HHS Public Access

Author manuscript

Am J Phys Anthropol. Author manuscript; available in PMC 2017 April 01.

Published in final edited form as:

Am J Phys Anthropol. 2016 April ; 159(4): 639–645. doi:10.1002/ajpa.22923.

Vitamin D heritability and effect of pregnancy status in vervet monkeys (*Chlorocebus aethiops sabaeus*) under conditions of modest and high dietary supplementation

Geetha Chittoor[1,2,*], **Nicholas M. Pajewski**[3,*], **V. Saroja Voruganti**[1,2], **Anthony G. Comuzzie**[4,5], **Thomas B. Clarkson**[6], **Matthew Nudy**[7,8], **Peter F. Schnatz**[7,8,9,10], **Jay R. Kaplan**[6], and **Matthew J. Jorgensen**[6,#]

[1]Department of Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, Kannapolis, North Carolina

[2]UNC Nutrition Research Institute, University of North Carolina at Chapel Hill, Chapel Hill, Kannapolis, North Carolina

[3]Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston-Salem, North Carolina

[4]Department of Genetics, Texas Biomedical Research Institute, San Antonio, Texas

[5]Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, Texas

[6]Department of Pathology, Section of Comparative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina

[7]Department of Obstetrics and Gynecology, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, Pennsylvania

[8]Department of Internal Medicine, Sidney Kimmel Medical College at Thomas Jefferson University, Philadelphia, Pennsylvania

[9]Department of Obstetrics and Gynecology, Reading Hospital, Reading, Pennsylvania

[10]Department of Internal Medicine, Reading Hospital, Reading, Pennsylvania

Abstract

Objectives—The two objectives of the current study were to: 1) investigate the genetic contributions to variations in serum vitamin D concentrations under two dietary conditions (a standard monkey biscuit diet vs. a diet designed to model typical American consumption) and; 2) explore the interaction of vitamin D with pregnancy status using a cohort of pedigreed female vervet/African green monkeys.

[#]**Correspondence to:** Matthew Jorgensen, Ph.D., Department of Pathology, Section of Comparative Medicine, Wake Forest School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157-1040, mjorgens@wakehealth.edu, Phone: 336-716-6935, Fax: 336-716-1515.

[*]Equal contribution of both authors

Materials and Methods—This study includes 185 female (≥3.5 years) vervet/African green monkeys (*Chlorocebus aethiops sabaeus*) from a multi-generational, pedigreed breeding colony. The $25(OH)D_3$ concentrations were first measured seven to eight weeks after consuming a "typical American" diet (TAD), deriving 37%, 18%, and 45% of calories from fat, protein sources, and carbohydrates, and supplemented with vitamin D to a human equivalent of 1,000 IU/day. Vitamin D concentrations were assessed again when animals were switched to a low-fat, standard biscuit diet (LabDiet 5038) for eight months, which provided a human equivalent of approximately 4,000 IU/day of vitamin D. All statistical analyses implemented in SOLAR.

Results—Pregnancy was associated with reduced $25(OH)D_3$ concentrations. Heritability analyses indicated a significant genetic contribution to $25(OH)D_3$ concentrations in the same monkeys consuming the biscuit diet (h^2=0.66, p=0.0004) and TAD (h^2=0.67, p=0.0078) diets, with higher $25(OH)D_3$ concentrations in animals consuming the biscuit diet. Additionally, there was a significant genotype-by-pregnancy status interaction on $25(OH)D_3$ concentrations ($p<0.05$) only among animals consuming the TAD diet.

Discussion—These results support the existence of a genetic contribution to differences in serum $25(OH)D_3$ concentrations by pregnancy status and emphasize the role of diet (including vitamin D supplementation) in modifying genetic signals as well as vitamin D concentrations.

Keywords

$25(OH)D_3$; Vervet/African green monkeys; genotype-by-diet; genotype-by pregnancy

Introduction

Vitamin D (25-hydroxy vitamin D [25(OH)D]) is essential for calcium and phosphorus homeostasis, bone health, and other physiologic functions (Dawodu and Akinbi 2013; Jehan 2014). Recently, there has been interest in evaluating the associations between vitamin D concentrations and extra-skeletal outcomes including cardiovascular and autoimmune disease, cancer, type 2 diabetes, obesity, and cognitive impairment (McGreevy and Williams 2011; Jorgensen et al., 2012; Theodoratou et al., 2014), although most studies to date have been inconclusive. In addition, animal and human studies suggest that vitamin D may be involved in many functions of the reproductive system in both males and females, but mainly in females with in vitro fertilization outcome and association with endometriosis and polycystic ovary syndrome (Zanatta et al., 2011; Anagnostis et al., 2013; Mahmoudi et al., 2013; Irani and Merhi 2014).

Studies have reported that the typically low vitamin D concentrations found in pregnant vs. non-pregnant women are further associated with multiple potentially adverse maternal (pregnancy induced hypertension, preeclampsia, gestational diabetes, infectious and inflammatory disorders), fetal (gestational duration), and infant (juvenile diabetes, developmental and psychological disorders) outcomes (Dror 2011; Dawodu and Akinbi 2013; Karras et al., 2014). There is also contradictory evidence regarding the associations between maternal vitamin D status and bone development in offspring, while supplementation studies with vitamin D have shown improvement in neonatal calcium homeostasis (Karras et al., 2014, 2015). The mechanism for the beneficial effects of vitamin

D on the adult skeletal health has been documented, but the adverse effects and role in non-bone health outcomes in pregnancy remains unclear (Brannon 2012; Glendenning and Inderjeeth 2015).

Vitamin D concentrations are under considerable genetic influence. Various candidate gene and genome-wide association studies (GWAS) have consistently implicated a role for genetic variation in vitamin D metabolism, with loci that include group-specific component (vitamin D binding protein [*GC*]), 7-dehydrocholesterol reductase (*DHCR7*), cytochrome P450, family 2, subfamily R, polypeptide 1 (*CYP2R1*), and cytochrome P450, family 24, subfamily A, polypeptide 1 (*CYP24A1*) (Ahn et al., 2010; Berry and Hyppönen 2011; Zhang et al., 2012; Hiraki et al., 2013; Zhang et al., 2013). However, variation in these genes appears to explain only a small proportion of the estimated heritability (additive genetic effects) for vitamin D. In addition, GWAS studies have focused on the direct effect of genetic variants on vitamin D concentrations, leaving the potential for interactions with vitamin D intake or synthesis relatively unexplored.

However, human genetic studies on vitamin D can be problematic in that they are typically based on observational data with highly variable environmental conditions. This lack of control over diet and other environmental determinants such as vitamin D supplementation, seasonal variation, and geographic location suggests broad utility for genomic investigations in animal models that share a considerable part of their genome with humans and in which controlled, human-like diets and environments can be manipulated. Many of these concerns can be addressed using animal models, especially nonhuman primates (NHP). Studies with vervet monkeys (*Chlorocebus aethiops sabaeus*) (Kavanagh et al., 2007; Jasinska et al., 2012; Jorgensen et al., 2012; Voruganti et al., 2013) and other species of NHP have already shown the practical use of such animal models in genetic studies of metabolic diseases including type 2 diabetes and dyslipidemia (Comuzzie et al., 2003; Cox et al., 2009, 2013; Jasinska et al., 2013).

In contrast to human observational studies, NHP models provide an opportunity to conduct genomic research that controls for environmental factors such as diet, climate, housing as well as individual differences in reproductive condition. The objectives of the current study were to (1) investigate the genetic contributions to variations in serum vitamin D concentrations under two dietary conditions (a standard monkey biscuit diet vs. a diet designed to model typical American consumption) and; (2) explore the interaction of vitamin D with pregnancy status using a cohort of pedigreed female vervet/African green monkeys.

Materials and Methods

Subjects

The study population consisted of 270 female vervet/African green monkeys (*Chlorocebus aethiops sabaeus*) from the Vervet Research Colony (VRC) of the Wake Forest Primate Center (WFPC). Study animals were of age (2.5 to 24.7 years old) and were all US-colony born. Details of the VRC have been previously described (Jorgensen et al., 2012; Jasinska et al., 2013). In brief, the VRC is a multi-generational, pedigreed, and genotyped colony

originally founded in 1975 with 57 animals imported from St. Kitts and Nevis. In early 2008, the VRC was transferred from Los Angeles to the WFPC.

Housing

Subjects were housed in 16 indoor-outdoor pens at the WFPC. Each pen consisted of a large outdoor area (~1200 square-feet) and a divided indoor area (300 square-feet). All pens were fitted with elevated perches, platforms, and climbing structures. Each of the 16 pens contained a matrilineal breeding group consisting of one to two adult males and a varying number of adult females and immature offspring. Each group housed between 11 and 36 animals greater than 3 years of age (19.31 ± 1.82). In general, females remain in their natal groups for their entire lives. Males are removed at four years of age to prevent sibling or son-mother mating and adult males are replaced every three-five years to maintain genetic diversity and prevent father-daughter matings. Males in some groups were vasectomized to control population growth.

Diet

Prior to 2004, the colony had been fed a standard primate diet (LabDiet 5038, Purina, St Louis MO), which is a standard biscuit diet. In 2004, the standard biscuit diet was replaced with a high-fiber, high-protein diet (LabDiet 5052; Fairbanks et al., 2010). In January and February 2008, the colony was transferred to WFPC and the diet was switched back to standard biscuit (LabDiet 5038) for approximately 11 months. Then, as part of a dietary challenge study described in Voruganti et al. (2013), the diet was switched to LabDiet 5L0P or TAD. After a 6-month challenge period, all animals returned to consuming the 5038-biscuit diet. The challenge diet (5L0P, TAD) was formulated to mimic a ‘typical’ American consumption, deriving 37% of calories from fat, primarily saturated and monounsaturated fatty acids from animal fat, 18% from protein sources (mostly animal), 45% from carbohydrates (~20% starches, and ~19% simple sugars), and containing 0.18 mg/Cal cholesterol (Table 1). The TAD diet contained 3.0 IU/g of vitamin D_3, a moderate level of supplementation equivalent to a 1000 IU/day for humans. In contrast, the biscuit diet contained 6.6 IU/g of vitamin D_3, representing substantial supplementation of vitamin D to the human equivalent of 4000 IU/day. See Table 1 for details on the diet formulations. Throughout the study, animals were supplemented with fresh fruits and vegetables. All animals had ad libitum access to food, water, and opportunities to exercise.

Experimental Procedures

Of the 270 animals, 217 had 25(OH)D_3 data collected while fed a biscuit diet and 26% (56/217) of individuals were pregnant at the time of vitamin D determination, 17 animals were lactating, and 3 animals had a miscarriage or stillbirth, and 238 had data collected while fed a TAD diet and 28% (67/238) of individuals were pregnant, 7 animals were lactating, and 12 animals had a miscarriage or stillbirth. For all the analyses, only animals that have information on both diets were used (N =185). Animals were sedated using ketamine (10–15 mg/kg) for all blood-sampling procedures, which are usually in the mornings between 9am and noon. Blood samples for 25(OH)D_3 were collected on two occasions: 1) seven to eight weeks after they began consuming the TAD diet in April, 2009; and 2) eight months after they had been switched back to the biscuit diet in May, 2010.

Body weight and abdominal circumference at the level of the umbilicus were also measured at each time period. We utilized the Reading Hospital Vitamin D Testing Center's Shimadzu liquid chromatography - mass spectrometry/mass spectrometry (LC-MS/2) technology. The HPLC/tandem mass spectrometry was used for the 25(OH)D assays with a determination for vitamin D_3. Detailed methods for the collection of the samples and assays for 25(OH)D (vitamin D_2 and D_3) concentrations have been described previously (Jorgensen et al., 2012). Since $25(OH)D_2$ concentrations were often below the level of detectability (related to dietary intake), only $25(OH)D_3$ data were analyzed. Ultrasounds were performed on all females at date of sampling to determine pregnancy status and to help identify possible early miscarriages. Pregnancy status was further validated based on birth records such that any animal that gave birth within 165 days of the sampling date was determined to be pregnant at the time of sampling.

Approvals

All procedures were approved by the Wake Forest Institutional Animal Care and Use Committee (IACUC). Wake Forest University is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and all animal care procedures follow the NIH Guide.

Statistical Analyses—All genetic analyses were implemented in the software Sequential Oligogenic Linkage Analysis Routines (SOLAR) (Almasy and Blangero 1998). Minitab Express version 1.1.0 (Minitab Inc. State College, PA) was used to conduct two-sample t-tests for comparing vitamin D concentrations in pregnant vs. non-pregnant vervets consuming two different diets.

Estimation of heritability

To estimate the genetic contribution to $25(OH)D_3$ concentrations under each of the dietary conditions (biscuit and TAD), quantitative genetic analyses were performed using SOLAR both for unadjusted model and for a model with adjustment of age and pregnancy status effects. Heritability (h^2) is defined as the total phenotypic variance (V_P) in a trait (e.g., $25(OH)D_3$) attributed to additive genetic factors (V_A) (Falconer and Mackay 1996), which is $h^2 = V_A/V_P$.

Genotype-by-pregnancy status interaction

To examine whether genetic influences on the $25(OH)D_3$ varied by pregnancy status (yes (PY) or no (PN)) under each of the dietary conditions, we conducted genotype-by-pregnancy (G × P) status interaction analyses. This approach is an extension of the variance components decomposition approach (Blangero and Almasy 1997; Almasy and Blangero 1998), which tests two hypotheses: 1) whether variance due to genetic factors was significantly different according to the pregnancy status under each of the dietary conditions *i.e.*, $\sigma g(PY) = \sigma g(PN)$, and 2) whether the genetic correlation of vitamin D phenotype measured between pregnant and non-pregnant vervets under two different diets differed significantly from 1.0 *i.e.*, $\rho G(PY,PN) = 1$. To test for genotype-by-diet (G × D) influences on the $25(OH)D_3$ concentrations, we used a similar approach as for vitamin D phenotype

and tested two hypotheses — $\sigma g(biscuit) = \sigma g(TAD)$, and $\rho G(biscuit,TAD) = 1$. The details of this interaction model are described in Mahaney et al., (1999) and Voruganti et al. (2013).

Results

Table 2 summarizes the descriptive characteristics and heritability estimates for all traits considered for analysis. For the biscuit diet (n = 185), the mean ± SD of age and 25(OH)D_3 concentrations of study animals were 10.80 ± 4.43 years and 80.63 ± 24.40 ng/ml, respectively. In comparison, for the TAD diet (n = 185), the mean ± SD of age and 25(OH)D_3 concentrations were 9.73 ± 4.43 years and 62.38 ± 20.81 ng/ml, respectively. Significant heritability ($p < 0.05$) of 25(OH)D_3 concentrations was detected for biscuit diet when unadjusted (66%), but not significant when adjusted for age and pregnancy status (21%). However, 25(OH)D_3 concentrations during the TAD dietary exposure were significantly heritable for both models at $p < 0.05$, 67% for un-adjusted and 51% for age and pregnancy status-adjusted (Table 2). Body weight and abdominal circumference tended to be higher in pregnant animals (TAD: 5.15 ± 0.75 kg, 35.06 ± 4.18 cm; biscuit: 5.35 ± 0.90 kg, 36.70 ± 5.00 cm) than non-pregnant (TAD: 5.06 ± 0.62 kg, 33.26 ± 3.37 cm; biscuit: 5.47 ± 0.79 kg, 36.50 ± 4.17 cm) animals in both diets, but only abdominal circumference was significantly different between pregnant and non-pregnant vervets consuming TAD diet ($P = 0.0038$). We also estimated phenotypic, genetic, and environmental correlations between vitamin D concentrations and both body weight and abdominal circumference (a better surrogate for obesity if >=40.5cm; Kavanagh et al., 2007). Yet, body weight and abdominal circumference did not exhibit statistically significant correlations with other traits in the current study, indicating that they do not share genetic effects with traits including 25(OH)D_3 concentrations (data not shown). On the other hand, heritabilities of both body weight and abdominal circumference were clearly influenced by age and pregnancy status when monkeys were on TAD diet, but not on biscuit (Table 2).

Figure 1 depicts the variability of 25(OH)D_3 concentrations measured in pregnant vs. non-pregnant monkeys, differentiated by consumption of the biscuit and TAD diets. It is clear that 25(OH)D_3 concentrations were higher in monkeys while consuming the biscuit diet ($p < 0.0001$) and also higher in non-pregnant (biscuit (n =137): 86.00 ± 21.85 ; TAD (n = 121): 64.99 ± 22.05; $p < 0.0001$) compared to pregnant monkeys (biscuit (n = 48): 65.30 ± 25.00; TAD (n = 64): 57.45 ± 17.36; $p = 0.0659$).

To assess the genetic contribution to differences in 25(OH)D_3 concentrations in response to pregnancy, we conducted a G × P interaction analysis (Table 3). As described in the Methods, we assessed two interaction models. We found genetic variances of 25(OH)D_3 concentrations while consuming the TAD diet to be significantly different ($p < 0.02$) between pregnant and non-pregnant animals. The second interaction model tested the hypothesis that the genetic correlation between the phenotypes under the two conditions is significantly different from one. This interaction model showed that genetic correlations between pregnant and non-pregnant animals were significantly different while consuming the TAD diet ($p < 0.03$) condition; these interaction effects did not extend to animals consuming the biscuit diet.

Table 4 gives the results of G × D interaction analysis. We found genetic variances of $25(OH)D_3$ concentrations to be not significantly different between the biscuit and TAD diets, and also, the genetic correlation between biscuit and TAD diets for $25(OH)D_3$ concentrations was not significantly different from one *i.e.*, $\rho G(biscuit,TAD) = 1$.

Discussion

The main findings of this study were that substantial additive genetic effects (heritability) on the variation in $25(OH)D_3$ concentrations were observed under both the biscuit and TAD diet condition in this NHP model. These outcomes extend our previous report showing large variations in serum vitamin D concentrations in monkeys with identical living situations, and diets (Jorgensen et al., 2012). In humans, heritability estimates for vitamin D concentrations range from 29% to 80% (Berry and Hypponen 2011; Hiraki et al., 2013). Similarly, our results showed that $25(OH)D_3$ concentrations were significantly heritable and in the same range as observed in people, and were observed regardless of diet (66% for biscuit and 67% for TAD), and that the greater vitamin D supplementation in the biscuit formulation significantly increased vitamin D concentrations. These observations comprise the first in-depth report of vitamin D genetics in an Old World monkey. One other study (Marx et al., 1989) reported differences in vitamin D metabolism among four different anthropoid species (crab-eating macaques (*Macaca fascicularis*), rhesus macaques (*M. mulatta*), squirrel monkeys (*Saimiri sciureus*), and night monkeys (*Aotus vociferans*)); however that investigation did not report on the genetics of variation in vitamin D concentrations.

Globally, the prevalence of inadequate vitamin D concentrations in pregnant women range from 5 to 84% (Brannon 2012), and evidence supports a role for reduced maternal vitamin D in pregnancy-related complications such as preeclampsia, gestational diabetes mellitus, preterm birth, and small size during gestation (Wei et al., 2013; Bodnar et al., 2013, 2014). Several studies reported low vitamin D levels in pregnant women (Dawodu and Wagner 2012; Wagner et al., 2012); however, role of vitamin D in pregnancy outcomes is yet to be fully described. Some studies speculate that given the presence of vitamin D receptors in gestational tissues, there is biological plausibility for variation of vitamin D concentrations during pregnancy (Thota et al., 2014). Interestingly, in the present study, pregnancy status was associated with reduced vitamin D concentrations regardless of diet. Further study will be required to determine whether adverse outcomes occur in relation to individual differences in the degree of vitamin D reduction observed in this colony. It is also known that maternal vitamin D metabolism changes during pregnancy in women, but this phenomenon was not studied here. Nor are the mechanisms linking variation in vitamin D to maternal or fetal outcomes well understood (Brannon 2012; Theodoratou et al., 2014).

As a secondary outcome, we also found significant G × P interaction effects on $25(OH)D_3$ concentrations. This interaction indicates that phenotypic variation in $25(OH)D_3$ concentrations is attributed to genetic architecture based on pregnancy status in this species. We found that the genetic correlation was significantly different from 1, but only while monkeys consumed the TAD diet. This finding indicates that different genes influence expression of vitamin D concentrations in pregnant vs non-pregnant individuals in this

dietary condition. Similarly, variance due to genetic factors was significantly different in pregnant vs. non-pregnant animals while they consumed the TAD diet, indicating an effect of pregnancy on the magnitude of genetic influence on the $25(OH)D_3$. We did not find any significant G × P interaction effects for biscuit diet, may be because the effects were masked by the heavy supplementation of diet with vitamin D.

Also, non-significant G × D interaction for $25(OH)D_3$ indicates that the genes affecting vitamin D in monkeys consuming biscuits may not be different from the genes affecting vitamin D in monkeys eating a more human-like TAD diet. However, previous genetic analyses of cardiometabolic traits in the VRC have suggested a differential genetic architecture comparing the TAD and standard biscuit diet (Voruganti et al., 2013). These observations highlight the importance of mimicking a human dietary environment for translational genomic research in NHP model systems (Cox et al., 2013; Jasinska et al., 2013; Kavanagh et al., 2013). This is especially the case for biological processes and pathways related to vitamin D, where a standard biscuit diet like the one used here reflects a degree of vitamin D supplementation well beyond what is currently recommended for humans (4,000 IU compared to the National Academy of Medicine's current recommendation of 600 IU for adults under 70 years of age and 800 IU for those 70 years and older; Institute of Medicine).

Several limitations of the current study deserve consideration. First, only a small number of male animals in the VRC pedigree were assayed for vitamin D, thus restricting our analyses to females. Also, while our statistical models controlled for pregnancy, we were not able to control for gestational age due to insufficient sample size. Therefore, it is unclear whether this adjustment is sufficient to address expected fluctuations in vitamin D throughout the course of pregnancy in vervets (based on evidence observed in humans by Milman et al., 2011). Furthermore, we have not investigated how the effects of specific genetic variants may change in response to dietary vitamin D supplementation. In the future, the availability of whole-genome sequence for the VRC will provide a complete catalogue of vervet genetic variation (Jasinska et al., 2013), facilitating association-mapping experiments across the allele frequency spectrum. In addition, it will also enable more accurate estimates of genetic variance based on the realized relationship matrix derived from marker genotypes (Lee et al., 2010). Therefore, one immediate future research direction is to utilize VRC whole genome data to investigate genetic interactions with dietary and/or other environmental manipulations. We also note that vitamin D was assessed at only a single time point in each dietary condition, a fact that matches typical clinical practice but that also likely increased error variance. Despite these limitations, the current investigation highlights in a widely used NHP model the significant influence of genes on variation in vitamin D concentrations, the reduction of vitamin D concentrations in pregnant vs. non-pregnant individuals, the fact that pregnancy modifies genetic influences on individual variation in vitamin D concentrations, and finally the observation that different genes influence vitamin D variation in monkeys consuming a standard biscuit diet vs. those fed the more human like TAD diet seems unlikely from our findings.

In summary, our study is the first to report that vitamin D concentrations are under significant genetic influence in a nonhuman primate model studied under controlled dietary,

housing, and climate conditions. The presence of gene-by-pregnancy status interaction indicates that differential genetic effects are involved in the regulation of these vitamin D concentrations in pregnant and non-pregnant vervets under biscuit and TAD diets, respectively. These observations highlight the importance of mimicking a human dietary environment for translational genomic research in model systems such as the NHP. In addition, vervets that were pregnant had decreased serum vitamin D concentrations regardless of which diet they received. The knowledge gained from this study has to be utilized in investigating genetic mechanisms underlying these changes and the role of vitamin D during pregnancy.

Acknowledgements

The VRC is supported in part by grant number P40 OD010965 from the National Institutes of Health, the Department of Veterans Affairs, the UCLA Semel Institute, Wake Forest School of Medicine, and the Reading Hospital.

Literature Cited

Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet. 2010; 19(13):2739–2745. [PubMed: 20418485]

Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet. 1998; 62(5):1198–1211. [PubMed: 9545414]

Anagnostis P, Karras S, Goulis DG. Vitamin D in human reproduction: a narrative review. Int J Clin Pract. 2013; 67(3):225–235. [PubMed: 23294318]

Berry D, Hyppönen E. Determinants of vitamin D status: focus on genetic variations. Curr Opin Nephrol Hypertens. 2011; 20(4):331–336. [PubMed: 21654390]

Blangero J, Almasy L. Multipoint oligogenic linkage analysis of quantitative traits. Genet Epidemiol. 1997; 14(6):959–964. [PubMed: 9433607]

Bodnar LM, Rouse DJ, Momirova V, et al. Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network. Maternal 25-hydroxyvitamin d and preterm birth in twin gestations. Obstet Gynecol. 2013; 122(1):91–98. [PubMed: 23743453]

Bodnar LM, Simhan HN, Catov JM, et al. Maternal vitamin D status and the risk of mild and severe preeclampsia. Epidemiology. 2014; 25(2):207–214. [PubMed: 24457526]

Brannon PM. Vitamin D and adverse pregnancy outcomes: beyond bone health and growth. Proc Nutr Soc. 2012; 71(2):205–212. [PubMed: 22260841]

Comuzzie AG, Cole SA, Martin L, et al. The baboon as a nonhuman primate model for the study of the genetics of obesity. Obes Res. 2003; 11(1):75–80. [PubMed: 12529488]

Cox LA, Glenn J, Ascher S, Birnbaum S, VandeBerg JL. Integration of genetic and genomic methods for identification of genes and gene variants encoding QTLs in the nonhuman primate. Methods. 2009; 49(1):63–69. [PubMed: 19596448]

Cox LA, Comuzzie AG, Havill LM, et al. Baboons as a model to study genetics and epigenetics of human disease. ILAR J. 2013; 54(2):106–121. [PubMed: 24174436]

Dror DK. Vitamin D status during pregnancy: maternal, fetal, and postnatal outcomes. Curr Opin Obstet Gynecol. 2011; 23(6):422–426. [PubMed: 21986726]

Dawodu A, Akinbi H. Vitamin D nutrition in pregnancy: current opinion. Int J Womens Health. 2013; 5:333–343. [PubMed: 23843706]

Dawodu A, Wagner CL. Prevention of vitamin D deficiency in mothers and infants worldwide - a paradigm shift. Paediatr Int Child Health. 2012; 32(1):3–13. [PubMed: 22525442]

Fairbanks LA, Blau K, Jorgensen MJ. High-fiber diet promotes weight loss and affects maternal behavior in vervet monkeys. Am J Primatol. 2010; 72(3):234–241. [PubMed: 19937736]

Falconer, DS.; Mackay, TFC. Introduction to Quantitative Genetics. 4th ed.. Longman; 1996.

Glendenning P, Inderjeeth CA. Controversy and consensus regarding vitamin D: Recent methodological changes and the risks and benefits of vitamin D supplementation. Crit Rev Clin Lab Sci. 2015; 17:1–16.

Hiraki LT, Major JM, Chen C, et al. Exploring the genetic architecture of circulating 25-hydroxyvitamin D. Genet Epidemiol. 2013; 37(1):92–98. [PubMed: 23135809]

Institute of Medicine, Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. Dietary reference intakes for calcium and vitamin D. 2010

Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertil Steril. 2014; 102(2):460.e3–468.e3. [PubMed: 24933120]

Jasinska AJ, Lin MK, Service S, et al. A non-human primate system for large-scale genetic studies of complex traits. Hum Mol Genet. 2012; 21(15):3307–3316. [PubMed: 22556363]

Jasinska AJ, Schmitt CA, Service SK, et al. Systems biology of the vervet monkey. ILAR J. 2013; 54(2):122–143. [PubMed: 24174437]

Jehan F, Voloc A. Classical actions of vitamin D: insights from human genetics and from mouse models on calcium and phosphate homeostasis. Biol Aujourdhui. 2014; 208(1):45–53. [PubMed: 24948018]

Jorgensen MJ, Rudel LL, Nudy M, et al. 25(OH)D3 and cardiovascular risk factors in female nonhuman primates. J Womens Health (Larchmt). 2012; 21(9):959–965. [PubMed: 22876774]

Karras SN, Anagnostis P, Bili E, et al. Maternal vitamin D status in pregnancy and offspring bone development: the unmet needs of vitamin D era. Osteoporos Int. 2014; 25(3):795–805. [PubMed: 23907574]

Karras SN, Anagnostis P, Petroczi A, Annweiler C, Naughton DP, Goulis DP. Maternal vitamin D status in pregnancy: a critical appraisal of current analytical data on maternal and neonatal outcomes. Hormones (Athens). 2015; 14(2):224–231. [PubMed: 26158654]

Kavanagh K, Fairbanks LA, Bailey JN, et al. Characterization and heritability of obesity and associated riskfactors in vervet monkeys. Obesity (Silver Spring). 2007; 15(7):1666–1674. [PubMed: 17636084]

Kavanagh K, Wylie AT, Tucker KL, et al. Dietary fructose induces endotoxemia and hepatic injury in calorically controlled primates. Am J Clin Nutr. 2013; 98(2):349–357. [PubMed: 23783298]

Lee SH, Goddard ME, Visscher PM, van der Werf JH. Using the realized relationship matrix to disentangle confounding factors for the estimation of genetic variance components of complex traits. Genet Sel Evol. 2010; 42:22. [PubMed: 20546624]

Mahaney MC, Blangero J, Rainwater DL, Mott GE, Comuzzie AG, MacCluer JW, VandeBerg JL. Pleiotropy and genotype by diet interaction in a baboon model for atherosclerosis: a multivariate quantitative genetic analysis of HDL subfractions in two dietary environments. Arterioscler Thromb Vasc Biol. 1999; 19(4):1134–1141. [PubMed: 10195946]

Mahmoudi AR, Zarnani AH, Jeddi-Tehrani M, et al. Distribution of vitamin D receptor and 1α-hydroxylase in male mouse reproductive tract. Reprod Sci. 2013; 20(4):426–436. [PubMed: 23188491]

Marx SJ, Jones G, Weinstein RS, Chrousos GP, Renquist DM. Differences in mineral metabolism among nonhuman primates receiving diets with only vitamin D3 or only vitamin D2. J Clin Endocrinol Metab. 1989; 69(6):1282–1290. [PubMed: 2555385]

McGreevy C, Williams D. New insights about vitamin D and cardiovascular disease: a narrative review. Ann Intern Med. 2011; 155(12):820–826. [PubMed: 22184689]

Milman N, Hvas AM, Bergholt T. Vitamin D status during normal pregnancy and postpartum. A longitudinal study in 141 Danish women. J Perinat Med. 2011; 40(1):57–61. [PubMed: 22098305]

Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. BMJ. 2014; 348:g2035. [PubMed: 24690624]

Thota C, Laknaur A, Farmer T, Ladson G, Al-Hendy A, Ismail N. Vitamin D regulates contractile profile in human uterine myometrial cells via NF-κB pathway. Am J Obstet Gynecol. 2014; 210(4):347.e1–347.e10. [PubMed: 24262718]

Voruganti VS, Jorgensen MJ, Kaplan JR, et al. Significant genotype by diet (G × D) interaction effects on cardiometabolic responses to a pedigree-wide, dietary challenge in vervet monkeys (Chlorocebus aethiops sabaeus). Am J Primatol. 2013; 75(5):491–499. [PubMed: 23315630]

Wagner CL, Taylor SN, Dawodu A, Johnson DD, Hollis BW. Vitamin D and its role during pregnancy in attaining optimal health of mother and fetus. Nutrients. 2012; 4(3):208–230. [PubMed: 22666547]

Wei SQ, Qi HP, Luo ZC, Fraser WD. Maternal vitamin D status and adverse pregnancy outcomes: a systematic review and meta-analysis. J Matern Fetal Neonatal Med. 2013; 26(9):889–899. [PubMed: 23311886]

Zanatta L, Zamoner A, Gonçalves R, et al. Effect of 1α,25-dihydroxyvitamin D3 in plasma membrane targets in immature rat testis: ionic channels and gamma-glutamyl transpeptidase activity. Arch Biochem Biophys. 2011; 515(1–2):46–53. [PubMed: 21933661]

Zhang Y, Wang X, Liu Y, et al. The GC, CYP2R1 and DHCR7 genes are associated with vitamin D levels in northeastern Han Chinese children. Swiss Med Wkly. 2012; 142:w13636. [PubMed: 22801813]

Zhang Y, Yang S, Liu Y, Ren L. Relationship between polymorphisms in vitamin D metabolism-related genes and the risk of rickets in Han Chinese children. BMC Med Genet. 2013; 14:101. [PubMed: 24073854]

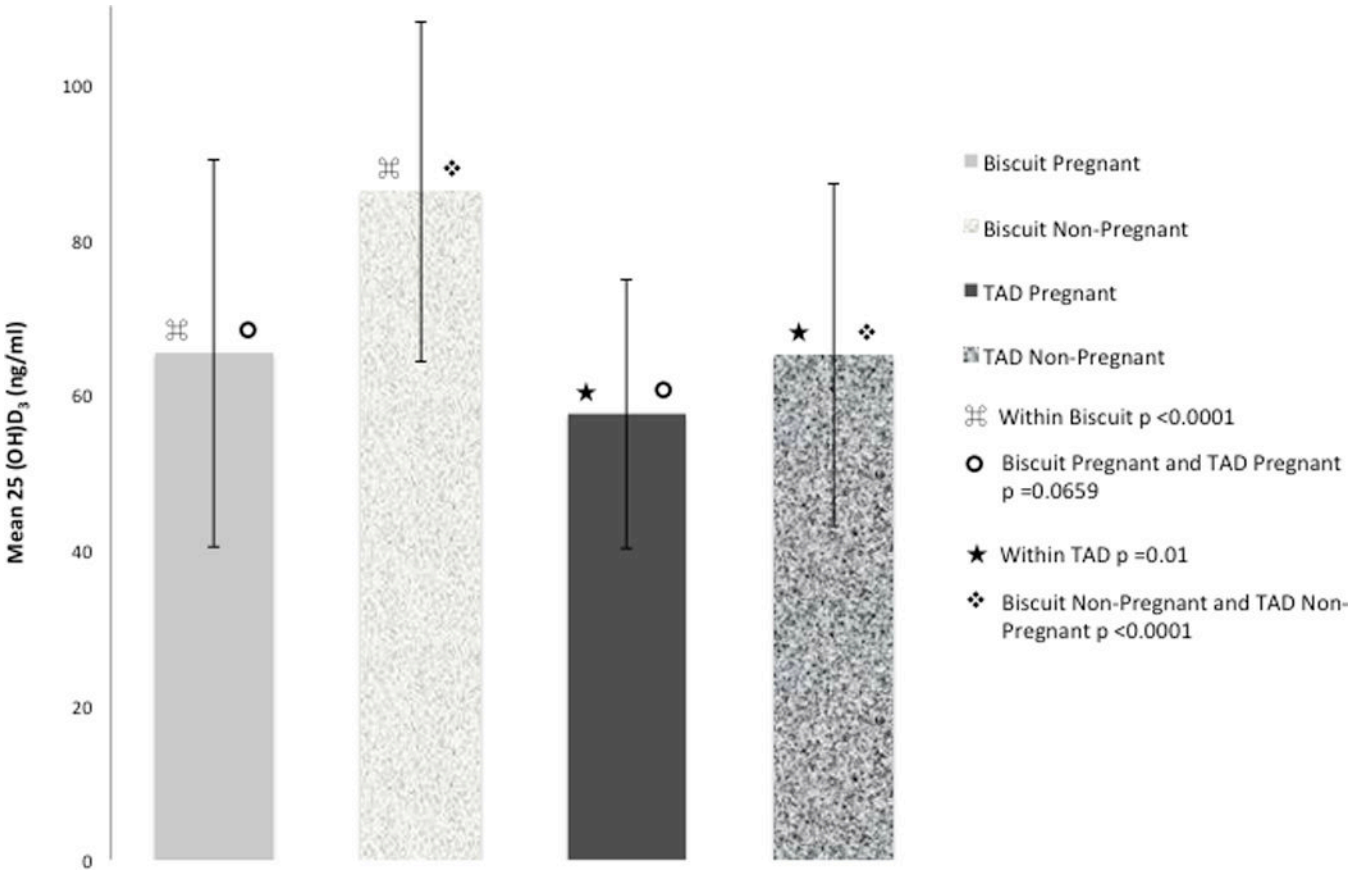

Figure 1.

$25(OH)D_3$ mean values for pregnant vs. non-pregnant vervet monkeys on Biscuit and TAD diets

Table 1

Diet formulations for the “standard” Biscuit diet and the “typical” American diet (TAD) including the women’s equivalent of daily vitamin D

	Biscuit (Purina LabDiet 5038)	TAD (Purina LabDiet 5L0P)
Fiber (crude, %)	5	9
Calories by protein (%)	18	18
Calories by fat (%)	13	37
Calories by carbohydrates (%)	69	45
Metabolizable energy (kcal/g)	3.22	3.34
Vitamin D_3 (IU/g)	6.6	3.0
Women’s equivalent of Vitamin D_3 (IU/day)	4000	1000

Table 2

Descriptive characteristics and heritability (h^2) estimates

Variable (N = 185)	Mean ± SD	h^2 ± SE	*P* Value[c]	h^2 ± SE	*P* Value[c]	Significant Covariates
		Model 1[a]		Model 2[b]		
Biscuit Diet						
Age (years)	10.80 ± 4.43	-		-	-	-
Body weight (kg)	5.44 ± 0.82	0.68 ± 0.21	**0.0021**	0.68 ± 0.21	**0.0021**	-
Abdominal Circumference (cm)	36.56 ± 4.39	0.33 ± 0.22	0.0526	0.31 ± 0.21	0.0560	Age
25(OH)D_3(ng/ml)	80.63 ± 24.40	0.66 ± 0.21	**0.0004**	0.21 ± 0.24	0.1285	Age, Pregnancy Status
TAD Diet						
Age (years)	9.73 ± 4.43	-		-	-	-
Body weight (kg)	5.09 ± 0.67	0.57 ± 0.22	**0.0071**	0.56 ± 0.22	**0.0059**	Age, Pregnancy Status
Abdominal Circumference (cm)	33.89 ± 3.76	0.58 ± 0.23	**0.0065**	0.50 ± 0.22	**0.0102**	Age, Pregnancy Status
25(OH)D_3 (ng/ml)	62.38 ± 20.81	0.67 ± 0.26	**0.0078**	0.51 ± 0.29	**0.0389**	Age, Pregnancy Status

SD – standard deviation; SE - standard error

[a] Model 1 – unadjusted

[b] Model 2 – adjusted for age and pregnancy status

[c] $p<0.05$ considered to be significant

Table 3

Summary of the genotype-by-pregnancy status interaction for 25(OH)D_3 phenotype

Trait	σg(PY) = σg(PN); p value	ρG(PY,PN) =1; p value
Biscuit-25(OH)D_3	0.3275	0.2532
TAD-25(OH)D_3	**0.0138**	**0.0206**

p values in bold significant at<0.05 level

σg: Variance due to genetic factors
ρG: Correlation due to genetic factors
PY, PN: Pregnancy status (yes or no)

Table 4

Summary of the genotype-by-dietary supplementation interaction for 25(OH)D_3 phenotype

Trait	σg(Biscuit) = σg(TAD); p value	ρG(Biscuit,TAD) =1; p value
Diet-25(OH)D_3	0.6994	0.1515

p values in bold significant at<0.05 level

σg: Variance due to genetic factors
ρG: Correlation due to genetic factors