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Seasonal variation in the degu (Octodon degus) endocrine stress response



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ABSTRACT

Many wild animals show seasonal variation in circulating levels of stress hormones. Seasonal changes in the stress response may help animals better cope with the different challenges faced during each life history stage. We determined the seasonal stress profile of wild, free-living degus in Chile. Female degus were sampled during non-breeding (January), mating/early gestation (July), late gestation (August), and lactation (1st litter-September, 2nd litter-January). Male degus were sampled during the first three time-points. We measured baseline cortisol (CORT), stress-induced CORT, and negative feedback efficacy using a dexamethasone suppression test. While we found that neither males nor females showed seasonal variation in baseline CORT or negative feedback levels, we did find significant seasonal variation in stress-induced CORT levels of both sexes. Male stress-induced CORT was lowest during mating while female stress-induced CORT was highest during late gestation and lactation. Overall, females had higher stress-induced CORT compared to males. Our data suggest that stress-induced levels of CORT are highest during periods with increased chance of stressor exposure or times of positive energy balance. Consequently, CORT responses to stress appear to be regulated according to different life history needs.

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1. Introduction

Baseline and stress-induced glucocorticoid levels have been shown to seasonally vary in many wild, free-living vertebrates (Romero, 2002). This seasonal variation has been relatively well studied in birds, reptiles, and amphibians. In these three taxonomic groups, most species show significant seasonal variation in baseline and stress-induced glucocorticoid levels, with peak glucocorticoid levels usually occurring during breeding (Romero, 2002). Seasonal variation of cortisol or corticosterone (CORT) has not been as well studied in free-living mammals, and only a handful of these studies have attempted to determine whether baseline CORT levels are seasonally modulated. Additionally, there is a lack of consensus on which life history stages have been examined; some studies assessed seasonal differences in baseline CORT between the breeding and non-breeding seasons (e.g., snowshoe hares; Boonstra and Singleton, 1993: talas tuco-tuco: Vera et al. 2012) while other studies measured baseline CORT at specific time-points during the breeding season such as mating, gestation, and lactation (e.g., yellowpine chipmunks, Place and Kenagy, 2000; Kenagy and Place, 2000: female ring-tailed lemurs, Carvigelli, 1999). This study addresses this gap in our knowledge by determining the seasonal CORT profiles of male and female degus (*Octodon degus*) at several different life history stages.

Degus are diurnal, semi-fossorial rodents native to central Chile. Unlike most mammals for which seasonal CORT profiles have been determined, degus are highly social, precocial mammals. Kenagy et al. (1999) determined that cortisol (CORT) is the main or predominant glucocorticosteroid in degus, and that degus seasonally modulate stress-induced CORT levels. We elaborated on Kenagy et al.'s study by measuring baseline CORT levels and negative feedback efficacy at several times of year, in addition to measuring stress-induced CORT concentrations. Because degus are highly social, precocial mammals, an additional purpose of our study was to better understand why degus seasonally modulate CORT levels.

In response to a stressor, the endocrine portion of the stress response increases secretion of glucocorticoids into the bloodstream. CORT affects many different physiological processes such as metabolism, immune function, reproduction, and the cardiovascular stress response (Sapolsky et al., 2000). At baseline levels, CORT has mostly permissive effects, such as priming the cardiovascular system to respond more fully to catecholamine action (Sapolsky et al., 2000). At stress-induced levels, CORT can have suppressive, stimulatory, or preparative effects, depending upon the nature of the stressor and the context in which it occurs.

Two hypotheses have been proposed to explain seasonal patterns in glucocorticoid secretion: the Energy Mobilization Hypothesis and the Preparative Hypothesis (Romero, 2002). The

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Energy Mobilization Hypothesis predicts that CORT levels will be highest during energetically expensive life history stages (Romero, 2002). Because baseline CORT levels are important for regulating normal metabolic demands (Sapolsky et al., 2000), it is predicted that these levels will be elevated during energetically expensive times of year. The Preparative Hypothesis predicts that CORT levels will be highest during times of year when there is a greater likelihood of encountering stressors (Romero, 2002). Both baseline and stress-induced CORT levels are predicted to increase during these times of year since both levels of CORT help mediate the stress response. It should be noted that animals are not preparing for a season; rather, they are preparing for potential stressors within that season. Determining the times of year when there is a greater likelihood of encountering stressors, however, is not readily apparent for most species. Romero (2002) posits that risk of predation and mate competition are factors that may cause seasonal variation in the frequency of adverse events. It should be noted that the Energy Mobilization and Preparative Hypotheses are not mutually exclusive.

Under the Energy Mobilization Hypothesis, we predicted that baseline CORT levels would be highest during the most energetically expensive times of year. For most female mammals, lactation is considered the most energetically expensive life history stage (Speakman, 2008). This is true for degus, as the resting metabolic rate of captive females is higher during lactation than during breeding or pregnancy (Veloso and Bozinovic, 2000). For male mammals, the most energetically expensive life history stage is generally considered to be mating due to increased frequency of aggressive interactions and territorial disputes (Boswell et al., 1994). While there is no direct, metabolic evidence supporting this in degus, male testosterone levels are highest during the breeding season (Kenagy et al., 1999; Soto-Gamboa et al., 2005) and male degus allocate significantly more time to aggressive interactions during the breeding season compared to the non-breeding season (Ebensperger and Hurtado, 2005b: Soto-Gamboa et al., 2005).

Under the Preparative Hypothesis, we predicted that baseline and stress-induced CORT levels would be highest during times of year with increased predation risk and mate competition. For degus, risk of predation is most likely higher in the spring due to several factors. Firstly, degu density is lower and degus form smaller groups aboveground during the spring compared to other times of year, so individuals are less likely to benefit from enhanced predator detection via con-specific alarm calls (Ebensperger and Wallem, 2002; Ebensperger and Hurtado, 2005b). Secondly, there is more herbaceous vegetation during the spring compared to other times of year, and lateral cover may impair detection of predators (Ebensperger and Hurtado, 2005a). Thirdly, the landscape is greener during the spring compared to other seasons, and during this time the degus brown fur does not act as good camouflage. And finally, Meserve et al. (1993) has shown that predation has greater effects when degu populations are low. While predator density has been shown to be lower in the spring compared to other times of year (Ebensperger and Hurtado, 2005b), predators most likely increase their hunting effort during the spring in order to feed their own offspring. This is supported by data showing that radiocollared degus at our field site are more likely to be depredated in the spring compared to the autumn (C. León, personal communication). Since female body mass increases during pregnancy and daily energy expenditure increases during lactation (Bozinovic et al., 2004; Veloso and Bozinovic, 2000), females may spend more time foraging during the spring and thus experience increased predation risk. Therefore, under the Preparative Hypothesis, we predicted that for female degus, the highest levels of baseline and stress-induced CORT would occur during spring and that these levels may be higher than in males.

For male degus, we also predicted that they would have high levels of baseline and stress-induced CORT during the spring due to increased predator risk. However, because the mating season may also be a time of increased agonistic interactions, we predicted that male degus would also have elevated levels of baseline and stress-induced CORT during this time of year. Male degus increase testosterone levels during the mating season and more frequently engage in aggressive interactions (Ebensperger and Hurtado, 2005b; Kenagy et al., 1999; Soto-Gamboa et al., 2005). Soto-Gamboa et al. (2005) found that male degus with high testosterone levels also had high stress-induced CORT levels, perhaps related to increased agonistic encounters. This supports the hypothesis that, for males, the mating season is a time of year with an increased frequency of stressors. Therefore, under the Preparative Hypothesis, we predicted that males would have elevated baseline and stress-induced CORT levels during spring and the mating season.

While the Energy Mobilization Hypothesis only makes predictions concerning baseline CORT, the Preparative Hypothesis makes predictions for both baseline and stress-induced CORT. If the Energy Mobilization Hypothesis best explains seasonal variation in CORT levels, then we expected to see increased baseline CORT levels during lactation for females and during mating for males. If the Preparative Hypothesis best explains seasonal variation in CORT levels, then we expected to see increased baseline and stress-induced CORT during spring for both sexes, and during mating for males.

2. Methods

2.1. Study animals

We studied the common degu (Octodon degus), a caviomorph, semi-fossorial rodent native to central Chile. Study animals were captured and sampled near Santiago. Chile at Estación Experimental Rinconada de Maipú (33°23'S, 70°31'W, altitude 495 m), a field station owned and managed by the Universidad de Chile. Rinconada de Maipú, hereafter referred to as Rinconada, is characterized by an open, matorral habitat. Degus typically mate in the late austral autumn (June/July) (Yáñez and Jaksic, 1978) and then undergo a 90-day gestation period (Morales, 1982). Parturition coincides with the early austral spring (September), when food availability is at its peak (Fulk, 1976; Yáñez and Jaksic, 1978). After parturition, females lactate for approximately 40 days (Becker et al., 2007; Veloso and Kenagy, 2005). At about four weeks of age, pups emerge from burrows and forage on their own (Bauer, unpublished data). Female degus will often get pregnant immediately after parturition, but second litters of pups (December) have only been found during years of exceptionally good food availability (Ebensperger et al., 2013; Kenagy et al., 1999; Meserve et al., 1995), which occurred during the last year of our study. We trapped female degus during five life history stages: non-breeding (December 31st, 2012-January 13th, 2013), early gestation (July 5th-7th, 2011), late gestation (August 24th-September 11th, 2011), first litter lactation (September 21st-30th, 2012), and second litter lactation (January 2nd-13th, 2013). Male degus were trapped during the first three life history stages listed above. It should be noted that non-breeding degus and female degus nursing their second litter were trapped during the same time; non-breeding degus were approximately 4 months old while degus nursing their second litter were over a year old. Because we caught different degus at each time-point, we were unable to confirm that January females were nursing their second litter. However, we felt that we could safely assume that females had two litters because every female degu we captured that spring was pregnant or

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lactating. Additionally, during 2005–2008, Ebensperger et al. (2011) found that 99% of female degus at Rinconada bred during the spring.

2.2. Trapping and blood sampling

Tomahawk live traps (Tomahawk Live Trap Company, Hazelhurst, WI, USA) were used to live trap degus. Traps were baited with plain, rolled oats and were placed near burrows where degu activity had been previously observed. Because degus show seasonal variation in activity patterns, traps were opened during the most active part of the day (Ebensperger and Hurtado, 2005a,b; Kenagy et al., 2002). This meant that degus were trapped within the first few hours after sunrise in the austral spring and summer, and in the middle of the day during the austral winter. We have previously shown that baseline CORT does not correlate with time of day (Bauer et al., 2013). In order to determine the exact time of capture, 1-5 observers with binoculars continuously monitored traps. Degus were then bled for a baseline blood sample ($\sim 60 \mu$ L) within three minutes of capture since CORT has been shown to increase after this amount of time (Romero and Reed, 2005). Degus were bled from the saphenous vein and blood was collected in heparinized microhematocrit capillary tubes. Any degus trapped unnoticed were removed from baseline CORT analysis.

After collecting baseline blood samples, degus were returned to their traps and carried to a shady area. Each degu was then sexed, ear-tagged for identification purposes, and weighed to the nearest 0.1 g. Females were noted as lactating if milk could be expressed from the teats. Stress-induced blood samples (\sim 30 µL) were then taken 30 min after capture (Bauer et al., 2013). After collecting stress-induced blood samples, degus were given an intra-peritoneal injection of dexamethasone (DEX) at a dose of 1 mg/kg of body weight. DEX is a synthetic glucocorticoid and thus inhibits endogenous CORT release if negative feedback is functioning normally (Carroll et al., 1981; Sapolsky and Altmann, 1991); DEX suppression tests are a common way to measure negative feedback (Boonstra and Singleton, 1993; Romero and Wikelski, 2010). A final blood sample (\sim 60 µL) was taken 90 min after DEX injection (Bauer et al., 2013).

Degus were then returned to the burrow system at which they were captured. Removal of lactating females for 2 h was most likely not detrimental for the offspring because degus are precocial mammals (Long and Ebensperger, 2010), thus pups can thermoregulate on their own. Additionally, because degus are plural breeders that practice communal care (Ebensperger et al., 2004), other group members may care for pups during the mother's absence.

Blood samples were stored in a cooler with cold packs and were centrifuged within nine hours of collection at approximately 230 G for 2–3 min. Plasma was drawn off and stored at -20 °C until further analysis. Samples were measured for CORT with a commercial I¹²⁵ radioimmunoassay kits (Corti-Cote Solid Phase Component System, MP Biomedicals LLC, Irvine, CA, USA). This assay kit was validated for measuring cortisol in degu samples by confirming that serial plasma dilutions were parallel to standard curves (data not shown). Distilled water was added to bring total sample volume up to 50 µL and samples were assayed in duplicate. Assay sensitivity was 0.7 ng/mL and intra- and inter-assay variation were 3.3% and 9.5%, respectively.

2.3. Statistical analyses

Statistical analyses were performed in SPSS (Version 20.0). Negative feedback was calculated as the percent decrease in CORT from stress-induced levels to levels 90 min after DEX injection: (stress-induced CORT-post-DEX CORT)/(stress-induced CORT) * 100 (Lattin et al., 2012). Because some animals escaped

during processing or were trapped unnoticed, we do not have all three samples for each individual. Therefore, sample sizes are different for each variable. Because baseline CORT, stress-induced CORT, and negative feedback are regulated independently, we analyzed each CORT variable separately (Hodgson et al., 2007; Landys et al., 2006; Reul et al., 1987; Romero, 2006). Because we sampled different degus for each sampling period, seasonal time-points were considered independent of one another. We used two-way ANOVAs to determine the effects of season, sex and a season by sex interactive effect on body mass, baseline CORT, stress-induced CORT, and negative feedback at the first three seasonal timepoints. Because two time-points (1st litter lactation and 2nd litter lactation) had only female data, we ran one-way ANOVAs on all five time-points to determine the effect of season on female body mass, baseline CORT, stress-induced CORT, and negative feedback. If there was a significant season by sex interactive effect in a twoway ANOVA, we also ran a one-way ANOVA to determine the effect of season on male parameters at the first three seasonal timepoints. Baseline CORT and stress-induced CORT were log-transformed to meet normality and homogeneity of variance assumptions. All significant interactions were analyzed with post hoc tests with a step-down sequential Bonferroni correction (Garcia, 2004; Hochberg, 1988). Data transformation failed to obtain a normal distribution for negative feedback data, so we used a Kruskal-Wallis test with the Scheirer-Ray-Hare extension (SRHE Kruskal-Wallis, the non-parametric equivalent of a twoway ANOVA) to determine the effects of season, sex, and a season by sex interactive effect on negative feedback (Scheirer et al., 1976).

3. Results

3.1. Body mass

Body mass did not significantly differ between males and females across seasons, but there was a significant interaction between season and sex (two-way ANOVA, sex: $F_{1,73} = 0.04$, p = 0.85; season * sex: $F_{2,73} = 4.71$, p < 0.001). Males were significantly heavier in January (non-breeding) and August (late gestation) compared to July (early gestation) (one-way ANOVA, $F_{2,38} = 11.70$, p < 0.001, post hoc tests with step-down sequential



Fig. 1. Mean (\pm SE) body mass of female and male degus during non-breeding (n = 16 and 16), mating/early gestation (n = 12 and 17), late gestation (n = 9 and 8), the first bout of lactation (n = 9), and the second bout of lactation (n = 10), respectively. Bars on the *x*-axis indicate the approximate time and length of the different life history stages; note that since female degus had two litters, they were pregnant during their first bout of lactation. Also note that mating/early gestation and late gestation samples were collected in 2012, and the second bout of lactation and non-breeding samples were collected in December, 2012–January, 2013. Also note that different degus were sampled for each life history stage.

Bonferroni corrections all ps < 0.01) (Fig. 1). Females were heaviest during their first bout of lactation, and were also significantly heavier during late gestation and their second bout of lactation compared to non-breeding and early gestation (one-way ANOVA, $F_{4,52}$ = 41.92, p < 0.001, all ps < 0.01) (Fig. 1).

3.2. Baseline CORT

There was no significant interaction between season and sex on baseline CORT (two-way ANOVA, season * sex: $F_{2,68} = 0.68$, p = 0.51). Baseline CORT did not significantly differ across seasons for males (two-way ANOVA, season: $F_{2,68} = 0.23$, p = 0.80) or females (one-way ANOVA, $F_{4,47} = 0.51$, p = 0.73) (Fig. 2). There was a significant effect of sex on baseline CORT (two-way ANOVA, sex: $F_{1,68} = 9.33$, p < 0.01), most likely driven by males having lower baseline CORT than females during non-breeding (January) (Fig. 2).

3.3. Stress-induced CORT

Compared to males, females had higher stress-induced CORT, but there was no significant interaction between season and sex on stress-induced CORT (two-way ANOVA, sex: $F_{1,72} = 15.86$, p < 0.001, season * sex: $F_{2,72} = 1.51$, p = 0.23). Males had significantly higher stress-induced CORT during January (non-breeding) and August (late gestation) compared to July (early gestation) (two-way ANOVA, $F_{2,72} = 13.17$, p < 0.001, all ps < 0.01) (Fig. 2). Females had significantly higher stress-induced CORT during late gestation and the first bout of lactation, and also had significantly higher levels during non-breeding compared to the second bout of lactation (one-way ANOVA, $F_{4,51} = 16.25$, p < 0.001, all ps < 0.02) (Fig. 2).

3.4. Negative feedback

There was no significant sex or season by sex interactive effect on negative feedback (SRHE Kruskal–Wallis test, sex: $H_1 = 0.62$, p = 0.42, season * sex: $H_2 = 0.41$, p = 0.82). Negative feedback did not significantly differ across seasons for males (SRHE Kruskal– Wallis test, sex: $H_2 = 5.55$, p = 0.06) or females (Kruskal–Wallis test, $H_4 = 6.87$, p = 0.14) (Fig. 3).



Fig. 2. Solid lines represent mean (\pm SE) plasma baseline (BL) cortisol (CORT) concentrations of female and male degus during non-breeding (n = 16 and 16), mating/early gestation (n = 10 and 14), late gestation (n = 10 and 8), the first bout of lactation (n = 6), and the second bout of lactation (n = 10), respectively. Dashed lines represent mean (\pm SE) plasma stress-induced (SI) CORT concentrations of female and male degus during non-breeding (n = 16 and 16), mating/early gestation (n = 12 and 17), late gestation (n = 9 and 8), the first bout of lactation (n = 9), and the second bout of lactation (n = 9), and the second bout of lactation (n = 10, respectively.



Fig. 3. Mean (±SE) percent decrease in cortisol (CORT) levels after DEX injection for female and male degus during non-breeding (n = 15 and 16), mating/early gestation (n = 9 and 15), late gestation (n = 9 and 8), the first bout of lactation (n = 9), and the second bout of lactation (n = 10), respectively.

4. Discussion

Although there is evidence of seasonal CORT rhythms in several wild, free-living mammal species (Romero, 2002), there has been little previous research examining the seasonality of multiple endocrine stress response parameters (Romero et al., 2008). This study provides a comprehensive profile of the degu stress response by measuring not only stress-induced levels of CORT, but also baseline CORT levels and negative feedback efficacy. While we found seasonal differences in body mass and stress-induced CORT in both sexes, we found no seasonal changes in baseline CORT or negative feedback. These results suggest that degus seasonally modify the magnitude of their CORT response to stressors but not other aspects of CORT regulation.

Our results partially agree with the findings of Kenagy et al. (1999). Female degu body mass is similar between our study and Kenagy et al.'s except at the mating/early gestation time point; female degus sampled in 1997 (Kenagy et al., 1999) were heavier than those sampled in our 2011 study. Kenagy et al. (1999) also found that females were significantly heavier than males in June, while we did not find a significant sex difference at this time point. These differences could be due to the fact that animals were sampled from different locations. Additionally, degus from the Kenagy et al. study may have had better food availability. This is supported by the fact that degus underwent a second pregnancy during 1997 but not 2011.

Stress-induced CORT data from our study support the findings of Kenagy et al. (1999). In both studies, stress-induced CORT was lowest during mating (June/July), highest during lactation (September), and at medium levels during early non-breeding (December/January). Kenagy et al. (1999) found no sex differences in stress-induced CORT levels during mating/early gestation, which our data also supports. We cannot compare male and female stress-induced CORT differences at other time points since we failed to capture males during lactation and Kenagy et al. (1999) were unable to trap males during non-breeding. While we took stress-induced blood samples 30 min after capture, Kenagy et al. (1999) sampled degus that had been trapped for unknown amounts of time (up to 2 h). Kenagy et al. (1999) determined that after removing degus from traps and holding them for 15 min, CORT levels increased by about 20%. Taking this into account, the stress-induced CORT levels from our study match Kenagy et al.'s very well.

Seasonal CORT rhythms have been well studied in birds and reptiles compared to mammals (Romero, 2002). Of the few studies

on mammals, the majority found seasonal variation in both baseline and stress-induced CORT levels (87% and 70%, respectively) (Romero, 2002). Our results only support variation in stress-induced CORT levels. Although many wild mammals show seasonal variation of stress-induced CORT concentrations, there is no clear consensus on which life history stages have the highest or lowest concentrations (Romero, 2002; Romero et al., 2008). This suggests that seasonal changes in the endocrine stress response are driven by multiple factors or stressors that peak at different life history stages across species (Romero et al., 2008).

We also examined whether degus show seasonal variation in negative feedback efficacy. Negative feedback is an important part of the stress response and has been shown to be a good indicator of animal health (Sapolsky, 1983) and resistance to stressors (Romero and Wikelski, 2010). Lattin et al. (2012) found in wild free-living house sparrows that negative feedback efficacy was lower during the pre-laving stage compared to early winter and breeding. However, we failed to find any seasonal variation in degu negative feedback efficacy. Considering the potential importance of negative feedback to the overall stress response, future studies should continue to examine seasonal variation of negative feedback efficacy.

The Energy Mobilization Hypothesis predicts that baseline CORT levels will be highest during energetically expensive life history stages (Romero, 2002). In other words, CORT levels would be highest when allostatic load was highest (Wingfield, 2005). If seasonal variation in degu CORT levels were tied to energetic needs, we predicted that baseline CORT levels would be highest for female degus during lactation and highest for males during the mating season. However, our data do not fit either of these predictions; neither male nor female degus showed seasonal variation in their baseline CORT levels. This suggests that for degus, baseline CORT levels do not change in concert with seasonally predictable metabolic demands. This is surprising given that several other mammals (voles: Boonstra and Boag, 1992; chipmunks: Kenagy and Place, 2000; bats: Reeder et al., 2004; whales: Hunt et al., 2006; squirrels: Dantzer et al., 2010) show increased levels of baseline CORT during gestation and lactation. In addition, female talas tuco-tucos, a caviomorph rodent, show increased baseline CORT during the reproductive season (Vera et al. 2012). It is possible that corticosteroid-binding globulins (CBG) may be seasonally fluctuating (Breuner et al., 2003) in degus, thus affecting levels of free CORT. However, there is debate as to whether free CORT is the only active portion of CORT in the bloodstream (Schoech et al., 2013). Seasonal variation of other components of the downstream stress response, such as glucocorticoid receptors (Breuner and Orchinik, 2001) and 11^β-hydroxysteroid dehydrogenase levels (Monder and Shackleton, 1984), could be affecting glucocorticoid action as well.

The Preparative Hypothesis predicts that both baseline and stress-induced CORT levels are highest during times of year when there is a greater likelihood of encountering stressors (Romero, 2002). Based upon this hypothesis, we predicted that for female degus, the highest levels of baseline and stress-induced CORT would occur during spring due to increased predator risk. Female degus had their highest stress-induced CORT levels during late gestation and the first bout of lactation, which supports the prediction that CORT levels are highest during periods of increased predation risk. Since degus have been shown to change their behavior in response to perceived predation risk (Lagos et al., 1995), it is likely that degus are also changing their ability to respond physiologically to potential predation attempts. Other sources of stressors during this period are less likely. In contrast to males (see below), to our knowledge there is no evidence that females engage in more aggressive encounters during the mating season. Soto-Gamboa et al. (2005) did not find any male-female agonistic interactions during the breeding season, and since females are plural breeders that practice communal care (Ebensperger et al. 2002, 2004), it is

unlikely that there's significant female-female aggression. It is thus likely that, compared to mate competition, predation risk better explains the pattern of stress-induced CORT release in females.

Under the Preparative Hypothesis, we predicted that male baseline and stress-induced CORT levels would be elevated during spring due to predation risk and during the mating season due to increased mate competition. However, male degu stressinduced CORT levels were not highest during the spring; August (spring) and January (non-breeding) stress-induced CORT levels were not significantly different. As for mating competition, we found that male degus had their lowest levels of stress-induced CORT during the mating season (July), which does not support the prediction that male stress-induced CORT levels are highest during times of increased mate competition. However, we sampled degus during a low-density year (Ebensperger, unpublished data), so it is possible that there was decreased competition for mates during our sampling period.

We also predicted that female baseline and stress-induced CORT levels would be higher than males during the spring since high energy demands during pregnancy and lactation may cause increased foraging time, which could then increase predation risk. Females had higher stress-induced CORT levels compared to males during August (late gestation), which supports our predictions. However, female rats naturally have higher corticosterone concentrations than males, which is thought to be linked to pregnancy and lactation (Kudielka and Kirschbaum, 2005), so elevated stress-induced CORT levels in female degus may be related to internal rather than external differences. It is also possible that CORT increases in response to trapping may be due to contextual differences (Boyce and Ellis, 2005). There could be seasonal changes in how degus perceive and respond to different types of stressors.

Interestingly, degu body mass and stress-induced CORT patterns seem to mirror each other. For males, body mass and stress-induced CORT trends correspond exactly. For females, the same trends match well, although stress-induced CORT peaks during late gestation (August) while body mass does not. Additionally, female body mass is still quite high during second lactation while stress-induced CORT is at its nadir. Instead of considering body mass, it may be more appropriate to compare energy balance with stress-induced CORT. Because CORT's multiple metabolic effects all essentially work to increase blood glucose levels, high levels of stress-induced CORT may inflict substantial energy costs. During times of year with low food availability, it may be advantageous to keep stress-induced CORT levels low in order to prevent an energy deficit (McEwen and Wingfield, 2003). This leads to the hypothesis that degus only secrete high levels of stress-induced CORT when they have a positive energy balance. Our data fit this hypothesis well; for females, stress-induced CORT is highest when food is most plentiful (late gestation and first lactation). Female stress-induced CORT is lowest during second lactation (December), a time of low energy balance since females don't have as much food to buffer the calories they lose via lactation. However, the "energy balance hypothesis" does not explain why female stressinduced CORT is higher than males during August. Because food availability is high during this time of year, both females and males should have equivalent, positive energy balances. It is also possible that there are inherent differences in the stress response between the sexes, however, and this may explain differences in stress-induced CORT between males and females (Kudielka and Kirschbaum, 2005).

5. Conclusions

Results from this study support the findings of Kenagy et al. (1999) and add new information on the baseline CORT levels and

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negative feedback efficacy of wild, free-living degus. While we found seasonal variation in stress-induced CORT levels of both male and female degus, we did not find seasonal variation in baseline CORT levels. These results do not support the Energy Mobilization Hypothesis but partially support the Preparative Hypothesis. We also propose that stress-induced CORT levels may be seasonally regulated according to times of year with predictable high or low energy balance. To test this hypothesis, future studies should examine whether stress-induced CORT patterns are regulated on an ultimate, seasonal level or on a proximate, food-availability and condition level.

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