

## ASSESSMENT OF SERUM 25-HYDROXYVITAMIN D CONCENTRATIONS IN TWO COLLECTIONS OF CAPTIVE GORILLAS (*GORILLA GORILLA GORILLA*)

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**Abstract:** Serum 25-hydroxyvitamin D concentrations were assessed in subadult to adult captive lowland gorillas (*Gorilla gorilla gorilla*) ( $n = 26$ ) at two institutions with different husbandry and management practices. Serum 25-hydroxyvitamin D (25[OH]D) concentrations for gorillas managed predominantly indoors was low ( $14.2 \pm 5.9$  ng/ml), despite consuming commercial biscuits fortified with vitamin D<sub>3</sub>. Concentrations of 25(OH)D in gorillas with near daily outdoor access were significantly higher than gorillas managed indoors, although many individuals still had serum values below concentrations recommended for adult humans. Consideration should be given to assessing 25(OH)D concentrations in all captive gorillas and providing specific supplementation, particularly to juveniles without access to direct sunlight.

**Key words:** 25-Hydroxyvitamin D, cholecalciferol, *Gorilla gorilla gorilla*, primate, vitamin D.

### INTRODUCTION

Although physicians have been aware of the importance of vitamin D concentrations in bone health and calcium homeostasis in humans for nearly a century, only more recently have vitamin D receptors been documented to be present in most tissues and cells in the body.<sup>13</sup> The role of vitamin D has been elucidated to extend far beyond skeletal development and maintenance of calcium concentrations, including influence on immune function and on modulation of cell proliferation and differentiation, thereby contributing to the prevention of certain types of cancer.<sup>10</sup> Low vitamin D concentrations have been associated with increased risk of type I diabetes and cardiovascular disease.<sup>10</sup> Serum 25-hydroxyvitamin D (25[OH]D) is generally accepted as the best indicator of vitamin D status as it reflects vitamin D that is cutaneously synthesized (25[OH]D<sub>3</sub>) as

well as orally absorbed (25[OH]D<sub>2</sub>).<sup>26</sup> Normal serum concentrations of vitamin D have not been established for wild gorillas, and only rare documentation of vitamin D concentrations in captive gorillas is available. In one study, single serum 25(OH)D concentrations from two captive gorillas with no access to direct sunlight were 15 and 35 ng/ml.<sup>8</sup> In a second study assessing 25(OH)D concentrations in nonhuman primates at four different institutions, the mean value for 25 gorillas was  $16.7 \pm 1.16$  ng/ml.<sup>9</sup>

In 2005, concern about vitamin D concentrations in captive gorillas at a northeastern U.S. zoological institution (ZNE) arose when an infant gorilla that was not exposed to sunlight presented with clinical signs of metabolic bone disease. This animal was successfully parent-reared with supplementation of oral cholecalciferol (Sigma-Aldrich, St. Louis, Missouri 63103, USA; 8,000 IU/ml; 5,000 IU q. 72 hr for 5 doses, then 5,000 IU weekly for 5 yr). To achieve oral supplementation of the offspring, the dam was trained to present varying body parts. Since the mother always carried the offspring, treatment was achieved by asking the mother to present the body part in closest proximity to the offspring. Keepers then placed the supplement directly in the infant's mouth using a syringe with or without a catheter extension. In 2010, a subsequent offspring was parent-reared successfully by prophylactic supplementation with 1,000 IU vitamin D<sub>3</sub> orally per day. No clinical signs of hypovitaminosis D had been recognized in the adult gorillas of this troop. Due to their lack of exposure to sunlight, as the troop was housed

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nearly exclusively indoors, an investigation was performed to assess baseline concentrations of serum 25(OH)D. To serve as a comparison, serum 25(OH)D concentrations were measured from gorillas housed at a southeastern U.S. institution (ZA); these gorillas had frequent outdoor access of at least 4 hr per day.

## MATERIALS AND METHODS

### Animals

At ZNE the gorillas were provided access to indoor exhibit and holding spaces, which did not provide access to natural sunlight, and an outdoor chute (61 ft long × 6 ft wide × up to 7 ft, 7 in tall) with one side of steel bars, one side and both ends of cement, and the top covered with 2-in steel mesh. Little direct sunlight penetrated into the chute due to the thick foliage cover above and on the side. The females were on exhibit daily. Two younger, nonbreeding males alternated with the adult breeding male every other day going on exhibit with the females. When weather conditions were appropriate (outdoor ambient temperatures >50°F [>10°C]) the male gorilla(s) held off exhibit for the day had access to the outdoor chute. From May to September they had access from 9 AM to 5 PM, while from October to April they had access from 8 AM to 4 PM. Generally due to temperature constraints, gorillas did not have chute access from November to April. When they were given access to the chute, they spent an average of 1–2 hr in the chute per day.

At ZA the gorillas were placed in an outdoor exhibit during the day and in an indoor holding area at night. The gorillas went outdoors and had direct access to natural light every day when the temperatures were above 40°F (4.4°C), although animals less than 1 yr old and greater than 45 yr old only went outside if the temperatures were above 50°F (10°C). It was rare that the gorillas did not get access to the outdoor exhibit for an average minimum of 4 hr a day for more than a few days consecutively.

The gorillas at ZNE were fed extruded biscuits (Primate Browse Biscuit, 5MA4, Mazuri, PMI Nutrition International, LLC, Brentwood, Missouri 63144, USA), a mix of fresh vegetables (string beans, cucumber, carrots, celery, kale, romaine, collard greens), fruit (apples, oranges), and oatmeal. According to the U.S. Department of Agriculture (USDA) National Nutrient Database, none of the produce items fed contained vitamin D<sub>2</sub>.<sup>25</sup> Browse (including Russian olive

[*Elaeagnus angustifolia*], knotweed [*Fallopia japonica*], Austree willow [hybrid of *Salix babylonica* and *Salix alba*], sugar maple [*Acer saccharum*], ficus [*Ficus* spp.] and banana leaves (*Musa* spp.) were offered at least three times a week when in season. Documentation of the vitamin D<sub>2</sub> content of these browse items could not be identified. One adult gorilla received Mazuri primate maintenance biscuit 5MA2 in addition to 5MA4, for reasons of individual preference. Both Mazuri diets contained 3,325 IU/kg of vitamin D<sub>3</sub>. Typically, no vitamin or mineral supplement was provided to the adults. However, daily prenatal vitamins (Spring Valley Prenatal Multivitamin, Wal-Mart Stores Inc., Bentonville, Arizona 72716, USA; 400 IU vitamin D<sub>3</sub>/tablet; one tablet p.o. s.i.d. for the duration of the pregnancy) were provided to pregnant gorillas.

At ZA the gorillas were offered Purina High Protein Monkey Diet 5045 (Purina Animal Nutrition, LLC, Gray Summit, Missouri 63039, USA) from 1992 to 2000, at which time the biscuit was changed to HMS High Fiber Primate Diet (Republic Mills, Okalona, Michigan 43350, USA). The Purina diet contained 6,600 IU/kg vitamin D<sub>3</sub>, whereas the HMS diet contained 3,542 IU/kg. The gorillas were also offered a mix of fresh vegetables (greens, cabbage, sweet potato, carrot, corn, celery), fruit (citrus, apple, banana, tomato, kiwi, melon), and browse (bamboo [*Bambusa vulgaris*], collard ends [*Brassica oleracea*], banana, elm [*Ulmus* spp.], and mulberry [*Morus rubra*]). According to the USDA National Nutrient Database, none of the produce items fed contained vitamin D<sub>2</sub>, but the vitamin D<sub>2</sub> content of the browse is not known.<sup>25</sup> A subset of gorillas was on vitamins that may have contained vitamin D at the time of their blood collection. However, due to record keeping and the extensive period over which samples were collected, the exact type of vitamin could not always be documented for each gorilla.

### Serum/plasma 25-hydroxyvitamin D assay

Concentrations of 25(OH)D (including both 25[OH]D<sub>2</sub> and 25[OH]D<sub>3</sub>) from two serum samples opportunistically collected from each of the six adult gorillas ranging from 9.5 to 39 yr old at ZNE were compared with those from 20 subadult to adult gorillas ranging in age from 6 to 44.5 yr old at ZA. Note that for one gorilla at ZNE, 25(OH)D was obtained from a plasma sample, but no difference is expected in 25(OH)D concentrations in serum compared to plasma.<sup>23</sup> The time interval between the two samples ranged from 4

mo to 5.5 yr. Samples, which were collected between August 1992 and December 2013, were stored frozen at  $-80^{\circ}\text{C}$  until analysis. Samples were analyzed for 25(OH)D concentrations using a serum vitamin D binding protein method as described previously.<sup>5</sup> In order to confirm the data generated by this method, a high-performance liquid chromatographic (HPLC) method was also utilized to analyze a representative group of samples.<sup>4</sup> Briefly, an aliquot of 3H-25(OH)D<sub>3</sub> (PerkinElmer/New England Nuclear, Boston, Massachusetts 02118, USA) was added into serum or plasma samples prior to extraction with acetonitrile for recovery calculation purpose. The extract was then applied to a Bond-Elute C-18 reversed phase cartridge (Agilent Technologies, Santa Clara, California 95051, USA) to remove neutral lipids and other vitamin D metabolites as described.<sup>6</sup> The fraction containing both 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> as detected at 265 nm was eluted with 1% 2-propanol in hexane, and was dried down under a stream of nitrogen gas, redissolved in acetonitrile, and then applied to an HPLC system (Waters Corporation, Milford, Massachusetts 01757, USA) equipped with a C-18 reversed-phase column (5- $\mu\text{m}$  particle size, 250  $\times$  4.6 mm, Agilent Technologies) using 100% acetonitrile as a mobile phase with a flow rate of 1.0 ml/min to separate 25(OH)D from polar and nonpolar contaminants. The fraction containing 25(OH)D was collected, dried, and reconstituted in the same running solvent consisting of 0.35% methanol and 3.5% 2-propanol in n-hexane, and then applied to a normal phase silica HPLC column (5  $\mu\text{m}$ , 250  $\times$  4.6 mm, Alltech Associates Inc., Deerfield, Illinois 60015, USA), with a flow rate of 1.8 ml/min for the final purification and to separate 25(OH)D<sub>3</sub> from 25(OH)D<sub>2</sub>. The eluent containing 25(OH)D<sub>3</sub> was collected in a counting vial, dried down, and dissolved in 5 ml scintillation cocktail and counted in a Wallac Micro Beta TriLus counter (PerkinElmer, Shelton, Connecticut 06484, USA) to obtain the percentage of recovery values for each sample after a lengthy preparation procedure. The final concentration of 25(OH)D<sub>3</sub> in the unknown serum was calculated first by comparing its integrated peak area obtained from HPLC against a standard curve obtained by running a series of increasing concentrations of a standard 25(OH)D<sub>3</sub> solution to generate a series of increasing ultraviolet (UV) 265-nm absorbance peak areas, and then the concentrations were corrected with percentage of recovery.

### Statistical analysis

All statistical analyses were performed using JMP® 8.0.2.2 (SAS Institute Inc., Cary, North Carolina 27513, USA). For all results, both parametric and complementary nonparametric analyses were conducted. In all cases, statistical significance did not differ between the parametric and nonparametric tests. Thus, parametric values (average  $\pm$  95% confidence interval) were presented for ease of interpretation.

To assess for an association between serum 25(OH)D concentrations and seasonality, the ZA data were divided into "summer" and "winter" periods. Summer was defined as April through October, when the average high temperature at the southeastern facility was  $>70^{\circ}\text{F}$ , ( $21.1^{\circ}\text{C}$ ).<sup>28</sup> The average annual UV index for this location from 2011 to 2015 was 6.15. For 2015, the average UV index for the summer months was 8.75 versus 3.00 for the winter months.<sup>17</sup> Winter was defined as November through March, when the average high temperature was  $<70^{\circ}\text{F}$ , ( $21.1^{\circ}\text{C}$ ).

### RESULTS

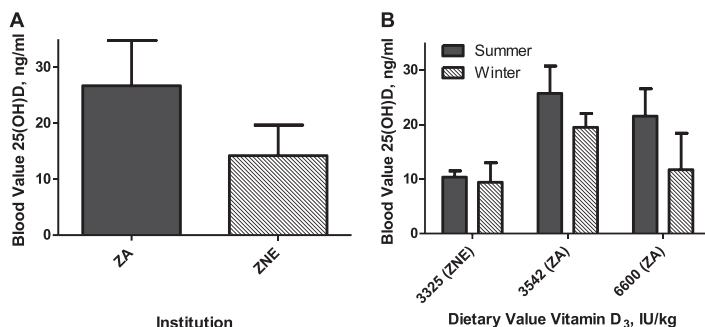
The binding assay used in this study was positively correlated to a HPLC assessment of the same sample ( $r^2 = 0.89$ ,  $n = 7$ , binding assay range 16–150 ng/ml). A total of 26 gorillas were sampled for 25(OH)D: 20 from ZA and 6 from ZNE with each animal being sampled twice (Table 1). However, the second sample from one ZNE gorilla was excluded from statistical analysis due to a markedly elevated (150 ng/ml) 25(OH)D value, likely a result of consumption of Mazuri biscuit 5MA2 tainted with high concentrations of vitamin D<sub>3</sub> (Tollefson, pers. comm.). It should be noted that only one animal was affected since only this individual was receiving the 5MA2 biscuit. For the remaining 25 gorillas the individual difference between the first and second measurement of 25(OH)D was not different from 0. The percentage of difference of 25(OH)D between replicated individual first and second samples was  $-11.3 \pm 27.1\%$ , average  $\pm$  95% confidence interval, while the time between samples averaged  $902.6 \pm 285.2$  days. However, no association was identified between the absolute value of the difference between individual samples, or the time interval between the samples ( $r^2 = 0.02$ ).

Overall the average 25(OH)D value per gorilla was greater at ZA ( $26.7 \pm 16.8$  ng/ml) than at ZNE ( $14.2 \pm 5.9$  ng/ml) (unpaired  $t$ -test = 2.06,  $P < 0.01$ ; Fig. 1A). The ZA gorillas differed from those at ZNE in that they had access to the

**Table 1.** Serum 25-hydroxyvitamin D (25[OH]D) concentration in blood samples from gorillas (*Gorilla gorilla gorilla*) at a northeastern (ZNE) and a southeastern (ZA) U.S. institution.

Gorilla	Institution <sup>a</sup>	Age when sampled (yr)	Sex	Diet	25(OH)D (ng/ml)
1	ZNE	39.3	0.1	5MA2/5MA4	22
1	ZNE	40.3	0.1	5MA2/5MA4	150 <sup>a</sup>
2	ZNE	29.3	0.1	5MA4	17
2	ZNE	31.2	0.1	5MA4	16
3	ZNE	15.2	1.0	5MA4	<5
3	ZNE	20.9	1.0	5MA4	16
4	ZNE	15.3	1.0	5MA4	<5
4	ZNE	20.6	1.0	5MA4	<5
5	ZNE	9.6	0.1	5MA4	9
5	ZNE	13.6	0.1	5MA4	11
6	ZNE	21.5	1.0	5MA4	16
6	ZNE	25.6	1.0	5MA4	18
7	ZA	37.4	1.0	Purina 5045	25
7	ZA	42.0	1.0	HMS	12
8	ZA	30.6	1.0	Purina 5045	15
8	ZA	31.1	1.0	Purina 5045	16
9	ZA	37.2	0.1	Purina 5045	50
9	ZA	44.5	0.1	HMS	40
10	ZA	40.0	0.1	HMS	98
10	ZA	40.3	0.1	HMS	58
11	ZA	35.5	1.0	Purina 5045	54
11	ZA	36.1	1.0	Purina 5045	40
12	ZA	21.3	0.1	Purina 5045	35
12	ZA	21.7	0.1	Purina 5045	17
13	ZA	13.2	0.1	Purina 5045	14
13	ZA	13.7	0.1	Purina 5045	19
14	ZA	30.3	0.1	Purina 5045	<5
14	ZA	30.6	0.1	Purina 5045	7
15	ZA	12.6	1.0	HMS	16
15	ZA	14.3	1.0	HMS	17
16	ZA	9.7	0.1	Purina 5045	18
16	ZA	14.0	0.1	HMS	16
17	ZA	6.1	1.0	Purina 5045	16
17	ZA	8.7	1.0	HMS	11
18	ZA	10.1	0.1	HMS	15
18	ZA	13.5	0.1	HMS	17
19	ZA	31.1	1.0	HMS	6
19	ZA	31.6	1.0	HMS	19
20	ZA	11.7	0.1	HMS	35
20	ZA	13.4	0.1	HMS	21
21	ZA	13.9	1.0	HMS	22
21	ZA	17.2	1.0	HMS	20
22	ZA	22.2	0.1	HMS	25
22	ZA	22.6	0.1	HMS	20
23	ZA	6.4	0.1	HMS	21
23	ZA	11.0	0.1	HMS	37
24	ZA	11.0	1.0	HMS	53
24	ZA	13.5	1.0	HMS	35
25	ZA	14.0	1.0	HMS	31
25	ZA	15.6	1.0	HMS	22
26	ZA	23.8	0.1	Purina 5045	26
26	ZA	24.4	0.1	Purina 5045	27

<sup>a</sup> This vitamin D concentration was not included in the statistical analysis because there was an accidental manufacturer's oversupplementation that occurred in diet 5MA2.



**Figure 1.** A. The average serum 25-hydroxyvitamin D (25[OH]D) concentration for gorillas (*Gorilla gorilla gorilla*) at a southeastern U.S. institution (ZA) as compared to a northeastern U.S. institution (ZNE). B. Comparison of 25(OH)D concentrations in gorillas in summer versus winter at ZNE versus ZA with notation of the dietary level of vitamin D<sub>3</sub> supplementation in the biscuit.

outdoors and were fed biscuits with higher concentrations of vitamin D<sub>3</sub> (6,600 IU/kg until 2000, and 3,542 IU/kg after 2000). While a trend for summer values to be greater than winter values was observed (Fig. 1B), this difference was not identified to be significant (two-way analysis of variance [ANOVA],  $F_{1,36} = 1.89$ ,  $P > 0.15$ ). Likewise diet was not associated with a significant difference in 25(OH)D concentrations (two-way ANOVA,  $F_{1,36} = 0.43$ ,  $P > 0.5$ ).

## DISCUSSION

Vitamin D<sub>3</sub> promotes calcium absorption from the intestine, reabsorption from the kidney, and resorption from the bone to increase serum calcium concentrations. Animals with inadequate 25(OH)D concentrations will become hypocalcemic. In response to hypocalcemia, parathyroid hormone is released, which promotes bone resorption and renal phosphorus excretion.

Sources of vitamin D for gorillas include diet and endogenous production following exposure to UV light. In humans, placental transfer of vitamin D occurs and is dependent on maternal concentrations of vitamin D. The fetus can potentially form stores of vitamin D, but these reserves will wane within the first several weeks postpartum.<sup>11</sup> Supplementing lactating mothers with 4,000–6,000 IU vitamin D<sub>3</sub> per day can provide sufficient concentrations in the milk to satisfy the infant's requirements.<sup>16</sup> However, most prenatal vitamins only contain 400 IU vitamin D<sub>3</sub>, resulting in inadequate concentrations of vitamin D in the breast milk. Human infants, therefore, generally rely on direct supplementation and endogenous production of vitamin D.<sup>11</sup> Exposure to natural sunlight causes photo-conversion of 7-dehydrocholesterol to previtamin D<sub>3</sub>, which is

then thermo-isomerized to vitamin D<sub>3</sub>. The vitamin D<sub>3</sub> is hydroxylated first in the liver to form 25(OH)D<sub>3</sub>, then in the kidney to form 1,25 dihydroxyvitamin D<sub>3</sub> (1,25[OH]<sub>2</sub>D<sub>3</sub>), the biologically active form. Commercially produced formulated diets for nonhuman primates are typically fortified with vitamin D<sub>3</sub>. For primates that need vitamin D supplementation, vitamin D<sub>3</sub> is generally preferred to vitamin D<sub>2</sub>. Although successful treatment of hypovitaminosis D was achieved using vitamin D<sub>2</sub> in juvenile chimpanzees, vitamin D<sub>3</sub> is more effective at increasing 25(OH)D serum concentrations in humans.<sup>18,19</sup> The contribution of vitamin D<sub>2</sub> in overall vitamin D status in wild and captive gorillas has not been established.

The mean 25(OH)D concentration of ZNE gorillas was significantly lower than that of gorillas at ZA. Although dietary supplementation may have played a role, the difference is most likely attributed to ZA gorillas typically having daily access to direct sunlight while ZNE gorillas were housed indoors. Although at times during the study interval, the ZA gorillas were offered biscuits supplemented with higher concentrations of cholecalciferol than they receive now, the vitamin D<sub>3</sub> concentration of the biscuit currently being offered at ZA (3,542 IU/kg) is similar to that of ZNE (3,325 IU/kg), yet the serum 25(OH)D concentrations of the ZA gorillas are still markedly higher (Fig. 1B). It is also noteworthy that the animals at ZA fed the diet with higher vitamin D<sub>3</sub> concentrations (Purina High Protein Monkey Diet 5045; 6,600 IU/kg vitamin D<sub>3</sub>) actually had lower serum 25(OH)D concentrations than the gorillas fed the diet with lower concentrations of vitamin D<sub>3</sub> supplementation (HMS High Fiber Primate Diet; 3,542 IU/kg vitamin D<sub>3</sub>) (Fig. 1B). Possible reasons for this include inaccurate fortification of biscuits, as well



as variation in the palatability and consumption of the biscuit offered. The gorillas at the two institutions were offered different produce and browse items. However, according to the USDA National Nutrient Database, none of the produce items fed at either institution contain vitamin D<sub>2</sub>.<sup>25</sup> It is possible that there are differing concentrations of vitamin D<sub>2</sub> in the browse.

While ideal concentrations of 25(OH)D in humans have not been firmly established, strong evidence supports that serum concentrations should be at least 30 ng/ml.<sup>15,21</sup> Concentrations from 21 to 29 ng/ml are considered insufficient; 10–20 ng/ml, deficient; and less than 10 ng/ml, severely deficient.<sup>21</sup> Despite provision of fortified biscuits, none of the ZNE animals had concentrations of 25(OH)D that would be considered sufficient in an adult human. Six of 11 samples would be considered deficient, and 4 of 11 samples would be considered severely deficient. Considering the lack of direct sunlight exposure, these results were not surprising. However, it was unexpected that 17 of 40 samples from ZA gorillas also would be considered deficient by adult human standards with 3 of 40 samples considered severely deficient. One of the gorillas with serum 25(OH)D concentrations <10 was confirmed with hypothyroidism and was overweight at the time of sampling. Vitamin D is a fat-soluble vitamin, and increased adiposity has been associated with lower vitamin D concentrations in humans.<sup>26,29</sup> However, another gorilla that was noted to be in heavy body condition actually had a high 25(OH)D concentration. Gorillas also were measured with higher 25(OH)D concentrations when they were in overweight body condition as compared to samples taken when they were assessed in good body condition. In humans, increased skin pigment is associated with reduced synthesis of vitamin D<sub>3</sub>.<sup>7</sup> It is possible that the pigment in gorilla skin also contributes to lower concentrations of serum 25(OH)D. Although humans and gorillas share 98% of their DNA, it is possible that normal 25(OH)D concentrations differ between the two species. As forest-dwelling animals, wild gorillas may have less direct UV-B exposure and lower 25(OH)D concentrations as compared to humans. Nocturnal animals with minimal to no direct UV-B exposure, such as certain chiropterans, can have low to nondetectable levels of 25(OH)D, yet maintain adequate serum mineral concentrations.<sup>3</sup>

In humans, low serum concentrations of 25(OH)D can cause increased renin synthesis, leading to hypertension and cardiovascular dis-

ease, including left ventricular hypertrophy.<sup>21</sup> Low 25(OH)D concentrations are associated with decreased gastrointestinal calcium absorption and resultant increase in parathyroid hormone, which is also associated with increased blood pressure, and hypertrophy and fibrosis of the left ventricle.<sup>21</sup> Several types of cardiovascular disease, including congestive heart failure, aortic dissection, and atherosclerotic coronary artery disease, have been documented in gorillas, but the most common manifestation is myocardial replacement fibrosis, also known as fibrosing cardiomyopathy.<sup>23,24,27</sup> In many apes with myocardial fibrosis, left ventricular hypertrophy is present.<sup>23</sup> Vitamin D deficiency should be considered as a possible contributor to some of these conditions. Further research is warranted to investigate associations between 25(OH)D concentrations in gorillas and the presence of hypertension, cardiac disease, cancer, diabetes, metabolic bone disease, and immune function.

To treat vitamin D deficiency in humans, initially high doses of oral vitamin D (50,000 IU weekly for up to 12 wk) are administered, followed by a maintenance dose of 1,000–2,000 IU/day of vitamin D<sub>3</sub>.<sup>13,21</sup> Wide therapeutic ranges of supplemental vitamin D<sub>3</sub> have been used with no toxic effects seen with doses up to 10,000 IU/day for 5 mo.<sup>12</sup> To increase serum 25(OH)D concentrations in gorillas, oral supplementation appears to be an efficient method that is easy to administer. Additionally, for those institutions that cannot provide gorillas access to direct sunlight year-round, skylights and windows that utilize UV-B-transmitting material could be used. However, these materials still block many UV-B rays and become less effective at transmission over time.<sup>1</sup> Providing UVB light bulbs can be impractical since the animal typically needs to be within 18 in of the light, although newer 160-W light bulbs can now project UVB for several feet. UV-B-transmitting skylights and UV-B-emitting bulbs, however, only provide a fraction of the UV-B provided by direct exposure to natural sunlight.<sup>1</sup> Further research is warranted to determine the reference range for serum 25(OH)D concentrations and/or UV-B exposure of wild gorillas. Although obtaining serum samples would be ideal, it may be logistically more feasible to ascertain their UV-B exposure. By observing their natural basking, feeding, and resting behavior—and measuring the UV-B exposure at those sites—an overall assessment of their daily UV-B exposure could be made. This could then be used to provide

appropriate UV-B exposure in captivity, as has been done for a number of reptile species.<sup>2</sup> Although providing gorillas with UV-B irradiation in a large indoor exhibit or holding space may seem impractical, it is possible that the gorillas may seek the exposure if offered. Human skin cells exposed to UV-A and UV-B radiation produced  $\beta$ -endorphins, and humans have significant increases in serum  $\beta$ -endorphin levels after sunning in tanning beds.<sup>14</sup> Mice also had increased  $\beta$ -endorphin levels after UV-B exposure.<sup>14</sup> It is possible that gorillas may be attracted to UV-B light sources if it increases their endorphin levels. In one study panther chameleons (*Furcifer pardalis*) with different levels of dietary vitamin D appeared to alter their basking habits in correlation with their level of supplementation, basking for longer periods if supplementation levels were low.<sup>20</sup> Perhaps gorillas with low serum 25(OH)D concentrations would seek UV-B basking opportunities as well.

### CONCLUSIONS

In conclusion, it is advisable for 25(OH)D concentrations to be opportunistically monitored in captive gorillas. Although gorillas in all but three Association of Zoos and Aquariums-accredited zoos in the United States housing gorilla ( $n = 47$ ) obtain access to natural sunlight, many have only seasonal access. Additionally, in this study even gorillas with access to direct, and nearly daily, sunlight appear to have concentrations of 25(OH)D that would be considered low as compared to human standards. Therefore strong consideration should be given to supplementing adult gorillas with serum concentrations less than 30 ng/ml, and all infant and juvenile gorillas while they are nursing. Infant and juvenile gorillas appear to maintain 25(OH)D concentrations considered adequate for humans when receiving 1,000 IU vitamin D<sub>3</sub> per day. At ZNE the adult male gorillas are currently supplemented with 4,000 IU daily, while the females are supplemented with 2,000 IU daily. Serum concentration obtained from one female 4 mo after initiating supplementation was considered adequate (54 ng/ml). Reference values for 25(OH)D concentrations in wild gorillas would provide context for more specific recommendations.

*Acknowledgments:* The authors would like to thank the Zoo New England gorilla keepers and veterinary technicians, and Jeannine Jackle and

Stephanie Earhart for their assistance with data research and preparation of the manuscript.

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*Received for publication 19 February 2016*