Effect of Housing Type on 25 OH Vitamin D in Serum of Rhesus Monkeys

Alan M. Preston, PhD*; José Rodríguez-Orengo, PhD*;
Lorena González-Sepúlveda, MS†; Sylvette Ayala-Peña, PhD‡;
Elizabeth Maldonado-Maldonado, MS¶

Objective: Vitamin D status is primarily dependent upon sun exposure and dietary sources, however genetic, cultural, and environmental factors can have a modulating role in the measured amount. One under-reported factor is the effect of regular living quarters on the degree of sun exposure. Herein, we assess vitamin D status in the blood of Rhesus monkeys (*Macaca mulatta*) housed in high amounts of sunlight (corn-cribs), medium sunlight (corrals with shaded areas), and minimal sunlight (quarantine cages).

Methods: Fifty-five male Rhesus monkeys, aged 1 to 31 years were housed in varying amounts of sun exposure at the Caribbean Primate Research Center. Serum was collected and analyzed for 25 OH Vitamin D which is the preferred metabolite for determination of Vitamin D using High Performance Liquid Chromatography (HPLC).

Results: 25 OH Vitamin D levels in blood were significantly greater in corn-crib-housed monkeys than in corral or quarantine-housed animals ($p > 0.01$ and $p > 0.001$ respectively). Significant differences of serum levels were not found when ages of animals housed in the same environment were compared.

Conclusion: Monkeys housed in a tropical environment with the greatest amount of exposure to sunlight maintain the highest serum levels of 25 OH vitamin D independent of age. These findings emphasize the importance of documenting the environment in which subjects typically spend their time when Vitamin D results are interpreted. [*P R Health Sci J 2018;37:124-127*]

Key words: 25 OH vitamin D, Housing type, Rhesus monkeys, Sun exposure

Vitamin D status in all vertebrates is primarily dependent upon sunlight exposure and dietary intake but blood levels of 25 OH Vitamin D, the preferred bio-indicator, can be modulated by several genetic, environmental, and cultural factors (1, 2). Age, gender, season of the year, time of day, latitude, skin color, protective clothing, use of sunblock among other variables have all been shown to alter levels of 25 OH vitamin D (3). One factor, which is less mentioned when determining vitamin D status, is the environment in which the subject generally spends time. The focus of our report is directed to how different types of housing which limit exposure to sunlight can alter serum levels of 25 OH vitamin D. To reduce the number of confounding variables typically found in human studies which relate to genetic, gender, and cultural factors, we concentrated our study on male Rhesus monkeys, all part of the Caribbean Primate Research Center (C-PRC) with serum collected at the same time of day in the same month.

Methods

All work related to this study was carried out with approval of the Medical Sciences Campus Institutional Care and Use Committee (IACUC).

Animals

The study population consisted of 55 male rhesus monkeys (*Macaca mulatta*), classified as Old World primates, ages 1 to 31 years, living at the Sabana Seca Field Station, Puerto Rico (SSFS). Animals were housed in 3 environments: quarantine cages (QC) with minimal sunlight (n = 27), open corrals (OC) with intermediate sunlight (n = 10), and corn crib cages (CC) with ample sunlight (n = 18). These environments are shown in Figure 1. Exact specifications for the housing types are available from the first author upon request. It should be mentioned that quarantine-housed monkeys were isolated for social adjustment purposes and not for reasons of poor health. Blood samples were collected between 7 and 10 am in May 2011. The

*Department of Biochemistry, School of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, PR; †Biostatistics, Puerto Rico Clinical and Translational Research Consortium, San Juan, PR; ‡Department of Pharmacology and Toxicology, School of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, PR; ¶Caribbean Primate Research Center, San Juan, PR

The author/s has/have no conflict/s of interest to disclose.

Address correspondence to: Alan M. Preston, PhD, Department of Biochemistry, School of Medicine, University of Puerto Rico Medical Sciences Campus, San Juan, PR. Email: alan.preston@upr.edu

Figure 1. Types of Housing at the Caribbean Primate Research Center, Sabana Seca, Puerto Rico. Rhesus monkeys housed in corn cribs, corrals and quarentine cages received ample, medium and minimal amounts of sunlight respectively.

collection procedure has been described previously (4,5). Blood withdrawals were performed from healthy monkeys, obtained from the femoral vein with the animal under anesthesia using ketamine HCl (12 mg/kg) and stored at -70o C until analysis.

Chemical determination

Serum 25(OH) D levels (D2 and D3) were measured by liquid chromatography-tandem mass spectrometry (UPLC-Agilent 1290-QQQ-Agilent 6460) in duplicate (inter-assay coefficient of variation 9–15%, intra-assay coefficient of variation - 10%).

Statistical analysis

Measures of central tendency and variability were used to summarize 25 OH Vitamin D levels. To assess normal distribution and variance homogeneity a Shapiro-Wilk test and Levene's test were respectively performed. A Mann-Whitney U test was performed to compare 25 OH Vitamin D levels between age groups within each type of housing. Furthermore, a Kruskal-Wallis test was used to compare the 25 OH Vitamin D levels between types of housing; pairwise comparisons were performed using Dunn's test with Bonferroni correction. Values under 0.05 were considered statistically significant. All statistical analyses were performed using Stata version 14.

Results

Figure 2 shows the association between housing environment and 25 OH vitamin D levels ($p < 0.001$). Monkeys housed in

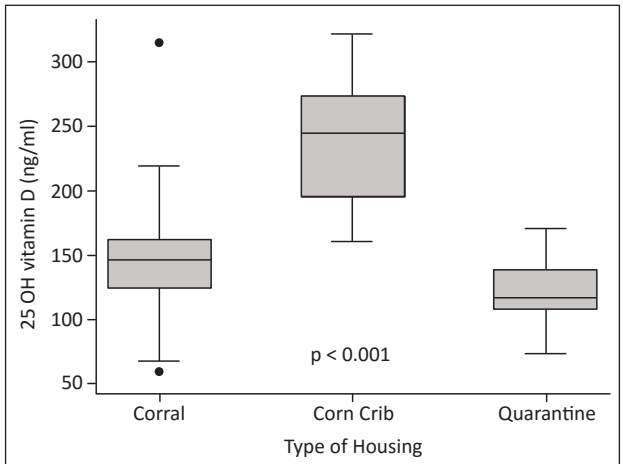

Figure 2. 25OH Vitamin D in monkeys according to type of housing

CC cages (median = 244.5 ng/mL) had greater vitamin D levels as compared to monkeys housed in OC (median = 146.3 ng/mL, $p < 0.01$) and to those housed in QC (median = 117.2 ng/mL, $p < 0.001$). Compared to recently published values all are much higher than in free ranging toque macaques (6) and values for CC-housed monkeys were above those reported in New World primates while being in reported range for OC and QC housed animals (7,8). In Table 1 we report no significant difference in 25(OH)D levels according to age 25(OH)D levels according to age in monkeys within the same type of housing. This finding is in agreement with human studies in which no

age-related decline was observed (8) but is in disagreement with a small previous study of corral housed monkeys at the SSFS which showed increased levels in older animals (9) as well as with results from Ziegler et al which showed an age-related decrease (7).

Table 1. Mean of 25OH Vitamin D among monkeys according to age and type of housing

		25 OH Vitamin D (ng/ml)		
	n	Mean (± SD)	Median (P25-P75)	P-value*
Corral				
≤15 years	6	141.4 (33.0)	130.4 (124.3-162.2)	0.67
>15 years	4	172.8 (105.7)	158.7 (108.4-237.3)	
Corn Crib				
≤9 years	12	226.7 (50.8)	237.7 (176.8-260.6)	0.09
>9 years	6	260.2 (35.9)	273.1 (245.1-278.3)	
Quarantine				
≤9 years	14	121.1 (20.2)	115.8 (107.3-134.0)	0.56
>9 years	13	123.9 (26.8)	118.0 (112.3-138.2)	

*Mann-Whitney test was performed to determine association between age group and vitamin D levels.

Discussion

Among the many factors that can determine vitamin D status, typical living environment can have a major role in the amount of sun exposure. Populations adapting sun protective behaviors through cultural beliefs or fear of skin cancer have low 25 OH vitamin D levels (8). Even when adequate exposure time occurs, cutaneous synthesis from sunlight is compromised in latitudes above 35° and above 51° no vitamin D synthesis occurs during winter months (9). Our study was carried out in a fully tropical environment (latitude 18° N) which allows cutaneous photo-production year-round (9). Use of monkeys obviates several human-associated factors related to vitamin D status so focus can be directed at sun exposure due to housing type. Herein, we report that monkeys housed in corn cribs, which allow the greatest degree of sun exposure, also maintain statistically highest levels of 25 OH vitamin D. Coral housed animals have the benefit of some shade in which rhesus in warm climates are known to spend more time (11) hence reducing sun exposure but well above that of quarantined housed animals. These findings concur with similar results with captive marmoset monkeys in which 25 (OH) D was evaluated according to percent of sunlight exposure (6).

Age of the animals is not a determining factor; however other non-environmental influences should be considered. Dietary intake was not measured. Vitamin D_3 in Teklad NIB primate diet is 8000 IU/kg and animals consume 3-4% of their body weight/day (10) which could contribute to differences, however it is unlikely that housing type should cause major changes in eating behavior. Likewise, body composition can affect vitamin D status. Human studies indicate obese individuals sequester the fat-soluble vitamin so maintain lower blood levels than lean persons (11). Percent body fat was not measured in this study.

In summary, we have observed that monkeys housed in a tropical environment with the greatest amount of exposure to sunlight maintain the highest serum levels of 25 OH Vitamin D. While these findings are not unexpected, they emphasize the importance of documenting the environment in which subjects typically spend their time when Vitamin D results are interpreted.

Resumen

Objetivo: Los niveles de vitamina D en la sangre depende principalmente de la exposición al sol y de fuentes dietéticas, sin embargo, factores genéticos, culturales y ambientales pueden tener un papel modulador en la cantidad medida. Un factor que ha sido poco reportado es el efecto de las viviendas regulares en el grado de exposición al sol. Por esta razón, evaluamos el estado de vitamina D en la sangre de monos Rhesus (Macaca mulatta) alojados bajo altas cantidades de luz solar ("corn-crib"), exposición mediana de luz solar media (corrales con áreas sombreadas) y mínima luz solar (jaulas de cuarentena). Métodos: Cincuenta y cinco monos Rhesus machos, de 1 a 31 años de edad fueron alojados en cantidades variables de exposición al sol en el Centro de Investigación de Primates del Caribe. Se tomaron muestras de sangre y mediante HPLC se determinaron en el suero los niveles de 25 OH Vitamin D, el metabolito preferido para la determinación de Vitamina D. Resultados: Los niveles de 25 OH vitamina D en la sangre fueron significativamente más altos en los monos alojados en "corn-crib" que en los animales alojados en corral o en cuarentena ($p> 0.01$ y $p> 0.001$, respectivamente). No se encontraron diferencias significativas en los niveles séricos cuando se compararon las edades de los animales alojados en el mismo ambiente. Conclusiones: Monos alojados en un ambiente tropical donde hay una mayor cantidad de exposición a la luz solar mantienen niveles más elevados de 25 OH vitamina D en suero independiente de su edad. Estos hallazgos enfatizan la importancia de documentar el entorno en el que los sujetos normalmente pasan su tiempo cuando se interpretan los resultados de la vitamina D.

Acknowledgments

The authors would like to thank the staff of the Caribbean Primate facility especially Armando Burgos, DVM for providing information and photos of animal housing as well as Franceska Corujo for analytical support and Maria del R. Castro for technical assistance. Research reported in this publication was supported by the National Institute on Minority Health and Health Disparities of the National Institutes of Health under Award Number 2U54MD007587, NIHP40RR003640 and P400D012217. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Holick MF. Vitamin D deficiency. N Eng J Med 2007; 357:266-281.
2. DeLuca HF. Evolution of our understanding of vitamin D. Nutr Rev 2008;66(Suppl):S73-S87.
3. Wacker M, Holick MF. Sunlight and vitamin D –A global perspective for health. Dermato Endocrinology 2013;5:51-108.
4. Castro, M del R, Suarez E, Kraiselburd E, Isidro A, Paz J, Ferder L, Ayala-Torres S. Aging increases mitochondrial DNA damage and oxidative stress in liver of rhesus monkeys. Experimental Gerentol 2012;47: 29-37.
5. Preston AM, Bercovitch FB, Rodriguez CA, Lebron MR, Rivera CE. Plasma ascorbic acid concentrations in a population of rhesus monkeys (Macaca mulatta). Contemp Topics Lab Animal Sci 2001;40:30-32.
6. Power ML, Dittus WPJ. Vitamin D status in wild toque macaques (Macaca sinica) in Sri Lanka. Am J Primatol 2017;79:e22655.
7. Teixiera DS, Castro LCG, Nobrega YKM, Almeida RC, Gondolfi L, Pratesi R. 25-Hydroxy-vitamin D levels among Callithrix penicillata primate species raised in captivity. J Med Primatol 2010;39:77-82.
8. Ziegler TE, Kapoor A, Hedman CJ, Binkley N, Kemnitz JVV. Measurement of 25-hydroxyvitamin D(2&3) and 1,25-dihydroxyvitamin D(2&3) by tandem mass spectrometry: A primate multispecies comparison. Am J Primatol 2015;77:801-810.
9. Sherman SS, Hollis BW, Tobin JD. Vitamin D status and related parameters in a healthy population:The effects of age, sex and season. J Clin Endocrinol Metab 1990;71:405-413.
10. Vieth R, Kessler MJ, Pritzker KPH. Serum concentrations of vitamin D metabolites in Cayo Santiago rhesus macaques. J Med Primatol 1987;16:349-357.
11. Linos E, Keiser E, Kanzler M, Sainani KL, Lee W, Vittinghoff E, Chen MM, Tang JY. Sun protective behaviors and vitamin D levels in the UAS population: NHANES 2003-2006. Cancer Causes Control 2012;23:133-140.
12. Webb AR. Who, what where and when-influences on cutaneous vitamin D synthesis. Prog Biophys Mole Biol 2006;92:17-25.
13. Cawthorn Lang KA. Primate Factsheets: Rhesus macaque (Macaca mulatta). Toxicol, Morphology & Ecology (July 20, 2005).
14. Teklad NIB Primate Diet. Information sheet.
15. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Hollick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690-693.