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Ovarian acyclicity in zoo African elephants (*Loxodonta africana*) is associated with high body condition scores and elevated serum insulin and leptin

Kari A. Morfeld[A,B] *and Janine L. Brown*[A]

[A]Center for Species Survival, Smithsonian Conservation Biology Institute, National Zoological Park, 1500 Remount Road, Front Royal, VA 22630, USA.
[B]Corresponding author. Email: morfeldk@si.edu

Abstract. The purpose of the present study was to determine whether excessive body fat and altered metabolic hormone concentrations in the circulation were associated with ovarian acyclicity in the world's largest land mammal, the African elephant. We compared body condition, glucose, insulin and leptin concentrations and the glucose-to-insulin ratio (G : I) between cycling ($n = 23$; normal 14–16 week cycles based on serum progestagens for at least 2 years) and non-cycling ($n = 23$; consistent baseline progestagen concentrations for at least 2 years) females. A validated body condition score (BCS) index (five-point scale; 1 = thinnest, 5 = fattest) was used to assess the degree of fatness of the study elephants. The mean BCS of non-cycling elephants was higher than that of their cycling counterparts. There were differences in concentrations of serum metabolic biomarkers, with non-cycling elephants in the BCS 5 category having higher leptin and insulin concentrations and a lower G : I ratio than cycling BCS 5 females. Using 'non-cycling' as the outcome variable in regression models, high BCS was a strong predictor of a non-cycling status. This study provides the first evidence that ovarian acyclicity in zoo African elephants is associated with body condition indicative of obesity, as well as elevated, perturbed biomarkers of metabolic status.

Additional keywords: infertility, metabolic hormones, obesity, reproduction.

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Introduction

Poor reproductive success due to ovarian cycle disruptions in zoo-maintained African elephants (*Loxodonta africana*) is a problem that has been recognised for more than a decade (Brown *et al.* 2004*a*). In a recent survey, 16% of reproductive-age (11–35 years) female African elephants exhibited irregular cycles and 30% had a complete lack of cyclicity (Dow and Brown 2012). Studies of cycle abnormalities have yet to find a definitive cause, although it appears they are not related to elevated cortisol (Proctor *et al.* 2010), altered androgen production (Mouttham *et al.* 2011), thyroid problems (Brown *et al.* 2004*b*) or pituitary gonadotropin dysfunction (Brown *et al.* 2004*b*).

There is growing recognition and concern that obesity and metabolic conditions are negatively impacting the health of many species, including humans, companion and domestic animals. A similar health concern exists for zoo-held species, including elephants that often are fed diets high in calories and given inadequate exercise (Ange *et al.* 2001; Hatt and Clauss 2006; Clubb *et al.* 2009; Lewis *et al.* 2010). A recent study found that a high body mass index (BMI) is positively correlated with ovarian inactivity in African elephants (Freeman *et al.* 2009), suggesting that reproductive problems may be caused, in part, by metabolic derangements associated with excessive body fat. This seems plausible given that studies in horses and humans have shown that obesity can lead to metabolic changes that impair fertility (Hartz *et al.* 1979; Bray 1997; Clark *et al.* 1998; Norman and Clark 1998; Irvine and Shaw 2003; Pasquali *et al.* 2003; Chang 2007). For example, obese mares experience an extended interval between successive ovulations (Vick *et al.* 2006), not unlike the irregular cycles observed in elephants (Brown *et al.* 2004*b*). Stillbirths and dystocias also are common in obese women and horses (Clubb and Mason 2002; Althabe 2012) and are causes of significant calf mortality in zoo elephants (Clubb and Mason 2002; Clubb *et al.* 2009; Dale 2010; Mason and Veasey 2010). Such evidence suggests elephants may be experiencing reproductive problems associated with excessive bodyweight, including ovarian acyclicity. Thus, investigations that compare obesity-related metabolic hormones in cycling and non-cycling elephants may help identify factors that impact on reproductive potential.

For equids, various methods have been developed to subjectively assess obesity, including visual body condition scoring (BCS), calculating BMI and using ultrasound to measure subcutaneous fat thickness to predict total body fat content (Henneke *et al.* 1983; Kearns *et al.* 2002; Donaldson *et al.* 2004).

In particular, BCS has become an integral part of assessing body fat in veterinary practices as an indirect, effective and inexpensive method for quantifying patient condition. Other methodologies to assess altered metabolic states associated with obesity include measuring glucose, insulin and leptin concentrations and calculating a glucose-to-insulin ratio (G : I). Impaired insulin sensitivity (insulin resistance) leads to a multitude of ailments related to high blood sugar (glucose) and diabetes (e.g. kidney disease, stroke, heart attack, blindness, nerve damage, poor circulation and wound healing), some of which are reported causes of morbidity and mortality in zoo elephants (Clubb and Mason 2002; Clubb *et al.* 2009; Mason and Veasey 2010). The relationship between insulin resistance and infertility has been well documented in women and horses (Hartz *et al.* 1979; Johnson 2002; Pasquali *et al.* 2003; Vick *et al.* 2006) but has not been examined to date in elephants.

Leptin, a protein produced by adipose tissue, is important in the regulation of food intake and energy balance (Considine *et al.* 1996) and also plays a role in reproduction, with excess levels being associated with obesity and ovarian dysfunction (Mácajová *et al.* 2004; Brewer and Balen 2010). Leptin has been shown to vary directly with the percentage of body fat in several species (Pasquali and Casimirri 1993; Fors *et al.* 1999; Banks *et al.* 2001; Garnsworthy *et al.* 2008) and, in women, elevated serum leptin is a sign of energy imbalance associated with excess energy, poor diet, insulin resistance or changes in other metabolic risk factors (Leyva *et al.* 1998; Martins *et al.* 2012). Taking these observations into consideration together, it is not unreasonable to question whether obesity and altered metabolic states may also be responsible, at least in part, for the reproductive problems observed in a high percentage of female elephants.

The aim of the present study was to determine whether excessive body fat and altered metabolic hormone levels are associated with ovarian acyclicity in zoo-maintained African elephants. This study was unique because it compared two large cohorts of intensively managed African elephants where reproductive status, including ovarian activity, was clearly known from at least 2 years of progestagen monitoring. The hypothesis was that non-cycling African elephant females exhibit more characteristics of metabolic aberrations than their normal cycling zoo counterparts. The aims of the study were to: (1) compare measures of body and metabolic condition (BCS, leptin, glucose, insulin, G : I ratio) between cycling and non-cycling elephants; (2) compare metabolic hormone concentrations across BCS categories in cycling and non-cycling elephants; and (3) determine whether obesity and altered metabolic hormone concentrations are risk factors for abnormal ovarian function. An improved knowledge of factors contributing to poor reproduction may enable the development of management strategies to mitigate obesity and health or reproductive problems associated metabolic conditions.

Materials and methods

Animals

This study was approved by the Institution Animal Care and Use Committees of the Smithsonian Conservation Biology Institute (SCBI) and all participating zoos. Female African elephants housed in Association of Zoo and Aquariums (AZA)-accredited zoos that exhibited normal ovarian cycles served as the control, reference population ($n = 23$ elephants at 12 zoos; mean age 31.6 years; age range 22–44 years). Non-cycling female elephants ($n = 23$ elephants at 10 zoos; mean age 33.7 years; age range 24–44 years) served as the experimental population. Details on categorising elephants as cycling or non-cycling to define our study groups are given below. Blood was collected from an ear vein by collaborating veterinarians at the facilities in which the animals resided. All elephants were well conditioned to the blood sampling procedure, which was part of the normal management routine. Blood was maintained at approximately 4°C and centrifuged in refrigerated centrifuge (4°C) at 2000*g* for 15 min within 3 h of collection. Serum samples were stored at −20°C or below until analysis.

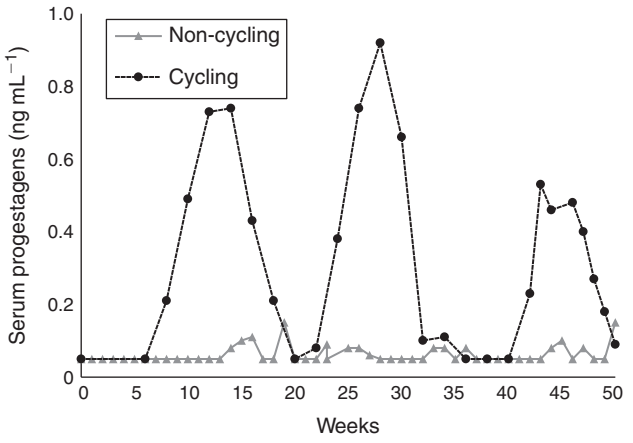

Fig. 1. Representative profiles of serum progestagen concentrations for a cycling and non-cycling female African elephant.

Determination of reproductive cyclicity status

Serum progestagens (most circulating luteal steroid in elephants are reduced pregnanes, not progesterone, so the generic term 'progestagens' is used herein) were analysed using a solid-phase progesterone ^{125}I radioimmunoassay (RIA; Seimens Medical Solutions Diagnostics, Los Angeles, CA, USA) validated for elephant serum (Brown and Lehnhardt 1995). At least 2 years of current weekly progestagen data were used to categorise the ovarian cycle status of each elephant ('cycling' or 'non-cycling'), with normal cycling represented by a 14- to 16-week cycle, consisting of an 8- to 12-week luteal phase and a 4- to 6-week follicular phase (Brown *et al.* 2004*b*). Non-cycling females exhibited consistent baseline concentrations (<0.15 ng mL^{-1}) for at least 2 years before the study. Representative profiles for a cycling and non-cycling female African elephant are shown in Fig. 1.

Body condition scoring

To assess body condition, participating zoos were provided a photographic guide containing detailed instructions as to how to obtain three standardised photographs for each elephant for visual body condition assessment. Photographs were collected

after the 2 years of progestagen data collection for categorising ovarian cycle status of each elephant. Zoos submitted the photographs for scoring, which was done by one author (KAM), using a visual BCS index recently developed and validated with ultrasound measurement of subcutaneous fat in African elephants (Morfeld *et al.* 2014). The visual BCS method consisted of a list of key body regions (ribs, backbone and pelvic bone) and the physical criteria used for assigning an overall score on a five-point scale, with 1 representing the lowest and 5 representing the highest levels of body fat. Ultrasound was used in a previous study to validate the BCS index and showed that as BCS increased, ultrasound measures of subcutaneous fat thickness also increased significantly, indicating that the scores closely coincide with physical measures of fat reserves (Morfeld *et al.* 2014).

Serum analyses

To assess metabolic hormone status, five weekly serum samples were collected within approximately 1 month of obtaining photographs for BCS.

Leptin

Serum leptin concentrations were measured using a multi-species double-antibody RIA (Xl-85K; Linco Research, St Louis, MO, USA) that relies on a ^{125}I-human leptin tracer and a guinea-pig anti-human leptin antiserum. Serum samples (100 μL), analysed in duplicate, were incubated with 100 μL antiserum at 4°C overnight; then, 100 μL of ^{125}I-leptin was added to each tube and samples were incubated at 4°C for 18–24 h. The following day, 1.0 mL cold precipitating reagent (goat anti-guinea-pig IgG serum, 3% polyethylene glycol and 0.05% Triton X-100 in 0.05 M phosphate-buffered saline) was added to all tubes except those for measurements of total counts. Tubes were vortexed, incubated at 4°C for 20 min, centrifuged at 4°C for 20 min at 2500*g* and the resulting supernatant decanted without disturbing the precipitate. Bound radioactivity was measured for 1 min in a gamma counter (IsoData 20, New York, NY, USA). The leptin RIA was validated for use in African elephant serum by demonstrating: (1) parallelism between the leptin standard curve and serial serum samples (100 μL; 1 : 2, 1 : 4 and 1 : 8) diluted with kit assay buffer; and (2) significant recovery (94%; $y = 1.089x - 0.531$) of leptin standard (50 μL; 1.56, 3.13, 6.25, 12.5, 25 and 50 ng mL^{-1}) added to 50 μL low leptin concentration serum. The inter- and intra-assay CV were <10%.

Insulin

Serum insulin concentrations were measured using a solid-phase, two-site bovine insulin enzyme immunoassay (EIA; 10-1113-01; Mercodia, Uppsala, Sweden). Bovine insulin standards, controls and serum samples (25 μL) were incubated in duplicate in a 96-well anti-bovine insulin antibody-coated plate after immediate addition of 100 μL enzyme conjugate solution containing peroxidase-conjugated anti-bovine insulin antibodies. Plates were incubated for 2 h on a shaker at room temperature. To remove unbound enzyme-labelled antibody, plates were washed six times by hand with wash buffer. Bound conjugate was detected by reaction with 200 μL of 3,3′5,5′-tetramethylbenzidine (TMB) added to each well. After 15 min incubation at room temperature, 50 μL of 0.5 M H_2SO_4 was added to stop the enzymatic reaction and the optical density was determined spectrophotometrically (450 nm filter) with an Opsys MR Microplate Reader (Dynex Technologies, Chantilly, VA, USA). The insulin EIA was validated for use in African elephant serum by demonstrating: (1) parallelism between the insulin standard curve and serial 25 μL serum samples (1 : 2, 1 : 4 and 1 : 8) diluted with 25 μL zero calibrator; and (2) significant recovery (88%; $y = 0.976x + 0.019$) of insulin standard (12.5 μL; 0.05, 0.15, 0.50, 1.5 and 3.0 mg mL^{-1}) added to 12.5 μL low insulin concentration serum. The inter- and intra-assay CVs were <10%.

Glucose

Serum glucose was determined using an automated glucose analyser (One Touch Ultra; LifeScan, Milpitas, CA, USA) and the G : I was calculated.

Data analysis

Statistical analyses were performed using SAS software (SAS Institute, Cary, NC, USA). Power analysis was conducted (80% power; GPOWER) to determine that the study sample size ($n = 23$ non-cycling and $n = 23$ cycling elephants) was sufficient for 95% confidence in the utility of the models (*F*-test). The Kolmogorov–Smirnov test was used to test for normality of the hormone data and the Levene median test was used to measure for equal variances. Data are expressed as the mean ± s.d. Five serum samples from each elephant were analysed for glucose, insulin and leptin concentrations, and a mean value calculated for each elephant. Metabolic parameters (BCS, leptin, insulin, glucose, G : I) were compared between cycling and non-cycling elephants using unpaired *t*-tests. The Cochran–Mantel–Haenszel statistic was used to test for linear trend by testing the null hypothesis that BCS was randomly distributed between the cycling and non-cycling groups. Relationships between BCS and hormone concentrations were analysed using Spearman correlation coefficients. Correlations were considered to be different from zero at $P < 0.10$. To predict cycling status from the study variables, 'non-cycling' was used as the outcome variable in regression models and BCS, leptin, insulin and G : I were examined as predictors.

Results

The number of cycling and non-cycling elephants in each BCS category is given in Table 1. There was a strong association between the physical condition of a given animal and whether it was reproductively active on the basis of serum progestagen cyclicity. The BCS values for non-cycling elephants was higher than for their cycling counterparts ($P = 0.0228$; Table 1). The most frequent (mode) BCS observed in cycling elephants was 3 (35%), whereas the most frequent BCS in the non-cycling group was 4 (52%). The category representing the most body fat (i.e. BCS 5) was observed in 44% of non-cycling and 26% of cycling elephants. Two cycling elephants had a BCS of 2

Table 1. Number of cycling and non-cycling female African elephants in each body condition score (BCS) category, where 1 = thinnest and 5 = fattest

BCS	Cycling elephants	Non-cycling elephants	*P*-value
1	0	0	
2	2	0	
3	8	1	
4	7	12	
5	6	10	
Total	23	23	
Median (range)	4 (2–5)	4 (3–5)	
Mean ± s.d.	3.73 ± 0.93	4.39 ± 0.58	0.0228[A]
Mode	3	4	

[A]Cochran–Mantel–Haenszel test for linear trend.

Table 2. Comparison of metabolic hormone concentrations between cycling and non-cycling zoo African elephants according to body condition score category

Data are the mean ± s.d. Significant values are bolded. Body condition score (BCS) was assessed on a five-point scale, where 1 = thinnest and 5 = fattest. NA, not applicable (sample size too small for statistical analysis and/or comparisons between groups); G : I, glucose-to-insulin ratio

Hormone	BCS*	Cycling	Non-cycling	*P*-value
Leptin (ng mL^{-1})	2	2.90 ± 0.71	NA	NA
	3	2.89 ± 0.93	3.30	NA
	4	3.34 ± 0.98	3.57 ± 0.71	0.62
	5	3.58 ± 0.64	4.75 ± 2.12	**0.05**
Insulin (mg mL^{-1})	2	0.59 ± 0.42	NA	NA
	3	0.45 ± 0.19	0.23	NA
	4	0.45 ± 0.10	0.56 ± 0.23	0.19
	5	0.49 ± 0.18	0.78 ± 0.33	**0.04**
Glucose (mg dL^{-1})	2	105 ± 17	NA	NA
	3	93 ± 18	70	NA
	4	87 ± 25	92 ± 15	0.56
	5	104 ± 17	100 ± 13	0.59
G : I ratio	2	219 ± 125	NA	NA
	3	206 ± 65	299	NA
	4	199 ± 54	182 ± 42	0.37
	5	227 ± 64	143 ± 43	**0.02**

compared with none in the non-cycling cohort; no elephants in this study had a BCS of 1.

When comparing mean serum hormone concentrations between reproductive categories (i.e. non-cycling vs cycling elephants), serum leptin (4.03 ± 1.63 vs 3.16 ± 0.88 ng mL^{-1}, respectively; $P = 0.041$) and insulin (0.65 ± 0.31 vs 0.48 ± 0.18 mg mL^{-1}, respectively; $P = 0.032$) concentrations were higher in the non-cycling group. Serum glucose levels did not differ between the two groups (95.0 ± 19.9 vs 94.3 ± 15.3 mg /dL^{-1} in non-cycling vs cycling elephants, respectively; $P = 0.892$). However, the G : I was lower in non-cycling than cycling females (169.82 ± 53.21 vs 227.18 ± 96.23, respectively; $P = 0.019$). Comparisons of metabolic hormones according to cyclicity and BCS categories are given in Table 2, with significant differences observed in all hormones except glucose between cycling and non-cycling elephants with a BCS of 5. Because so few elephants had a BCS of 2 or 3 (and no elephants had a BCS of 1), we were unable to perform statistical comparisons for those categories.

Table 3. Logistic regression results of metabolic parameters predicting a non-cycling status in female African elephants

BCS, body condition score; G : I, glucose-to-insulin ratio; SE, standard error of β coefficient; OR, odds ratio; CI, confidence interval

Predictors	SE	*P*-value	OR (95% CI)
BCS	0.43	0.01	3.15 (1.36–7.26)
Leptin	0.35	0.04	2.05 (1.03–4.08)
Glucose	0.017	0.89	0.99 (0.97–1.03)
Insulin	1.45	0.04	19.50 (1.08–350.84)
G : I	0.006	0.04	0.99 (0.98–0.99)

There were positive correlations between BCS and mean serum leptin ($r^2 = 0.39$; $P = 0.005$) and insulin ($r^2 = 0.30$; $P = 0.06$) concentrations. Glucose was not correlated with BCS ($r^2 = 0.18$; $P = 0.34$); however, the G : I ratio was negatively correlated with BCS ($r^2 = -0.19$; $P = 0.09$).

A logistic regression was performed to determine which model variables (BCS, leptin, glucose, insulin, G : I) contributed most to the occurrence of a non-cycling ovarian status (Table 3). The odds ratio for BCS predicts that with each 1-point increase in BCS, an elephant was approximately threefold more likely to be non-cycling. Leptin, insulin and the G : I ratio were also significant predictors of a non-cycling status.

Discussion

It is well established that ovarian activity is compromised in zoo-maintained African elephants, largely expressed as baseline levels of progestagens, indicative of complete ovarian inactivity, or as irregular cycles. The incidence of acyclicity appears to be on the rise, having increased from 22% in 2002 (Brown *et al.* 2004*a*) to 46% in 2008 (Dow *et al.* 2011). If these trends continue, the US zoological collection of African elephants will be reproductively non-viable in approximately 50 years (Faust and Marti 2011). Not surprisingly, understanding causes of morbidity, mortality and poor reproductive performance to prevent further population declines are high priorities of the AZA/Elephant Species Survival Plan (SSP) Management Committee (Keele and Ediger 2011). The AZA/SSP takes steps to ensure females of reproductive age are given the opportunity to breed and works closely with zoos to promote reproduction for population sustainability in zoos.

The present study examined one suspected cause of ovarian inactivity in the African elephant, namely obesity-related metabolic conditions, and found significant relationships between reproductive function and both body condition and circulating concentrations of insulin and leptin. Similar to Freeman *et al.* (2009), who found a relationship between high BMI and ovarian acyclicity in this species, we discovered that higher BCS values were associated with ovarian inactivity in females of breeding age. Most importantly, we determined that there were significant differences in metabolic hormone concentrations between

cycling and non-cycling females, with non-cycling elephants having higher concentrations of insulin and leptin and a lower G : I ratio. Our logistic regression analysis predicted that an elephant was approximately threefold more likely to be non-cycling with each 1-point increase in the BCS value. Together, these results are highly suggestive that excessive body condition, or body fat, and related metabolic perturbations are significant risk factors for provoking ovarian inactivity in African elephants managed in *ex situ* conditions.

The most frequently observed BCS value in cycling elephants was 3, compared with a value of 4 in the non-cycling group. However, there were cycling elephants in both the BCS 4 (30% of all animals evaluated) and BCS 5 (26%) categories, so higher body condition does not necessarily mean a female will be acyclic. This is similar to finding in humans, whereby approximately 75% of ovulatory infertility is not associated with being overweight (Grodstein *et al.* 1994; Rich-Edwards *et al.* 1994). However, obesity can exert effects on the hypothalamic–pituitary–ovarian (HPO) axis and hence disturb menstrual cyclicity and ovulation (Hartz *et al.* 1979; Lake *et al.* 1997), and overweight women are threefold more likely to suffer from infertility than women with a normal BMI (Rich-Edwards *et al.* 1994). Although sometimes having normal menstrual cycles, obese women often experience other reproductive problems, such as reduced fecundity (Fedorcsák *et al.* 2004) and an increased incidence of miscarriage (Gesink Law *et al.* 2007) compared with normal size counterparts. These are common reproductive problems in zoo elephants; for example, stillbirths and dystocias are causes of significant calf mortality in zoo elephants (Clubb and Mason 2002; Clubb *et al.* 2009; Mason and Veasey 2010). Therefore, although cyclicity *per se* may not always be affected because of excess weight, other reproductive functions could be altered. Ovarian cyclicity was the only reproductive parameter analysed in the present study, so additional studies are certainly warranted to determine how high body condition affects other aspects of reproductive health. Furthermore, in women, the impact of excess weight on reproductive function is influenced by age at obesity onset, time taken to become obese and diet (Pasquali *et al.* 2003), and these may well be important factors to consider for elephants.

In women, losing excess weight can improve fertility and lead to conception (Clark *et al.* 1995; Bray 1997; Pasquali *et al.* 2003; Norman *et al.* 2004). However, food restriction to facilitate weight loss does not always alter oestrous cycle activity, as evidenced in other species, such as horses (Vick *et al.* 2006) and rats (Marín-Bevins and Olster 1999). These latter studies suggest that excess bodyweight by itself may not directly cause reproductive dysfunction, but rather may be a contributing factor (Wade *et al.*1996; Marín-Bevins and Olster 1999; Vick *et al.* 2006). Although the exact mechanisms by which bodyweight contributes to reproduction is unknown, one proposed mechanism is that metabolic cues detected in the viscera are transmitted to the gonadotrophin-releasing hormone (GnRH)-secreting neurons in the brain, thus altering cycle activity. Whether acyclicity in elephants is due to direct or indirect effects of high BCS, the results of the present study provide the first science-based evidence that the bodyweight of zoo elephants may be related to ovarian activity, perhaps through mechanisms related to perturbations in biomarkers of metabolic status, including leptin, insulin and glucose concentrations. The next step is to determine whether high BCS is a result of excessive caloric intake (Hatt and Clauss 2006), inadequate exercise or both.

Of particular interest was the finding that non-cycling elephants with a BCS of 5 exhibited significant alterations in metabolic hormones compared with cycling elephants in that category. Specifically, insulin concentrations were higher and the G : I ratio was lower in non-cycling compared with cycling elephants with a BCS of 5. The G : I ratio was included to account for the non-fasting state of our study animals and is a common proxy for counteracting the effects of changes in glucose and/or insulin due to feeding status (Ralston 2002). Under normal conditions, insulin enhances the recruitment and growth of preovulatory follicles (Poretsky *et al.* 1999) and promotes follicle maturation and ovarian growth (Hsueh *et al.* 1994; Poretsky *et al.* 1999). The ovary is a target for insulin, acting via insulin and insulin-like growth factor (IGF) receptors (Poretsky *et al.* 1999). Insulin is an important regulator of the gonadotropic axis; reproductive dysfunction due to abnormal insulin and glucose levels results from perturbations at different levels of the gonadotropic axis, including the hypothalamus, pituitary and ovary (Codner and Cassorla 2009). Obesity induces a state of hyperinsulinaemia and insulin resistance in humans (Matthaei *et al.* 2000), which leads to hyperandrogenaemia and perturbations in the IGF system, thereby increasing the likelihood of menstrual and ovulatory disturbance in obese women (Butzow *et al.* 2000). Excess insulin may also lead to overstimulation of insulin receptors in the ovary, resulting in androgen secretion that causes premature follicle atresia and anovulation and, in some cases, the development of polycystic ovarian syndrome (PCOS; Willis *et al.* 1996; Poretsky *et al.* 1999). However, this condition is not believed to be a significant problem in captive African elephants. Ovarian cysts occur in only approximately 15% of captive female elephants (Hildebrandt *et al.* 2006) and there has been only one case study of ovarian cysts being associated with acyclicity (Brown *et al.* 1999*a*). No assessments of ovarian cysts were performed on the elephants in the present study. Ovarian cysts are a major cause of infertility in dairy cows, likely involving the IGF family system or imbalances between IGFs and IGF-binding proteins (Rodríguez *et al.* 2011). In horses, often used as a model for elephants, insulin resistance (i.e. elevated insulin) and obesity compromise reproduction through a direct action on the ovaries, rather than at the level of the hypothalamic–pituitary axis (Vick *et al.* 2007; Johnson *et al.* 2012). This assumption is based on the finding that no differences in secretion of LH or FSH are observed in mares with insulin resistance (Sessions *et al.* 2004; Vick *et al.* 2006). Acyclic African elephants have normal baseline concentrations of LH and FSH, which respond normally to GnRH challenge (Brown 2000; Brown *et al.* 2004*b*), suggesting pituitary function in this species is also not compromised. Nevertheless, our findings of normal glucose but elevated serum insulin concentrations provides evidence of disrupted insulin regulation in non-cycling African elephant females with a high BCS, which could indicate that these females may either have or be at risk of developing insulin resistance.

Leptin concentrations were also higher in non-cycling female elephants. In women, leptin plays a major role in energy balance and reproduction, with excess levels being associated with ovarian cycle problems and amenorrhoea (Mácajová *et al.* 2004; Tena-Sempere 2007; Hill *et al.* 2008; Brewer and Balen 2010). Leptin also has stimulatory effects on the HPO axis at normal serum concentrations, but can exert inhibitory effects on folliculogenesis when levels are elevated, such as those caused by obesity (Tamer Erel and Senturk 2009). In rats, leptin at high concentrations interferes with normal follicular development, including dominant follicle selection, oocyte maturation and ovulation (Duggal *et al.* 2000). In one study, just a 10% reduction in body fat led to a 53% reduction in serum leptin concentrations (Considine *et al.* 1996). Perhaps weight loss in elephants with a high BCS and elevated leptin levels could improve reproductive potential in part by reducing circulating leptin concentrations and its detrimental effects on the HPO axis. Because leptin is a hormone produced by adipose cells, concentrations vary directly with the percentage of body fat in several species, including humans (Pasquali and Casimirri 1993; Fors *et al.* 1999; Banks *et al.* 2001; Garnsworthy *et al.* 2008). We found a similar correlation with leptin and BCS in female African elephants. Hence, assessing serum leptin concentrations and/or BCS could serve as useful screening tools to identify overweight elephants in zoos. Furthermore, the gonadal steroid hormone oestrogen plays a central role in the regulation of reproduction and has a metabolic role by contributing to the regulation of energy balance (Gao and Harvath 2008). It has been shown that oestrogen levels are higher in the adipose tissue of obese compared with non-obese humans due to enhanced aromatase activity in the fatty tissue (Strong *et al.* 2013). These extragonadal steroids in excess associated with human obesity can explain the negative impacts on reproduction, including hyperandrogenic anovulation (Goudas and Dumesic 1997). Although mechanisms of metabolic hormone perturbations in elephants are unknown, based on insulin and leptin data, we theorise that derangements in metabolic activity, rather than the amount of body fat alone, may be altering ovarian activity in the fattest elephants.

In conclusion, the potential negative effects of excess bodyweight and poor metabolic status on reproductive and health function in zoo elephants could be significant. This is the first study to assess body condition and metabolic hormones in relation to ovarian activity in the elephant. This issue could also be true for other zoo-maintained animals, which warrants a need to re-examine diets and food-based enrichment programs in many zoo species that are suspected of being overweight but also need to reproduce to support the species. We found that BCS, leptin and insulin concentrations, as well as the G : I ratio, were all significantly altered in non-cycling compared with normal cycling females, suggesting that factors related to obesity may be compromising reproductive function. Studies in humans and horses indicate that similar changes in bodyweight and metabolic markers can severely compromise fertility, and that even small reductions in body fat and weight loss may effectively restore ovarian activity (Clark *et al.* 1995; Hill *et al.* 2008; Miller *et al.* 2008). In fact, overweight and obese women are advised to participate in lifestyle intervention programs to lose weight before entering fertility programs (National Institute for Clinical Excellence 2004). Given that the zoo African elephant population is not self-sustaining, a logical next step would be to promote weight loss in non-cycling females by changes in diet and/or exercise with the goal of improving metabolic health and resumption of ovarian activity. We also need to assess body condition, insulin, glucose and the G : I ratio in the context of other causes of morbidity and mortality in zoo elephants (e.g. kidney disease, foot and joint problems; Clubb and Mason 2002; Clubb *et al.* 2009; Lewis *et al.* 2010; Mason and Veasey 2010), which are commonly associated with insulin–glucose dysfunction in other species (Bailey *et al.* 2008; Geor and Frank 2009; Dimitroulas *et al.* 2013; Roberts *et al.* 2013). Because of potential health effects, facilities housing elephants should monitor body condition and metabolic status regularly and, if necessary, implement mitigating management strategies for at risk individuals.

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