Hormonal and Behavioral Responses to Stress in Lactating and Non-lactating Female Common Marmosets (*Callithrix jacchus*)

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Abstract

In several mammalian species, hypothalamic-pituitary-adrenal (HPA) and behavioral responses to stressors are down-regulated in lactating females, possibly preventing stress-induced disruptions of maternal care. Experimental elevations of HPA axis hormones have been found to inhibit maternal behavior in lactating common marmoset monkeys (*Callithrix jacchus*), raising the question of whether lactating female marmosets also have blunted endogenous responses to stress. Therefore, we compared HPA and behavioral responses to standardized stressors in reproductively experienced female common marmosets that were undergoing ovulatory cycles and that either were (N=7) or were not lactating (N=8). Each marmoset underwent (1) a restraint stressor during the early follicular phase of the ovarian cycle (approximately 5 weeks postpartum for lactating females) and (2) exposure to a simulated hawk predator during the early to mid-luteal phase (approximately 7 weeks postpartum for lactating females). Lactating females were tested in the presence of one of their infants. Blood samples were collected before, during, and immediately after each test for determination of plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations. Both stressors caused significant elevations in plasma ACTH and cortisol levels, and significant decreases in cortisol:ACTH ratios; however, lactating and non-lactating females showed no significant differences in their endocrine or behavioral responses to either stressor, or in baseline ACTH or cortisol levels. These findings suggest that in contrast to several other mammalian species, lactating female marmosets maintain full behavioral and HPA responsiveness to stress, at least in the presence of their infants.

Keywords

adrenocorticotropic hormone; cortisol; lactation; maternal care; parental care; stress

1. Introduction

The glucocorticoid hormones (e.g., cortisol and corticosterone) and other hormones of the hypothalamic-pituitary-adrenal (HPA) axis are thought to mediate tradeoffs between

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survival and reproduction [1–3]. Specifically, under stressful conditions, glucocorticoids promote individual survival by modulating such essential functions as metabolism, immunity, and cardiovascular activity, while simultaneously suppressing such non-essential functions as sexual and parental behavior and reproductive physiology [4]. Consequently, several authors have suggested that in some individuals, populations, or species, the HPA response to stress might be down-regulated during periods of high investment in reproduction, to protect against stress-induced disruptions of reproductive effort [1,2,5].

Consistent with this hypothesis, female mammals of several species show blunted HPA responses to stress during the lactational period. Compared to virgin females, for example, lactating rats (*Rattus norvegicus*) show suppressed corticosterone and adrenocorticotropic hormone (ACTH) responses to numerous psychological and physiological stressors (reviewed by [6,7]), as well as reduced expression of corticotropin-releasing hormone (CRH) mRNA in the brain following exposure to stressors [8–10]. In addition to their blunted HPA responses, lactating females of several species also show reduced behavioral responses to stressors and diminished behavioral indices of anxiety. Postpartum rats, for example, show reduced acoustic startle responses, increased locomotion in the open field, increased time in the open arms of an elevated plus maze, reduced shock-induced aggression, and reduced fleeing from an intruder, compared to nulliparous females [11–18]. Thus, the postpartum/lactational period has been referred to as a period of stress-hyporesponsiveness (e.g., [19,20]). Blunted HPA stress responses during lactation have also been reported in sheep (*Ovis aries*), variable flying foxes (*Pteropus hypomelanus*), Columbian ground squirrels (*Spermophilus columbianus*), and, inconsistently, women [6,7,21–25]. Studies of other species, however, have yielded little or no evidence of lactational stress hyporesponsiveness (e.g., mouse, *Mus*: [26]; rhesus macaque, *Macaca mulatta*: [27,28]). Thus, the extent to which lactational stress hyporesposiveness can be generalized across mammalian taxa remains unclear.

In the present study, we tested the hypothesis that HPA and behavioral responses to stress are blunted during lactation in a nonhuman primate that shows extremely high investment in reproduction, the common marmoset (*Calllithrix jacchus*). This small-bodied (~350 g), neotropical monkey has unusually high annual reproductive output, both in the field and in captivity, as compared to other primates: after a gestation lasting roughly 144 days, females typically give birth to fraternal twins or triplets (or, less commonly, singletons or quadruplets), undergo a postpartum ovulation, and are likely to conceive again within 2–4 weeks (reviewed by [29–31]). Thus, marmosets do not undergo lactational inhibition of ovulation, and are often simultaneously lactating and either cycling or pregnant [29,32]. Combined litter mass can approach or even exceed 20% of maternal mass [29,33]. All group members, including adults and juveniles of both sexes, assist with infant care; however, mothers spend substantial amounts of time carrying their infants (approximately 30–40% of observation time during the first month postpartum and approximately 10–20% during the second month postpartum; [34,35]). Infants nurse exclusively for the first 3–4 weeks of life and are completely weaned by 8–10 weeks [29].

Previously we have found that hormones of the HPA axis can inhibit maternal behavior in common marmoset mothers. First, treatment of multiparous females with high levels of exogenous cortisol over an 8-day period, which elevated circulating cortisol levels into or above the stress range for this species, significantly reduced infant-carrying [36]. Second, acute, intracerebroventricular infusion of CRH in primiparous mothers reduced infant-carrying and approaches to infants [37]. In view of the high reproductive investment routinely made by marmoset mothers, as well as the ability of both cortisol and CRH to disrupt maternal behavior in this species, we predicted that lactating female marmosets would exhibit blunted HPA and behavioral responses to stressors. To test this hypothesis, we

characterized the ACTH, cortisol, and behavioral responses to stressors during both the mid-lactational period, when mothers were in the early follicular phase of the ovarian cycle, and the late lactational period, when they were in the early to mid-luteal phase of the cycle or early pregnancy, and compared these responses to those of multiparous but non-lactating females.

2. Materials and Methods

2.1. Animals

Subjects were 15 reproductively experienced, adult female common marmosets, including seven lactating and eight non-lactating females. Power analysis (JMP, SAS Institute, Cary, NC) based on corticosterone responses to stressors in lactating and non-lactating female rats [11] indicated that this sample size yielded power of >87% to detect significant differences between groups. Lactating and non-lactating animals did not differ in age (57.1 ± 6.7 vs. 65.4 ± 4.1 months, respectively, mean ± SEM; T[7,8]=1.060, P=0.314) or parity (4.4 ± 1.0 vs. 5.1± 0.4 litters; T[7,8]=0.649, P=0.535) at the outset of data collection, and all females were undergoing ovulatory cycles during the study (see below). Non-lactating females had not given birth for 128–488 days prior to data collection as a result of treatment with cloprostenol, a prostaglandin analog, after each ovulation (see below), and were housed with an adult male pairmate and up to eight of their offspring. Lactating females were housed with an adult male pairmate, 1–2 infants, and up to four older offspring.

Animals were housed indoors at the National Primate Research Center at the University of Wisconsin (UW) – Madison (WNPRC), in aluminum and wire mesh cages (61 × 91 × 183 cm or 122 × 61 × 183 cm) that permitted visual, auditory and olfactory contact between animals in different groups. The animals were fed Mazuri Hi-Fiber Callitrichid Diet (Mazuri, Richmond, IN) supplemented with vitamin D, at 12:30–13:30 h; however, food was typically available in the cages at all times. Water was available ad libitum. Lights were on from 6:30 to 18:30 h, and room temperature and humidity were maintained at approximately 23°C and 30–70%, respectively.

All procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* and were reviewed and approved by the Graduate School Animal Care and Use Committee of UW-Madison. WNPRC is accredited by AAALAC as part of the UW-Madison Graduate School.

2.2. Design

Each adult female marmoset underwent two stress tests: a restraint test and a simulated predator (hawk) test. With the exception of one lactating female (see below), all animals underwent the restraint test first and the hawk test 9–20 (14.8 ± 0.7) days later. Marmosets underwent the restraint test during the early follicular phase of the ovarian cycle, 2–3 days following treatment with cloprostenol to induce luteolysis (see below), and the hawk test approximately 6–10 days following the subsequent ovulation, during the early to mid-luteal phase or early pregnancy (lactating: 8.2 ± 0.5 days post-ovulation, non-lactating: 7.9 ± 0.5 days post-ovulation). Lactating females underwent restraint tests at 29–37 days postpartum (with one exception; see below), and hawk tests at 45–50 days postpartum. Beginning several weeks (24.5 ± 2.3 days) before the first stress test, we collected blood samples (0.1 ml) from each animal twice per week, at 3- to 4-day intervals, to monitor plasma progesterone levels for characterization of ovulatory activity (see below).

One lactating female had an unusually late postpartum ovulation, 36 days after giving birth. Therefore, this female underwent her luteal-phase hawk test first (46 days postpartum) and her restraint test during the follicular phase of the subsequent cycle (52 days postpartum).

Her behavioral and hormonal data fell within the range of other lactating females and so were included in all analyses except comparisons of baseline hormone concentrations between groups (lactating vs. non-lactating) and between reproductive conditions (mid lactational period/early follicular phase vs. late lactational period/luteal phase). Another lactating female underwent the restraint test without her infant present, due to logistical constraints. Again, this animal's cortisol, ACTH, and behavioral data (except infant-related behaviors) were comparable to those of other lactating females and so were included in the analyses.

2.3. Restraint tests

Restraint tests were performed at 14:00–15:00 h in a room containing no other animals. Marmosets were restrained for a total of 50 min on a polycarbonate restraint board that was padded with flannel; velcro straps were wrapped firmly around the animal's abdomen, chest, thighs, arms, and wrists. The female's nipples were left exposed, allowing infants of lactating females to suckle during the test. The restraint board was stationed at a 30° angle in a stainless steel stand, which was placed inside a test cage ($45 \times 60.5 \times 60.5$ cm). For tests on six of the seven lactating females, one of the female's infants was released into the test cage immediately after the mother was restrained, and remained with its mother throughout the test. The seventh lactating female was tested individually, as described above.

We collected blood samples (0.4 ml) immediately before the restraint period, and 20 and 50 minutes after the beginning of restraint. All samples were collected from unanesthetized animals by femoral venipuncture using heparinized syringes. For the initial (baseline) sample, animals were captured from their home cages and placed in a marmoset blood-sampling restraint tube [38]; blood was collected within 3 minutes of initial disturbance (investigator's entry into the home cage). For the subsequent samples, marmosets remained on the restraint board, and blood was collected within 3 minutes of the investigator's emergence from the observation blind. We have previously demonstrated that our routine blood-sampling procedures do not elevate plasma cortisol levels in female marmosets that have undergone these procedures on a regular basis [39]. Blood samples were immediately placed on ice and subsequently processed as described by Orth [40] for ACTH assay: samples were initially centrifuged at 4200 rpm for 15 min at 4°C, and the plasma fraction was removed and centrifuged again at 9000 rpm for 10 min at 4°C. The plasma fraction was again removed and stored at −20°C (plasma to be assayed for cortisol) or −80°C (plasma to be assayed for ACTH) until assayed.

Behavioral data were collected for 5 min at each of four time points during restraint tests (beginning 5, 15, 30 and 45 min after the beginning of restraint) by a trained observer seated inside a blind with a one-way viewing shade. Behavior was recorded on a laptop computer using the JWatcher event-recorder program [41]. Table 1 describes the behaviors that were scored in the restraint test and/or the hawk test (see below). A number of additional behaviors, including approach infant, solicit infant, inspect infant, lick infant, reject infant, cuff infant, bite infant, attack infant, suckle, facial submit, genital present, vocal threat (erh-erh), long-call (phee) and chirp (see [36,42] for descriptions), were included in our ethogram but were observed too infrequently to permit statistical analysis.

2.4. Hawk tests

Hawk tests were performed at 14:00–15:00 h. A female marmoset and, in the case of lactating females, one of her infants was placed in a test cage ($45 \times 60.5 \times 60.5$ cm), in a room containing no other marmosets, for 30 min. Two, 8 and 12 min later, a realistic-looking, life-size hawk model (hawk-replica kite [119 cm wingspan; Peaceful Valley Farm Supply, Grass Valley, CA] mounted on Plexiglas) attached to a 0.91 m-long dowel, was

manually "flown" over the test cage three times in rapid succession by an investigator who remained out of the animals' view. A red-tailed hawk (*Buteo jamaicensis*) call (http://fsc.fernbank.edu/Birding/birdID/red_tailed.htm) was simultaneously played from a laptop computer. Blood samples (0.4 ml) were collected from the adult female immediately before and 15 and 30 min after her release into the test cage. For each sample, the marmoset was placed in a marmoset blood-sampling restraint tube [38] and blood was collected by femoral venipuncture into a heparinized syringe, within 3 minutes of the investigator's entry into the marmoset's home cage (first sample) or emergence from the observation blind (second and third samples). Behavioral data were collected continuously during the first 15 minutes after the marmoset was released into the test cage, using the methods described above (Table 1).

2.5. Hormone assays

Hormone assays were fully validated for marmoset plasma (cortisol, progesterone: [39]; ACTH: [43]). Samples were assayed in duplicate for plasma cortisol using an antibody-coated-tube radioimmunoassay (RIA) kit (GammaCoat, DiaSorin Corp., Stillwater, MN [39]). Assay sensitivity at 90% binding was 0.1 ng/tube (1.0 µg/dl), and inter- and intra-assay coefficients of variation (CVs) of a plasma pool assayed in quadruplicate in each assay were 7.5% and 5.3%, respectively.

Plasma ACTH concentrations were measured by RIA as described previously [43]. Assay sensitivity at 90% binding was 0.5 pg/tube (6.7 pg/ml), and inter- and intra-assay CVs of a plasma pool assayed in triplicate in each assay were 13.7% and 1.9%, respectively.

Plasma progesterone concentrations were measured in duplicate using a heterologous enzymeimmunoassay [39]. Assay sensitivity at 90% binding was 3.6 pg/tube (2.7 ng/ml), and inter- and intra-assay CVs of a plasma pool assayed in duplicate in each assay were 13.9% and 4.4%, respectively.

2.6. Monitoring and control of reproductive function

Reproductive status of non-lactating females prior to the beginning of the experiment was monitored on the basis of uterine palpation and plasma progesterone concentrations in monthly blood samples, and reproductive status of lactating and non-lactating females during the experiment was monitored on the basis of plasma progesterone levels in twice-weekly blood samples. Marmosets were considered to be in the luteal phase of the ovarian cycle or early pregnancy when progesterone concentrations exceeded 10 ng/ml, and in the follicular phase when plasma progesterone concentrations were <10 ng/ml [39,44]. Ovulation was considered to have occurred the day before a rise in plasma progesterone levels above 10 ng/ml.

To prevent non-lactating females from sustaining pregnancies before or during the experiment, and to prevent lactating females from sustaining postpartum pregnancies during the study, marmosets were treated with cloprostenol sodium (Estrumate; Schering-Plough, Pointe Claire, Quebec; 0.75–1.0 µg, IM), a prostaglandin F2α analog, approximately 15–30 days after ovulation. Cloprostenol causes luteolysis and termination of the luteal phase or early pregnancy [45], but has not been found to alter baseline cortisol levels in adult female common marmosets [46].

2.7. Analysis

Plasma cortisol and ACTH concentrations and cortisol:ACTH ratios were log-transformed to increase normality and linearity, and to reduce heterogeneity of variance; however, raw values (mean ± SE) are presented in the text and figures for clarity. Hormonal data were

analyzed by ANOVA, with lactational status (lactating vs. non-lactating) as a between-groups factor and reproductive phase (mid lactation/follicular phase vs. late lactation/luteal phase) and time (pre, 20 min, and 50 min from onset of restraint, or pre, 15 min, and 30 min from start of hawk test) as repeated measures. Post hoc pairwise comparisons following significant main effects of time utilized the Bonferroni correction for multiple comparisons. Behavioral data were analyzed nonparametrically, and hormone-behavior associations were evaluated using Spearman rank correlations. Analyses were performed using Systat (Chicago, IL) and were evaluated at the .05 level (2-tailed).

3. Results

3.1. Baseline hormone concentrations

A 2-way (lactational status x reproductive phase) ANOVA revealed that afternoon baseline plasma ACTH concentrations, determined immediately before the stress tests, did not differ between lactating and non-lactating females ($F[1,11]=0.708$, $P=0.418$) or between the early follicular phase of the ovarian cycle (corresponding to the mid-lactational period in lactating females) and the early to mid-luteal phase/early pregnancy (corresponding to the late lactational period in lactating females) ($F[1,11]=0.006$, $P=0.938$; Fig. 1A, 2A). Moreover, baseline ACTH levels were not significantly influenced by an interaction between lactational status and reproductive phase ($F[1,11]=0.000$, $P=0.995$).

Afternoon baseline plasma cortisol concentrations, like ACTH, did not differ between lactating and non-lactating females ($F[1,12]=0.341$, $P=0.570$) or between the early follicular phase (mid-lactational period) and the early to mid-luteal phase/early pregnancy (late lactational period) ($F[1,12]=1.285$, $P=0.279$; Fig. 1B, 2B). Again, we did not find a significant interaction between lactational status and reproductive phase ($F[1,12]=0.003$ $P=0.960$).

We analyzed the ratio of plasma cortisol levels to plasma ACTH levels in the same sample as an index of adrenocortical responsiveness to ACTH. Under baseline conditions, the cortisol:ACTH ratio was not significantly influenced by lactational status ($F[1,11]=0.808$, $P=0.388$), reproductive phase ($F[1,11]=0.100$, $P=0.758$), or a lactation x reproductive phase interaction ($F[1,11]=0.005$, $P=0.948$; Fig. 1C, 2C).

3.2. Restraint test

As expected, female marmosets' circulating concentrations of both ACTH and cortisol increased markedly in response to restraint (ACTH: $F[2,26]=59.996$, $P<0.001$, Fig. 1A; cortisol: $F[2,26]=42.504$, $P<0.001$, Fig. 1B). Post hoc pairwise comparisons indicated that ACTH levels increased significantly from immediately before to 20 minutes after the start of restraint ($P<0.001$), but did not show a further elevation after 50 minutes. In contrast, cortisol concentrations rose significantly from before to 20 minutes after the start of restraint ($P<0.001$), and increased further at the 50-minute time point ($P<0.001$). Neither overall hormone concentrations (ACTH: $F[1,13]=1.783$, $P=0.205$; cortisol: $F[1,13]=0.173$, $P=0.684$) nor the pattern of change over time (ACTH: $F[2,26]=0.577$, $P=0.569$; cortisol: $F[2,26]=0.819$, $P=0.452$) differed between lactating and non-lactating females.

The ratio of plasma cortisol to ACTH concentrations decreased significantly across time during the restraint test ($F[2,26]=28.297$, $P<0.001$; Fig. 1C). Post hoc pairwise comparisons indicated that the ratio declined significantly from before to 20 minutes after the beginning of restraint ($P<0.001$), but did not show a further decrease from 20 to 50 minutes. Again, neither absolute values of the cortisol:ACTH ratio ($F[1,13]=1.003$, $P=0.335$) nor the pattern of change over time ($F[2,26]=0.492$, $P=0.617$) differed between lactating and non-lactating females.

All of the adult females struggled during the restraint period, and all but two of them chewed on the restraint apparatus; however, the total amounts of time spent struggling and chewing did not differ between lactating and non-lactating females (Table 2). Very few females vocalized (moan, long-call, chirp, vocal threat) during restraint tests. Behavior of infants during their mothers' restraint was highly variable, in terms of the amount of time spent on the mother and the number of vocalizations emitted (Table 2). Only one infant was observed to suckle.

To investigate the relationship between females' behavioral responses to restraint (struggling, chewing on the restraint apparatus) and HPA activity, we performed a series of Spearman rank correlations using data from all 15 lactating and non-lactating females. Marmosets' incremental cortisol responses at the end of the restraint period (cortisol concentration after 50 min of restraint minus baseline cortisol concentration immediately before restraint) showed a moderate negative correlation with the total amount of time that the animals spent struggling in the restraint ($r_s = -0.689$, N=15, P<0.01). In contrast, the total amount of time that the animals spent chewing on the restraint apparatus was not significantly correlated with their cortisol responses to restraint ($r_s = -0.304$, N=15, NS), and neither struggling ($r_s = 0.039$, N=15, NS) nor chewing ($r_s = 0.034$, N=15, NS) was correlated with incremental ACTH responses to the stressor.

To determine whether the lactating females' hormonal responses to restraint were associated with their infants' behavior during the same time period, we performed Spearman rank correlations between mothers' incremental ACTH and cortisol responses to restraint and their infants' total behavioral scores across the four 5-min observations. None of the resulting correlations were statistically significant, possibly due to the small sample size (ACTH and number of "nga" vocalizations by infant: $r_s = 0.714$, N=6, NS; ACTH and amount of time that the infant spent on the mother: $r_s = -0.600$, N=6, NS; cortisol and number of "nga" vocalizations by infant: $r_s = -0.371$, N=6, NS; cortisol and amount of time that the infant spent on the mother: $r_s = 0.829$, N=6, NS).

3.3. Hawk test

Confinement of adult female marmosets in a novel cage plus exposure to a simulated predator (hawk model + hawk vocalization) elicited pronounced increases in plasma ACTH and cortisol concentrations (ACTH: $F_{[2,24]} = 32.694$, P<0.001, Fig. 2A; cortisol: $F_{[2,26]} = 32.764$, P<0.001, Fig. 2B). As in the restraint test, plasma ACTH levels were significantly elevated 15 min after the start of the hawk test, as compared to pre-test baseline levels (P<0.05), but did not show a further increase from 15 to 30 minutes, whereas cortisol concentrations showed progressive, significant increases across the three blood samples (Ps≤0.001). Lactating and non-lactating females showed no significant differences in overall hormone levels (ACTH: $F_{[1,12]} = 1.450$, P=0.252; cortisol: $F_{[1,13]} = 0.795$, P=0.389) or in the pattern of change over time (ACTH: $F_{[2,24]} = 0.542$, P=0.589; cortisol: $F_{[2,26]} = 0.422$, P=0.660).

As in the restraint test, cortisol:ACTH ratios during the hawk test declined significantly over time ($F_{[2,24]} = 12.617$, P<0.001, Fig. 2C). Values of this ratio were significantly lower at 15 minutes after the start of the hawk test than before the test began (P=0.001) but did not show a further change at 30 minutes. Once again, the cortisol:ACTH ratio did not differ between lactating and non-lactating females (main effect of lactation: $F_{[1,12]} = 2.198$, P=0.164, lactation x time interaction: $F_{[2,24]} = 0.179$, P=0.838).

Most of the adult female marmosets responded to simulated hawk exposures by flinching and visually scanning the area above the cage. Neither of these behaviors differed significantly between lactating and non-lactating females (Table 3). The proportion of time

spent bristle strutting varied widely among animals but, again, did not differ between lactating and non-lactating females. Each lactating female retrieved her infant within 13 sec after the beginning of the hawk test and carried it continuously for the remainder of the test. These females engaged in very few other overt interactions with their infants during this time.

For all females combined, neither incremental cortisol nor incremental ACTH responses to the stressor after 30 minutes were significantly correlated with the number of visual scans performed (cortisol: $r_s=-0.041$, ACTH: $r_s=0.128$, N=15, NS), the number of flinches performed (cortisol: $r_s=0.029$, ACTH: $r_s=0.168$, N=15, NS), or the amount of time spent bristle strutting (cortisol: $r_s=0.093$, ACTH: $r_s=-0.498$, N=15, NS). The lactating females' infants showed very little variation in their behavior during the hawk test; therefore, we did not perform correlational analyses using infant behaviors.

4. Discussion

This experiment is one of the first systematic investigations into the effects of lactation on hypothalamic-pituitary-adrenal and behavioral responses to stressors in nonhuman primates. None of our measures, including plasma ACTH and cortisol concentrations, cortisol:ACTH ratios, and behavior, differed reliably between lactating and non-lactating females in response to either a restraint stressor (administered during the mid-lactational period) or simulated predator exposure (administered during the late lactational period). These results suggest that common marmosets may differ from several other mammals in maintaining full HPA and behavioral responsiveness to stress during lactation.

Several features of our experiment might have contributed to the absence of significant differences in stress-responsiveness between lactating and non-lactating marmosets. First, lactating females underwent both the restraint stressor and the simulated predator stressor in the presence of one of their infants. In other species, the immediate presence of the mother's infant(s) has been found to affect the mother's stress response, although the direction of this effect can differ with stressor and/or species. Lactating ewes temporarily housed away from their lambs had blunted cortisol and ACTH responses to a psychological stressor, as compared to non-lactating females. These hormonal responses were further attenuated if the ewes were permitted to interact with (but not to nurse) their lambs during exposure to the stressor, and were essentially abolished if the lambs were permitted to suckle during stress-testing [24]. Among rats, in contrast, corticosterone and ACTH responses to predator odor (fox urine) were attenuated in lactating females tested without their pups, as compared to virgin females, but HPA responsiveness was fully restored when lactating females were tested with their pups present [19]. In women, the effect of the immediate presence or absence of the infant is unclear, but breastfeeding has been suggested to exert only transient inhibitory effects on HPA activity [47,48]. Although we did not find significant correlations between mothers' cortisol or ACTH responses to stressors and their infants' behaviors, these findings in women suggest that HPA stress responses in primate mothers may be modulated by nursing or other interactions with their infants.

Another variable that can influence responsiveness to stress in lactating females is phase of lactation. In rats, hyporesponsiveness is more pronounced (i.e., responses to stress are smaller) during the late lactational period than the early lactational period [19,49]. We tested marmosets during both mid-lactation, when they were in the early follicular phase of the ovarian cycle, and late lactation, when they were in the early to mid-luteal phase or early pregnancy. Hormonal and behavioral responses to stress did not differ between lactating and non-lactating females at either time point.

We did not systematically compare marmosets' responses to stressors between the two reproductive phases, because different stressors were used at the two time points. Nonetheless, both lactating and non-lactating females showed more robust ACTH and cortisol responses to the restraint test during the early follicular phase than to the hawk test during the luteal phase or early pregnancy. This pattern is likely to reflect the difference between the two stressors used, as our restraint paradigm appears to be a particularly potent stressor for marmosets (unpub. data). In contrast, the hawk test, which was developed specifically for this experiment, elicited distinct but relatively modest hormonal and behavioral responses. Thus, this test may provide a useful mild-stress paradigm for future studies of stress responsiveness in marmosets.

Lactating and non-lactating female marmosets in our study were similar not only in their HPA responses to stress, but also in their baseline ACTH and cortisol concentrations. In women, breastfeeding causes acute reductions in circulating ACTH and cortisol levels, but does not appear to have chronic effects on baseline ACTH or cortisol concentrations [50,51]. Lactating rats, on the other hand, exhibit elevated baseline concentrations of corticosterone and, in some studies, ACTH, reflecting alterations in the circadian rhythm of hormone release [6,25,52]. In the present study, we characterized baseline hormone levels at only a single time of day (14:00 h), when circulating ACTH and cortisol concentrations were approaching their nadir (unpub. data), and we were not able to control for acute effects of suckling. ACTH and cortisol levels will need to be characterized at more time points across the circadian cycle, as well as before and after nursing bouts, to more fully investigate possible effects of lactation on baseline HPA activity in marmosets and other nonhuman primates.

Because lactating marmosets do not appear to have blunted hormonal responses to stress, as compared to non-lactating (but reproductively experienced) females, it might be hypothesized that other mechanisms exist to prevent the stress response from inhibiting the expression of maternal behavior in this species [2]. This does not, however, appear to be the case. In previous studies we found that chronic, experimental elevation of circulating cortisol levels, as well as acute, intracerebroventricular infusions of CRH (which stimulated increases in plasma ACTH and cortisol levels), inhibited aspects of maternal behavior, especially infant-carrying, in lactating marmosets [36,37]. Thus, not only do lactating female marmosets mount full HPA responses to stress, but these HPA responses appear to suppress maternal behavior. Such stress-induced inhibition of maternal behavior in a broad range of species is thought to be adaptive in promoting the mother's survival under energetically challenging conditions, and ultimately in maximizing lifetime reproductive success, although at the cost of current reproductive investment [2].

In the only other published study on HPA responses to stress in lactating nonhuman primates, Maestripieri et al. [27] found that plasma cortisol concentrations of free-ranging rhesus macaques were higher in lactating than non-lactating, non-pregnant females both after an acute stressor (capture, handling, and anesthetization with ketamine) and after a more prolonged stressor (approximately 22 hours of confinement in a small cage, either alone [non-lactating females] or with their infant [lactating females]). The results of this study are somewhat difficult to interpret, as baseline cortisol levels could not be determined. Moreover, several methodological differences between the present study on marmosets and Maestripieri et al.'s [27] study on rhesus macaques – including differences in the type and time course of the stressors imposed and in the time of day at which blood samples were collected – preclude direct comparison between the two experiments. Nonetheless, the present findings in marmosets, combined with these results in rhesus macaques and inconsistent findings in women, suggest that lactational hyporesponsiveness to stress may be expressed less consistently in primates than in rats and several other mammals, if at all.

The apparent differences among taxa should, however, be interpreted with caution. First, whereas both the marmosets in this study and the rhesus macaques studied by Maestripieri et al. [27] were stressed in the presence of their infants, most studies of rodents have tested dams that were temporarily isolated from their pups. The possible significance of this difference is underscored by findings that the immediate presence of pups significantly increases rat dams' HPA responses to acute stressors, as described above [19]. Second, studies on rats have typically compared stress responses between lactating and virgin females, whereas those on humans and nonhuman primates have usually compared lactating females with non-lactating but parous females. This methodological difference might be especially relevant, as findings from both rats and women indicate that reproductive experience or lactation can have long-term effects on HPA activity. Among rats that were not currently lactating, for example, fecal corticosterone concentrations, as well as fear-related or anxiety-like behavior, were reduced in parous compared to nulliparous females [53]. Moreover, among postmenopausal women, circulating cortisol concentrations were significantly higher in individuals who had previously breast-fed for a total of more than 12 months than in those who had not [54; but see 55]. Thus, apparent taxonomic differences in the occurrence of lactational hyporesponsiveness to stress could potentially result, at least in part, from differences in the types of control groups used, stress-test conditions, and/or other aspects of methodology.

It remains unclear, therefore, to what extent mammalian species differ in the occurrence of lactational hyporesponsivenss to stress. Such differences might be linked to differences in the timing of infant neural development (e.g., altricial vs. precocial), relative maternal investment in each infant or litter, type or frequency of maternal care (e.g., parking vs. carrying), or availability of additional caregivers (e.g., fathers or alloparents). Additional studies are needed to identify the functional significance of lactational hyporesponsiveness and to characterize its distribution among mammals.

Research Highlights

- In some mammals, lactating females have blunted behavioral responses to stress.

- In some mammals, hormonal responses to stress are blunted in lactating females.

- Cortisol and ACTH stress responses were similar in lactating and non-lactating female marmosets.

- Behavioral responses to stress did not differ between lactating and non-lactating female marmosets.

- This primate species shows no evidence of lactational hyporesponsiveness to stress.

Acknowledgments

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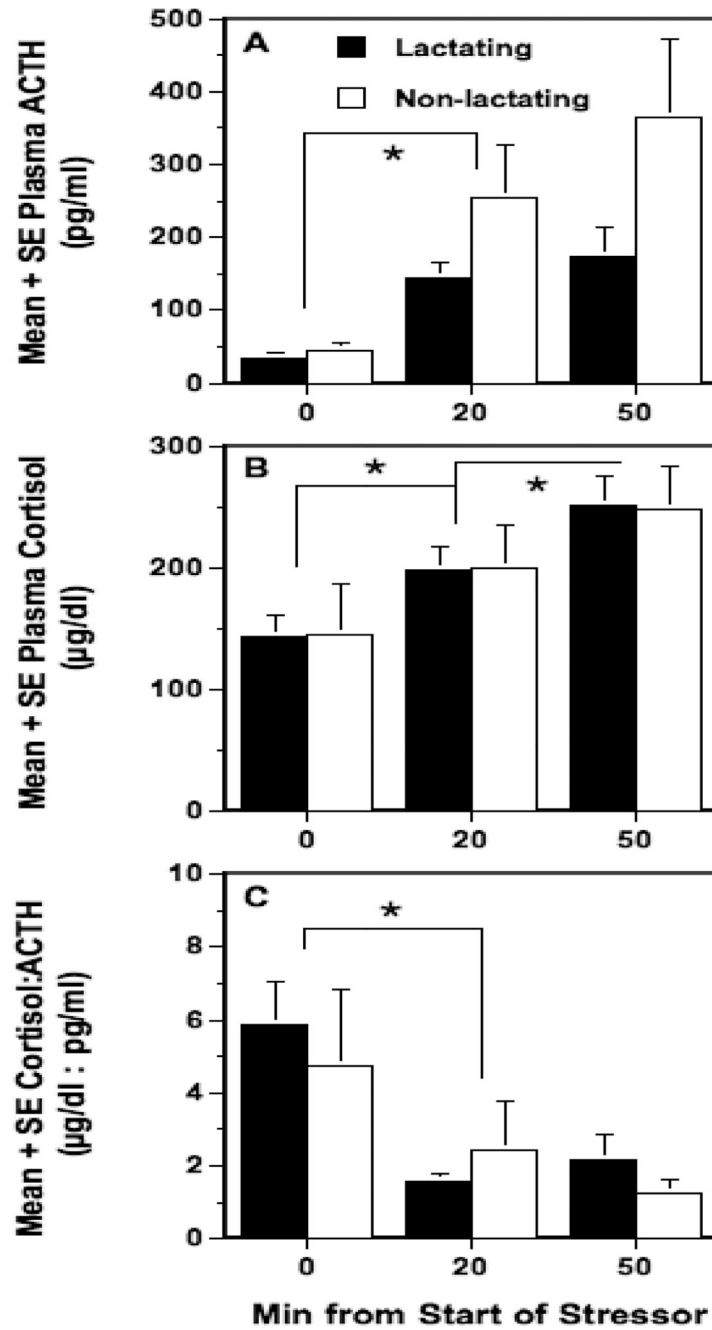**Fig. 1.**
Hormonal responses of lactating and non-lactating female common marmosets to restraint. A: plasma ACTH, B: plasma cortisol, C: plasma cortisol:ACTH ratio. All three measures differed significantly across time, but none differed reliably between lactating and non-lactating animals. * - P≤0.001.
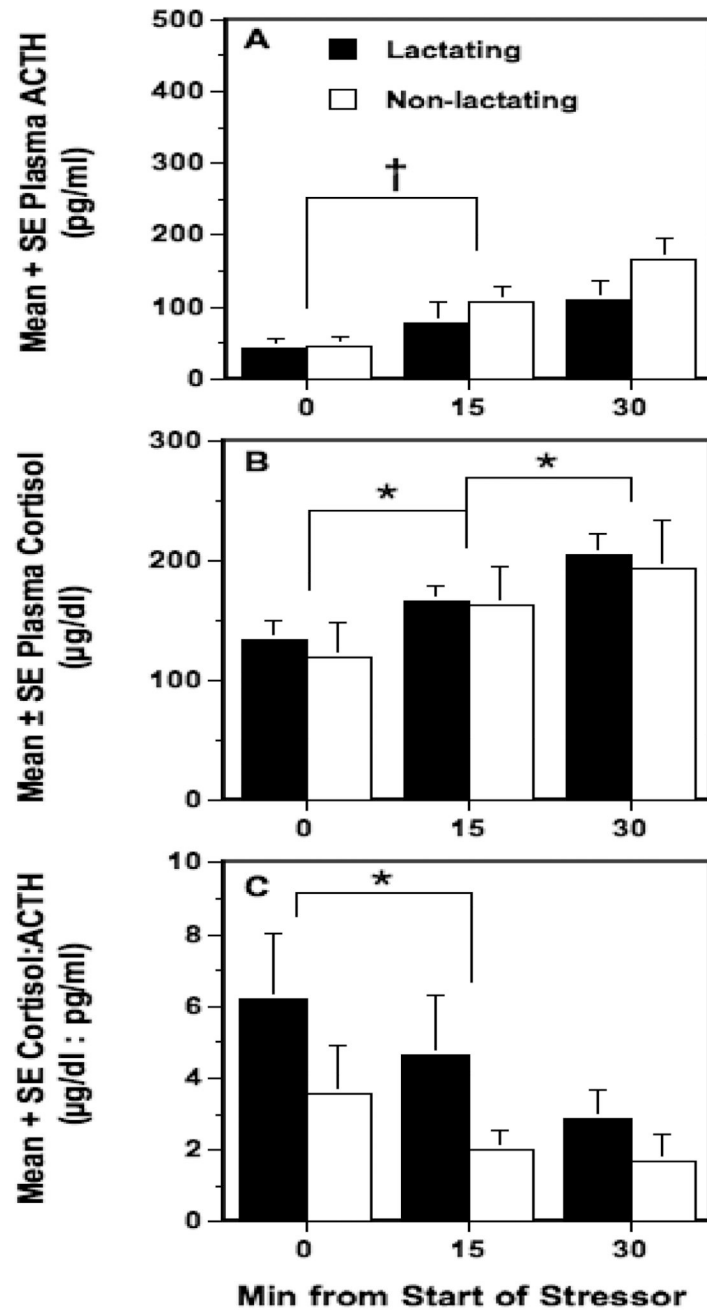
Fig. 2.
Hormonal responses of lactating and non-lactating female common marmosets to simulated predator (hawk) exposure. A: plasma ACTH, B: plasma cortisol, C: plasma cortisol:ACTH ratio. All three measures differed significantly across time, but none differed reliably between lactating and non-lactating animals. * -P≤0.001, †–P<0.05.

Table 1

Behaviors scored in restraint tests and hawk tests. All behaviors were scored when performed by adult females unless otherwise specified.

Behavior	Test	Measure	Definition
Struggle	Restraint	Duration	Strain or pull against restraint and/or twist body in restraint
Chew	Restraint	Duration	Chew or gnaw at restraint, including padding or Velcro straps.
Infant on	Restraint, Hawk	Duration	Infant has all four limbs on mother's body
Bristle strut	Hawk	Duration	Arching posture and/or strut locomotion and/or general piloerection
Scan above cage	Hawk	Frequency	Look to top of cage and/or move head from side to side to monitor top of cage
Flinch	Hawk	Frequency	Flinch during presentation of hawk stimuli
Moan	Restraint	Frequency	Moaning, crying or whining vocalization (usually associated with struggling)
Infant ngä	Restraint, Hawk	Frequency	Relatively low-pitched, atonal, infantile squeal by infant

Table 2

Behavior (median, range) of lactating (N=7) and non-lactating (N=8) female common marmosets during 50-minute restraint.

Behavior	Lactating	Non-Lactating	Mann-Whitney U	P
Struggle[a]	0.067 (0.042, 0.192)	0.078 (0.018, 0.200)	31.000	0.728
Chew[a]	0.030 (0.000, 0.180)	0.011 (0.000, 0.101)	30.500	0.772
Moan[b]	0.000 (0, 29)	2.000 (0, 6)	23.000	0.529
Infant on[a]	0.248 (0.000, 0.973)	---	---	---
Infant nga[b]	17.000 (0, 385)	---	---	---

[a] Proportion of time across all four 5-minute observations

[b] Total number of occurrences across all four 5-minute observations

Table 3

Behaviors (median, range) of lactating (N=7) and non-lactating (N=8) female common marmosets exposed to a simulated predator (hawk model + vocalization).

Behavior	Lactating	Non-Lactating	Mann-Whitney U	P
Bristle strut[a]	0.990 (0.000, 0.997)	0.444 (0.000, 0.995)	37.000	0.296
Scan above cage[b]	17.000 (1, 28)	6.500 (0, 20)	37.000	0.296
Flinch[b]	3.000 (1, 7)	2.500 (0, 8)	30.000	0.815
Infant on[a]	0.991 (0.986, 0.997)	---	---	---

[a]Proportion of time during the first 15 minutes of the 30-minute test

[b]Total number of occurrences during the first 15 minutes of the 30-minute test