Effect of Season on 25-OH Vitamin D in Serum of Rhesus Monkeys Housed in Puerto Rico

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Objective: Vitamin D blood levels have been shown to be partially dependent upon season in temperate climates, however this same evaluation has not yet been reported in fully tropical climates. Herein, we assessed the vitamin D levels in the blood of Rhesus monkeys housed at the Puerto Rico Caribbean Primate Research Center collected in the island’s “summer” (May-October) and “winter” (November-April) months.

Materials and Methods: In 2006 through 2014, repeated measurements of blood samples were collected from 5 Rhesus monkeys (IACUC-approved) during “summer” and “winter” months to assess 25-OH vitamin D, determined via HPLC. UV-B and UV-A (KJ/m²/day) were measured using a ground based radiometer for these time periods. A paired t-test and a multilevel mixed-effect model approach was performed for data analysis.

Results: The difference of the mean serum values of 25-OH vitamin D between seasons showed lower levels during “winter” than “summer” months. About 23% of the variance in levels can be attributed to difference between the monkeys. The means of UV-B and UV-A, as a proxy for sunlight intensity were greater (over the entire study interval) during the “summer” as opposed to “winter” months (p < 0.001).

Conclusion: Vitamin D levels were substantially higher in the “summer” rather than the “winter” months. This observation implies that even in fully tropical regions, such as Puerto Rico, time of year can have an influence on vitamin D status. While comparable studies have not been undertaken in humans, it would not be unreasonable to suggest that similar results would be obtained should such a study be done. [PR Health Sci J 2018;37:143-147]

Key words: 25-OH vitamin D, Sunlight exposure, Rhesus monkeys, Season

Vitamin D status in humans is a topic of concern, given that low levels have been associated with increased risk of not only to altered bone metabolism but also to a plethora of other medical conditions among them cancer, diabetes, cardiovascular disease and a variety of autoimmune conditions (1, 2). To avoid sub-optimal status, which is generally recognized as ≤20 ng/ml (≤50 nmol/L) of the blood bioindicator, 25-OH vitamin D (3), one must have adequate dietary sources of the vitamin (including supplement form) and/or receive sufficient exposure to UV light (4), however cutaneous synthesis from sunlight is further dependent on altitude, time of year and day, cloud cover, latitude and factors such as age, skin pigmentation, adiposity, clothing and sunscreen (5). The focus of this investigation was the combination of latitude and season. Vitamin D photo-production is compromised in latitudes above 35°, and above 51°, no vitamin D synthesis occurs during winter months (6). The finding that 25-OH vitamin D levels tend to be particularly low at the end of winter months in temperate climates around the globe is amply documented (7) however in tropical climates (below 23.5° N-S) sunlight can induce vitamin D synthesis throughout the year (6). The question raised is—are levels of 25-OH vitamin D consistent throughout the year or can they vary according to season in a fully tropical environment? While human studies assessing this issue are not available, we had the opportunity to investigate this subject using Rhesus monkeys being housed at the Puerto Rico Primate Center (latitude 18° N) in which animals we compared levels of 25-OH vitamin D in blood collected in “summer” months (May-October) with those of blood collected in “winter” months (November-April) and report our findings herein.
Materials and Methods

Animals
The study population consisted of 5 rhesus monkeys (Macaca mulatta), classified as Old World primates, 3 females and 2 males living at the Sabana Seca Field Station (SSFS), Puerto Rico. The animals were identified individually and a census record was maintained for tracking demographic changes. The monkeys were housed in conubribs cages which are rectangular (10’ x 20’) structures, 10’ high which rest on a concrete platform. The conubribs cages contain perches, feeders, ladders and several enrichment devices and were in compliance with all federal and local regulations. There was ample sunlight exposure for most of the day. The monkeys were fed Teklad NIB primate diet ("Envigo", formerly "HarlanTeklad", Madison, WI). This diet contained 8000 IU/Kg (200 ug/Kg) of Vitamin D3. Care was taken to ensure that none of the feedbags had expired milling dates. Water was available ad libitum. Blood samples were collected from 7 to 10 AM from animals 2 times per year, once in “summer” and once in “winter”. Sampling was done from 2006 thru 2014 with some animals providing more data points than others. The monkeys ranged from juvenile to those in young-adult stages of development. The collection procedure has been described previously (8). Approximately 5 ml blood were obtained per animal, placed on ice and stored at -70°C until analysis. All procedures were authorized by the Medical Sciences IACUC as well as by the director of the SSFS.

Analytical

UV Determination: Environmental UV-A and UV-B data were obtained at 1 minute intervals from a GUV 511 ground-based radiometer (Biospherical Instruments, Inc., San Diego, CA) that records photosynthetically available radiation (PAR) and UV radiation at 4 channels centered at 305, 320, 340, and 380 nm respectively (9). This instrument forms part of a aerosol and UV radiation monitoring station (AERADNET) located at the Isla Magueyes field station of the Department of Marine Sciences in La Parguera, southwestern Puerto Rico, about 70 miles from the SSFS. The equations of Orce and Helbling (1997) were used for the spectral reconstruction of the 4 channel GUV-511 data into daily downwelling irradiance in the UV range. The resulting data were integrated to ascertain daily doses of UV-A and UV-B, expressing them in KJ/m²/day.

Chemical determination

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

Statistical analysis

Summary measurements were used to describe the distribution of UV-A and UV-B intensities and the 25-OH vitamin D levels. A paired t-test was performed to compare the mean differences of UV-A and UV-B intensities between seasons (“summer” vs. “winter”). Furthermore, a multilevel mixed effect model approach was used to determine the difference in 25-OH vitamin D levels among monkeys; these differences were adjusted for potential confounders and the effect of repeated measures within the monkeys. The likelihood ratio test was used to assess the effect of random intercept and random coefficients mixed models. P-values less than 0.05 were considered statistically significant. All statistical analyses were performed using the statistical software Stata version 14.1 (College Station, Texas 77845).

Results

Table 1 presents values for UV-B and UV-A by season for the years in which the monkeys had blood samples collected. While significant statistical differences were not generally observed between seasons within individual years, the overall mean exposure (from 2006 – 2014) for the monkeys was significantly different seasonally for both UV-B (100 vs 82 KJ/m²/day) and UVA (1066 vs 920 KJ/m²/day) (both p < 0.0001). This level of UV-B intensity is sufficient to stimulate the cutaneous route of vitamin D synthesis throughout the year (10). The impact of UV-A on vitamin D synthesis is less well understood but in any case has much less influence on vitamin D production (11).

Table 2 presents the average values of 25-OH vitamin D for the 5 monkeys by season using all the collected samples. After adjusting for sex, season and the repeated measures effect in each monkey, the overall mean of 25-OH Vitamin D levels during summer was 342.76 ng/ml (95% CI: 282.87, 402.65) and 224.33 ng/ml (95% CI: 175.68, 272.99) for winter. There were no significant differences among monkeys in the serum vitamin D levels between seasons; a paired t-test was performed to determine if the different seasons were the same (Table 2).

Table 1. UV-B and UV-A intensities according to season of the year.

<table>
<thead>
<tr>
<th>Year</th>
<th>UV-B(KJ/m² per day)</th>
<th>UV-A(KJ/m² per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
<td>Summer</td>
</tr>
<tr>
<td>2006</td>
<td>79.96 ± 20.71</td>
<td>98.81 ± 12.43</td>
</tr>
<tr>
<td>2007</td>
<td>80.91 ± 21.58</td>
<td>100.46 ± 14.27</td>
</tr>
<tr>
<td>2008</td>
<td>70.67 ± 13.42</td>
<td>98.82 ± 12.05</td>
</tr>
<tr>
<td>2009</td>
<td>97.62 ± 26.91</td>
<td>104.16 ± 10.26</td>
</tr>
<tr>
<td>2010</td>
<td>80.27 ± 24.33</td>
<td>97.89 ± 11.66</td>
</tr>
<tr>
<td>2011</td>
<td>65.62 ± 12.43</td>
<td>101.37 ± 4.38</td>
</tr>
<tr>
<td>2012</td>
<td>62.33 ± 16.56</td>
<td>83.14 ± 7.88</td>
</tr>
<tr>
<td>2013</td>
<td>114.40 ± 11.67</td>
<td>99.47 ± 9.41</td>
</tr>
<tr>
<td>2014</td>
<td>81.98 ± 22.44</td>
<td>99.77 ± 12.56</td>
</tr>
</tbody>
</table>

*Paired t-test was performed.
The adjusted difference of the mean values of 25-OH vitamin D between seasons ($\Delta \_\text{adj} = -57.14 \text{ ng/ml}; 95\% \text{ CI: } -103.87, -10.43$) shows that there were significantly lower levels of vitamin D during winter than summer. Random effects (LR test vs. linear model = 3.78; $p = 0.03$) showed that for any season, the monkeys had distinct intercepts of vitamin D levels that were up to 82.9 ng/ml higher or lower about 95% of the time. The variability of monkey’s vitamin D levels during the 2 different seasons was 75.99 ng/ml around the regression lines for each monkey. The estimated variance partition coefficient was 0.229; this indicates that approximately 22.9% of the variance in 25-OH Vitamin D levels can be attributed to differences between monkeys.

Figure 1 presents individual values of 25-OH vitamin D for the 5 monkeys by season using all collected samples. The mean values of 25-OH vitamin D of monkeys ranged from 255.7 to 443.1 ng/ml during the “summer” and from 210.6 to 320.7 ng/ml during the “winter”.

**Discussion**

The photosynthesis of vitamin D occurs in all vertebrates resulting from exposure to solar UV-B photons which penetrate the skin causing the photolysis of 7-dehydrocholesterol to precholecalciferol. Once formed, precholecalciferol undergoes a thermally induced rearrangement of its double carbon bonds to form cholecalciferol (D3). Further modification takes place in the liver to 25-OH D3 and finally in the kidney to 1,25 dihydroxy D3, the active form (12). Most basic knowledge of factors related to vitamin D status has been obtained in human studies however not all apply to non-human primates.

Regarding this topic, it would be instructive to compare these factors and to elaborate further on those that have most influenced the data presented in the tables and figure. While the focus of this study was the influence of latitude and UV radiation on vitamin D status, the diet of the sampled population was also a contributor to blood levels. Diets for Old World primates typically contain 6,000 to 8,200 IU/kg of vitamin D3 (13). In humans about 80% of orally ingested vitamin D3 is absorbed by the lymphatic system (14), and daily food consumption for non-human primates is about 3 to 4% of their body weight (15). Monkeys at the SSFS are fed a diet containing 8,000 IU/kg (200 ug/kg) so it is not unexpected that the blood levels of 25-OH vitamin D are multiples of human values where daily recommendation is only 400 IU’s/day (16). Our average values of about 350 ng/ml for 25-OH vitamin D compare closely to those previously obtained by Vieth et al at the SSFS for similarly aged animals (17). Other non-geographic factors influencing vitamin D status in human studies are skin color, use of protective clothing, use of sunscreen and/or certain religious and cultural beliefs (18) none of which elements apply to non-human primates however factors such as age, gender and body fat content do apply and will be further discussed.

Geographic factors are the prime focus of this investigation. The intensity of UV-B radiation in Puerto Rico which is located at 18° N latitude (presented in Table 1) is sufficient to stimulate the cutaneous route of vitamin D synthesis all year round however when the year is divided into seasons, “summer” intensity can be seen to be significantly stronger ($p < 0.001$) than “winter” intensity over the period of time our measurements were taken. This observation has been suggested to partially explain results in Figure 1 and Table 2 which results show that 25-OH vitamin D

**Table 2. Multilevel mixed effect model for predicting 25-OH vitamin D levels among monkeys.**

<table>
<thead>
<tr>
<th></th>
<th>Estimated Coefficient</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixed Effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>342.76</td>
<td>282.87 ; 402.64</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Summer</td>
<td>-57.14</td>
<td>-103.87; -10.43</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>Males</td>
<td>-54.93</td>
<td>-144.60; 34.74</td>
</tr>
<tr>
<td><strong>Random Effect</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept variance</td>
<td>1717.89</td>
<td>274.34 ; 10757.19</td>
</tr>
<tr>
<td>Intercept residual</td>
<td>5774.12</td>
<td>3654.76 ; 9122.45</td>
</tr>
</tbody>
</table>

Abbreviations: CI, Confidence Intervals. Note: Coefficients with 95% CI not including zero were considered statistically significant with $p<0.05$. 

![Figure 1. Values of 25-OH vitamin D for the 5 monkeys by season.](image)
levels in blood are higher (as a group average and in individual monkeys) in “summer” vs “winter”.

We believe that this conclusion is reasonable but should be evaluated further and its strengths and weaknesses delineated. The strengths are the accurate measurements of UV intensities and 25-OH vitamin D levels as well as the use of comparisons of blood values in the same animal at different times in the year. We observed a gender effect, that in that both males and females demonstrated higher values in “summer” than values in “winter” with females expressing higher overall levels than males. A possible factor which might help explain the variance of 25-OH vitamin D values exhibited by individual monkeys (Figure 1) could be related to age, since samples were collected over a broad time span and it has been reported that values increase as an individual grows from juvenile to young adulthood (17). Weaknesses include the small sample size and other factors that could influence the results which are lack of animal observation (which might account for gender differences), dietary intake, body fat content, the latter of which was not measured. Regarding the diet, monkeys consume about 3-4% of their body weight in food daily however this amount is consistent in both “summer” and “winter” months and so should have had minimal influence on the blood levels of 25-OH vitamin D. The intensity of UV-B radiation is of greater concern since (in humans) it is the principle source of vitamin D (1). The Isla Magueyes Field Station where the UV measurements were taken is about 70 miles SSW of SSFS, however the cloud cover and ozone layer are similar and so any differences between the 2 should not be enough to have a significant impact. Time of day that samples are collected can result in large differences in vitamin D status (2). Our samples were collected from 7-10 AM which might account for some of the variations in individual animals. Likewise, UV intensity is not uniform within the ranges that we have classified as “summer” and “winter” months. In terms of the former, the months of June through July tend to have higher UV values; for the latter, the span of December through January received less intense UV radiation.

In conclusion, we used the vitamin D biomarker 25-OH vitamin D, to evaluate vitamin D status for both “summer” and “winter” months in non-human primates living in a completely tropical environment. The greater the intensity of UV-B radiation received in the “summer” months correlates to an increase in the levels of the biomarker in the blood of the sampled monkeys over that in blood collected in the “winter” months, both as a group average and in individual animals. While equivalent studies have not been carried out in humans it would not be unreasonable to suggest that similar results might be obtained were such a study to be undertaken.

Acknowledgments

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References

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Resumen

Objetivo: Se ha demostrado que los niveles de vitamina D en la sangre son parcialmente dependientes de la época del año en las regiones templadas; sin embargo, esta evaluación aún no ha sido realizada para climas completamente tropicales. Por tal razón, evaluamos la relación entre niveles de vitamina D en la sangre y la estación del año en monos Rhesus alojados en el Puerto Rico Caribbean Primate Research Center. Métodos: En el periodo 2006-2014, se tomaron muestras repetidas de sangre en cinco monos Rhesus (aprobado por IACUC) durante el verano (mayo-octubre) y el invierno (noviembre-abril) para evaluar la 25-OH vitamina D, determinada por HPLC. Además, las dosis de UV-B y UV-A (KJ/m²/día) fueron determinadas usando un radiómetro en tierra para estos periodos de tiempo. Se realizó una prueba T pareada y un modelo multinivel de efectos mixtos para analizar los datos. Resultados: La diferencia de la media de 25-OH vitamina D entre las estaciones mostró niveles más bajos de vitamina D durante el invierno que el verano. Alrededor del 23% de la varianza en los niveles de 25-OH vitamina D se puede atribuir a las diferencias entre los monos. Los valores de las dosis de UV-B y UV-A como indicador de la intensidad de la luz solar fueron mayores en todo el intervalo de estudio para verano e invierno (p<0.001). Conclusión: Los niveles de vitamina D fueron sustancialmente más altos en los meses de verano y de invierno. Estas observaciones implican que incluso en regiones totalmente tropicales, como Puerto Rico, la época del año puede tener una influencia en el estado de vitamina D. Aunque no se han reportado estudios comparables en seres humanos, no sería irrazonable sugerir que se obtendrían resultados similares.
15. Teklad NIB primate diet. Information sheet.