Hormonal changes related to paternal and alloparental care in common marmosets (*Callithrix jacchus*)

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Abstract

The physiological mechanisms of parental and alloparental care in cooperatively breeding nonhuman primate species such as the common marmoset (*Callithrix jacchus*) are poorly known. In this study, we examined prolactin and cortisol plasma levels of fathers and older offspring of both sexes, with and without previous experience in infant carrying, around parturition and during infant carrying. Blood samples were collected from fathers and older offspring and prolactin and cortisol were measured by RIA and EIA, respectively. Prolactin levels of both caretakers were not influenced by infant's birth, previous experience or proximity to parturition. However, prolactin levels increased in both caretakers while in physical contact with infants and also with the number of infants being carried in older offspring. These findings suggest that increased prolactin seems to be mainly due to physical effort rather than a physiological trigger of paternal and alloparental care in common marmosets. Cortisol levels were higher for experienced fathers shortly before parturition which could act to reinforce affiliative bonds between breeding males and females at this time or in the ability of males to detect the proximity of the parturition or both.
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Introduction

The regulation of maternal care in mammals is related to hormonal (Rosenblatt, 1990) and somatosensory stimuli such as sight, sound, and odor, which contribute to contact seeking (Stern, 1997). In this sense, the hormonal correlates of maternal behavior, especially in rats (Rosenblatt, 1990; Rosenblatt et al., 1979; Bridges, 1990), are better understood. The close association between the young and their mothers, particularly during pregnancy and nursing, allows them to share a physiological environment that might be essential both to maintenance of the fetus and initiation of mother−infant interaction after parturition. A regulation model of maternal behavior in marmosets and tamarins was developed by Pryce (1993) and includes the hormones that control pregnancy and parturition, genital stimulation during parturition, previous experience with infants at or after it, and the synergistic interaction of these three factors at birth. Among the factors mentioned above, previous experience has an important role in establishing effective mothering (rats: Rosenblatt, 1967; Fleming and Sarker, 1990; sheep: Poindron and Le Neidren, 1980; Lévy and Poindron, 1987; nonhuman primates: Holman and Goy, 1995), where recognition of the infant as a familiar individual is a way of preventing avoidance behavior or even infanticide as reported for male rodents (Huck et al., 1982; Elwood and Kennedy, 1991). Thus, maternal behavior might have two different regulators: a physiological one, which is mainly related to progesterone, estradiol, prolactin, and oxytocin levels throughout pregnancy and lactation, and a non-physiological one related to pup stimulation for the maintenance of maternal behavior post-partum.

Among mammals, paternal care is relatively uncommon and has been observed in carnivores, rodents, and primates

(Kleiman and Malcolm, 1981). The participation of reproductive males seems to be related to the availability of females, access to the female during the estrus period, predator avoidance, and defense of specific resources (Kleiman, 1977). In contrast, the older offspring, as social group members, might also be involved in carrying the infants, especially because this would have an indirect benefit on their inclusive fitness. Members of the Callitrichidae family are described as communal breeders, with all individuals assisting in infant care (Box, 1977; Cleveland and Snowdon, 1984; Stevenson and Rylands, 1988; Yamamoto, 1993), which is related to the costs of carrying behavior (for a review, see Tardif, 1997), particularly for adult females regarding the high litter–maternal weight ratio at birth (Garber, 1994), post-partum estrus (Epple, 1970; Dixson and Lunn, 1987; Ziegler et al., 1990), and lactation. The carrying behavior pattern among callitrichids is variable between genera and among species, with breeding males being the main carrier (Epple, 1975; Hoage, 1982; Rothe, 1978; Sommer, 1984; Rylands, 1986; Goldizen, 1987; Ferrari, 1992) and non-breeding adult animals carrying more than subadult and juvenile siblings (Terborgh and Goldizen, 1985; Yamamoto, 1993; Albuquerque, 1994). In this sense, one critical question must be raised: is there a difference in the benefits and costs regarding infant care between callitrichid fathers and older siblings? For fathers, their participation in infant care might increase their access to the breeding female (Price, 1990). For older offspring, it might improve their infant care skills by gaining experience with siblings (Hoage, 1978; Epple, 1978; Tardif et al., 1984; Cleveland and Snowdon, 1984; Pryce, 1988, 1993; Baker and Woods, 1992; Snowdon, 1996; Sousa et al., 1997), increase the likelihood of inheriting a breeding position by staying in the social group (Goldizen and Terborgh, 1989; Ferrari and Diego, 1992; Baker et al., 1993), especially in saturated areas, since breeding opportunity outside the group may be limited and increase inclusive fitness gains through caring for and improving the survival of relatives (Garber et al., 1984). On the other hand, the costs for fathers and older offspring related to caring behavior seem to be the energetic cost of infant transport during traveling (Kirkwood and Underwood, 1984; Goldizen, 1987; Price, 1992; Sanchez et al., 1999), the increased risk of predation leading to the use of anti-predator strategy by caretakers (Caine, 1993; Tardif, 1994, 1997; Snowdon, 1996), and reduced foraging time during infant transport (Goldizen, 1987; Price, 1992; Digby and Barreto, 1996). The final outcome of infant care is the reproductive success of the father and inclusive fitness of the siblings. Thus, we might expect that both types of caretakers would increase their prolactin levels associated with caring behavior, but considering the reproductive investment by breeding males, it should be higher among fathers.

The study of the hormonal response mediating parental and alloparental behavior in cooperatively breeding nonhuman primates has shown that an elevation of estradiol levels in females is related to maternal care (red-bellied tamarins: Pryce et al., 1988; common marmosets: Pryce, 1993; see also Saltzman and Abbott, 2005), while an inverse correlation was found for black tufted-ear marmosets (Fite and French, 2000). Prolactin was also associated with maternal care (Dixson and George, 1982; Roberts et al., 2001). In males, estradiol and testosterone levels were lower among males who carried infants at high rates (black tufted-ear marmosets: Nunes et al., 2001; Fite et al., 2005). In cotton-top tamarins, estradiol may play a role in promoting paternal care (Ziegler et al., 2004; see also Trainor and Marler, 2002). An association between prolactin levels and paternal and alloparental care has been investigated in birds (Garcia et al., 1996; Schoech et al., 1996), rodents (Gubernick and Nelson, 1989; Brown et al., 1995), New World primates (Dixson and George, 1982; Ziegler and Snowdon, 2000; Ziegler et al., 1996; Mota and Sousa, 2000), and humans (Storey et al., 2000; Fleming et al., 2002), showing an elevation in prolactin levels among animals participating in infant care. Among callitrichids, prolactin levels increases following infant carrying (common marmosets: Dixson and George, 1982; Mota and Sousa, 2000; Roberts et al., 2001). Furthermore, the more births a father has experienced, the higher his prolactin levels (cotton-top tamarins: Ziegler and Snowdon, 2000; Ziegler et al., 2000). The hormonal response of caretakers was not related to age (Ziegler et al., 2000; Schradin and Anzenberger, 2004; Schradin et al., 2003). Moreover, some authors have associated glucocorticoid cortisol with parental response (black tufted-ear marmosets: Nunes et al., 2001; cotton-top tamarins: Ziegler et al., 2004) and prior experience raising young (cotton-top tamarins: Ziegler et al., 1996; black tufted-ear marmosets: Nunes et al., 2001).

Despite the evidence that non-breeding animals participate in infant care, so far, little is known about the endocrine correlate of alloparental care in callitrichids. The main hormone investigated has been prolactin, and limited information is available for steroid modulation of both paternal and alloparental behavior. Therefore, the aims of this study were to investigate (a) whether prolactin and cortisol plasma levels change in common marmoset fathers and older offspring in association with the birth of infants and (b) to determine if previous experience in infant care, carrying episode duration, and the number of infants being carried influenced the hormonal levels of caretakers.

Material and methods

Animals

Seventeen common marmosets (*Callithrix jacchus*), fourteen males and three females, were used. They were housed in the breeding colony of the Physiology Department of Universidade Federal do Rio Grande do Norte, Natal-RN, Brazil (05°46′S 35°12′W). All animals were

categorized as either experienced or non-experienced in carrying behavior, based on the number of sets of offspring they carried previously as parents or siblings. They were studied under the following categories:

Fathers

Ten adult common marmoset males were used. Eight were living with their pregnant mates only. Two were living with both their pregnant partners and a set of offspring, throughout the data collection period, as shown in Table 1. The males ranged in age from 2 to 6 years. Five males were experienced in infant carrying: two of them experienced with 1 set of infants, two others with 2 sets, and the last two with 3 sets of infants. The other five males had no previous experience in carrying as fathers or siblings during this study and were raising their first set of infants as parents.

Older offspring

Seven older offspring (male: $n = 4$; female: $n = 3$) living with their parents (adult male and pregnant female) before parturition were used (Table 1). Their ages ranged from 12 to 21 months. Four older offspring were experienced in carrying one set of infants (male: $n = 3$; female: $n = 1$), and three were experiencing the birth of their first siblings (male: $n = 1$; female: $n = 2$). No group had multiple subordinate caretakers, and no inexperienced subordinates had experienced older siblings in their cages. Since no statistical difference was found in mean baseline prolactin levels between male and female helpers (independent t test: $t = -0.803$, $P > 0.05$) and since a similar pattern of carrying behavior between genders using the same captive population was found by Yamamoto and Box (1997), they were combined in a single group.

The study was developed according to the animal welfare guidelines of Universidade Federal do Rio Grande do Norte, Natal-RN, Brazil.

Husbandry

The animals were kept in wire mesh and brick wall outdoor cages measuring $2.00 \times 1.00 \times 2.00$ m under natural light–dark cycles. The animals were fed twice a day around 9:00 AM with a protein mixture (powdered milk, corn syrup, eggs, bread, soy bean protein, and bone flour supplemented with Vitamin A, D, and E) and around 2:00 PM with a portion of regional tropical fruits. The marmosets also received additional food, which included chicken, special powdered milk for pregnant women, monkey chow pellets, and worms, at least three times a week. Water was provided ad libitum.

Experimental design

Behavioral measure

We assessed prolactin and cortisol plasma levels of fathers and older offspring after 10, 20, 30 min, and longer (from 30 min to 1 h) carrying bouts. To achieve the necessary episodes, we either interrupted the carrying bouts at the desired time or took advantage of spontaneous carrying bouts with the desirable duration, which allowed us to evaluate the effect of physical contact on the hormonal response. A 50-min time lapse was observed prior to data collection and between two successive episodes of carrying to prevent a possible cumulative effect of the preceding carrying episode/physical contact on prolactin levels and because little is known about the metabolic clearance rate and half-life of this hormone in the plasma of nonhuman primates. For humans, it has been demonstrated that the prolactin increase lasts around 50 min (Sassin et al., 1972; Sievertsen et al., 1980).

Blood sample collection was made either interrupting the carrying bout or waiting for the bout to end and removing the focal animal and the newborns from their cage.

Table 1
Information about experimental animals

Experimental groups	Age (years/months)	Previous experience in carrying infants	Observation period
F443*/M434	2y5m/2y4m	Yes	May/Sep97
F497*/M567	2y/2y1m	Yes	Oct97/Jan98
F217*/M438	6y5m/2y9m	Yes	Sep97/Jan98
F477*/M470	2y2m/2y2m	No	Nov96/Feb97
F447*/M436	2y5m/2y5m	No	May/Sep97
F445*/F460	2y2m/2y7m	No	Oct97/Jan98
F313*/M310/MO459*/FO452*[a]	4y/5y/16m/16m	Yes/Yes/Yes	Jun/Oct96
F415*/M366/MO441*/FO434*[a]	2y/5y/12m/12m	No/No/No	Jan/Feb96
F441*/M440/MO567*[b]	2y4m/2y5m/12m	Yes/No	May/Sep97 (F441) and Dec97/Mar98 (MO567)
F337*/M400/MO499*/FO484*[c]	5y8m/2y1m/1y9m/1y9m	No/Yes/Yes	Nov95/Feb96 (F337) and Out97/Jan98 (MO499, FO484)

F: father; M: mother; MO: male older offspring; FO: female older offspring; *: experimental subject.
 [a] Father and older offspring were used as experimental animals at the same time.
 [b] F441 was used as experimental animal between May and September 1997. In the following pregnancy period, MO567 was used as a juvenile helper.
 [c] F337 carried infants (MO499 and FO484) in 1996. In 1998, both older offspring were used as experimental subjects.

Additionally, the carrying behavior of caretakers was recorded using the focal continuous method, three times a week, for 30 min, throughout the first 8 weeks after parturition, on days without blood collection, in the morning (between 8:00 AM and 11:00 AM) and afternoon (1:00 PM and 4:00 PM).

Blood collection

Blood sample collection was carried out twice a week (on Tuesdays and on Thursdays) starting 4 weeks before and 8 weeks after birth for fathers and older offspring (8 weeks/2 months). All blood samples (1 mL) were collected between 10:00 AM and 1:00 PM by femoral venipuncture to minimize the circadian variation in prolactin plasma concentrations. A 1-mL heparinized syringe (Liquemine, Roche Pharmacy Laboratory) was attached to a 27.5G needle. Before blood collection, the subjects were habituated to the capture and handling procedure in order to diminish their stress responsiveness to the restraint situation. The animals were held using gloved hand and were removed from the cage twice a week. They were placed in a restraint device for small primates (Hearn, 1983), and blood samples were obtained. Using this procedure, it was possible to have blood samples within 80–150 s after handling the subjects. Following each procedure, the animals received an iron supplement and additional vitamins and were rewarded with sweet milk. After this, samples were centrifuged for 10 min at 3000 rpm and frozen at -20 °C for subsequent determination of prolactin and cortisol concentrations by radioimmunoassay (RIA) and enzyme immunoassay (EIA) methods, respectively.

Hormonal assays

Prolactin

The assays were carried out using a human prolactin [^{125}I] double monoclonal antibody (Prolactin Testkit IRMA-coated tubes, Euro-Diagnostica B.V., The Netherlands). The first antibody is radio-iodinated, and the other is bound to the tube wall. Eight prolactin standards were used 65, 125, 240, 540, 900, 1800, and 4500 mIU/L, calibrated according to the 1st International Reference Preparation (75/504), 1 ng = 20 mIU/L. Purified human prolactin was used as control in doses ranging from 0 to 225.0 ng/tube. A 50-μL sample of standard and control was mixed with 200 μL of anti-PRL IgG [^{125}I] tracer in all tubes. The tubes were vortexed and incubated for 2 h at 37 °C in a waterbath. After this procedure, 2 ml of wash solution (PBS/Tween wash solution) was added (except to Total count tubes) and the supernatant decanted. The last procedure was repeated twice. The bound fractions were counted in duplicates. The antigen–antibody reaction was estimated with 50, 88, and 158 mIU/L prolactin concentrations. The recovery of varying prolactin concentrations was between 93 and 107%. The coefficient of variation within-run ranged from 2.0 to 3.2% and between-run ranged from 2.7 to 5.6%. The

sensitivity as judged by 2 SD changes from zero standard was 5 mIU/L (0.25 ng/mL). The prolactin assay was validated for marmoset plasma by determining parallelism and accuracy. Serial dilutions of pooled marmosets samples ($n = 7$) paralleled the standard curve ($n = 7$) with no difference in slope (low pool: $t = 0.140$, $P = 0.8911$; high pool: $t = 0.049$, $P = 0.9611$). Accuracy was $101 \pm 14.9\%$.

Prolactin analysis was run at the Medical School Radioimmunoassay Laboratory of Universidade Federal do Rio Grande do Norte, Natal-RN and at the Physiology Department of the Ribeirão Preto Medical School, Universidade de São Paulo-SP, Brazil for convenience. The procedures are described as follows:

Three assays used a 50 μL volume of plasma. In this case, the sensitivity was 0.1164 to 0.3538 ng/mL. Intra-assay coefficients of variation of low and high pools were 5.21 and 5.29%, respectively; inter-assay coefficients of variation were 5.42 and 6.47% for low and high pools, respectively. Bound fractions were counted using a Gambty Cr DPC counter. Additionally, three assays were performed using dilution of 1:2.5 (once 20 μL of marmoset plasma was available) plus 30 μL of a phosphate 0.001 M buffer (16.3 g of NaCl, 0.2 g of NaN$_3$, 6.5 mL of NaH$_2$PO$_4$, and 43.3 mL Na$_2$HPO$_4$ added with 2 L of distilled water and pH regulated to 7.6) and 0.1% gelatin in the coated tube containing the anti-prolactin IgG [I^{125}] monoclonal tracer. Assay sensitivity was 0.0984 to 0.1257 ng/mL. Intra-assay coefficients of variation for a low and high pool were 9.12 and 11.56%, respectively; and inter-assay coefficients of variation were 20.02 and 13.39% for low and high pools, respectively. The reliability between the two laboratories was determined to be $5.32 \pm 4.49\%$ by using the same pools.

Cortisol

The analysis was run at the Hormonal Measures Laboratory at the Physiology Department of Universidade Federal do Rio Grande do Norte, Natal-RN, Brazil. Cortisol plasma levels were determined using an enzyme immunoassay (EIA). The technique was performed following the protocol developed by Munro and Stabenfeldt (1984) and modified by the Wisconsin Regional Primate Research CTR. Intra- and inter-assay coefficients of variation were 4.5 and 16.9% for low pool and 3.1 and 13.8% for high pool, respectively. Serial dilutions of marmoset-pooled plasma were used to demonstrate parallelism (no difference between slopes, $P > 0.05$) and accuracy ($92.1 \pm 5.8\%$).

Statistical analyses

The Statistics for Windows Statistical Package was used (version 5.5). Before statistical analysis was carried out, both prolactin and cortisol levels were evaluated with respect to their normal distribution, and a log transformation was performed. To evaluate the effect of birth, ANOVA one-way post-hoc Tukey test was used to compare the hormonal levels of fathers and older offspring separately, under three

conditions: before birth, not carrying, and carrying the infants. Additionally, repeated-measures ANOVA (post-hoc Tukey test) was used to analyze prolactin and cortisol levels 1 month before and 2 months after delivery—data were averaged respectively into 2 and 4 fortnights. The same test was used to compare the carrying episodes duration of fathers and older offspring throughout the week following parturition. To evaluate the influence of the type of caretakers (fathers or older offspring) and previous experience in carrying infants (yes or no) on hormones levels, an independent t test was used. The effect of the number of infants being carried was evaluated by a dependent t test. P values < 0.05 were considered significant.

Results

Prolactin and cortisol response to birth

Prolactin levels of fathers and older offspring were compared separately to verify the effect of parturition. Prolactin levels were significantly higher for both fathers (ANOVA, $F_{2,18} = 6.99$, $P = 0.00$) and older offspring (ANOVA, $F_{2,26} = 4.56$, $P = 0.02$) when carrying infants as compared to not carrying ($P < 0.05$) and to levels before the birth of infants ($P < 0.05$), as shown in Fig. 1A. Cortisol levels did not change for fathers (fathers: ANOVA, $F_{2,26} = 0.20$, $P =$
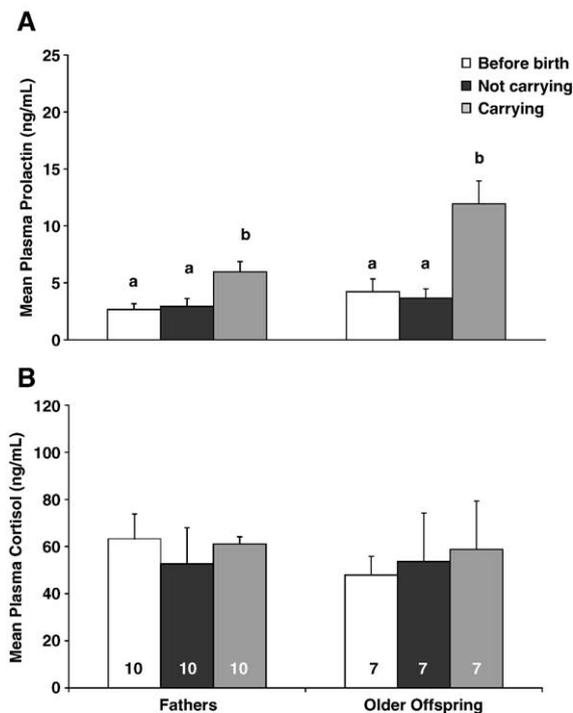

Fig. 2. Prolactin (A) and cortisol (B) plasma levels (mean ± SE) of non-experienced and experienced fathers and older offspring. Independent t test, $P \leq 0.05$. Non-experienced (white bars) and experienced (black bars) caretakers for prolactin (A) and cortisol (B). t test, $P < 0.05$. Letter a is significantly different from b for cortisol levels. Sample size is within the bars.

0.82) or older offspring ($F_{2,18} = 0.19$, $P = 0.85$) among the three conditions (Fig. 1B). Thus, carrying behavior, but not the birth of infants, influenced prolactin levels.

The prolactin levels of the fathers did not show a significant variation (repeated-measure ANOVA: $F_{5,40} = 1.56$, $P = 0.21$) throughout 2 fortnights before and 4 fortnights after birth. However, they increased before parturition (fortnights 1 and 2). Older offspring showed higher prolactin levels in the first two fortnights after birth compared to the ones before (repeated-measure ANOVA: $F_{5,28} = 5.71$, $P = 0.00$). Cortisol levels did not vary between fortnights for both caretakers (repeated-measure ANOVA, fathers: $F_{5,40} = 0.98$, $P = 0.44$; older offspring: $F_{5,28} = 0.90$, $P = 0.48$).

Effects of previous experience in infant carrying and carrying episode duration

No difference in prolactin levels was found between fathers and older offspring (independent t test: $t = -0.69$, $P = 0.50$; mean ± SE: father: 2.61 ± 0.64 ng/mL; older offspring: 2.26 ± 0.31 ng/mL) in the last month before parturition. A comparison within each type of caretaker regarding their previous experience in carrying infants revealed that prolactin levels were not influenced by prior contact with newborns (fathers: independent t test: $t = -0.23$, $P = 0.82$; older offspring: independent t test: $t = -0.53$, $P = 0.61$), as shown in Fig. 2A.

Fig. 1. Plasma prolactin (A) and cortisol (B) levels (mean ± SE) for caretakers before (white bars) and after infant's birth (not carrying: black bars and carrying the infants: gray bars). One-way ANOVA, $P < 0.05$, post-hoc Tukey test. Letter a is significantly different from b for prolactin levels in fathers and older offspring. Sample size is within the bars.
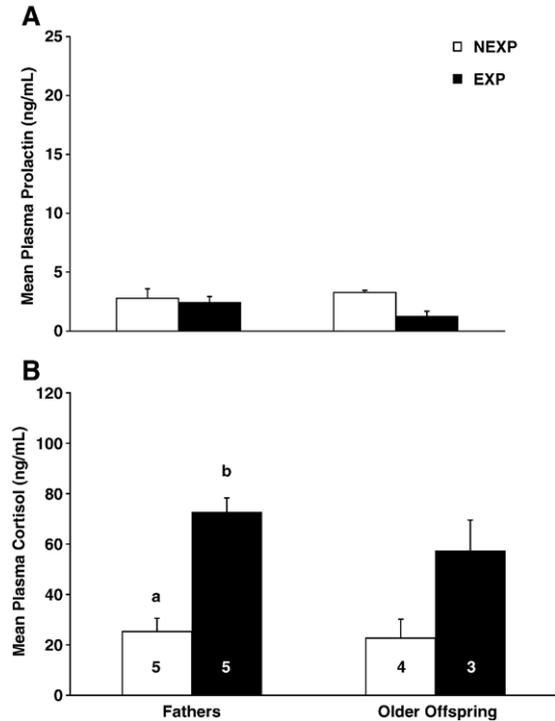
No difference in cortisol levels was found between fathers and older offspring (independent t test: $t = 1.28$, $P = 0.22$; mean ± SE: father: $49.95 ± 5.5$ ng/mL; older offspring: $40.0 ± 9.85$ ng/mL). When previous experience in carrying infants was taken into account, experienced fathers had higher mean cortisol levels compared to non-experienced ones (independent t test: $t = 2.25$, $P = 0.05$). However, no difference was found between non-experienced and experienced older offspring (independent t test: $t = 1.47$, $P = 0.20$; Fig. 2B).

Prolactin levels of caretakers differed between before birth (BB) and after birth conditions (ANOVA, $F_{5,81} = 7.26$, $P = 0.00$, post-hoc Tukey test). For older offspring ($F_{5,33} = 3.29$, $P = 0.02$), prolactin levels increased significantly following carrying bouts (C10, C20, C30, and C > 30) compared to BB ($P < 0.05$) and not carrying infant conditions (C0: $P < 0.05$). For fathers, prolactin increase was significantly different (ANOVA, $F_{5,42} = 4.77$, $P ≤ 0.00$) only between BB and C20 ($P < 0.05$), C30 ($P < 0.05$), and C > 30 ($P < 0.05$), and between C0 and C + 30 ($P < 0.05$), as shown in Fig. 3A.

No significant difference was found for plasma cortisol for fathers (ANOVA, $F_{5,42} = 0.65$, $P = 0.72$) and older offspring (ANOVA, $F_{5,33} = 0.50$, $P = 0.77$) among BB, C0, and carrying episodes (Fig. 3B).

Effect of the number of infants being carried

No difference in prolactin levels was observed when both caretakers were carrying one or two infants (dependent t
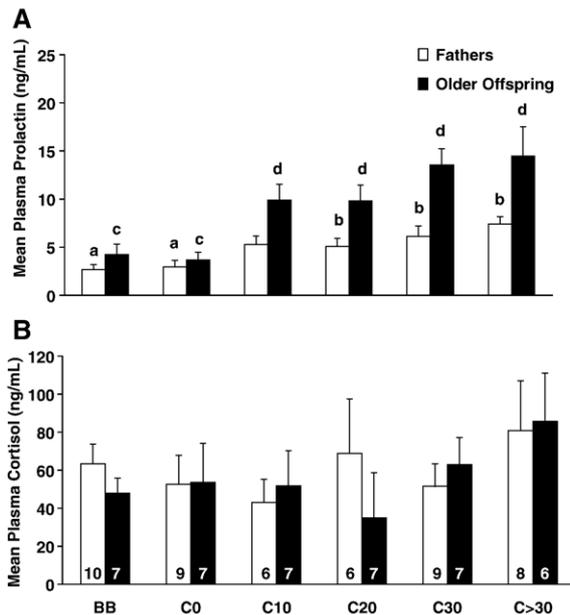

Fig. 3. Plasma prolactin (A) and cortisol (B) levels (mean ± SE) of fathers (white bars) and older offspring (black bars) before birth (BB) and after birth when not carrying (C0) and carrying infants (C10, C20, C30, and C > 30 min). Repeated-measures ANOVA, $P < 0.05$. Letter a is significantly different from b for fathers, and letter c is significantly different from d for older offspring. Sample size is within the bars.

test: -2.09, $P = 0.06$). However, the evaluation of prolactin levels of each type of caretaker individually showed an increase in older offspring prolactin levels when carrying two infants compared to when carrying one (dependent t test: fathers: $t = 0.14$, $P = 0.89$; older offspring: $t = -4.38$, $P = 0.00$). No interaction was found between the number of infants being carried and the duration of carrying episodes in prolactin levels for both types of caretakers (ANOVA, fathers: $F_{3,27} = 0.54$, $P = 0.66$; older offspring: $F_{3,23} = 1.05$ $P = 0.39$). Cortisol levels were not influenced by the number of infants being carried by either caretaker (dependent t test: fathers: $t = 0.69$, $P = 0.52$, older offspring: $t = -0.32$, $P = 0.75$). No interaction was found between the number of infants being carried and the duration of carrying episodes for fathers (ANOVA $F_{3,27} = 0.62$, $P = 0.60$) and older offspring (ANOVA, $F_{3,23} = 0.23$ $P = 0.87$).

The percentage of time spent carrying infants by fathers and older offspring was significantly different over the post-partum weeks (repeated-measure ANOVA: $F = 16.36$, $P ≤ 0.05$). Fathers carried newborns twice as much as older offspring in their first 4-week period of life. Afterwards, the mean time spent carrying infants diminished at week 8 for both caretakers.

Discussion

In the study presented, we found that the prolactin levels of common marmosets significantly changed in response to participation in infant care and the number of infants being carried but not by the birth of infants and previous experience in carrying infants. This supports the assumption that prolactin may be modulated by physical contact with infants and, probably, by the physical effort following carrying behavior. Moreover, the social changes in group dynamics related to the birth of infants seem not to be a stressful event since the cortisol levels of caretakers did not change between experimental conditions. The prolactin pattern of older offspring was more responsive to transitions between before (BB) and not carrying (C0) and the participation in infant care conditions (carrying for 10, 20, 30, and more than 30 min). Prolactin levels of fathers were also related to carrying duration. Additionally, experienced fathers had higher cortisol levels.

The change in prolactin levels of fathers and older offspring when carrying infants suggests that physical contact influenced caretaker hormonal response in this species. A preliminary report from our laboratory suggested both the number of infants being carried and the duration of carrying as increasing factors of prolactin (Mota and Sousa, 2000). The lack of a hormonal preparation among fathers and older offspring, as suggested for female rodents (Rosenblum, 1972), New World primates (Pryce et al., 1995), and humans (Fleming et al., 1997) over the course of gestation and lactation, points to different mechanisms for initiating and maintaining responsiveness to infants among

reproductive males and extra helpers. For rodents, stimuli from females, previous social experience, stimuli from neonates, and hormonal changes in response to females influence paternal behavior (Brown, 1993). Among callitrichids, paternal responsiveness is related to affiliative relationship strength within breeding pairs before parturition (Evans and Poole, 1984; Price, 1992; Silva and Sousa, 1997) because cooperation is needed for infant care. Male callitrichids may receive olfactory cues from breeding females during pregnancy or parturition (Cebuella pygmaea: Carlson et al., 1996; C. jacchus: Sousa et al., 1997), which could ensure male participation in carrying. Regarding prolactin levels, although the social environment of caretakers seems not to be the main factor modulating prolactin levels before and after parturition, the hormonal levels of fathers showed an increase in the periparturitional period. A study using C. jacchus (Schradin, 2001, apud Schradin et al., 2003) showed an association between increased prolactin in fathers before the breeding female gave birth. Cotton-top tamarin (Saguinus oedipus) fathers showed an elevation in urinary prolactin levels before birth (Ziegler et al., 1996, 2004), which could be related to olfactory signaling within breeding pairs.

The changes in prolactin levels of fathers and older offspring when carrying infants, found in this study, support previous studies using common marmoset, which indicated that physical contact with newborns (Dixson and George, 1982; Mota and Sousa, 2000; Roberts et al., 2001) influences their hormonal response. Prolactin levels of older offspring also increased when two infants were being carried. The present results may be related to the physical effort following carrying behavior. Callitrichid carrying behavior demands high metabolic cost, proportional to high infant birth weight (Garber, 1994) with an increase of 21% per minute when carrying two 30-day-old infants during traveling (Tardif, 1997). In this case, young animals carry less (Yamamoto and Box, 1997) because they cannot bear the weight of infants for longer time periods. In cotton-top tamarins, fathers and brothers weighed less after parturition while carrying the infants (Sanchez et al., 1999). However, a study using common marmosets did not find a relationship between extensive participation in infant care and changes in energy intake or weight loss after parturition in fathers (Nievergelt and Martin, 1999). It is worth emphasizing in this study that, even though they carried the infants less, mean prolactin levels of juvenile and subadult helpers were higher than those of fathers. It is possible that both caretakers responded to tactile stimulation by infants rather than to a stressful condition, a fact that is reinforced by the absence of cortisol increases during long carrying episodes. These findings also suggest that prolactin and cortisol are probably not working as physiological modulators (inductors) of parental care in common marmosets. A study using inexperienced common marmosets measuring infant retrieval and carrying behavior showed an increase in prolactin levels following repeated testing in an infant retrieval paradigm and according to carrying episode duration (Roberts et al., 2001). However, urinary prolactin levels were not associated with the amount of paternal carrying in common marmoset fathers (Schradin and Anzenberger, 2004; Schradin et al., 2003). In this case, a direct correlation between physical contact and prolactin levels may not be possible since the daily first-morning urine sample measures accumulated prolactin rather than the immediate changes that occur in the blood while caretakers carry the newborns, as shown by Dixson and George (1982) and Roberts et al. (2001) and the present study. Furthermore, the metabolic clearance rate and half-life of plasma prolactin in nonhuman primates are poorly understood, but, in humans, its increase lasts around 50 min (Sassin et al., 1972; Sievertsen et al., 1980), supporting the association between the plasma prolactin levels and physical contact during carrying found here.

The prolactin levels of caretakers (fathers and helpers) were not influenced by previous experience in infant carrying, corroborating the findings of Schradin and Anzenberger (2004) in common marmosets. In contrast, an increase in prolactin levels for fathers, as the number of sets of infants being previously carried increased, was found for cotton-top tamarins fathers (Ziegler et al., 1996, 2000). The difference among callitrichid species in the timing of infant care after birth is consistent with a possible role of prolactin in carrying behavior. Infants of Cebuella and Callithrix genus are carried until the 8th week of life (C. jacchus: Ingram, 1977; Arruda et al., 1986; C. pygmaea: Wamboldt et al., 1988), while Saguinus and Leontopithecus infants are still intensively carried beyond this time (Leontopithecus rosalia: Hoage, 1982; S. oedipus: Tardif et al., 1986). Moreover, previous experience seems to be essential for future reproductive success mainly in tamarins (S. oedipus: Tardif et al., 1984; Saguinus fuscicollis: Epple, 1978; Goldizen, 1988; L. rosalia: Baker and Woods, 1992). Hence, it would be expected that common marmosets also show this effect. However, it should be noted that cortisol levels seem to be influenced by previous experience in infant care for fathers. In Callithrix kuhli (Nunes et al., 2001), urinary cortisol was lower among fathers who carried infants at higher rates and was negatively correlated with infant-carrying behavior but decreased after the birth of the second litter. It seems that neither infant care nor contact time evoked a stress response, suggesting that contact with infants minimized their physiological stress response, which is also influenced by previous reproductive experience in this species. In nonhuman mammals, glucocorticoids were related to the establishment of social bonds in mated pairs (Carter and Altemus, 1997). Moreover, an increase in cortisol levels was found in human fathers before parturition, which may help fathers to focus on and bond with their newborns (Storey et al., 2000). In cotton-top tamarins, experienced fathers showed an elevation in cortisol levels before parturition (Ziegler et al., 1996, 2004), which might stimulate an increased interest in female reproductive status.

Recently, Ziegler et al. (2004) found no change in cortisol levels among male cotton-top tamarins carrying infants in response to ovulatory scent from novel females, suggesting an association between their diminished hormonal response and their monogamous social system. In the current study, no dependent infant was present before parturition, and the elevation in cortisol levels before birth could be part of the sexual strategy of breeding males to pair bond strength.

In summary, this study showed that prolactin is related to the duration of carrying behavior in fathers and older offspring in common marmosets. Regarding older offspring, prolactin increase is also associated with the number of infants being carried, suggesting that infant carrying demands higher energetic cost for callitrichid non-adult helpers than for fathers. The absence of cortisol changes during carrying episodes in both caretakers raises the possibility that prolactin increases are not related to stressful situations and that the dissociation between both hormonal levels needs further investigation. Cortisol in experienced fathers tends to increase before parturition, pointing to a possible role in pair bond, detection of parturition, or both. Thus, cortisol and prolactin seem not to trigger parental and alloparental care in this species but are probably released by social interactions with pregnant females and newborns.

Acknowledgments

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