Serum Vitamin D Concentrations in Baboons (Papio spp.) during Pregnancy and Obesity

Natalia E Schlabritz-Loutsevitch,1,7 Anthony G Comuzzie,2 Michael M Mahaney,3 Gene B Hubbard,4 Edward J Dick, Jr,5 Mehmet Kocak,4 Sonali Gupta,7 Maira Carrillo,1 Mauro Schenone,7 Arnold Postlethwaite,4 and Andrzej Slominski9,10

Obesity is associated with vitamin D deficiency, which can lead to serious problems during pregnancy. However, the mechanisms of the deficiency and guidelines for vitamin D supplementation during pregnancy are not established yet, and variations in environmental exposures combined with the difficulties of performing research in pregnant women are obstacles in the evaluation of vitamin D metabolism. Baboons (Papio spp.) are an excellent, well-established model for reproductive research and represent a unique opportunity to study vitamin D metabolism in a controlled environment. This study used secondary data and specimen analysis as well as a novel experimental design to evaluate pregnant and nonpregnant baboons that were or were not exposed to sunlight while they were obese and after weight reduction. Daily D3 intake was 71% higher in nonpregnant obese baboons than in their nonobese counterparts, but serum vitamin D concentrations did not differ between these populations. In addition, serum 25-hydroxyvitamin D concentrations correlated negatively with the obesity index. This report is the first to show the effect of obesity and pregnancy on vitamin D concentrations in a NHP population. These data underline the importance of adequate vitamin D supplementation in obese animals.

Original Research

Vitamin D deficiency during pregnancy is a major public health problem50 that is associated with increased maternal and neonatal morbidity,5,6,22,31 Even though vitamin D is actively involved in placental function and fetal growth,5,11,43 the dosage of vitamin D supplementation during pregnancy has not been established.30 Maternal serum concentrations of 25-hydroxyvitamin D are directly related to vitamin D intake and sunlight exposure in humans.27 Obesity is an important risk factor for vitamin D deficiency for which the mechanisms remain unknown, and the effect of weight loss on vitamin D status is unclear.14,15,33,34 In addition, obesity itself is a significant public health problem.18,31 Several mechanisms linking obesity and vitamin D deficiency have suggested, including decreased exposure to sunlight, sequestration of vitamin D in the adipose tissue,50 and other factors related to obesity.34,49 In pregnancy an additional factor involved in vitamin D activation and metabolism is the placenta—a temporary endocrine organ whose function is linked to the metabolism of the adipose tissue.7

An understanding of the relationship between vitamin D, obesity, and pregnancy is critical for reducing maternal and neonatal morbidity. Baboons (Papio spp.) have been used extensively in pregnancy-related research.17,40 The advantages of this model include the similarity of its placentation to that in humans and the ability to create uniform exposures to environmental factors (for example, sunlight, dietary composition) and thus study underlying metabolic pathways, which are impossible to study in the heterogeneous human population. However, only a very few studies related to the vitamin D requirements in these NHP species are available,35,39 and none of them addresses the effect of obesity and pregnancy on vitamin D metabolism. The objective of the current study was to evaluate the effects of obesity, weight reduction, and pregnancy on the systemic vitamin D status of baboons, by using data collected during prior studies as well as a novel study design.

Materials and Methods

Animal housing and handling. All baboons were maintained in a social environment with partly controlled climate conditions. The baboons had unrestricted access to a commercial diet identical in composition to Purina 5038 (LEO 5, Purina, St Louis, MO) and water. The data from nonpregnant and near-term pregnant baboons (Papio spp.) were used in this study. The research complied with protocols approved by the Animal Care and Use Committee of the Texas Biomedical Research Institute (10/29/2007 no. 1129 and 12/21/2005 no. 1015). The research adhered to the regulations underlined in the AALAS position statements.

Group composition and procedures. The data were collected from the following nonpregnant animals: obese baboons (n = 3; weight [mean ± SEM], 18.8 ± 0.7 kg), the obese animals after weight reduction (n = 3; weight, 17.9±1.02), and nonobese baboons (n = 4; weight, 13.5±0.7 kg; Table 1). Obesity in nonpregnant

1Texas Tech University HSC School of Medicine at the Permian Basin, Odessa, Texas; Departments of 2Genetics and 3Pathology, Texas Biomedical Research Institute, San Antonio, Texas; The University of Texas Rio Grande Valley, Brownsville, Texas; 4Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, Texas; 5Division of Biostatistics, Department of Preventive Medicine; 6Department of Obstetrics and Gynecology; University of Tennessee Health Science Center, Memphis, Tennessee; 7Department of Biostatistics, Department of Preventive Medicine; 8Division of Connective Tissue Diseases, Department of Medicine, University of Tennessee Health Science Center, and Department of Veterans Affairs Medical Center, Memphis, Tennessee; 9Department of Dermatology and Pathology, University of Alabama at Birmingham, and 10VA Medical Center, Birmingham, Alabama
*Corresponding author. Email: Natalia.schlabritz-loutsevitch@uthsc.edu
baboons was defined according to weight, waist circumference, and skinfold thickness; the cut off for baboons to be characterized as obese was a waist circumference of 50 cm.12 Weight loss was achieved by using individualized reduction of total daily food consumption by 30% over 3 mo, as detailed previously for pregnant baboons.40 Venous blood was collected as previously described.41

In nonpregnant baboons, the number of biscuits eaten during individual feeding sessions was recorded daily and the total weight consumed was calculated.40 Supplemental vitamin D3 was added to the diet at 6.6 IU/g, according to the diet manufacturer’s recommendation.

Secondary analysis was performed for 6 pregnant baboons which were housed in outdoor cages.20 Three of these animals had direct exposure to sunlight (3585 Lux), whereas the other 3 did not (85.5 Lux).20 The calculation of the UV light exposure in both groups during pregnancy (duration of pregnancy 175 d of gestation) was performed based on the information provided by the National Oceanic and Atmospheric Administration for daily UV light exposure in the baboons’ geographic area. For sunlight-exposed baboons, the average daily erythemally weighted dose rate during pregnancy was 213.2 ± 33.7 mW/m² on sunny days and 174.5 ± 28.8 mW/m² on cloudy days. Sunlight-exposed baboons received 180.9 ± 27 mW/m² daily on sunny days and 148.0 ± 22.8 mW/m² on cloudy days. All 6 of these baboons underwent cesarean sections at 175 d gestational age (0.97 gestation); maternal and umbilical cord plasma were collected, flash frozen in liquid nitrogen, and stored at –80 °C until evaluated for 1,25-dihydroxyvitamin D3.20

Secondary analysis was performed for the data from 4 pregnant baboons, the daily food consumption (and thus calculated vitamin D intake) were lower in nonobese baboons (42.7 ± 5.6 biscuits) than in obese animals (72.9 ± 0.3 biscuits; P < 0.05). Serum vitamin D concentration did not differ between obese and nonobese nonpregnant baboons. Food restriction in the obese animals resulted in weight losses of 0.28, 0.96, and 1.36 kg, leading to postrestriction differences in serum vitamin D levels of –9.38, 8.52, and –9.99 ng/mL, respectively, relative to the level before food restriction. Vitamin D concentrations before and after food restriction did not differ statistically.

Results

Nonpregnant baboons. Among nonpregnant baboons, the daily food consumption (and thus calculated vitamin D intake) were lower in nonobese baboons (42.7 ± 5.6 biscuits) than in obese animals (72.9 ± 0.3 biscuits; P < 0.05). Serum vitamin D concentration did not differ between obese and nonobese nonpregnant baboons. Food restriction in the obese animals resulted in weight losses of 0.28, 0.96, and 1.36 kg, leading to postrestriction differences in serum vitamin D levels of –9.38, 8.52, and –9.99 ng/mL, respectively, relative to the level before food restriction. Vitamin D concentrations before and after food restriction did not differ statistically.

Pregnant baboons. Plasma concentrations of 1,25-dihydroxyvitamin D, did not differ between sunlight-exposed and -unexposed pregnant baboons (325.24 ± 58.57 ng/L compared with 229.82 ± 33.33 ng/L, respectively) or their fetuses (102.35 ± 11.54 ng/L compared with 93.40 ± 3.68 ng/L). In addition, the maternal/fetal ratio of 1,25-dihydroxyvitamin D, plasma concentrations did not differ between the 2 groups (sunlight-exposed, 2.2 ± 0.2; sunlight-unexposed, 3.5 ± 0.6).

Serum concentrations of 25-hydroxyvitamin D concentrations were higher (P = 0.077) in pregnant, nonobese baboons than in their obese counterparts. The group sample sizes (pregnant obese, n = 4; pregnant nonobese, n = 3) achieved 80.0% power to reject the null hypothesis of equal means for vitamin D serum concentration when the population mean difference is μ1 – μ2 = 142.4 – 93.2 ng/mL, with standard deviations of 7.6 ng/mL for pregnant

Table 1. Description of study baboons

<table>
<thead>
<tr>
<th>Description</th>
<th>Nonpregnant</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obsese (n = 3)</td>
<td>Nonobese (n = 4)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>11.7 ± 2.7</td>
<td>13.4 ± 1.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>18.8 ± 1.5</td>
<td>13.5 ± 1.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>52.9 ± 5.9</td>
<td>43.5 ± 5.1</td>
</tr>
<tr>
<td>Abdominal skinfold thickness (mm)</td>
<td>6.0 ± 1.0</td>
<td>4.2 ± 0.8</td>
</tr>
<tr>
<td>Obesity index (Rb) (kg/m²)</td>
<td>not shown</td>
<td>not shown</td>
</tr>
<tr>
<td>Serum 25-hydroxyvitamin D (ng/mL)</td>
<td>61.4 ± 15.3</td>
<td>83.0 ± 40.8</td>
</tr>
<tr>
<td>Daily vitamin D intake (IU)</td>
<td>2836.9 ± 10.3</td>
<td>1661.9 ± 218.6</td>
</tr>
</tbody>
</table>

P values are derived from Wilcoxon–Mann–Whitney analyses of differences between nonobese and obese baboons of the same pregnancy status.

*Data obtained from reference 17.

The obesity index was documented in 4 baboons but was not included in this table.

Vitamin D intake was estimated according to the number of biscuits eaten.

Serum 1,25-dihydroxyvitamin D3 concentrations before and after food restriction did not differ between nonobese and obese nonpregnant baboons of the same pregnancy status. The group sample sizes (pregnant obese, n = 4; pregnant nonobese, n = 3) achieved 80.0% power to reject the null hypothesis of equal means for vitamin D serum concentration when the population mean difference is μ1 – μ2 = 142.4 – 93.2 ng/mL, with standard deviations of 7.6 ng/mL for pregnant

Statistical analyses. Data were analyzed by using the nonparametric Wilcoxon signed-rank test. The association between 2 continuous variables of interest was assessed according to Spearman correlation. Data are presented as means ± SEM. Significance was set at P value of less than 0.05; the P values reported were not adjusted for multiplicity. All analyses were conducted using SAS software (version 9.4, Cary, NC).

138
obese baboons and 30.2 ng/mL for pregnant nonobese animals and a significance (α) level of 0.050 by using a 2-sided 2-sample unequal-variance t test. In the pooled data set (combining pregnant and nonpregnant baboons), vitamin D concentrations were significantly negatively rank-correlated with the Obesity index (Rh) \((P = 0.01)\) and showed a trend toward positive rank-correlation with kidney weight in pregnant animals \(P = 0.11\); Table 2.

### Discussion

In general, data regarding the vitamin D status in baboons are sparse. One of the limitations of the current study, the small population size, is associated with the study design, secondary data analyses. Taking into consideration the uniform social and physical environments, dietary composition, and animals’ ages, these data provide information that would require larger numbers in human population studies. In addition, despite the few baboons studied, our dataset represents the largest one in adult \(Papio\) baboons and the only data set that describes consumed (not provided) vitamin D amounts and corresponding vitamin D serum concentrations\(^9\), but we could not analyze the data for pregnant baboons at term. In addition, the placenta has an active role in vitamin D metabolism.\(^5\) In addition, the placenta has an active role in the D3 metabolism, which includes both the classic pathway \((D3\rightarrow25S-hydroxyvitamin\ D ,D\rightarrow1,25\text{-dihydroxyvitamin}\ D )\) and a CYP11A1-activated pathway \((D\rightarrow20S-hydroxyvitamin\ D \rightarrow(OH)_n\cdot D )\).\(^{45-48}\) The differences in the vitamin D3 metabolism between obese and nonobese baboons should be considered when interpreting the data from experimental studies involving pregnant animals and determining their dietary supplementation.

The serum 25-hydroxyvitamin D concentration of the nonpregnant baboons in our study is higher to that obtained in D3-sufficient human subjects (40 to 60 ng/mL)\(^9\) and is within the range reported for \(M.\ mulatta\) (from 50 ± 4 ng/mL to 154.8 ± 5.5 ng/mL).\(^{45,46}\) The D3 levels among New World primates reportedly are much higher than those of Old World Primates and humans due to organ resistance to D3 and that this resistant state could be compensated by maintenance of high 1,25-dihydroxyvitamin D levels, for example, 478 ± 108 ng/mL in common marmosets \((Callithrix\ jacchus)\)\(^9\),\(^{48}\) to 236 ng/mL in cottontop tamarins \((Saguinus\ oedipus)\)\(^8\),\(^{50}\) and 104.8 to 137.1 ng/mL in black-faced marmosets \((C.\ penicillata)\)\(^5\).

In summary, to our knowledge, the current study is the first to address the effect of obesity and pregnancy on vitamin D concentrations in any NHP species. These data underline the importance of adequate vitamin D supplementation in obese animals.

### Table 2. Association of serum 25-hydroxyvitamin D concentrations with parameters of maternal morphometry

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(n)</th>
<th>Spearman’s (\rho)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity index (Rh)(^a)</td>
<td>11</td>
<td>−0.74</td>
<td>0.01</td>
</tr>
<tr>
<td>Age(^b)</td>
<td>14</td>
<td>−0.35</td>
<td>0.22</td>
</tr>
<tr>
<td>Kidney weight(^d)</td>
<td>6</td>
<td>0.71</td>
<td>0.11</td>
</tr>
<tr>
<td>Fat gain during pregnancy(^d)</td>
<td>7</td>
<td>−0.36</td>
<td>0.43</td>
</tr>
<tr>
<td>Weight gain during pregnancy(^d)</td>
<td>7</td>
<td>−0.21</td>
<td>0.64</td>
</tr>
<tr>
<td>Placental weight(^d)</td>
<td>7</td>
<td>−0.11</td>
<td>0.82</td>
</tr>
<tr>
<td>Weight loss in nonpregnant obese animals(^e)</td>
<td>3</td>
<td>−0.50</td>
<td>0.67</td>
</tr>
</tbody>
</table>

\(^a\)Includes both pregnant and nonpregnant baboons with available data.

\(^b\)Data obtained from reference 17.

\(^c\)Nonpregnant obese baboons underwent 30% food reduction over 3 mo.

\(^d\)Obesity profoundly influences fetal and maternal vitamin D metabolism.\(^5\) In addition, the placenta has an active role in the D3 metabolism, which includes both the classic pathway \((D3\rightarrow25\text{-hydroxyvitamin}\ D ,D\rightarrow1,25\text{-dihydroxyvitamin}\ D )\) and a CYP11A1-activated pathway \((D\rightarrow20S\text{-hydroxyvitamin}\ D \rightarrow(OH)_n\cdot D )\).\(^{45-48}\) The differences in the vitamin D3 metabolism between obese and nonobese baboons should be considered when interpreting the data from experimental studies involving pregnant animals and determining their dietary supplementation.

The serum 25-hydroxyvitamin D concentration of the nonpregnant baboons in our study is higher to that obtained in D3-sufficient human subjects (40 to 60 ng/mL)\(^9\) and is within the range reported for \(M.\ mulatta\) (from 50 ± 4 ng/mL to 154.8 ± 5.5 ng/mL).\(^{45,46}\) The D3 levels among New World primates reportedly are much higher than those of Old World Primates and humans due to organ resistance to D3 and that this resistant state could be compensated by maintenance of high 1,25-dihydroxyvitamin D levels, for example, 478 ± 108 ng/mL in common marmosets \((Callithrix\ jacchus)\)\(^9\),\(^{48}\) to 236 ng/mL in cottontop tamarins \((Saguinus\ oedipus)\)\(^8\),\(^{50}\) and 104.8 to 137.1 ng/mL in black-faced marmosets \((C.\ penicillata)\)\(^5\).

In summary, to our knowledge, the current study is the first to address the effect of obesity and pregnancy on vitamin D concentrations in any NHP species. These data underline the importance of adequate vitamin D supplementation in obese animals.
Table 3. Published studies regarding vitamin D status in baboons (*Papio* spp.) and values from selected human studies

<table>
<thead>
<tr>
<th>Species</th>
<th>25-hydroxyvitamin D (ng/mL)</th>
<th>1,25-dihydroxyvitamin D$_3$ (pg/mL)</th>
<th>n</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Daily dietary vitamin D (IU/d)$^a$</th>
<th>Feeding mode</th>
<th>Housing characteristics</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baboons (<em>P. anubis</em>)</td>
<td>NR</td>
<td>NR</td>
<td>7</td>
<td>3 male, 4 female</td>
<td>NR</td>
<td>400</td>
<td>Unrestricted</td>
<td>Outdoor, uncontrolled conditions</td>
<td>Trust Research Laboratories, Nairobi, Kenya</td>
<td>19</td>
</tr>
<tr>
<td>Baboons (<em>Papio</em> spp.)</td>
<td>NR</td>
<td>NR</td>
<td>2</td>
<td>male</td>
<td>3.5</td>
<td>1000</td>
<td>Individual unrestricted</td>
<td>Indoor year-round</td>
<td>Regional Primate Research Center</td>
<td>39</td>
</tr>
<tr>
<td>Baboons (<em>P. ursinus</em>)</td>
<td>14.07 ± 4.05</td>
<td>NR</td>
<td>28</td>
<td>20 male, 8 female</td>
<td>0.5-1</td>
<td>100 IU/100 g diet</td>
<td>NR</td>
<td>Indoor during reported time period</td>
<td>University of the Witwatersrand, Johannesburg, South Africa</td>
<td>35</td>
</tr>
<tr>
<td>Baboons (<em>P. cynocephalus</em>)</td>
<td>48.1 (36.6–59.6)</td>
<td>66 (55–77)</td>
<td>2</td>
<td>NR</td>
<td>7–21</td>
<td>NR</td>
<td>Group</td>
<td>Indoor year-round</td>
<td>Brookfield Zoo</td>
<td>13</td>
</tr>
<tr>
<td>Humans$^b$</td>
<td>15–40</td>
<td>15–80</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Turkey</td>
</tr>
<tr>
<td>Baboons (<em>Papio</em> spp.)</td>
<td>35.0 ± 5.127</td>
<td>82.2 ± 14.0</td>
<td>6</td>
<td>male, female</td>
<td>NR</td>
<td>34.9</td>
<td>Group</td>
<td>Some exposure to natural light as well as to indoor lighting</td>
<td>Brookfield Zoo, Fort Worth Zoo, Lincoln Park Zoological Gardens, and North Carolina Zoological Park</td>
<td>52</td>
</tr>
<tr>
<td>Humans$^b$</td>
<td>24.3 ± 16.9</td>
<td>34.3 ± 18.0</td>
<td>20</td>
<td>male, female</td>
<td>56.6 ± 5.1</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
<td>Turkey</td>
<td>1</td>
</tr>
<tr>
<td>Baboons (<em>Papio</em> spp.)</td>
<td>72.2 ± 28.1</td>
<td>NA</td>
<td>7</td>
<td>female, non-pregnant</td>
<td>12.6 ± 1.1</td>
<td>2249.4</td>
<td>Individual unrestricted</td>
<td>Outdoor, partially controlled</td>
<td>Southwest Primate Research Center, San Antonio, Texas</td>
<td>Present study</td>
</tr>
<tr>
<td>Baboons (<em>Papio</em> spp.)</td>
<td>66.6 ± 18.9</td>
<td>NA</td>
<td>7</td>
<td>female, pregnant</td>
<td>10.2 ± 1.3</td>
<td>NR</td>
<td>Individual unrestricted</td>
<td>Outdoor, partially controlled</td>
<td>Southwest Primate Research Center, San Antonio, Texas</td>
<td>Present study</td>
</tr>
<tr>
<td>Baboons (<em>Papio</em> spp.)</td>
<td>NA</td>
<td>277 ± 0.04</td>
<td>6</td>
<td>female, pregnant</td>
<td>9.5 ± 1.0</td>
<td>NR</td>
<td>Group unrestricted</td>
<td>Outdoor, partially controlled$^c$</td>
<td>Southwest Primate Research Center, San Antonio, Texas</td>
<td>Present study</td>
</tr>
</tbody>
</table>

NA, not applicable; NR, not reported.

$^a$The amount provided but not necessarily consumed.

$^b$Data from a human study performed during the same time period as the NHP studies in the preceding row(s).

$^c$Combined average daily erythemally weighted dosage rate during pregnancy for baboons exposed to and deprived of sunlight: 179.15 ± 28.25 mWt/m$^2$.

---

**Acknowledgments**

We acknowledge the help and dedication of J J Gomez, S Chambers, and the many excellent animal caretakers, technicians, and veterinarians of the Southwest National Primate Center and the help of Craig Long (NOAA), who provided the data regarding the UV light exposure in San Antonio during 2002 and 2003. The excellent assistance of Patricia Wheller is appreciated. This investigation used resources that were supported by the Southwest National Primate Research Center grant P51 RR013986 from the National Center for Research Resources (NIH) and that currently are supported by the Office of Research Infrastructure Programs through grant P51 OD011133. This investigation was conducted in facilities constructed with support from the Office of Research Infrastructure Programs (NIH) through grants C06 RR015456 and C06 RR014578. The research also was supported by a New Investigator (UTHSCSA) grant and Southwest National Primate Center Pilot study grant (to N S-L), NIH grant HD21350 (to PN, UTHSC–San
Antonio), the Good Chair of Excellence in Medicine at UTHSC (AEP), and research funds from the Department of Veterans Affairs (to AEP) and grants 2R01AR052190, 1R21AR063242-01A1, and R21AR066501 from NIH/NIAMS (to AS).

References


April 2016

42. Extremely high circulating levels of 1α,25-dihydroxyvitamin D, in the marmoset, a New World monkey. Biochem Biophys Res Commun 114:452–457.


