Maternal stress increases cooperative behaviour of offspring in wild meerkats

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Abstract

The phenotype of parents can have long-lasting effects on the development of offspring as well as on their behaviour, physiology, and morphology as adults. In some cases, these changes may increase offspring fitness but, in others, they can elevate parental fitness at a cost to the fitness of their offspring. Here, we show that in Kalahari meerkats (*Suricata suricatta*) the circulating glucocorticoid hormones of pregnant females affect the growth and cooperative behaviour of their offspring. We performed a 3-year experiment in wild meerkats to test the hypothesis that stress-mediated maternal effects reduce the potential for offspring to reproduce directly and therefore cause them to exhibit more cooperative behaviour. Daughters (but not sons) born to mothers treated with cortisol during pregnancy were smaller early in life and exhibited significantly more of two types of cooperative behaviour (pup rearing and feeding) once they were adults compared to offspring from control mothers. They also had lower measures of stress hormones as they aged, which could explain the observed increases in cooperative behaviour. Because early growth is a crucial determinant of fitness in female meerkats, our results indicate that stress-mediated maternal effects may reduce the fitness of offspring but may elevate parental fitness as a consequence of increasing the cooperative behaviour of their daughters.

Significance Statement

Maternal characteristics can have a profound influence on offspring, which are referred to as maternal effects. It is widely assumed that selfish maternal effects, where mothers modify offspring to benefit their own fitness and decrease the fitness of offspring, are rare but this assumption is rarely tested. We show that a stress-mediated maternal effect in meerkats altered the growth, behaviour, and stress physiology of daughters in such a way that benefitted mothers at the expense of their daughters. Daughters from stressed mothers grew more slowly early in life, which should reduce their future reproductive opportunities, but exhibited more cooperative behaviour that should directly benefit their mother. This highlights the important role of sociality in the evolution of maternal effects.

Introduction

Parental effects are a mechanism of trans-generational phenotypic plasticity that occurs when the parental phenotype or parental environment modifies offspring characteristics (1). Parental effects can increase the survival or reproduction of offspring, thereby elevating the direct fitness of both offspring and parents (2-6). Alternatively, parental effects can increase parental fitness, but at some cost to the fitness of their offspring (7-8) – a process regarded as a type of parental manipulation (9-12) or 'selfish parental effect' (13). For example, in mammals, the optimal birth weight or litter size often differs between mothers and offspring (14) and pregnant females experiencing stressful environments may reallocate resources away from offspring and towards themselves, so that their offspring are smaller or grow more slowly before weaning (15). Despite these observations, it has also been suggested that selfish parental effects may be rare and unstable because selection would be expected to favour the evolution of resistance mechanisms in offspring (7, 11, 13, 16, 17).

Selfish parental effects may in fact be more likely in cooperatively breeding species where philopatric offspring (subordinates) help to rear the subsequent offspring of their parents or other close relatives. This is because parents may gain substantial direct fitness benefits from delaying the development of their offspring if this causes them to invest in alloparental care directed at the parent's subsequent offspring (9-10). In addition, the costs of selfish parental effects to offspring could be reduced in these circumstances, as offspring will gain indirect fitness benefits by contributing to raising the subsequent offspring of their parents (18). For example, laboratory studies of eusocial insects suggest the possibility that selection will favour the evolution of alleles

that enable mothers to increase the helping behaviour of their offspring while simultaneously reducing their capabilities of reproducing on their own (19-20; but see 21). In addition, it is often the case that the probability that parents will rear young successfully is substantially higher than the probability that inexperienced and subordinate females will do so, increasing the inclusive fitness benefits of helping versus breeding to offspring (22).

To date, empirical field tests of how parental effects shape the helping behaviour of offspring are rare (23) and studies of selfish parental effects have mostly focused on non-social species (13, 24). Here, we report the results of experiments designed to test the hypothesis that elevated maternal glucocorticoid (stress hormone) levels reduce the potential for offspring to have direct reproductive opportunities and causes them to exhibit more cooperative behaviour. In a 3-year field study, we experimentally elevated maternal stress by treating pregnant dominant female meerkats with glucocorticoids (cortisol) and tracking the growth, stress physiology, and cooperative behaviour of their offspring from birth until ~18 months of age, compared to those from control litters. We manipulated maternal glucocorticoids because they are known to cause mothers to reallocate energy away from offspring and towards themselves (15), indicating that they may function as a mediator of selfish maternal effects. Changes in maternal glucocorticoids have also previously been shown to delay the dispersal of offspring as well as influence the parental care behaviour of offspring (25-26), both traits that are important in cooperative breeders where philopatric offspring exhibit alloparental care behaviour towards juveniles.

95 To identify if the exposure of mothers to heightened glucocorticoids reduced
96 reproductive success of their offspring, we examined if offspring from mothers treated
97 with cortisol during pregnancy grew slower or were smaller early in life. In meerkats, the
98 rate of early life growth and body mass is closely linked to future direct fitness through
99 its effects on survival, foraging success, adult body mass (27-29), as well as the
100 probability of acquiring dominance (30-31) and other direct reproductive opportunities
101 (32). As elevated exposure to maternal glucocorticoids in some mammals may reduce
102 offspring size and growth early in life (15), we predicted that offspring from mothers
103 treated with cortisol during pregnancy would be smaller early in life and would
104 consequently contribute more to cooperative activities than controls.
105 Secondly, we determined if offspring from mothers treated with cortisol during
106 pregnancy subsequently increased their contributions to two types of cooperative
107 behaviours: pup rearing ("babysitting": 33) and food provisioning during the period when
108 the pups are foraging with their natal group but are not yet nutritionally independent
109 ("pup feeding": 34). If offspring from mothers treated with cortisol during pregnancy
110 exhibit more of either of these two types of alloparental care, this should increase both
111 parental fitness and the indirect fitness of offspring because subsequent offspring that
112 receive more alloparental care should grow faster or have higher early life survival (27,
113 32, 35, 36).
114 To assess the mechanism by which elevated exposure to maternal stress may
115 affect the alloparental care behaviour of offspring, we repeatedly measured plasma
116 cortisol and faecal glucocorticoid metabolite (fGCM) concentrations of offspring from
117 when they were approximately 1 to 18 months of age to identify how our manipulations

118 affected their neuroendocrine stress axes (glucocorticoid output). Elevated maternal
119 glucocorticoids can cause long-term changes in the neuroendocrine stress axis of
120 offspring (37) and elevated activity of the neuroendocrine stress axis in meerkats can
121 reduce their contributions to alloparental care (38). We therefore predicted that if
122 offspring born to mothers treated with cortisol during pregnancy exhibited more
123 alloparental care behaviour compared to controls, they would also have reduced plasma
124 cortisol and fGCM concentrations.
125
126 **Results**
127 *Effects of treatments on litter characteristics and offspring survival*
128 There was no evidence that the treatment of pregnant females with cortisol
129 affected their ability to maintain litters to term or the survival of their pups prior to
130 emergence from the natal burrow (Tables S1-S2). The number of pups surviving to
131 emergence from the natal burrow or 3, 6, or 12 months of age and the litter sex ratio
132 were not different among litters from cortisol-treated (31 pups from 10 litters produced
133 by 9 dominant females), control (25 pups from 6 litters produced by 6 females), or
134 untreated (195 pups from 52 litters produced by 21 dominant females) females (Table
135 S1-S2).
136 *Effects of treatments on offspring early life body mass*
137 The effects of the treatments on body mass from initial emergence to nutritional
138 independence (1-3 months) differed between daughters and sons and depended upon
139 the age of offspring (Table S3). Daughters (but not sons) from cortisol treated litters
140 were significantly smaller from 1-3 months than those from control litters especially as

they approached nutritional independence (daughters: age × treatment, t = -4.17, P < 0.0001; sons: t = -1.48, P = 0.14), but were of similar body mass compared to those from untreated litters (daughters: age × treatment, t = 0.65, P = 0.51; sons: t = -0.52, P = 0.6, Table S3, Fig. 1). This difference in body mass between daughters from mothers treated with cortisol during pregnancy and those from control litters increased from one to three months, as indicated by the significant interaction between age and control treatment (Table S3). Daughters, but not sons, from control litters were larger than those from untreated litters, especially as they got older (daughters: age × treatment, t = -4.24, P < 0.0001; sons: t = 1.35, P = 0.18).

Effects of treatments on offspring cooperative behaviour

The effects of the maternal treatments on babysitting behaviour of offspring depended upon the age and sex of the offspring (Table S4). Babysitting contributions in daughters from mothers treated with cortisol during pregnancy slightly, but significantly increased and rose more rapidly with increasing age of the babysitter than those from control litters (age × treatment, z = -2.89, P = 0.0039) but not untreated litters (age × treatment, z = 1.88, P = 0.06; Table S4, Fig. 2). Babysitting contributions in sons from mothers treated with cortisol during pregnancy showed a similar tendency to slightly increase more rapidly with age compared to those from control litters, but this difference was not significant (age × treatment, z = -1.92, P = 0.055). Further, age-related increases in babysitting contributions between males from mothers treated with cortisol during pregnancy and untreated litters did not differ (age × treatment, z = -0.03, P = 0.97; Table S4, Fig. 2). Comparisons of the magnitude of effect sizes showed that the interaction between age and litter treatment had a larger effect on babysitting

contributions in daughters but not sons than other variables known to impact babysitting contributions, such as foraging success, age-related body mass, or group size (Table S4).

The effects of the maternal treatments on pup feeding depended upon the sex of the offspring, but not their age (Table S5). Daughters but not sons from mothers treated with cortisol during pregnancy exhibited significantly more pup feeding contributions than those from control litters (females: z = -3.12, P = 0.00018; males: z = -1.14, P = 0.25) or untreated litters (females: z = -3.49, P = 0.0005, sons: z = -1.03, P = 0.3, Table S5, Fig. 3). Notably, the magnitude of effect size of litter treatment for daughters was much larger than other variables known to impact babysitting contributions such as foraging success, age-related body mass, and group size (Table S5).

Effects of treatments on offspring stress physiology

Daughters and sons from mothers treated with cortisol during pregnancy had lower plasma cortisol and fGCM concentrations as they became older compared to those from control litters. Plasma cortisol concentrations in daughters from mothers treated with cortisol during pregnancy had lower plasma cortisol concentrations as they became older compared to those from control litters but these differences were not significant (age × treatment, t = -1.61, P = 0.11, Table S6, Fig. 3A). fGCM concentrations in daughters were significantly lower than those from control litters as they became older (age × treatment, t = -2.95, P = 0 .0033, Table S7, Fig. 4A). Sons from mothers treated with cortisol during pregnancy had significantly lower plasma cortisol concentrations as they became older compared to those from control litters (age × treatment t = -2.59, P = 0.01, Table S6, Fig. 3B) but similar fGCM concentrations

187 compared to those from control litters as they became older (age × treatment, t = -0.49,
188 P = 0.62, Table S7, Fig. 4B).
189
190 **Discussion**
191 We found some support for our hypothesis that elevated maternal stress would
192 reduce the potential for offspring to have direct reproductive opportunities and would
193 therefore shift them towards exhibiting more cooperative behaviour that should increase
194 their indirect fitness. Daughters, but not sons, from mothers treated with cortisol during
195 pregnancy grew more slowly early in life and exhibited more babysitting and pup
196 feeding behaviour as they became older compared to controls. Other than offspring
197 survival (Table S2), we were unable to quantify the direct and indirect fitness of
198 offspring from control or cortisol-treated litters, but early life growth or body mass (which
199 we measured here) is closely linked to direct fitness opportunities in daughters.
200 Previous studies in meerkats show that female, but not male, offspring that grow faster
201 from 1-3 months are more likely to acquire the dominant breeding position (31), perhaps
202 because offspring that grow faster from 1-3 months are heavier later in life (32, 39, 40),
203 and heavier females are more likely to acquire a vacant dominant breeding position (30,
204 32). As such, daughters, but not sons, from mothers with elevated cortisol levels during
205 pregnancy should have reduced future direct fitness opportunities and therefore
206 increase their investment in behaviours that elevate their indirect fitness. Our results are
207 consistent with studies in other taxa that suggest that individuals adjust their
208 contributions to cooperative behaviour according to their future reproductive potential.
209 For example, wasps whose probability of acquiring the dominant breeding position was

210 experimentally increased exhibited significantly less helping behaviour (41). Similarly, in
211 cooperatively breeding fish, subordinates will reduce their helping investment
212 immediately prior to dispersal from their natal group where they attempt to reproduce on
213 their own rather than stay in their natal group and queue for dominance (42).

214 The effects of maternal stress on offspring should lead to substantial fitness
215 benefits to mothers. Daughters from mothers treated with cortisol during pregnancy
216 exhibited more alloparental care compared to controls, such that subsequent offspring
217 produced in groups with offspring from cortisol-treated litters should have received more
218 alloparental care behaviours. Because offspring that receive more alloparental care
219 grow faster early in life or are larger later in life (32, 39), the presence of offspring from
220 cortisol-treated litters should increase the direct fitness of dominant breeders and the
221 indirect fitness of the cortisol-treated offspring. Taken together, our results suggest that
222 this stress-mediated maternal effect reduced the direct fitness opportunities of
223 daughters by reducing their early life growth but they compensated by increasing their
224 investment in indirect fitness opportunities (helping to rear non-descendent offspring).
225 This is in line with theoretical predictions that parental manipulation of the cooperative
226 behaviour of offspring can evolve if the costs of resisting the parental effect are high and
227 inclusive fitness benefits of helping rear subsequent offspring are high (18).

228 Control dominant females that were fed during pregnancy produced daughters
229 that were larger during early development (1-3 months) compared to daughters from
230 cortisol-treated or untreated litters. Although mothers that were treated with cortisol
231 during pregnancy received the same amount of food as controls, daughters and sons
232 from mothers fed cortisol during pregnancy did not differ in body mass compared to

233 those from untreated litters. This indicates that the additional food provided to dominant
234 females during pregnancy had the potential to increase growth, but the added cortisol
235 prevented those gains in body mass. This has implications for understanding the fitness
236 consequences of maternal stress on offspring growth trajectories (15, 43) because our
237 results show that elevated circulating glucocorticoid levels in pregnant females in the
238 absence of energetic constraints induced reductions in the early life growth of offspring.
239 This supports the hypothesis that maternal stress levels during offspring development
240 act as a cue that induces plasticity in offspring growth rather than simply mediating the
241 effects of energetic constraints.

242 The reductions in the activity of the neuroendocrine stress axis of daughters may
243 have potentiated the increased alloparental care behaviour that we observed.
244 Compared to daughters from control litters, daughters from mothers treated with cortisol
245 during pregnancy exhibited more babysitting as they became older, more overall pup
246 feeding, and they also had lower plasma cortisol and fGCM concentrations. Males from
247 mothers treated with cortisol during pregnancy had significantly lower plasma cortisol
248 concentrations, but not fGCM concentrations as they got older and also tended to
249 exhibit more babysitting as they aged. The activity of the neuroendocrine stress axis is
250 closely linked to an array of social behaviours (44) and our recent work shows that
251 elevated activity of the neuroendocrine stress axis reduces babysitting in both females
252 and males and decreases pupfeeding in females (38). Together, this supports the
253 hypothesis that the mechanism by which early life stress increases the cooperative
254 behaviour of daughters is by dampening the activity of their neuroendocrine stress axis.

255 Our results show that the effects of maternal stress on offspring growth,
256 physiology, and behaviour were greater in daughters than in sons, which adds to
257 biomedical (45-46) and ecological (47-49) studies that highlight how early life conditions
258 or maternal stress levels can have sex-specific consequences for offspring. In
259 meerkats, there may be added benefits for the dominant female for altering the
260 cooperative behaviour of daughters compared to sons; daughters exhibit more
261 cooperative behaviour than sons (35) and are more responsive to the begging calls of
262 subsequent offspring that they provision with food (50). More broadly, sex-differences in
263 natal dispersal may cause these differential responses to parental effects. In meerkats,
264 subordinate males voluntarily disperse from their natal group to look for receptive
265 females but can return to their natal group whereas subordinate females rarely
266 voluntarily disperse from their natal group (51). In our case and in others (43), the more
267 philopatric sex (females) is more sensitive to early life conditions, which may be due to
268 differential costs of parental modification between the philopatric and dispersing sex. If
269 parental effects have long-term consequences on offspring characteristics, as we show
270 here, there may be an increased degree of mismatch between the phenotype of the
271 dispersing sex and the postnatal environment where individuals eventually settle. If this
272 mismatch has fitness costs, this should select for individuals from the dispersing sex to
273 be less responsive to cues from the parental phenotype or environment.
274 Our results provide some support for the hypothesis that parents may alter the
275 cooperative tendencies of their offspring by manipulating the characteristics of their
276 offspring (9-10). Explanations regarding the evolutionary origins of cooperative
277 behaviour involve nepotism or kin selection (52), mutualisms or reciprocity (53), but few

studies have tested the “parental manipulation” hypothesis proposed by Alexander (9). Some studies show that alleles that increase maternal fitness at the expense of the direct fitness of offspring can evolve (19) and that cooperative breeders may bias investment towards offspring that exhibit more cooperative behaviour (54). Our study provides evidence that environmental stressors and parental effects can modify the cooperative tendencies of their offspring.

Finally, our results have two implications for theoretical models examining the evolution of parental effects. First, given the sex-specificity of parental effects, our results challenge the conclusions of models examining the evolution of parental effects that assume that all offspring are equally sensitive to the parental effect (16), or those that assume that the benefits of exhibiting the phenotype resulting from the parental effect are equal for all offspring (18). Second, selfish parental effects are thought to be relatively rare (8, 13) and theory (16-17) and empirical studies showing sex-specific responses to early life stress (45-46) indicate that offspring can become resistant to such selfish parental effects. However, the evolution of selfish parental effects may be dependent upon the social environment (24) especially if the selfish parental effect influences the expression of alloparental care behaviour of offspring and therefore increases the indirect fitness of offspring. Our results provide an example whereby a stress-mediated maternal effect reduces the direct fitness of daughters (by reducing their early life growth), but presumably increases the direct fitness of mothers and indirect fitness of daughters by elevating their cooperative behaviour.

301 **Methods**

302 *Study site*

303 We studied free-living meerkats at the Kuruman River Reserve (26° 58' S, 21°
304 49' E) in the Northern Cape, South Africa from 2014-2017. Individuals were marked
305 uniquely (with PIT tags: Identipet®, Johannesburg, South Africa) as well as dye marks
306 so that they could be identified. Groups were visited for ~4-8 hours per day ~4-6 times
307 per week throughout each year of study and sometimes more frequently such as when
308 there were pups being babysat.

309 *Experimental manipulations of dominant females*

310 Dominant females in each group were identified via behavioural observations
311 (55). During each visit, we recorded which individuals were present as well as their body
312 mass. Meerkats were weighed on a portable balance each morning before foraging, 2-4
313 hrs after foraging was initiated, and immediately prior to when foraging ended (56). The
314 pregnancy status of dominant females was determined visually (distended abdomen) as
315 well as noting a constant increase in their body mass.

316 Dominant females were treated with either a cortisol solution or a control oil
317 vehicle when they were pregnant by feeding them food containing one of these two
318 treatments. We initially offered experimental animals hard boiled egg with added cortisol
319 but found that they rejected all foods that contained added cortisol with the exception of
320 scorpions. We consequently fed experimental females with cortisol (10 mg/kg of
321 hydrocortisone, Sigma H4126), that were dissolved in 100 μl of 100% coconut oil and
322 injected into a dead scorpion (*Opistophthalmus* spp.). Control females were fed a dead
323 scorpion that was injected with 100 μl of 100% coconut oil. A previous study using the

324 same protocol showed that meerkats that were fed cortisol had significantly higher
325 plasma cortisol and fGCM concentrations than control animals and these increases
326 were within a biologically-relevant range (38). This indicated that our treatment causes
327 the exogenous glucocorticoids that we feed the meerkats to enter their bloodstream and
328 leads to sustained increases in their circulating glucocorticoid concentrations.

329 We aimed to experimentally increase the glucocorticoid concentrations of
330 pregnant dominant females from when they were first confirmed to be pregnant (second
331 half of gestation) until parturition. Meerkats have a ~70 d gestation period so we aimed
332 to treat them with glucocorticoids from approximately 35-70 d during gestation. In
333 reality, females that successfully produced a litter where pups emerged from the natal
334 burrow were treated with cortisol for 12-36 days prior to birth (n=10 litters from 9
335 females, mean = 23.7 d, median = 23.5 d) whereas controls were fed for 12-58 d prior
336 to birth (n=6 litters from 6 females, mean = 30 d, median = 20.5 d).

337 Females were randomly allocated to the treatments. Across the three years of
338 this study, we produced a total of 16 cortisol-treated or control litters produced by 12
339 dominant females in 12 different social groups. Three of the females experienced both
340 the control and cortisol treatments at different time points of the experiment, whereas
341 one female experienced the control treatment once and the cortisol treatments twice.
342 For these latter females treated twice, the order of treatments was randomly selected.
343 We conducted these experiments over the course of three years: 13 litters from 10
344 females in 2014 (April-December 2014, 3 litters aborted), 5 litters from 5 females in
345 2015 (February-July 2015, 1 litter aborted), and 3 litters from 3 females in 2016 (July
346 2016).

347 To provide an additional comparison group to investigate how our treatments (fed
348 during pregnancy or fed cortisol during pregnancy) affected offspring survival, body
349 mass, and cooperative behaviour, we also monitored these traits in offspring produced
350 by dominant females that were untreated during pregnancy (n = 52 litters from 21
351 dominant females). For our analyses of how the treatments affected offspring survival
352 and body mass, the untreated offspring were those from litters produced by dominant
353 females in other meerkat groups in our same study area and were born during our study
354 (hereafter called 'untreated' litters). We assessed the contributions of offspring from
355 mothers treated with cortisol during pregnancy to two cooperative behaviours
356 (babysitting and pup feeding) compared to those from control litters but also to other
357 group members from untreated litters. We did not have data from offspring from
358 untreated litters when we assessed how our treatments affected their plasma cortisol or
359 fGCM concentrations.

360 *Quantifying early life growth of offspring*

361 Meerkat pups typically first emerge from their natal burrow approximately 21-30 d
362 after birth. Meerkat groups and dominant females were monitored daily around the
363 estimated date of parturition and birth dates were estimated according to the change in
364 the physical appearance of the dominant female, a large drop in body mass overnight,
365 and group members exhibiting babysitting behaviour at the sleeping burrow. Burrows
366 containing pups were monitored each day and, when pups emerged, they were uniquely
367 marked by trimming small sections of hair before permanent PIT tags could be applied.
368 Pups were weighed each time we visited the groups on a portable balance in the
369 morning after group members emerged from their sleeping burrow (as above).

370 *Quantifying cooperative behaviour of offspring*

371 We measured the babysitting (controls: 195-655 d; cortisol: 184-655 d; untreated:
372 155-655 d) and pup feeding (controls: 220-635 d; cortisol: 184-655 d; untreated: 155-
373 626 d) contributions of offspring from cortisol-treated and control litters when they were
374 >6 months of age until death or disappearance. We visited sleeping burrows containing
375 pups every day in the morning and recorded the identity of the attending babysitters. As
376 we have done previously (33, 57), we calculated relative babysitting contributions of
377 each individual meerkat for each litter by dividing the total number of days an individual
378 babysat a litter over the total number of days that this specific litter had a babysitter.
379 Pup feeding behaviour for each pup produced by the dominant females in the different
380 treatment groups was estimated using *ad libitum* sampling (34). When the social group
381 contained pups (up to 90 d of age), we recorded all pup-feeding events, which are
382 visually and acoustically conspicuous to observers (58). We used the probability of pup
383 feeding occurring and corrected for variation in observation time (see below).

384 *Quantifying plasma cortisol concentrations from offspring*

385 We obtained plasma samples from offspring from cortisol-treated and control
386 litters approximately every 3 months from first emergence from the burrow (~1 month)
387 until ~18 months of age (controls: 20-548 d; cortisol-treated: 25-559 d). Capture and
388 blood processing procedures are described elsewhere (59-60). We measured total
389 plasma cortisol concentrations using a previously validated assay (Coat-a-Count,
390 Siemens Diagnostic Products Corporation, Los Angeles, USA: validation described in
391 59). The sensitivity of the assay was 1.9 ng/ml and cross reactivity to other hormones
392 was 76% with prednisolone, 11.4% with 11-deoxycortisol, 2.3% with prednisone and

393 <1% with aldosterone, corticosterone, cortisone, estriol, estrone and pregnenolone.
394 Intra-assay coefficient of was 7% (n = 20 samples). Inter-assay coefficient of variation
395 for a low control was 8% (78.5 ± 6.3 nmols/l, n = 5 assays) and 2.8% for a high control
396 (187 ± 5.3 ng/ml, n = 5 assays).

397 *Quantifying fGCM concentrations from offspring*

398 We collected faecal samples from offspring from cortisol-treated and control
399 litters opportunistically during behavioural observations over the course of the study
400 (controls: 25-356 d; cortisol-treated: 32-326 d). Faecal samples were processed as
401 described previously using a methanol extraction to remove fGCMs (61-62).
402 Immunoreactive fGCM concentrations were determined using an enzyme immunoassay
403 measuring cortisol metabolites with a 5β-3α,11β-diol-structure (11β-
404 hydroxyetiocholanolone) already established for monitoring fGCM alterations in
405 meerkats (62). fGCMs measured reflect average adrenal cortisol production over the
406 previous ~24 to 48 hr period (62). Detailed assay characteristics, including full
407 descriptions of the assay components and cross-reactivities, are found elsewhere (63)
408 The sensitivity of the assay was 1.2 ng/g dry weight and intra-assay coefficients of
409 variation (CV) determined by repeated measurements of high and low value quality
410 controls were 6.9% and 7.4%, respectively, and inter-assay CV values were 11.5% and
411 15.9% (n = 29 assays). fGCM concentrations below are expressed as ln ng/g dry faecal
412 matter.

413 *Statistical analyses*

414 We used generalized (binomial errors) or linear mixed-effects models (LMMs) to
415 examine how our treatments affected the probability that the litter was aborted, litter size

416 and sex ratio at emergence from the burrow, and the proportion of the litter that survived
417 to emergence from the burrow, independence (90 d of age: 29), and 6 or 12 months of
418 age. We focused on addressing whether the cortisol-treated litters differed from our
419 control or untreated litters. These models included a fixed effect for date of birth of the
420 litter and random intercept terms for dominant female identity and year (as the
421 experiments were conducted over 3 years). None of the GLMMs were overdispersed
422 (Table S2).

423 We used a LMM to investigate how the maternal treatments affected offspring
424 body mass from first emergence from their natal burrow (~1 month) to 3 months of age
425 when the pups are typically foraging independently (29, 34). Morning body mass (in
426 grams) was the response variable with the fixed effects of maternal treatment (cortisol-
427 treated, control, or untreated), pup sex, pup age, litter size at burrow emergence, group
428 size, group size^2, total rainfall in the previous 60 days, two measures of seasonality
429 (sine and co-sine functions of day of weight measure: see 56), and two three-way
430 interactions between sex, treatment, age or age^2. Random intercept terms for year and
431 the identity of the individual nested in litter, nested in dominant female identity, nested in
432 group were also included in this model. Fixed and random effects included in these
433 models were based upon previous studies investigating meerkat body mass and growth
434 from 1-3 months (28, 31, 56). Only individuals that survived to 90 d were included in
435 these analyses.

436 We assessed how the treatments affected the relative babysitting and pup
437 feeding contributions of subordinates when they were >6 months (as they rarely do
438 alloparental care behaviour <6 months: 35) from cortisol-treated, control, and untreated

439 litters. In these generalized linear mixed-effects models (GLMM, binomial errors), we
440 included a three-way interaction between treatment, sex, and age of the subordinate to
441 assess if the effects of the treatments on babysitting or pup feeding varied according to
442 the sex or age of the subordinate, as contributions to cooperative behaviour in meerkats
443 are known to vary according to subordinate sex and age (35). To account for
444 differences in observation time, we included a co-variate for the number of days the
445 litter was babysat (babysitting length) and the number of days the subordinate was
446 observed in the group during babysitting as well as the total time spent observing the
447 group during pup feeding (observation time). We included a range of co-variates (see
448 Tables S4-S6) that have been previously documented to affect contributions to
449 babysitting and pup feeding, including age, foraging success, body mass, and group
450 size (Clutton-Brock et al., 2000, 2002, 2003; Carlson et al., 2006*a*; Dantzer et al.,
451 2017*a*). Relatedness between the subordinate and the litter being babysat was not
452 included as it has not been shown to impact babysitting or pup feeding contributions
453 (27, 57) and most of the litters in our entire dataset were produced by the mother or full
454 sibling of the subordinate. Random intercept terms for year and the identity of the
455 individual, and litter being babysat or pup fed nested within the group where the litter
456 was being babysat or pup fed. Overdispersion was not an issue for our GLMM for
457 babysitting as indicated by the goodness of fit test (Pearson χ^2 = 147.1, df = 154, P =
458 0.64, using package aods3: 64) but our GLMM for pup feeding was initially
459 overdispersed (Pearson χ^2 = 310, df=165, P < 0.0001) so we included an observation
460 level random intercept term.

We used two separate LMMs to assess how our manipulations affected plasma cortisol and fGCMs in offspring from cortisol-treated and control litters. Each model included fixed effects for maternal treatment, pup sex and age, time of day and year that the sample was acquired (2014 or 2015), and random intercept terms for identity of individual nested in their birth litter and group. In the model for plasma cortisol concentrations, we also included a linear and second order fixed effect for the time it took to acquire the blood sample to control for any variation in plasma cortisol concentrations due to restraint stress (60). Year was included as a fixed effect here because we only had samples from two separate years. We included covariates associated with the individual meerkat and weather or social group characteristics that are known to affect plasma cortisol (60) or fGCM (62) concentrations (see Tables S6-S7).

We used R (version 3.4.3: 65) for all of our statistical analyses. R package lme4 (version 1.1-14: 66) was used for LMMs and P values were estimated using lmerTest (version 2.0-33: 67). A graphical approach was used to confirm normality and homoscedasticity of residuals and to confirm there were no observations with high leverage (68). All continuous variables were standardized to a mean of 0 and SD of 1.

Ethics: All protocols used in our experiments were approved by the Animal Ethics Committee at the University of Pretoria (Pretoria, South Africa: #EC031-13, #EC047-16) and the Northern Cape Department of Environment and Nature Conservation Research (South Africa: FAUNA 050/2013, FAUNA 192/2014, FAUNA 1020/2016).

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Authors' Contributions: B.D. and T.H.C-B planned the study, B.D. designed the experiments, B.D., C. Dubuc, D.L.C, T.H.C-B, D.G., I.BG., N.B., M.H., A.G., and C. Duncan coordinated and collected data, B.D. conducted analyses and produced figures, B.D. and T.H.C-B authored manuscript with contributions from all authors.

Date, code and materials: All data will be archived on Data Dryad. All code for statistical analyses is available from first author.

Competing interests: We have no competing interests.

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697 **Figure Legends**

698

699 **Figure 1.** (A) Daughters but not (B) sons from mothers treated with cortisol during
700 pregnancy were significantly smaller from initial emergence from their natal burrow to
701 nutritional independence (~1-3 months) compared to those from control litters
702 (daughters: age × treatment, t = -4.17, P < 0.0001; sons: t = -1.48, P = 0.14), but not
703 untreated litters (daughters: age × treatment, t = 0.65, P = 0.51; sons: t = -0.52, P = 0.6,
704 Table S3). Data are body mass measures from offspring from cortisol-treated (females:
705 n = 373 estimates; males: n = 488), control (females: n = 215; males: n = 241), and
706 untreated litters (females: n = 1121; males: n = 2238). Raw data and regression lines
707 are shown (full results in Table S3).

708

709 **Figure 2.** Babysitting contributions of (A) daughters and (B) sons from mothers treated
710 with cortisol during pregnancy increased with age at a faster rate than those from
711 control (females: z = -2.89, P = 0.0039; males: z = -1.92, P = 0.055), but not untreated
712 ("None") litters (females: z = 1.88, P = 0.06; males: z = -0.03, P = 0.97, Table S4). Data
713 are relative babysitting contributions from offspring from cortisol-treated (females: n = 15
714 estimates; males: n = 24), control (females: n = 15; males: n = 10), and untreated litters
715 (females: n = 49; males: n = 69). Raw data and regression lines are shown (full results
716 in Table S4).

717

718 **Figure 3.** Pup feeding contributions of (A) daughters, but not (B) sons, from mothers
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720 (females: z = -3.09, P = 0.0004; males: z = -1.15, P = 0.25) or untreated ("None") litters
721 (females: z = -3.47, P = 0.0005; males: z = -0.89, P = 0.37, Table S5). Data are relative
722 pup feeding contributions from offspring from cortisol-treated (females: n = 16
723 estimates; males: n = 26), control (females: n = 16; males: n = 10), and untreated litters
724 (females: n = 64; males: n = 71). Raw data are shown (full results in Table S5). Boxplots
725 show median (solid horizontal line), mean (grey diamonds), and first (25%) and third
726 (75%) quartiles.
727
728 **Figure 4.** (A) Daughters and (B) sons from mothers treated with cortisol during
729 pregnancy had lower plasma cortisol concentrations as they became older compared to
730 those from control litters, though the difference was only significant in males (daughters:
731 age x treatment, t = -1.61, P = 0.11; sons: age x treatment t = -2.59, P = 0.01, Table
732 S6). Data are residual plasma cortisol concentrations from offspring from cortisol-
733 treated (females: n = 64 samples; males: n = 92) and control (females: n = 89; males: n
734 = 104) litters. Residuals from a linear mixed-effects model (Table S6) are shown on y-
735 axis.
736
737 **Figure 5.** Faecal glucocorticoid metabolite (fGCM) concentrations in (A) daughters but
738 not (B) sons from mothers treated with cortisol during pregnancy were significantly
739 lower than those from control litters as they became older (daughters: age x treatment, t
740 = -2.95, P = 0 .0033; sons: age x treatment, t = -0.49, P = 0.62, Table S7. Data are
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743 Residuals from a linear mixed-effects model (Table S7) are shown on y-axis.

744

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746 **Figure 1.** (A) Daughters but not (B) sons from mothers treated with cortisol during
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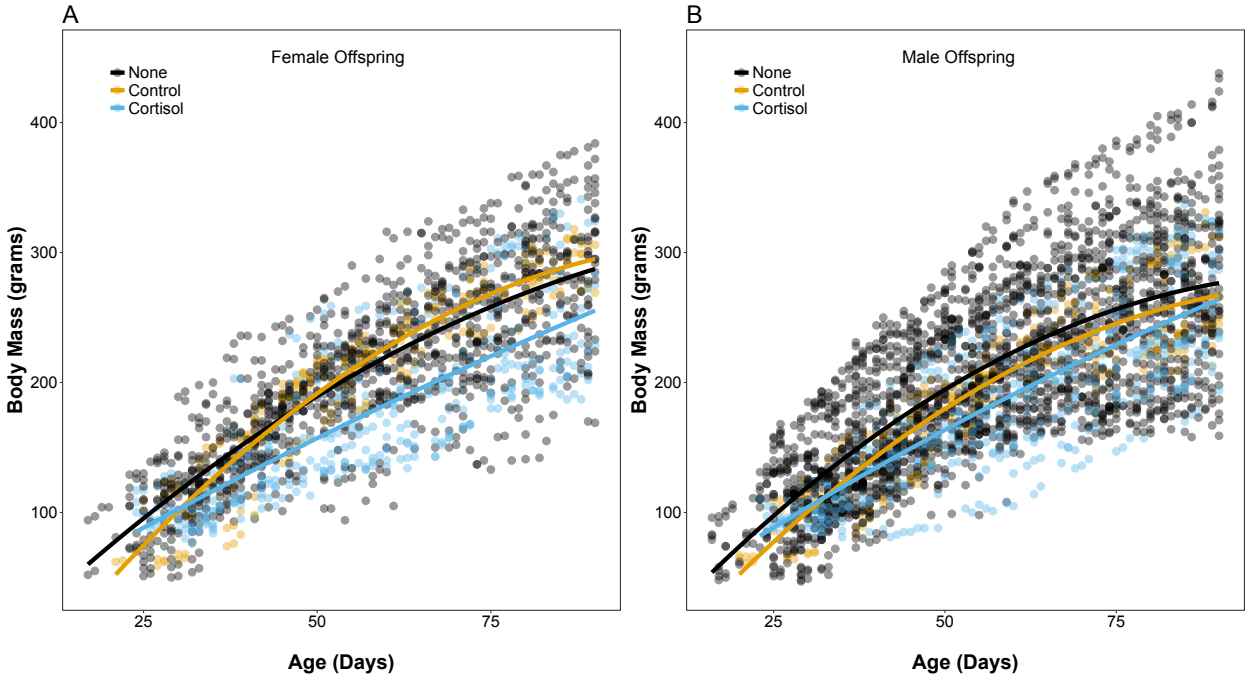

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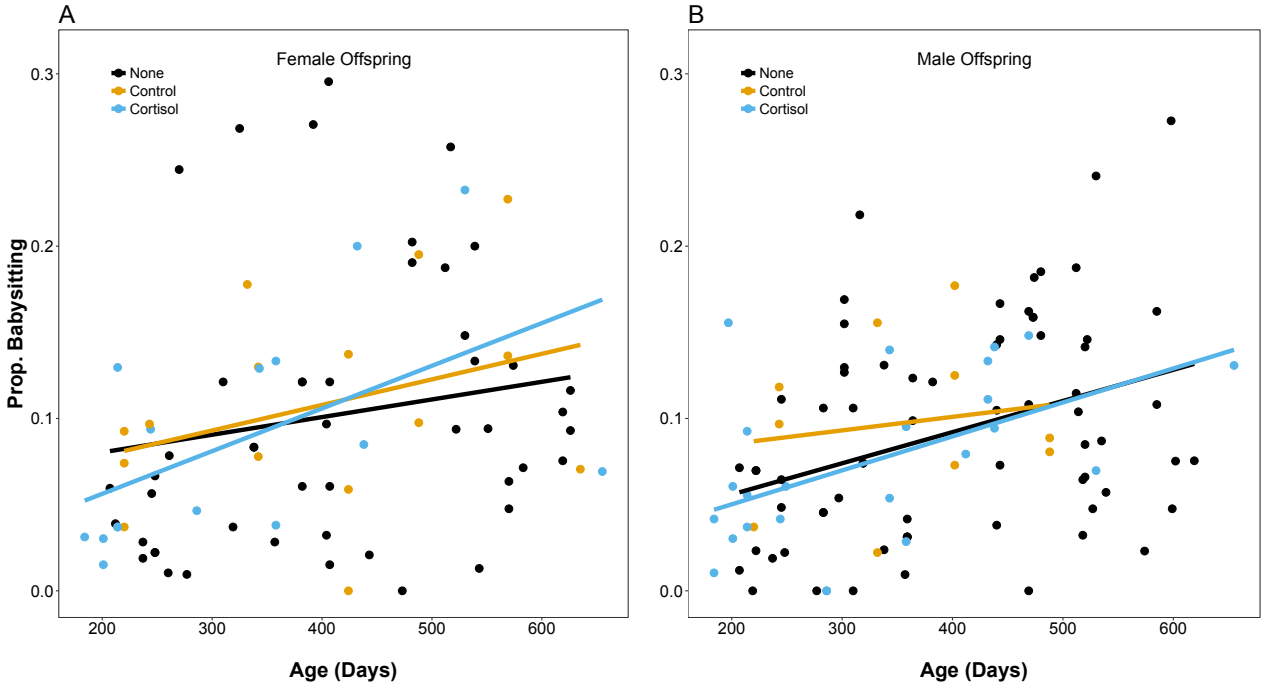


765

766

Figure 3. Pup feeding contributions of (A) daughters, but not (B) sons, from mothers treated with cortisol during pregnancy were significantly higher compared to from control (females: z = -3.09, P = 0.0004; males: z = -1.15, P = 0.25) or untreated ("None") litters (females: z = -3.47, P = 0.0005; males: z = -0.89, P = 0.37, Table S5). Data are relative pup feeding contributions from offspring from cortisol-treated (females: n = 16 estimates; males: n = 26), control (females: n = 16; males: n = 10), and untreated litters (females: n = 64; males: n = 71). Raw data are shown (full results in Table S5). Boxplots show median (solid horizontal line), mean (grey diamonds), and first (25%) and third (75%) quartiles.

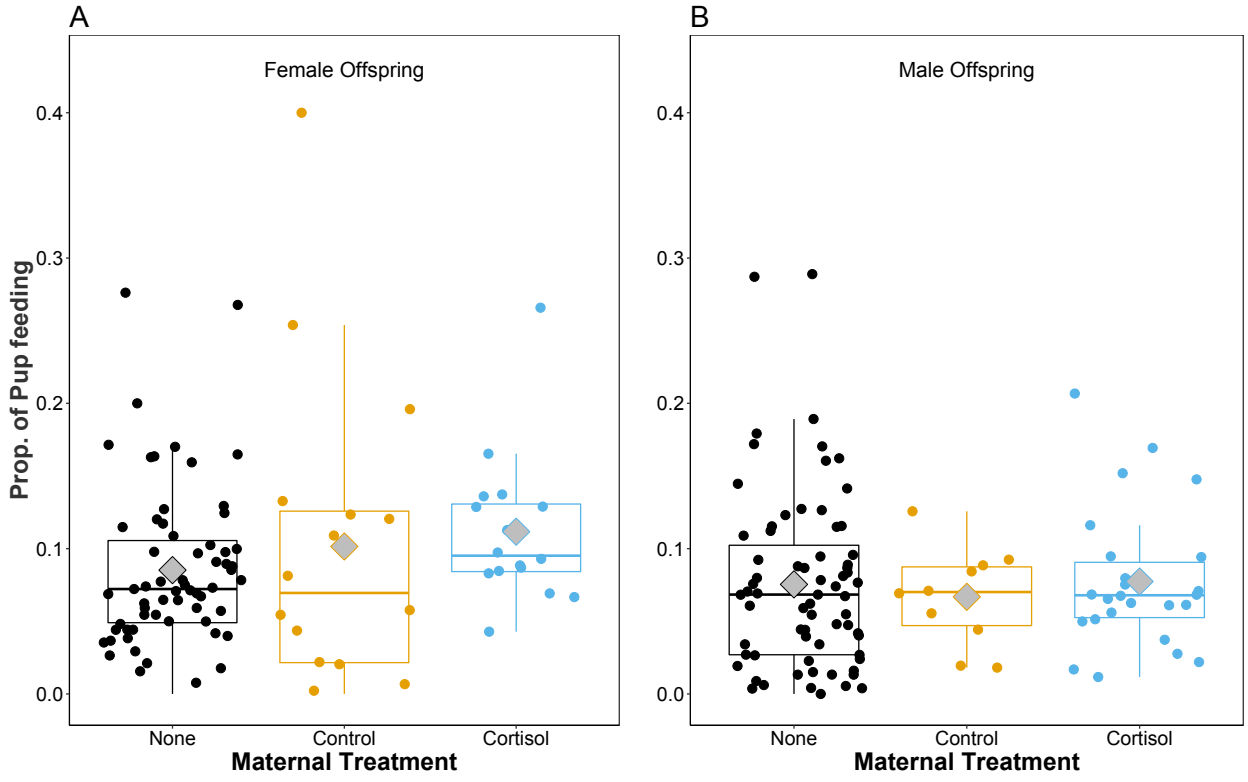


Figure 4. (A) Daughters and (B) sons from mothers treated with cortisol during pregnancy had lower plasma cortisol concentrations as they became older compared to those from control litters, though the difference was only significant in males (daughters: age x treatment, t = -1.61, P = 0.11; sons: age x treatment t = -2.59, P = 0.01, Table S6). Data are residual plasma cortisol concentrations from offspring from cortisol-treated (females: n = 64 samples; males: n = 92) and control (females: n = 89; males: n = 104) litters. Residuals from a linear mixed-effects model (Table S6) are shown on y-axis.

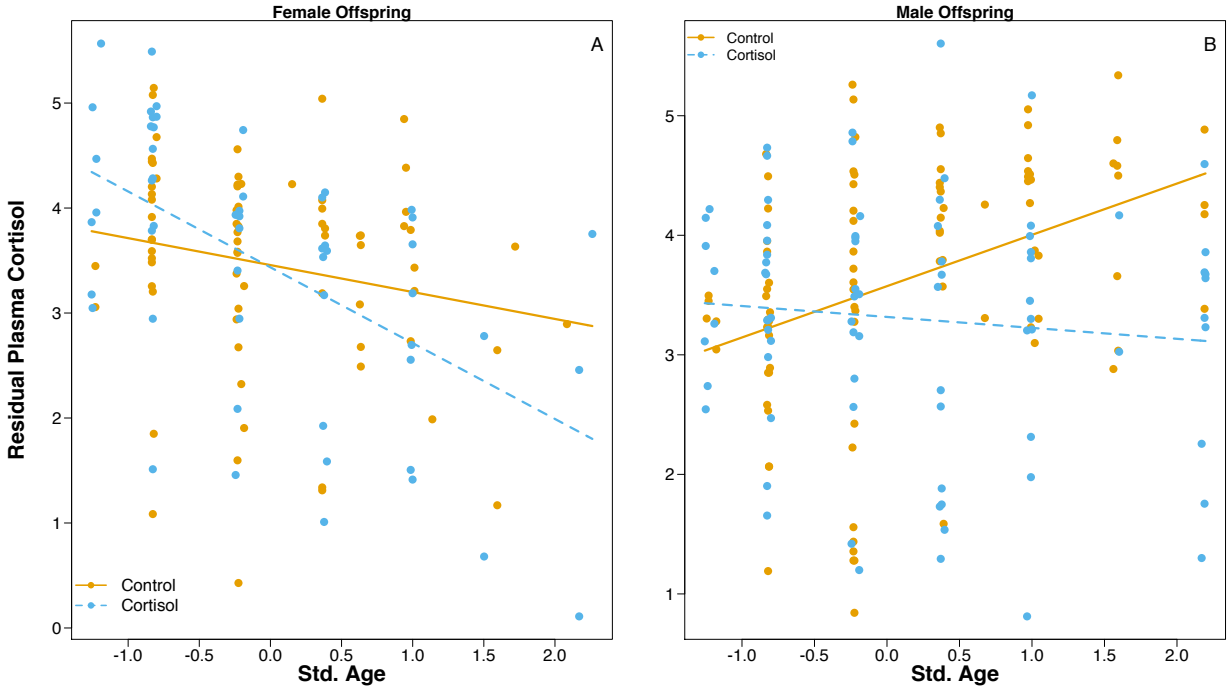
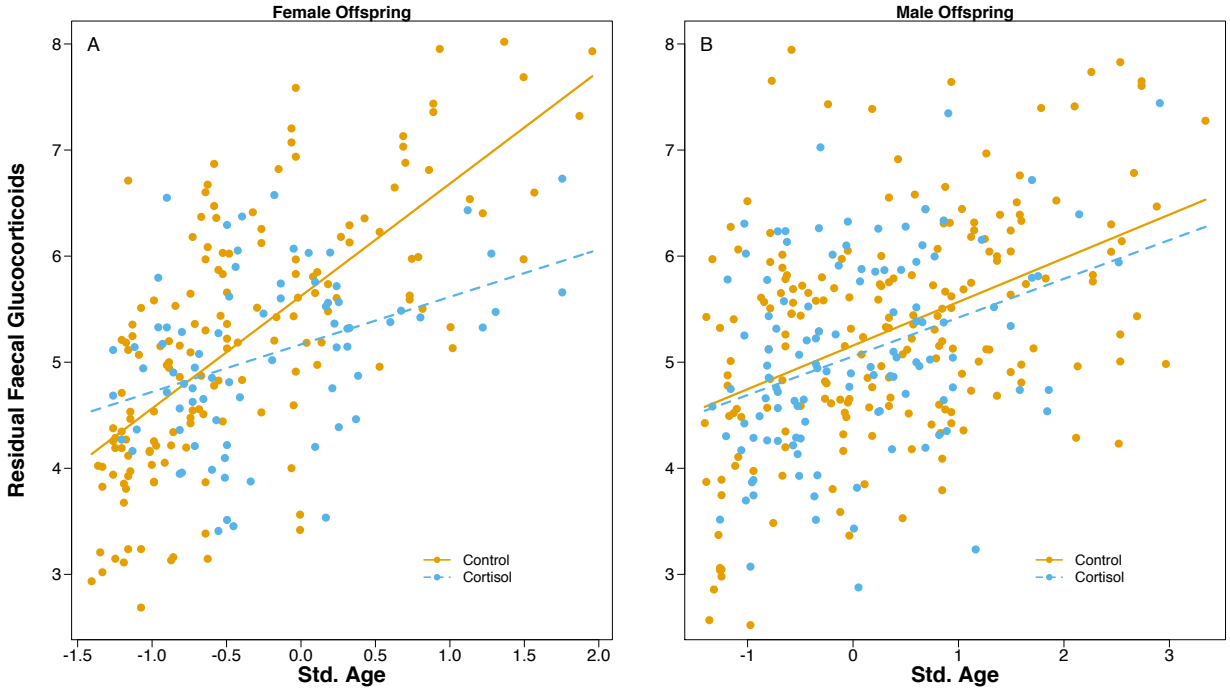


Figure 5. Faecal glucocorticoid metabolite (fGCM) concentrations in (A) daughters but not (B) sons from mothers treated with cortisol during pregnancy were significantly lower than those from control litters as they became older (daughters: age x treatment, t = -2.95, P = 0 .0033; sons: age x treatment, t = -0.49, P = 0.62, Table S7. Data are residual fGCM concentrations from offspring from cortisol-treated (females: n = 79 samples; males: n = 118) and control (females: n = 154; males: n = 201) litters. Residuals from a linear mixed-effects model (Table S7) are shown on y-axis.



Electronic Supplementary Material

Maternal stress increases cooperative behaviour of offspring in wild meerkats

Ben Dantzer, Constance Dubuc, Ines Braga Goncalves, Dominic L. Cram, Nigel C. Bennett, Andre Ganswindt, Chris Duncan, David Gaynor, Marta B. Manser, Tim H. Clutton-Brock

Contents:

Tables S1-S7

Table S1. Sample sizes and effects of dominant female treatments on litter characteristics and offspring survival. Means and standard deviations are shown. The number of litters aborted for untreated females was not known. Number of pups emerged correspond to those that emerged from the natal burrow. Three cortisol-treated litters and 1 control litter were aborted prior to birth and are not shown below. Litter sex ratio is the proportion of males in the litter. In some of the untreated litters, sex of the pups was not known (they died before sexing) and so are listed as unknown ("Unk").

Treatment	Total # litters & females treated	Total # pups emerged (F, M, Unk)	Avg. Pups emerged	Avg. Pups Surviving to 3 months	Avg. Pups Surviving to 6 months	Avg. Pups Surviving to 12 months
Untreated	52 (21 females)	49 F, 84 M, 52 Unk	3.78 ± 1.23	2.68 ± 1.58	2.1 ± 1.63	1.62 ± 1.54
Control	7 (6 females)	12 F, 13 M	4.17 ± 0.98	3.83 ± 1.17	3.83 ± 1.17	2.83 ± 1.94
Cortisol	13 (10 females)	13 F, 18 M	3.87 ± 0.83	3.25 ± 1.03	2.75 ± 1.28	1.75 ± 1.75

Table S2. Effects of dominant female treatments on litter characteristics and pup survival. Results are from a linear mixed-effects model (# pups emerged) or generalized linear mixed-effects models (GLMMs, all other response variables) that each contained random intercept terms for dominant female identity and year. No GLMM was overdispersed as indicated by goodness of fit tests (R package aods3, P-values from Pearson χ^2 tests ranged from 0.13 to 1).

Response variable	Fixed effect	b	SE	t or z	P-value
# Litters aborted					
	Intercept	-1.21	0.66	-1.83	0.07
	Birthdate	-0.17	0.59	-0.29	0.77
	Treatment				
	Control	-0.59	1.27	-0.46	0.64
# Pups emerged					
	Intercept	**3.9**	**0.42**	**9.36**	**<0.0001**
	Birthdate	0.02	0.16	0.11	0.91
	Treatment				
	Control	0.3	0.62	0.49	0.63
	Untreated	-0.11	0.45	-0.25	0.8
Litter sex ratio					
	Intercept	-0.54	0.3	-1.84	0.066
	Birthdate	0.02	0.12	0.15	0.88
	Treatment				
	Control	-0.11	0.45	-0.23	0.81
	Untreated	0.09	0.33	0.27	0.78
Prop. litter surviving to 3 months					
	Intercept	-0.16	0.27	-0.62	0.53
	Birthdate	-0.12	0.11	-1.09	0.27
	Treatment				
	Control	0.06	0.39	0.16	0.87
	Untreated	-0.19	0.3	-0.64	0.52
Prop. litter surviving to 6 months					
	Intercept	-0.32	0.28	-1.16	0.24
	Birthdate	-0.19	0.11	-1.65	0.099
	Treatment				
	Control	0.21	0.4	0.52	0.6
	Untreated	-0.28	0.31	-0.91	0.36
Prop. litter surviving to 12 months					
	Intercept	**-0.91**	**0.46**	**-1.999**	**0.046**
	Birthdate	-0.22	0.14	-1.58	0.11
	Treatment				
	Control	0.36	0.46	0.79	0.43
	Untreated	0.096	0.38	0.25	0.8

Reference for Treatment was cortisol-treated litters. Data other than # litters aborted are based upon an initial sample size of offspring from untreated (195 pups from 52 litters produced by 21 dominant females), control (25 pups from 6 litters produced by 6 females), or cortisol-treated (31 pups from 10 litters produced by 9 females) litters that produced pups that emerged from the burrow.

Table S3. Effect of dominant female treatments on offspring body mass from emergence to nutritional independence (1-3 months of age). Data are from a linear mixed-effects model where the response variable was morning body mass that contained random intercept terms for individual identity nested in birth litter nested in mother nested in natal group (σ^2 = 116.7) and year (σ^2 = 0).

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**184.6**	**9.75**	**18.93**	**53**	**<0.0001**
Males	**189.1**	**9.5**	**19.8**	**49**	**<0.0001**
Litter size	-5.31	3.9	-1.37	48	0.18
First weight	**15.96**	**2.52**	**6.344**	**166**	**<0.0001**
Sex (M)	4.54	4.32	1.05	171	0.29
Age	**53.3**	**1.06**	**50.4**	**4569**	**<0.0001**
Age^2	**-4.76**	**1.15**	**-4.12**	**4516**	**<0.0001**
Rainfall	**2.81**	**0.61**	**4.57**	**4550**	**<0.0001**
Season (Sine)	**-67.2**	**3.3**	**-20.6**	**4238**	**<0.0001**
Season (Co-sine)	**66.22**	**3.4**	**19.2**	**4191**	**<0.0001**
Group size	2.3	2.66	0.86	109	0.39
Group size^2	-0.09	2.21	-0.04	178	0.97
Treatment (Control)					
Females	22.6	15.6	1.45	52	0.15
Males	12.9	15.5	0.83	50	0.41
Treatment (None)					
Females	**23.6**	**10.6**	**2.23**	**54**	**0.029**
Males	**22.9**	**10.4**	**2.22**	**50**	**0.031**
Age x Sex (M)	1.64	1.37	1.19	4567	0.23
Age^2 x Sex (M)	1.02	1.53	0.67	4543	0.5
Sex (M) x Treatment (Control)	-9.73	7.7	-1.27	164	0.21
Sex (M) x Treatment (None)	-0.63	5.1	-0.12	172	0.9
Age x Treatment (Control)					
Females	**7.6**	**1.8**	**4.17**	**4595**	**<0.0001**
Males	-2.3	1.5	-1.48	4548	0.14
Age x Treatment (None)					
Females	0.79	1.2	0.65	4574	0.51
Males	-0.53	1.01	-0.52	4580	0.6
Age^2 x Treatment (Control)					
Females	-0.13	1.93	-0.07	4521	0.94
Males	-1.05	1.6	-0.65	4547	0.52
Age^2 x Treatment (None)					
Females	-2.6	1.3	-1.96	4534	0.051
Males	**-6.9**	**1.1**	**-6.23**	**4556**	**<0.0001**
Age x Treatment (Control) x Sex (M)	**-9.9**	**2.3**	**-4.23**	**4574**	**<0.0001**
Age x Treatment (None) x Sex (M)	-1.3	1.6	-0.84	4581	0.4
Age^2 x Treatment (Control) x Sex (M)	-0.93	2.5	-0.37	4552	0.71
Age^2 x Treatment (None) x Sex (M)	**-4.3**	**1.7**	**-2.5**	**4552**	**0.01**

Data used in these analyses were 4676 measures of body mass from 195 meerkats produced by 21 dominant females across 53 different litters in 16 different social groups in three different years. Only offspring that survived to 90 days of age were included in these analyses.

Table S4. Effect of dominant female treatments on relative babysitting contributions. Data are from a generalized linear mixed-effects model where the response variable is the proportion of babysitting exhibited by the subordinate meerkat relative to the total babysitting contributions the litter received. The model contained random intercept terms for individual ($\sigma^2 = 0.12$), litter nested within group ($\sigma^2 = < 0.0001$), and year ($\sigma^2 = 0.000$).

Fixed Effect	b	SE	z	P-value
Intercept				
Females	**-2.1**	**0.22**	**-9.37**	**< 0.0001**
Males	**-2.14**	**0.19**	**-11.01**	**< 0.0001**
Babysitting length	**0.24**	**0.08**	**2.77**	**0.0056**
Observation time	**-0.28**	**0.08**	**-3.4**	**0.0007**
Litter size	0.015	0.05	0.32	0.75
Mixed Litter?	-0.04	0.12	-0.31	0.76
Sex (M)	-0.03	0.27	-0.13	0.9
Age				
Females	0.13	0.17	0.75	0.45
Males	**0.4**	**0.19**	**2.77**	**0.0056**
Foraging success	-0.05	0.05	-0.99	0.32
Mass				
Females	**-0.32**	**0.12**	**-2.61**	**0.009**
Males	-0.098	0.14	-0.7	0.48
Group size				
Females	**-0.35**	**0.09**	**-3.97**	**< 0.0001**
Males	**-0.27**	**0.08**	**-3.28**	**0.001**
Treatment (Control)				
Females	**-0.67**	**0.31**	**-2.14**	**0.032**
Males	-0.12	0.28	-0.43	0.67
Treatment (None)				
Females	-0.06	0.23	-0.25	0.8
Males	-0.16	0.18	-0.85	0.39
Foraging success x Mass	0.036	0.06	0.57	0.57
Age x Mass				
Females	**-0.47**	**0.09**	**-5.08**	**< 0.0001**
Males	**-0.29**	**0.08**	**-3.79**	**0.0001**
Age x Sex (M)	0.28	0.23	1.18	0.24
Mass x Sex (M)	0.22	0.17	1.26	0.21
Group size x Sex (M)	0.07	0.1	0.77	0.44
Treatment (Control) x Sex (M)	0.55	0.42	1.31	0.19
Treatment (None) x Sex (M)	-0.1	0.28	-0.36	0.72
Age x Treatment (Control)				
Females	**-0.62**	**0.21**	**-2.89**	**0.0039**
Males	-0.52	0.27	-1.92	0.055
Age x Treatment (None)				
Females	0.34	0.18	1.88	0.059
Males	-0.006	0.16	-0.03	0.97
Age x Mass x Sex	0.18	0.12	1.5	0.13
Age x Treatment (Control) x Sex (M)	0.09	0.34	0.28	0.78
Age x Treatment (None) x Sex (M)	-0.34	0.24	-1.45	0.14

Data used in these analyses were 182 observations of relative babysitting contributions to 28 litters produced in 9 groups across 3 years recorded from 105 subordinate meerkats.

Table S5. Effect of dominant female treatments on relative pup feeding contributions. Data are from a generalized linear mixed-effects model where the response variable is the proportion of pup feeds exhibited by the subordinate meerkat relative to the total pup feeds the litter received. The model contained random intercept terms for individual ($\sigma^2 = 0.000$), litter nested within group ($\sigma^2 = 0.2$), year ($\sigma^2 = 0.08$), and an observational level random intercept term to control for overdispersion ($\sigma^2 = 0.19$).

Fixed Effect	b	SE	z	P-value
Intercept				
Females	**-2.27**	**0.28**	**-8.2**	**<0.0001**
Males	**-2.66**	**0.25**	**-10.45**	**<0.0001**
Observation time	**0.59**	**0.06**	**9.41**	**<0.0001**
Litter size	-0.055	0.1	-0.55	0.58
Mixed litter (Y)	-0.03	0.29	-0.1	0.92
Sex (M)	-0.39	0.2	-1.93	0.053
Age				
Females	**-0.37**	**0.16**	**-2.33**	**0.019**
Males	**0.29**	**0.14**	**2.07**	**0.039**
Foraging success	**0.14**	**0.06**	**2.12**	**0.033**
Mass				
Females	0.1	0.11	0.91	0.36
Males	**-0.37**	**0.11**	**-3.4**	**0.0007**
Group size				
Females	**-0.48**	**0.13**	**-3.57**	**0.0003**
Males	**-0.32**	**0.13**	**-2.46**	**0.014**
Treatment (Control)				
Females	**-0.73**	**0.23**	**-3.12**	**0.0018**
Males	-0.24	0.21	-1.14	0.25
Treatment (None)				
Females	**-0.64**	**0.18**	**-3.49**	**0.0005**
Males	-0.14	0.13	-1.03	0.3
Foraging success x Mass	0.06	0.06	0.97	0.33
Age x Mass				
Females	-0.04	0.07	-0.62	0.54
Males	**-0.19**	**0.07**	**-2.68**	**0.007**
Age x Sex (M)	**0.66**	**0.19**	**3.49**	**0.00048**
Mass x Sex (M)	**-0.47**	**0.13**	**-3.63**	**0.0003**
Group size x Sex (M)	0.16	0.08	1.89	0.059
Treatment (Control) x Sex (M)	0.49	0.31	1.58	0.11
Treatment (None) x Sex (M)	**0.5**	**0.22**	**2.23**	**0.023**
Age x Treatment (Control)				
Females	-0.11	0.22	-0.5	0.61
Males	0.03	0.29	0.11	0.91
Age x Treatment (None)				
Females	0.17	0.16	1.03	0.3
Males	-0.02	0.14	-0.12	0.9
Age x Mass x Sex	-0.14	0.09	-1.51	0.13
Age x Treatment (Control) x Sex (M)	0.14	0.34	0.42	0.67
Age x Treatment (None) x Sex (M)	-0.19	0.21	-0.91	0.36

Data used in these analyses were 192 observations of relative pup feeding contributions to 26 litters produced in 7 groups across 3 years recorded from 101 subordinate meerkats.

Table S6. Effect of dominant female treatments on plasma cortisol concentrations. Data are from a linear mixed-effects model where the response variable is plasma cortisol concentrations (ln transformed) of the subordinate meerkat. The model contained random intercept terms for individual nested within their birth litter ($\sigma^2 = 0.042$), and capture group ($\sigma^2 = 0.000$).

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**3.18**	**0.29**	**11.14**	**120**	**<0.0001**
Males	**3.32**	**0.26**	**12.68**	**111**	**<0.0001**
Sampling time	**1.15**	**0.10**	**11.74**	**260**	**<0.0001**
Sampling time^2	**-0.24**	**0.03**	**-7.27**	**267**	**<0.0001**
Time of day	-0.2	0.14	-1.37	275	0.17
Sample year (2015)	0.46	0.24	1.89	220	0.06
Sex (M)	0.19	0.2	0.91	37	0.37
Age					
Females	-0.1	0.24	-0.41	266	0.68
Males	0.12	0.21	0.57	250	0.57
Body mass	-0.19	0.21	0.89	230	0.37
Foraging success					
Females	-0.1	0.12	-0.84	226	0.4
Males	-0.11	0.11	-0.96	262	0.34
Group size	0.055	0.11	0.49	240	0.62
Group size^2	0.048	0.08	0.58	211	0.56
Pups in group	-0.28	0.18	-1.53	275	0.12
Group sex ratio	**0.19**	**0.08**	**2.35**	**183**	**0.02**
Relatedness	-0.1	0.18	-0.57	41	0.57
Weather (PC1)	-0.1	0.11	-0.94	275	0.35
Treatment (Cortisol)					
Females	-0.03	0.25	-0.11	39	0.91
Males	-0.31	0.19	-1.59	33	0.12
Sex (M) x Age	0.22	0.22	1	272	0.32
Sex (M) x Foraging success	-0.01	0.14	-0.07	257	0.94
Sex (M) x Treatment (Cortisol)	-0.29	0.3	-0.93	34	0.36
Age x Treatment (Cortisol)					
Females	-0.39	0.24	-1.61	275	0.11
Males	**-0.49**	**0.19**	**-2.59**	**274**	**0.01**
Group size x Pups Present (Yes)	-0.05	0.15	-0.37	215	0.71
Group size2 x Pups Present (Yes)	0.07	0.12	0.55	264	0.58
Group size x Weather (PC1)	-0.04	0.08	-0.49	273	0.62
Age x Sex (M) x Treatment (Cortisol)	-0.097	0.31	-0.31	274	0.75

Data used in these analyses were 299 measures of plasma cortisol concentrations from 49 subordinate meerkats produced in 14 litters from 10 different groups. Reference levels (intercept) for "Sex" was female, "Pups in group" was Yes, and for Relatedness was "No parent was dominant".

Table S7. Effect of dominant female treatments on faecal glucocorticoid metabolite (fGCM) concentrations. Data are from a linear mixed-effects model where the response variable fGCM concentrations (ln+1 transformed) of the subordinate meerkat. The model contained random intercept terms for individual nested within their birth litter ($\sigma^2 = 0.10$) and collection group ($\sigma^2 = 0.000$).

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**5.49**	**0.27**	**20.1**	**32**	**<0.0001**
Males	**5.4**	**0.25**	**21.6**	**18**	**<0.0001**
Time of day	**-0.14**	**0.04**	**3.27**	**514**	**0.001**
Sample year (2015)	0.02	0.18	0.13	142	0.89
Sex (M)	-0.9	0.21	-0.44	21	0.67
Age					
Females	**0.81**	**0.27**	**3.03**	**167**	**0.0029**
Males	0.33	0.2	1.68	131	0.096
Body mass	-0.3	0.19	-1.5	317	0.13
Foraging success					
Females	0.21	0.13	1.57	394	0.12
Males	0.06	0.06	1.13	518	0.26
Group size	**0.37**	**0.12**	**3.04**	**113**	**0.003**
Group size2	-0.04	0.11	-0.37	462	0.71
Pups in group	-0.14	0.17	-0.84	325	0.4
Group sex ratio	**0.15**	**0.07**	**2.03**	**190**	**0.04**
Relatedness	0.18	0.29	0.6	14	0.55
Weather (PC1)	0.1	0.07	250	1.47	0.14
Treatment (Cortisol)					
Females	-0.15	0.33	-0.46	14	0.65
Males	-0.06	0.28	-0.22	7	0.83
Sex (M) x Age	**-0.48**	**0.18**	**-2.69**	**230**	**0.008**
Sex (M) x Foraging success	-0.14	0.14	-1.02	395	0.3
Sex (M) x Treatment (Cortisol)	0.09	0.34	0.26	18	0.79
Age x Treatment (Cortisol)					
Females	**-0.65**	**0.22**	**-2.95**	**481**	**0.0033**
Males	-0.07	0.15	-0.49	507	0.62
Group size x Pups Present (Yes)	-0.17	0.12	-1.39	272	0.17
Group size2 x Pups Present (Yes)	0.11	0.12	488	0.96	0.34
Group size x Weather (PC1)	0.06	0.06	1	202	0.32
Age x Sex (M) x Treatment (Cortisol)	**0.57**	**0.25**	**2.3**	**456**	**0.022**

Data used in these analyses were 542 faecal samples (n= 355 from controls, n = 187 from cortisol treated litters) from 34 subordinate meerkats (control: n = 12 females, n = 11 male2; cortisol-treated: n = 5 females, n = 6 males) produced in 10 litters from 7 different groups. Reference levels (intercept) for "Sex" was female, "Pups in group" was Yes, and for Relatedness was "No parent was dominant".