Vitamin D Content in Milk of the Rhesus Monkey

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Objective: Milk is the first and continued source of ingested Vitamin D. Extensive studies have been carried out in humans measuring Vitamin D in lactating mothers but to date few values have been obtained for milk of non-human primates and none for rhesus monkeys. Consequently. we have determined Vitamin D and antirachitic activity (ARA) in milk samples obtained from 21 rhesus monkeys.

Methods: Lactating dams were sampled by hand-stripping. 25(OH)D2, Vitamin D2, 25(OH)D3, Vitamin D3 and ARA were assessed in foremilk using LC-MS/MS techniques.

Results: 25(OH)D2 and Vitamin D2 were below detectible limits (<0.5 ng/g), 25(OH) D3 =4.2 ± 1.8 ng/ml, Vitamin D3 = 6.1 ± 3.1 ng/ml and ARA = 1080 ± 480 IU/L.

Conclusions: This is the first report of content of Vitamin D and ARA activity in foremilk of the rhesus monkey and can serve as a reference for future studies. [*P R Health Sci J 2021;40:50-52*]

Key words: Vitamin D, Milk, Rhesus monkey, Antirachitic activity

Natural sources of vitamin D for rhesus monkeys are relatively limited since they are omnivores and feed on a wide variety of plants and invertebrate product few of which contain vitamin D_3 (1). Animals in this study are part of the population on the island of Cayo Santiago Field Station of the Caribbean Primate Research Center (CPRC) in Puerto Rico. Since 1938 the island has served as a laboratory for studying free-range rhesus macaques. In addition to consumption of fruits, seeds, roots and insects which have no vitamin D_3 , food patterns show that 50% of total ingestion is commercial feed which contains 8000IU/kg vitamin D3 (2). In addition like all vertebrates, monkeys have the capacity to form vitamin D_3 from exposure to sunlight (3). Consequently, diet and sunlight exposure will determine the animal's vitamin D status which will, in turn determine the amount of vitamin D that will be incorporated into the dam's milk. In this study we have determined the content of vitamin D and the antirachitic activity (ARA) in milk of lactating rhesus monkeys. By content of Vitamin D, we refer to Vitamin D_2, Vitamin D_3, 25(OH) D_2 , and 25(OH) D_3 which comprise close to 100 % of the total.

Methods

All work related to this study was carried out with approval of the Medical Sciences Campus Institutional Animal Care and Use Committee (IACUC). Animals: The study population consisted of 21 lactating female rhesus monkeys (Macaca mulatta) classified as Old World primates, selected from a group of culled animals transported from Cayo Santiago to the Sabana Seca Field Station of the CPRC. Sampling was carried out in December, 2016. Milk was collected by hand-stripping based on procedures described by Hinde et al (4) with modifications (see below). Mothers were separated from infants and lightly sedated using 5 mg ketamine hydrochloride per kg of body weight administered by intramuscular (IM) injection. The animals were held upright in a sitting position and the nipple area was cleaned, hair removed and milk was collected from a mammary gland by gentle hand-stripping. The amount collected sufficed for analysis but was far less than obtained in reference 4 to absence of a holding jacket for the monkey and use of oxytocin to stimulate flow of milk which resulted in only foremilk being collected. Samples were stored at -20o until analysis. It should be noted that fat and vitamin D content of milk increase by more than 2 times from foremilk to hindmilk in breast-feeding mothers (5), however, neither of which were pertinent in the study reported here. Chemical Determinations: Milk samples were weighed out along with assay controls containing Vitamin D_2/D_3 and 25(OH)D_2/D_3. Samples and controls were then spiked with d2-vitamin D^3/d_3, 25(OH)D_2/d_3, 25(OH)D_3 internal standards. Methanolic potassium hydroxide was then added to all samples and controls and saponified in a water bath at 60oC. Samples and controls were vortexed and then liquid-liquid extracted with hexane methylene chloride solution. The organic layer was then applied

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to 1.0 gram silica solid phase extraction columns for purification and isolation. All samples and controls were then injected into an Agilent 1100 High Pressure Liquid Chromatography (HPLC) for further purification across straight phase column chemistry. Samples were then dried down in a savant vacuum dryer, then reconstituted with LCMSMS mobile phase containing methanol and water and 0.1% formic acid and then loaded into the auto-sampler for analysis. The LC/MS/MS system used was an Agilent 1290 HPLC coupled to an Agilent 6460 MS/MS with ESI source and monitored in positive ion mode. All controls were found to be >95% accurate with %CV for intra-assay of <5.0%. All analyses had R2 values >0.99 with assay range from 0.5 to 62.5 ng/g. Orally consumed 25(OH)D_3 and 25(OH)D_2 have been shown to be about 5 times more effective in raising circulating concentration of 25(OH)D_3 than an equivalent amount of vitamin D_3 (5.) Total anti-rachitic activity (ARA) in milk samples is the sum of vitamin D_2, vitamin D_3, 25(OH)D_2 and 5 x 25(OH D_2 + 25(OH)D_3) expressed as IU/L (6).

Results

Results are presented in Table 1. The finding that D3 was greater than 25(OH)D_3 was in agreement with some studies but not with others (5). Our values for nmol/L of vitamin D_3 and for 25(OH)D_3 match closely to ones determined for vitamin D- supplemented lactating women (6). Our ARA value expressed as IU/L was well in excess over those determined in human studies which was about 60 IU/L (7). This finding is consistent with results from lactating women who had 25(OH)D_3 levels of 0.8 nmol/L in foremilk (6) compared to our value of 1.7 nmol/L or a factor of 2.1 times.

Table 1. Vitamin D and Antirachitic Activity (ARA) in Milk of Rhesus Monkeys

Component	Value
25(OH) D2	<0.5ng/g (below detectible limit)
25(OH) D3	4.2 ± 1.8ng/ml* (range 1.8 -10.1ng/ml) or 1.7 ± 0.7 nmol/L
Vitamin D2	<0.5ng/g (below detectible limit)
Vitamin D3	6.1 ± 3.1 ng/ml* (range 1.5 - 11.8 ng/ml) or 2.3 ± 1.1 nmol/L
ARA	27.1 ± 12.1 ng/ml or 1080 ± 480 IU/L (range 435 – 2256 IU/L)

*ng/g was changed to ng/ml by mutiplying by the specific gravity of primate milk (1.03 g/ml (8). Conversion Factors: 25(OH)D_3 : 1 nmol/L = 0.4 ng/ml. Vitamin D_3 : 1 nmol/L = 0.38 ng/ml. 1 IU = 25 ng Vitamin D_3

Discussion

Although intake of food for the monkeys was not measured, a reasonable estimate of Vitamin D intake can be calculated knowing the weight of the dam. It is known that chow is consumed at about 3.5% of body weight/day and that absorption of Vitamin D is about 80% (9). Mean weight of the dams in this study was 6.73 kg so consumption would be 0.24 kg and absorption 0.19 kg/day. This calculation is close to the value of 0.23 kg/ day obtained by Marriott et al (2) who measured average intake in monkeys from Cayo Santiago Field Station. Monkey chow is very high in Vitamin D_3 (containing 8000 IU or 200 ng/kg) so mean intake is about 1520 IU/day or 2.5 times the suggested level of 600 IU/day for adults. In addition, exposure to sunlight (exposure time not measured) will stimulate endogenous synthesis so total activity would be even greater. Consequently we would expect the milk to have a high ARA value, which was the case.

A significant correlation has been observed between 25(OH)D_3 in milk and in plasma of lactating mothers (5,10). Again, while 25(OH)D_3 was not measured in serum of dams in this study, we have data collected in same age and weight animals that could give approximate values for comparison with findings in humans. Streym et al (5) observed a correlation of 1.35% between maternal plasma concentration of 25(OH)D_3 found in foremilk. Our previous study for equivalent-aged female rhesus monkeys, sampled in "winter" gave an average of 106.4 nmol/L for 25(OH)D_3 (9). This value corresponds to a correlation of 3.8% , however, given all the conjectures leading to this value, it is at best a rational estimate. Considering the high ARA value and the correlation result, a reasonable expectation would be that newborns would have ample Vitamin D status, the levels of which beg testing.

In conclusion, we report herein the first determination of Vitamin D_3, 25(OH)D_3 and ARA in milk of the rhesus monkey. These values can serve as references but should be restricted to sampling conditions including similar diet, only foremilk, "winter" collection and absence of Vitamin D status of the dam.

Resumen

Objetivo: La leche es la fuente número uno de Vitamina D consumida y la más sostenida. Se han realizado extensos estudios entre seres humanos para medir la Vitamina D en madres lactantes, pero al día de hoy muy pocos estudios han obtenido leche materna de seres- no humanos primitivos y ninguno ha obtenido leche materna perteneciente a Monos Rhesus. Porconsecuencia, hemos determinado la Vitamina D y la actividad antirraquítica (ARA) en muestras obtenidas de leche materna perteneciente a 21 Monos Rhesus. Metodos: Las muestras de obtuvieron de forma manual, ordeñando a la presa. 25(OH)D_2, Vitamina D_2, 25(OH)D_3, Vitamina D3 y ARA obtenida en la leche materna, utilizando las técnicas de LC-MS/MS. Resultados: Los niveles de 25(OH)D2 y Vitamina D2 se encontraron por debajo de los limites detectable (<0.5 ng/g). 25(OH)D_3 = 4.2 ± 1.8 ng/ml, Vitamina D_3 = 6.1 ± 3.1 ng/ml y ARA = 1080 ± 480 IU/L Conclusion: Este es el primer estudio reportado sobre el contenido de Vitamina D y actividad antirraquítica (ARA) en la leche materna de los Monos Rhesus y puedo servir de referencia para estudios futuros.

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