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RESEARCH ARTICLE

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Vitamin D status in wild toque macaques (*Macaca sinica*) in Sri Lanka

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The vitamin D receptor is found on most cells, including active immune cells, implying that vitamin D has important biological functions beyond calcium metabolism and bone health. Although captive primates should be given a dietary source of vitamin D, under free-living conditions vitamin D is not a required nutrient, but rather is produced in skin when exposed to UV-B light. The circulating level of 25 hydroxyvitamin D (25-OH-D) considered adequate for humans is a topic of current controversy. Levels of circulating 25-OH-D sufficient for good health for macaques and other Old World anthropoids are assumed to be the same as human values, but data from free-living animals are scant. This study reports values for 25-OH-D and the active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25$[OH]_2$ D) for wild, forest-ranging toque macaques (*Macaca sinica*) in Sri Lanka. Plasma samples were obtained from eight adult males, seven juvenile males, six young nulliparous females, nine adult females not pregnant or lactating, eleven lactating adult females, and four pregnant females. Mean values for the complete sample were 61.3 ± 4.0 ng/ml for 25-OH-D and 155.6 ± 8.7 pg/ml for 1,25$[OH]_2$ D. There were no significant differences for either metabolite among age and sex classes, nor between lactating and non-reproductive females. Values from the literature for circulating 25-OH-D in captive macaques are three times higher than those found in this wild population, however, 1,25$[OH]_2$ D values in captive animals were similar to the wild values. The data from this study indicate that anthropoid primates exposed to extensive sunlight will have circulating values of 25-OH-D generally above 30 ng/ml, providing some support for the Endocrine Society recommendations for humans. Current dietary vitamin D supplementation of captive macaques likely exceeds requirement. This may affect metabolism and immune function, with possible consequences for macaque health and biomedical research results.

KEYWORDS
captive management, health, nutrition, primate

1 | INTRODUCTION

The primary known biological actions of vitamin D are in support of calcium metabolism and bone health (Pludowski et al., 2013). However, the vitamin D receptor is found on most cells, including not only those cells important in calcium homeostasis (e.g., intestine, kidney, parathyroid gland, and bone), but also active immune cells, pancreatic beta cells, bronchial epithelial cells, skin epithelial cells, testes, and mammary gland (Wang, Zhu, & DeLuca, 2012). Varying evidence indicates potential roles for vitamin D in immune function, metabolism, and cell differentiation (Pludowski et al., 2013; Rosen et al., 2012). Inadequate vitamin D status is implicated in increased susceptibility to tuberculosis (Nielsen et al., 2010; Nnoaham & Clarke, 2008) and colorectal cancer (Rosen et al., 2012). Adequate vitamin D status appears to be beneficial to health in more ways than for calcium metabolism and bone.

Although it is recommended that all captive primates be given a dietary source of vitamin D, under wild, free-living conditions vitamin D is not a required nutrient, but rather is an endogenously produced substance. When exposed to unfiltered natural sunlight, a large number of vertebrates, including primates, produce vitamin D in the skin by photoconversion of 7-hydrocholesterol. It is only relatively recently in human history that vitamin D became a required nutrient. Our species evolved under circumstances of extensive sun exposure, so in the evolutionary sense vitamin D was not a true vitamin but rather an endogenously photosynthetically

produced precursor to the active steroid hormone, 1,25-dihydroxyvitamin D (1,25$[OH]_2$ D). The geographic expansion of humans, the modern environment, and medical concerns over the sequela of extensive sun exposure (e.g., melanoma) have created conditions where for much of modern humans endogenous vitamin D production is likely far below that of our ancestors. Similarly, for many of the captive animals in our care sun exposure is less than in the evolutionary past. This is especially true for tropical primates, which are often housed indoors in facilities located in temperate areas. The importance of dietary vitamin D, both naturally occurring in foods and via supplements added to foods or consumed directly, has increased.

Vitamin D itself has limited biological activity. Two sequential hydroxylations are required to produce the most biologically active metabolite, 1,25$[OH]_2$ D. The first is an unregulated hydroxylation that takes place in the liver to produce 25 hydroxyvitamin D or 25-OH-D. This is the primary circulating vitamin D metabolite, and serves as the substrate for the regulated hydroxylation in the kidney that produces the active metabolite. Thus, 25-OH-D can be considered the main storage form of vitamin D. Low serum concentrations of 25-OH-D is considered diagnostic of vitamin D deficiency (Pludowski et al., 2013).

There is continuing controversy over the appropriate levels of circulating 25-OH-D that represent good health for humans. The result has been conflicting recommendations from the Institute of Medicine (IOM) (IOM, 2011; Rosen et al., 2012; Ross et al., 2011) and the Endocrine Society (Holick et al., 2011). Based on data regarding bone health, the IOM panel concluded that a circulating level above 20 ng/ml 25-OH-D is sufficient (Rosen et al., 2012; Ross et al., 2011). Other researchers have questioned this finding, citing both a concern that circulating levels of 25-OH-D at 20 ng/ml have not been shown to be sufficient for bone health for all populations and that other non-skeletal functions of vitamin D potentially important for health, which the IOM report discounts based on inadequate evidence, may yet be shown to be important and require higher circulating levels of 25-OH-D (Heaney & Holick, 2011; Hollis & Wagner, 2013). The Endocrine Society defined deficiency as circulating 25-OH-D of less than 20 ng/ml but also defined 20–29 ng/ml as insufficiency, with a recommended level of above 30 ng/ml (Holick et al., 2011).

In support of the Endocrine Society recommendations, some researchers point to the likely higher circulating levels of 25-OH-D in our ancestors that relied primarily on photosynthetic production of vitamin D, arguing that our vitamin D metabolism is adapted to high endogenous production due to extensive exposure to sunlight (Heaney & Holick, 2011; Hollis & Wagner, 2013). For example, circulating levels of 25-OH-D in traditionally-living people in Tanzania (mean 44 ng/ml; range of 23–69 ng/ml) were double the IOM suggested 20 ng/ml value for sufficiency (Luxwolda et al., 2013).

Because many captive primates cannot be kept under conditions where they will receive sufficient unfiltered sunlight, vitamin D is routinely provided in the diet. Levels of circulating 25-OH-D sufficient for good health for Old World anthropoids are assumed to be the same as the human values. Higher levels have been suggested to be necessary for New World monkeys. However, there are few published studies that provide data on natural levels of circulating 25-OH-D in wild primate populations.

In this study we examined serum concentrations of 25-OH-D and 1,25$[OH]_2$ D collected from wild toque macaques (*Macaca sinica sinica*) from Sri Lanka, including adult and juvenile animals of both sexes. Among adult females, samples were obtained from four pregnant females, eleven lactating females, and nine females that were neither lactating nor pregnant. These data provide information on levels of 25-OH-D under natural, evolutionarily relevant circumstances, inform our recommendations for appropriate circulating levels of 25-OH-D in captive macaques, and are relevant to the continuing discussions regarding appropriate values in human populations.

2 | METHODS

2.1 | Study site and subjects

The animals ($n = 45$) in this study were from nine different social groups of wild toque macaques (B, CF, IHS, IHN, IH3, IH4, IH5, N, and M) that were part of a long-term study (1968–2017) located in the natural dry evergreen forest of the Archeological Reserve and Nature Sanctuary at Polonnaruwa, Sri Lanka, at 7.9403° N, 81.0188° E (e.g., Dittus, 2013). The floral composition and structure of the forest has been described earlier (Dittus, 1977) and was subject to cyclone damage in November 1978 (Dittus, 1985). The area has contrasting seasons of heavy rainfall during the North-East monsoon (November to January), and drought (May to September) during the South-West monsoon, and light rain in the intervening months (Dittus, 1977). The macaques in the present study were sampled in 1994, on 11 different days (between 13 July and 27 August), during the peak of the dry season when foliation in the upper canopy layer was minimal, or insolation was maximal. Macaques, however, thermoregulate by shunning direct exposure to the sun.

Longevity in this population of macaques can exceed 30 years of age, mean lifespan, however, is less than 8 years owed to high mortality among infants and juveniles, and males and females differ markedly in survivorship schedules (Dittus, 2004). Compared to captive or food provisioned populations of other species of macaques, the wild toque macaques show slower population growth and generally greater mortality owed primarily to the effects of a limited food supply in the wild (Dittus, 1980, 2012).

The research was in compliance with all legal and ethical requirements of the government of Sri Lanka, the Smithsonian Institution, and with the American Society of Primatologists Principles for the Ethical Treatment of Non Human Primates. All animals were individually recognized, and were of known age (Dittus & Thorington, 1981). Procedures for the capture and release of macaques had been described earlier (Hoelzer, Dittus, Ashley, & Melnick, 1994). Animals were baited into live traps, anesthetized with ketamine hydrochloride (Ketalar, Park-Davis Co.), and a blood sample was drawn. Animals were categorized by sex, age, and reproductive status into the following groups: adult male

(*n* = 8, age range 9.2–22.4 years), juvenile male (*n* = 6, 4.4–6.2 years), nulliparous female (*n* = 7, 3.6–6.0 years), adult female not pregnant or lactating (*n* = 7, 7.2–24.4 years), lactating adult female (*n* = 13, 6.3–20.5 years), or pregnant adult female (*n* = 4, 9.5–21.4 years).

2.2 | Sample collection and analyses methods

The blood was centrifuged, the plasma removed to a cryovial, placed in liquid nitrogen and shipped to the Nutrition Laboratory of the Smithsonian National Zoological Park in Washington DC. The plasma samples were stored at −20°C until they were shipped on dry ice to the laboratory of Dr. Michael F. Holick, Boston University School of medicine where they were analyzed for 25 OH-D using a competitive protein binding assay as described in Chen, Turner, and Holick (1990a) and $1,25[OH]_2$ D by the methods described in Chen, Turner, and Holick, (1990b).

2.3 | Statistical analyses

Concentrations of 25-OH-D (ng/ml) and $1,25[OH]_2$ D (pg/ml) are presented as median and mean ± SEM. The relation between the two vitamin D metabolites was assessed using Pearson correlation. Differences among age-sex categories were assessed using analysis of variance. The values for 25 OH-D were compared with values from 18 adult wild cotton-top tamarins (Power et al., 1997), a New World monkey native to Colombia, using ANOVA. Qualitative comparisons were also made with published data from free-ranging rhesus macaques on Cayo Santiago supplemented with vitamin D fortified food (Vieth, Kessler, & Pritzker, 1987) and captive macaques with minimal sunlight exposure but fed vitamin D fortified diets (Marx, Jones, Weinstein, Chrousos, & Renquist, 1989; Shinki et al., 1983; Ziegler, Kapoor, Hedman, Binkley, & Kemnitz, 2015).

3 | RESULTS

The maximum concentrations the assays could measure were 150 ng/ml for 25-OH-D and 260 pg/ml for $1,25[OH]_2$ D. One adult male had a 25-OH-D concentration above 150 ng/ml; his value was set to 151 ng/ml. Four adult females had $1,25[OH]_2$ D concentrations above 260 pg/ml (for statistical purposes conservatively set to 261 pg/ml). Three of the subjects with off-scale $1,25[OH]_2$ D were females in mid or late pregnancy and the other was a small adult female whose infant had died; there is no evidence that she was pregnant at the time the blood sample taken.

The ranges of values for the concentrations of both 25-OH-D (16–151 ng/ml) and $1,25[OH]_2$ D (71–261 pg/ml) were substantial. The mean values for the complete sample were 61.3 ± 4.0 ng/ml for 25-OH-D and, excluding the pregnant females, 149.3 ± 8.2 pg/ml for $1,25[OH]_2$ D. There were no significant differences in serum concentrations for either metabolite among the age and sex classes, nor between lactating and non-reproductive females (Table 1). The values for 25-OH-D and $1,25[OH]_2$ D were not correlated ($r = -0.187$, $p = 0.248$).

The four pregnant females did not differ from the other animals in circulating 25-OH-D; one of them had the lowest value of the sample (16 ng/ml) but the other three pregnant females had 25-OH-D values of 39, 58, and 120 ng/ml. Their circulating $1,25[OH]_2$ D concentrations were higher than for the other animals, even assuming a conservative value of 261 pg/ml for three of the values ($p = 0.001$). The pregnant female with lower $1,25[OH]_2$ D (97 pg/ml) was in early pregnancy; the other three with $1,25[OH]_2$ D values above 260 ng/ml were in mid or late pregnancy.

The mean value for 25-OH-D for wild toque macaques was slightly but significantly lower than the value obtained from wild cotton-top tamarins (*N* = 18, 76.4 ± 5.6 ng/ml, $p = 0.043$; Power et al., 1997), although there was extensive overlap in the ranges

TABLE 1 Median and mean ± standard error (SEM) for 25-OH-D and $1,25[OH]_2$ D by different age, sex, and reproductive classes for free-living toque macaques (*Macaca sinica*)

	25-OH-D (ng/ml)	$1,25[OH]_2$ D (pg/ml)
Juvenile male	62	152
N = 7	71.3 ± 8.4	177.3 ± 17.1
Juvenile female	63	150.5
N = 6	60.0 ± 10.0	155.7 ± 19.6
Adult male	66	117.5
N = 8	72.1 ± 12.8	121.0 ± 15.7
Adult female neither pregnant nor lactating	56	150
N = 9	53.6 ± 6.2	149.4 ± 21.7
Lactating adult female	53	142
N = 11	55.4 ± 7.0	148.4 ± 15.5
Pregnant adult female	48.5	Above 260
N = 4	58.3 ± 22.3	Cannot be calculated
All animals excluding pregnant females	60	142
N = 41	61.6 ± 4.0	149.3 ± 8.2

TABLE 2 Mean ± SEM values for 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D for captive rhesus (*Macaca mulatta*) and cynomologus (*M. fascicularis*) macaques

	25-OH-D (ng/ml)	1,25[OH]$_2$ D (pg/ml)
Rhesus macaque (Vieth et al., 1987)		
Juvenile males (*N* = 10)	165 ± 26	201 ± 48
Juvenile females (*N* = 12)	163 ± 43	145 ± 33
Adult males (*N* = 10)	218 ± 21	125 ± 14
Adult females (*N* = 13)	221 ± 22	163 ± 57
*Rhesus macaque (Ziegler et al., 2015)		
Adult males and females (*N* = 25)	155 ± 5.5	206 ± 19
*Cynomologus macaques (Ziegler et al., 2015)		
Adult males (*N* = 25)	165 ± 6.9	193 ± 15
Rhesus macaque (Marx et al., 1989)		
Adult males and females; diet with 1.5 IU/g vitamin D (*N* = 3)	68 ± 8	Not measured
Diet with 6 IU/g vitamin D_3 (*N* = 6)	144 ± 10	Not measured
Cynomologus macaques (Marx et al., 1989)		
Adult males and females; diet with 1.5 IU/g vitamin D (*N* = 3)	44 ± 3	Not measured
Diet with 6 IU/g vitamin D_3 (*N* = 6)	96 ± 6	Not measured
Rhesus macaques (Shinki et al., 1983)		
Adult females (*N* = 6); diet with 2.4 IU/G vitamin D	50 ± 4	100 ± 5

*Diets for both macaque species in Ziegler et al. (2015) had 8 IU/g vitamin D.

(25.5–120 ng/ml for cotton top tamarins). Circulating concentrations of 25-OH-D in captive macaques fed diets with moderate levels of vitamin D (e.g., 1.5 IU/g) were similar to the values for wild toque macaques, however, values for feral (Vieth et al., 1987) and captive (Marx et al., 1989; Ziegler et al., 2015) macaques fed diets supplemented with high levels of vitamin D were two-to-four times higher than the values found in this wild population (Table 2). Values from the literature for circulating 1,25[OH]$_2$ D values in managed feral and captive macaques were not different from the values for the free-ranging animals from this study, regardless of the level of vitamin D supplementation (Table 2).

4 | DISCUSSION

The known functions of 1,25[OH]$_2$ D relative to calcium metabolism include enhancing intestinal absorption of both calcium and phosphate, decreasing urinary excretion of calcium and phosphate by acting on the kidney to increase reabsorption by tubules, and, in concert with the peptide parathyroid hormone (PTH), regulating the mobilization of calcium from bone (Pludowski et al., 2013; Power et al., 1999). Vitamin D deficiency is associated with low serum ionized calcium, bone mineral loss, and eventual metabolic bone disease. Substantial evidence suggests that 1,25[OH]$_2$ D has biological functions in addition to those for whole-body calcium regulation (Pludowski et al., 2013). Most nucleated cells express the vitamin D receptor, including cardiac, brain, skin, gonadal, and immune cells (Fraser, 1995; Holick, 1994). Epidemiological evidence suggests that vitamin D deficiency is associated with an increased risk for colon, breast, and prostate cancers (Anderson & Toverud, 1994; Holick et al., 2011). However, trials of supplementation with vitamin D have shown little success in reducing disease and mortality, except in elderly women (Autier, Boniol, Pizot, & Mullie, 2014). It has been suggested that low 25-OH-D status may be a marker of ill health, especially stemming from or resulting in inflammation, as opposed to a direct causal factor (Autier et al., 2014).

The levels of circulating 25-OH-D recommended by IOM for adequate human health (above 20 ng/ml) and, by extension, to other anthropoid primates in captivity have been called too conservative and driven by a concern regarding health risks of over supplementation and high circulating levels of 25-OH-D for which there is scant evidence and that appear implausible from an evolutionary perspective (Heaney & Holick, 2011). The data from feral (Vieth et al., 1987) and captive (Marx et al., 1989; Ziegler et al., 2015) macaques fed diets with high (above 6 IU/g) vitamin D appear to bear out that the risk of high supplementation may be overestimated, as these populations were both healthy and had levels of circulating 25-OH-D about 7–10 times higher than the IOM minimal level. However, the data from this study imply that captive macaques likely are being over supplemented with vitamin D, as their circulating 25-OH-D levels exceed the wild values by several-fold. Diets with 1.5 and 2.4 IU/g of vitamin D resulted in circulating levels of 25-OH-D that matched the values from the wild toque macaques from this study (Table 2). Although we know of no reports of concerns regarding vitamin D toxicity in captive macaques, animal care staff, and veterinarians might consider whether maintaining animals at levels of circulating 25-OH-D apparently well above "natural" circulating levels is appropriate. In humans, both low (less than 30 ng/ml) and moderate-to-high circulating 25-OH-D (above 56 ng/ml) were associated with a higher risk of tuberculosis (Nielsen et al., 2010). Human epidemiological studies have found an

association of increased health risk at high levels of circulating 25-OH-D, including all-cause mortality (Sempos et al., 2013), though causality has not been shown. In most cases the increase in risk at high levels of 25-OH-D is much less than the increase in risk for levels below 30 ng/ml (Sempos et al., 2013), indicating that high 25-OH-D (below toxic levels) is less a health risk than very low levels. Finally, the fact that 25-OH-D levels in wild populations generally are lower than that of captive animals does not necessarily indicate that the high levels in captivity are detrimental to health and well-being in the captive environment.

The high concentrations of $1,25[OH]_2$ D in female macaques during mid-to-late pregnancy is consistent with human data that show that circulating $1,25[OH]_2$ D is increased after the first trimester (Pludowski et al 2013; Power et al., 1999). Vitamin D metabolism differs during pregnancy, with a substantial increase in the production of the active form (1,25-dihydroxyvitamin D), likely due in part to placental production, resulting in elevated levels relative to the non-pregnant state (Pludowski et al., 2013; Power et al., 1999). During pregnancy there is also a direct association between circulating 25-OH-D and $1,25[OH]_2$ D, which is not the case outside of pregnancy, suggesting both higher substrate (25-OH-D) turnover and a greater effect of 25-OH-D levels on vitamin D actions on physiology and metabolism (Pludowski et al., 2013). Lower circulating $1,25[OH]_2$ D is associated with several pregnancy complications in humans, including preterm birth (Thota et al., 2014). The association between 25-OH-D and $1,25[OH]_2$ D appears to plateau above 40 ng/ml of 25-OH-D in humans (Hollis, DJohnson, Hulsey, Ebeking, & Wagner, 2011), leading some researchers to conclude the Endocrine Society recommended levels for circulating 25-OH-D are more metabolically appropriate during pregnancy (Hollis & Wagner, 2013). Unfortunately, the values for $1,25[OH]_2$ D for the three pregnant females were above the level the assay could measure, so we cannot determine if the wide range in 25-OH-D levels in these females (16, 58, and 120 ng/ml) also resulted in differing $1,25[OH]_2$ D values, nor what "normal" levels for pregnant macaques would be, other than likely higher than 260 ng/ml.

Evidence from captive New World primates, especially callitrichid monkeys, suggests that these species typically have significantly higher levels of both circulating 25-OH-D and $1,25[OH]_2$ D compared to Old World anthropoids (reviewed in Ziegler et al., 2015). However, the circulating concentrations of 25-OH-D in wild cotton top tamarins (Power et al., 1997) and free-living black-tufted marmosets not supplemented with dietary vitamin D (20.1–103.3 ng/ml, Teixeira et al., 2012) exhibit broad overlap with the values from wild toque macaques in this study. Unfortunately, there are no data for circulating $1,25[OH]_2$ D concentrations for wild New World monkeys. The similarity between the circulating values for $1,25[OH]_2$ D in the wild macaques from this study compared to captive/managed macaques despite differences in circulating 25-OH-D concentrations suggests some support for the hypothesis that some New World primates likely exhibit high circulating $1,25[OH]_2$ D compared to Old World anthropoids. Whether these New World monkeys truly require higher circulating 25-OH-D is uncertain.

The data from this study support the idea that anthropoid primates under the evolutionary-norm conditions of extensive sunlight exposure typically will have circulating values of 25-OH-D well above 20 ng/ml. Three quarters of the animals in this study had circulating levels of 25-OH-D above 40 ng/ml and 90% had values above 30 ng/ml. Only one animal in this study had a value below 20 ng/ml and that was a female in mid-to-late pregnancy. Pregnancy is a time of upregulation of the conversion of 25-OH-D to $1,25[OH]_2$ D, possibly accounting for the lower level in this individual. Only four animals (8.9%) had circulating levels above 100 ng/ml, in stark contrast to recently measured levels in a captive population where the mean values were above 150 ng/ml (Ziegler et al., 2015).

If free-living macaques are an appropriate model for circulating 25-OH-D in humans, then these data suggest that the IOM lower limit for human circulating 25-OH-D is well below what the "natural" level would have been for most of our evolutionary history. In contrast to low levels of 25-OH-D in human populations likely representing low levels of sun exposure and minimal dietary intake, captive macaques currently appear to be fed diets supplemented with vitamin D at a level to maintain circulating 25-OH-D levels well above the "natural" level. Although we know of no health concerns related to possible vitamin D toxicity in captive macaques, this fact might be a concern for biomedical studies where macaques are used as models for human diseases in which vitamin D may play a role.

The takeaway messages from this study are: (1) median and mean values for circulating 25-OH-D in wild macaques under natural sunlight conditions are three fold higher than the IOM minimal levels for human health; (2) current dietary supplementation of captive macaques results in circulating 25-OH-D levels at least two fold higher than the "natural" levels; and (3) this potential over supplementation raises mild concerns regarding possible issues of vitamin D-related changes in metabolism and immune function that may affect health and/or influence the results of biomedical studies.

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5 | CONFLICT OF INTEREST

The authors acknowledge no conflict of interest in the submission.

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