The development of individual differences in cooperative behaviour: maternal glucocorticoid hormones alter helping behaviour of offspring in wild meerkats

Ben Dantzer[1,2,3*], Constance Dubuc[1,2], Ines Braga Goncalves[2,4], Dominic L. Cram[1,2], Nigel C. Bennett[5], Andre Ganswindt[5,6], Michael Heistermann[7], Chris Duncan[2], David Gaynor[2,5], Tim H. Clutton-Brock[1,2,5]

[1]Department of Zoology, University of Cambridge, Cambridge, United Kingdom

[2]Kalahari Meerkat Project, Kuruman River Reserve, Northern Cape, South Africa

[3]Department of Psychology, Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109, USA

[4]Department of Evolutionary Biology and Environmental Studies, Animal Behaviour, University of Zurich, Switzerland

[5]Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Pretoria, South Africa

[6]Endocrine Research Laboratory, Department of Anatomy and Physiology, University of Pretoria, 0110, Onderstepoort, South Africa

[7]Endocrinology Laboratory, German Primate Center, Leibniz Institute for Primate Research, Kellnerweg 4, D-37077 Göttingen, Germany

*Corresponding Author: dantzer@umich.edu

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. We show that in Kalahari meerkats (*Suricata suricatta*), the circulating glucocorticoid (GC) 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 GC-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 grew more slowly 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 GCs as they aged, which could explain the observed increases in cooperative behaviour. Because early life growth is a crucial determinant of fitness in female meerkats, our results indicate that GC-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.

Keywords: Cooperation, Early life adversity, Glucocorticoids, Growth, Maternal stress

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 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 could be especially likely under low food or high stress conditions as 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).

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 levels (GCs) 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 GCs by treating pregnant dominant female meerkats with 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 GCs 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 GCs 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.

To identify if the exposure of mothers to heightened GCs reduced reproductive success of their offspring, we examined if offspring from mothers treated with cortisol during pregnancy grew more slowly early in life. In meerkats, the rate of early life growth and body mass is closely linked to future direct fitness through its effects on survival, foraging success, adult body mass (27-29), as well as the probability of acquiring

dominance (30-31) and other direct reproductive opportunities (32). As elevated exposure to maternal GCs in some mammals may reduce offspring size and growth early in life (15), we predicted that offspring from mothers treated with cortisol during pregnancy would be smaller or grow more slowly early in life. Because the rate of early life growth is predictive of future direct fitness in meerkats (27-32), we predicted that if offspring from mothers treated with cortisol did grow more slowly, they would consequently invest more in indirect fitness opportunities by contributing more to cooperative activities than controls.

Secondly, we determined if offspring from mothers treated with cortisol during pregnancy subsequently increased their contributions to two types of cooperative behaviours: pup rearing ("babysitting": 33) and food provisioning during the period when the pups are foraging with their natal group, but are not yet nutritionally independent ("pup feeding": 34). We chose these two behaviours as they appear to be most costly from an energetic perspective (35) and are most closely tied to the probability of parents successfully rearing offspring. If offspring from mothers treated with cortisol during pregnancy exhibit more of either of these two types of alloparental care, this should increase both parental direct fitness (the number of offspring that they subsequently produce) and the indirect fitness of offspring, because subsequent offspring that receive more alloparental care should grow faster or have higher early life survival (27, 32, 36, 37). Previous studies in meerkats show that offspring with more helpers or those that receive more alloparental care grow faster or have early life survival (27, 32, 36, 37).

To assess the mechanism by which elevated exposure to maternal stress may affect the alloparental care behaviour of offspring, we repeatedly measured plasma

cortisol and faecal glucocorticoid metabolite (fGCM) concentrations of offspring from when they were approximately 1 to 18 months of age to identify how our manipulations affected their neuroendocrine stress axes (GC output). Elevated maternal GCs can cause long-term changes in the neuroendocrine stress axis of offspring (38) and elevated activity of the neuroendocrine stress axis in meerkats can reduce their contributions to alloparental care (39). We therefore predicted that if offspring born to mothers treated with cortisol during pregnancy exhibited more alloparental care behaviour compared to controls, they would also have reduced plasma cortisol and fGCM concentrations.

Methods

Study site & basic data collection

We studied free-living meerkats at the Kuruman River Reserve (26° 58' S, 21° 49' E) in the Northern Cape, South Africa from 2014-2017. Individuals were marked uniquely with PIT tags (Identipet®, Johannesburg, South Africa) as well as dye marks so that they could be identified. Groups were visited for ~4-8 hours per day ~4-6 times per week throughout each year of study and sometimes more frequently such as when there were pups being babysat. Groups were visited at sunrise before meerkats emerged from their sleeping burrow. After all the meerkats had emerged, but prior to when they started going foraging, we counted the total number of meerkats in the group (to get estimates of group size) and recorded which individuals were present (using their unique combinations of dye marks). We recorded their body mass on a portable balance each morning before foraging, 2-4 hrs after foraging was initiated, and

134 immediately prior to when foraging ended (40). These measures of body mass provided
135 our estimates of growth, body mass, and foraging success that are used in our analyses
136 described below.

137 *Experimental manipulations of dominant females*

138 Dominant females in each group were identified via behavioural observations
139 (41). The pregnancy status of dominant females was determined visually (distended
140 abdomen) as well as noting a constant increase in their body mass. Dominant females
141 were treated with either a cortisol solution or a control oil vehicle when they were
142 pregnant by feeding them food containing one of these two treatments. We initially
143 offered experimental animals hard boiled eggs with added cortisol but found that they
144 rejected all foods that contained added cortisol with the exception of scorpions. We
145 consequently fed experimental females with cortisol (10 mg/kg of hydrocortisone, Sigma
146 H4126), that were dissolved in 100 μl of 100% coconut oil and injected into a dead
147 scorpion (*Opistophthalmus* spp.). Control females were fed a dead scorpion that was
148 injected with 100 μl of 100% coconut oil. A previous study using the same protocol
149 showed that meerkats that were fed cortisol had significantly higher plasma cortisol and
150 fGCM concentrations than control animals and these increases were within a
151 biologically relevant range (39). This indicated that our treatment causes the exogenous
152 glucocorticoids that we feed the meerkats to enter their bloodstream and leads to
153 sustained increases in their circulating glucocorticoid concentrations.

154 Females were randomly allocated to the treatments. Across the three years of
155 this study, we produced a total of 13 cortisol-treated litters from 10 females and 7
156 control litters produced by 6 females (Table 1). Three of the females experienced both

157 the control and cortisol treatments at different time points of the experiment, whereas
158 one female experienced the control treatment once and the cortisol treatments twice.
159 For these latter females treated twice, the order of treatments was randomly selected.
160 We conducted these experiments over the course of three years: 13 litters from 10
161 females in 2014 (April-December 2014, 3 litters aborted), 5 litters from 5 females in
162 2015 (February-July 2015, 1 litter aborted), and 3 litters from 3 females in 2016 (July
163 2016). Three cortisol-treated mothers and one control mother aborted their offspring
164 prior to birth and were excluded from any analyses except for assessing differences in
165 the frequency of abortion between control and cortisol-treated mothers (Table 2). This
166 provided final sample sizes of 31 pups from 10 litters from 9 cortisol-treated females
167 and 25 pups from 6 litters from 6 control females (Table 1).

168 We aimed to experimentally increase the glucocorticoid concentrations of
169 pregnant dominant females from when they were first confirmed to be pregnant (second
170 half of gestation) until parturition. Gestation in meerkats is ~70 days so we aimed to
171 treat them with glucocorticoids from approximately 35-70 d during gestation. In reality,
172 females that successfully produced a litter where pups emerged from the natal burrow
173 were treated with cortisol for 12-36 days prior to birth (n=10 litters from 9 females, mean
174 = 23.7 d, median = 23.5 d), whereas controls were fed for 12-58 d prior to birth (n=6
175 litters from 6 females, mean = 30 d, median = 20.5 d). Although controls were treated
176 for slightly longer, there was no significant difference in treatment duration between
177 control and cortisol-treated females (general linear model, $t = 1.05$, $P = 0.31$).

178 To provide an additional comparison group to investigate how our treatments (fed
179 during pregnancy or fed cortisol during pregnancy) affected offspring survival, growth,

and cooperative behaviour, we also monitored these traits in offspring produced by dominant females that were untreated during pregnancy (n = 52 litters from 21 dominant females, Table 1). These females were not fed or treated with cortisol (hereafter, "untreated mothers"). For our analyses of how the treatments affected offspring survival and growth, the untreated offspring were those from litters produced by dominant females in other meerkat groups in our same study area and were born during our study. We assessed the contributions of offspring from mothers treated with cortisol during pregnancy to two cooperative behaviours (babysitting and pup feeding) compared to those from control mothers, but also to other group members from untreated mothers. We did not have data from offspring from untreated mothers when we assessed how our treatments affected their plasma cortisol or fGCM concentrations.

Quantifying early life growth of offspring

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

Quantifying cooperative behaviour of offspring

We measured the babysitting (controls: 195-655 d; cortisol: 184-655 d; untreated: 155-655 d) and pup feeding (controls: 220-635 d; cortisol: 184-655 d; untreated: 155-626 d) contributions of offspring from cortisol-treated and control mothers when they were >6 months of age until death or disappearance. We visited sleeping burrows containing pups every day in the morning and recorded the identity of the attending babysitters. As we have done previously (33, 36, 39, 42), we calculated relative babysitting contributions of each individual meerkat for each litter by dividing the total number of days an individual babysat a litter over the total number of days that this specific litter had a babysitter. Pup feeding behaviour for each pup produced by the dominant females in the different treatment groups was estimated using *ad libitum* sampling (34, 39). When the social group contained pups (up to 90 d of age), we recorded all pup-feeding events from all individuals, which are visually and acoustically conspicuous to observers (43). We then used these data to estimate the proportion of pup-feeding events exhibited by an individual relative to all others in the group (i.e., relative pup feeding). Because the total amount of time devoted to the *ad libitum* recording sessions varied, we corrected for variation in observation time (see below).

Quantifying plasma cortisol concentrations from offspring

We obtained plasma samples from offspring from cortisol-treated and control mothers approximately every 3 months from first emergence from the burrow (~1 month) until ~18 months of age (controls: 20-548 d; cortisol-treated: 25-559 d). Capture and blood processing procedures are described elsewhere (44-45). The amount of time it took to obtain the blood samples varied (median = 10.6 min, SD = 7.2 min), but we included co-variates for sampling time and sampling time^2 to control for effects of

225 sampling time (described in 45). We measured total plasma cortisol concentrations
226 using a previously validated assay (Coat-a-Count, Siemens Diagnostic Products
227 Corporation, Los Angeles, USA: validation described in 44). The sensitivity of the assay
228 was 1.9 ng/ml and cross-reactivity to other hormones was 76% with prednisolone,
229 11.4% with 11-deoxycortisol, 2.3% with prednisone and <1% with aldosterone,
230 corticosterone, cortisone, oestriol, estrone and pregnenolone. Intra-assay coefficient of
231 variation (CV) was 7% (n = 20 samples). Inter-assay CV for a low control (78.5 ± 6.3
232 ng/ml n = 5 assays) was 8% and 2.8% for a high control (187 ± 5.3 ng/ml, n = 5
233 assays).

234 *Quantifying fGCM concentrations from offspring*

235 We collected faecal samples from offspring of cortisol-treated and control
236 mothers opportunistically during behavioural observations over the course of the study
237 (controls: 25-356 d; cortisol-treated: 32-326 d). Faecal samples were processed as
238 described previously using a methanol solution to extract fGCMs for analysis (46-47).
239 Immunoreactive fGCM concentrations were determined using a group-specific enzyme
240 immunoassay measuring cortisol metabolites with a 5β-3α,11β-diol-structure (11β-
241 hydroxyetiocholanolone), already validated and established for monitoring fGCM
242 alterations in meerkats (47). Faecal GCMs measured reflect average adrenal cortisol
243 production over the previous ~24 to 48 hr period (47). Detailed assay characteristics,
244 including full descriptions of the assay components and cross-reactivities, are found
245 elsewhere (48). The sensitivity of the assay was 1.2 ng/g dry weight and intra-assay CV
246 determined by repeated measurements of high and low value quality controls were

247 6.9% and 7.4% and inter-assay CV values were 11.5% and 15.9% (n = 29 assays),
248 respectively.

249 *Statistical analyses*

250 We used generalized (binomial errors) or linear mixed-effects models (LMMs) to
251 examine how our treatments affected the probability that the litter was aborted, litter size
252 and sex ratio at emergence from the burrow, and the proportion of the litter that survived
253 to emergence from the burrow, independence (~90 d of age: 29), and 6 or 12 months of
254 age. We focused on addressing whether the offspring from cortisol-treated mothers
255 differed from control or untreated mothers. These models included a fixed effect for date
256 of birth of the litter and random intercept terms for dominant female identity and year (as
257 the experiments were conducted over 3 years). None of the GLMMs were
258 overdispersed (Table 2).

259 We used a LMM to investigate how the maternal treatments affected offspring
260 growth from first emergence from their natal burrow (~1 month) to 3 months of age
261 when the pups are typically foraging independently (29, 34). Morning body mass (in
262 grams) was the response variable with the fixed effects of maternal treatment (cortisol-
263 treated, control, or untreated), pup sex, pup age, litter size at burrow emergence, first
264 measure of body mass when the pups first emerged from the burrow (to control for
265 possible differences in age or development when they entered our study population),
266 group size, group size^2, total rainfall in the previous 60 days, two measures of
267 seasonality (sine and co-sine functions of day of weight measure: see 40), and two
268 three-way interactions between sex, treatment, age or age^2. Group size was defined as
269 the average number of subordinate meerkats >6 months of age in the group during the

entire period of offspring growth. Random intercept terms for year and the identity of the individual nested in litter, nested in dominant female identity, nested in group were also included in this model. Fixed and random effects included in these models were based upon previous studies investigating meerkat body mass and/or growth from 1-3 months (28, 31, 40). To prevent any issues associated with selective disappearance of specific individuals, only individuals that survived to 90 d were included in these analyses.

We assessed how the treatments affected the relative babysitting and pup feeding contributions of subordinates when they were >6 months (as they rarely do alloparental care behaviour when <6 months: 36) from cortisol-treated, control, and untreated mothers. Relative babysitting and pup feeding contributions are defined as the proportion of babysitting or pup feeding contributions exhibited by a specific individual compared to the total number of babysitting or pup feeding contributions for that litter exhibited by all individuals in the group that were >6 months of age at the time of the birth of the litter (36, 39, 42). In these generalized linear mixed-effects models (GLMM, binomial errors), we included a three-way interaction between treatment, sex, and age of the subordinate to assess if the effects of the treatments on babysitting or pup feeding varied according to the sex or age of the subordinate, as contributions to cooperative behaviour in meerkats are known to vary according to subordinate sex and age (36). To account for differences in observation time, we included a co-variate for the number of days the litter was babysat (babysitting length) and the number of days the subordinate was observed in the group during babysitting as well as the total time spent observing the group during pup feeding (observation time). We included a range of co-variates (see Tables 4-6) that have been previously documented to affect relative

293 contributions to babysitting and pup feeding, including age, foraging success, body
294 mass, and group size (34-36, 42, 49; 50). Group size was defined as the average
295 number of subordinate meerkats >6 months of age in the group while the litter was
296 being babysat or pup fed. Foraging success was defined as the average weight gained
297 per hour estimated as the change in body mass from morning weight to evening weight
298 over the total number of hours that had elapsed since those two weights (45).
299 Relatedness between the subordinate and the litter being babysat was not included as it
300 has not been shown to impact babysitting or pup feeding contributions (27, 42) and
301 nearly all of the litters in our dataset were produced by the mother or full sibling of the
302 subordinate. Random intercept terms for year and the identity of the individual, and litter
303 being babysat or pup fed were nested within the group where the litter was being
304 babysat or pup fed. Overdispersion was not an issue for our GLMM for babysitting as
305 indicated by the goodness of fit test (Pearson χ^2 = 147.1, df = 154, P = 0.64, using
306 package aods3: 51) but our GLMM for pup feeding was initially overdispersed (Pearson
307 χ^2 = 310, df=165, P < 0.0001) so we included an observation level random intercept
308 term.

309 We used two separate LMMs to assess how our manipulations affected plasma
310 cortisol and fGCMs in offspring from cortisol-treated and control mothers (we did not
311 have these data from offspring from untreated mothers). Each model included fixed
312 effects for maternal treatment, pup sex and age, time of day and year that the sample
313 was acquired (2014 or 2015), and random intercept terms for identity of individual
314 nested in their birth litter and group. In the model for plasma cortisol concentrations, we
315 also included a linear and second order fixed effect for the time it took to acquire the

316 blood sample to control for any variation in plasma cortisol concentrations due to
317 restraint stress (45). Year was included as a fixed effect because we only had samples
318 from two separate years. We included covariates associated with the individual meerkat
319 and weather or social group characteristics that are known to affect plasma cortisol (45)
320 or fGCM (47) concentrations (see Tables 6-7).

321 We used R (version 3.4.3: 52) for all of our statistical analyses. R package lme4
322 (version 1.1-14: 53) was used for LMMs and P values were estimated using lmerTest
323 (version 2.0-33: 54). A graphical approach was used to confirm normality and
324 homoscedasticity of residuals and to confirm there were no observations with high
325 leverage (55). Collinearity among predictor variables included in our models was
326 assessed by calculating variance inflation factors (55) or generalized variance inflation
327 factors (for variables that had a second order term or those included in an interaction:
328 56). Collinearity was not a problem as indicated by our variance inflation factors (VIFs)
329 as VIFs or generalized VIFs were less than ~4 for all variables. In our model for how our
330 treatments affected offspring growth (Table 3), the generalized VIF for the two
331 measures of seasonality (sine and co-sine functions of day of weight measure) were <6
332 but these two variables were included a priori given their previously documented effects
333 on body mass and growth in meerkats (40). All continuous variables were standardized
334 to a mean of 0 and SD of 1.
335
336 **Results**
337 *Effects of treatments on litter characteristics and offspring survival*

There was no evidence that the treatment of pregnant females with cortisol affected their ability to maintain litters to term or the survival of their pups prior to emergence from the natal burrow (Tables 1-2). The number of pups surviving to emergence from the natal burrow or 3, 6, or 12 months of age and the litter sex ratio were not different among litters from cortisol-treated, control, or untreated females (Tables 1-2).

Effects of treatments on offspring early life growth

The effects of the treatments on offspring growth from initial emergence to nutritional independence (1-3 months) differed between daughters and sons, as reflected in the significant three-way interaction between treatment, sex, and age (Table 3). Daughters (but not sons) from cortisol treated mothers grew more slowly from 1-3 months compared to those from control (fed) mothers (daughters: age x treatment, t = -4.17, $P < 0.0001$; sons: t = -1.48, P = 0.14), but exhibited similar growth compared to those from untreated (unfed) mothers (daughters: age x treatment, t = 0.65, P = 0.51; sons: t = -0.52, P = 0.6, Table 3, Fig. 1). Daughters, but not sons, from control (fed) mothers grew faster than those from untreated mothers (daughters: age x 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 4). Babysitting contributions in daughters from mothers treated with cortisol during pregnancy were slightly, but significantly higher with increasing age of the babysitter compared to those from control mothers (age x treatment, z = -2.89, P = 0.0039) but not untreated mothers (age x

361 treatment, z = 1.88, P = 0.06; Table 4, Fig. 2). Babysitting contributions in sons from
362 mothers treated with cortisol during pregnancy showed a similar tendency to slightly
363 increase with age compared to those from control mothers, but this difference was not
364 significant (age x treatment, z = -1.92, P = 0.055). Further, age-related increases in
365 babysitting contributions between males from mothers treated with cortisol during
366 pregnancy and untreated mothers did not differ (age x treatment, z = -0.03, P = 0.97;
367 Table 4, Fig. 2). Comparisons of the magnitude of effect sizes showed that the
368 interaction between age and maternal treatment had a larger effect on babysitting
369 contributions in daughters but not sons than other variables known to impact babysitting
370 contributions, such as foraging success, age-related body mass, or group size (Table
371 4).

372 The effects of the maternal treatments on pup feeding depended upon the sex of
373 the offspring, but not their age (Table 5). Daughters, but not sons from mothers treated
374 with cortisol during pregnancy exhibited significantly more pup feeding contributions
375 than those from control mothers (females: z = -3.12, P = 0.00018; males: z = -1.14, P =
376 0.25) or untreated mothers (females: z = -3.49, P = 0.0005, sons: z = -1.03, P = 0.3,
377 Table 5, Fig. 3). Notably, the magnitude of effect size of maternal treatment for
378 daughters was much larger than other variables known to impact babysitting
379 contributions such as foraging success, age-related body mass, and group size (Table
380 5).

381 *Effects of treatments on offspring stress physiology*

382 Daughters from mothers treated with cortisol during pregnancy had lower
383 plasma cortisol concentrations (age x treatment, t = -1.76, P = 0.08, Table 6, Fig. 4A)

and lower fGCM concentrations (age x treatment, t = -2.9, P = 0 .004, Table 7, Fig. 5A) as they became older compared to those from control mothers but these differences were only significant for fGCM concentrations. Sons from mothers treated with cortisol during pregnancy had significantly lower plasma cortisol concentrations as they became older compared to those from control mothers (age x treatment t = -2.68, P = 0.008, Table 6, Fig. 4B) but similar fGCM concentrations compared to those from control mothers as they became older (age x treatment, t = -0.1, P = 0.49, Table 7, Fig. 5B).

Discussion

We found some support for our hypothesis that elevated maternal GCs would reduce the potential for offspring to have direct reproductive opportunities and would therefore shift them towards exhibiting more cooperative behaviour that could increase their indirect fitness. Daughters, but not sons, from mothers treated with cortisol during pregnancy grew more slowly early in life and exhibited more babysitting and pup feeding behaviour as they became older compared to controls. Other than offspring survival (Table 2), we were unable to quantify the direct and indirect fitness of offspring from control or cortisol-treated mothers, but early life growth or body mass (which we measured here) is closely linked to direct fitness opportunities in daughters (27-32). Previous studies in meerkats show that female, but not male, offspring that grow faster from 1-3 months are more likely to acquire the dominant breeding position (31), perhaps because offspring that grow faster in their first 3 months of life are heavier later in life (32, 57, 58), and heavier females are more likely to acquire a vacant dominant breeding position (30, 32). As such, daughters, but not sons, from mothers treated with cortisol

levels during pregnancy should have reduced future direct fitness opportunities and therefore increase their investment in behaviours that elevate their indirect fitness. Our results are consistent with studies in other taxa that suggest that individuals adjust their contributions to cooperative behaviour according to their future reproductive potential. For example, in cooperatively breeding birds, when the chances of direct reproduction are elevated, subordinates often stop helping at the nest (59). Studies of social wasps show that individuals whose probability of acquiring the dominant breeding position was experimentally increased exhibited significantly less helping behaviour (60, 61). Finally, in cooperatively breeding fish, subordinates will reduce their helping investment immediately prior to dispersal from their natal group where they attempt to reproduce on their own rather than stay in their natal group and queue for dominance (62).

Our results show that increases in maternal GCs can increase the cooperative behaviour of daughters, which should lead to substantial direct fitness benefits to mothers. Daughters from mothers treated with cortisol during pregnancy exhibited more alloparental care compared to controls, such that subsequent offspring produced in groups with offspring from cortisol-treated mothers should have received more alloparental care. Because offspring that receive more alloparental care grow faster early in life or are larger later in life (32, 57), the presence of offspring from cortisol-treated mothers should increase the direct fitness of dominant breeders and the indirect fitness of the offspring from cortisol-treated mothers. Taken together, our results suggest that this GC-mediated maternal effect reduced the direct fitness opportunities of daughters by reducing their early life growth, but they compensated by increasing their investment in indirect fitness opportunities (helping to rear non-descendent offspring).

This is in line with theoretical predictions that parental manipulation of the cooperative behaviour of offspring can evolve if the costs of resisting the parental effect are high and inclusive fitness benefits of helping rear subsequent offspring are increased (18), as is the case in cooperative breeders.

Control females that were fed during pregnancy produced daughters that grew faster during early development (1-3 months) compared to daughters from cortisol-treated or untreated mothers. Although mothers that were treated with cortisol during pregnancy received the same amount of supplemental food as controls, daughters and sons from mothers fed cortisol during pregnancy did not differ in early life growth compared to those from untreated mothers. This indicates that the additional food provided to dominant females during pregnancy had the potential to increase growth, but the added cortisol prevented those gains in body mass. This has implications for understanding the fitness consequences of maternal stress on offspring growth trajectories (15, 63) because our results show that elevated circulating GC levels in pregnant females in the absence of energetic constraints induced reductions in the early life growth of offspring. This supports the hypothesis that maternal GC levels during offspring development act as a cue that induces plasticity in offspring growth rather than simply mediating the effects of energetic constraints. Alternatively, elevated maternal GCs could alter patterns of maternal investment in offspring. Identifying whether offspring or mothers are driving these effects is a major challenge in studies of maternal stress effects in wild animals.

The reductions in the activity of the neuroendocrine stress axis of daughters may have potentiated the increased alloparental care behaviour that we observed.

Compared to daughters from control mothers, daughters from mothers treated with cortisol during pregnancy exhibited more babysitting as they became older, more overall pup feeding, and they also had lower plasma cortisol and fGCM concentrations. Males from mothers treated with cortisol during pregnancy had significantly lower plasma cortisol concentrations, but not fGCM concentrations as they got older and also tended to exhibit more babysitting as they aged. The activity of the neuroendocrine stress axis is closely linked to an array of social behaviours (64) and our recent work shows that elevated activity of the neuroendocrine stress axis reduces babysitting in both females and males and decreases pup feeding in females (39). Together, this supports the hypothesis that the mechanism by which early life stress increases the cooperative behaviour of daughters is by dampening the activity of their neuroendocrine stress axis.

Our results show that the effects of maternal GCs on offspring growth, physiology, and behaviour were greater in daughters than in sons, which adds to biomedical (65-66) and ecological (67-69) studies that highlight how early life conditions or maternal GC levels can have sex-specific consequences for offspring. In meerkats, there may be added benefits for the dominant female for altering the cooperative behaviour of daughters compared to sons; daughters exhibit more cooperative behaviour than sons (36) and are more responsive to the begging calls of subsequent offspring that they provision with food (70). More broadly, sex-differences in natal dispersal may cause these differential responses to parental effects. In meerkats, subordinate males voluntarily disperse from their natal group to look for receptive females but can return to their natal group whereas subordinate females rarely voluntarily disperse from their natal group (71). In our case and in others (63), the more

476 philopatric sex (females) is more sensitive to early life conditions, which may be due to
477 differential costs of parental modification between the philopatric and dispersing sex. If
478 parental effects have long-term consequences on offspring characteristics, as we show
479 here, there may be an increased degree of mismatch between the phenotype of the
480 dispersing sex and the postnatal environment where individuals eventually settle. If this
481 mismatch has fitness costs, this should select for individuals from the dispersing sex to
482 be less responsive to cues from the parental phenotype or environment.

483 Our results provide some support for the hypothesis that parents may alter the
484 cooperative tendencies of their offspring by manipulating the characteristics of their
485 offspring (9-10), though we note that it is uncertain if the transfer of maternal GCs to
486 offspring was passive or active. Explanations regarding the evolutionary origins of
487 cooperative behaviour involve nepotism or kin selection (72), mutualisms or reciprocity
488 (73), but few studies have tested the “parental manipulation” hypothesis proposed by
489 Alexander (9). Some studies show that alleles that increase maternal fitness at the
490 expense of the direct fitness of offspring can evolve (19) and that cooperative breeders
491 may bias investment towards offspring that exhibit more cooperative behaviour (74).
492 Our study supports the hypothesis that environmental stressors may induce a parental
493 effect that can modify the cooperative tendencies of their offspring.

494 Finally, our results have two implications for theoretical models examining the
495 evolution of parental effects. First, given the sex-specificity of parental effects, our
496 results challenge the conclusions of models examining the evolution of parental effects
497 that assume that all offspring are equally sensitive to the parental effect (16), or those
498 that assume that the benefits of exhibiting the phenotype resulting from the parental

499 effect are equal for all offspring (18). Second, selfish parental effects are thought to be
500 relatively rare (8, 13) and theory (16-17) and empirical studies showing sex-specific
501 responses to early life stress (65-66) indicate that offspring can become resistant to
502 such selfish parental effects. However, some models indicate that the evolution of
503 selfish parental effects may be dependent upon the social environment (24), especially
504 if the selfish parental effect influences the expression of alloparental care behaviour of
505 offspring and therefore increases the indirect fitness of offspring. Our results provide an
506 example whereby a GC-mediated maternal effect should decrease the direct fitness of
507 daughters (by reducing their early life growth), but should increase the direct fitness of
508 mothers and indirect fitness of daughters by elevating their cooperative behaviour.
509
510 **Ethics:** All protocols used in our experiments were approved by the Animal Ethics
511 Committee at the University of Pretoria (Pretoria, South Africa: #EC031-13, #EC047-16)
512 and the Northern Cape Department of Environment and Nature Conservation Research
513 (South Africa: FAUNA 050/2013, FAUNA 192/2014, FAUNA 1020/2016).
514
515 **Acknowledgements:** We thank M. Manser for her contributions to maintain the long-
516 term Kalahari Meerkat Project (KMP) and her contributions to this specific project.
517 Thanks to P. Roth, S. Bischoff-Mattson, L. Howell, J. Samson, N. Thavarajah, and
518 numerous KMP volunteers who contributed to data collection. Thanks to M. Manser and
519 S. Guindre-Parker for helpful comments. Thanks to the Northern Cape Conservation
520 Authority for permission to conduct research and the Kotze family for allowing us to
521 study meerkats on their property.

522

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

527

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

530

531 **Competing interests:** We have no competing interests.

532

533 **Funding:** Research was supported by grants from National Environment Research
534 Council (RG53472) and the European Research Council (294494) to T.H.C-B. I.B.G.
535 was funded by the Swiss National Science Foundation (grant no. 31003A_13676 to M.
536 Manser). NCB was supported by a DST-NRF SARCHI chair of Mammal Behavioural
537 Ecology and Physiology. The KMP has also been financed by the University of
538 Cambridge (T.H.C.-B) and University of Zurich (M. Manser).

539

References

1) Wolf JB, Wade MJ (2009) What are maternal effects (and what are they not)? *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1520), 1107-1115.

2) Kirkpatrick M, Lande R (1989) The evolution of maternal characters. *Evolution*, *43*(3), 485-503.

3) Mousseau TA, Fox CW (Eds.) (1998) *Maternal Effects as Adaptations*. Oxford University Press.

4) Agrawal AA, Laforsch C, Tollrian R (1999) Transgenerational induction of defences in animals and plants. *Nature* 401, 60-63

5) Dantzer B, Newman AE, Boonstra R, Palme R, Boutin S, Humphries MM, McAdam AG (2013) Density triggers maternal hormones that increase adaptive offspring growth in a wild mammal. *Science*, 1235765.

6) Duckworth RA, Belloni V, Anderson SR (2015) Cycles of species replacement emerge from locally induced maternal effects on offspring behavior in a passerine bird. *Science*, *347*(6224), 875-877.

7) Uller T (2008) Developmental plasticity and the evolution of parental effects. *Trends in Ecology & Evolution*, *23*(8), 432-438.

8) Uller T, Nakagawa S, English S (2013) Weak evidence for anticipatory parental effects in plants and animals. *Journal of Evolutionary Biology*, *26*(10), 2161-2170.

9) Alexander, RD, (1974) The evolution of social behavior. *Annual Review of Ecology and Systematics*, *5*(1), 325-383.

10) Michener CD, Brothers DJ 1974 Were workers of eusocial Hymenoptera initially

563 altruistic or oppressed? *Proceedings of the National Academy of Science* USA
564 71, 671–674
565 11) Müller W, Lessells CKM, Korsten P, Engelhardt NV (2007) Manipulative signals in
566 family conflict? On the function of maternal yolk hormones in birds. *The American*
567 *Naturalist*, *169*(4), E84-E96.
568 12) Schwarzkopf L, Andrews RM (2012) Are moms manipulative or just selfish?
569 Evaluating the “maternal manipulation hypothesis” and implications for life-history
570 studies of reptiles. *Herpetologica*, *68*(2), 147-159.
571 13) Marshall DJ, Uller T (2007) When is a maternal effect adaptive?. *Oikos*, *116*(12),
572 1957-1963.
573 14) Wilson AJ, Pilkington JG, Pemberton JM, Coltman DW, Overall AD, Byrne KA,
574 Kruuk, LEB (2005) Selection on mothers and offspring: whose phenotype is it
575 and does it matter? *Evolution*, *59*(2), 451-463.
576 15) Berghänel A, Heistermann M, Schülke O, Ostner J (2017) Prenatal stress
577 accelerates offspring growth to compensate for reduced maternal investment
578 across mammals. *Proceedings of the National Academy of Sciences*, *114*(50),
579 E10658-E10666.
580 16) Uller T, Pen I (2011) A theoretical model of the evolution of maternal effects under
581 parent–offspring conflict. *Evolution*, *65*(7), 2075-2084.
582 17) Kuijper B, Johnstone RA (2018) Maternal effects and parent–offspring
583 conflict. *Evolution*, *72*(2), 220-233.
584 18) González-Forero M, Gavrilets S (2013) Evolution of manipulated behavior. *The*
585 *American Naturalist*, *182*(4), 439-451.

586 19) Wade MJ (2001) Maternal effect genes and the evolution of sociality in haplo-diploid
587 organisms. *Evolution*, *55*(3), 453-458.
588 20) Linksvayer TA, Wade MJ (2005) The evolutionary origin and elaboration of sociality
589 in the aculeate Hymenoptera: maternal effects, sib-social effects, and
590 heterochrony. *The Quarterly Review of Biology*, *80*, 317-336.
591 21) Bourke AF, Franks NR (1995). *Social Evolution in Ants*. Princeton University Press.
592 22) Clutton-Brock TH (2016) *Mammal Societies*. Wiley-Blackwell
593 23) Russell A F, Lummaa V (2009) Maternal effects in cooperative breeders: from
594 hymenopterans to humans. *Philosophical Transactions of the Royal Society B:*
595 *Biological Sciences*, *364*(1520), 1143-1167.
596 24) Taborsky B (2017) Developmental plasticity: preparing for life in a complex
597 world. *Advances in the Study of Behavior*, *49*, 49-99.
598 25) Carter CS, Roberts RL. (1997) The psychobiological basis of cooperative breeding
599 in rodents. In *Cooperative Breeding in Mammals* (Eds.: NG Solomon, JA
600 French), Cambridge University Press, Pp. 231-266.
601 26) De Fraipont M, Clobert J, Alder HJ, Meylan S (2000) Increased pre-natal maternal
602 corticosterone promotes philopatry of offspring in common lizards *Lacerta*
603 *vivipara*. *Journal of Animal Ecology*, *69*(3), 404-413.
604 27) Clutton-Brock TH, Brotherton PNM, O'riain MJ, Griffin AS, Gaynor D, Kansky R,
605 Sharpe L, McIlrath GM (2001) Contributions to cooperative rearing in
606 meerkats. *Animal Behaviour*, *61*(4), 705-710.
607 28) Russell AF, Clutton-Brock TH, Brotherton PN, Sharpe LL, Mcilrath G, Dalerum FD,
608 Cameron EZ, Barnard JA (2002). Factors affecting pup growth and survival in co-

operatively breeding meerkats *Suricata suricatta*. *Journal of Animal Ecology*, *71*(4), 700-709.

29) Thornton A (2008) Social learning about novel foods in young meerkats. *Animal Behaviour*, *76*(4), 1411-1421.

30) Hodge SJ, Manica A, Flower TP, Clutton-Brock TH (2008) Determinants of reproductive success in dominant female meerkats. *Journal of Animal Ecology*, *77*(1), 92-102.

31) English S, Huchard E, Nielsen JF, Clutton-Brock TH (2013) Early growth, dominance acquisition and lifetime reproductive success in male and female cooperative meerkats. *Ecology and Evolution*, *3*(13), 4401-4407.

32) Russell AF, Young AJ, Spong G, Jordan NR, Clutton-Brock TH (2007) Helpers increase the reproductive potential of offspring in cooperative meerkats. *Proceedings of the Royal Society of London B: Biological Sciences*, *274*(1609), 513-520.

33) Clutton-Brock TH, Gaynor D, Kansky R, MacColl ADC, McIlrath G, Chadwick P, Brotherton PNM, O'rian JM, Manser M, Skinner JD (1998) Costs of cooperative behaviour in suricates (*Suricata suricatta*). *Proceedings of the Royal Society of London B: Biological Sciences*, *265*(1392), 185-190.

34) Brotherton PNM, Clutton-Brock TH, O'riain MJ, Gaynor D, Sharpe L, Kansky R, McIlrath GM (2001) Offspring food allocation by parents and helpers in a cooperative mammal. *Behavioral Ecology*, *12*(5), 590-599.

35) Russell AF, Sharpe LL, Brotherton PNM, Clutton-Brock TH (2003) Cost

631 minimization by helpers in cooperative vertebrates. Proceedings of the National
632 Academy of Sciences USA 100(6), 3333-3338.
633 36) Clutton-Brock TH, Russell AF, Sharpe LL, Young AJ, Balmforth Z, McIlrath GM
634 (2002). Evolution and development of sex differences in cooperative behavior in
635 meerkats. *Science*, *297*(5579), 253-256.
636 37) Bell MBV, Cant MA, Borgeaud C, Thavarajah N, Samson J, Clutton-Brock TH
637 (2014) Suppressing subordinate reproduction provides benefits to dominants in
638 cooperative societies of meerkats. *Nature Communications* 5, 4499
639 38) Harris A, Seckl J (2011) Glucocorticoids, prenatal stress and the programming of
640 disease. *Hormones and Behavior*, *59*(3), 279-289.
641 39) Dantzer B, Goncalves IB, Spence-Jones HC, Bennett NC, Heistermann M,
642 Ganswindt A, Dubuc C, Gaynor D, Manser MB, Clutton-Brock TH 2017. The
643 influence of stress hormones and aggression on cooperative behaviour in
644 subordinate meerkats. *Proc. R. Soc. B*, *284*(1863), 20171248.
645 40) English S, Bateman AW, Mares R, Ozgul A, Clutton-Brock TH (2014) Maternal,
646 social and abiotic environmental effects on growth vary across life stages in a
647 cooperative mammal. *Journal of Animal Ecology*, *83*(2), 332-342.
648 41) Kutsukake N, Clutton-Brock TH (2006) Aggression and submission reflect
649 reproductive conflict between females in cooperatively breeding meerkats
650 *Suricata suricatta*. *Behav. Ecol. Sociobiol.* **59**, 541-548. (doi: 10.1007/s00265-
651 005-0079-7)
652 42) Clutton-Brock TH, Brotherton PNM, O'Riain MJ, Griffin AS, Gaynor D, Sharpe L,

653 Kansky R, Manser MB, McIlrath GM (2000). Individual contributions to
654 babysitting in a cooperative mongoose, *Suricata suricatta*. *Proceedings of the*
655 *Royal Society of London B: Biological Sciences*, *267*(1440), 301-305.
656 43) Kunc HP, Madden JR, Manser MB (2007) Begging signals in a mobile feeding
657 system: The evolution of different call types. *The American Naturalist* 170, 617-
658 624.
659 44) Carlson AA, Young AJ, Russell AF, Bennett NC, McNeilly AS, Clutton-Brock T
660 (2004). Hormonal correlates of dominance in meerkats (*Suricata*
661 *suricatta*). *Hormones and Behavior*, *46*(2), 141-150.
662 45) Dantzer B, Bennett NC, Clutton-Brock TH (2017) Social conflict and costs of
663 cooperation in meerkats are reflected in measures of stress
664 hormones. *Behavioral Ecology*, *28*(4), 1131-1141.
665 46) Heistermann M, Finke M, Hodges JK (1995) Assessment of female reproductive
666 status in captive-housed Hanuman langurs (*Presbytis entellus*) by measurement
667 of urinary and fecal steroid excretion patterns. *Am. J. Primatol.* 37, 275-284. (doi:
668 10.1002/ajp.1350370402)
669 47) Braga Goncalves I, Heistermann M, Santema P, Dantzer B, Mausbach J,
670 Ganswindt A, Manser, MB (2016) Validation of a fecal glucocorticoid assay to
671 assess adrenocortical activity in meerkats using physiological and biological
672 stimuli. *PloS One*, *11*(4), e0153161.
673 48) Heistermann M, Palme R, Ganswindt A (2006) Comparison of different
674 enzyme immunoassays for assessment of adrenocortical activity in primates
675 based on fecal analysis. *American Journal of Primatology* 68, 257-273.

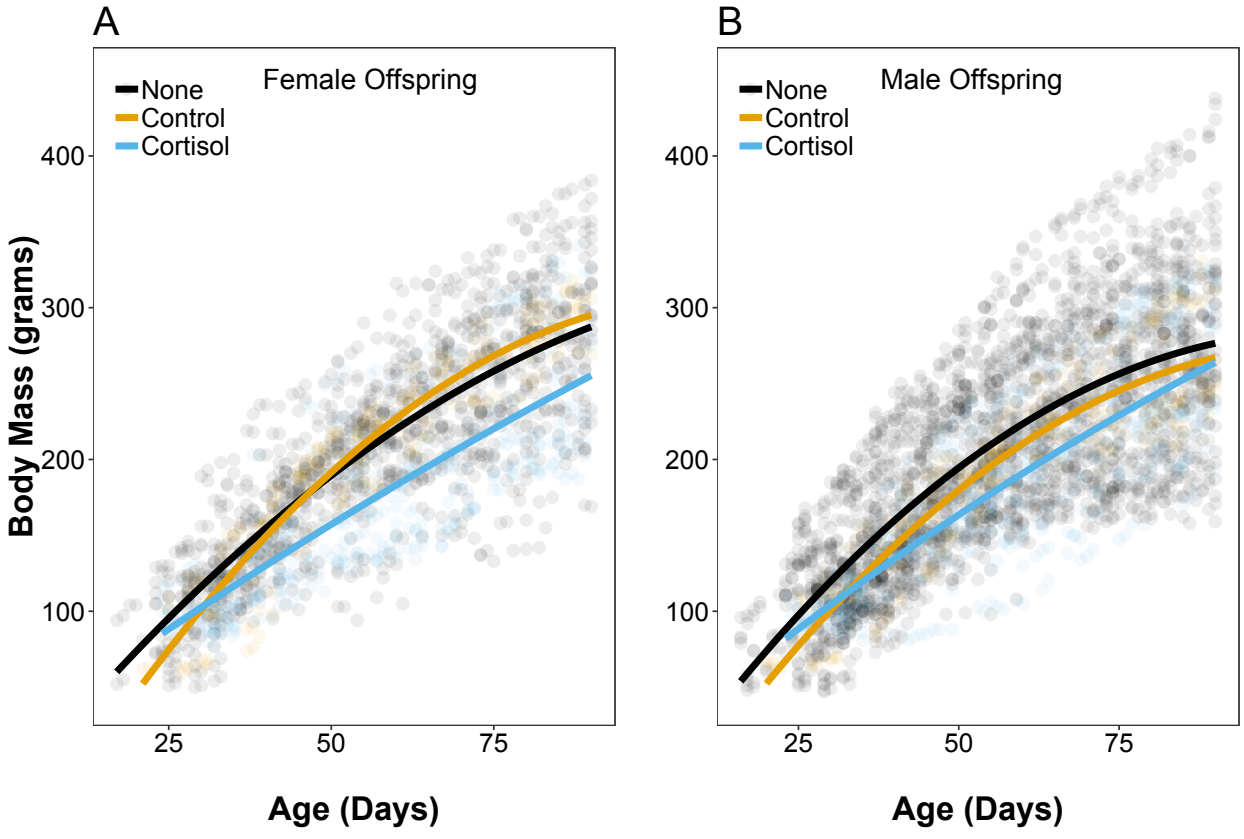
676 49) Clutton-Brock TH, Russell AF, Sharpe LL (2003) Meerkat helpers do not specialize
677 in particular activities. *Animal Behaviour* 66(3), 531-540.
678 50) Carlson AA, Manser MB, Young AJ, Russell AF, Jordan NR, McNeilly AS, Clutton-
679 Brock T (2006) Cortisol levels are positively associated with pup-feeding rates in
680 male meerkats Proceedings of the Royal Society B 273(1586), 571-577.
681 51) Lesnoff M, Lancelot R (2013) aods3: analysis of overdispersed data using S3
682 methods. aods3 package version 0.4-1.
683 52) R Development Core Team. 2013. R: A language and environment for statistical
684 computing. R Foundation for Statistical Computing, Vienna, Austria; available at
685 www.r-project.org/.
686 53) Bates D, Maechler M, Bolker B, Walker S (2014) lme4: Linear mixed-effects models
687 using Eigen and S4. *R Package Version 1*(7), 1-23.
688 54) Kuznetsova A, Brockhoff PB, Christensen RHB (2016) lmerTest: Tests in Linear
689 Mixed Effects Models. R package version 2.0-30; available at http://CRAN.R-
690 project.org/package=lmerTest
691 55) Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid
692 common statistical problems. *Meth. Ecol. Evol.* **1**, 3-14. (doi: 10.1111/j.2041-
693 210X.2009.00001.x)
694 56) Fox J, Monette G (1992) Generalized collinearity diagnostics. *Journal of the*
695 *American Statistical Association* 87(417), 178-183.
696 57) Clutton-Brock TH, Russell AF, Sharpe LL, Brotherton PNM, McIlrath GM, White S,
697 Cameron EZ (2001) Effects of helpers on juvenile development and survival in
698 meerkats. *Science*, *293*(5539), 2446-2449.

699 58) Huchard E, English S, Bell MB, Thavarajah N, Clutton-Brock T (2016) Competitive
700 growth in a cooperative mammal. *Nature*, *533*(7604), 532.
701 59) Hatchwell BJ, Komdeur J (2000) Ecological constraints, life history traits and the
702 evolution of cooperative breeding. *Animal Behaviour* 59(6), 1079-1086.
703 60) Cant MA, Field J (2001) Helping effort and future fitness in cooperative animal
704 societies. Proceedings of the Royal Society B 268, 1959-1964.
705 61) Field J, Cronin A, Bridge C (2006) Future fitness and helping in social
706 queues. *Nature*, *441*(7090), 214.
707 62) Zöttl M, Chapuis L, Freiburghaus M, Taborsky M (2013). Strategic reduction of help
708 before dispersal in a cooperative breeder. *Biology Letters*, *9*(1), 20120878.
709 63) Sheriff MJ, Bell A, Boonstra R, Dantzer B, Lavergne SG, McGhee KE, MacLeod KJ,
710 Winandy L, Zimmer C, Love OP (2017) Integrating ecological and evolutionary
711 context in the study of maternal stress. *Integrative and Comparative*
712 *Biology*, *57*(3), 437-449.
713 64) Raulo A, Dantzer B. (2018) Associations between glucocorticoids and sociality
714 across a continuum of vertebrate social behaviour. *Ecology and Evolution* in
715 press (doi: 10.1002/ece3.4059).
716 65) Weinstock M (2007) Gender differences in the effects of prenatal stress on brain
717 development and behaviour. *Neurochemical Research*, *32*(10), 1730-1740.
718 66) Mueller, BR, Bale, TL (2008) Sex-specific programming of offspring emotionality
719 after stress early in pregnancy. *Journal of Neuroscience*, *28*(36), 9055-9065.
720 67) Wilkin TA, Sheldon BC (2009) Sex differences in the persistence of natal
721 environmental effects on life histories. *Current Biology*, *19*(23), 1998-2002.

722 68) Metzger DC, Schulte PM (2016) Maternal stress has divergent effects on gene
723 expression patterns in the brains of male and female threespine
724 stickleback. *Proc. R. Soc. B*, *283*(1839), 20161734.
725 69) Marshall HH, Vitikainen EI, Mwanguhya F, Businge R, Kyabulima S, Hares MC,
726 Inzani E, Kalema-Zikusoka G, Mwesige K, Nichols HJ, Sanderson JL, Thompson
727 FJ, Cant MA (2017) Lifetime fitness consequences of early-life ecological
728 hardship in a wild mammal population. *Ecology and Evolution*, *7*(6), 1712-1724.
729 70) English S, Kunc HP, Madden JR, Clutton-Brock TH (2008) Sex differences in
730 responsiveness to begging in a cooperative mammal. *Biology Letters*, *4*(4), 334-
731 337.
732 71) Clutton-Brock TH, Manser M (2016) Meerkats: cooperative breeding in the Kalahari.
733 *Cooperative Breeding in Vertebrates* (Eds.: WD Koenig, JL Dickinson).
734 Cambridge University Press, Pp. 294-317
735 72) Hamilton WD (1964) The genetical evolution of social behaviour. II. *Journal of*
736 *Theoretical Biology*, *7*(1), 17-52.
737 73) Trivers RL (1971) The evolution of reciprocal altruism. *The Quarterly Review of*
738 *Biology*, *46*(1), 35-57.
739 74) Griffin AS, Sheldon BC, West SA (2005) Cooperative breeders adjust offspring sex
740 ratios to produce helpful helpers. *The American Naturalist*, *166*(5), 628-632.

741
742

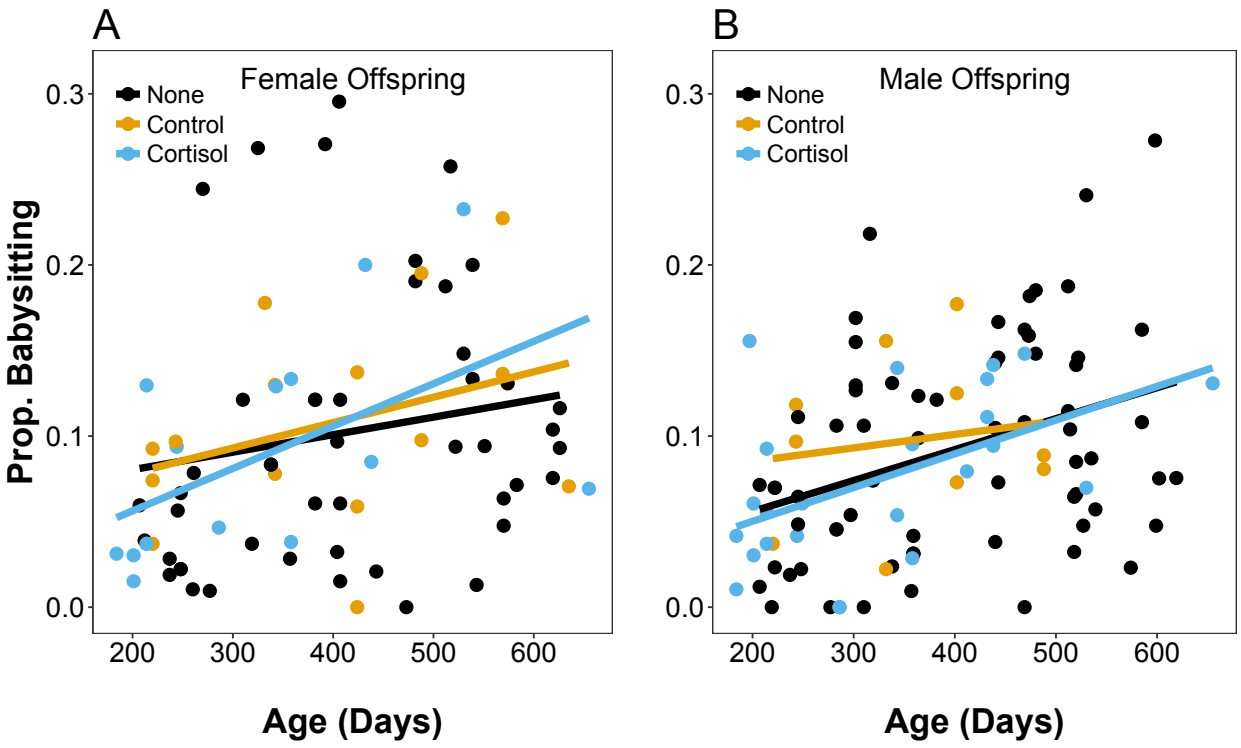
743 **Figure 1.** (A) Daughters but not (B) sons from mothers treated with cortisol during
744 pregnancy were significantly smaller from initial emergence from their natal burrow to
745 nutritional independence (~1-3 months) compared to those from control mothers
746 (daughters: age x treatment, t = -4.17, P < 0.0001; sons: t = -1.48, P = 0.14), but not
747 untreated mothers (daughters: age x treatment, t = 0.65, P = 0.51; sons: t = -0.52, P =
748 0.6, Table 3). Data are body mass measures from offspring from cortisol-treated
749 (females: n = 373 estimates; males: n = 488), control (females: n = 215; males: n =
750 241), and untreated mothers (females: n = 1121; males: n = 2238). Raw data and
751 regression lines are shown (full results in Table 3).



752

753

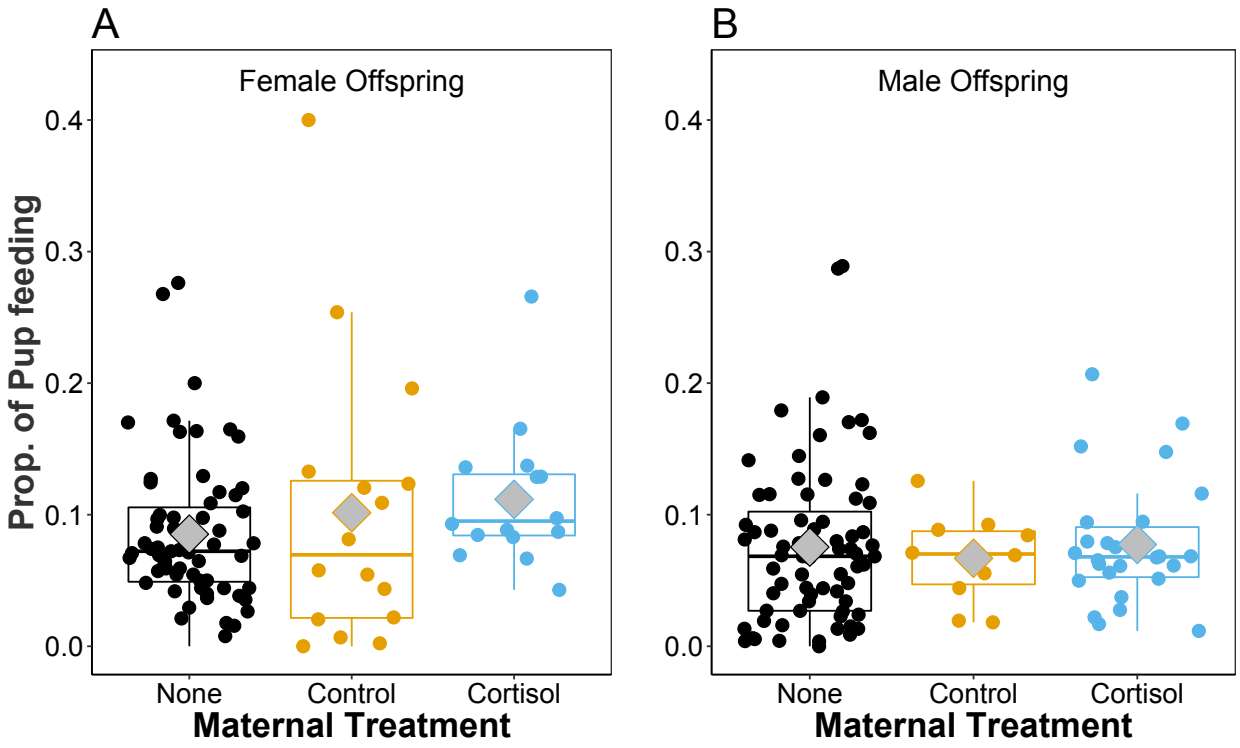
754 **Figure 2.** Babysitting contributions of (A) daughters and (B) sons from mothers treated
755 with cortisol during pregnancy increased with age at a faster rate than those from
756 control (females: z = -2.89, P = 0.0039; males: z = -1.92, P = 0.055), but not untreated
757 ("None") mothers (females: z = 1.88, P = 0.06; males: z = -0.03, P = 0.97, Table 4).
758 Data are relative babysitting contributions from offspring from cortisol-treated (females:
759 n = 15 estimates; males: n = 24), control (females: n = 15; males: n = 10), and untreated
760 mothers (females: n = 49; males: n = 69). Raw data and regression lines are shown (full
761 results in Table 4).



762

763

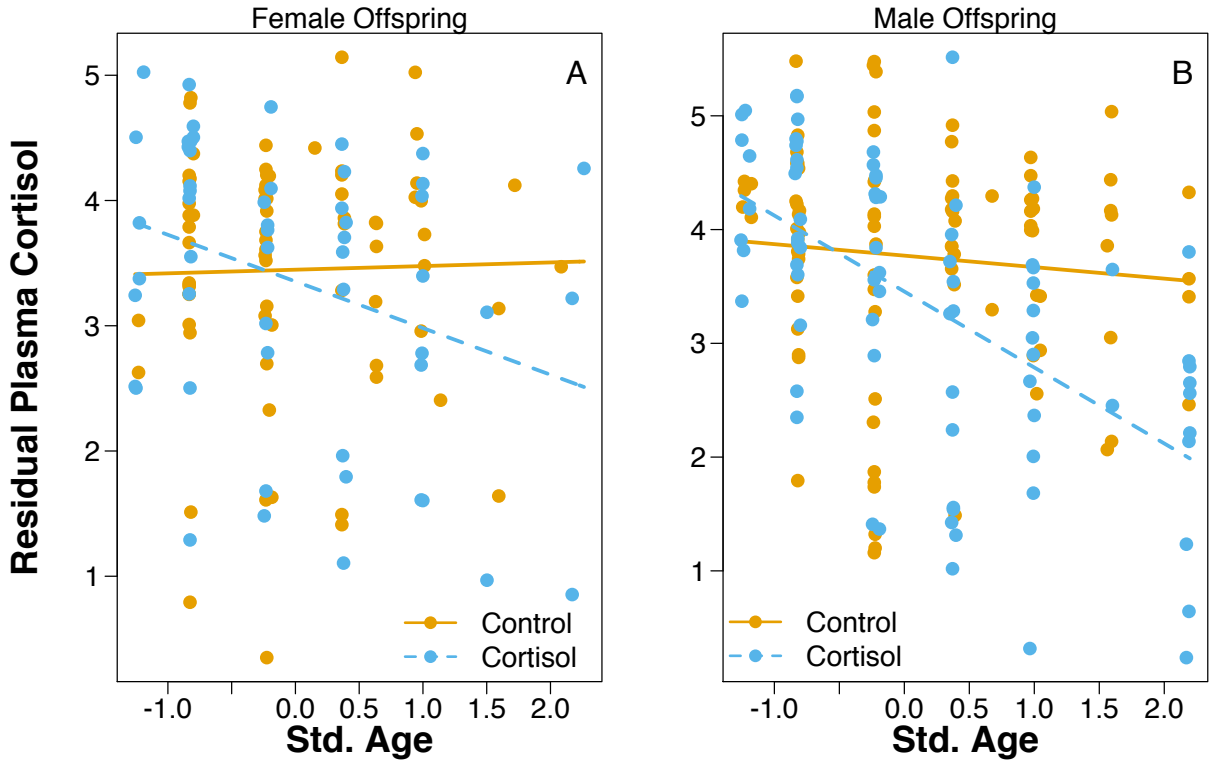
764 **Figure 3.** Pup feeding contributions of (A) daughters, but not (B) sons, from mothers
765 treated with cortisol during pregnancy were significantly higher compared to from control
766 (females: z = -3.09, P = 0.0004; males: z = -1.15, P = 0.25) or untreated ("None")
767 mothers (females: z = -3.47, P = 0.0005; males: z = -0.89, P = 0.37, Table 5). Data are
768 relative pup feeding contributions from offspring from cortisol-treated (females: n = 16
769 estimates; males: n = 26), control (females: n = 16; males: n = 10), and untreated
770 mothers (females: n = 64; males: n = 71). Raw data are shown (full results in Table 5).
771 Boxplots show median (solid horizontal line), mean (grey diamonds), and first (25%)
772 and third (75%) quartiles.



773

774

775 **Figure 4.** (A) Daughters and (B) sons from mothers treated with cortisol during
776 pregnancy had lower plasma cortisol concentrations as they became older compared to
777 those from control mothers, though the difference was only significant in males
778 (daughters: age x treatment, t = -1.76, P = 0.08; sons: age x treatment t = -2.68, P =
779 0.008, Table 6). Data are residual plasma cortisol concentrations from offspring from
780 cortisol-treated (females: n = 64 samples; males: n = 92) and control (females: n = 89;
781 males: n = 104) mothers. Residuals from a linear mixed-effects model (Table 6) are
782 shown on y-axis.



783

784

785 **Figure 5.** Faecal glucocorticoid metabolite (fGCM) concentrations in (A) daughters but
786 not (B) sons from mothers treated with cortisol during pregnancy were significantly
787 lower than those from control mothers as they became older (daughters: age x
788 treatment, t = -2.9, P = 0.004; sons: age x treatment, t = -0.1, P = 0.49, Table 7. Data
789 are residual fGCM concentrations from offspring from cortisol-treated (females: n = 79
790 samples; males: n = 118) and control (females: n = 154; males: n = 201) mothers.
791 Residuals from a linear mixed-effects model (Table 7) are shown on y-axis.

792

793

794

795

Table 1. Summary of effects of dominant female treatments on litter characteristics and offspring survival. Number of pups emerged correspond to those that emerged from the natal burrow and in some cases these pups died before their sex could be determined (shown as "Unk"). Three of the 13 litters treated with cortisol and one of the 7 control (fed) litters were aborted prior to birth.

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 2. Effects of dominant female treatments (cortisol or control) 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). The number of litters aborted by untreated females was not known so we only assessed the effects of cortisol vs. control treatments on the number of litters aborted. Litter sex ratio is the proportion of males in the litter.

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 mothers. 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 3. Effect of dominant female treatments on offspring growth 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 ($\sigma^2 = 116.7$) and year ($\sigma^2 = 0$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

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			
Age	53.3	1.06			
Age^2	-4.76	1.15			
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			
Males	12.9	15.5			
Treatment (None)					
Females	23.6	10.6			
Males	22.9	10.4			
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 4. 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 ($\sigma^2 = 0.000$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

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		
Males	0.4	0.19		
Foraging success	-0.05	0.05	-0.99	0.32
Mass				
Females	-0.32	0.12		
Males	-0.098	0.14		
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		
Males	-0.12	0.28		
Treatment (None)				
Females	-0.06	0.23		
Males	-0.16	0.18		
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 5. 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 ($\sigma^2 = 0.19$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

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		
Age				
Females	-0.37	0.16		
Males	0.29	0.14		
Foraging success	**0.14**	**0.06**	**2.12**	**0.033**
Mass				
Females	0.1	0.11		
Males	-0.37	0.11		
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 6. 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 ($\sigma^2 = 0.034$), and capture group ($\sigma^2 = 0.000$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**3.18**	**0.29**	**11.31**	**128**	**<0.0001**
Males	**3.37**	**0.25**	**13.37**	**113**	**<0.0001**
Sampling time	**1.16**	**0.10**	**11.95**	**259**	**<0.0001**
Sampling time^2	**-0.24**	**0.03**	**-7.24**	**269**	**<0.0001**
Time of day	-0.2	0.14	-1.38	276	0.17
Sample year (2015)	0.41	0.24	1.72	228	0.09
Sex (M)	0.2	0.2	0.97	39	0.34
Age					
Females	-0.24	0.18			
Males	-0.01	0.14			
Foraging success					
Females	0.13	0.11	-1.22	231	0.22
Males	-0.15	0.1	-1.42	269	0.16
Group size	0.07	0.11	0.62	242	0.54
Group size^2	0.05	0.08	0.63	211	0.53
Pups in group	-0.31	0.18	-1.79	276	0.075
Group sex ratio	**0.2**	**0.08**	**2.53**	**180**	**0.01**
Relatedness	-0.09	0.18	-0.5	40	0.62
Weather (PC1)	-0.08	0.1	-0.75	276	0.45
Treatment (Cortisol)					
Females	-0.02	0.24			
Males	-0.32	0.19			
Sex (M) x Age	0.22	0.22	1.04	272	0.3
Sex (M) x Foraging success	-0.013	0.14	-0.09	257	0.93
Sex (M) x Treatment (Cortisol)	-0.3	0.3	-1.01	35	0.32
Age x Treatment (Cortisol)					
Females	-0.42	0.24	-1.76	276	0.08
Males	**-0.5**	**0.19**	**-2.68**	**274**	**0.008**
Group size x Pups Present (Yes)	-0.05	0.15	-0.36	216	0.72
Group size^2 x Pups Present (Yes)	0.07	0.12	0.6	263	0.55
Group size x Weather (PC1)	-0.02	0.08	-0.30	273	0.76
Age x Sex (M) x Treatment (Cortisol)	-0.08	0.31	-0.26	275	0.8

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 7. 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 ($\sigma^2 = 0.094$) and collection group ($\sigma^2 = 0.000$). If fixed effects by themselves were involved in significant higher order interactions with other variables, only parameter estimates are shown.

Fixed Effect	b	SE	t	df	P-value
Intercept					
Females	**5.4**	**0.25**	**21.36**	**50**	**<0.0001**
Males	**5.33**	**0.23**	**23.4**	**23**	**<0.0001**
Time of day	**-0.14**	**0.04**	**-3.29**	**516**	**0.001**
Sample year (2015)	0.07	0.17	0.43	120	0.67
Sex (M)	-0.06	0.2	-0.31	22	0.76
Age					
Females	0.48	0.16			
Males	0.06	0.1			
Foraging success					
Females	0.16	0.13	1.29	355	0.2
Males	0.04	0.06	0.81	520	0.42
Group size	**0.41**	**0.12**	**3.52**	**96**	**0.0007**
Group size^2	-0.05	0.11	-0.78	267	0.43
Pups in group	-0.13	0.16	-0.78	267	0.43
Group sex ratio	**0.16**	**0.07**	**2.27**	**158**	**0.024**
Relatedness	0.28	0.25	1.1	14	0.29
Weather (PC1)	0.09	0.07	1.4	203	0.16
Treatment (Cortisol)					
Females	-0.12	0.29			
Males	-0.03	0.24			
Sex (M) x Age	**-0.42**	**0.17**	**-2.47**	**221**	**0.014**
Sex (M) x Foraging success	-0.12	0.13	-0.87	375	0.38
Sex (M) x Treatment (Cortisol)	0.1	0.33	0.29	18	0.77
Age x Treatment (Cortisol)					
Females	**-0.63**	**0.22**	**-2.9**	**447**	**0.004**
Males	-0.1	0.14	-0.69	491	0.49
Group size x Pups Present (Yes)	-0.17	0.12	-1.46	216	0.14
Group size^2 x Pups Present (Yes)	0.11	0.12	456	0.97	0.33
Group size x Weather (PC1)	0.04	0.05	0.69	129	0.49
Age x Sex (M) x Treatment (Cortisol)	**0.53**	**0.25**	**2.15**	**413**	**0.032**

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 males; 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".