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Elevated prolactin levels immediately precede decisions to babysit by male meerkat helpers

Anne A. Carlson [a,*], Andrew F. Russell [b], Andrew J. Young [a], Neil R. Jordan [a], Alan S. McNeilly [c], Al F. Parlow [d], Tim Clutton-Brock [a,e]

[a] *Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK*
[b] *Department of Animal and Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK*
[c] *MRC HRSU, Centre for Reproductive Biology, University of Edinburgh, 49 Little Crescent, Old Dalkeith Road, Edinburgh EH16 4SB, UK*
[d] *National Hormone and Peptide Program, Harbor-UCLA Medical Center, 1000 W. Carson Street, Torrance, CA 90509, USA*
[e] *Mammal Research Institute, University of Pretoria, Pretoria 0002, South Africa*

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Abstract

Recent studies suggest that decisions to care for the offspring of others in societies of cooperative vertebrates may have a hormonal basis. The crucial question of whether changes in hormone levels immediately precede or merely follow bouts of offspring care, however, remains largely unanswered. Here, we show that in wild groups of cooperatively breeding meerkats, male helpers that decided to babysit for the day had significantly higher levels of prolactin, coupled with lower levels of cortisol, before initiating a babysitting session compared with similarly aged individuals that decided to forage. In addition, these hormonal differences disappeared over the course of the day, suggesting that hormone levels changed in a fundamentally different way in meerkats that babysat versus those that foraged. In contrast, long-term contributions to babysitting were not significantly associated with plasma levels of prolactin, cortisol, or testosterone in individual male helpers. Our results show, for the first time, that elevated levels of prolactin may immediately precede bouts of helping behavior but differ from recent findings on the same study population in which plasma levels of cortisol, but not prolactin, were significantly and positively associated with rates of pup feeding by male helpers. Together, these results lend significant weight to the idea that decisions to help in cooperative vertebrates have a hormonal basis, although different hormones appear to be associated with different types of care.

Keywords: Cooperative care; Cooperative breeding; Prolactin; Cortisol; Testosterone

In some species of cooperative vertebrates, individuals (here termed helpers) provide care despite not having delivered/sired any offspring. Helpers within the same group can vary substantially in their contributions to cooperative care, however, with some individuals contributing nothing while others provide up to 90% of the care for a particular helping behavior (Clutton-Brock et al., 1998, 2002, 2004). Knowledge of the underlying causes of helping behavior, as well as the reasons behind the marked variation in individual contributions to alloparental care, remain two of the greatest challenges in understanding the adaptive nature of vertebrate cooperative breeding systems (Cockburn, 1998; Heinsohn and Legge, 1999; Schoech et al., 2004).

Recent studies have investigated whether decisions to care for the offspring of others may have a hormonal basis. Experimental manipulations of prolactin and testosterone levels suggest that these hormones have a causal influence on parental investment in non-cooperative vertebrates (Buntin et al., 1991; Ketterson and Nolan, 1994; Ketterson et al., 1992), but it is not known if the same is true of helpers in societies of cooperative vertebrates (see reviews by Schoech et al., 2004; Russell, 2004). In birds, for example, helpers in groups of Harris' hawks (*Parabuteo unicinctus*) had higher levels of prolactin than non-helpers (Vleck et al., 1991), while helper provisioning frequency in Florida scrub jays (*Aphelocoma coerulescens*)

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* Corresponding author. Fax: +1 518 689 0224.
E-mail address: zambiawilddog@yahoo.com (A.A. Carlson).

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was positively correlated with individual levels of prolactin (Schoech et al., 1996). In contrast, rates of provisioning were unrelated to individual prolactin levels in red cockaded woodpeckers (*Picoides borealis*) (Khan et al., 2001). In mammals, no evidence of a relationship between prolactin levels and infant-carrying frequency was detected in male cotton-top tamarin helpers (*Saguinus oedipus*) (Ziegler et al., 1996). In addition, although plasma prolactin levels of subordinate common marmosets (*Callithrix jacchus*) were positively related to their duration of contact with offspring during infant carrying (Roberts et al., 2001a), infant retrieval by helpers was eliminated in only four of eight helpers receiving bromocriptine, a dopamine agonist that significantly reduces prolactin levels (Roberts et al., 2001b). Research examining correlations between testosterone levels and helping behavior has produced mixed results as well. In birds, the administration of exogenous testosterone decreased helper feeding rates in superb fairy wrens (*Malurus cyaneus*) (Peters et al., 2002), but there is, as yet, little evidence that increased levels of testosterone reduce rates of helping behavior in mammals (Carlson et al., 2006).

Thus, in spite of recent studies on the topic, our knowledge of the physiological correlates of helping behavior is too inadequate to produce coherent generalizations for either birds or mammals. Moreover, two crucial questions regarding the effect of hormones on levels of care remain largely unanswered. First, although there is evidence that the average prolactin levels of helpers in some cooperatively breeding societies increase before exposure to the dominants' offspring (Schoech et al., 1996; Kreeger et al., 1991), it is currently unknown whether further, short-term increases in hormone levels immediately precede bouts of helping behavior or merely follow them. In Florida scrub jays, for example, Schoech et al. (1996) found that prolactin levels from plasma samples collected *after* helping bouts were significantly correlated with both the number of visits to the nest and the amount of food that the helpers had previously brought to the nestlings; whether the prolactin levels of individual helpers immediately *prior* to the onset of a bout of nestling provisioning were significantly correlated with subsequent rates of helping behavior is not known, however. This is an important point given that the results of several common marmoset studies suggest that elevated prolactin levels may occur in response to physical contact with infants, rather than as a "physiological trigger" of cooperative care (Mota et al., 2006; Dixson and George, 1982; Almond et al., in press). Second, we now know that hormone levels are significantly associated with a wide range of non-hormonal factors also known to influence levels of care, such as helper age, physical condition, and sex (Ziegler et al., 1996; Carlson et al., 2006). To date, few studies have used a multivariate statistical framework to consider whether hormone levels account for some of the variation in individual contributions to cooperative care after controlling for these non-hormonal correlates of helping behavior (Carlson et al., 2006).

The goal of this study is to examine the hormonal basis of babysitting in adult male helpers of the cooperatively breeding meerkat (*Suricata suricatta*). First, we investigate whether decisions to babysit or forage on a given day are associated with hormonal differences between individuals before the bouts of babysitting or foraging have begun. Second, we investigate whether babysitting or foraging sessions are associated with hormonal differences between individuals at the end of the day. Third, we determine whether hormones explain part of the variation in individual contributions to the entire babysitting period after using multivariate statistics to control for significant, non-hormonal effects. The hormones considered are prolactin, cortisol, and testosterone, all of which have been found to be either positively or negatively associated with levels of alloparental care in cooperative vertebrates (see above, Carlson et al., 2006).

Meerkats are small (<1 kg), desert-dwelling carnivores that live in family groups of up to 50 (mean = 19) individuals in Southern Africa. Reproduction is typically limited to the dominant pair (Griffin et al., 2003; Russell et al., 2004), with dominant females typically delivering two to four litters per year of three to six pups per litter (Russell et al., 2003a). Pups remain in their natal burrow for their first month of life. During this period, they are supervised on 90% of days by one group member typically, which we term a babysitter (Clutton-Brock et al., 1998, 2004). Babysitting is energetically costly: group members seldom babysit when young, light, or on consecutive days, and weight loss over the babysitting period is proportional to the amount of babysitting conducted (Clutton-Brock et al., 1998; Russell et al., 2003b). Individuals within the same group vary substantially in their contributions to babysitting, with some individuals contributing nothing while others babysit up to 90% of the time (Clutton-Brock et al., 1998, 2002, 2004). Although some of this variation can be explained by helper number, sex, age, weight, foraging efficiency, and investment in the previous litter, the explanatory power of these variables is limited (Clutton-Brock et al., 2000, 2002; Russell et al., 2003b). Here we examine the possibility that variation in hormone levels accounts for some of this unexplained variation (Carlson et al., 2006).

Methods

We studied the association between the babysitting behavior of adult male meerkats and individual levels of prolactin, cortisol, and testosterone near VanZyl's Rus in the South African Kalahari desert (26°59′S 21°59′E) between 1998 and 2003. All protocols in this study adhered to the animal welfare standards established by the National Institutes of Health. The study site was established in 1993, and the life history of all animals has been recorded subsequently. Details of the study site, population, and climate have been described elsewhere (Clutton-Brock et al., 1998; Russell et al., 2002). Individuals were identified from emergence at 3 weeks of age using unique 'hair cuts' or dye patches on their fur, which were easily administered and maintained while the animals rested, and without the need for capture. All animals used in this study were individually known adult (>1 year) males and were habituated both to observation within a meter and repeated weighing each day using crumbs of hard-boiled egg to entice them onto electronic balances (±1 g) (Clutton-Brock et al., 1998).

Hormonal basis for individual decisions to babysit or forage

Our first two aims were to investigate whether individuals that decided to babysit on a given day had different hormonal profiles from those that decided to forage at the beginning of that day (i.e., before either babysitting or foraging had begun) and at the end of that day (i.e., after 12 h of either babysitting or foraging). Babysitters can be captured before they begin to babysit because they usually emerge from the burrow in the morning to warm up in the sun with all other group members before returning to the burrow interior once the group has departed to forage. Similarly, babysitters can be captured at the end of the day because they often re-emerge from the burrow after the group returns from foraging.

Overall, we collected 40 blood samples from 30 adult male meerkats during the babysitting period of 22 litters in 10 different groups. Individuals that babysat were of similar age to those that foraged (mean = 782 days ± 460 (SD) (babysitter) versus 797 days ± 475 (SD) (forager); $t_{38} = -0.10$, $P = 0.92$) and in 80% of samples, the babysitter and forager were taken from the same group. Sampled animals fell into one of four categories: (i) those that we sampled before they started to babysit on a given day ($n = 12$ individuals), (ii) those that we sampled before they began to forage on a given day ($n = 12$ individuals), (iii) those that we sampled after they babysat for the entire day (>12 h) ($n = 8$ individuals), and (iv) those that we sampled after they foraged for the entire day (>12 h) ($n = 8$ individuals). Seven individuals were sampled both in categories (iii) and (iv) (4–276 days between samples, median = 10 days), and one individual was sampled in each of categories (i) and (iv) (671 days between samples), (ii) and (iii) (1036 days between samples), and (i) and (i) in two different litters (75 days between samples). Only a small number of samples ($n = 6$ of 40; 15%) arose from re-sampling animals within 4–10 days after they were first sampled; these intervals are consistent with the "washout period" used to avoid any residual effects of anesthesia drugs on hormone levels in livestock that are sampled more than once during long-term experiments (Ranheim et al., 2000). Repeated sampling (within individuals, litters, and groups) was controlled statistically by fitting each as random terms in the models (Schall, 1991).

Three separate analyses were conducted, one for each of the three hormones, using GENSTAT 6 (Rothamsted Experimental Station, Harpendon, UK). In each analysis, hormone levels were fitted as the response term, the interaction between activity (babysitting/foraging) and time (before/after) was fitted as the fixed effect. Prolactin (after square-root transformation) and testosterone data were normally distributed (Kolmogorov–Smirnov $P > 0.15$) and hence were analyzed using a general linear mixed model with normal error structure. Cortisol data were significantly skewed to the right ($P < 0.01$) and so were analyzed using a generalized linear mixed model with Poisson error structure. Capture-to-bleed times, group size, helper age, and levels of the other two hormones were fitted as covariates in all three analyses. The residuals from the final models were normally distributed in all analyses ($P > 0.15$).

Hormonal correlates of long-term contributions to babysitting

Our third aim was to investigate whether variance in individual contributions to babysitting over the entire (ca. 1 month) babysitting period of a litter was related to individual hormone levels. During the babysitting period of each litter, groups are visited daily and the identity of babysitters and non-babysitters determined. We obtained 62 blood samples from 40 adult male meerkats (1–4 measures per individual) contributing to 25 litters (1–7 measures per litter) in 10 groups (1–18 measures per group). All blood samples were collected during the late afternoon or early evening from individuals that were foraging ($n = 62$) during babysitting periods between 1998 and 2002.

For each blood sample, we fitted the number of days that the individual was observed to babysit as the response term and the number of days that it could have spent babysitting as the binomial denominator in a generalized linear mixed model with binomial error structure and logit link function. We then investigated the non-hormonal correlates of contributions to babysitting by fitting helper number, age, age-corrected weight, rate of daily weight gain, and relatedness to the litter as fixed effects, and individual, litter, and group identities as random effects (see above). Those terms that were significant were retained, while those that were not were dropped. We then fitted levels of prolactin, cortisol, and testosterone as the main fixed effects, as well as sampling time, whether the animal was sampled as a babysitter or as a forager, and capture-to-bleed times to the minimal model obtained to determine whether hormone levels accounted for significant amounts of additional variation in the model.

Blood sampling

To obtain a blood sample for hormonal analysis, the target animal was approached on foot, gently picked up by the base of the tail and placed inside a capture bag before being immobilized immediately with a single intra-muscular injection of Anaket V at 5 mg/kg (100 mg/ml Ketamine hydrochloride) and Dormitor at 0.03 mg/kg (1 mg/ml medetomidine hydrochloride), as described by O'Riain et al. (2000). As soon as the animal was completely anesthetized (2–3 min), a 0.3–1.5 ml blood sample was collected from the jugular vein using a 24 1/2-gauge needle and 1-ml syringe. The effects of the Dormitor were reversed with an intra-muscular injection of Antisedan at 0.2 mg/kg (5 mg/ml atipamezole hydrochloride), and the animals returned to their group once fully conscious and mobile (15–30 min after administration of Antisedan). Blood samples were immediately placed on ice until they were centrifuged at 500 *g* on return to the laboratory; there the plasma was pipetted into a vial and frozen at −5°C until analysis. All blood samples were collected between 1700 and 2030 except for those samples used to assess pre-babysitting hormonal levels; these samples were collected between 0530 and 0830.

All blood samples were collected 4–7 min after the moment of capture. To examine the effects of bleed times on hormone levels, we plotted stress profiles for 15 subordinate meerkats bled between two and four times each between 3 and 40 min (Carlson et al., 2003, 2004, 2006). These profiles revealed that prolactin levels in blood samples collected 10–20 min post-capture were significantly higher than blood samples drawn within 10 min of capture and after 20 min of capture (Carlson et al., 2003, 2006). Additionally, levels of plasma cortisol rose steadily and significantly with increasing capture-to-bleed time (Carlson et al., 2004, 2006). Hence, the empirical evidence showed no significant, stress-induced changes in either prolactin or cortisol levels for the blood sampling times used in the study. As a result, we interpret the hormone levels in our studies as baseline but recognize that despite anesthetizing our animals within a minute of capture and obtaining blood samples within 7 min, slight-stress-induced elevations of plasma hormone are possible. Finally, it is worth noting that the capture-to-bleed times in this study are among the shortest obtained for a cooperative breeder (e.g., Reyer et al., 1986; Mays et al., 1991; Schoech et al., 1996), and consequently, we view our results as comparable to those from previous studies on the subject.

Hormonal assays

We assayed our samples for prolactin using a highly specific heterologous radioimmunoassay (RIA) of rabbit antiserum to human prolactin (final dilution 1:105,000) and canine $[^{125}\mathrm{I}]$iodo-prolactin (National Hormone and Pituitary Program Harbor-UCLA Medical Center, Torrance, California), validated for meerkat plasma (Carlson et al., 2003). Assay sensitivity was 0.05 ng/ml. Intra- and inter-assay coefficients for a meerkat plasma pool were 8.3 and 12.6% for the multivariate analysis ($n = 6$ assays). We assayed our samples for cortisol using Coat-a-Count Cortisol kits (Diagnostic Products Corporation, Los Angeles, California) validated for meerkat plasma (Carlson et al., 2004). Assay sensitivity was 0.25 μl/dl. Intra- and inter-assay coefficients for a meerkat plasma pool were 4.5 and 6.8%, respectively, for the multivariate analysis ($n = 6$). We assayed our samples for testosterone using Coat-a-Count Total Testosterone kits (Diagnostic Products Corporation, Los Angeles, California) validated for meerkat plasma (Carlson et al., 2004). Assay sensitivity was 20 ng/dl, while cross-reactivity of the Coat-a-Count testosterone antibody was 16% with 11-ketotestosterone, <5% with dihydrotestosterone and 19-hydroxyandrostendione, and <1% with all other steroids. Intra- and inter-assay coefficients for a meerkat plasma pool were 4.4 and 7.5% ($n = 6$ assays).

Results

Hormonal basis for individual decisions to babysit or forage

We found a significant interaction between whether individuals were sampled at the beginning of the day or at the end of the day, and whether they babysat or foraged, on levels of prolactin ($\chi^2 = 7.74$, $df = 1$, $P = 0.005$; Fig. 1a). Individuals that were about to spend the day babysitting had significantly higher levels of prolactin than similarly aged individuals that were about to spend the day foraging ($\chi^2 = 7.50$, $df = 1$, $P = 0.006$). In contrast, by the end of the day, levels of prolactin in males that had spent the day babysitting did not differ from levels in males that spent the day foraging ($\chi^2 = 0.30$, $df = 1$, $P = 0.58$). Significantly lower levels of prolactin at the end of the day (compared with the beginning of the day) in babysitters ($\chi^2 = 9.40$, $df = 1$, $P = 0.002$), coupled with no change in prolactin levels in foragers during the same time period ($\chi^2 = 0.08$, $df = 1$, $P = 0.78$), suggest that babysitting is associated with reductions in prolactin levels.

Similarly, we found a significant interaction between whether individuals were sampled at the beginning of the day or the end of the day, and whether they had babysat or foraged, on levels of cortisol ($\chi^2 = 7.83$, $df = 1$, $P = 0.005$; Fig. 1b). In contrast to the prolactin results described above, individuals that were about to spend the day foraging showed a tendency to have higher levels of cortisol than similarly aged individuals that were about to spend the day babysitting ($\chi^2 = 3.60$, $df = 1$, $P = 0.058$). By the end of the day, individuals that had spent the day foraging tended to have lower levels of cortisol than those that had spent the day babysitting ($\chi^2 = 3.31$, $df = 1$, $P = 0.069$). Significantly higher levels of cortisol at the end of the day (compared with the beginning of the day) in babysitters ($\chi^2 = 4.01$, $df = 1$, $P = 0.045$), coupled with no change in cortisol in foragers ($\chi^2 = 0.55$, $df = 1$, $P = 0.46$), suggest that babysitting is associated with increased levels of cortisol.

In contrast, we found no interaction between whether individuals were sampled at the beginning of the day or at the end of the day, and whether or not they had babysat or foraged, on levels of testosterone ($\chi^2 = 0.22$, $df = 1$, $P = 0.64$; Fig. 1c). Testosterone levels were similar between babysitters and foragers irrespective of whether samples were collected in the morning ($\chi^2 = 0.92$, $df = 1$, $P = 0.34$) or evening ($\chi^2 = 0.32$, $df = 1$, $P = 0.57$). Additionally, levels of testosterone did not differ between morning and evening sampling periods, regardless of whether individuals were babysitters ($\chi^2 = 0.04$, $df = 1$, $P = 0.85$) or foragers ($\chi^2 = 0.12$, $df = 1$, $P = 0.74$).

Hormonal correlates of individual contributions to babysitting

Individual contributions to the entire babysitting period decreased with increasing helper number and increased with increasing helper age. After controlling for these significant effects, we found no evidence to suggest that levels of prolactin, cortisol, or testosterone explained a significant proportion of the variation in total individual contributions to the babysitting period (Table 1).

Discussion

Results from this study suggest that decisions by adult male meerkat helpers to babysit have a hormonal basis. Specifically,

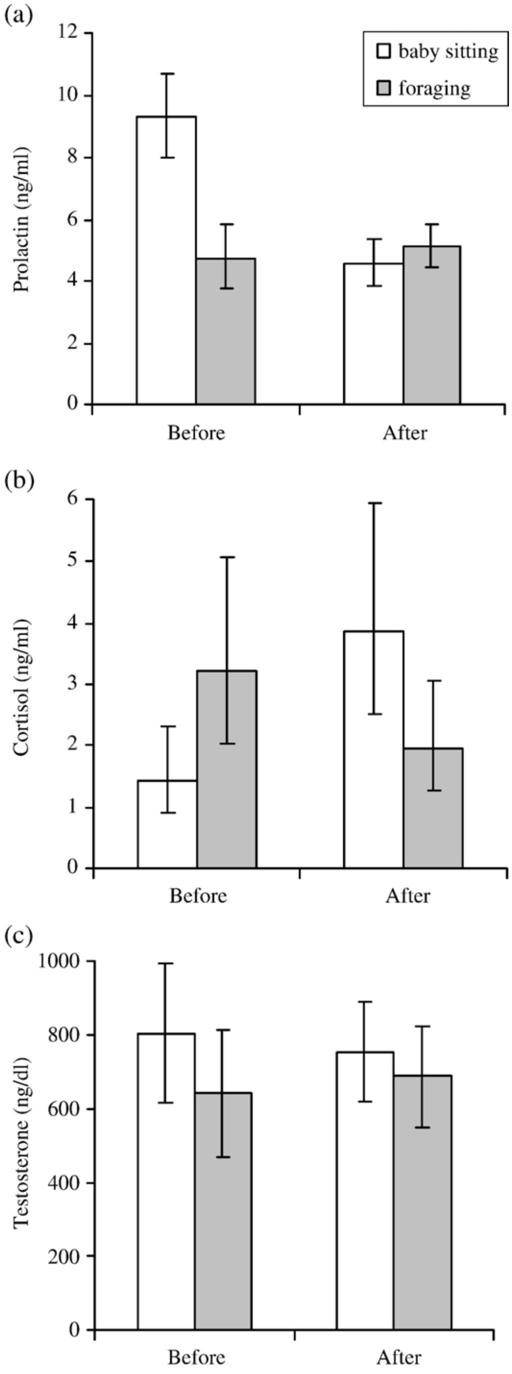

Fig. 1. Association between activity (babysitter versus forager) and timing of sampling (at the beginning of the day before individuals babysat or foraged versus at the end of the day when they had babysat for 12 h or foraged for 12 h) and level of circulating (a) prolactin; (b) cortisol; and (c) testosterone. Graphs show predicted means from the statistical models ±1 standard error. In the case of prolactin and cortisol, the means are presented back-transformed using a square and exponential function, respectively. Prolactin results are presented after controlling for positive effects of cortisol levels ($\chi^2 = 7.80$, $df = 1$, $P = 0.005$), capture to bleed time ($\chi^2 = 3.46$, $df = 1$, $P = 0.063$), helper numbers ($\chi^2 = 2.43$, $df = 1$, $P = 0.12$), and negative effects of testosterone levels ($\chi^2 = 0.71$, $df = 1$, $P = 0.40$). Cortisol results are presented after controlling for positive effects of testosterone levels ($\chi^2 = 7.41$, $df = 1$, $P = 0.006$), prolactin levels ($\chi^2 = 4.33$, $df = 1$, $P = 0.037$), and capture to bleed time ($\chi^2 = 2.05$, $df = 1$, $P = 0.15$), and negative effects of helper numbers ($\chi^2 = 11.26$, $df = 1$, $P = 0.001$). Testosterone results are presented after controlling for positive effects of cortisol levels ($\chi^2 = 6.61$, $df = 1$, $P = 0.010$) and helper numbers ($\chi^2 = 2.65$, $df = 1$, $P = 0.10$). Sample sizes differed from those provided in the methods by up to three because hormones could not be obtained from all samples.

Table 1
Effect of hormone levels on contributions to babysitting by adult male meerkats over the 1-month babysitting period

Model terms	Effect	SE	Wald statistic (χ^2)	*P* value
Age	0.0013	0.00042	10.01	0.002
Helper number	−0.062	0.017	13.65	<0.001
Weight (g)	0.00021	0.0042	0.01	0.96
Daily weight gain (g/h)	0.0011	0.0013	0.88	0.35
Immigrant status ($I > N$)	−0.74	0.54	1.92	0.17
Prolactin	0.014	0.046	0.09	0.76
Cortisol	0.13	0.096	1.88	0.17
Testosterone	0.00037	0.00036	1.06	0.30
Constant	−2.03	0.17		

Whether the male was natal or an immigrant was used as a proxy for relatedness (Griffin et al., 2003). Effect and SE indicate the predicted effect sizes of each term on contributions to babysitting and their standard error obtained from the GLMM after setting the minimum value of each term to zero. Degrees of freedom were 1 in each case.

individuals that babysat or foraged on a given day had different prolactin and cortisol profiles immediately before bouts of babysitting or foraging began. Furthermore, the endocrine profiles that characterized babysitters and foragers at the start of the day differed from those that characterized them 12 h later, suggesting that the hormonal levels of helpers changed over the course of the day depending on the activity performed. Despite these findings, there was no evidence of an association between endocrine levels and long-term contributions to babysitting after controlling for the positive effects of helper age and the negative effects of helper number within the group. Finally, we provide no evidence to suggest that testosterone levels significantly influenced decisions to babysit by male meerkats (reviewed by Ziegler, 2000).

Why did individuals that decided to spend the day babysitting have significantly higher levels of circulating prolactin than individuals that decided to spend the day foraging? Prolactin levels are believed to increase in adults exposed to auditory, visual, olfactory, and/or tactile stimuli from pups (Grosvenor et al., 1990; Freeman et al., 2000). For example, physical contact with infants during carrying has been linked to increased levels of circulating prolactin in male callitrichid primate helpers (Dixson and George, 1982; Roberts et al., 2001a; Mota et al., 2006). Hence, one explanation for our results is that male helpers that spent the night huddled next to pups experienced significant increases in prolactin levels and consequently were more likely to babysit the following day.

There are two problems with this hypothesis, however. First, we know that prolactin levels do not increase in male meerkat helpers exposed to playbacks of pup-begging calls (Carlson et al., 2006). Second, it is unclear why prolactin levels would decrease over the course of the day in babysitters but increase overnight in those meerkats adjacent to pups in the burrow, since both are exposed to the same visual, auditory, olfactory, and tactile cues from pups. This point is especially relevant given that babysitters are likely to receive stronger and more frequent stimulation from hungry pups during the day than they are during the night when the pups are less hungry and the rest of the group is also present. For these reasons, it seems unlikely that infant cues are the primary reason that prolactin levels become elevated in those meerkat helpers about to begin a babysitting session, and we have yet to identify other factors likely to stimulate prolactin secretion in male meerkats immediately prior to a babysitting bout.

The results from the babysitting studies described here differ substantially from recent work on the hormonal correlates of pup feeding from the same meerkat population. Carlson et al. (2006) found that cortisol levels of individual male meerkat helpers were significantly and positively associated with both long- and short-term patterns of pup feeding. The long-term study, which identified the factors affecting the total number of food items adult subordinate male helpers provided to pups during the peak pup-feeding period (when pups were 35–75 days old), showed that individual contributions to pup-feeding were significantly and positively correlated with plasma levels of cortisol, but not with prolactin or testosterone. A playback experiment confirmed these results by using pup-begging calls to stimulate pup provisioning by male helpers; the playbacks gave rise to parallel increases in plasma cortisol levels, while levels of prolactin and testosterone remained unchanged (Carlson et al., 2006). Thus, a combination of long-term datasets, observational studies, and experiments have confirmed that prolactin levels are associated with babysitting in the short-term, while cortisol levels are associated with pup-feeding in the long-term and the short-term.

Why are different hormones associated with different types of helping behavior in meerkats? The correlation between prolactin levels and babysitting in meerkat helpers is consistent with numerous studies documenting the role of prolactin in both maternal and paternal care over the past 40 years (see reviews by Numan, 1994; Wynne-Edwards and Reburn, 2000). The connection between cortisol and pup care is more surprising but may be due to the physiological effects of hunger on glucocorticoid concentrations and subsequent patterns of pup feeding. Specifically, frequent babysitting entails repeated short-term fasting because helpers rarely eat during babysitting sessions (Clutton-Brock et al., 1998). Short-term fasting has been shown to trigger increases in circulating glucocorticoids (Lea et al., 1992; Lynn et al., 2003; Koch et al., 2002, 2004). Over the course of an entire babysitting period then, the top babysitters in each group would experience substantial amounts of weight loss (Clutton-Brock et al., 1998; Russell et al., 2003b) as well as frequent, short-term increases in circulating glucocorticoids. Small but statistically significant increases in plasma cortisol levels could facilitate later pup-feeding by "increasing the foraging behavior that underlies … provisioning" by parents (Koch et al., 2004), or, in this case, helpers. This hypothesis is supported by the fact that all of the blood samples for the pup-feeding study were collected prior to the pup-feeding period (Carlson et al., 2006). That is, plasma cortisol levels from the babysitting period, not the pup-feeding period, predicted long-term contributions to pup feeding among male helpers. Furthermore, adult meerkat helpers that contributed heavily to babysitting typically contributed heavily to pup-feeding for the same litter (Clutton-Brock et al., 2003; Russell et al., 2003b).

Our pup-feeding results are consistent with an increasing number of studies documenting positive links between rates of caring behavior and small, but significant, increases in glucocorticoid levels (e.g., Fleming et al., 1997; Stallings et al., 2001; DeVries, 2002). Not surprisingly, however, evidence from avian studies has shown that extremely high concentrations of circulating glucocorticoids can lead to abandonment of both the young and territory by the breeding pair (Silverin, 1986). Thus far, our studies of the physiological basis of caring behavior have not included artificial manipulations of glucocorticoid levels, as was the case in Silverin (1986); hence, we do not know if a similar relationship exists in meerkats.

In conclusion, we provide the first evidence that increased prolactin levels immediately precede bouts of helping behavior in a cooperatively breeding vertebrate. Furthermore, our studies of pup feeding and babysitting in meerkats show that, although helping behavior appears to have an endocrine basis, different hormones may be associated with different kinds of caring behavior. We suggest that future research on the effects of hunger levels and food intake on individual prolactin and cortisol levels over different time scales, coupled with detailed investigations of the mechanisms that stimulate short-term increases in prolactin levels in free-ranging vertebrates, will lead to significant improvements in our understanding of the physiological basis of cooperative care.

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