J Comp Physiol B (2010) 180:919–925
DOI 10.1007/s00360-010-0467-9

ORIGINAL PAPER

Sex differences in the excretion of fecal glucocorticoid metabolites in the Syrian hamster

Marie-Odile M. Chelini · Emma Otta · Clarissa Yamakita · Rupert Palme

Received: 4 August 2009 / Revised: 7 March 2010 / Accepted: 8 March 2010 / Published online: 25 March 2010
© Springer-Verlag 2010

Abstract We verified the relevance of measuring fecal glucocorticoid metabolites (FGM) to assess the stress response of the Syrian hamster. Male and female hamsters ($n = 10$ each) were submitted to an adrenocorticotropic hormone (ACTH) challenge test, whereas animals in the control group received 0.5 mL of sterile isotonic saline solution. All feces voided by each animal were collected at 4 h intervals from 24 h before (baseline) until 48 h after injections. FGM were quantified using an 11-oxoetiocholanolone enzyme immunoassay (EIA). Basal concentrations of FGM were almost four times higher in males than in females. Following ACTH administration, FGM levels started rising from 8 h onwards, reaching peak concentrations 20 or 28 h post injection in males and females, respectively. Despite the much higher absolute concentrations present in males, the relative increase (500%) in response to the ACTH stimulation was similar in both sexes. Sex differences in FGM levels are in accordance with results reported by others regarding the hamster adrenal physiology. The comparison of the adrenocortical response of males and females to an ACTH challenge provided new information about the amplitude and the timing of such a response and the excretion of glucocorticoids in both sexes. We demonstrated for the first time in the Syrian hamster that adrenocortical activity can be monitored in fecal samples in a noninvasive way. Our study provides a humane, practical, and noninvasive alternative to blood removal and therefore a powerful tool for stress-related studies in a species frequently used as an animal model in medical research.

Keywords Hamster · Cortisol · Noninvasive monitoring · Fecal metabolites · ACTH challenge test

Communicated by G. Heldmaier.

M.-O. M. Chelini (✉) · E. Otta
Department of Experimental Psychology,
Institute of Psychology, University of São Paulo,
Av. Prof. Mello Moraes, 1721, Cidade Universitária,
São Paulo 05508-030, Brazil
e-mail: marodile@usp.br

C. Yamakita
Faculty of Veterinary Medicine and Zootechnology,
University of São Paulo, São Paulo, Brazil

R. Palme
Department of Biomedical Sciences/Biochemistry,
University of Veterinary Medicine, Vienna, Austria

Introduction

Since the capture of the first litter in 1930, Syrian hamsters (*Mesocricetus auratus*) have become one of the species mostly used in biological and medical research due to some unique features these animals present. For instance, hamsters can reproduce very fast and actually present the shortest gestation period (16 days) among all eutherian mammals. Additionally, hamsters are extensively used in cardio-vascular research (Bajusz 1969; Missihoun et al. 2009) because their circulatory system shares some similarities with the human system. Moreover, these solitary-living and strictly territorial animals (Gattermann et al. 2001) have been widely used as models for behavioral and stress-related studies (e.g. Cain et al. 2004; Cordner et al. 2004; Gebhardt-Henrich et al. 2007; Taravosh-Lahn and Delville 2004; Touma and Palme 2005; Wommack et al. 2004; Zhang et al. 2008) for which measuring the activity of the hypothalamic–pituitary–adrenal axis is a basic tool (Möstl and Palme 2002). Classically, such studies have

relied on blood sampling and the measurement of serum cortisol or corticosterone, depending upon which glucocorticoid is predominantly secreted in each species (Palme et al. 2005). Whereas in the rabbit and most rodents, including the rat and the mouse, corticosterone is well-recognized as the major glucocorticoid, a controversy exists about this point in the hamster. Lehoux et al. (1992) and Cloutier et al. (1997), for instance, reported that hamsters, as human beings, synthesize more cortisol. In contrast, Ottenweller et al. (1985) measured three or four times higher basal concentrations of corticosterone than cortisol. Therefore, these authors suggested that both glucocorticoids should be measured when assessing adrenocortical function in the hamster. Methods for blood removal from laboratory mammals and birds have been reviewed by the BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement (1993). The Group was unable to recommend a preferred route for venepuncture in the gerbil and hamster. Actually, blood collection in hamsters is generally performed by cardiac puncture under general anesthesia or decapitation. Noninvasive methods of quantifying fecal glucocorticoid metabolites (FGM) are now broadly used with free-ranging and intractable animals (Bosson et al. 2009; Charbonnel et al. 2008; Franceschini et al. 2007; Huber et al. 2003; Monclús et al. 2009; Rehnus et al. 2010; Soto-Gamboa et al. 2009; Strauss et al. 2007; Thiel et al. 2008; Wang et al. 2009) as well as with laboratory animals (Bauer et al. 2008; Lepschy et al. 2007; Nováková et al. 2008; Touma et al. 2003) to investigate hormone–behavior relationships, as well as questions in the fields of animal welfare, ecology, conservation biology, and biomedicine (Touma and Palme 2005). These techniques avoid effects associated with blood sampling, which can seriously confound the experimental results. They enable frequent sampling of the same animal (and thus longitudinal studies), using individuals as their own controls. Moreover, no animal material is easier to collect than feces, and sampling can be done without disturbing the animals. All these advantages justify the increasing success of the analysis of fecal steroids. However, because the metabolism and the excretion of steroids differ significantly between species, and sometimes even between sexes (Palme et al. 1996, 2005; Touma et al. 2003), these methods must be rigorously validated for each species and sex before application, to demonstrate that the assay technique is able to detect changes in the levels of fecal steroid metabolites related to respective changes of steroid concentrations in the blood (Palme 2005). Despite the large number of studies dealing with FGM measurement in a wide range of animal species, validation experiments have been performed in only a few species (Touma and Palme 2005) and never, to our knowledge, in the Syrian hamster. Therefore, the objective of this work was (1) to detect whether diurnal changes in endogenous glucocorticoid levels are reflected in FGM concentrations, (2) to examine the effect of an adrenocorticotropic hormone (ACTH) challenge test on fecal glucocorticoid metabolite concentrations, and (3) to compare such effects in male and female Syrian hamsters.

Materials and methods

Animals and housing conditions

A total of 41 heterogenic, adult (10–15 weeks of age), sexually mature Syrian hamsters (20 males and 21 females) were obtained from the Laboratory Animal Facility of the Departamento de Patologia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, Brazil, whose Ethics Committee approved the experimental design. The animals were individually housed in standard propylene cages in the same animal facility under conventional conditions (12:12-h light:dark, lights on at 03:00 h; room temperature: 22 ± 2°C; 20 changes of air per hour; air pouch filters) according to guidelines from the Institute of Laboratory Animal Research (ILAR) (1996). Specific pelleted food (Nuvilab CR1, Nuvital, Curitiba, Brazil) and filtered bottled tap water were supplied ad libitum. In order to facilitate feces collection, absorbent paper pads were substituted for the traditional wood-shaving bedding.

ACTH challenge test

The ACTH injection was used in ten males and ten females randomly chosen (ACTH groups) to stimulate adrenocortical activity (i.e., to increase circulating glucocorticoid levels). On the second day (13:00 h) of the experiment, these hamsters were injected (i.m.) with 100 µg of synthetic ACTH (Synacthène Retard, 1 mg/mL, Novartis Pharma SAS, Rueil-Malmaison, France) dissolved in 0.5 ml sterile isotonic solution. To investigate the potentially stressful effects of the injection procedure itself on the pattern of excreted FGM, another 11 females and 10 males (Control groups) received an injection of 0.5 mL sterile isotonic saline solution.

Feces collection

All feces voided by each animal were collected at 4-h intervals from 24 h before until 48 h after the injections. In this way, we were able to determine baseline concentrations and the normal circadian pattern of FGM in each group and each group was used as its own control. All samples ($n = 711$) were weighed, immediately identified, and stored in a freezer at −20°C.

Steroid extraction and quantification

Fecal steroids were extracted using the methanol-based procedure described by Palme (2005). Because hamster feces are very dry, lyophilization was not necessary. After the homogenization of each fecal sample, we shook an aliquot of 0.5 g or the whole sample for 20 min on a multivortex with 5 mL of 80% methanol for samples heavier than 0.25 g, 2.5 mL for samples whose weight was between 0.1 and 2.5 g and 1 mL for samples lighter than 0.1 g. The suspension was then centrifuged at 500×g for 10 min. The supernatant was stored at −20°C until assayed. Fecal glucocorticoid metabolites were quantified in an aliquot of the extract (50 µl diluted 1:10) using a group-specific 11-oxoetiocholanolone enzyme immunoassay (EIA measuring glucocorticoid metabolites with a 5β-3α-ol-11-one structure) first described by Möstl et al. (2002) and successfully validated for the common hamster (*Cricetus cricetus*; Franceschini et al. 2007). Intra- and inter-assay coefficients of variation were 8.6 and 9.7, respectively. All samples were assayed in duplicate. Concentrations of glucocorticoid metabolites were expressed as nanograms per gram fecal dry matter.

Statistics

Data were analyzed using the software SPSS 13.0 for Windows. As they did not fulfill the assumptions of normality and homogeneity of variance FGM data were log transformed after replacing missing values ($n = 68$; no feces produced in the respective interval) by linear interpolation. Repeated measure General Linear Model (rmGLM) was used to detect sex, treatment (ACTH or saline), and time effects on these concentrations. We performed Wilcoxon signed ranks tests (Bonferroni corrected) as a post hoc test to compare concentrations measured in different intervals within a sex. Means are given with standard deviations and significance levels for all tests were set at $p < 0.05$ unless otherwise noted.

Results

Baseline levels

The rmGLM analysis of FGM concentrations measured on the day preceding injections showed a strong effect of sex ($F = 288.5$, $p < 0.005$) but no effect of treatment ($F = 0.193$, $p = 0.663$) as between subject factors. In fact, male baseline concentrations (320 ± 16 ng/g feces) were almost four times higher than female ones (89 ± 50 ng/g feces; Fig. 1). However, despite individual differences in FGM concentrations (range: 30–283 ng/g feces for

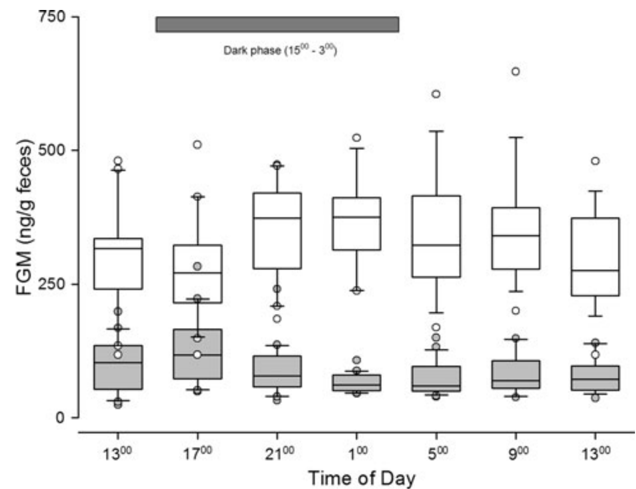

Fig. 1 Variation of fecal glucocorticoid metabolites (FGM) over 24 h in male ($n = 20$; *white boxes*) and female ($n = 21$; *light gray boxes*) Syrian hamsters

females; 135–605 ng/g feces for males), the homogeneity of the groups was confirmed by similar baseline concentrations among females and males (Females: ACTH 88.6 ± 43.8 ng/g feces; Control: 88.6 ± 49.3 ng/g feces; Males: ACTH: 306 ± 69 ng/g feces; Control: 335 ± 65 ng/g feces). A clear effect of the interaction between sex and time (Wilks' Lambda $F = 11.004$, $p < 0.0005$) indicated that concentrations varied in a different way in females and males. As no effect of time of day as a within subject factor was detected (Wilks' Lambda $F = 1.207$, $p = 0.328$), no circadian rhythm of FGM excretion was documented by our assay.

ACTH challenge test

FGM values (of the 4 h intervals) from the moment of injections onwards were submitted to rmGLM with time as a within subject factor. FGM concentrations changed significantly in males and females in response to the ACTH stimulation, and the time course of FGM excretion differed between the sexes. There were marked time (Wilks' Lambda $F = 25.009$, $p < 0.0005$) and interaction effects (time × sex: Wilks' Lambda $F = 13.108$, $p < 0.0005$; time × treatment: Wilks' Lambda $F = 22.009$, $p < 0.0005$; time × sex × treatment: Wilks' Lambda $F = 2.968$, $p = 0.010$). There were also strong effects of both, sex ($F = 456$, $p < 0.0005$) and treatment ($F = 142$, $p < 0.0005$) as between subject factors. As found with basal concentrations, absolute values were much higher in feces of males, but the relative increase was quite similar for both sexes (509 ± 131%).

Wilcoxon signed ranks test with Bonferroni correction (significance level $p < 0.016$) confirmed that FGM

concentrations in males of the ACTH group were significantly higher than the ones measured at the same time on the day prior to injection from 8 h after injection ($Z = -2.66$) onwards, and reached its highest value (median: 1,408 ng/g feces) 20 h after administration (Fig. 2). It was also from 8 h after the injection in females that the ACTH group showed significantly higher concentrations compared with the corresponding baseline values ($Z = -2.803$), but the highest concentrations (median: 346 ng/g of feces) were detected only after 28 h (Fig. 3). In contrast, FGM were still higher in ACTH males at the end of the sampling period (48 h after injection) than baseline values ($Z = -2.547$). In the female group, FGM had already returned to baseline levels 44 h after injections ($Z = -1.826$, $p = 0.068$).

Following the saline injection no significant (Wilcoxon signed ranks test, Bonferroni corrected $p > 0.016$ for all comparisons between concentrations measured at the same time intervals on the different days) increase in FGM was found in males. In females only FGM values 32 h after the injection (median: 154 ng/g feces) were significantly higher (Wilcoxon signed ranks test, $Z = -2.934$, $p = 0.003$).

Discussion

Our study describes and successfully validates a non-invasive method for evaluating adrenocortical activity in hamsters by measuring fecal glucocorticoid metabolites (FGM) for the first time. It demonstrates that in hamsters

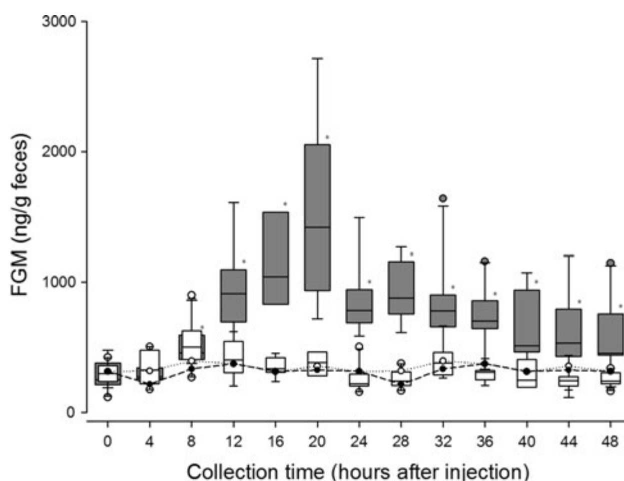

Fig. 2 *Boxplots* of fecal glucocorticoid metabolites (FGM; ng/g) of male Syrian hamsters after an i.m. injection of adrenocorticotropic hormone (ACTH; *dark gray boxes*) or saline solution (Control; *white boxes*). Respective median baseline values of the ACTH (*filled circle with broken lines*) and control group (*open circle with dotted lines*) at the same time of the day are also shown. *Asterisks* beside the *box* indicate significant differences compared with the baseline levels of the same group

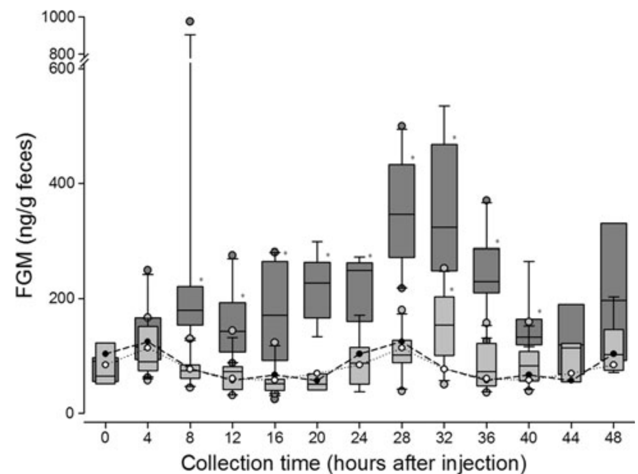

Fig. 3 *Boxplots* of fecal glucocorticoid metabolites (FGM; ng/g) of female Syrian hamsters after an injection of adrenocorticotropic hormone (ACTH; *dark gray boxes*) or saline solution (Control; *light gray boxes*). Respective median baseline values of the ACTH (*filled circle with broken lines*) and control group (*open circle with dotted lines*) at the same time of the day are also shown. *Asterisks* beside the *box* indicate significant differences compared with the baseline levels of the same group

adrenocortical activity is well reflected in concentrations of FGM, measured with an 11-oxoetiocholanolone EIA. This indispensable validation (Touma and Palme 2005), the lack of which some authors already deplored (Gebhardt-Henrich et al. 2007), provides the basis for a reliable and powerful non-invasive tool for further investigations involving monitoring of hamsters' glucocorticoids. Beyond this, there are other important findings in our study. One of them, which has already been reported for cortisol concentrations in hamster blood samples (Gebhardt-Henrich et al. 2007; Lucas et al. 1999) is the presence of large inter-individual differences regarding baseline FGM concentrations. For example, levels between females varied almost tenfold during the day prior to the injection. This confirms one more time the importance of longitudinal studies, in which each animal or group is its own control, which is almost impossible in small rodents, if blood is sampled.

In most laboratory rodents, females have larger adrenal glands and greater steroid production than males (Huhman et al. 2003; Nikicicz et al. 1984). This dimorphism is reversed in Syrian hamsters and the adrenal glands of male hamsters are larger and secrete more cortisol than those of the females (Huhman et al. 2003; Nikicicz et al. 1984). Our data show that, as expected, this sex difference is reflected in FGM concentrations: Males exhibited four times higher baseline and peak concentrations than females. Glucocorticoid excretion was shown to differ significantly between sexes in a related species, the Common hamster (*C. cricetus*; Franceschini et al. 2007). However, when these authors compared FGM concentration before, during and

after the reproductive period, they only found significant differences during the reproductive phase, when male FGM levels increased significantly, whereas FGM concentrations in females remained relatively constant throughout the active season. Therefore, they associated higher male levels to competition for females and general high mating effort. Our study was performed in laboratory conditions with constant temperature (22 ± 2°C) and photoperiod (12:12-h light:dark), conditions considered as photostimulatory (Ottenweller et al. 1985) all year around. In fact, our hamsters reproduce all year around and may present high glucocorticoid levels related to reproduction at every time.

In the feces we were unable to detect the significant circadian rhythmicity of corticoid secretion reported in male hamsters (Albers et al. 1985; Ottenweller et al. 1987; we did not find any description of the female circadian rhythm in the literature). However, Ottenweller et al. (1987) reported that, whereas plasma corticosterone and cortisol rhythms are present on long days (14:10-h light–dark cycle), only cortisol has a significant rhythm on short days (10:14-h light–dark cycle). Even its amplitude was suppressed compared with the cortisol rhythm on long days. Our animals were housed under an intermediate 12:12-h light–dark photoperiod which may have blunted the amplitude of the diurnal glucocorticoid rhythm. In addition, because fecal hormone metabolite levels reflect the cumulative secretion and excretion of hormones over several hours, unlike blood samples, fecal samples are less affected by the pulsatility of hormone secretion, and fluctuations in the blood plasma are smoothed in the feces (Palme 2005). Similar, a rhythm in FGM levels was absent in guinea-pigs (Bauer et al. 2008), but in contrast, well expressed in rats and mice (Lepschy et al. 2007; Touma et al. 2004).

Numerous researchers investigated the adrenal response of the Syrian hamster to a wide range of stressors and to an ACTH challenge test, and various concentrations of cortisol and corticosterone in this species have been published (e.g. Gebhardt-Henrich et al. 2007; Lehoux et al. 1992; Lucas et al. 1999; Ottenweller et al. 1985). However, as with most rodent models, male hamsters are usually preferred for non-reproductive research purposes, because they are less aggressive than females and there is less hormonal interference due to cyclic fluctuations of hormone levels. Therefore, most studies including glucocorticoid measurement are performed with male hamsters, and we could not find any record of females' endocrine reaction to an ACTH challenge. Identical treatment applied to both male and female hamsters in our study allows for the first time a reliable assessment of sex differences in adrenal responsiveness and glucocorticoid excretion in the Syrian hamster. A first difference occurred in control animals: whereas the injection procedure caused a transient but significant peak (153% above level at the same time of the precedent day) in females 32 h after the injection, no significant response could be measured in the male group. Again, it is possible that the signal of a short rise in circulating glucocorticoid had been dampened in feces of males. It seems, however, that the whole process of capture, immobilization, and injection represented only a short mild stress in laboratory animals used to routine handling. This was underlined by results in mice and rats, or guinea pigs (Touma et al. 2004; Lepschy et al. 2007; Bauer et al. 2008), where the injection procedure itself did not cause a significant increase in FGM. In contrast, an ACTH administration induced long-lasting increases in our hamsters, and peak values were in both sexes about 500% above the baseline. These increases in FGM were lower than those measured for plasma cortisol concentrations by Lehoux et al. (1992) after injecting ACTH in male hamsters (approximately 700% above basal level 5 h after the injection) or the tenfold increase observed by Gebhardt-Henrich et al. (2007). However, the latter injected 12 male hamsters and decapitated them one by one in ascending time intervals for glucocorticoid measurement, so the real significance of the resulting data may be problematic. Again in males, Ottenweller et al. (1985) measured both cortisol and corticosterone blood concentrations following ACTH administration. They reported a significant increase (about 350% above basal levels) in total glucocorticoid concentrations. Interestingly, these authors observed that, although corticosterone and cortisol concentrations increased significantly, the rise in cortisol was greater. Altogether, the comparison of our results with Ottenweller's data suggests that the fivefold increase in FGM concentrations we detected following ACTH stimulation reflects total variation in plasma glucocorticoids and that our EIA actually detected both cortisol and corticosterone metabolites both the feces. of both males and females This is also in accordance with the characteristics of the antibody used in the 11-oxoetiocholanolone EIA. It measures glucocorticoid metabolites with a 5β-3α-ol-11-one structure (Möstl et al. 2002), which can be derived from both, cortisol or corticosterone. This is an additional advantage of our analytic technique for assessing adrenocortical function in this species, since the measurement of both glucocorticoids is recommended (Ottenweller et al. 1987).

The route of steroid excretion varies among species, as well as among steroids within the same species (Palme et al. 1996, 2005). We did not find a study about glucocorticoid metabolism and excretion in the Syrian hamster, but Chelini et al. (2005) suggested delay times between 5 and 25 h for the excretion of gonadal steroids. In the present study, the time lag between ACTH injections and the appearance of peak FGM levels was 20 h (males) or 28 h (females). These delay times are longer than those

found in mice and rats (4–12 h; Touma et al. 2003; Lepschy et al. 2007), but comparable to those reported in chinchillas (30 h; Ponzio et al. 2004). In the guinea-pig, Bauer et al. (2008) observed FGM concentrations reaching peak values between 14 and 20 h after ACTH administration, but, in contrast with these authors, we observed a clear difference between sexes in this timing, which has never been reported in any other species so far. In males the increase in FGM concentrations peaked (20 h vs. 28 h) earlier, but lasted longer (until the end of the experiment) than in females. If these time differences reflect different adrenal responses to ACTH or differences in glucocorticoid metabolism and excretion or more likely both, we are not able to decide, because more accurate investigations such as a radiometabolism study with a characterization of the excreted glucocorticoid metabolites in both sexes of this species are still lacking.

In conclusion, our results show for the first time in the Syrian hamster that adrenocortical activity can be monitored in fecal samples in a non-invasive way. Actually, the satisfactory results generated by our EIA, measuring FGM with a 5β-3α-ol-11-one structure demonstrated clearly that changes in glucocorticoid concentrations in the blood are well reflected in changes in FGM in both sexes. The difference we observed between male and female FGM levels are in accordance with the sex difference reported by others regarding the hamster adrenal physiology. Therefore, our study provides a humane, practical, and noninvasive alternative to blood removal, and therefore a powerful tool for stress-related studies in a species where venepuncture has traditionally been regarded as problematic. This is especially important, as hamsters are used as an animal model for medical research.

Acknowledgments We thank Edith Klobetz Rassam for excellent technical assistance in the laboratory. This work was supported by grants awarded by the Fundação de Amparo à Pesquisa do Estado de São Paulo to Emma Otta (06/57257-0) and Marie Odile Monier Chelini (05/59377-0) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico to Emma Otta.

Conflict of interest statement The authors declare no competing interests that might be perceived to influence the results and discussion reported in this manuscript.

References

Albers HE, Yogev L, Todd RB, Goldman BD (1985) Adrenal corticoids in hamsters: role in circadian timing. Am J Physiol Regul Integr Comp Physiol 248:434–438

Bajusz E (1969) Hereditary cardiomyopathy: a new disease model. Am Heart J 77:686–696

Bauer B, Palme R, Machatschke IH, Dittami J, Huber S (2008) Non-invasive measurement of adrenocortical and gonadal activity in male and female guinea pigs (*Cavia aperea f. porcellus*). Gen Comp Endocrinol 156:482–489

Bosson CO, Palme R, Boonstra R (2009) Assessment of the stress response in Columbian ground squirrels: laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. Physiol Biochem Zool 82:291–301

BVA/FRAME/RSPCA/UFAW Joint Working Group on Refinement (1993) Removal of blood from laboratory mammals and birds. Lab Anim 27:1–22

Cain SW, Karatsoreos I, Gautam N, Konar Y, Funk D, McDonald RJ, Ralph MR (2004) Blunted cortisol rhythm is associated with learning impairment in aged hamster. Physiol Behav 82:339–344

Charbonnel N, Chaval Y, Berthier K, Deter J, Morand S, Palme R, Cosson JF (2008) Stress and demographic decline: a potential effect mediated by impairment of reproduction and immune function in cyclic vole populations. Physiol Biochem Zool 81:63–73

Chelini MOM, Souza NL, Rocha AM, Felippe ECG, Oliveira CA (2005) Quantification of fecal estradiol and progesterone metabolites in Syrian hamster (*Mesocricetus auratus*). Braz J Med Biol Res 38:1711–1717

Cloutier M, Fleury A, Courtemanche J, Ducharme L, Mason JI, Lehoux JG (1997) Characterization of the adrenal cytochrome P450C17 in the hamster, a small animal model for the study of adrenal dehydroepiandrosterone biosynthesis. DNA Cell Biol 16:357–368

Cordner AP, Herwood MB, Helmreich DL, Parfitt DB (2004) Antidepressants blunt the effects of inescapable stress on male mating behaviour and decrease corticotropin-releasing hormone mRNA expression in the hypothalamic paraventricular nucleus of the Syrian hamster (*Mesocricetus auratus*). J Neuroendocrinol 16:628–636

Franceschini C, Siutz C, Palme R, Millesi E (2007) Seasonal changes in cortisol and progesterone secretion in Common hamsters. Gen Comp Endocrinol 152:14–21

Gattermann R, Fritzsche P, Neumann K, Al-Hussein I, Kayser A, Abiad M, Yakti R (2001) Notes on the current distribution and the ecology of wild golden hamsters (*Mesocricetus auratus*). J Zool Lond 254:359–365

Gebhardt-Henrich SG, Fischer K, Hausenberger AR, Keller P, Steiger A (2007) The duration of capture and restraint during anesthesia and euthanasia influences glucocorticoid levels in male golden hamsters. Lab Anim 36:41–46

Huber S, Palme R, Arnold W (2003) Effects of season, sex, and sample collection on concentrations of fecal cortisol metabolites in red deer (*Cervus elaphus*). Gen Comp Endocrinol 130:48–54

Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, Israel JE, Jasnow AM (2003) Conditioned defeat in male and female Syrian hamsters. Horm Behav 44:293–299

Institute of Laboratory Animal Research (ILAR) (1996) Guide for the care and use of laboratory animals. National Academy Press, Washington

Lehoux JG, Mason JI, Ducharme L (1992) In vivo effects of adrenocorticotropin on hamster adrenal steroidogenic enzymes. Endocrinology 131:1874–1882

Lepschy M, Touma C, Hruby R, Palme R (2007) Non-invasive measurement of adrenocortical activity in male and female rats. Lab Anim 41:372–387

Lucas RJ, Stirland JA, Darrow JM, Menaker M, Loudon ASI (1999) Free running circadian rhythms of melatonin, luteinizing hormone, and cortisol in Syrian hamsters bearing the circadian tau mutation. Endocrinology 140:758–764

Missihoun C, Zisa D, Shabbir A, Lin A, Lee T (2009) Myocardial oxidative stress, osteogenic phenotype, and energy metabolism are differentially involved in the initiation and early progression

of δ-sarcoglycan-null cardiomyopathy. Mol Cell Biochem 321:45–52

Monclús R, Palomares F, Tablado Z, Martínez A, Palme R (2009) Testing the threat-sensitive predation avoidance hypothesis: physiological responses and predator pressure in wild rabbits. Oecologia 158:615–623

Möstl E, Palme R (2002) Hormones as indicators of stress. Dom Anim Endocrinol 23:67–74

Möstl E, Maggs JL, Schrötter G, Besenfelder U, Palme R (2002) Measurement of cortisol metabolites in faeces of ruminants. Vet Res Commun 26:127–139

Nikicicz H, Kasprzak A, Malendowicz LK (1984) Sex differences in adrenocortical structure and function. XIII. Stereologic studies on adrenal cortex of maturing male and female hamsters. Cell Tissue Res 235:459–462

Nováková M, Palme R, Kutalová H, Janský L, Frynta D (2008) The effects of sex, age and commensal way of life on levels of fecal glucocorticoid metabolites in spiny mice (*Acomys cahirinus*). Physiol Behav 95:187–193

Ottenweller JE, Tapp WN, Burke JM, Natelson BH (1985) Plasma cortisol and corticosterone concentrations in the golden hamster, (*Mesocricetus auratus*). Life Sci 37:1551–1558

Ottenweller JE, Tapp WN, Pitman DL, Natelson BH (1987) Adrenal, thyroid, and testicular hormone rhythms in male golden hamsters on long and short days. Am J Physiol 253:321–328

Palme R (2005) Measuring fecal steroids: guidelines for practical application. Ann NY Acad Sci 1046:75–80

Palme R, Fischer P, Schildorfer H, Ismail MN (1996) Excretion of infused ^{14}C-steroid hormones via faeces and urine in domestic livestock. Anim Reprod Sci 43:43–63

Palme R, Rettenbacher S, Touma C, El-Bahr SM, Möstl E (2005) Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion and noninvasive measurement in fecal samples. Trends in comparative endocrinology and neurobiology. Ann NY Acad Sci 1040:162–171

Ponzio MF, Monfort SL, Busso JM, Dabbene VG, Ruiz RD, Cuneo MF (2004) A non-invasive method for assessing adrenal activity in the chinchilla (*Chinchilla lanigera*). J Exp Zool 301:218–227

Rehnus M, Hackländer K, Palme R (2010). A non-invasive method for measuring glucocorticoid metabolites (GCM) in Mountain hares (*Lepus timidus*). Eur J Wildl Res. doi:10.1007/s10344-009-0297-9)

Soto-Gamboa M, Gonzalez S, Hayes LD, Ebensperger LA (2009) Validation of a radioimmunoassay for measuring fecal cortisol metabolites in the hystricomorph rodent, *Octodon degus*. J Exp Zool 311A:496–503

Strauss A, Mascher E, Palme R, Millesi E (2007) Sexually mature and immature yearling male European ground squirrels: a comparison of behavioral and physiological parameters. Horm Behav 52:646–652

Taravosh-Lahn K, Delville Y (2004) Aggressive behavior in female golden hamsters: development and the effect of repeated social stress. Horm Behav 46:428–435

Thiel D, Jenni-Eiermann S, Braunisch V, Palme R, Jenni L (2008) Ski tourism affects habitat use and evokes a physiological stress response in capercaillie *Tetrao urogallus*: a new methodological approach. J Appl Ecol 45:845–853

Touma C, Palme R (2005) Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of a biological validation. Ann NY Acad Sci 1046:54–74

Touma C, Sachser N, Möstl E, Palme R (2003) Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 130:267–278

Touma C, Palme R, Sachser N (2004) Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. Horm Behav 45:10–22

Wang D, Wang Z, Zhang J, Zhang J, Zhang Z (2009) Fecal hormone variation during prolonged social interaction in male Tscheskia triton. Physiol Behav 97:347–352

Wommack JC, Salinas A, RHJr Melloni, Delville Y (2004) Behavioural and neuroendocrine adaptations to repeated stress during puberty in male golden hamsters. J Neuroendocrinol 16:767–775

Zhang JZ, Rao XP, Sun L, Wang DW, Liu D, Zhao C (2008) Cohabitation impaired physiology, fitness and sex-related chemosignals in golden hamsters. Physiol Behav 93:1071–1077