Published in final edited form as:

J Am Coll Nutr. 2013 ; 32(4): 215–223. doi:10.1080/07315724.2013.821886.

Vitamin D deficiency in cord plasma from multiethnic subjects living in the tropics

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Abstract

Background—Vitamin D deficiency is commonly reported in high latitude areas and in dark pigmented individuals. However, nothing is known about vitamin D in cord blood from multiethnic subjects living in the tropics.

Objective—Our study objective was to determine the prevalence of vitamin D deficiency in summer and winter in cord blood from multiethnic individuals in Hawai'i where sufficient sun irradiance occurs year-round for cutaneous vitamin D production.

Methods—25-hydroxyvitamin D (25(OH)D) levels were quantified by enzyme-immunoassay in 100 cord plasma samples from apparently healthy full term newborns and their mothers. Stratification was performed by birth season and ethnicity.

Results—Mean 25(OH)D levels were 24.5 ng/mL (9.1–68.3 ng/mL). Overall, 28% of samples were Vitamin D deficient (<20 ng/mL) and 50% were insufficient (20–30 ng/mL). 25(OH)D levels (ng/mL) were highest in Caucasians (30.5, n=19) then Asians (25.1, n=43), Hispanics (21.5, n=3), Pacific Islanders (20.0, n=25), and African Americans (19.6, n=2). Differences among groups were significant (p=0.008). Cord plasmas from summer versus winter were higher overall (p=0.001) and among Asians (p=0.0003). Seasonal changes were correlated with sun irradiance overall (r=0.43, p=0.0001), among Caucasians (r=0.45, p=0.05), and among Asians (r=0.45, p=0.0001).

Conclusion—Our results suggest that prenatal supplement recommendations of 400 IU vitamin D/day does not protect against vitamin D deficiency, even in subjects living in the tropics where ample sun irradiance exists for cutaneous vitamin D synthesis. The high prevalence of vitamin D deficiency we observed emphasizes the necessity for regular 25(OH)D monitoring, particularly during pregnancy and lactation, in dark pigmented individuals, and during winter months.

Keywords

vitamin D insufficiency; seasonal variation; cord blood; Hawaii

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Financial Disclosure and Conflict of Interest: All authors state that they have no conflict of interest.

INTRODUCTION

The relevance of vitamin D for maintaining health throughout life is supported by its vital involvement in controlling approximately 3% of the human genome in addition to affecting numerous other essential functions and conditions such as calcium and bone metabolism, rheumatoid arthritis, infections, multiple sclerosis, type 1 and 2 diabetes mellitus, autoimmune disease, cardiovascular disease, Crohn's disease, and many cancers [1-8].

Poor vitamin D status has been shown to be more prevalent during pregnancy [9] and is a major risk factor for preeclampsia, delivery via cesarean section in pregnant women, and for infant rickets, respiratory infections, wheezing, diabetes, and possibly schizophrenia and autism in early life and childhood [10-16]. In utero, the fetus is completely dependent on the mother for vitamin D with the vitamin D status of the fetus closely related to that of the mother [17]. Recent studies have shown 25-dihydroxy vitamin D (25(OH)D) levels in cord blood to correlate well with those in maternal blood [18-20]. Thus, during pregnancy, the maintenance of sufficient 25(OH)D levels remains of utmost importance.

Several studies in adults have reported low vitamin D status among individuals with dark skin pigmentation [21], in people living at high latitudes [22], and during winter months [18, 23-26], while a few studies using umbilical cord blood have found widespread vitamin D insufficiency at latitudes 24 to 67° [18, 19, 27, 28]. However, the prevalence of vitamin D deficiency and insufficiency in cord blood or venous blood from multiethnic individuals living in a tropical climate is not known.

Our study objectives were to determine vitamin D status in cord blood from a convenience sample of 100 subjects and to examine whether cord blood vitamin D status varies during different seasons and within different ethnic groups in Honolulu, Hawai'i, a low-latitude (21°N latitude) tropical location with ample year-round sun exposure and extensive ethnic diversity.

PATIENTS AND METHODS

Sample Collection

This study was part of a prospective investigation on antenatal seafood exposure and neurodevelopmental outcomes in infants. Healthy pregnant women admitted for delivery to Kapi'olani Medical Center for Women and Children in Honolulu, Hawai'i (island of Oahu) between June 2010 and March 2011 were assessed for study eligibility. Patients were considered eligible if they were between 18 and 45 years old, pregnant with a singleton live fetus and gestational age $\geq$ 37 weeks. A total of 107 cord blood samples were collected after cord clamping but only 100 samples were used in the final analysis after excluding mothers who could not provide consent or whose infants were not able to be examined prior to discharge. Routine newborn examinations were performed immediately after birth in addition to a Ballard scale and a Neonatal Intensive Care Unit Network Neurobehavioral Scale exam. Exclusion criteria included reported tobacco, alcohol, or illicit drug use; English illiteracy; pregnancy with multiple gestation; women who wanted to take home their placentas; women with diabetes, hypertension or other chronic diseases; and women pregnant with a fetus with growth restriction or any known congenital anomalies.

After delivery, approximately 20 mL of whole blood from the umbilical cord was collected into EDTA tubes and processed within two hours. Personal and demographic data were obtained through questionnaire. Infant birth weight, APGAR scores, head circumference, length, gestational age, sex, date of birth, and maternal daily intake of 400 IU Vitamin D supplementation were also documented. The Institutional Review Board at Kapi'olani

Medical Center for Women and Children and the Western IRB approved this study and all participants signed a consent form.

Solar ground radiation data (or global horizontal irradiance (GHI), the sum of direct normal irradiance, diffuse horizontal irradiance, and the negligible ground-reflected radiation) were retrieved online from the National Renewable Energy Laboratory (NREL) website (http://www.nrel.gov/midc/kalaeloa_oahu/). The data were recorded at Kalaeloa, Hawai'i (island of Oahu, latitude 21.3° N, longitude 158.1° W, altitude 11 m AMSL) by a Rotating Shadowband Radiometer that uses a silicon-based photodiode pyranometer. Averaged 24-hour readings of total solar radiation (W/m²) for the 17 days prior to and including the birthdate were used to calculate correlations with cord plasma 25(OH)D concentrations.

Distinction of seasons

Due to the tropical location of Hawai'i, its seasons are limited to winter and summer. For the purpose of this study, summer season was extended by 17 days to adjust for the 2-3 week half-life of circulating 25(OH)D that results in a 'spillover' of the 25(OH)D produced during summer season into the beginning of winter season. Thus, for analysis purposes, the summer season lasted from May 1 to November 17. The winter season was not extended due to the fast 25(OH)D synthesis and distribution into the circulation after UV exposure and lasted from November 18 to April 30. Only one cord blood sample was collected in the 'spill-over' period Nov 1-17 (from a Pacific Islander) and removing that data point from that collection led to trivial and entirely insignificant changes of all findings. Thus, all 100 data points were used for analysis.

Biochemical analysis

Cord blood samples were centrifuged and plasma aliquots were kept at -80 °C until use. Duplicate 25(OH)D levels were measured using a commercial enzyme immunoassay kit (Immunodiagnostic Systems, Inc Cat no AC-57F1, Scottsdale, AZ) following manufacturer's instructions. The inter-assay coefficient of variation was 9.9% at mean concentrations of 31.9 ng/mL. This assay was compared to the gold standard HPLC method [29] and validated by participation in quality assurance programs organized by the Vitamin D External Quality Assessment Scheme (DEQAS, London, UK) and the US National Institute of Standards and Technologies (NIST, Gaithersburg, MD).

Vitamin D deficiency, insufficiency and sufficiency was defined as traditionally recommended in the literature and currently applied using cut-offs (traditional cut-offs, TC) of <20 ng/mL, 20-30 ng/mL, and >30 ng/mL, respectively [30-37]. These definitions also concur with recommendations from the 2011 Endocrine Society's Practice Guidelines, which define vitamin D deficiency and insufficiency cutoffs as <20 ng/ml and 21-29 ng/ml, respectively [38]. To convert ng/mL to nmol/L, multiply ng/mL by 2.5.

Grouping of ethnicities

The 17 reported ethnicities were condensed into 6 groups to allow better inter-ethnic evaluations: Caucasian (white, non-Hispanic), African American (black, non-Hispanic), Hispanic (includes Spanish), Asians (Japanese, Chinese, Korean, Filipino, other Asian), Pacific Islander (Hawaiian, Samoan, Tongan, Micronesian, Chamorro, Cook Islander), and Other (Indian, American Indian, unknown). Participants were assigned to one of these 6 groups if they possessed combined 50% or more ethnic makeup present in that group.

Statistical evaluations

The SAS 9.3 statistical software (SAS Institute, Cary, NC) was used for all analyses. The GLM procedure was used to assess the effects of ethnicity, season (summer vs. winter), and the ethnicity by season interaction on 25(OH)D concentrations. The Tukey-Kramer method was used for subsequent group comparisons. Because the seasonal dichotomy may have been too broad, we also applied nonlinear regression with the NLIN procedure. We fit the model equation shown below, where y is the value of 25(OH)D and x the date of observation. The parameters a , b , and c are calculated to give the best possible fit, minimizing the error (e) variance.

$$y = a + b * \sin \left(2\pi * \left(\frac{x}{365.25} + c \right) \right) + e$$

The parameter of primary interest is b , the amplitude. If statistically different from zero, it would indicate the presence of sine wave; if not, it would suggest a straight line. Because of possible differences in skin pigmentation, we stratified the sine wave models by ethnic group. Only three ethnic groups (Caucasian, Asian, and Pacific Islander) had sufficient numbers for this modeling approach. Finally, the CORR procedure was used to calculate Pearson correlations between radiation and 25(OH)D concentrations.

RESULTS

Demographics

The mean age of participants was 28 years (range 18-44 years; Table 1). All but 3 participants resided on Hawai'i (island of Oahu) for the entire duration of their pregnancy (data not shown). Other demographic characteristics are summarized in Table 1. According to the entries in the medical records recorded at the prenatal visits, participants were compliant with Vitamin D supplementation of 400 IU/day (data not shown). The ethnic diversity among our 100 subjects was very extensive (Table 1). Only half of the participants reported to be of a single ethnicity while the other half were mixtures of two (22%), three (17%), four (8%), or five (3%) ethnicities in varying combinations and degrees.

25(OH)D levels

ANOVA with cord plasma 25(OH)D concentration as the dependent variable was performed with ethnic group, season, and the ethnic group by season interaction as the independent variables. The interaction was not statistically significant [$F(5,88) = 0.45$, $p = 0.81$] and was omitted from the model. In the revised model, ethnicity [$F(5,93) = 3.36$, $p = 0.008$] and season [$F(1,93) = 11.6$, $p = 0.001$] were both statistically significant. The overall mean cord plasma 25(OH)D level was 24.5 ng/mL (9.1-68.3 ng/mL; Table 2). Mean concentrations were highest in Caucasians (30.5 ng/mL) followed by Asians (25.1 ng/mL), Hispanics (21.5 ng/mL), Pacific Islanders (20.0 ng/mL), and African Americans (19.6 ng/mL). Using the Tukey-Kramer multiple group comparison test, mean concentrations of 25(OH)D were significantly different between Caucasians and Pacific Islanders ($p = 0.002$). Other comparisons were not statistically significant.

Overall, the prevalence of deficiency (<20ng/mL) and insufficiency (20-30 ng/mL) was 28% and 50%, respectively (Table 2). Only 13% of the subjects had 25(OH)D levels between 30-36 ng/mL, while 3% possessed levels between 36-40 ng/mL, and just 6% had levels between 40 and 75 ng/mL (data not shown). Within ethnic groups, the prevalence of vitamin D deficiency and insufficiency was 16% and 32% in Caucasians, 21% and 60% in Asians,

33% and 67% in Hispanics, 48% and 40% in Pacific Islanders, and 50% and 50% in African Americans, respectively.

Vitamin D status by season

Overall, the mean cord plasma 25(OH)D levels were 30% higher in samples collected in the summer compared to winter season (27.1 vs. 20.8 ng/mL; $p=0.001$, Table 2). Among all ethnicities (except for Hispanic which remained the same), 25(OH)D levels were higher in the summer compared to winter (highly significant in Asians; $p=0.0003$). Seventeen of a total of 41 samples collected in winter (41%) were deficient and 21 (51%) were insufficient. Eleven of a total of 59 samples collected in summer (19%) were deficient and 29 (49%) were insufficient.

Correlations of 25(OH)D concentrations with solar radiation

Seasonal changes in 25(OH)D concentrations were correlated with solar ground radiation readings in all subjects ($r=0.43$, $p<0.0001$), in Caucasians ($r=0.45$, $p=0.05$), in Asians ($r=0.55$, $p=0.0001$), but not in Pacific Islanders ($r=0.18$, $p=0.39$; figure 1). The sine wave model was statistically significant only for the Asian group ($b=5.1$, $p=0.0005$).

No correlations were observed between 25(OH)D concentrations and maternal age, income, education, gravida, baby gender, birth weight, AGPAR score, head circumference, or body length (data not shown) which is in agreement with recent findings [20].

DISCUSSION

High prevalence of insufficiency

Vitamin D insufficiency and seasonal variation in vitamin D status are commonly reported in populations living at high latitudes or in countries where purdah is observed [18, 20, 23-25, 34, 36, 39], but not from individuals residing in the tropics. To our knowledge, this is the first report investigating seasonal and ethnical variation in vitamin D status in umbilical cord blood collected from apparently healthy multiethnic subjects living in the tropics. For at least one month prior to delivery, all participants resided on the island of Oahu (state of Hawai'i), a tropical location located at 21°N latitude where sufficient sun and UV-B radiation occurs year-round for cutaneous vitamin D synthesis. Also, in Hawai'i, pregnant women are advised to take daily prenatal vitamins containing 400 IU vitamin D. Given these conditions favoring adequate vitamin D status, we unexpectedly found a high prevalence of vitamin D deficiency (<20 ng/mL) and insufficiency (20-30 ng/mL) that was particularly high in samples derived in the winter season when sun irradiance was lower and, as expected, higher in ethnicities with dark versus light skin pigmentation: in Caucasians, 16% were deficient and 32% were insufficient compared to other ethnic groups where 21-50% deficiency and 40-67% insufficiency was observed respectively, when the entire year was considered. These findings are in good agreement with a recent study in minority groups [36] that found a fairly high percentage (14-34%) of vitamin D insufficiency in 6-12 year-old children despite them having mean dietary intakes of 420-450 IU/day during the summer and 430-500 IU/day during the winter. Both intakes were fairly close to the recently recommended dietary intake from the Endocrine Society's Clinical Practice Guideline of 600 IU/day [38]. Our findings are also in agreement the vitamin D deficiency observed in 50% of mothers and 65% of their newborns at delivery despite the mother's daily ingestion of approximately 600 IU/day –receiving 400 IU from prenatal supplements and 200 IU from the consumption of two glasses of milk [40].

The need to consider higher vitamin D dosing particularly during pregnancy is highlighted by two supplementation trials. In the first USA based trial, only 50% of mothers

supplementing with 400 IU/day vitamin D achieved circulating 25(OH)D levels of 32 ng/mL at delivery [34], a level needed in order for cord blood to reach 25(OH) levels of 20 ng/mL [17]. Moreover, approximately 60% of the infants born to these mothers had 25(OH)D levels considered vitamin D insufficient. For neonates of mothers supplementing with 2,000 IU/day, approximately 40% had 25(OH)D levels considered less than sufficient. In the second trial, 160 pregnant minority women in the UK supplementing with 800-1,600 IU vitamin D/day during pregnancy were only able to increase 25(OH)D levels from approximately 8 ng/mL at baseline to 11 ng/mL at delivery.

While several studies have reported similar 25(OH)D concentrations in maternal and cord blood [18-20], one study found maternal concentrations to be lower (by approximately 4.8 ng/mL) than in cord blood [41], while two other studies reported the opposite [18, 28]. If we presume maternal blood 25(OH)D concentrations are 4.8 ng/mL lower than in cord blood, the prevalence of vitamin D deficiency plus insufficiency in our adult population using TC would worsen (61% plus 30% (data not shown) *versus* the current 28% plus 50%). Alternatively, if we presume maternal blood 25(OH)D concentrations are 4.8 ng/mL higher than in cord blood, the prevalence of vitamin D deficiency plus insufficiency in our adult population, despite improving somewhat (11% plus 50%, data not shown), would still warrant great concern. In either scenario, the vitamin D status of both mother and newborn would still be alarmingly low because, as explained below, cord blood reflects a mixture from both mother and fetus.

Use of cord blood

Umbilical cord blood represents a mixture of maternal and fetal blood (presumably 1:1) as the umbilical vein supplies the fetus with nutrient-rich blood from the mother *after* it has crossed the placenta barrier at the same time the two umbilical arteries transport nutrient-depleted blood from the fetus back to the placenta. Therefore, unless blood is exclusively collected from the umbilical vein or arteries [42], 25(OH)D concentrations measured from umbilical cord blood reflect contributions from the maternal blood supply crossing the placental barrier together with those from the neonate.

For our study, only healthy full-term newborn infants born to healthy mothers were included. However, given that maternal vitamin D deficiency has been shown to increase the risk of preeclampsia, possibly preterm delivery during pregnancy [43, 44], poor neonatal vitamin D status [45], and neonatal small-for-gestational age births [46], it is likely the actual prevalence of vitamin D deficiency and insufficiency at delivery is higher in Hawai'i than observed in our study.

The lack of correlation between 25(OH)D concentrations and maternal age and other often quoted determinants of vitamin D status is in agreement with studies investigating other cord blood investigations [20] and was probably due to the relative young population, the narrow age range, and low number of participants.

Ethnic differences

Racial differences have been noted as a factor for varying vitamin D concentrations and the underlying consensus is that dark pigmented individuals synthesize less vitamin D in the skin than their Caucasian counterparts owing to the higher content of melanin [21, 27, 47, 48]. Our findings are in excellent agreement with this consensus. In our study, significant differences in 25(OH)D levels between ethnic groups were observed with the highest levels observed in Caucasians and lowest levels observed in African Americans (Table 2).

Seasonal differences

25(OH)D levels were significantly higher in the summer than winter season among all subjects and also within Caucasians and Asians (Figure 1) but not within Pacific Islanders, which have been due to their relatively dark skin pigmentation and/or lifestyle factors that prevent sun exposure.

Correlation with sun irradiance

Positive correlations were found between solar radiation and 25(OH)D levels among all participants and also within Caucasians and Asians (Figure 1). Solar radiation data used in this study included overall direct plus indirect solar irradiance energies (i.e. GHI). While cutaneous vitamin D synthesis depends solely on UV-B radiation in the range of 280-320 nm [33], GHI data can act as a reliable proxy for UV-B dose. Positive correlations between GHI and UV-B energies have been shown previously [49] and were also observed during the period of this study ($r=0.54$; $p<0.0001$) using data from NREL obtained from the Baseline Measurement System in Colorado and located at 39.7° North, 105.2° West, 1828.8 m AMSL which measured 280-315 nm energies (data available at http://www.nrel.gov/midc/srrl_bms/).

GHI concentrations on Oahu were found to be much higher in the summer than winter season (Figure 1). This summer-high trend is in excellent agreement with: (i) solar UV irradiance measurements from the Hawai'i National Park (19.4° North, 1243 m AMSL) where, in 2000, 700-900 W/m² were reported in the summer and 200-400 W/m² in the winter for 286.5-363.0 nm energies [50]; (ii) the UV index trends on Oahu measured by the National Weather Service with averages of 11-12 in the summer and 4-6 in the winter; and (iii) theoretical daily vitamin D production calculations at latitude 21° estimating approximately 10 hours during the summer and 6 hours in the winter [51].

Analytical issues

The validity of vitamin D assays need to be demonstrated in order to have confidence in produced data and should be examined by participation in reputable quality assurance programs such as DEQAS or NIST. The 25(OH)D immunoassay employed in this study not only participated in both programs, but was also compared to an HPLC assay, the accepted gold standard procedure for 25(OH)D measurement [29]. We acknowledge the limitation of the 25(OH)D immunodiagnostic assay of having only 70% for recovery of 25(OH)D₂. However, this is of little relevance due to the usually negligible levels of 25(OH)D₂ in cord blood [17] and of entire irrelevance when comparing 25(OH)D₂ between groups within this study.

Dilemma of acquiring adequate vitamin D

Over the last several decades, negative publicity surrounding sun exposure coincided with an epidemic of vitamin D deficiency and insufficiency. For this reason and based on results from this study, we recommend that circulating 25(OH)D levels be monitored regularly, particularly during pregnancy and lactation. This should be followed by personalized dosing of sun exposure and/or vitamin D supplements in order to attain circulating 25(OH)D concentrations that will assure optimal health for both mother and newborn during the most critical period of life [12, 52]. The 2011 Endocrine Society's Practice Guidelines suggests that pregnant and lactating women ingest a minimum of 600 IU vitamin D per day, 200 IU/d higher than the amount currently available in prenatal supplements. The society also recognizes that maintenance of 25(OH)D blood levels above the level deemed sufficient (30 ng/ml) may require vitamin D dosing of at least 1,500-2,000 IU/day [38].

Limitations of our study include missing data on confounders such as sun exposure, clothing worn, sunscreen use, dietary and supplement intake (aside from the prenatal intake of daily 400 IU vitamin D), and quantitative measures of skin pigmentation (e.g. by skin reflectometry). In addition, we lacked information regarding pre-pregnancy BMI and tanning booth use, albeit the latter being extremely rare on Oahu. These potential determinants may have played a role in the low prevalence of vitamin D sufficiency observed along with seasonal 25(OH)D changes. It is also possible that ethnic differences may be due to these unaccounted confounders. However, we have no reason to suspect that these factors were distributed differently among the study participants compared to the general population in Hawai'i. While our study population was relatively small (n=100), the ethnic distribution of our participants was almost identical to the ethnic distribution in Hawai'i, according to the most recent 2010 census -Asian 43% vs. 44%, Pacific Islander 25% vs. 26%, Caucasian 19% vs. 25% (available at www.census.gov), which is a considerable strength over previous studies that lack generalizability. Additionally, another strength in our study is our uniquely ethnically diverse population and high proportion (~75%) of non-Caucasian individuals. This diversity not only allowed comparisons among several diverse ethnic groups (with ethnic mixtures often not observed outside of Hawai'i) but also allowed us to observe differences in 25(OH)D concentrations in these groups living at a low-latitude tropical environment favorable for cutaneous vitamin D synthesis all year round.

CONCLUSION

In conclusion, we found a high prevalence of vitamin D deficiency and insufficiency in cord blood from 100 participants living in the tropical climate of Hawai'i. Our results parallel those from others with milk [40] in that over two-thirds of our study population had vitamin D concentrations indicative of deficiency or insufficiency despite daily recommendations to take 400 IU of vitamin D daily. This is of great concern, particularly for the fetus and the young child who requires this important vitamin for intestinal calcium absorption, the development of vital functions, and the protection against serious diseases. Based on results from our study, we recommend that circulating 25(OH)D concentrations be monitored regularly, particularly during pregnancy and lactation, so that personalized dosing of sun exposure and/or vitamin D supplements can be determined and ultimately lead to circulating 25(OH)D concentrations that are optimal for health.

Acknowledgments

This project was supported by grants from the National Cancer Institute P30 CA71789, the National Center for Research Resources (NCRR) S10 RR020890 and from the RMATRIX award (award No. U54RR026136) NCRR, all part of the National Institutes of Health (NIH). The content in this paper is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. The authors would like to thank Thomas Giambelluca, Ryan Longman and Pat Caldwell (University of Hawaii) for their assistance in retrieving the solar radiation data and Gary Lensmeyer (University of Wisconsin) for measuring 25(OH)D levels in our quality control plasma sample by HPLC. We acknowledge NREL for the generation of the solar radiation data and making them available for this study. We thank Stephen Wilcox (NREL) for the retrieval of solar and UV data from the Baseline Measurement System in Colorado.

ABBREVIATIONS

25(OH)D	25 hydroxyvitamin D
AMSL	above mean sea level
DEQAS	Vitamin D External Quality Assessment Scheme
DRI	dietary reference intake

GHI	global horizontal irradiance
NIST	National Institute of Standards and Technologies
NREL	National Renewable Energy Laboratory
SPF	sun protection factor
TC	traditional cut-offs for circulating 25(OH)D
UVB	ultraviolet-B

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Figure 1. 25(OH)D levels and solar ground radiation over time in Caucasians (n=19), Asians (n=43) and Pacific Islanders (n=25). Curves were generated by a sine wave model.

Table 1

Demographic characteristics of participants

Characteristic	Mean (SD)
Mother's age, y	28.3 (6.2)
Baby's birth weight, g	3411.6 (464.3)
Baby's head circumference, cm	34.7 (1.5)
Baby's length, cm	51.5 (2.1)
Gravida	3.0 (2.0)
Parity	1.0 (1.0)
APGAR at 1 min, score	8.0 (1.0)
APGAR at 5 min, score	9.0 (0)
<u>Mother's Ethnicity, n (% within subgroup)</u>	<u>Single ethnicity</u>
Total	50 (100%)
Caucasian (white, non-Hispanic)	6 (12%)
Asian (Japanese, Chinese, Korean, Filipino, other Asians)	26 (52%)
Pacific Islander (Hawaiian, Samoan, Tongan, Micronesian, Chamorro, Cook Islander)	15 (30%)
Hispanic (includes Spanish)	3 (6%)
African American (Black, non-Hispanic)	0 (0%)
Other (Indian, American Indian unknown)	0 (0%)
2 or more ethnicities	50 (100%)
2 ethnicities	22 (44%)
3 ethnicities	17 (34%)
4 ethnicities	8 (16%)
5 ethnicities	3 (6%)
6 ethnicities	0 (0%)
<u>Education, n</u>	
< High school	9
High school	91
<u>Income, n</u>	
\$50,000	46
> \$50,000	36
not stated	18
<u>Years lived in Hawai'i, n</u>	
< 1 year	3
2-5 years	19
6-10 years	16
11+ years	62
<u>Baby gender, n</u>	
Male	62
Female	38

n= number of subjects; also % since total number is 100

Table 225(OH)D levels and prevalence of deficiency, insufficiency, and sufficiency^{*}

	n	Mean (SD) (ng/mL)	Range (ng/mL)	Deficient (<20 ng/mL)	Insufficient (20-30 ng/mL)	Sufficient (>30 ng/mL)
All year all subjects	100	24.5 (9.0)	9.1-68.3	28%	50%	22%
Summer ^a all subjects ^b	59	27.1 (9.8)	11.2-68.3	19%	49%	32%
Winter ^a all subjects ^b	41	20.8 (6.3)	9.1-41.0	41%	51%	7%
All year^c						
Caucasian ^{d,e}	19	30.5 (11.8)	12.1-68.3	16%	32%	53%
Asian ^{d,f}	43	25.1 (7.9)	9.1-44.4	21%	60%	19%
Pacific Islander ^{e,f}	25	20.0 (7.5)	9.2-40.5	48%	40%	12%
Hispanic	3	21.5 (2.0)	19.6-23.5	33%	67%	0%
African American	2	19.6 (3.0)	17.5-21.7	50%	50%	0%
other	8	23.7 (6.4)	13.7-34.2	25%	63%	13%
Summer^a						
Caucasian	14	31.5 (12.8)	12.1-68.3	14%	29%	57%
Asian ^g	25	28.6 (7.4)	17.5-44.4	4%	68%	28%
Pacific Islander	15	21.2 (8.7)	11.2-40.5	47%	33%	20%
Hispanic	1			0%	100%	0%
African American	1			0%	100%	0%
other	3	27.0 (7.7)	18.9-34.2	33%	33%	33%
Winter^a						
Caucasian	5	27.7 (8.8)	18.9-41.0	20%	40%	40%
Asian ^g	18	20.2 (5.8)	9.1-31.2	44%	50%	6%
Pacific Islander	10	18.1 (5.3)	9.2-25.0	50%	50%	0%
Hispanic	2	21.5 (2.8)	19.6-23.5	50%	50%	0%
African American	1			100%	0%	0%
other	5	21.8 (5.5)	13.7-28.2	20%	80%	0%

To convert ng/mL to nmol/L, multiply ng/mL by 2.5

If no superscript is shown then the difference in 25(OH)D concentrations is not significant

Sum of percentage can deviate from 100 due to rounding

^{*} based on traditionally recommended cut-offs used in the literature (see Methods)^a Summer= May 1 to November 17, Winter= November 18 to April 30 (removing data obtained from 1 cord blood sample collected in the period of Nov 1-17 (from a Pacific Islander) led to trivial and entirely insignificant changes of all findings)^b p=0.001 for difference in 25(OH)D levels by season^c p=0.008 for difference between all ethnicities^d p=0.05 for difference between Caucasians and Asians

^ep=0.0001 for difference between Caucasians and Pacific Islanders

^fp=0.01 for difference between Pacific Islanders and Asians

^gp=0.0003 for difference between Summer and Winter in Asians