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Adiposity and reproductive cycling status in zoo African elephants

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Abstract

Objective—The majority of zoo African elephants exhibit abnormal reproductive cycles, but it is unclear why. Acyclicity has been positively associated with body condition scores. The objective of this study was to measure body composition and examine the relationship between adiposity and cyclicity status, mediated by glucose, insulin, leptin, and inflammation.

Methods—Body composition was assessed by deuterium dilution in 22 African elephants. Each elephant was weighed and given deuterated water orally (0.05 mL/kg), and blood was collected from the ear prior to and five time after deuterium administration. Glucose, insulin, leptin and pro-inflammatory biomarker concentrations in serum were determined.

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DISCLOSURES

The opinions expressed herein are those of the authors and not necessarily those of any other organization with which the authors are affiliated.

Results—Body fat percentage ranged from 5.24% to 15.97%. Fat adjusted for fat free mass (FFM) and age was not significantly associated with cyclicity status (P=0.332). Age was the strongest predictor of cyclicity status (P=0.040). Fat was correlated with weight (ρ=0.455, P=0.044) and when adjusted for FFM with circulating glucose (ρ=0.520, P=0.022), and showed a trend for association with leptin (unadjusted: ρ=0.384, P=0.095; adjusted for FFM: ρ=0.403, P=0.087).

Conclusions—Deuterium dilution appears to be an available technique to measure body composition in African elephants. In this sample, fat was not associated with cyclicity status and age may be more important to cyclicity status.

Keywords

African elephant; body composition; reproductive cycling; fat

Introduction

Obesity is an epidemic not only affecting humans but animals associated with humans, including companion and domestic animals [1] and, possibly, zoo animals. Approximately 33% of North American female zoo African elephants (*Loxodonta africana*) are classified as obese, based on the body condition score (BCS) [2]. BCS is a visual assessment of key skeletal structures and is, therefore, a subjective assessment of subcutaneous body fat stores [3]. Although BCS provides an overall assessment of elephant body condition, it does not quantify body composition (fat [FM] or fat free mass [FFM]).

Over 50% of zoo African elephants in the United States exhibit irregular reproductive cycles or are acyclic [4], yet the causes of acyclicity remain unknown. Previous studies have demonstrated a positive association between condition indices (i.e., BCS and body mass index) with rates of reproductive acyclicity in zoo African elephants [5, 6], and there is evidence in other species linking body composition to reproductive impairments [7–9]. Thus, it is not unreasonable to posit that zoo elephants with increased FM may be more likely to exhibit abnormal reproductive cycles than are elephants with lower FM.

Assessing elephant adiposity is a necessary next step in evaluating this species' reproductive and overall health. Similar reproductive impairments to those observed in zoo elephants have been noted in other species and shown to be associated with excess FM [9, 10]. The relationship between excess FM and reproductive problems may be mediated through the animal's metabolic health. For instance, leptin and insulin are positively associated with FM [11, 12]. Excess fat is also mechanistically linked with inflammation [13]. Leptin, hyperinsulinemia, and chronic inflammation have all been shown to play a role in reproduction and related dysfunction [14, 15].

Given the elephant's size, deuterium dilution offers a tenable solution to estimate body composition *in vivo*. Deuterated water has been used to measure total body water (TBW) (i.e., the combination of intra- and extracellular water) in animals ranging in body size from the bumblebee to the Atlantic Walrus [16, 17]. Yet, to our knowledge, no published studies have been conducted in the largest terrestrial mammal, the elephant. Deuterium (^{2}H), a non-

radioactive isotope of hydrogen (^{1}H), when administered to animals, is diluted by the ^{1}H in water molecules, providing an estimate of TBW [18]. TBW is assumed to be restricted to the animal's FFM compartment; thereby, using the standard mammalian hydration constant, based on TBW of 0.73, FFM can be calculated [19]. FM is then inferred by subtracting FFM from weight.

Thus, the purpose of the present study is to measure body composition via deuterium dilution and then to evaluate the relationship between body composition and reproductive cyclicity status in zoo African elephants and to investigate the relationship between body composition, circulating glucose, insulin, leptin and inflammatory markers.

Materials and methods

Animals

This study was approved by the Institution Animal Care and Use Committee of the University of Alabama, Birmingham (UAB), and the participating zoos. Of 19 zoos contacted, 13 zoos participated and were visited between November 2014 and June 2016. Data from five of those zoos were omitted because the plateau method for determining TBW was found to be invalid (Supporting Information Table S1). A total of 22 female African elephants of reproductive-age (≥16 years of age; Table 1) housed in eight accredited U.S. institutions were studied. Elephants were not pregnant. All isotopes were analyzed blind to the animal status.

Body Composition

Elephants were weighed to the nearest 5 pounds, pound, or kilogram, depending on the institution's scale. Venous blood was collected from an ear vein by zoo personnel prior to deuterated water administration to determine background isotope enrichment. Subsequently, an oral dose of (99.9% APE) deuterium oxide (0.05 mL D_2O/kg of weight; DLM-4-1000, Cambridge Isotopes, Tewksbury, Massachusetts) was administered using bread (Publix®, Birmingham, Alabama) as a vehicle. Bread was weighed (to the closest 0.01 g, Pioneer, Ohaus, Pine Brook, New Jersey), deuterated water was carefully added, and the bread was then reweighed. The difference in weight represented the dose of deuterated water. Each elephant received four to five pieces of bread with approximately 40–50 g of deuterated water per piece. Blood (~9 mL) was sampled at regular intervals (~24, 120, 240, 360, and 480 hours) post deuterium administration. All samples sat up to 30 minutes in an airtight container to allow for coagulation. Whole blood was centrifuged and serum was collected, aliquoted, and frozen at a minimum of −20 °C until shipped on dry ice overnight to UAB. Samples were then kept in a frost-free −80 °C freezer until analysis.

Isotope ratio mass spectroscopy (Finningan Delta V Advantage, Thermo Fisher Scientific, Waltham, Massachusetts) analysis was carried out by UAB's Nutrition Obesity Research Center's Metabolism Core with guidance and support from the Energetics Research Group at the University of Aberdeen, Aberdeen, Scotland. In brief, the ^{2}H/^{1}H delta value was converted to parts per million, and used to calculate deuterium dilution space size [18]. The

dilution space (N_d) is considered to reflect TBW content, which is then converted to FFM using the hydration constant (see Supplemental Information).

To determine blood sampling intervals, in one elephant, blood was collected from an ear vein prior to deuterated water administration and then daily up to 391 hours post administration (Figure 1). These samples were analyzed by liquid water isotope analysis (Los Gatos Research, San Jose, California, USA) at the Energetics Research Group (University of Aberdeen, Scotland).

The water turnover rate was calculated as provided in the Supporting Information.

Determination of Reproductive Cyclicity Status

Reproductive cyclicity status was provided by participating zoos and was based on progestogen analyses of longitudinal serum samples [20]. When possible, cycling status was confirmed through longitudinal samples (45% of samples) at the Smithsonian Conservation Biology Institute Endocrinology Laboratory.

Body Condition Score BCS

BCS was based on photographs and taken as previously described [3]. Briefly, photographs of each elephant were taken from three angles (side, rear, and rear-angle view). Two assessors (DEC and KM) visually scored the elephant on a 5-point scale based on key skeletal regions (ribs, pelvic bone, and backbone), where a score of 1 represents the least amount of subcutaneous fat and 5 represents the most amount of subcutaneous fat (see Supplemental Information).

Serum analyses

Assays were conducted on the blood samples taken prior to administration of deuterium. The blood was collected in the morning before elephants received their first meal of the day. Elephants' last meals were given during the late afternoon or evening the day before blood collection. Samples were typically run neat (i.e., not diluted), but if above the assay range, they were diluted in reagent diluent until they fell within the detectable range of the assay.

Serum glucose was measured by an automated glucose analyzer (Stanbio Sirrus, Stanbio Laboratories, Boerne, Texas, USA). Samples (3 μL) were done in singlicate and analyzed at the same time.

Serum insulin concentrations were determined with one assay using a solid-phase, two-site bovine insulin enzyme immunoassay (EIA; 10-1201-01; Mercodia, Uppsala, Sweden) validated for use in African elephants [5]. Samples were done in duplicate. The intra-assay CV (coefficient of variation) was 5.2%. See Supporting Information for detailed methods.

Serum leptin concentrations were measured with one assay using a multi-species double-antibody RIA (XL-85K; Millipore, Billerica, Massachusetts, USA) validated for African elephants [5], which utilized a ^{125}I-human leptin tracer and a guinea-pig anti-human leptin antiserum. Samples were done in duplicate. The intra-assay CV was 4.29%. See Supporting Information for detailed methods.

Serum Amyloid A (SAA) was determined using a RX Daytona automated clinical chemistry analyzer (Randox Industries-US Ltd., Kearneysville, West Virginia, USA) and commercially available reagents (150 μL), calibrators (0.1–500 mg/L) with two-level controls (Eiken Chemical Co. Ltd, Tokyo, Japan). Samples (4 μL) were run in singlicate and analyzed at the same time.

Serum tumor necrosis factor alpha (TNF-α) concentrations were measured using an equine TNF-α sandwich enzyme immunoassay kit (EIA; ESS0017; Thermo Fisher Scientific, Waltham, Massachusetts; Edwards et al., 2016 unpublished data) according to manufacturer's instructions. Inter-assay CVs were 6.4% and 2.6% for low and high concentration controls, respectively. CVs for all duplicates were between 0.2–13.4%. See Supporting Information for detailed methods.

Statistical analyses

All statistical analyses were performed using SAS 9.4 statistical software (SAS Institute, Cary, North Carolina, USA) and specified prior to examining data, unless otherwise stated. Although 22 elephants were included in this study, two elephants were excluded from body composition statistical analyses because blood sample time points were unknown and therefore accurately calculating body composition was not possible.

The primary model to address our main hypothesis was a generalized estimating equation, regressing cyclicity status on FM adjusting for FFM, and age to the power lambda (age^{λ}). The lambda value for age was calculated by fitting a nonlinear model based on data previously collected on 95 female African elephants, for which age and cyclicity status were known. The best estimate of lambda was 1.62. FM, FFM, and $age^{1.62}$ were included as continuous variables. To adjust for factors related to residing in the same zoo, the zoo ID was treated as random effect in all the models. The second logistic model added FM^2 and FFM^2 to the primary model, as the relationship between body composition and cyclicity status may be nonlinear. Secondary sensitivity analyses were conducted on the primary logistic model after looking at the data. Theses analyses included the addition of nulliparous status, dominance status, and whether the elephants were housed with male elephants. Nulliparous and dominance status were included as dichotomized variables, while elephants were characterized as either not housed with male elephants, housed with males with direct contact, or housed with males without direct contact.

The mediation of FM in predicting cyclicity status was tested using a 2-sided Sobel test [21]. Mediators tested were glucose, insulin, SAA, and TNF-α (see Supporting Information).

Pearson correlations between FM and weight, BCS, leptin, glucose, and insulin were conducted. Spearman correlations were conducted between FM and SAA and TNF-α because of the inflammatory biomarkers' non-normal distribution. Correlations between body weight and water turnover, in addition to BCS and weight, FFM, FM, $age^{1.62}$ and percent body fat, were determined. Partial correlations between FM and leptin, glucose, and insulin, adjusted for FFM, were conducted. Relative fat was determined by the residual for each elephant when FM was regressed on weight.

Descriptive statistics between cycling and non-cycling groups were assessed. See Supporting Information for details. Significance level was accepted at P<0.05 (2-tailed).

Results

Fifty-nine percent of the elephants exhibited normal reproductive cycles at the time of body composition assessment (*n*=13 from eight zoos; mean age 31.3 ± 2.1 years; age range 16–48 years), while the remaining 41% exhibited abnormal reproductive cycles (*n*=9 from six zoos; mean age 39.9 ± 1.9 years; age range 33–51 years). Descriptive statistics by cyclicity status are in Table 2.

Body composition and reproductive cyclicity status

Body composition was estimated by deuterium dilution (Table 1) based on the washout curve for each elephant (Supporting Information Figure S1). Body fat percentage averaged 9.69% (SD: 3.18, range: 5.24% –15.97%; *n*=20). Generalized estimating equation models analyzing predictors of cyclicity status are in Table 3. FM was not shown to be associated with cyclicity status, either unadjusted (P=0.131) or adjusted for FFM and age$^{1.62}$ (P= 0.332; Figure 2). When FFM^2 and FM^2 were included in the model, FM was not significantly associated with cyclicity status (P=0.350). When nulliparous status was included, the primary model improved but remained non-significant (P=0.158). The addition of male interaction to the primary model did not improve significance (P=0.337) unless included with nulliparous status (P=0.075). When nulliparous and dominance status were included in the primary model, FM was not associated with cyclicity status (P=0.172). BCS did not predict cyclicity status either unadjusted (P=0.234) or adjusted for age$^{1.62}$ (P=0.750). Age$^{1.62}$ was a significant predictor of cyclicity status (P=0.040). Weight, unadjusted, was a significant predictor of cyclicity status (P=0.038), but was not significant when adjusted for age$^{1.62}$ (P=0.647).

BCS was strongly correlated with age (ρ=0.603; P=0.003), weight (ρ=0.759; P<0.0001; Figure 3A), FFM (ρ=0.702, P=0.001; Figure 3B) and FM (ρ=0.583; P=0.007; Figure 3C) but not with relative fat (ρ=0.256; P=0.276; Figure 3D) or percent body fat (ρ=0.337; P=0.146).

Body composition and glucose, insulin, leptin and inflammatory biomarkers

The correlation between FM and relative fat with glucose (ρ=0.379; P=0.100; ρ=0.555; P=0.011, respectively; Figure 4A–B), insulin (ρ=0.369, P=0.110; ρ=0.352; P=0.128, respectively; Figure 4C–D), and leptin (ρ=0.384; P=0.095; ρ=0.399; P=0.081, respectively; Figure 4E–F) nearly reached significance. FM, adjusted for FFM, was correlated with glucose (ρ=0.520; P=0.022) and trended towards significance with insulin (ρ=0.371; P=0.118) and leptin (ρ=0.403; P=0.087). FM was not correlated with SAA (ρ=0.007; P=0.979; Figure 4G) or TNF-α (ρ=−0.0353; P=0.883; Figure 4H). Weight was not correlated with water turnover rate (ρ=0.357; P=0.123). Glucose was correlated with insulin (ρ= 0.430; P=0.046).

Cyclicity status and glucose, insulin, leptin and inflammatory biomarkers

Glucose, (P=0.366), insulin (P=0.406), leptin (P=0.991), SAA (P=0.095) and TNF-α (P=0.349) did not significantly differ by cyclicity status (Table 1). Although there was no overall association between FM and cyclicity status, mediation analyses were still conducted to better understand why the variables did not relate. Glucose (P=0.276) and insulin (P=0.220) were not mediators between FM and cyclicity status. SAA, analyzed as a dichotomous (P=0.564) and continuous variable (P=0.477), and TNF-α, analyzed as a dichotomous (P=0.841) and continuous variable (P=0.432), were not mediators between FM and cyclicity status.

Discussion

This study investigated the association between adiposity with reproductive cyclicity status, mediated through glucose, insulin, leptin and pro-inflammatory biomarkers. We did not find that FM was significantly associated with cyclicity status in zoo African elephants, nor was the relationship mediated through inflammation, glucose, or insulin. FM was correlated with glucose and nearly with leptin levels. Noncycling elephants were more likely to be older.

Deuterium dilution by the intercept method appears to be a useful, noninvasive technique that can be used to estimate body composition of African elephants. Deuterium dilution measures TBW by using either the plateau or the intercept method [22]. The plateau method assumes equilibrium is reached rapidly and neither deuterium nor body water are metabolized during equilibrium [18]. These assumptions may be violated in elephants because of their large body volume and slow heart rate [23] (Supporting Information Table S1). The intercept method does not rely on a measurement during equilibrium and therefore allows for a longer equilibrium period and continuous water turnover over an extended period of time through the collection of several samples over time and back extrapolating to when deuterated water was administered [22]. The intercept method was therefore used in the present study, in which we observed a log-linear elimination in deuterium enrichment over time in the elephants.

The derivation of FM from TBW estimated by deuterium dilution is based on the ratio of TBW to FFM, termed the hydration constant [19], which to our knowledge has not been determined in elephants. Therefore, we used the most regularly used hydration constant of ~0.73 [24]. Pace and Rathbun [25] first recommended this hydration constant based on several species of mammals, and since then it has been reinvestigated and confirmed [24]. However, the hydration constant may vary by body size. Pitts and Bullard [26] demonstrated that as body size increases, there is a slight decrease in the TBW/FFM. This may be related to larger mammals having a larger proportion of their body mass comprised of bone, as skeletal tissue is comparatively “dry” [26]. This could certainly pertain to the African elephant, which may have a hydration constant different from 0.73. For instance, if the hydration constant was 0.71, body fat for this sample would be ~3–14%. If the hydration constant was 0.75, body fat for this sample would be ~8–18%. Even though the exact hydration constant of the elephant is unknown, it should not affect the primary results, as any change in the hydration constant would result in a linear transformation of the FM values.

Body fat percentages in this study sample ranged from approximately 5% to 16%. Most African ungulates have little subcutaneous fat, which may help facilitate heat dissipation [27]. Most published studies examine only small areas of the body, which show limited quantities of fat, and may be inappropriate to extrapolate to total body fat. For example, female giraffes are found to have 0.52% to 1.39% rump fat [27]. Similarly, the rump fat percentage of male kudu and blesbok is 1.3% and 1.4%, respectively [28]. Comparatively more work has been done with domesticated animals. For instance, horses have on average 5.1% to 22.3% body fat, which is correlated with rump fat [29]. Elephants have a low surface area to volume ratio, so it would be expected elephants may have little subcutaneous fat; however, the study sample is from zoos where food is plentiful and of high quality. To determine the accuracy of our body composition measures would require comparing our technique to carcass analysis, which is not feasible.

Obesity has been associated with anovulation in other mammalian species [7, 30, 31], and in previous studies a relationship between body condition indices (e.g., BMI and BCS) and cyclicity status has been observed in zoo African elephants [5, 6]. However, the current study did not find such a relationship between adiposity and cyclicity status, which could be due to a variety of factors. For instance, our sample size (n = 20) was smaller than our previous work [9], which examined 50 elephants (half cycling, half not). Based on our data, to have 0.80 power to detect an association between FM and cyclicity status would require approximately 170 elephants. Differences in results may be age related, which is supported by a recent study demonstrating age is positively associated with acyclicity [4]. We found noncycling elephants to be significantly older and heavier than those that were cycling, with age and weight strongly positively correlated. In our previous study [9], we did not adjust for age, which might explain the discordance in results. Further, BCS correlated most strongly with weight. Therefore, it appears BCS may be capturing the overall weight and not the amount of fat of the elephant. As age and weight are highly correlated (ρ=0.679), age may be driving the relationship between BCS and cyclicity status and should be considered in future studies.

The elephant's metabolic state, rather than absolute FM, may be more important to consider in relation to reproductive and overall health. FM, adjusted for FFM, was positively correlated with glucose levels, and FM, adjusted and unadjusted, almost reached significance with insulin levels. There was one insulin outlier, and although there was no reason to exclude it, running the analyses without this data point resulted in a significant correlation between unadjusted and adjusted FM and insulin (ρ= 0.479, P= 0.038; ρ=0.516, P=0.028, respectively). In a post-prandial state, elevated blood glucose levels stimulate insulin secretion [32], in turn promoting glucose uptake and utilization and suppressing gluconeogenesis [32]; therefore, abnormal insulin secretion, in addition to insulin resistance, can lead to hyperglycemia [32]. Hyperglycemia and hyperinsulinemia are both associated with obesity and comorbidities [32, 33]. Although FM may not be associated with acyclicity, it may impact health in other ways, such as increasing the risk of arthritis and calving problems [34].

Hyperglycemia and hyperinsulinemia stimulate an inflammatory state [35, 36], and in other species, inflammation is associated with reproductive impairments and obesity [15].

However, our results do not indicate an inflammatory state. The SAA reference interval for clinically healthy Asian elephants is 0 to 47.5 mg/L [37]. No elephant, cycling or non-cycling, had levels greater than 3.5 mg/L, suggesting all the elephants in this study had low levels of inflammation. Further, SAA levels were not correlated with FM, adjusted or unadjusted, even though SAA is the most sensitive acute phase protein in elephants [37]. TNF-α was not associated with FM, adjusted or unadjusted. Taken together, results suggest these elephants did not have chronic inflammation, as observed in other species that exhibit obesity and reproductive impairments [15, 38], at least measured by these inflammatory factors; thus, these elephants may not be obese.

FM should be monitored as it is an active endocrine organ. In addition to other adipokines, white adipose tissue produces leptin [39]. Leptin plays a permissive role in activating the reproductive axis and, when levels are abnormally high as observed in an obese state [39], prevents ovarian steroidogenesis, inhibiting proper follicle development [40]. This is likely not a reason for acyclicity in these elephants, as leptin levels were similar between the two groups; however, as leptin nearly correlated significantly with FM, there may be other related health issues to consider. For example, similar to girls with obesity [39], zoo elephants reach puberty at earlier ages than their wild counterparts [10]. Earlier puberty will likely expose the elephants to more reproductive cycles and associated endogenous hormones over their lifetime, which may lead to the development of reproductive tract pathologies [10]. With known endocrine function, continued fat accrual may possibly lead to a point at which elephants show similar metabolic dysfunction exhibited by humans and domestic animals with obesity.

This study demonstrated that deuterium dilution can be used to estimate body composition in African elephants. We used a method that has proven accurate over the decades in other species with a 10^5 times difference in body size. Thus, although assumptions were made (e.g., hydration constant) and there was an inability to validate the technique by total carcass analysis, the method appears to be robust over time and species. Results open up a new avenue of research questioning for large herbivores and the effects of diet and exercise on adiposity and its relation to health and reproduction.

In conclusion, obesity and inflammation were not unequivocally found to be significant factors for this study sample regarding acyclicity status. Regardless, there does appear to be a relationship between FM and metabolic health in African elephants. The majority of these elephants appear to be metabolically healthy, but some could be categorized as overweight or at risk for metabolic dysfunction. This supports the need to individualize management strategies, as each elephant responds uniquely to the environment and diet. This is of paramount consideration, as the zoo elephant population is currently not self-sustaining.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Importance Questions

What is already known about this subject?

- The North American zoo African elephant population is not self-sustaining.
- Over half of the North American zoo female African elephant population exhibits abnormal reproductive cycles, but it is unclear why.
- Body condition scores and body mass index have been shown to be positively associated with reproductive cyclicity status.

What does your study add?

- To date, no work has assessed the body composition of the African elephant to investigate the relationship between adiposity and reproductive impairments.
- Adiposity does not appear to be associated with reproductive cyclicity status.
- Adiposity is associated with glucose and nearly reached significance with leptin, but not with a chronic inflammatory state.

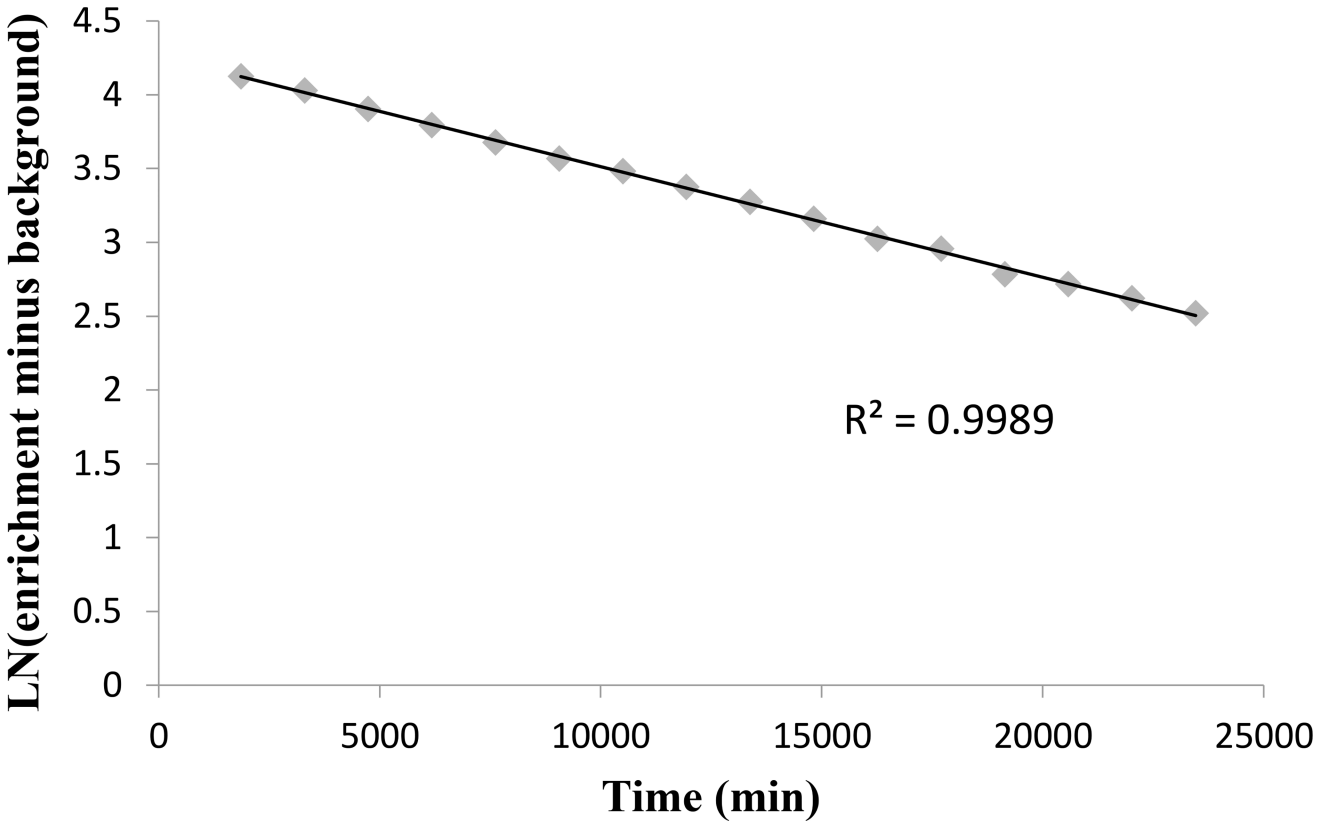

Figure 1.

Natural log deuterium concentration in venous blood in one reproductive-age female zoo African elephant after enriched orally with deuterated water at Time = 0.

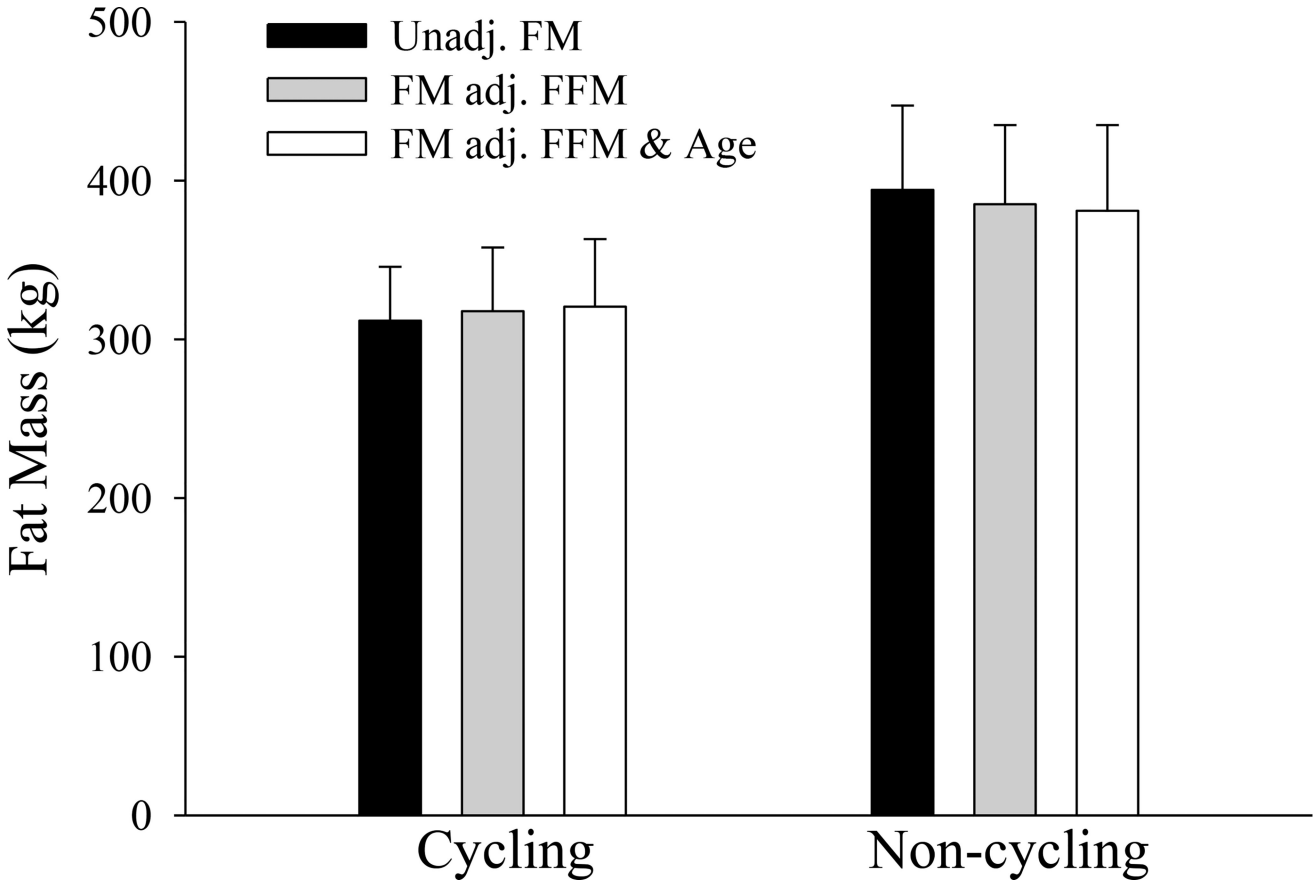

Figure 2.

Fat mass, fat mass adjusted by fat free mass, and fat mass adjusted by fat free mass and age by cycling status.

FM: Fat mass; FFM: Fat free mass.

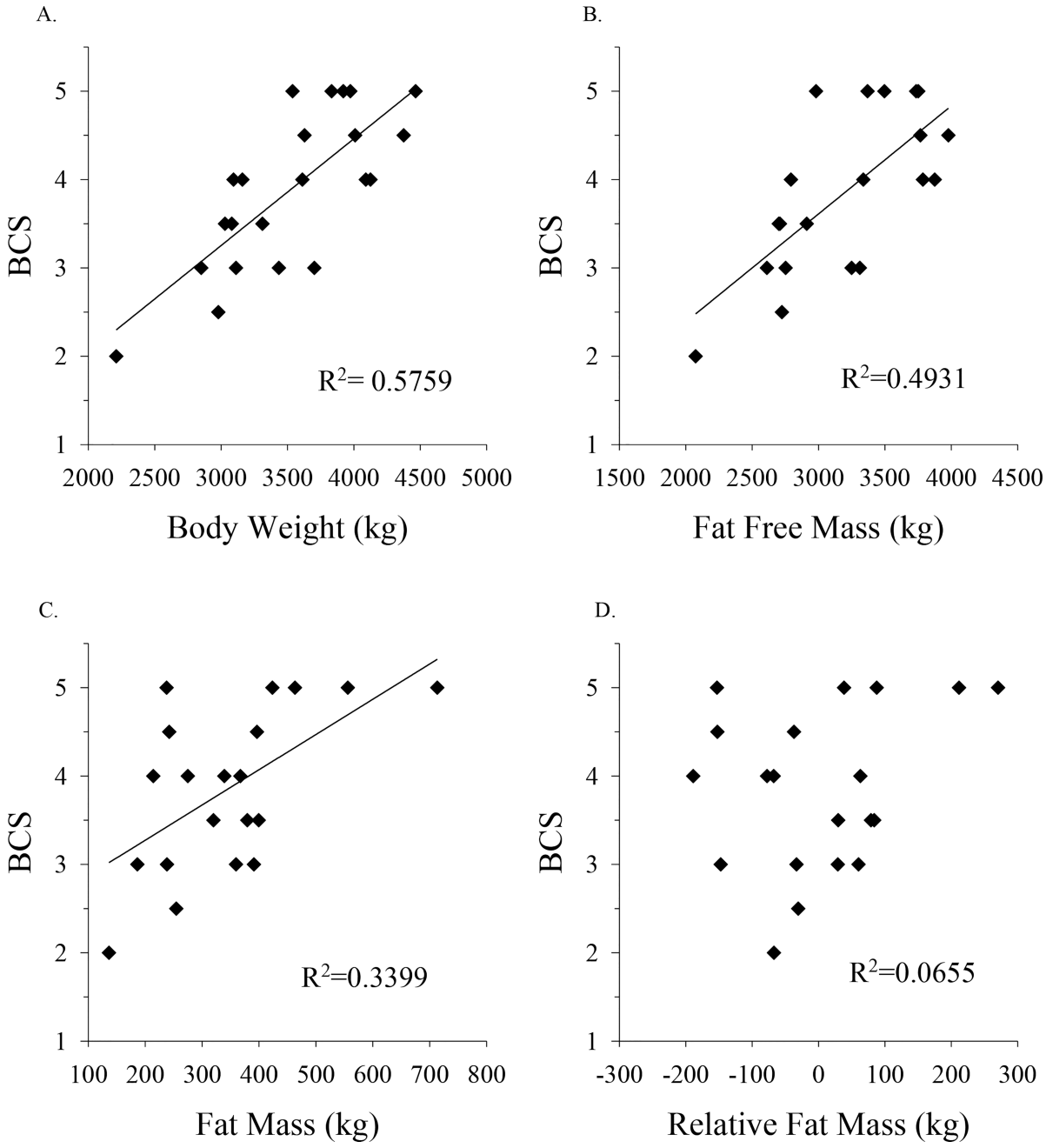

Figure 3.

A: The relationship between BCS and body weight; B: Relationship between BCS and FFM; C: Relationship between BCS and fat mass; D: Relationship between BCS and relative fat. Relative fat was determined by the residual for each elephant when fat mass was regressed on body weight.

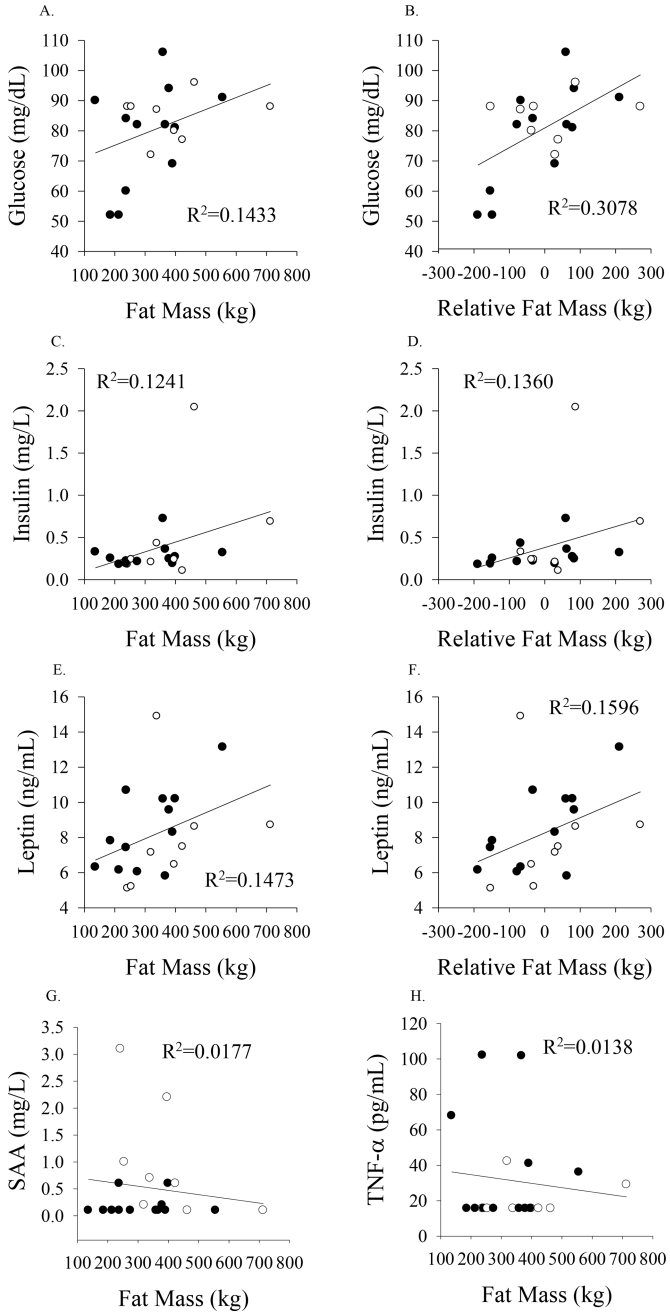

Figure 4.

A: The relationship between fat mass and glucose; B: Relationship between relative fat mass and glucose; C: Relationship between fat mass and insulin; D: Relationship between relative fat mass and insulin; E: Relationship between fat mass and leptin; F: Relationship between relative fat mass and leptin; G: Relationship between fat mass and SAA; and H: Relationship between fat mass and TNF-α.

Closed circles: cycling elephants; Open circles: non-cycling elephants. Relative fat was determined by the residual for each elephant when fat mass was regressed on body weight.

Table 1

Body composition of reproductive-aged female zoo African elephants using deuterium dilution by intercept method.

ID	Age	BW (kg)	Nd (kg)	TBW (kg)	TBW (%TBM)	FFM (kg)	FM (kg)	WTR (L/day)
A	32	3436	2467.22	2372.32	69.04	3249.75	186.25	301.01
B	51	4125	2874.53	2763.97	67.00	3786.26	338.74	260.40
C	30	3311	2210.63	2125.61	64.19	2911.79	399.21	214.77
D	26	2850	1982.87	1906.61	66.90	2611.79	238.21	458.38
E	26	3080	2178.09	2094.32	68.00	2868.93	211.07	317.24
F	26	3112	2089.72	2009.35	64.57	2752.53	359.47	385.71
G	35	3833	2420.62	2327.52	60.73	3188.38	644.62	291.87
H	37	4090	2942.45	2829.28	69.17	3875.73	214.27	441.79
I	43	4010	2860.40	2750.39	68.59	3767.66	242.34	489.45
L	33	2979	2068.54	1988.99	66.76	2724.64	254.36	341.28
M	33	3538	2263.92	2176.84	61.53	2981.98	556.02	234.60
N	37	3613	2534.21	2436.74	67.45	3337.99	275.01	262.13
P	33	3973	2835.94	2726.87	68.63	3735.43	237.57	285.99
R	32	3703	2612.84	2512.35	67.85	3441.57	261.43	338.25
S	34	3029	2056.79	1977.68	65.29	2709.15	319.85	215.63
T	16	2211	1575.02	1514.44	68.50	2074.58	136.42	169.45
U	43	4375	3020.52	2904.34	66.38	3978.55	396.45	319.15
W	48	3160	2120.27	2038.72	64.52	2792.77	367.23	297.92
X	40	3920	2654.58	2552.48	65.11	3496.54	423.46	366.13
Y	40	4465	2848.31	2738.76	61.34	3751.72	713.28	399.08

BW: body weight; Nd: dilution space; TBW: total body water in kg and the percent of total body mass (TBM); FFM: fat free mass; FM: fat mass; WTR: water turnover rate.

Table 2

Sample characteristics of the study sample.

	Cycling (N=13)	Non-cycling (N=9)
Age (years)	31.3 ± 2.1	39.9 ± 1.9[a]
Body Weight (kg)	3321 ± 137	3818 ± 176[b]
Fat Mass (kg)	312 ± 34	394 ± 53
Fat Free Mass (kg)	3028 ± 145	3448 ± 173
Height (in)	99 ± 1	101 ± 2
Length (in)	88 ± 2	96 ± 1[b]
Nulliparous	8/13	7/9
BCS	3.6 ± 0.2	4.3 ± 0.3
Glucose (mg/dL)	78.62 ± 4.52	83.44 ± 2.60
Insulin (μg/L)	0.309 ± 0.043	0.490 ± 0.202
Leptin (ng/mL)	8.53 ± 0.61	8.51 ± 1.12
SAA (mg/L)	0.2 ± 0.1	0.9 ± 0.4
TNF-α (pg/mL)	36.4 ± 9.1	20.6 ± 3.5

Data, except for nulliparous, presented as mean ± SE. Nulliparous data presented as number of elephants that were nulliparous out of total number of elephants. BCS: body condition score.

[a] $P<0.01$ significance between cycling and non-cycling elephants.

[b] $P<0.05$ significance between cycling and non-cycling elephants.

Table 3

Estimates for FM in statistical models to predict cycling status.

Model	Estimate	SE	95% CI		P
Cycling = FM	0.005	0.003	−0.002	0.012	0.131
Cycling = FFM FM	0.005	0.004	−0.004	0.014	0.249
Cycling = $age^{1.62}$ FFM FM	0.004	0.004	−0.004	0.013	0.332
Cycling = $age^{1.62}$ FFM^2 FM^2	0.000	0.000	−0.000	0.000	0.350
Cycling = $age^{1.62}$ nulliparous FFM FM	0.016	0.011	−0.006	0.037	0.158
Cycling = $age^{1.62}$ dominant FFM FM	0.004	0.004	−0.004	0.012	0.359
Cycling = $age^{1.62}$ dominant nulliparous FFM FM	0.014	0.010	−0.006	0.034	0.172
Cycling = $age^{1.62}$ male FFM FM	0.004	0.004	−0.004	0.013	0.337
Cycling = $age^{1.62}$ male nulliparous FFM FM	0.046	0.026	−0.005	0.097	0.075

Cycling: cycling status; FFM: fat free mass; FM: fat mass; Dominant: dominance status; Nulliparous: nulliparous status; Male: housed with males with direct contact, housed with males with indirect contact, or not housed with males.