5
	Treatment (GCs)	0.61	0.34	1.79	18	0.09
	Treatment (GCs) x Growth	-0.23	0.32	-0.72	18	0.48

(C)

Offspring trait	Variable	b	SE	t	df	*P*-value
Liver PC1	**Intercept (2015, Control, Female)**	**-1.34**	**0.43**	**-3.11**	**12.1**	**0.009**
	Year (2016)	0.99	0.63	1.56	8.7	0.15
	Year (2017)	**2.32**	**0.46**	**9.91**	**5.08**	**0.0005**
	Sex (Male)	-0.03	0.3	-0.1	14.2	0.92
	Birth date	-0.23	0.3	-0.79	10.4	0.44
	Growth	-0.22	0.23	-0.95	17.9	0.35
	Litter size	-0.47	0.38	-1.24	9.8	0.24
	Treatment (GCs)	-0.08	0.37	-0.22	9.9	0.83
	Treatment (GCs) x Growth	-0.04	0.32	-0.12	15.6	0.9

811 Results from 27 offspring from 18 litters over 3 years.
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