**TITLE**

**Body composition is not associated with reproductive cycling status, but with metabolites, in zoo African elephants**

**AUTHORS**

Daniella E. Chusyd1,2, Janine L. Brown3, Catherine Hambly4, Maria S. Johnson1, Kari Morfeld5, Amit Patki6, John R. Speakman4,7, David B. Allison1,2,8,9,10 and Tim R. Nagy1,2,9,10\*

**AFFILIATIONS**

1Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, USA; 2Nutrition Obesity Research Center, Birmingham, AL, USA; 3Department of Reproductive Sciences, Conservation & Research Center, National Zoological Park, Smithsonian Institution, Front Royal, VA, USA; 4Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, Scotland, UK; 5Kansas City Zoo, Kansas City, KS, USA; 6Department of Biostatistics, University of Alabama at Birmingham, Birmingham, AL, USA; 7Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; 8Office of Energetics, University of Alabama at Birmingham, Birmingham, AL, USA; 9Nathan Shock Center, University of Alabama at Birmingham, Birmingham, AL, USA; 10Diabetes Research Center, University of Alabama at Birmingham, Birmingham, AL, USA

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**\*CORRESPONDING AUTHOR**

Tim R. Nagy, PhD

University of Alabama at Birmingham

Department of Nutrition Sciences

Webb 421, 1675 University Blvd.

Birmingham, AL 35294

Email: [tnagy@uab.edu](mailto:tnagy@uab.edu)

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**Study Importance Questions**

* Although it is unclear why close to half of the North American zoo female African elephant population exhibits abnormal reproductive cycles, non-cycling elephants have been shown to have higher body condition scores.
* To date, no work has assessed the body composition of the African elephant in this context to determine if adiposity is associated with reproductive cycling status.

**Abstract**

**Objective**

It is unclear why more than half of the U.S. zoo African elephant population exhibits abnormal reproductive cycles, yet no studies have quantified fat mass of African elephants to examine its relationship with reproductive and overall health. The objective of this study was to determine whether body fatness was associated with reproductive cycling, metabolites and inflammation.

**Methods**

Body composition was assessed by deuterium dilution in 22 African elephants. Each elephant was weighed, given deuterated water orally (0.05mL/kg), and blood was collected from the ear prior to and five times after deuterium administration within a three-week period. Metabolite and proinflammatory biomarker concentrations in serum were determined.

**Results**

Fat mass adjusted for fat free mass (FFM) and age were not significantly associated with cycling status (P=0.332). Age was the strongest predictor of acyclicity (P=0.040). Fat mass was correlated with body weight (ρ=0.455, P=0.044) and circulating glucose (unadjusted: ρ=0.379, P=0.100; adjusted for FFM: 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**

In this sample, fat mass was not associated with cycling status. These findings suggest that age may be a more important contributor to cycling status. Fat may negatively influence the elephant’s metabolic health.

**Introduction**

Obesity is an epidemic not only affecting humans, but also animals associated with humans, including companion and domestic animals [[1](#_ENREF_1), [2](#_ENREF_2)], and possibly, animals under human care in zoological institutions. In North America, approximately 33% of female zoo African elephants (*Loxodonta africana)* have obesity, based on the body condition score (BCS) [[3](#_ENREF_3)]. BCS is a subjective measurement of the subcutaneous body fat stores, based on a visual assessment of key skeletal structures [[4](#_ENREF_4)]. Although BCS provides a quick overall assessment of elephant body condition, it does not quantify either fat (FM) or fat free mass (FFM).

Over 50% of female zoo African elephants in the United States exhibit irregular reproductive cycles or are acyclic [[5](#_ENREF_5)], yet the causes of this acyclicity remains unknown [[6](#_ENREF_6)]. 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 [[7](#_ENREF_7), [8](#_ENREF_8)] and there is evidence in other species linking body composition to reproductive impairments [[9-11](#_ENREF_9)]. Thus, it is not unreasonable to posit that zoo elephants with increased FM may be more likely to exhibit metabolic perturbations and abnormal reproductive cycles than are elephants with lower FM.

Given the elephant’s size, deuterium dilution offers a tenable solution to estimate the elephant’s 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 [[12](#_ENREF_12), [13](#_ENREF_13)]. Yet, to our knowledge, no published studies have been conducted in the largest terrestrial mammal, the African elephant. Deuterium (2H), a non-radioactive isotope of hydrogen (1H), when administered to animals, is diluted by the 1H in water molecules, providing an estimate of TBW [[14](#_ENREF_14)]. 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 [[14](#_ENREF_14)]. FM is then inferred by subtracting FFM from total body weight.

Assessing elephant body composition is a necessary next step in assessing 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 [[11](#_ENREF_11), [15](#_ENREF_15)]. This relationship between excess FM and reproductive impairments may be mediated through the animal’s metabolic health. For instance, leptin and insulin are positively associated with FM [[16](#_ENREF_16), [17](#_ENREF_17)]. Excess fat is also mechanistically linked with inflammation [[18](#_ENREF_18)]. Leptin, hyperinsulinemia, and chronic inflammation have all been shown to play a role in reproduction and related dysfunction [[19](#_ENREF_19), [20](#_ENREF_20)].

Thus, the purpose of the present study is to assess the relationship between FM and reproductive cycling status in reproductive-aged, zoo African elephants and to investigate the relationship between FM, several circulating metabolites and markers of inflammation.

**Materials and methods**

**Animals**

This study was approved by the Institution Animal Care and Use Committee of the University of Alabama, Birmingham (UAB). In addition, this study was authorized by each participating zoo. A total of 19 zoos were contacted to participate in the study, and 13 zoos were visited between November 2014 and June 2016, of which eight were included in the present study. The remaining five zoos were not included because we estimated body composition at these zoos using deuterium dilution by the plateau method, which appeared not to work (Table S1, File “Chusyd-Supp-0002”). Female African elephants of reproductive-age (≥16 years of age, N=22) housed in accredited U.S. institutions served as the study sample. Elephants were excluded if they were not of reproductive age, showed stress to any part of the study, or were pregnant. There were roughly equal numbers in the reproductive cycling groups; however, we did not know which elephants cycled normally or not until after data collection. All isotopes were analysed blind to the animal status.

**Body Composition**

Elephants were weighed to the nearest five pounds, pound, or kilogram, depending on the institutions’ scale. Venous blood was collected from the ear by participating institution’s trained personnel. Blood was collected prior to deuterated water administration to determine the background isotope enrichment. Subsequently, an oral dose of (99.9% APE) deuterium oxide (0.05mL D2O/kg of body weight; DLM-4-1000, Cambridge Isotopes, Tewksbury, MA) was administered using bread as a vehicle. All bread was of the same type and purchased from the same store (Publix®, Birmingham, AL). The use of bread allowed establishment of the accurate amount of deuterated water ingested by the elephant. Bread was first weighed on the scale (to the closest 0.01g, Pioneer, Ohaus, Pine Brook, NJ), deuterated water was then slowly added to the bread making sure none was spilled nor leaked out. The bread was then reweighed. The difference in weight represented the dose of deuterated water. Each elephant typically received four to five pieces of bread with approximately 40-50g of deuterated water per piece. No aversion to the dosed bread was observed. Blood (~9mL) was sampled from the ear at regular intervals (~24, 120, 240, 360, and 480 h) post deuterium administration. All samples were allowed to sit 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 shipment 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, USA) 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 2H/1H delta value was converted to parts per million (ppm), and used to calculate deuterium dilution space size according to Speakman (1997) [[21](#_ENREF_21)]. The dilution space is considered to reflect TBW content, which is then converted to FFM using the hydration constant (file “Chusyd-Supp-0001”).

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

Body water turnover is the replacement of body water lost during a given period of time and indicates water homeostasis [[22](#_ENREF_22)]. The water turnover rate was calculated as provided in the supporting information (file “Chusyd-Supp-0001”).

**Determination of Reproductive Cycling Status**

Reproductive cycling status was determined based on each participating zoo reporting the cycling status for their elephants. Elephant reproductive cycling status is normally monitored to allow for optimal timing of breeding or artificial insemination. Based on progestagen monitoring, cycling elephants showed a 14- to 16-week cycle (8- to 12-week luteal phase) and non-cycling elephants were characterized by baseline progestagen concentrations (<0.15ng/mL) [[7](#_ENREF_7)]. Three elephants that previously exhibited long luteal phases were characterized as cycling as all three elephants were cycling at the time of sampling. When possible, cycling status was confirmed through longitudinal samples at the Smithsonian Conservation Biology Institute Endocrinology Laboratory.

**Body Condition Score (BCS)**

BCS was based on standardized photographs and taken as previously described [[4](#_ENREF_4)]. Briefly, a set of standardized photographs of each elephant was taken from three angles (side view, rear view, and rear-angle view). Using the photographs, 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 least amount of fat and 5 represents the most amount of fat (See file “Chusyd-Supp-0001” for details).

**Serum analyses**

Metabolites were assessed from the serum samples collected prior to deuterated water administration). Samples were collected in the morning prior to the elephants receiving their first meal of the day. Elephants’ last meals were typically given during the late afternoon, evening the day before blood collection. Samples were typically run neat (i.e., not diluted), but if above the assay range, the samples were serially diluted in reagent diluent until detectable within the range of the assay.

**Glucose**

Serum glucose was measured by an automated glucose analyzer (Stanbio Sirrus, Stanbio Laboratories, Boerne, TX, USA). Samples (3µL) and run in single. All samples were analyzed at the same time. Intra-assay CV was 1.28%.

**Insulin**

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). The insulin EIA was previously validated for use in African elephants [[7](#_ENREF_7)]. The intra-assay CV was 5.2%. See file “Chusyd-Supp-0001” for detailed methods.

**Leptin**

Serum leptin concentrations were determined with one assay using a multi-species double-antibody RIA (XL-85K; Millipore, Billerica, MA, USA) using a 125I-human leptin tracer and a guinea-pig anti-human leptin antiserum. Leptin RIA was previously validated for use in African elephants [[7](#_ENREF_7)]. The intra-assay CV was 4.29%. See file “Chusyd-Supp-0001” for detailed methods.

**Serum amyloid A**

Serum Amyloid A (SAA) was determined using a RX Daytona automated clinical chemistry analyzer (Randox Industries-US Ltd., Kearneysville, WV, USA) and commercially available reagents (150µL), calibrators (0.1-500mg/L) and two-level controls (Eiken Chemical Co. Ltd, Tokyo, Japan). Samples (4µL) were run in single. SAA has previously been validated in Asian elephants [[23](#_ENREF_23)].

**TNF-α**

Serum tumor necrosis factor alpha (TNF-α) concentrations were measured using an equine TNF-α sandwich enzyme immunoassay kit (EIA; ESS0017; Thermo Fisher Scientific, Frederick, MD; Edwards et al. 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 below 10%. See file “Chusyd-Supp-0001” for detailed methods.

**Statistical analyses**

All statistical analyses were performed using SAS 9.4 statistical software (SAS Institute, Cary, NC, USA) and specified prior to examining data*,* unless otherwise stated.

The primary model to address our main hypothesis was a generalized estimating equation (GEE), regressing cycling status on FM adjusting for FFM and age to the power lamba (ageλ). The lambda value for age was calculated by fitting a non-linear model based on data previously collected on 95 female African elephants, where age and cycling status were known. The best estimate of lambda was 1.62. FM, FFM, and ageλ were included as continuous variables. To adjust for relatedness among elephants from the same zoo, the zoo ID was treated as random effect in all the models. The second logistic model added FM2 and FFM2 to the primary model as the relationship between body composition and cycling 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 cycling status was tested using a 2-sided Sobel test. Mediators tested were glucose, insulin, SAA, and TNF-α. Regardless of the treatment effect (i.e., association) of the primary model, it is still useful to conduct mediation analyses to further examine whether a treatment is affecting the outcome through any mediator [[24](#_ENREF_24)]. Because approximately half of the TNF-α and SAA samples were below detection, these variables were analyzed two ways: (1) dichotomized: above and below detection; and (2) as continuous variables, whereby the lowest detectable value was used if the sample was below detection, effectively winsorizing the data.

Pearson correlations between FM and body weight, BCS, leptin, glucose, insulin were assessed, while Spearman correlations were conducted between FM and SAA and TNF-α because of their non-normal distributions. Correlations between body weight and water turnover, in addition to BCS and body weight, FFM, FM, and percent body fat were assessed. Partial correlations between FM and leptin, glucose, and insulin, adjusted for FFM, were conducted.

Descriptive statistics between the cycling and non-cycling groups were assessed. *T-tests* were used to compare the means of age, body weight, FM, FFM, height, body length, BCS, glucose, insulin and leptin by cycling status. Wilcoxon test was used to compare the means of SAA and TNF-α by cycling status because of their non-normal distributions. Fisher’s exact test was used to compare the proportion of nulliparous elephants by cycling status. Although 22 elephants were included in this study, two elephants were excluded from statistical analyses pertaining to body composition measurements because the time points of their blood samples were unknown and therefore accurately calculating body composition was not possible. 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 at 8 zoos; mean age 31.3 years; age range 16-48 years), while the remaining 41% exhibited abnormal reproductive cycles (*n=*9 at 6 zoos; mean age 39.9 years; age range 33-51 years). Descriptive statistics by cycling status are in Table 1.

**Body composition and reproductive cycling status**

Body composition was estimated by deuterium dilution (Table 2) based on the washout curve for each elephant (Figures S1, File “Chusyd-Supp-0001”). Body fat percentage averaged 9.69% (SD: 3.18, range: 5.24 -15.97%; N=20). GEE models analyzing predictors of cycling status are in Table 3. FM was not shown to be associated with cycling status, unadjusted (P=0.131) or adjusted for FFM and age1.62 (P= 0.332; Figure 2) in this study sample. When FFM2 and FM2 were included in the model, FM was not significantly associated with cycling status (p=0.350). When nulliparous status was included in the primary model, the model improved but was not significant (P=0.158). The addition of male interaction to the primary model did not improve significance (P=0.337), but did so when included with nulliparous status (P=0.075). When nulliparous and dominance status were included in the primary model FM was not associated with cycling status (P=0.172). BCS did not predict cycling status, unadjusted (P=0.234) or adjusted for age (P=0.750). Age was a significant predictor of cycling status (P=0.040). Body weight, unadjusted, was a significant predictor of cycling status (P=0.038), but was not significant when adjusted for age (P=0.647).

**Body composition and metabolites 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 3A&B), insulin (ρ= 0.369, P=0.110; ρ=0.352; P=0.128, respectively; Figure 3C&D), and leptin (ρ= 0.384; P=0.095; ρ=0.399; P=0.081, respectively; Figure 3E&F) trended towards significance. Fat mass, 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). Fat mass was not correlated with SAA (ρ=0.007; P=0.979; Figure 3G) or TNF-α (ρ=-0.0353; P=0.883; Figure 3H). BCS was strongly correlated with body weight (ρ=0.759; P<0.0001; Figure 4A), FFM (ρ=0.702, P=0.001; Figure 4B) and FM (ρ=0.583; P=0.007; Figure 4C), but not with relative fat (ρ=0.256; P=0.276; Figure 4D) or percent body fat (ρ=0.337; P=0.146). Body weight was not correlated with water turnover rate (ρ=0.357; P=0.123). Glucose and insulin were correlated (ρ= 0.430; P=0.046).

**Cycling status and metabolites 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 cycling status (Table 1).

Although there was no overall association between FM and cycling 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 cycling 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 cycling status.

**Discussion**

This study investigated the association between body composition with reproductive cycling status, mediated through metabolites and pro-inflammatory biomarkers. We did not find that FM was significantly associated with cycling status in zoo African elephants, nor was the relationship mediated through inflammation, glucose, or insulin. FM was correlated with glucose, and there was a trend with leptin levels. Non-cycling elephants were more likely to be older.

Deuterium dilution by the intercept method appears to be a useful non-invasive technique that can be used to estimate body composition of the African elephant. Deuterium dilution is based on the measurement of TBW, and can be measured by two methods: 1) the plateau method; and 2) the intercept method (ref?). In the present study, TBW was measured by the intercept method. By collecting several samples over time, the intercept method accounts for a longer equilibrium period and continuous water turnover over an extended period of time. This is important because of the elephant’s large body volume and slow heart rate [[25](#_ENREF_25)], which could otherwise violate two of the major assumptions of the plateau method: (1) equilibrium is reached rapidly and (2) neither deuterium nor body water are metabolized during equilibrium (Table S1; File “Chusyd-Supp-0002”) [[21](#_ENREF_21)]. Based on our results, there was a log-linear elimination in deuterium enrichment over time. This indicates the intercept method is more suited for larger mammals, such as elephants.

The derivation of FM from TBW estimated by deuterium dilution hinges on the ratio of TBW to FFM, termed the hydration constant [[26](#_ENREF_26)]. To our knowledge, no one has published on the elephant’s hydration constant. Therefore, we relied on the most regularly used hydration constant of ~0.73 [[27](#_ENREF_27)]. Pace and Rathbun [[28](#_ENREF_28)] first recommended this hydration constant based on several species of mammals, and since then it has been reinvestigated and confirmed [[27](#_ENREF_27)]. However, the hydration constant may vary by body size. Pitts and Bullard [[29](#_ENREF_29)] 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” [[29](#_ENREF_29)]. This could certainly pertain to the African elephant, which may have a hydration constant different from 0.73. 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. You might however want to model what impact for example having a hydration constant of 0.7 or 0.67 would do to the %fatness values

Body fat percentages in this sample population ranged from approximately 5% to 16%, which appears to be similar to other large herbivores. Most African ungulates have little or no subcutaneous fat to help facilitate heat dissipation [[30](#_ENREF_30)]. Female giraffe are found to have 0.52-1.39% fat of the buttocks [[30](#_ENREF_30)], whereby the composition of the buttocks has been taken to reflect the composition of the entire body [[31](#_ENREF_31)]. Similarly, the fat percentage of the buttocks of male kudu and blesbok is 1.3% and 1.4%, respectively [[32](#_ENREF_32)]. Due to the elephant’s low surface area to volume ratio, it would be expected that elephants too have little overall fat in order to facilitate heat dissipation. To know the exact accuracy of our body composition measures would require a carcass analysis after dosing an elephant with deuterated water, something that is not feasible or ethical to do.

Obesity has been associated with anovulation in other mammalian species [[9-11](#_ENREF_9)], yet this was not found in the present study. Similar to our results, acyclicity was not associated with being overweight in zoo Asian elephants [[15](#_ENREF_15)], even though 65.6% of North American zoo Asian elephants appear to be obese compared to 40.7% of North American zoo African elephants, based on BCS [[3](#_ENREF_3)]. Our results, however, are not in accordance with previous studies showing a relationship between body condition indices (e.g., BMI and BCS) and cycling status in zoo African elephants [[7](#_ENREF_7), [8](#_ENREF_8)]. The discordance between results may be due to a few factors, including our small sample size. Morfeld and Brown (2014) showed 23 non-cycling elephants had higher BCSs compared to 23 cycling elephants. Based on effect size estimates in our data, to have 0.80 power to detect an association between FM and cycling status would require ~170 elephants. More reasonably, although it is generally acknowledged that elephants do not go through menopause [[15](#_ENREF_15)], the difference in results may be related to age, which is supported by a recent study demonstrating that age is positively associated with cycling status [[5](#_ENREF_5)]. We found non-cyclers were significantly older and heavier than cyclers, with age and body weight strongly positively correlated. Further, BCS correlated most strongly with body weight. It appears BCS may be capturing the overall weight, and not the elephant’s FM. As age and weight are highly correlated, age is likely driving the relationship between BCS and cycling status. Morfeld and Brown (2014) did not adjust for age, which could explain the discordance in results. Morfeld and Brown (2014) did indicate that age could contribute to acyclicity. Collectively, this suggests age should be an important factor to consider in breeding strategies and future research studies.

The elephant’s metabolic state, rather than absolute FM, may be more important to consider regarding reproductive and overall health. Similar to Morfeld and Brown’s (2014) findings, non-cycling elephants had numerically higher insulin levels compared to cycling elephants. Moreover, FM, adjusted for FFM, was positively correlated with glucose levels, and FM almost reached significance with insulin levels. Therefore, it may not only be absolute fat that is important to consider, but relative amounts of fat. In a post-prandial state, elevated blood glucose levels stimulate insulin secretion [[33](#_ENREF_33)], in turn promoting glucose uptake and utilization, and suppressing gluconeogenesis [[33](#_ENREF_33)]; therefore, abnormal insulin secretion can lead to hyperglycemia [[33](#_ENREF_33)]. Hyperglycemia and hyperinsulinemia both can lead to obesity and comorbidities [[33](#_ENREF_33), [34](#_ENREF_34)]. Increased FM may impact health independent of reproductive state, by increasing arthritis rates and calving problems, and through intrauterine programming. Therefore, monitoring FM accrual should be considered by zoological institutions.

Hyperglycemia and hyperinsulinemia stimulate an inflammatory state [[35](#_ENREF_35), [36](#_ENREF_36)] and in other species, inflammation is associated with reproductive impairments and obesity [[20](#_ENREF_20)]. However our results do not indicate an inflammatory state. The SAA reference interval for clinically healthy Asian elephants is 0-47.5mg/L [[37](#_ENREF_37)]. No elephant, cycling or non-cycling, had levels greater than 3.5mg/L, suggesting all the elephants in this study were clinically healthy. Further, SAA levels were not correlated with FM, even though SAA is the most sensitive APP in elephants [[23](#_ENREF_23), [37](#_ENREF_37)]. TNF-α was not associated with FM. Taken together, this would suggest these elephants are not characterized by chronic inflammation as seen in other species that exhibit obesity and reproductive impairments [[20](#_ENREF_20), [38](#_ENREF_38)], at least measured by these inflammatory factors, and may not even be categorized as obese.

FM should be monitored as it is an active endocrine organ. White adipose tissue mainly produces leptin [[39](#_ENREF_39)]. Leptin plays a permissive role in activating the reproductive axis, and when levels are abnormally high, as observed in an obese state [[39](#_ENREF_39)], prevents ovarian steroidogenesis, inhibiting proper follicle development [[40](#_ENREF_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](#_ENREF_39)], zoo elephants reach puberty at earlier ages than their wild counterparts [[15](#_ENREF_15)]. Earlier puberty will likely expose the elephants to more reproductive cycles and associated endogenous hormones over their lifetime, which may lead to asymmetric reproductive aging, demonstrated by reproductive tract pathologies [[41](#_ENREF_41)]. With known endocrine function, continued fat accrual will possibly lead to a point where elephants will show similar metabolic dysfunction exhibited by humans and domestic animals with obesity.

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. While the majority of these elephants appear to be metabolically healthy, for some, there are metabolic warning signs, and they 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 and there is a pronounced increase in poaching rates in range countries.

This study was the first to demonstrate that deuterium dilution can be used to estimate body composition in the African elephant. We used a novel method to estimate body composition for this population that has proven accurate in species with a 105 times difference in body size and has been used for several decades. Thus, although assumptions were made (e.g., hydration constant) and there was an inability to validate the technique, the method has proven robust over time and species. Even though this study had a small sample size, by using this technique, this study opened up a new avenue of research questioning for large herbivores.

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**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. The difference between fat mass, fat mass adjusted by fat free mass, and fat mass adjusted by fat free mass and age with cycling status.**

FM: Fat mass; FFM: Fat free mass.

**Figure 3. 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.

**Figure 4. 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.