DOI: 10.1002/vms3.1279

ORIGINAL ARTICLE

WILEY

Serum vitamin D in sanctuary chimpanzees (*Pan troglodytes*) in range countries: A pilot study

Yedra Feltrer-Rambaud[1] | Anneke Moresco[1,2,3,4] | Kimberly Ange-van Heugten[3] | Andrea Pizarro[5] | Beatriz Tomeo-Martín[6] | Lara Carrasco Pesquera[6] | Natalie Moresco[7] | Rebeca Atencia[1,8]

[1]International Primate Health & Welfare Group, Madrid, Spain

[2]Reproductive Health Surveillance Program, Morrison, Colorado, USA

[3]Department of Animal Science, North Carolina State University, Raleigh, North Carolina, USA

[4]Colorado Mesa Tech, Colorado Mesa university, Grand Junction, Colorado, USA

[5]Tacugama Chimpanzee Sanctuary, Freetown, Sierra Leone

[6]Unidad de Investigación Biomédica. Animal Physiology Departemtn., Universidad Alfonso X el Sabio (UIB-UAX), Madrid, Spain

[7]NMP consulting, Ashland, Massachusetts, USA

[8]Jane Goodall Institute (JGI) Republic of Congo, Pointe Noire, Congo

Correspondence
Yedra Feltrer-Rambaud, International Primate Health & Welfare Group, IPHWG, Avda Bruselas 43, 4Izq, 28028, Madrid, Spain.
Email: yedrafeltrer@hotmail.com

Funding information
Mazuri Exotic Nutrition; Animal Necessity; Fundació Barcelona Zoo

Abstract

Background: Vitamin D is essential for skeletal health, calcium homeostasis and general health. The major and more stable form of vitamin D in circulation is 25-hydroxyvitamin D (25-OH-D); this is the most valuable indicator of vitamin D status. There are studies on laboratory and zoo-housed chimpanzees; however, serum vitamin D status has not been documented in chimpanzees in range countries.

Objectives: (1) Determine the range of circulating 25-OH-D concentrations in chimpanzees in range countries. (2) Assess the influence of age, sex, and sun exposure on 25-OH-D serum concentrations.

Methods: Opportunistic blood samples were obtained from 127 clinically healthy chimpanzees. Serum 25-OH-D concentration was measured with a commercially available competitive ELISA.

Results: The median overall 25-OH-D concentration for chimpanzees in range countries was 46.24 nmol/L (range: 17.10–109.23 nmol/L). Males had a significantly lower concentration (40.15 nmol/L) than females (49.61 nmol/L), and infants (37.99 nmol/L) had a significantly lower concentration than adults (46.04 nmol/L). Concentrations of 25-OH-D in chimpanzees in sunnier habitats were significantly higher compared to thick tropical forest habitat.

Conclusion: The present constitutes a large dataset of serum 25-OH-D concentrations in range country sanctuary chimpanzees and contributes to document normal ranges. Age, sex, and sun exposure influenced serum concentrations of 25-OH-D in sanctuary chimpanzees.

KEYWORDS
Great apes, vitamin D, 25-OH-D, physiologic ranges, sun exposure, primate

1 | INTRODUCTION

Vitamin D is essential for skeletal health, calcium homeostasis and general health due to its extra-skeletal role (Bikle, 2016). Vitamin D (calciferol) is an essential lipo-soluble vitamin that is found in food and synthesised in the skin. It is available in two main forms: vitamin D_2 (ergocalciferol), found in some plants, and vitamin D_3 (cholecalciferol), which is naturally synthesized, in the skin from endogenous or dietary cholesterol upon exposure to sunlight with an ultraviolet light wavelength (290–315 nm; or UV-B) (Holick, 1981; Knapka, 2003), and is found in some food items of animal origin. These two forms, which are not biologically active, are bound to a vitamin D binding protein in the blood and transported to the liver, where they are subsequently converted to 25-hydroxy vitamin D (25-OH-D) and to the biologically active form 1-25-dihydroxyvitamin D_3 in the kidneys, which is then transported to target tissues, where it functions like a steroid, binding to the vitamin D receptor (VDR). The major circulating form of vitamin D is 25-OH-D, which has a half-life of approximately 2–3 weeks, and is the form generally measured to determine vitamin D status (Holick, 2009).

Although the major function of vitamin D is to maintain calcium–phosphorus homeostasis and proper bone formation, VDR are present in most tissues and cells in the body, thus, vitamin D has a wide range of biological actions affecting many cellular processes, including cell proliferation and differentiation, as well as apoptosis and inflammation (Bikle, 2016; Dusso et al., 2005; Fischer, 2020; Khammissa et al., 2018). There is an increasing recognition that vitamin D plays a crucial role in female reproduction (Xu et al., 2021) as well as in spermatogenesis (Zanatta et al., 2017). Low serum concentrations of 25-OH-D in humans have been associated with an increased risk of mortality and a variety of disorders including musculoskeletal diseases, diabetes mellitus, cardiovascular diseases, autoimmune diseases, cancer, gestational diabetes, and fertility issues (Dusso et al., 2005; Haidari et al., 2016; Holick, 2017; Pilz et al., 2018; Poel et al., 2012; Zhang & Naughton, 2010). Metabolic bone disease or rickets due to vitamin D deficiency has been documented in chimpanzees *(Pan troglodytes)*, and a wide range of non-human primates under human care (Junge et al., 2000; Knapka, 2003; Power et al., 1995).

For most animals under natural conditions, vitamin D is not a nutrient but an endogenously produced component of an endocrine-like hormonal system produced in the skin after exposure to sunlight or ultraviolet light. Thus, housing conditions are a determinant factor for 25-OH-D concentrations in primates under managed care (Videan et al., 2007). For the primate species that have been studied, vitamin D is not an essential component of the diet as long as they have adequate exposure to biologically active UV light (wavelength: 290–315 nm) (Holick, 1981). However, many chimpanzees under human care are exposed to suboptimal levels of UV-B radiation, requiring an exogenous source of vitamin D (AZA_Ape_TAG, 2010). Wild chimpanzees live in equatorial Africa and are exposed to higher amounts of sunlight and UV light year-round compared to zoo-housed chimpanzees in more northern and southern latitudes. For this reason, most zoo chimpanzees are fed a diet enriched with vitamin D or receive vitamin D supplements (Carlsen et al., 2022). Although there are several studies on serum vitamin D status on laboratory and zoo-housed chimpanzees (Crissey et al., 1999; Junge et al., 2000; Moittié et al., 2020, 2022; Videan et al., 2007), serum 25-OH-D concentrations have not been documented in chimpanzees living in range countries (either wild or under human care) despite their importance for overall health.

The purpose of this study was two-fold, first, to determine the range of circulating 25-OH-D concentrations in a population of sanctuary chimpanzees living in range countries in equatorial West Africa, and second to explore the influence of age, sex, and sun exposure on vitamin D serum concentrations.

2 | MATERIALS AND METHODS

Serum concentrations of 25-OH-D were measured in 127 chimpanzees, including 76 females and 51 males. Chimpanzee ages ranged from 1 to 36 years old, with 22 infants (I = 1 < 6 years old), 37 juveniles (J = 6 < 13 years old) and 68 adults (A = 13 < 40 years of age); no geriatric animals (G = 40 years and above) were present in the study population (age classes according to Vidal C., 2021 pers. comm.).

The chimpanzees were housed at two sanctuaries in their range countries in equatorial West Africa: Tacugama Chimpanzee Sanctuary (TCS), in Sierra Leone (n = 83, *Pan troglodytes verus*) and Jane Goodall Institute Tchimpounga Chimpanzee Rehabilitation Centre (TCRC), in the Republic of Congo (n = 44, *Pan troglodytes troglodytes*). Both sanctuaries are members of the Pan African Sanctuary Alliance (PASA) and the chimpanzees were cared for in accordance with the recommendations of the PASA operations manual (PASA, 2016). All habitats (enclosures) included a dormitory and a large outdoor area. Habitats were categorised according to sun exposure, from type 1 (least) to type 4 (most). At TCRC, one group was evaluated; it was composed of 33 adults, 9 juveniles and no infants. The outdoor habitat consisted of a large (106 ha) island on the Kouilou River, with natural dense tropical forest habitat (habitat type 1). At the time of this study at TCS, there were 33 adult, 28 juvenile and 22 infant chimpanzees divided into 10 mixed age and sex groups, with the exception of one group comprised only of infants. Three groups (n = 42; I = 7, J = 10, A = 25) had a large natural low secondary forest outdoor habitat (habitat type 2); two groups (n = 10; J = 3, A = 7) had smaller, low bush, outdoor habitats (habitat type 3); three groups had open grass, outdoor habitats; and two groups, which included a group of infants, had purpose-built outdoor habitats with climbing and shade structures (habitat type 4; n = 31; I = 15, J = 13, A = 3). All chimpanzees had all-day outdoor access; from 8 AM to 5 PM at TCS and from 7 AM to 4.30 AM at TCRC, all chimpanzees spent the night in the dormitories.

The diet consisted mostly of seasonal, local fresh fruits and vegetables, supplemented daily with a high-protein and energy cereal ball, and with hard-boiled eggs two to three times a week. The infants were also given human infant milk formula four times a day (NutriLac™ and SMA Pro™); the chimpanzees with outdoor forest habitats at TCS and the TCRC chimpanzees were also able to forage for wild fruits and vegetables throughout the day. No vitamin D supplements were given.

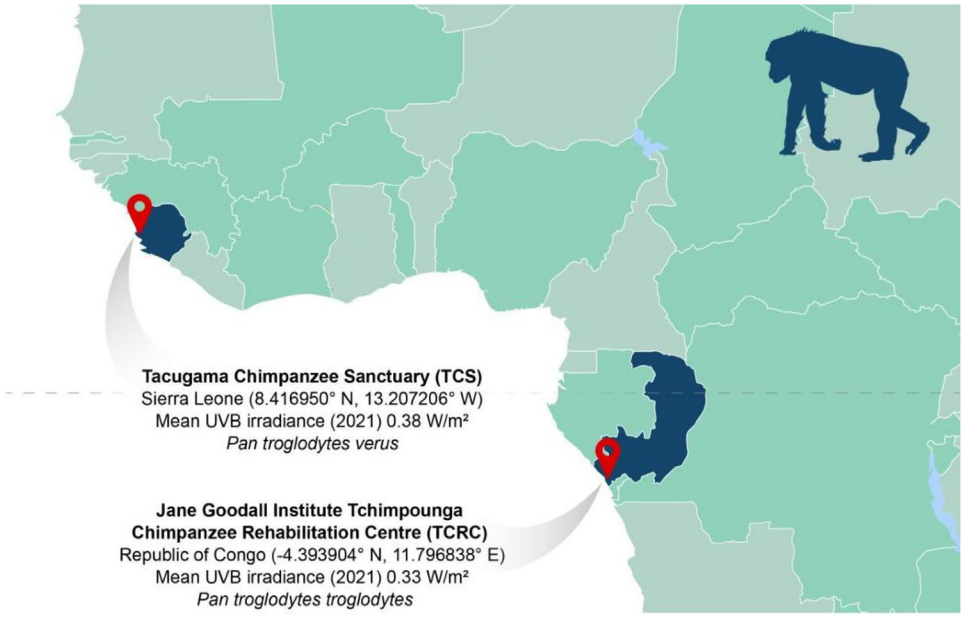

FIGURE 1 Geographic location and sanctuaries' characteristics. Map of western central Africa showing the location (latitude and longitude) and mean UV-B irradiance of Tacugama and Tchimpounga sanctuaries.

All sexually mature females (n = 60) were contracepted with long-acting progestagen implants (etonogestrel and levonorgestrel), with the exception of four females, two nursing females in TCS and two pregnant females (implant failure) at TCRC. Young females reaching sexual maturity were administered oral contraceptive pills for a short period of time until an implant could be placed.

Routine health checks are carried out approximately every 2 years by the attending veterinarian at each sanctuary and include full physical examinations, cardiac and reproductive assessments, morphometrics, faecal screening, haematology and biochemistry, tuberculosis testing and sample collection for virology and bacteriology for respiratory pathogens, among others. Radiographs were taken at TCRC on all chimpanzees (n = 44) and at TCS on two infants who had been recently rescued. Haematology and biochemistry analyses were carried out at local human laboratories on the day of sample collection: Choithram Memorial Hospital (Hill Station, Freetown, Sierra Leone) for TCS, and Laboratoires d'Analyses Medicales BioMedical (Pointe Noire, Republic of Congo). Both laboratories use automated colorimetry to measure phosphorus and calcium.

The health checks took place during the dry season at both sanctuaries, when the average UV index in both countries is 7 (Weather-atlas.com): end of January, beginning of February in TCS (mean UVB irradiance for that time of year of 0.36 W/m^2) and during December in TCRC (mean UVB irradiance for December of 0.38 W/m^2) (National-Aeronautics-and-Space-Administration-[NASA], 2022) (Figure 1).

For this study, blood samples were opportunistically obtained from the femoral vein during the health checks, and all individuals included were found to be clinically healthy. The blood samples were kept in a cool bag in the dark before transport to the sanctuary laboratory. The blood was allowed to clot and centrifuged at 3000 rpm for 5 min at the sanctuary laboratories, and serum was stored at −80°C (TCS) and at −20°C (TCRC) until CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) permits were obtained. Serum samples were shipped frozen within 8 weeks to the Unidad de Investigación Biomédica at the Universidad Alfonso X el Sabio (Madrid, Spain) for 25-OH-D analysis.

2.1 | Vitamin D detection by immunoassay

Serum 25-OH-D concentration was measured with a competitive Enzyme-Linked ImmunoSorbent Assay (EA300/96, DLD Diagnostika GMBH, Hamburg, Germany) commercially available for humans. This method was chosen for this study due to its reasonable cost, simplicity, and ease of use in the field, factors of key importance for future in-situ field and sanctuary studies.

Frozen serum samples were thawed without direct heat, and the reagents were prepared following the manufacturer's instructions. All samples were analysed in duplicate by the same researcher. Samples and standards were diluted 1:26 (by adding 20 μL of sample or standard in 500 μL of biotin), mixed, and incubated for 10 min at room temperature (RT) in polypropylene tubes. Diluted samples and standards (200 μL/well) were added to the plate in duplicate and incubated for 2 h at RT. The plate was washed three times with wash solution (300 μL/well each one); the enzyme-conjugate antibody was added (100 μL/well) and the plate was incubated for 30 min at RT. The plate was washed three times with a wash solution (300 μL/well), then, the chromogen and substrate solution was added (100 μL/well). The plate was incubated for 15 min at RT in the dark. Finally, the stop solution was added (100 μL/well) and the absorbance was measured at 450 nm

TABLE 1 Results of the influence of sex, age and sun exposure on concentrations of 25-OH-D by multiple regression model (25-OH-D nmol/L ~ intercept + sex + age class + sun exposure). Sex, age class and sun exposure were significant (*) predictor variables for serum concentrations of 25-OH-D in chimpanzees ($R^2 = 0.3691$).

Variables	Estimate	Standard error	95% CI (asymptotic)	|t|	*p* Value
Intercept	47.22	2.611	42.05 to 52.39	18.08	<0.0001
***Sex[M]**	−12.57	2.836	−18.19 to −6.957	4.433	<0.0001
Age class[Juvenile]	1.418	3.456	−5.425 to 8.261	0.4104	0.6823
***Age class[infant]**	−24.49	4.827	−34.05 to −14.93	5.074	<0.0001
***Sun exposure[2]**	10.22	3.382	3.528 to 16.92	3.024	0.0031
***Sun exposure[3]**	14.49	5.268	4.055 to 24.92	2.750	0.0069
***sun exposure[4]**	25.10	4.638	15.92 to 34.29	5.412	<0.0001

using the Varioskan LUX, version 1.00.37 (Thermo Fisher Scientific). The serum 25-OH-D concentrations (ng/mL) were measured using the SkanIt Software 5.0 for Microplate Readers RE, version 5.0.0.42 (Thermo Fisher Scientific) by interpolating the optical absorbance values obtained in the four-parameter logistic function standard curve that was generated using the reference standards. The individual sample value was calculated using the average of duplicates. The values of serum 25-OH-D quantified by ELISA (ng/mL) were converted to international system (IS) units in nmol per litre using the conversion factor 2.496 (1 ng/mL = 2.496 nmol/L).

2.2 | Statistical Analyses

Data were analysed using GraphPad Prism software (GraphPad Software) and SAS (SAS Institute). Continuous data (age and 25-OH-D concentration) were tested for normality with the Dt'Agostino & Pearson test. Summary statistics for data that did not pass the normality test are reported as median with interquartile range (IQR), normally distributed data are reported as mean ± SD. Multiple linear regression was used to assess the influence of sex, age class and sun exposure (habitat type). Significance was considered when *p* values were less than 0.05.

All *P. t. verus* were at TCS, and all *P. t. troglodytes* were at TCRC; also, all chimpanzees from TCRC resided in habitat type 1, while none of the TCS chimpanzees resided in habitat type 1, resulting in collinearity of location and subspecies with habitat. The resulting multicollinearity precluded the inclusion of location and subspecies in the multiple regression model.

2.3 | Ethical approval

The study was approved by the TCS, the Jane Goodall Institute, the Republic of Congo, and TCRC management. This was an opportunistic study during the regular health checks carried out at the sanctuaries and, as such, adheres to the PASA professional, ethical and welfare standards for such procedures.

3 | RESULTS

The median serum 25-OH-D concentration for all 127 chimpanzees was 46.24 nmol/L (IQR: 35.90–60.44 nmol/L) and the range was 17.10–109.23 nmol/L. The multiple regression model included age, sex and sun exposure or habitat type as variables of 25-OH-D concentrations (25-OH-D nmol/L ~ intercept + sex + age class + sun exposure). A robust regression (Chen, 2002) performed on the model indicated that there were two outliers; these were excluded from further analysis. Sex, age, class and sun exposure were significant predictor variables for serum concentrations of 25-OH-D in chimpanzees ($R^2 = 0.3691$; Table 1).

3.1 | Age and sex

Serum 25-OH-D concentrations were significantly ($p < 0.007$), lower in males (median: 37.91 nmol/L, IQR: 29.61–85.54) compared to females (49.61 nmol/L, IQR: 40.22–98.99) (Figure 2).

Infants had a significantly ($p < 0.0001$) lower concentration than adults (median: 37.77 noml/L; IQR: 32.09–45.59 vs. 45.95 mol/L IQR: 37.68–58.16), while the serum concentration in juveniles (53.97 nmol/L, IQR: 39.92–75.36 vs. 45.95 mol/L; IQR: 37.68–58.16) was not significantly different from adults (Figure 3).

3.2 | Habitat type

Serum concentrations of 25-OH-D in chimpanzees in habitats types 2–4 were significantly higher compared to habitat type 1, with an upward trend as the sun exposure increases until level 3, with no further increase between habitats 3 and 4 (Figure 4). As indicated by the wide IQRs (Table 2), there was a fair amount of variation in the concentration of 25-OH-D among the various habitats, potentially indicating a difference in habitat use by the different individuals.

Noteworthy cases were one individual that was nursing at the time of sampling (1-year-old) with a serum 25-OH-D concentration of 88.48 nmol/L, the lactating mother

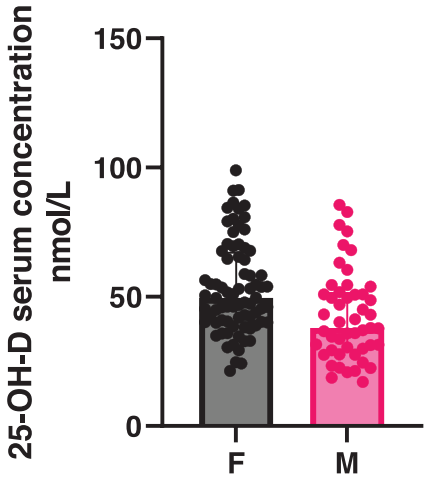

FIGURE 2 Median serum 25-OH-D concentrations for female (F) and male (M) chimpanzees in two African sanctuaries. Individual values are represented, shading indicates the median and the brackets indicate interquartile ranges.

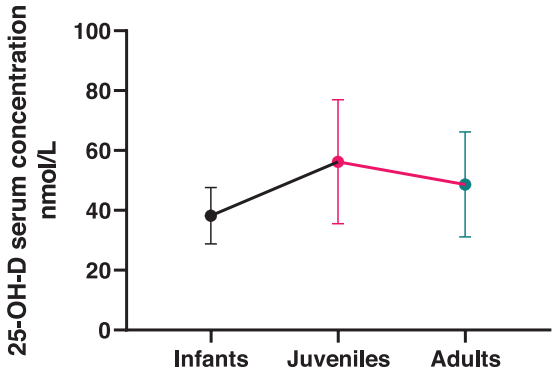

FIGURE 3 Median serum 25-OH-D concentrations for infant, juvenile and adult chimpanzees at two African sanctuaries. Brackets indicate interquartile ranges.

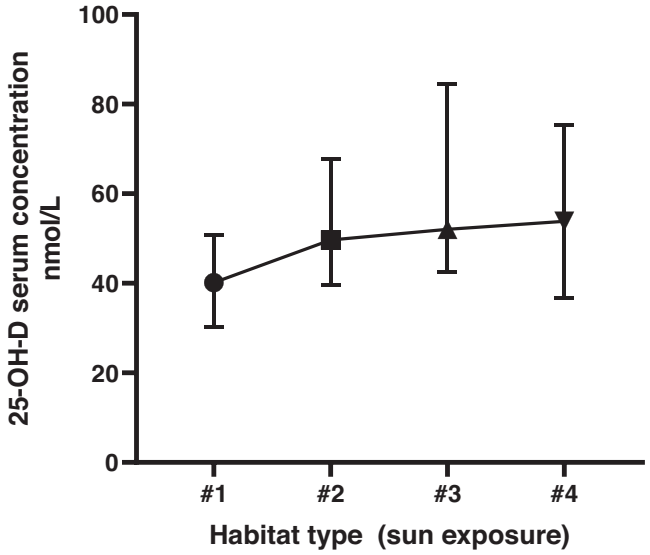

FIGURE 4 Sanctuary chimpanzee (*Pan troglodytes*) median 25-OH-D serum concentrations by habitat type: #1 natural dense tropical forest habitat; #2 natural low secondary forest outdoor habitat; #3 low bush habitat; #4 open grass, outdoor habitat with shade structures. Brackets indicate interquartile ranges.

TABLE 2 25-OH-D median serum concentrations and IQR in Sanctuary chimpanzees in range countries.

Habitat type	25-OH-D Median (IQR) nmol/L
1 Natural dense tropical forest habitat	40.16 (30.31–50.79)
2 Natural low secondary forest outdoor habitat	49.65 (39.67–67.84)
3 Low bush, outdoor habitat	52.04 (42.56–84.43)
4 Open grass, outdoor habitats and purpose-built outdoor habitats with climbing and shade structures	53.85 (36.69–75.36)

(14 years old) had a serum 25-OH-D concentration of 68.99 nmol/L, and another lactating female (14 years old) had a serum 25-OH-D concentration of 64.68 nmol/L. There were two pregnant females at TCRC who had serum 25-OH-D concentrations of 46.17 nmol/L (9 years old) and 76.03 nmol/L (16 years old).

The calcium (Ca) and phosphorus (P) concentrations were all, except for two females, within normal reference ranges for chimpanzees (Species360, 2022: Ca, 1.9–2.6 mmol/L & P, 0.35–2.33 mmol/L), and for the laboratory used in each country. The average total calcium was 2.22 ± 0.21 mmol/L for TCS and 2.14 ± 0.9 mmol/L for TCRC. Mean phosphorus was 1.26 ± 0.3 mmol/L for TCS and 1.36 ± 0.2 mmol/L for TCRC. A juvenile female (8 years old) at TCS had a total calcium concentration of 1.95 mmol/L, which was marginally below the reference range for the laboratory (2.00–2.74 mmol/L), but her phosphorus concentration was 1.43 mmol/L, within the reference interval, and a Ca:P ratio of 1.36:1 (normal 1–2:1) with a serum 25-OH-D concentration of 91.36 nmol/L. Another young adult female (13 years old) in TCS had a total calcium concentration of 3.09 mmol/L, which is above the reference range. With phosphorus concentrations of 1.28 mmol/L and a higher Ca:P ratio (2.4:1), the serum 25-OH-D concentration in this individual was 65.45 nmol/L.

No skeletal abnormalities were detected during the radiographic exams of the 44 TCRC chimpanzees. Except for two infants that showed no abnormalities, radiographs were not performed on the chimpanzees at TCS, but all chimpanzees presented with normal growth (Curry et al., 2022) and locomotion; additionally, no skeletal abnormalities were detected on physical examination.

Based on the clinical exam and laboratory results, all chimpanzees included in the study were clinically healthy except for two chimpanzees at TCS: a juvenile male and a recently rescued infant in quarantine. These two were excluded from the study.

4 | DISCUSSION

The present study reports on a large dataset of serum 25-OH-D concentrations in chimpanzees living in range countries and contributes data to document normal ranges. Age, sex and sun exposure (habitat type) were found to influence the serum concentrations of 25-OH-D in sanctuary chimpanzees.

Vitamin D deficiency and insufficiency are concerns for human health worldwide (Palacios & Gonzalez, 2014). Despite this, there is still no consensus about the optimal serum concentrations of 25-OH-D in humans, in part due to assay and laboratory variation. Generally, in humans, serum concentrations less than30 nmol/L are considered severely deficient, 30–50 nmol/L insufficient or deficient, and serum concentrations more than 50 nmol/L are considered sufficient (Amrein et al., 2020; Sempos et al., 2018). Vitamin D status and its impact on general health are also a concern for chimpanzees under human care (Moittié et al., 2022; Strong et al., 2020). The range of serum 25-OH-D concentrations for wild chimpanzees or chimpanzees under human care in range countries has not been studied, and reference ranges for circulating 25-OH-D in chimpanzees are lacking. Therefore, human reference ranges and cut-off values have historically been used to evaluate the vitamin D status of chimpanzees under human care (Junge et al., 2000; Moittié et al., 2022; Videan et al., 2007).

The authors recognise that there is a greater variability with the use of ELISA for the measurement of 25-OH-D compared to LC–MS, which is considered the gold standard technique in humans (Zelzer et al., 2018), and this variation constitutes a limitation of the present study. However, to improve reliability, all samples were run in duplicate by the same researcher. This ELISA was chosen for this study because of its reasonable cost, simplicity, and ease of use in the field. Even though the results of cannot be directly compared to studies that use different analytical techniques, 25-OH-D concentrations in the present study were lower (46.24 nmol/L) than the median concentrations found in a Europe-wide study of serum 25-OH-D concentrations (57.70 nmol/L) in zoo-housed chimpanzees (Moittié et al., 2022) and in laboratory chimpanzees (51.42 nmol/L) with indoor and outdoor access (Videan et al., 2007), as well as below the cut-off for sufficient 25-OH-D concentration for humans.

The median 25-OH-D serum concentration in this study was higher than reported in a study that compared nutrient metabolites across 9 species of primates in four North American zoos (Crissey et al., 1999), where the chimpanzee was the species with the lowest concentrations of 25-OH-D with 32.75 ± 9.80 nmol/L. The serum 25-OH-D concentrations reported in the present study were also higher than the ones reported as adequate by Junge et al. (2000) in adult chimpanzees (31 nmol/L) in a zoo in North America, but lower than for the same group of chimpanzees after being moved to Hawaii, where they had all year-round direct sunlight exposure, and their average concentrations of 25-OH-D increased to 97 nmol/L. Junge et al.'s findings reflect the importance of housing conditions for vitamin D status in chimpanzees under human care. Moittie et al. (2022) also found significant differences in Europe zoo-housed chimpanzees between winter 25-OH-D concentrations (median: 47.00 nmol/L) and summer (median: 71.80 nmol/L), and that the provision of unlimited daily outdoor access was associated with higher 25-OH-D concentrations year round. Interestingly, the winter concentrations of 25-OH-D were similar to the median concentration found in this study. Similarly, in the present study, the different habitat types and sun exposure had a significant influence of the on concentrations of 25-OH-D, with higher concentrations (53.85 nmol/L) in the sunnier more exposed habitat (type 4) in TCS and the lower 25-OH-D concentration (40.16 nmol/L) in the more covered tropical forest habitat (type 1) in TCRC. Nevertheless, there was large variation within habitat, particularly habitat type 1, that could possibly reflect habitat use, with some chimpanzees spending more time at the water border of the island or in the canopy with more sun exposure and higher vitamin D status than chimpanzees that spend more time in the ground or in the middle of the thick forest with less direct sunshine.

Chimpanzees inhabit a wide range of different habitats across equatorial Africa, they are found discontinuously across the forest belt of Africa, occupying primary and secondary moist lowland forest, swamp forest, submontane and montane forest, dry forest, forest galleries in savanna woodland and farmland (Oates, 2011). It is possible that these findings reflect natural variations on 25-OH-D concentrations that may occur in wild chimpanzees in their different natural habitat. Although unlikely, it might also be that different subspecies have varied requirements of 25-OH-D, unfortunately this could not be tested with the present dataset.

The summer and overall median concentrations of 25-OH-D in Europe zoo-housed chimpanzees are higher than in the present study where the chimpanzees had all day outdoor access in equatorial Africa with a relatively steady UV-B levels year round (Jablonski & Chaplin, 2010). This could possibly reflect the difference in diet as most zoos feed fortified commercial food or add vitamin supplements to the diet.

Age class was found to also influence vitamin D status and the results of the present study show that infant sanctuary chimpanzees had significantly lower 25-OH-D concentrations (37.77 nmol/L) than the juvenile chimpanzees (53.99 nmol/L) and it would be considered insufficient by human standards. This is despite most of the infants having an outdoor habitat with minimal vegetation and higher exposure to sunlight (type 4). However, the results of the health checks (including morphometrics, radiographs, total calcium and phosphorus concentrations and the normal growth rate of the infants) suggest that the concentrations of 25-OH-D in the sanctuary infant chimpanzees are adequate for normal development. Junge et al. (2000) report clinical rickets in two zoo infant chimpanzees with 25-OH-D concentrations equivalent to 4.0 and 24.25 nmol/L, and subclinical with 32 nmol/L, all below the median for the infants in this study. Human breastfed infants are at higher risk of vitamin D deficiency because the content of vitamin D in the breast milk is totally dependent of the mother's vitamin D status which is normally insufficient to provide the baby with their daily requirements (Institute-of-Medicine-Food-and-Nutrition-Board, 2010; við Streym et al., 2016). In this study, the nursing infant and lactating females in the present study had 25-OH-D serum above the

median concentration for this population. The two pregnant females at TCRC also had 25-OH-D concentrations higher than the median concentration for TCRC. As the present study did not include any geriatric chimpanzees (>40 years), no ageing effect could be observed or compared to the natural decrease in 25-OH-D production seen as humans age (Gallagher, 2013; Meehan & Penckofer, 2014).

Sex was also found to influence vitamin D status in sanctuary chimpanzees. The results of this study show significantly higher 25-OH-D concentrations in females than in males in contrast with several human studies that have found that 25-OH- D concentrations are higher in men than in women (Muscogiuri et al., 2019; Verdoia et al., 2015). The significance of the difference between males and females is uncertain and additional data and research is necessary. It is believed that contraceptives containing oestrogens or oestrogens combined with progestagens may affect 25-OH-D serum concentrations in women. Several studies have found that the use of oral combination contraceptive pill (oestrogen and progestagen) increases the circulating levels of 25-OH-D in premenopausal, healthy, adult women (Harmon et al., 2016). The contraceptive method used in both sanctuaries was long acting progestagen-only implants, and based on the current knowledge from human medicine, should not have any effect on the 25-OH-D status. However, our dataset does not allow further analysis on the effect of contraception and vitamin D status in female chimpanzee. Since 25-OH-D readily crosses the placenta (Shin et al., 2010) and vitamin D content in milk is dependent on the mother's concentrations, these findings could possibly be linked to successful reproduction and nursing in chimpanzees, similar to findings in humans (Luk et al., 2012).

Vitamin D deficiency in humans has been associated with an increased risk of mortality and a variety of disorders, including musculoskeletal diseases, diabetes mellitus, cardiovascular diseases, autoimmune diseases, cancer and with fertility problems (Heath et al., 2019; Pilz et al., 2018). Even though the chimpanzees in this study had, in general, lower serum concentrations of 25-OH-D than the cut-off for acceptable concentrations in humans, the population of the present study was clinically healthy, with no reproductive, cardiovascular or skeletal disease detected during the health check. There are important differences in bone structure between humans and chimpanzees, and genetic factors that affect bone regulation may be contributing factors to these differences (Tsegai et al., 2018). One such factor could be different requirements of 25-OH-D concentrations between humans and chimpanzees.

While the results of the present study cannot be directly compared to published studies that use different analytical techniques, they still provide valuable and unique information on vitamin D status in sanctuary chimpanzees. To the authors' knowledge, this is the first study of serum 25-OH-D concentrations in chimpanzees in range countries. With the exception of chimpanzees in sunnier habitats (types 3 and 4), the results from the present study show that sanctuary chimpanzees living in forest enclosures in range country have median 25-OH-D concentrations below what is considered adequate concentrations for humans (>50 nmol/L). These data suggest that using human values as reference ranges for 25-OH-D for chimpanzees under human care might not be appropriate. Although more research is needed, especially on the vitamin D status of wild chimpanzees, the current data is important to help establish reference values for serum 25-OH-D concentration in chimpanzees under human care.

AUTHOR CONTRIBUTIONS

Yedra Feltrer Rambaud: Conceptualization; Data curation; Funding acquisition; Methodology; Resources; Writing. Anneke Moresco: Conceptualization; Data curation; Funding acquisition; Methodology; Resources; Writing—review & editing. Kimberly Ange-van Heugten: Funding acquisition; Writing—review & editing. Beatriz Tomeo-Martin: Data curation; Formal anlysis. Andrea Pizarro: Conceptualization; Funding acquisition; Resources. Lara Carrasco Pesquera: Resources. Natalie Moresco: Formal analysis. Rebeca Atencia: Funding acquisition; Resources.

ACKNOWLEDGMENTS

The authors would like to thank the Barcelona Zoo Foundation (Fundació Zoo Barcelona, Barcelona, Spain), Mazuri® Exotic Nutrition (St. Louis, MO, USA), and Animal Necessity (Key Largo, FL, USA), for their financial support of this project.

Thank you as well to all the Tacugama and Tchimpounga chimpanzee care givers, other sanctuary staff, and colleagues who assisted with the health checks and laboratory work; without them, this study would not have been possible. And, of course, to the chimpanzees for whom we do this work.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

ETHICAL STANDARD

The study was approved by the Tacugama and the Jane Goodall Institute, Republic of Congo and Tchimpounga boards. This was an opportunistic study during the regular health checks carried out at the sanctuaries and as such adheres to the Pan African Sanctuary Alliance professional, ethical and welfare standards for such procedures.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted in the author guidelines, have been adhered to and that the appropriate ethical review approval by the board of both sanctuaries was received. This was an opportunistic study during the regular health checks carried out at the sanctuaries and as such also adheres to the Pan African Sanctuary Alliance professional, ethical and welfare standards for such procedures.

DATA AVAILABILITY STATEMENT

Data were not deposited in an official repository. The data that support the study findings are available upon reasonable request to reviewers.

ORCID

Yedra Feltrer-Rambaud https://orcid.org/0000-0002-1203-7367
Anneke Moresco https://orcid.org/0000-0002-1525-1166
Beatriz Tomeo-Martín https://orcid.org/0000-0002-7720-4162

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.1279.

REFERENCES

Amrein, K., Scherkl, M., Hoffmann, M., Neuwersch-Sommeregger, S., Köstenberger, M., Tmava Berisha, A., Martucci, G., Pilz, S., & Malle, O. (2020). Vitamin D deficiency 2.0: an update on the current status worldwide. *European Journal of Clinical Nutrition*, *74*, 1498–1513.

AZA_Ape_TAG. (2010). *Chimpanzee (*Pan troglodytes*) care manual*. Silver Spring, MD. Association of Zoos and Aquariums. 107 p.

Bikle, D. D. (2016). Extraskeletal actions of vitamin D. *Annals of the New York Academy of Sciences*, *1376*, 29–52.

Carlsen, F., de Jongh, T., & Pluháčková, J. 2022. EAZA best practice guidelines great ape taxon advisory group chimpanzees (*Pan troglodytes*)–1st edition (p. 146). European Associations of Zoos and Aquariums, Amsterdam, the Netherlands.

Chen, C. *Paper 265-27 Robust regression and outlier detection with the ROBUSTREG procedure*; 2002. Citeseer.

Crissey, S. D., Barr, J. E., Slifka, K. A., Bowen, P. E., Stacewicz-Sapuntzakis, M., Langman, C., Ward, A., & Ange, K. (1999). Serum concentrations of lipids, vitamins A and E, vitamin D metabolites, and carotenoids in nine primate species at four zoos. *Zoo Biology*, *18*, 551–564.

Curry, B. A., Drane, A. L., Atencia, R., Feltrer, Y., Howatson, G., Calvi, T., Palmer, C., Moittie, S., Unwin, S., & Tremblay, J. C. (2022). Body mass and growth rates in captive chimpanzees (*Pan troglodytes*) cared for in African wildlife sanctuaries, zoological institutions, and research facilities. *Zoo Biology*, *42*(1), 98–106..

Dusso, A. S., Brown, A. J., & Slatopolsky, E. A. 2005. *Vitamin D and renal failure* (pp. 1313–1338). Elsevier Inc.

Fischer, K. 2020. Vitamin D. In: R. De Caterina, A. J. Martinez, & M. Kohlmeier (Eds.), *Principles of nutrigenetics and nutrigenomics fundamentals of individualized nutrition* (p 245–254). Academic Press.

Gallagher, J. C. (2013). Vitamin D and aging. *Endocrinology and Metabolism Clinics*, *42*, 319–332.

Haidari, F., Zakerkish, M., Karandish, M., Saki, A., & Pooraziz, S. (2016). Association between serum vitamin D level and glycemic and inflammatory markers in non-obese patients with type 2 diabetes. *Iranian Journal of Medical Sciences*, *41*, 367.

Harmon, Q. E., Umbach, D. M., & Baird, D. D. (2016). Use of estrogen-containing contraception is associated with increased concentrations of 25-hydroxy vitamin D. *Journal of Clinical Endocrinology & Metabolism*, *101*, 3370–3377.

Heath, A. K., Kim, I. Y., Hodge, A. M., English, D. R., & Muller, D. C. (2019). Vitamin D status and mortality: A systematic review of observational studies. *International Journal of Environmental Research Public Health*, *16*, 383.

Holick, M. F. (1981). The cutaneous photosynthesis of previtamin D3: A unique photoendocrine system. *Journal of Investigative Dermatology*, *77*, 51–58.

Holick, M. F. (2009). Vitamin D status: Measurement, interpretation, and clinical application. *Annals of Epidemiology*, *19*, 73–78.

Holick, M. F. (2017). The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. *Reviews in Endocrine and Metabolic Disorders*, *18*, 153–165.

Institute-of-Medicine-Food-and-Nutrition-Board. (2010). *Dietary reference intakes: Thiamin R, niacin, vitamin B6, folate, vitamin B12, pantothenic acid*. Institute-of-Medicine-ood-and-Nutrition-Board.

Jablonski, N. G., & Chaplin, G. (2010). Human skin pigmentation as an adaptation to UV radiation. *Proceedings of Natural Academic Science USA*, *107*, 8962–8968.

Junge, R. E., Gannon, F. H., Porton, I., McAlister, W. H., & Whyte, M. P. (2000). Management and prevention of vitamin D deficiency rickets in captive-born juvenile chimpanzees (*Pan troglodytes*). *Journal of Zoo and Wildlife Medicine*, *31*(3), 361–369.

Khammissa, R., Fourie, J., Motswaledi, M., Ballyram, R., Lemmer, J., & Feller, L. (2018). The biological activities of vitamin D and its receptor in relation to calcium and bone homeostasis, cancer, immune and cardiovascular systems, skin biology, and oral health. *BioMed Research International*, *2018*, 9276380.

Knapka, J. (2003). Nutrient requirements of nonhuman primates. *Laboratory Animals*, *32*, 26.

Luk, J., Torrealday, S., Neal Perry, G., & Pal, L. (2012). Relevance of vitamin D in reproduction. *Human Reproduction*, *27*, 3015–3027.

Meehan, M., & Penckofer, S. (2014). The role of vitamin D in the aging adult. *Journal of Gerontology and Geriatrics*, *2*, 60–71.

Moittié, S., Graham, P. A., Barlow, N., Dobbs, P., Liptovszky, M., Redrobe, S., & White, K. (2020). Comparison of 25-hydroxyvitamin D concentration in chimpanzee dried blood spots and serum. *Veterinary Clinical Pathology*, *49*, 299–306.

Moittié, S., Jarvis, R., Bandelow, S., Byrne, S., Dobbs, P., Grant, M., Reeves, C., White, K., Liptovszky, M., & Baiker, K. (2022). Vitamin D status in chimpanzees in human care: A Europe wide study. *Scientific Reports*, *12*, 1–11.

Muscogiuri, G., Barrea, L., Somma, C. D., Laudisio, D., Salzano, C., Pugliese, G., de Alteriis, G., Colao, A., & Savastano, S. (2019). Sex differences of vitamin D status across BMI classes: An observational prospective cohort study. *Nutrients*, *11*, 3034.

National-Aeronautics-and-Space-Administration-(NASA). (2022). *Prediction of worldwide energy resource (POWER) project*. NASA.

Oates, J. F. (2011). *Primates of West Africa: A field guide and natural history*. Conservation International.

Palacios, C., & Gonzalez, L. (2014). Is vitamin D deficiency a major global public health problem? *Journal of Steroid Biochemistry and Molecular Biology*, *144*, 138–145.

PASA Operations Manual, 2nd edition (2016). https://pasa.org/wp-content/uploads/2016/04/PASA_Operations_Manual_2016.pdf

Pilz, S., Zittermann, A., Obeid, R., Hahn, A., Pludowski, P., Trummer, C., Lerchbaum, E., Pérez-López, F. R., Karras, S. N., & März, W. (2018). The role of vitamin D in fertility and during pregnancy and lactation: A review of clinical data. *International Journal of Environmental Research and Public Health*, *15*, 2241.

Poel, Y., Hummel, P., Lips, P., Stam, F., Van Der Ploeg, T., & Simsek, S. (2012). Vitamin D and gestational diabetes: A systematic review and meta-analysis. *European Journal of Internal Medicine*, *23*, 465–469.

Power, M. L., Oftedal, O. T., Tardif, S. D., & Allen, M. E. Vitamin D and primates: Recurring problems on a familiar theme; 1995.

Sempos, C. T., Heijboer, A. C., Bikle, D. D., Bollerslev, J., Bouillon, R., Brannon, P. M., DeLuca, H. F., Jones, G., Munns, C. F., & Bilezikian, J. P. (2018). Vitamin D assays and the definition of hypovitaminosis D: Results from the First International Conference on Controversies in Vitamin D. *British Journal of Clinical Pharmacology*, *84*, 2194–2207.

Shin, J. S., Choi, M. Y., Longtine, M. S., & Nelson, D. M. (2010). Vitamin D effects on pregnancy and the placenta. *Placenta*, *31*, 1027–1034.

Strong, V., Moittié, S., Sheppard, M. N., Liptovszky, M., White, K., Redrobe, S., Cobb, M., & Baiker, K. (2020). Idiopathic myocardial fibrosis in captive chimpanzees (*Pan troglodytes*). *Veterinary Pathology*, *57*, 183–191.

Tsegai, Z. J., Skinner, M. M., Pahr, D. H., Hublin, J. J., & Kivell, T. L. (2018). Systemic patterns of trabecular bone across the human and chimpanzee skeleton. *Journal of Anatomy*, *232*, 641–656.

Verdoia, M., Schaffer, A., Barbieri, L., Di Giovine, G., Marino, P., Suryapranata, H., De Luca, G., & Group, N. A. S. (2015). Impact of gender difference on vitamin D status and its relationship with the extent of coronary artery disease. *Nutrition, Metabolism and Cardiovascular Diseases*, *25*, 464–470.

við Streym, S., Højskov, C. S., Møller, U. K., Heickendorff, L., Vestergaard, P., Mosekilde, L., & Rejnmark, L. (2016). Vitamin D content in human breast milk: A 9-mo follow-up study. *American Journal of Clinical Nutrition*, *103*, 107–114.

Videan, E. N., Heward, C. B., Fritz, J., Murphy, J., Cortez, C., & Su, Y. (2007). Relationship between sunlight exposure, housing condition, and serum vitamin D and related physiologic biomarker levels in captive chimpanzees (*Pan troglodytes*). *Comparative Medicine*, *57*, 402–406.

Xu, F., Wolf, S., & Xu, J. (2021). Vitamin D in follicular development and oocyte maturation. *Reproduction*, *161*, R129–R137.

Zanatta, A. P., Brouard, V., Gautier, C., Goncalves, R., Bouraïma-Lelong, H., Mena Barreto Silva, F. R., & Delalande, C. (2017). Interactions between oestrogen and 1α, 25 (OH) 2-vitamin D3 signalling and their roles in spermatogenesis and spermatozoa functions. *Basic and Clinical Andrology*, *27*, 1–14.

Zelzer, S., Goessler, W., & Herrmann, M. (2018). Measurement of vitamin D metabolites by mass spectrometry, an analytical challenge. *Journal of Laboratory and Precision Medicine*, *3*, 1–14.

Zhang, R., & Naughton, D. P. (2010). Vitamin D in health and disease: current perspectives. *Nutrition Journal*, *9*, 1–13.

How to cite this article: Feltrer-Rambaud, Y., Moresco, A., Ange-van Heugten, K., Pizarro, A., Tomeo-Martín, B., Carrasco Pesquera, L., Moresco, N., & Atencia, R. (2023). Serum Vitamin D in sanctuary chimpanzees *(Pan troglodytes)* in range countries: A pilot study. *Veterinary Medicine and Science*, 1–9. https://doi.org/10.1002/vms3.1279