VITAMIN D STATUS IN WILD TOQUE MACAQUES (MACACA SINICA) IN SRI LANKA

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Abstract

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 human health is a topic of 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 8 adult males, 7 juvenile males, 6 young nulliparous females, 9 adult females not pregnant or lactating, and 11 lactating adult 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. 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.

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 active immune cells, pancreatic beta cells, bronchial epithelial cells, skin epithelial cells, testes and mammary gland (Wang et al., 2012). Varying evidence indicates potential roles for vitamin D in immune function, metabolism, and cell differentiation [Rosen et al., 2012; Pludowski et al., 2013). Adequate vitamin D status appears to be beneficial to health in more ways than for calcium metabolism and bone.

There is continuing controversy over the appropriate levels of circulating 25-OH-D that represent good health for humans. Based on data regarding bone health, the IOM panel concluded that a circulating level above 20 ng/ml 25-OH-D is sufficient (IOM, 2011; Ross et al., 2011; Rosen et al., 2012). 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).

Levels of circulating 25-OH-D sufficient for good health for macaques and other Old World anthropoid primates 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 toque macaques (*Macaca sinica*) in Sri Lanka. This paper provides partial results from the study; complete results and discussion can be found in Power & Dittus, 2017.

Materials & Methods

Plasma samples were obtained from 8 adult males, 7 juvenile males, 6 young nulliparous females, 9 adult females not pregnant or lactating, and 11 lactating adult females. The research was in compliance with all legal and ethical requirements of the government of Sri Lanka, the Smithsonian Institution. 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 et al., 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 9.2y – 22.4y), juvenile male (n=6, 4.4y - 6.2y), nulliparous female (n=7, 3.6y – 6.0 y), adult female not pregnant or lactating (n=7, 7.2y – 24.4y), lactating adult female (n=13, 6.3y – 20.5y), or pregnant adult female (n=4, 9.5y – 21.4y).

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 and colleagues (1990a) and 1,25$[OH]_2$ D by the methods described in Chen and colleagues (1990b).

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. Qualitative comparisons were made with published data from free-ranging rhesus macaques on Cayo Santiago supplemented with vitamin D fortified food (Vieth et al., 1987) and captive macaques with minimal sunlight exposure but fed vitamin D fortified diets (Shinka et al., 1983; Marx et al., 1989; Ziegler et al., 2015).

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. Values above those maximums were set to 151 ng/ml and 261 pg.ml for statistical purposes. The ranges of values for the concentrations of both 25-OH-D (16 to 151 ng/ml) and 1,25[OH]$_2$ D (71 to 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 nonreproductive females (Table 1). The values for 25-OH-D and 1,25[OH]$_2$ D were not correlated ($r = -0.187$, $p = 0.248$).

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 (Ziegler et al., 2016; Marx et al., 1989) 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).

Conclusions

The levels of circulating 25-OH-D recommended by the Institute of medicine (IOM, 2011) 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 IU/g 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 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; 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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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
All animals	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