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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

The stress of growing old: sex- and season-specific effects of age on allostatic load in wild grey mouse lemurs

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Abstract Chronic stress [i.e. long-term elevation of glucocorticoid (GC) levels] and aging have similar, negative effects on the functioning of an organism. Aged individuals' declining ability to regulate GC levels may therefore impair their ability to cope with stress, as found in humans. The coping of aged animals with long-term natural stressors is virtually unstudied, even though the ability to respond appropriately to stressors is likely integral to the reproduction and survival of wild animals. To assess the effect of age on coping with naturally fluctuating energetic demands, we measured stress hormone output via GC metabolites in faecal samples (fGCM) of wild grey mouse lemurs (*Microcebus murinus*) in different ecological seasons. Aged individuals were expected to exhibit elevated fGCM levels under energetically demanding conditions. In line with this prediction, we found a positive age effect in the dry season, when food and water availability are low and mating takes place, suggesting impaired coping of aged wild animals. The age effect was significantly stronger in females, the longer-lived sex. Body mass of males but not females correlated positively with fGCM in the dry season. Age or body mass did not influence fGCM significantly in the rainy season. The sex- and season-specific predictors of fGCM may reflect the differential investment of males and females into reproduction and longevity. A review of prior research indicates contradictory aging patterns in GC regulation across and even within species. The context of sampling may influence the likelihood of detecting senescent declines in GC functioning.

Keywords Coping · Glucocorticoid metabolites · Senescence · Sex difference · Trade-off

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Introduction

Glucocorticoid hormones (GC) are pivotal mediators of an array of physiological processes that allow an organism to respond adaptively to the energetic demands set by the environment, and to requirements associated with various life-history stages (Crespi et al. 2013). GCs facilitate physiological and behavioural mechanisms that promote survival directly (Sapolsky et al. 2000) by redirecting resources (mainly by regulating blood glucose levels). GCs can fluctuate adaptively in preparation for, or in response to, predictable energetic demands, such as changing ecological seasons and breeding activities (Boonstra et al. 2014; Romero 2002; Sapolsky et al. 2000). GCs also play an important role in responding to unpredictable, acute stressors (real or anticipated perturbations to homeostasis) such as predator attacks, which usually provoke rapid, short-term elevations in GCs above the baseline level (Sapolsky et al. 2000).

Normal, predictable variation in energetic demands should not lead to harmful effects in healthy individuals, as long as the variation stays within a range of conditions that the organism can cope with [termed "regulatory range" by Koolhaas et al. (2011)]. However, an individual's ability to react adaptively within this range might be influenced by illness, energy deficiency or maladaptive changes in GC functioning, leading to a decreased range of manageable conditions. If the energy demands placed on the system exceed the organism's capacity to react adaptively, excessive glucocorticoid activity leads to a negative energy balance and an increasing allostatic load, described as the "wear and tear that results from chronic overactivity or underactivity of allostatic systems" (McEwen 1998), such as the regulation of circulating glucocorticoid levels (McEwen and Wingfield 2003). Chronically elevated glucocorticoid levels can have negative consequences for physiological functioning (Boonstra 2005; McEwen 1998), including immune defences [e.g. humans (Juster et al. 2010)], reproductive performance [wild hares (Sheriff et al. 2009)], and survival probability [ring-tailed lemurs (Pride 2005) but see Cabezas et al. (2007)]. Therefore, although long-term elevation in GCs may in some circumstances adaptively promote survival at the expense of less essential functions (Boonstra 2013), it is often considered non-adaptive [studies in humans (McEwen 2008), laboratory rodents (Sapolsky et al. 1987) and wild animals (Pride 2005; Sapolsky and Altmann 1991; Sheriff et al. 2009)].

The ability to cope with challenges might also decline in old age due to senescent changes in the activity of the hypothalamic-pituitary-adrenal (HPA) axis that regulates the levels of GCs in circulation (Sapolsky et al. 1987; Veldhuis et al. 2013; Wilkinson et al. 1997). Many of the effects of chronic stress on physiological functioning parallel those associated with normal aging (Frolkis 1993; Veldhuis et al. 2013); hence, stress can accelerate age-related processes of deterioration. However, thus far the majority of studies on the combined effects of stress and aging has been conducted under captive conditions or in humans and the significance of these effects in the natural world remains unclear.

The regulatory range in GC levels of old individuals might narrow due to the deterioration of the HPA-axis and lead to impaired coping when unusually high demands are placed on the system. For example, aged humans suffer disproportionately severe consequences relative to younger individuals when faced with the same stressor (Frolkis 1993; Graham et al. 2006). Age-related changes in glucocorticoid functioning and the associated health consequences may also have implications for the functioning of aged individuals under varying environmental conditions, perhaps contributing to age-and condition-dependent mortality in natural populations. However, little is known about the effects of aging on HPA-activity in wild animals, and the influence of environmental challenges on the coping of aged wild individuals is unknown.

In this study, we evaluate support for the coping hypothesis in a natural population of grey mouse lemurs (*Microcebus murinus*)—a small-bodied primate—in which senescent declines begin after the age of 4–5 years in measures of physical functioning [e.g., senescent body mass decline in captivity but not in the wild (Hämäläinen et al. 2014a; Perret and Aujard 2001); declining physical strength in aged captive and wild animals (Hämäläinen et al. 2015)]. Season-specific patterns observed in these senescent changes might suggest compromised coping with environmental challenges at old age. In the dry season of western Madagascar, food and water availability are low (Dammhahn and Kappeler 2008) and the short mating season takes place at the end of the dry season (Eberle and Kappeler 2002, 2004b). As the body mass of both sexes also reaches its annual low at this time of year (Hämäläinen et al. 2014a), the dry season is presumably highly demanding energetically. In early rainy season, females incur the energetic costs of gestation and lactation, but food and water availability are high and offspring are weaned by late rainy season (Eberle and Kappeler 2004a). Adaptive responses to this seasonal variation likely have high significance for the reproduction and survival of individuals. It seems plausible that differences between individuals in coping are most pronounced at a time of higher environmental demands to the physiology, and the late dry season may therefore be a critical time for those individuals whose functioning is compromised. Seasonality was therefore used as an indicator of environmental demands in our study.

To evaluate seasonal changes in an individuals' allostatic load and the functioning of their HPA-axis (McEwen and Wingfield 2003; Sheriff et al. 2011) we quantified faecal GC metabolite (fGCM) levels in two dry and two rainy seasons. We sampled animals ranging from age at maturity to the maximum age in the population. The allostatic load would be expected to be generally higher in the dry relative to the rainy season due to the energetic demands likely imposed on all animals. However, following our predictions of an interaction between stress and aging, we expected to find elevated fGCM in aged animals in the dry season beyond the presumably adaptive fGCM levels of younger individuals, as an indication of a declined ability to respond adaptively to increased energetic stress. Elevated baseline GC levels are frequently associated with poor body condition [e.g. badgers (George et al. 2014) and kittiwakes (Kitaysky et al. 1999)], and animals in better body condition might be able to cope with the stressors better than leaner individuals; hence we also assessed the influence of body mass on individual GC profiles. We assumed prolonged nutritional stress or illness to be indicated by low

body mass, and therefore predicted body mass to correlate negatively with fGCM concentrations.

Based on results from our validation study (Hämäläinen et al. 2014b), we expected no overall sex-differences in fGCM levels, but aging might influence the sexes differentially, perhaps affecting females more, as found previously [e.g., humans (Veldhuis et al. 2013)]. The sexes typically differ in their HPA-activity due to the differing effects of gonadal steroid hormones (Handa et al. 1994; Kudielka and Kirschbaum 2005) as well as behavioural and life history differences on GC levels (Bokony et al. 2009). Therefore, both sexes were sampled to assess sex-specific effects of aging, body mass and seasonal fluctuations on GC profiles.

Materials and methods

Study population

The grey mouse lemur [Cheirogaleidae, small-bodied (60 g) strepsirrhine primate] reaches sexual maturity at 6–8 months of age (Némoz-Bertholet and Aujard 2003) and has a relatively short lifespan [average life expectancy after surviving their first dry season is ~2 years in the wild (Kraus et al. 2008), median adult longevity is 2.5 years and maximum age 11 years in our study population (personal observations)]. High extrinsic mortality risk [mainly by predation (Goodman et al. 1993)] leads to rapid selective disappearance of individuals in a declining condition (Hämäläinen et al. 2014a). Mortality in the wild is male biased, particularly during the mating season (Kraus et al. 2008). Males develop enlarged testes prior to the mating season (a few weeks at the end of the dry season in October–November) and during the mating season, males roam intensively in search of estrous females. Gestation length is 2 months and offspring are typically weaned by March (late rainy season) (Eberle and Kappeler 2004a). Adults in good enough body condition (and particularly females) use torpor in the dry season (Schmid and Kappeler 1998) when food and water availability are low. The species is a solitary foraging, nocturnal omnivore.

The study population (locally known as the "N5" population) has been monitored since 2000 in a 25 ha study site of dry deciduous forest in Kirindy/CNFEREF, central western Madagascar by researchers of the German Primate Center. The area experiences pronounced seasonal variation with a distinct dry season (~May–November), and rainy season (~November–April), with consequent variation in food availability (Dammhahn and Kappeler 2008). The energetic demands along with sex-specific behaviours [female-biased torpor use (Schmid and Kappeler 1998) and male roaming in the mating season, (Eberle and Kappeler 2004b)] lead to substantial seasonal, sex-specific body mass fluctuation in *M. murinus* (Hämäläinen et al. 2014a; Schmid and Kappeler 1998).

For long-term data collection, trapping and body mass measurements have been conducted at least 6 times per year (monthly in March–May and September–November) using Sherman live catch traps baited with small pieces of banana. Recapture rates are high [>0.5 for another subpopulation (Kraus et al. 2008)] during these months and no evidence has been found for long-term influences of the capture-and-handling protocols on the animals' stress physiology (Hämäläinen et al. 2014b). All captured animals are equipped with an individual subcutaneous transponder (Trovan EURO ID, Weilerswist, Germany) at first capture. Since most individuals are captured in their 1st year of life (age estimates confirmed by morphometrics), the age of all individuals can be estimated with a high level of accuracy. The capture and handling protocols have been detailed in e.g. Dammhahn and Kappeler (2008), and Eberle and Kappeler (2004a).

Sample collection and hormone analysis

A total of 464 faecal samples from 192 individuals was collected over four field seasons: in September–November (late dry season, mating season) 2010 and 2012 and in March–May (late rainy season, non-reproductive) 2011 and 2012. These included 73 individuals that were sampled in at least two distinct seasons, including 5 individuals that were sampled in all four seasons. Juveniles of the season were excluded from the rainy season sample and, therefore, all individuals were assumed to be sexually mature (age > 6 months). Animals were sampled either within a few hours after trap entry at night, or on the following morning after a night spent in a trap. Capturing induces an acute stress response that is measurable 24–72 h after capture (Hämäläinen et al. 2014b); hence samples were always collected within the "baseline" period (i.e. within 12 h of the first monthly capture) and should be unaffected by the capture event. The time of day of faecal sampling does not influence fGCM levels in the species [(Hämäläinen et al. 2014b), confirmed for the data used in this study].

The methods of collection, extraction and fGCM analysis have been validated previously (Hämäläinen et al. 2014b). Briefly, fresh faeces was collected from cleaned traps, avoiding urine contamination, weighed and stored in ethanol within a few hours of collection. Approximately 0.2 g fresh faeces (range 0.07–0.36 g) was subsampled and homogenized in 2 ml 90 % ethanol. The faecal suspension was then vortexed, centrifuged, and the supernatant stored for future hormone analyses. The pellet was dried to a constant mass to determine faeces water content. Duplicate aliquots of the faecal extracts were measured as detailed in (Heistermann et al. 2004) using a validated

enzymeimmunoassay for 11β-hydroxyetiocholanolone (Ganswindt et al. 2003)—a major metabolite of cortisol in grey mouse lemur faeces (Hämäläinen et al. 2014b). All hormone concentrations are given as nanograms per gram of faecal wet weight.

Statistical analyses

To test the basic assumption that the dry season is associated with a higher allostatic load, we first assessed seasonal differences in the fGCM using a linear mixed model [LMM, R-package lme4, (Bates et al. 2014)], with season (dry or rainy) as a fixed factor. Since seasonal effects may be mediated partially by body condition or water availability, we added the factors body mass and faeces water content [water%, to account for the known influence of water% on fGCM levels (Hämäläinen et al. 2014b)], and the interaction terms season × body mass and season × water%. The nuisance variable year of sampling (2010–2011 or 2012) was included in all models to account for yearly variation and individual identity was included as a random effect due to repeated sampling of the same individuals.

Given the known differences between the seasons in diet (Dammhahn and Kappeler 2008), water availability and body mass, we proceeded to analyze the influences of sex and age on fGCM levels separately for the dry and rainy season to reduce the complexity of the models. The fixed effects structure of each full model included the terms sex, age, body mass and the interaction terms sex × age and sex × body mass, year and water%. Individual identity was included as a random factor.

Due to the modest sample size and "noise" typical for hormone data, we removed non-significant interaction terms ($P > 0.1$) one at a time from the full model to avoid over-parameterization and to identify significant effects (Pinheiro and Bates 2000). Likewise, when body mass had a non-significant effect, it was removed from the model to enhance sample size, as not all samples could be matched to body mass measurements (data set including body mass $N = 374$ samples/173 individuals, excluding body mass $N = 464/192$). To account for the possibility that age effects are underestimated due to partially cross-sectional data and potential selective disappearance of individuals with a lower phenotypic quality [e.g. (Hämäläinen et al. 2014a; Nussey et al. 2008)], we also ran the same models after restricting the data set to longitudinal data, i.e. individuals that were sampled in two or more distinct seasons ($N = 297$ samples/74 individuals).

Age and body mass were log-transformed, centered and scaled (mean/SD) prior to the analyses to improve the interpretability of interactions (Schielzeth 2010) and fGCM was log-transformed. The normality and homogeneity of error assumptions were examined using residual plots for the most complex model for each data set. Satterthwaite estimation was used to compute P values [lmerTest-package, (Kuznetsova et al. 2014)]. Marginal and conditional R^2-scores (Nakagawa and Schielzeth 2013) were computed using the r.squaredGLMM-function of the MuMIn-package (Barton 2014). All analyses were performed in R version 3.1.1 (R Development Core Team 2014) and statistical significance was set at $P \leq 0.05$.

Review of existing data on sex-specific senescence in glucocorticoid regulation

To assess how our results on the effects of sex and age on fGCM compare with the trends observed in previous studies, we reviewed the literature on senescence in stress physiology, with a specific focus on studies conducted in the wild (Tables 3, 4). For this, we searched Web of Science and Google Scholar (in December 2014) for combinations of the following terms: wild population/natural population/wild/free-ranging; stress/glucocorticoid/HPA-axis/hypothalamic–pituitary–adrenal/stress response/corticosterone/cortisol; age/aging/ageing/senescence; and sex. We included only studies in which stress physiology was measured in old, known-age individuals [excluding studies that used age categories (e.g. subadult, adult) and ones using only young adults]. We additionally present a non-exhaustive sample of studies of captive animals, with the purpose of highlighting the variability of patterns found across studies.

Results

Substantially higher fGCM concentrations were found in the dry season relative to the rainy season (simplified model including the fixed terms season, water% and year: $N = 464$ samples/192 individuals, $\beta = -0.279$, SE = 0.097, $t = -2.870$, $P = 0.004$; Supplementary material Table S1), supporting our assumption that the animals experience higher allostatic loads in the late dry season. These seasonal differences were mediated by season-specific influences of water and body mass, indicated by the significance of the interaction terms (Table 1).

Results from the season-specific models indicate that fGCM-levels were higher at old age in the dry season (Fig. 1a) but not in the rainy season (Fig. 1b), as predicted based on the coping hypothesis. However, this relationship was much stronger in females, as indicated by the interaction term sex × age (Table 2; Fig. 1a). A positive association was found between body mass and fGCM levels in males in the dry season, whereas the trend for females was negative, as indicated by the interaction term sex × body mass (Table 2; Fig. 2a). No significant effects of either interaction term or body mass on fGCM were found in the

Table 1 Influence of seasonality on log [faecal glucocorticoid hormones (GC) metabolite (fGCM)] in the grey mouse lemur

	β	SE	t	P
Intercept	7.043	0.368	19.145	<0.001
Water%	−1.754	0.511	−3.432	<0.001
Year	0.473	0.122	3.868	<0.001
Season (ref. dry)	1.014	0.618	1.641	0.102
Body mass	0.551	0.127	4.349	<0.001
Season × water%	−2.429	0.826	−2.939	0.003
Season × body mass	−0.776	0.161	−4.814	<0.001

N = 374 samples/173 individuals, $R^2_{\text{conditional}}$ = 0.243, R^2_{marginal} = 0.550

rainy season (Table 2; Fig. 2b; full model shown in Supplementary material Table S2). The water% was negatively associated with fGCM levels in both seasons, and year of sampling had a significant effect in the rainy season (Table 2). As one sample from the oldest female in the dry season had a very high fGCM value and might therefore drive the overall effect, we re-ran the model after excluding this point, but the results remained unchanged and, therefore, the case was included in the final analyses.

When the same models were re-run using a smaller data set of only longitudinal data (Supplementary material, Tables S3, S4), both interaction terms and the main effect of body mass were non-significant and were removed from

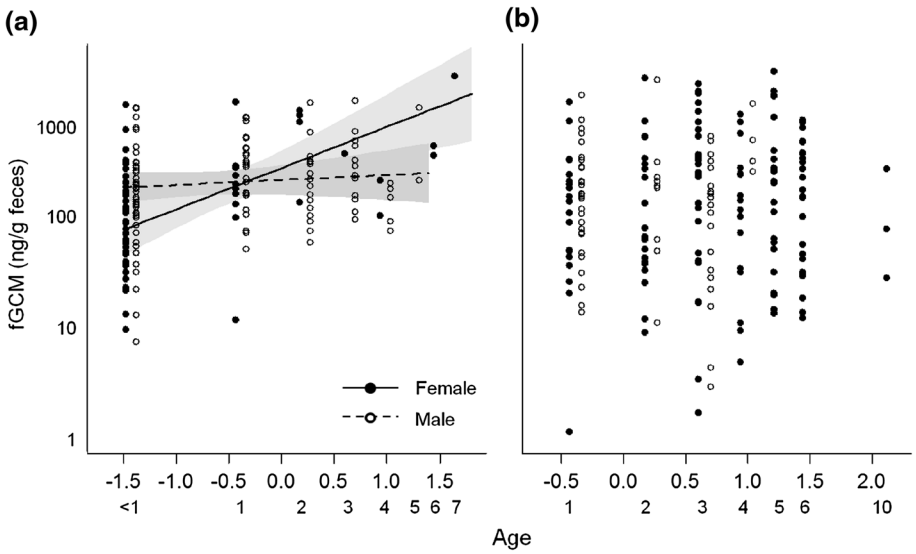

Fig. 1 **a** In the dry season, age was associated positively with faecal glucocorticoid hormones (GC) metabolite (fGCM)-levels of wild *Microcebus murinus,* but this effect was stronger for females, as indicated by an age × sex interaction term (P = 0.003). **b** No sex or age effects were found in the rainy season (P > 0.7). All data points are shown, prediction lines are only for year 2012 (predictions for 2010/2011 have slightly lower values but lines are parallel). *Lines* indicate predictions based on the final model for each season (Table 2) and *shading* shows the associated 95 % confidence intervals. The predictions are based on scaled and centered age (*x-axis top row*) but corresponding chronological age in years (*x-axis bottom row*) is indicated. Note log scale used on *y*-axis

Table 2 Predictors of fGCM in the dry and the rainy season based on the final model for each season

	Dry[a]				Rainy[b]			
	β	SE	t	P	β	SE	t	P
Intercept	6.442	0.437	14.760	<0.001	7.483	0.583	12.846	<0.001
Water%	−1.431	0.429	−3.335	0.001	−4.049	0.697	−5.809	<0.001
Year	0.247	0.130	1.899	0.059	0.792	0.190	4.179	<0.001
Sex (ref. Female)	0.483	0.320	1.508	0.134	0.065	0.209	0.313	0.756
Age	0.749	0.185	4.039	<0.001	0.035	0.147	0.238	0.813
Body mass	−0.464	0.337	−1.376	0.171				
Sex × age	−0.660	0.219	−3.011	0.003				
Sex × body mass	0.839	0.380	2.207	0.029				

[a] N = 170 samples/126 individuals, final model R^2_{marginal} = 0.243, $R^2_{\text{conditional}}$ = 0.550

[b] N = 218 samples/79 individuals, final model R^2_{marginal} = 0.272, $R^2_{\text{conditional}}$ = 0.426

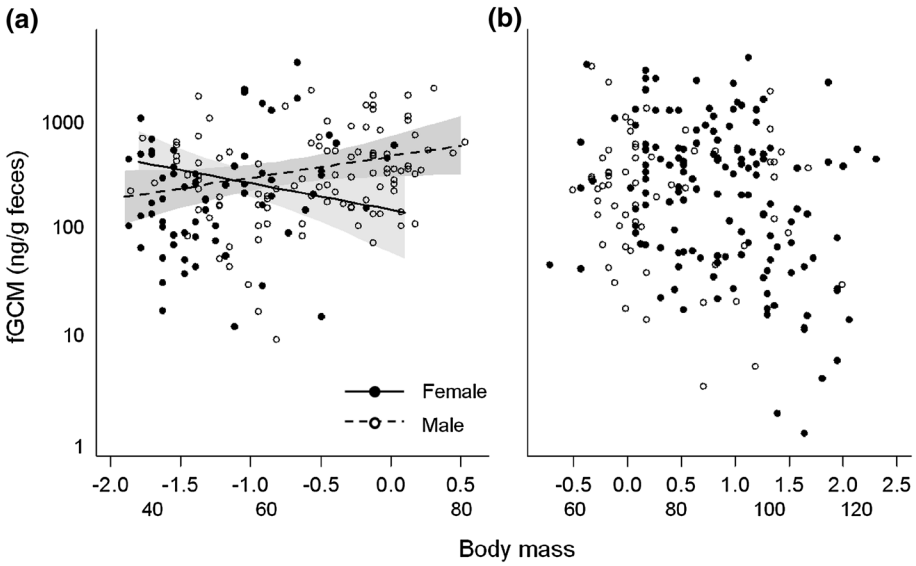

Fig. 2 Body mass as a predictor of fGCM-levels. **a** A significant interaction ($P = 0.029$) of sex × body mass was found in the dry season. **b** In the rainy season neither the interaction ($P > 0.2$) nor the main effect of body mass ($P > 0.5$ after removing interactions from the model, Table S2) was significant. All data points are shown, prediction lines are only for year 2012 (predictions for 2010/2011 have slightly lower values but lines are parallel). *Lines* indicate predictions from the best model for each season (Table 2), and *shading* shows the associated 95 % confidence intervals. The predictions are based on scaled and centered body mass (*x-axis top row*) but corresponding untransformed body mass in grams (*x-axis bottom row*) is indicated. Note log scale used on *y*-axis

the dry season model. The final model indicated a positive age effect in the dry season ($\beta = 0.221$, SE = 0.080, $t = 2.758$, $P = 0.007$), confirming the age effect found in the complete data set, but this effect was no longer sex-specific for this smaller data set. In the rainy season, none of the predictors had a significant effect on fGCM as found for the larger data set.

Discussion

In this study, we tested the coping hypothesis of aging, evaluating whether there are physiological indications that the performance of aged individuals in wild grey mouse lemurs is impaired during energetically demanding environmental conditions. In support of this hypothesis, we found a seasonal age effect, with old individuals showing significantly higher stress hormone (GC) levels than younger animals in the dry season, when both intrinsic and extrinsic factors may increase individual allostatic load. This effect was stronger for females, which are the longer-lived sex in the population. No age or sex effects were found in the non-reproductive, rainy season, when food availability is high.

Elevated fGCM in aged individuals during the dry season

Old individuals may be affected more than young ones when the energetic demands reach marginal levels along their regulatory range due to intensive or frequent stressors. Our finding of elevated fGCM in aged individuals during the dry season is consistent with this hypothesis, suggesting that the seasonal high energetic demands function as a more intensive stressor for older grey mouse lemurs. The adrenocortical regulation of GCs in circulation can change at old age via a reduced efficiency of the negative feedback mechanisms of the HPA-axis, or an increased duration of the stress response (Sapolsky et al. 1983, 1984). The increases in baseline GC levels observed in the aged animals in our study therefore likely reflect the deterioration of the negative feedback mechanisms (Mizoguchi et al. 2009; Sapolsky et al. 1983, 1986) that leads to a chronic GC elevation when high energetic demands are placed on the system. Evidence of impaired coping during chronic stress at old age has also been found in humans (Kudielka et al. 2009) and rats (Shoji and Mizoguchi 2010), but these results may be influenced by the settings that poorly reflect the ecological conditions that have shaped the physiology of the species over evolutionary time. Although several studies have assessed age-related changes in baseline, response or negative feedback levels of GC and found variable patterns of aging (Tables 3, 4), this is, to our knowledge, the first study showing age effects on coping with natural long-term stressors in wild animals.

While the pattern of high GCs in the dry season fits the coping hypothesis, alternative explanations for the observed pattern are conceivable. The highest fGCM concentrations in the dry season were found in females that had passed the

Table 3 Selective literature review of the (sex-specific) effects of aging on GC-levels at baseline and during acute stress response in humans and captive mammals and birds. *M* males, *F* females, *B* blood, *F* feces, *S* saliva, *U* urine

Species	Sex differences		Effect of aging[a]			Sex-specific aging	Matrix	References
	Baseline	Response	Baseline	Response	Feedback[b]			
Human (*Homo sapiens*)	F+	F+	+	+	NA	NA	B	Heuser et al. (1994)
	NA	NA	NA	+	NA	M + (free GC), F + (total GC)	B, S	Kudielka and Kirschbaum (2005)
	0	0	+	−	NA	F- (response)	S	Nicolson et al. (1997)
	0	NA	0	+	NA	F+	B & S	Otte et al. (2005)
	0/M+	0/F+/M+	NA	0/−/+	−	0/F+/M+	NA	Veldhuis et al. (2013)
Rhesus monkey (*Macaca mulatta*)	NA (F only)	NA (F only)	+	0	−	NA (F only)	B	Gust et al. (2000)
	NA (F only)	NA (F only)	0	0/+	−	NA (F only)	B	Goncharova and Lapin (2002, 2004)
Hamadryas baboon (*Papio hamadryas*)	NA (M only)	NA (M only)	0	NA	−	NA (M only)	B	Goncharova and Lapin (2004)
Dog (*Canis familiaris*)	NA	NA	+	+	0	NA	B	Reul et al. (1991)
	NA	NA	+	+	−	NA	B	Rothuizen et al. (1993)
Mouse (*Mus musculus*)	F+	NA	0/+	NA	NA	M + (baseline + begins earlier)	F	Touma et al. (2004)
Rat (*Rattus norvegicus*)	NA	NA	+	NA	−	0	B	Sapolsky et al. (1986) and Sapolsky (1992)
	F+	NA	NA	NA	NA	NA	B	Critchlow et al. (1963)
	NA	F+	NA	NA	NA	NA	B	Kitay (1961)
	NA (M only)	NA (M only)	0?	0/+	NA	NA (M only)	B	Herman et al. (2001)
	NA	NA	0	0/−	NA	F− (response − at old age)	B	Brett et al. (1983)
	NA (M only)	NA (M only)	0	NA	+/−	NA (M only)	B	Mizoguchi et al. (2009)
	NA (M only)	NA (M only)	0	0	+	NA (M only)	B	(Kasckow et al. 2005)
California mouse (*Peromyscus californicus*)	0	0	0	0	0/+ (M)	— (response + at old age)	B	Harris and Saltzman (2013)
Tree shrew (*Tupaia belangeri*)	NA (M only)	NA (M only)	0/+ (+ until 200 days, 0 after)	NA	NA	NA (M only)	U	Van Kampen and Fuchs (1998)

[a] +Denotes a positive association, − a negative one, 0 no difference or non-significant effect and NA lack of data

[b] − =Resistance to negative feedback at old age

Table 4 Literature review of the (sex-specific) effects of aging on GC-levels at baseline and during acute stress response in wild vertebrates. *M* males, *F* females, *B* blood, *F* feces, *S* saliva, *U* urine

Species	Sex differences		Effect of aging[a]			Sex-specific aging	Matrix	References
	Baseline	Response	Baseline	Response	Feedback[b]			
Yellow baboon (*Papio cynocephalus*)	0	0	+	NA	−	0	B	Sapolsky and Altmann (1991)
	NA	NA	+	NA	NA	0	F	Alberts et al. (2014)
Red-backed vole (*Myodes rutilus*)	NA (M only)	NA	0	NA	−	NA (M only)	B?	Fletcher et al. unpublished in Boonstra et al. (2014)
Eastern chipmunk (*Tamias striatus*)	NA	NA	0/+	NA	NA	F+	F	Montiglio et al. (2014)
Florida scrub-jay (*Aphelocoma coerulescens*)	NA (M only)	NA	0	NA	NA	NA (M only)	B	Wilcoxen et al. (2010)
	0	0	0	−/+ (general −, + in oldest age class)	NA	0	B	Wilcoxen et al. (2011)
Common tern (*Sterna hirundo*)	0	0	0	−	NA	NA	B	Heidinger et al. (2006, 2008)
Snow petrel (*Pagodroma nivea*)	M+	M+	0?[c]	0/+ (+in oldest animals)	NA	NA	B	Goutte et al. (2010)
	0	0	0	0	NA	0	B	Angelier et al. (2007a)
	0	NA	NA	0/+ (+ in oldest animals)	NA	NA	B	Angelier et al. (2007b)
Wandering albatross (*Diomedea exulans*)	0	NA	NA	0[d]	NA	NA	B	Angelier et al. (2006)
	NA	NA	0	NA	NA	0	B	Lecomte et al. (2010)
Thick-billed murre (*Uria lomvia*)	0	0	0?[e]	+ (+Begins after mid-life)	− (−Begins after mid-life)	NA	B	Elliott et al. (2014)
Black-legged kittiwake (*Rissa tridactyla*)	0	0	0?[e]	+ (+Begins after mid-life)[f]	+	NA	B	Elliott et al. (2014)
Common lizard (*Lacerta vivipara)*	NA (F only)	NA	0	NA	NA	NA (F only)	B	Massot et al. (2011)

[a] +Denotes a positive association, − a negative one, 0 no difference or non-significant effect and NA lack of data

[b] − = Resistance to negative feedback at old age

[c] Limited support from model selection for an age effect (based on graphics, negative trend at least in females), effects not reported

[d] No age effects, but a quadratic effect of breeding experience

[e] Increasing trend at old age (age^2 $P \leq 0.07$)

[f] Age effect found only during chick-rearing, no age effects during pre-breeding

average survival age in the wild and that most regularly utilize torpor in the dry season (Schmid 1999). Arising from torpor might be associated with high GC levels to induce re-feeding and boost metabolism after the long periods of anorexia, or in preparation for the impending mating season (Boonstra et al. 2014; Romero 2002). GC elevation in the dry season might therefore reflect an adaptive response to predictable seasonal fluctuation in energetic demands. However, arousal from torpor would be expected to elevate fGCM early in the dry season when most individuals terminate torpor use, and not during or after the mating season. We found no evidence of a temporal trend in fGCM levels over the course of the dry season (no significant interactions of month with age or sex, Supplementary material Table S5) and, thus, the coping hypothesis seems a more likely explanation for the observed pattern.

Sex and context influence the effects of aging on HPA-axis function

The patterns of age-related changes in the HPA-axis function differ substantially across vertebrate species and also across contexts and sexes within species, as demonstrated by a selection of captive studies in Table 3 and a review of studies of natural populations in Table 4. While almost every conceivable result is found on the (sex-specific) effects of age among, and even within, species, relatively consistent patterns were found in baseline GC (typically unchanged or elevated at old age) and negative feedback efficiency (unchanged or lowered at old age) in captive studies. Feedback efficiency has rarely been studied under natural conditions, presumably due to the logistic challenges, but based on the captive studies it might be a particularly informative indicator of HPA-axis aging. For the GC stress response, sex differences in baseline and response GC as well as sex-specific effects of aging on GC output, the main conclusion is a remarkable absence of general patterns in both the captive and wild setting. This variability might in part reflect the fact that the studies are rarely directly comparable due to differences in the methodology and medium used, parameters measured, demographic groups studied, and potential differences introduced by phylogeny or social/mating system of the species studied. This variability in study designs still impedes predictions on the directionality of age effects on HPA-axis aging across species and contexts.

Age-related changes in GC regulation tend to be more pronounced in females compared to males in the two most studied organisms: humans and laboratory rats (Table 3). This may follow sex differences in GC metabolism and the way that aging influences the HPA pathways of females relative to males (Kudielka and Kirschbaum 2005). In the wild, female-biased aging of GC functioning similar to this study has been found in eastern chipmunks (Montiglio et al. 2014), and no other study has found significant sex differences in aging (Table 4). Our results indicate that the age effects may be negligible if measured in the season of abundance, but in the lean season the same population can exhibit strong age effects that may be sex-specific. Similarly, seasonal differences in age trajectories of GC levels were found in black-legged kittiwakes (Elliott et al. 2014). The absence of patterns in the wild studies (Table 4) may reflect the manifestation of aging as difficulty of coping, as the detection of this effect would require sampling in energetically challenging circumstances. Furthermore, senescence may be more difficult to detect in natural populations compared to captive conditions (Hämäläinen et al. 2014a; Nussey et al. 2008, 2011) if the individuals that live to old age in nature are of high quality or have an HPA phenotype that promotes self-maintenance (perhaps at the expense of reproduction early in life). Due to the detrimental effects of chronic GC elevation on health (Glaser and Kiecolt-Glaser 2005), chronic stress may contribute to the selective disappearance of individuals (Pride 2005).

The smaller age-effects in grey mouse lemur males could be due partially to higher male mortality (Hämäläinen et al. 2014a; Kraus et al. 2008), which eliminates males from the population before they show senescent declines [senescence in other functional parameters begins at age 4–5 years (Hämäläinen et al. 2014a, 2015; Languille et al. 2012)]. Male mortality of grey mouse lemurs is highest in the mating season (Kraus et al. 2008), the time when GC levels are elevated in both sexes, and may indicate higher reproductive investment by males at the expense of survival, as predicted by life history theory (Bonduriansky et al. 2008; Williams 1957).

GCs have been implicated as possible mediators of survival-reproduction trade-offs due to their role in resource reallocation (Ricklefs and Wikelski 2002; Wingfield and Sapolsky 2003). Because an individual's future reproductive potential typically decreases with advancing age, it has been proposed that—in contrast to the coping hypothesis—fGCM of aged individuals should be lowered during the mating season to facilitate higher reproductive investment (sensu CORT-trade-off hypothesis (Boonstra et al. 2001; Patterson et al. 2014; Wingfield and Sapolsky 2003). The few studies that have thus far addressed this hypothesis have found limited support for it (Harris 2012; Harris and Saltzman 2013; Heidinger et al. 2006, 2008), see also (Elliott et al. 2014). Our study also fails to find evidence in favour of this idea, suggesting that grey mouse lemurs may not be able to compensate for their declining reproductive value by down-regulating their GC levels at old age, at least in the early stages of breeding. Further research is required on the differences between captive and wild studies, the influences of breeding status and annual fluctuations as

well as different mating systems on (sex-specific) aging to clarify the circumstances under which GC production is down- or up-regulated at old age.

Sex- and season-specific predictors of fGCM levels

Seasonal fluctuations are thought to be predictable for animals in their natural habitat due to the strong adaptive value of being able to anticipate and adjust rapidly to changing energetic needs (Landys et al. 2006; Romero 2002). Evidence has been found for elevated GC levels in preparation for, or in response to nutritional stress in e.g. baboons (Gesquiere et al. 2008, 2011) and sea birds (Kitaysky et al. 1999), and GCs tend to be elevated during the breeding season in many vertebrate taxa, although the evidence for this pattern is weakest for mammals (Romero 2002).

Our results indicate that heavier males have somewhat higher fGCM than lighter males, whereas a negative trend was observed for females. Heavier males may invest more resources into roaming and competing for females, or into testis growth prior to the mating season (Schmid and Kappeler 1998) to gain an advantage in sperm competition and thus enhance their reproductive success. Roaming males also tend to forego foraging (personal observations), hence they may have to utilize existing tissue for energy via elevated GC levels. The CORT-adaptation hypothesis (Bonier et al. 2009a, b) proposes that GC levels should be elevated during breeding to promote fitness when energetically expensive behaviours improve reproductive success, hence our results for males may offer support for this hypothesis. For females, the period of gestation and lactation (December–February) likely poses higher energetic costs. Unfortunately, this period could not be addressed in our study due to logistic and ethical limitations on capturing breeding females. All demographic groups appeared to experience similar GC exposure during the time of high food availability in the late rainy season.

The seasonal differences in fGCM might also be caused partially by hydration stress or overall "metabolic stress" due to harsh environmental conditions, as found in geladas (Beehner and McCann 2008). Seasonal fluctuations in predation pressure may also alter stress physiology (Clinchy et al. 2013; Sheriff et al. 2009), which may be amplified if mouse lemurs engage in more risky behaviours when foraging at times of low food availability and thus experience higher predation risk.

Conclusions

The seasonal age-effect found in this study for grey mouse lemurs (particularly the longer-lived females) likely reflects impairment of the physiological stress response to high energetic demands at old age. Since natural selection acts most strongly early in life, coping with stressors at old age may not be within the scope of selection and result in a narrowing regulatory range. However, to detect this effect, age trajectories of HPA-axis function must be studied across the range of energetic demands faced by the population. We invite further studies into the coping of aged animals across contexts to evaluate the significance of senescent changes in GC metabolism under natural conditions, ideally combining measures of fitness or performance with quantification of HPA-axis functioning. Glucocorticoids are thought to mediate survival-reproduction trade-offs and therefore, sex- and context-specific changes in GC regulation over the individual lifespan can potentially contribute to creating life-history variation between and within species.

Despite the increasing number of studies examining the effects of aging on GC metabolism, no clear patterns have thus far emerged on the directionality of age effects across the sexes, seasons or taxa [but see (Hau et al. 2010)]. Combined with the highly variable methodology and the young, evolving theory, this complexity manifests as the current disarray of results. Integrating the existing hypotheses into a single theoretic "stress of aging" framework would be essential for reconciling the causes underlying this variation.

Author contribution statement C.K. formulated the idea, M.H. implemented the hormone analyses, A.H.conducted fieldwork, A.H. analysed the data and wrote the paper with input from M.H. and C.K.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed.

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