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Serum Vitamin D, PTH, and Calcium Levels in Patients with and without Early Childhood Caries

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

by

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Abstract

SERUM VITAMIN D, PTH, AND CALCIUM LEVELS IN PATIENTS WITH AND WITHOUT EARLY CHILDHOOD CARIES.

By Susan Meinerz, DDS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

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Purpose: The purpose was to determine differences in serum vitamin D, parathyroid hormone (PTH), and calcium levels between patients with early childhood caries (ECC) and patients without dental decay.
Materials and Methods: Serum vitamin D, PTH, and calcium levels were obtained from 30 children without dental decay who acted as controls and 60 children with ECC. A questionnaire was filled out by the parent/guardian of each participant consisting of questions regarding medical and dental history, exposure to sources of vitamin D and demographic information.
Results: The difference in the vitamin D levels of the participants was most strongly associated with race. African American participants demonstrated lower levels of vitamin D than non-African Americans. After adjusting for race- related differences there was no significant difference in the Vitamin D levels in the ECC cases and the healthy controls.
Conclusions: The results of this study suggest that vitamin D levels, at least among non-African Americans, are unrelated to caries development. Future research in this area must control for important confounding factors such as skin pigmentation, season of measurement of serum vitamin D, sun exposure, fluoride exposure, water fluoridation status and tooth brushing in order to allow for vitamin D levels to be better tested against caries experience.

Introduction

Vitamin D has long been considered an important factor that affects overall health and well-being. It has also been shown to affect the oral health and caries risk of children.[1] At optimal levels, vitamin D has been shown to positively impact the immune system, gastrointestinal tract, genito urinary tract, the skin, and the oral health of individuals.[2] Yet, vitamin D deficiency and insufficiency continues to be a global issue.[2] Two of the more well-known outcomes involving vitamin D deficiency are rickets and osteoporosis. When a vitamin D deficiency occurs there is a reduction of absorbed dietary calcium and phosphorus, which reduces serum calcium levels. The calcium sensor in the parathyroid gland recognizes the drop in serum calcium and combats this by increasing the synthesis and secretion of parathyroid hormone (PTH).[3] The expression of PTH results in an increase in reabsorption of calcium and, similar to 1,25-dihydroxyvitamin D, increases the production of osteoclasts, which will act on the skeleton to mobilize calcium stores.[3] All of this then results in poor overall mineralization of the bones.[3] Depending on the duration of rickets and the age of the child, poor mineralization of the bones may also result in long term skeletal deformities.[3] Other manifestations of rickets induced severe hypocalcemia induced seizures, laryngospasm, hypocalcemic myocardiopathy, and even death.[3] Although rickets is typically defined as severe chronic vitamin D deficiency (25-hydroxyvitamin D < 15 ng/ml), there are still a large number of infants, children, and adolescents who are insufficient but do not present with any of the skeletal or calcium metabolism abnormalities.[3]

This important secosteroid hormone, vitamin D, is produced after ingestion of certain foods and supplements and in the skin after exposure to UVB light.[2] After sun exposure, vitamin D is produced from 7-dehydrocholesterol. It is then hydroxylated in the liver and again in the kidney to form 1,25 dihydroxyvitamin D.[2] This active hormone then impacts the amount of calcium and parathyroid hormone; a decrease in vitamin D will result in a decrease in serum calcium and an increase in PTH.[3]

1,25 dihydroxyvitamin D has also been implicated in the proper formation of the developing tooth bud.[4] In 1927, Lady Mellanby first demonstrated the effects of a vitamin D deficiency during the time of the developing teeth. She reported that teeth that developed in a vitamin D deficient state exhibited hypoplastic enamel and surface abnormalities.[5] Similarly, J.T. Irving, in his research with albino rats, demonstrated that the addition of vitamin D improved calcification in dentin. He showed that rats with a vitamin D deficiency had a wide predentin region bordering the hypocalcified dentin. After administration of vitamin D, the new predentin was of normal width as it bordered the new dentin.[6] In 1928, Lady Mellanby demonstrated the effect of vitamin D on preventing the initiation of new carious foci, limiting the spread of existing caries, and arresting the carious process in children.[1] She had established in a previous study that vitamin D was "undoubtedly responsible for promoting normal calcification, whether of the developing teeth or of the secondary dentine in erupted teeth."[1] In this study, she divided children with caries into groups with different diets, one of which was low in vitamin D and one of which was high in vitamin D. In the group of children who had the addition of vitamin D, there was a decrease in the initiation of new caries and in the spread of old caries and the infective process of caries was arrested in many instances.[1] In 1938, Dr. Bion East described an inverse relationship between caries rate and mean annual number of hours of sunshine.[7]

It is widely accepted that the development of dental caries is multifactorial. One of the central determinants for caries risk is diet; specifically, the length of time and frequency that the pH in the mouth is below “critical pH” at which demineralization of teeth begins.[8] “Critical pH” is defined as the pH of the oral plaque below 5.5-5.7, which leads to enamel dissolution.[9] Various therapies have been implicated in reducing caries risk, most notably, water fluoridation.[8] However, vitamin D supplementation has also been associated with a 47 percent reduction in caries risk.[10] Very little research has been done on vitamin D in comparison to the research on fluoride. Therefore, it is paramount that further research regarding the implications of vitamin D on oral health and caries risk is pursued.

A recent study has examined the serum vitamin D levels in patients with early childhood caries compared to patients of the same age with no caries.[11] The results demonstrated lower vitamin D levels in higher risk patients.[11] The specific aims of this study were:

- To determine if there is a difference between children with tooth decay and a control group of children according to the following serum values: vitamin D, parathyroid hormone (PTH), and calcium.
- To determine if there is a correlation between the following: serum vitamin D, PTH, calcium, and the decayed missing, filled teeth score (dmft).

Materials and Methods

This is a case control study investigating the relationship between vitamin D, PTH, and calcium levels in children with and without caries.

Inclusion and Exclusion Criteria

To be included or excluded in this study, the following conditioned needed to be met:

- Children ages 71 months or younger were recruited. Children were asked to participate if they were a classification of an American Society of Anesthesiologists (ASA) 1 patient, defined as healthy, or an ASA 2 patient, defined as a patient with mild systemic disease and no functional limitation; for instance, a patient with asthma or ADHD.[12]
- Children were excluded from the study if they were older than 71 months, were classified as an ASA 3 or greater, or had a complex metabolic or medical disorder.

Subject Recruitment

Two groups of patients were recruited to this study, cases and control. Data was collected at Virginia Commonwealth University Health System's Main Hospital and Ambulatory Care Center surgical units from January 1, 2015 to January 1, 2016.

Recruitment of Case Patients

Case patients for this study came from the patients at VCU Pediatric Dental Clinic that had the diagnosis of early childhood caries and were an ASA 1 or 2. Early childhood caries is defined as having one or more decayed, missing, or filled tooth surface in a child six years or younger. Of these patients, those that were scheduled for full mouth dental rehabilitation were asked the day of the dental surgery to participate in the study. This study was approved by the Institutional Review Board for Human Subjects at Virginia Commonwealth University. A total of 60 patients with early childhood caries and undergoing full mouth dental rehabilitation were recruited for this study.

Recruitment of Caries-Free Control Patients

The control patients, those without caries, were recruited from children 6 years and younger that were scheduled to undergo general anesthesia for an Ear, Nose, and Throat surgical procedure within the same surgical units at VCU. A dental exam was completed to ensure that patient had no visually detectable tooth decay. A total of 30 control patients with no decay and undergoing general anesthesia were recruited for this study.

Questionnaire

A questionnaire was filled out by the caregiver of each participant consisting of questions regarding medical and dental history, exposure to sources of vitamin D and demographic information. (Appendix) The questionnaire identified other risk for the development of tooth decay apart from the hypothesized serum vitamin D levels.

Blood Samples

After subjects' parents consented to participate, the operating room staff collected blood sample for both ECC and tooth decay-free participants. The two vials needed for determination of vitamin D, PTH, and calcium levels required 7cc of blood. As soon as the blood sample was collected and transferred to the vials, it was sent to the lab for testing. The blood samples were analyzed by VCU Hospital Health Systems Clinical Pathology Laboratory. The samples were analyzed for vitamin D, parathyroid hormone and calcium levels using liquid chromatography and tandem mass spectroscopy. Table 1 describes the accepted levels of deficient, insufficient, and sufficient serum vitamin D and gives the accepted ranges for low, normal, and high serum PTH and Calcium level, respectively.

Analytical Plan

The case (ECC) and control (non-caries) participants' demographic characteristics, dietary habits, and dental history were first compared using a chi-square or t-test, as appropriate. Significant differences in demographics were identified as potential confounding variables after which the case- and control-groups were compared on the outcome variables--serum Vitamin D, PTH, and Calcium--using a t-test and a chi-square analysis. The correlations between these outcomes and DMFT were also described. These preliminary, unadjusted analyses were used to describe the raw differences between the groups. The final adjusted analysis compared the groups using a multi-way ANOVA. The ANOVA model included all potential confounding factors in order to compare the vitamin D levels of the case- and control-groups after adjusting for different demographic characteristics. The differences between the groups were described using descriptive statistics and 95% confidence intervals.

Post hoc analyses were then used to describe the magnitude of differences between the groups. The post hoc analyses included an interaction test to determine if the differences between the case- and control-groups depended upon race and a two-way ANOVA were the case- and control-groups were compared in a subgroup analysis using race. All analyses were performed using SAS software (JMP Pro version 11, SAS Institute Inc., Cary NC).

Results

Subject Demographics

A total of 90 children were enrolled in the study. Of the 90 children, 60 were children with early childhood caries (cases) and 30 were children that were decay-free as determined by visual exam (controls). Table 2 shows the demographic characteristics of children recruited for this study. Note that not every caregiver responded to every question on the questionnaire and so the percentages in Table 2, and in all the other tables, are based on those who responded. The total responses in each group are shown in the shaded portion of the tables. The control children were significantly younger (mean 38 months) than the children with early childhood caries (ECC) (mean 49 months) (P=0.002). Ethnicity also varied widely amongst the children, as demonstrated in Table 2.

The caregiver responses regarding their child's sun exposure is presented in Table 3**Error! Reference source not found.**. Eighty-three percent of control caregivers claimed that their child had daily sun exposure; whereas 85% of the ECC children had daily sun exposure, and a few answered that their child had monthly (2%) or weekly sun exposure (8%). The child's sun exposure did not significantly differ between children with ECC and the control children.

Caregivers Demographics

The demographics of the caregivers are presented in Table 2. Most of the caregivers in the control group finished educational requirements beyond high school, with 43% earning a college degree and 25% completing graduate school. It is interesting that all control caregivers earned an education beyond elementary and middle school, whereas more than half of the caregivers in the ECC group only finished high school (56%) and a small fraction only went to elementary and middle school (5%). A decreased proportion of the ECC caregivers received a college or graduate school degree (27% and 12% respectively) when compared to the control group. In regards to employment status, approximately 82% of the control caregivers and 78% of the ECC caregivers were employed. Roughly 72% of the caregivers in the control group had a household income of $50,000 or more. A greater ratio of ECC caregivers had a total household income less than 50,000 (82%), while only 17% earned more than $50,000 per year. Although there was no significant difference between the two groups on employment (P>0.6), there was a significant difference in income level (P<0.001) and education level (P=0.026) with controls reporting high levels of education and income.

The control group had reported higher education (68% college and beyond) than the ECC group where 16% were high school or below (P = 0.026). The ECC group also had considerably less household income, with 65% indicating an income below $30,000 as compared to 72% of the Controls with income of at least $50,000 (P < .001).

Child's Diet Assessment

According to Table 4, the controls responded yes to using a sippy cup more often than the ECC children (52% of controls and 37% of ECC children). A considerable amount of controls (87%) and 73% of ECC patients did not bring a bottle to bed. Twelve percent of children with caries brought a bottle to bed daily, while 7% brought one to bed weekly. Of the decay-free children, 73% did not eat snacks before bedtime; contrastingly, 37% of the ECC group reported no snacking before bedtime. Eighty percent of the ECC group claimed to snack one or two times a day and another 18% had snacks three or more times a day. Of the control group, no children snacked three or more times a day and only 57% reported snacking one or two times a day. Further, two out of 30 control samples never had snacks throughout the day. Most of the children in both groups drank some form of a sugary drink one or two times a day, while 33% of children with caries and 21% of healthy children claiming to drink sugary drinks three or more times a day. Just over 80% of controls and 56% of ECC patients had tap water daily, whereas 10% of controls and 27% of the ECC group never drank tap water.

With regard to dietary habits, significant differences between ECC and controls were found in the following: snacks before bedtime (P=0.004), snacks throughout the day (P=0.002), sugary drinks (P=0.001), and tap water (P=0.037). As described in Table 4, the ECC children snacked before bedtime and throughout the day, had more sugary drinks, and drank tap water less often than the controls.

Child's Dental History

Table 5**Error! Reference source not found.** describes the dental history of the child. A greater number of control children lived in an area with fluoridated water (67%) compared to those who belonged to the ECC group (56%). The vast majority of both groups responded that their child did not take any additional fluoride supplements (90% of the control versus 84% of the ECC group). When asked if their child received fluoride treatment from a health professional, the majority of control patients (76%) replied "no." Similar percentages of ECC caregivers said that their child either received fluoride or did not receive fluoride from a health professional (48% said "yes" and 47% said "no."). The brushing frequency for the children of each group was also assessed. Around eighty-six percent of ECC caregivers reported that their child brushed their teeth at least daily, while only 90% of control children performed at least once daily brushing. Each group had several caregivers report that their children never brushed their teeth

(10% in the control and 7% in the ECC group). Many children in both groups claimed they never flossed (61% in the control and 46% in the ECC group).

Approximately 64% of ECC children visited their dentist twice a year, while only 45% of healthy children did. A substantial amount of healthy children had never been to the dentist (41%), whereas all the ECC children had been to the dentist whether yearly (31.4%) or only when in pain (8.6%). Remarkably, 97% the caregivers of the control children answered that they had no difficulties getting to the dentist, while 24% of the ECC children did. Around 80% of the ECC children and 47% of the control patients had Medicaid.

With regard to dental history, significant differences between ECC and controls were found in the following: dental visits ($P<0.001$) and having Medicaid ($P=0.002$). As described in Table 5, the ECC children saw the dentist more often and more often were enrolled in Medicaid than the controls.

Caregiver Dental Status

The bottom portion of Table 5 illustrates the dental history of the caregivers. A large proportion of the caregivers reported a history of dental caries (62% of the control, 69% of the ECC parents), and all, except one ECC caregiver, reported having their natural teeth. All caregivers in the control group stated they brushed their teeth daily, while 98% of the ECC caregivers completed daily brushing. Specifically, more control caregivers stated they brushed twice a day (69%) compared to ECC caregivers (41%). Fifty-two percent of ECC and 28% of control caregivers responded that they brushed once a day; 5% of ECC caregivers and 3% of control caregivers responded that they brushed three times a day.

With regard to caregiver dental status, significant differences between ECC and controls were found in the following: difficulty getting to the dentist (P=0.007). As described in Table 5, the ECC children had more difficulty getting to the dentist than the controls.

Serum Findings

This section compares the serum findings vitamin D, PTH, and calcium according to ECC (case) or decay-free (control) status. As displayed in Table 6 and Figure 2, the mean vitamin D level of the controls was shown to be vitamin D sufficient (31.47 ng/ml, SD±8.07) whereas the mean vitamin D level of the ECC group was vitamin D insufficient (26.80 ng/ml, SD±8.85). The results of our analysis indicate that the ECC has 4.67ng/ml less vitamin D than did the controls (95% confidence interval = 0.85 to 8.49ng/ml, P=0.0171). When these values were categorized into nominal values (vitamin D deficiency, insufficiency, or sufficiency), the chi-square test suggested a statistical significance (p=0.0691) in the number of vitamin D deficient, insufficient, and sufficient children in each group. This contingency table is located on the lower half of Table 6 and indicates that a greater number of ECC children may be vitamin D deficient (18% of the ECC compared to 7% of the controls) or vitamin D insufficient (50% of the ECC compared to 40% of the controls), and that nominally more control children exhibited vitamin D sufficient levels (53% of the controls compared to 32% of the ECC group), although the data does not reach statistical significance.

The PTH levels are shown in Table 7. Our serum measurements did not show a statistically significant difference in PTH, with a mean PTH level of 63.40 pg/ml (SD±37.78) for the control group and 64.27 pg/ml (SD±36.77) for the ECC group (p=0.9172). Although not significant, the PTH levels in the ECC group were generally higher when contrasted with the control group. Moreover, the contingency table did not find a statistical significance when the serum PTH values were divided into low, normal, and high values (p=0.3981). Most of the patients from both groups had a normal PTH value, with only one child in the ECC group with a low PTH level.

Similarly, the serum measurements of calcium did not demonstrate a statistically significant difference in the calcium levels between the two groups (Table 8, p=0.5325). In particular, the mean calcium level for the control group was 9.56 mg/dL (SD±0.40), while the mean calcium level for the ECC group was 9.61 mg/dL (SD±0.28). Results of the contingency table did not find a statistical significance when the serum calcium values were divided into low, normal, and high values (p=0.4819). The majority of children in both groups had normal calcium levels, with two children in each group presenting with low calcium values.

Multivariable Analysis

The initial, unadjusted analysis indicated that vitamin D levels were lower in ECC children compared to the control decay-free children. However, the controls and ECC groups differed on other characteristics, specifically the ECC children were more often African-American, their caregivers were less educated, had lower income, and they were older. The multiple regression analysis was performed with all of these factors included in the analysis. Specifically, the following factors were included in the analysis comparing decay-free children to ECC children: African-American (yes or no), Education level (≤high school, high school/GED, college, and beyond college), household income (in whole $ amounts, using the midpoint of the ranges indicated on the questionnaire), and age (in months).

The multiple-regression model was significant (P = .006, R-square = 22%) and indicated that, after adjusting for significant race-related differences (P = 0.0007), there was no significant difference in sufficient vitamin D levels between those children with ECC and the children in the control group (P = 0.1349,Table 9). No other factors were related to vitamin D levels in the multiple regression analysis. The bottom portion of Table 9 shows the estimated mean levels of vitamin D in the control and ECC groups and in non-African-American and African-American participants. As may be seen, African-American study participants had significantly lower vitamin D levels (difference = –7.8ng/ml, 96% CI = –12.2 to –3.4). After adjusting for this difference, the ECC participants had a nominally lower vitamin D levels (–3.842, 95% CI = –8.9 to +1.22), which was not significantly different (P = 0.1349).

Table 10 provides a stratified analysis according to race. A two-way ANOVA was used to analyze the ECC vs Control effect within each race group. Note that within the non-African-American groups there is not even a one ng/ml difference and that this difference is not significant (P > 0.9, **Error! Reference source not found.**). The lack of a difference is evident even though the sample size of non-African-Americans is larger than the sample size of African-Americans. And within the African-Americans, despite the smaller sample size, there is a significant difference (P = 0.0309). Within the subgroup of African-Americans the ECC participants had 6.5ng/ml less vitamin D. These two sub-group analyses were meant to illustrate the size of the differences although the two-way ANOVA indicates that the lack of a control vs ECC difference does not differ between the two race groups (interaction p-value = 0.1027), and equivalently that the race group difference does not vary between controls and ECC.

Discussion

The disease process of tooth decay is a complex and multifactorial process. There are individual factors such as diet or fermentable carbohydrates, microflora or a biofilm, and a susceptible tooth/host. In addition to caregiver/family and societal level factors such as, culture, community oral health environmental, physical environment, such as water fluoridation, socioeconomic status, and health status of parents.[13] Individual biochemical factors such as serum vitamin D levels have been implicated as one of those modifiers of the development of caries. It has been shown to impact dentin formation, thus strengthening the tooth against cariogenic bacteria.[6] A vitamin D rich diet has even been connected with arresting existing carious lesions.[1] This study attempted to corroborate previous studies that found that serum vitamin D levels in children with early childhood caries were lower than children without early childhood caries.[11]

Sufficient levels of serum vitamin D are defined as greater than 30 ng/ml, insufficient levels are between 20 and 30 ng/ml and anything under 20 ng/ml is deficient. Serum samples from each of 90 participants were obtained along with a questionnaire regarding dental history, medical history and demographic information Table 2 details significant differences between the ECC and control groups on the following demographic questions: race, education level, household income, and age. In Table 4, significant differences between the ECC and control groups are found in children's diet assessment for example children with ECC had increased snacking habits and sugary drinks. Differences were also noted in Table 5 between the two groups of children with regard to dental history such as fluoride treatment, dental visits and difficulty getting to a dentist, Medicaid enrollment, and parent insurance. From these differences, a multiple regression was completed using the following variables: caries, race, education level, income, and age. This study's findings do not corroborate similar studies completed by Schroth, et al. in which children with early childhood caries were found to have lower vitamin D levels than children without caries.[11] In this study, race was a significant modifier of the relationship between vitamin D levels and presence of ECC. After adjusting for race, education level, income, and age, there was not a significant difference in the vitamin D levels between the group of children with ECC compared to the group of decay-free children.

A difference in serum vitamin D levels as explained by race, or more specifically, a difference in skin pigmentation has been shown in several studies. In 2015, Xiang et al completed a systematic review of the influence of skin pigmentation on changes in concentrations of vitamin D and found convincing evidence to support that having pigmented skin reduces the effectiveness of UV-induced production of vitamin D.[14] The review goes on to describe the "perfect" study would measure skin pigmentation objectively using skin reflectance as the method of measurement and not relying on reported ethnicity.[14] As our study did not account for skin pigmentation apart from reported race, the significant or insignificant differences between specific races and their vitamin D level does not meet the "perfect" study standard as proposed by Xiang. A study by Libon, also supported the difference in vitamin D production between fair skinned and black skinned volunteers.[15] With regard to vitamin D and dental caries research, it would behoove future studies to control for skin pigmentation in order to better assess this relationship.

In our final analyses, seen in Table 10, the participants that self-identified as any race besides African American were divided into an ECC group and the control group. Similarly, the participants that self-identified as African American were divided into an ECC group and the control group. In a two-way AVOVA, no difference was shown in the vitamin D levels of not African American subgroup between the ECC and control group. Interestingly, there was a difference in the serum vitamin D levels of the ECC vs control group among the African American subgroup. The lack of significant difference in the non-African American subgroup is similar to findings from the National Health and Nutrition Examination Survey (NHANES). Herzog et al did not find an association between vitamin D and caries after adjusting for age, sex, race, ethnicity, ratio of income to poverty threshold, and sugar consumption.[16] However, the significance of vitamin D level difference among the African American subgroup complements Schroth's findings of serum vitamin D affecting caries level.[17] These findings also bring up an interesting biochemical question regarding the possibility of differences in the effects of serum vitamin D among different race groups.

Ultimately, an interaction test of the difference of the caries and controls of the African American subgroup and the difference of the caries and controls of the non-African American subgroup yielded no significant difference. Therefore, our data does not support that the difference between the caries groups depends on or differs by race. In other words, there is no evidence that the caries difference is different in the two race groups. Future studies could investigate the impact of race or skin pigmentation on vitamin D levels. Further, research regarding the impact of vitamin D levels on caries experience should attempt to control for race or skin pigmentation.

Conclusions

In summary, our study revealed the following:

- The difference in the vitamin D levels of the participants were most strongly associated with race
- African American participants demonstrated lower levels of vitamin D than non-African Americans.
- After adjusting for race- related differences there was no significant difference in the vitamin D levels in the ECC cases and the decay-free controls.
- In a post-hoc analysis we observed that African American participants with ECC had lower vitamin D levels than African American participants in the control group.
- In a post-hoc analysis we observed that non-African American participants did not have a difference in serum vitamin D between those with ECC and those in the control group.

The results of this study suggest that vitamin D levels, at least among non-African Americans, are unrelated to caries development. Future research in this area must control for important confounding factors such as skin pigmentation, season of measurement of serum vitamin D, sun exposure, fluoride exposure, water fluoridation status and tooth brushing in order to allow for vitamin D levels to be better tested against caries experience.

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Tables

Table 1. Vitamin D, PTH, and Calcium Levels

Vitamin D Status	Vitamin D Level (ng/ml)
Sufficient	> 30
Insufficient	20-30
Deficient	< 20
PTH Status	PTH Level (pg/ml)
Low	< 12.0
Normal	12.0-65.0
High	> 65.0
Calcium Status	Calcium Level (mg/dL)
Low	< 9.1
Normal	9.1-10.9
High	> 10.9

Table 2. Demographic Characteristics of Participants

Characteristic	Control N	Control (%)	ECC N	ECC (%)	P-value **
Race/ethnicity*	30		60		
African American	9	(30)	35	(58)	0.014
Asian	2	(7)	3	(5)	1.000
Caucasian	19	(63)	24	(40)	0.044
American Indian/Alaskan Native	0	(0)	2	(3)	0.546
Native Hawaiian or Pacific Islander	0	(0)	1	(2)	1.000
Hispanic	3	(10)	3	(5)	0.406
Education level	28		59		0.026
elementary and middle school	0	(0)	3	(5)	
high school, GED	9	(32)	33	(56)	
college	12	(43)	16	(27)	
graduate school beyond college	7	(25)	7	(12)	
Parent employed	28		54		0.612
yes	23	(82)	42	(78)	
no	5	(18)	12	(22)	
Household Income level	25		52		<.001
less than $30,000	6	(24)	34	(65)	
$30,000-49,999	1	(4)	9	(17)	
$50,000 or more	18	(72)	9	(17)	
Age	30		60		0.002
Mean (months)	38.13		48.69		
SD	17.04		13.31		

*Since race and ethnicity were indicated by "check all that apply", the percentages will not add to 100.

** Groups were compared using chi-square, or in the case of age using a t-test.

Table 3. Sun Exposure of the Participants

	Control		ECC	
Characteristic	N	(%)	N	(%)
Sun Exposure	23		48	
daily	2	(9)	14	(29)
monthly			1	(2)
weekly			4	(8)
more than two hours a day	5	(22)	9	(19)
one to two hours a day	5	(22)	8	(17)
at least one hour a day	7	(30)	10	(21)
none	4	(17)	2	(4)

Table 4. Dietary Habits of the Participants

	Control		ECC		
Behavior	N	(%)	N	(%)	P-value*
Bottle/ sippy cup	29		59		0.199
yes	15	(52)	22	(37)	
no	14	(48)	37	(63)	
Bed w/bottle	30		59		0.100
never	26	(87)	43	(73)	
monthly	0	(0)	1	(2)	
weekly	1	(3)	4	(7)	
daily	0	(0)	7	(12)	
one to two times a day	3	(10)	4	(7)	
Snacks before bedtime	30		57		0.004
never	2	(7)	4	(7)	
monthly	0	(0)	1	(2)	
weekly	0	(0)	9	(16)	
daily	1	(3)	9	(16)	
yes	5	(17)	13	(23)	
no	22	(73)	21	(37)	
Snacks throughout the day	30		51		0.002
three or more times a day	0	(0)	9	(18)	
one or two times a day	17	(57)	41	(80)	
weekly	10	(33)	10	(20)	
monthly	1	(3)	0	(0)	
never	2	(7)	0	(0)	
sugary drinks	24		45		0.001
three or more times a day	5	(21)	15	(33)	
one or two times a day	11	(46)	34	(76)	
weekly	4	(17)	10	(22)	
monthly	3	(13)	1	(2)	
never	6	(25)	0	(0)	
Tap water	29		59		0.037
daily	24	(83)	33	(56)	
weekly	2	(7)	10	(17)	
never	3	(10)	16	(27)	

*Groups were compared using chi-square.

Table 5. Dental History of the Participants

Characteristic	Control N	Control (%)	ECC N	ECC (%)	P-value*
Child's teeth or tooth care					
Fluoride in water	27		43		0.365
yes	18	(67)	24	(56)	
no	9	(33)	19	(44)	
Fluoride supplement	29		57		0.482
yes	3	(10)	9	(16)	
no	26	(90)	48	(84)	
Fluoride treatment	29		52		0.031
yes	7	(24)	25	(48)	
no	22	(76)	27	(47)	
Brushing frequency	30		58		0.114
weekly	0	(0)	4	(7)	
never	3	(10)	4	(7)	
once a day	11	(37)	32	(55)	
twice a day	15	(50)	17	(29)	
three times a day	1	(3)	1	(2)	
flossing frequency	28		59		0.140
daily	5	(18)	16	(27)	
weekly	6	(21)	11	(19)	
monthly	0	(0)	5	(8)	
never	17	(61)	27	(46)	
Parent and child's dental care					
Dental visits	29		58		<.001
never	12	(41)	0	(0)	
yearly	4	(14)	18	(31)	
only when in pain	0	(0)	3	(5)	
twice a year	13	(45)	37	(64)	
Difficulty getting to dentist	30		59		0.007
yes	1	(3)	14	(24)	
no	29	(97)	45	(76)	
Parent caries	29		58		0.522
yes	18	(62)	40	(69)	
no	11	(38)	18	(31)	
Parent have natural teeth	28		59		0.377
yes	28	(100)	58	(98)	
no	0	(0)	1	(2)	
Parent brushing	29		58		0.120
once a day	8	(28)	30	(52)	

	Control		ECC		
Characteristic	N	(%)	N	(%)	P-value*
twice a day	20	(69)	24	(41)	
three times a day	1	(3)	3	(5)	
never	0	(0)	1	(2)	
Medicaid	30		56		0.002
yes	14	(47)	45	(80)	
no	16	(53)	11	(20)	
Parent insurance	29		59		0.011
yes	21	(72)	24	(41)	
no	8	(28)	35	(59)	
Public assistance	30		58		0.353
yes	7	(23)	19	(33)	
no	23	(77)	39	(67)	

*Groups were compared using chi-square.

Table 6. **Serum Vitamin D Levels**

Group	n	Mean	SD	SE	P-value
Control	30	31.47	8.07	1.47	
ECC	60	26.80	8.85	1.14	
Difference		-4.67		1.92	0.0171

	n				
	D	I	S		
Control	2	12	16		
ECC	11	30	19		0.0866

Table 7. Serum PTH Levels

Group	n	Mean	SD	SE	P-value
Control	30	63.40	37.78	6.90	
ECC	60	64.27	36.77	4.75	
Difference		0.87		8.30	0.9172

	n				
	L	N	H		
Control	0	22	8		
ECC	1	37	22		0.3981

Table 8. Serum Calcium Levels

Group	n	Mean	SD	SE	P-value
Control	30	9.56	0.40	0.07	
ECC	60	9.61	0.28	0.04	
Difference		0.05		0.07	0.5325
	n				
	L	N	H		
Control	2	28			
ECC	2	58			0.4819

Table 9. Multiple Regression Results

Source	df	MS	F	P-value
Group	1	151.87	2.29	0.135
Race=AA	1	834.06	12.56	0.001
Education level	2	35.32	0.53	0.590
Income ($)	1	12.23	0.18	0.669
Age (months)	1	39.25	0.59	0.445
Whole model	6	221.06	3.33	0.006

Group	LS Mean	SE	95% CI		P-value
Control	30.564	2.038	26.50	34.63	
ECC	26.724	1.297	24.14	29.31	
Difference	-3.841	2.539	-8.90	1.22	0.1349
African-American					
No	32.548	1.568	29.42	35.67	
Yes	24.741	1.606	21.54	27.94	
Difference	-7.807	2.203	-12.20	-3.41	0.0007

Table 10. **Mean Serum Vitamin D Levels in Four Groups**

Group	N	Mean	SD	SE	P-value
	Not African-American				
Control	21	32.23	8.658	1.889	
ECC	25	31.96	8.497	1.699	
Difference		-0.27		2.340	0.9089
	African-American				
Control	9	29.69	6.602	2.201	
ECC	35	23.20	7.266	1.228	
Difference		-6.49		2.295	0.0309
interaction p-value = 0.1027					

* P-values from two-way ANOVA, nesting Group within AA.

Figures

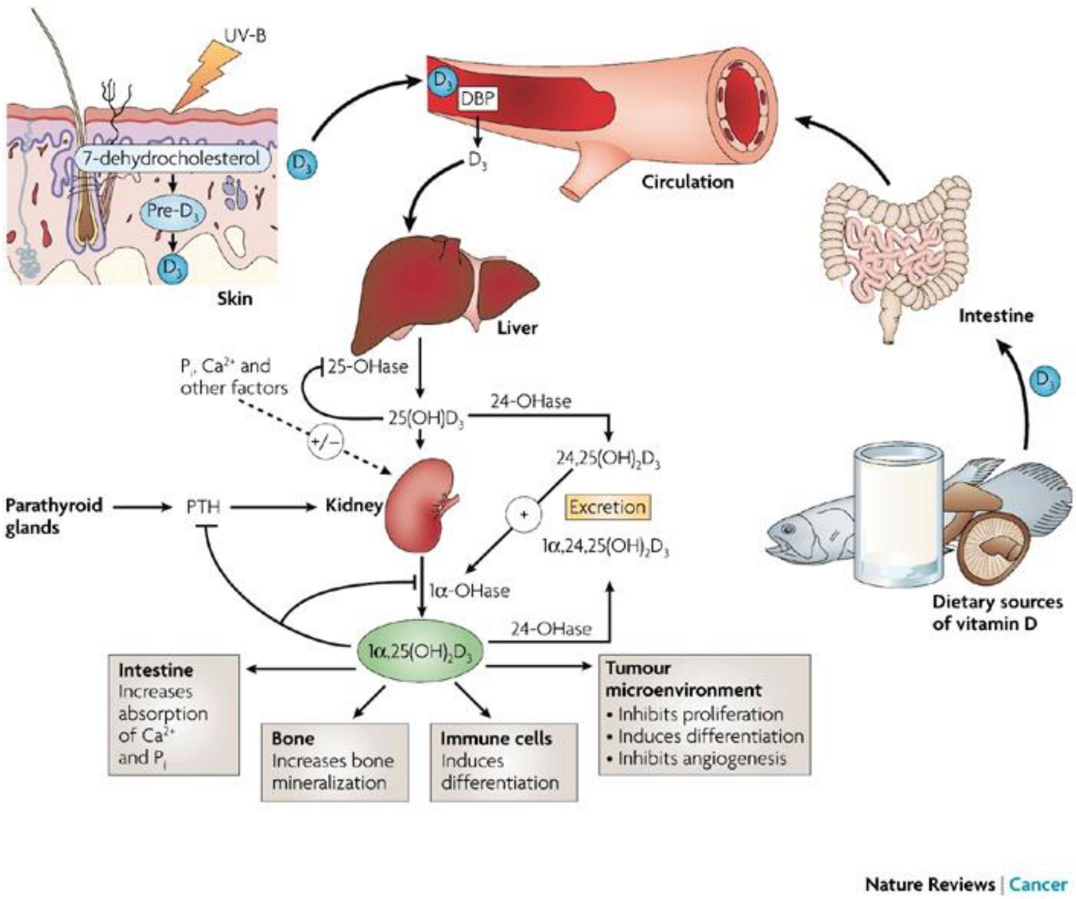

Figure 1. **Vitamin D Synthesis and Metabolism**[18]

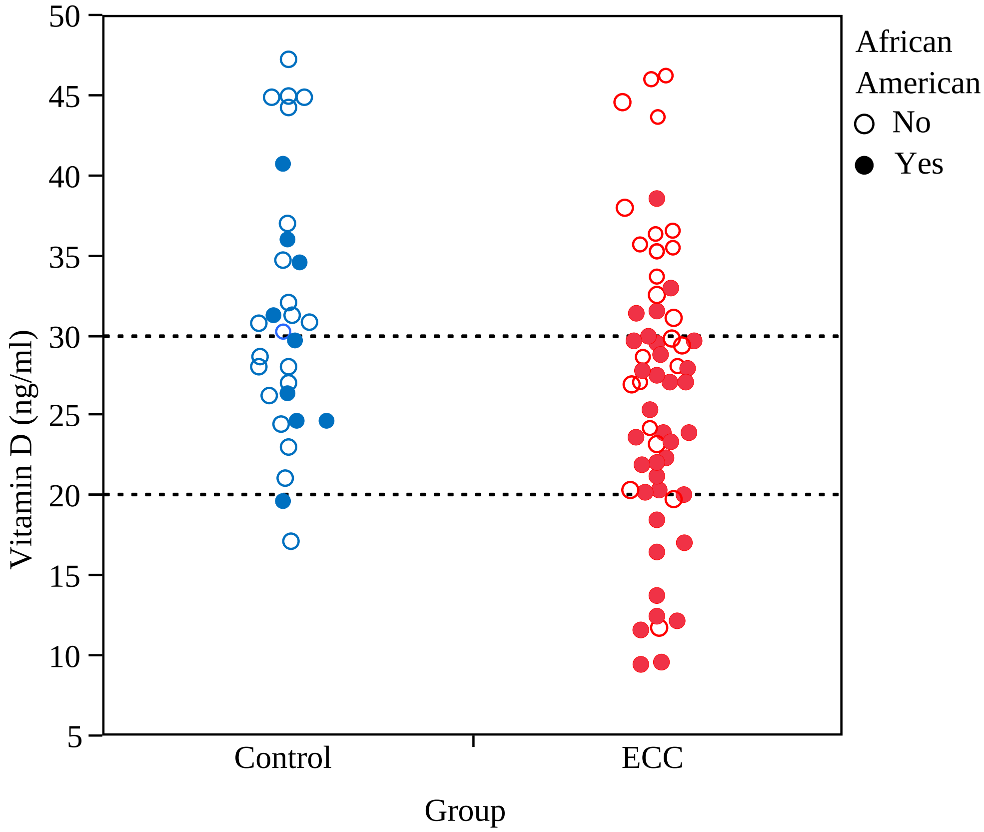

Figure 2. **Mean Serum Vitamin D Levels: Controls vs. ECC**

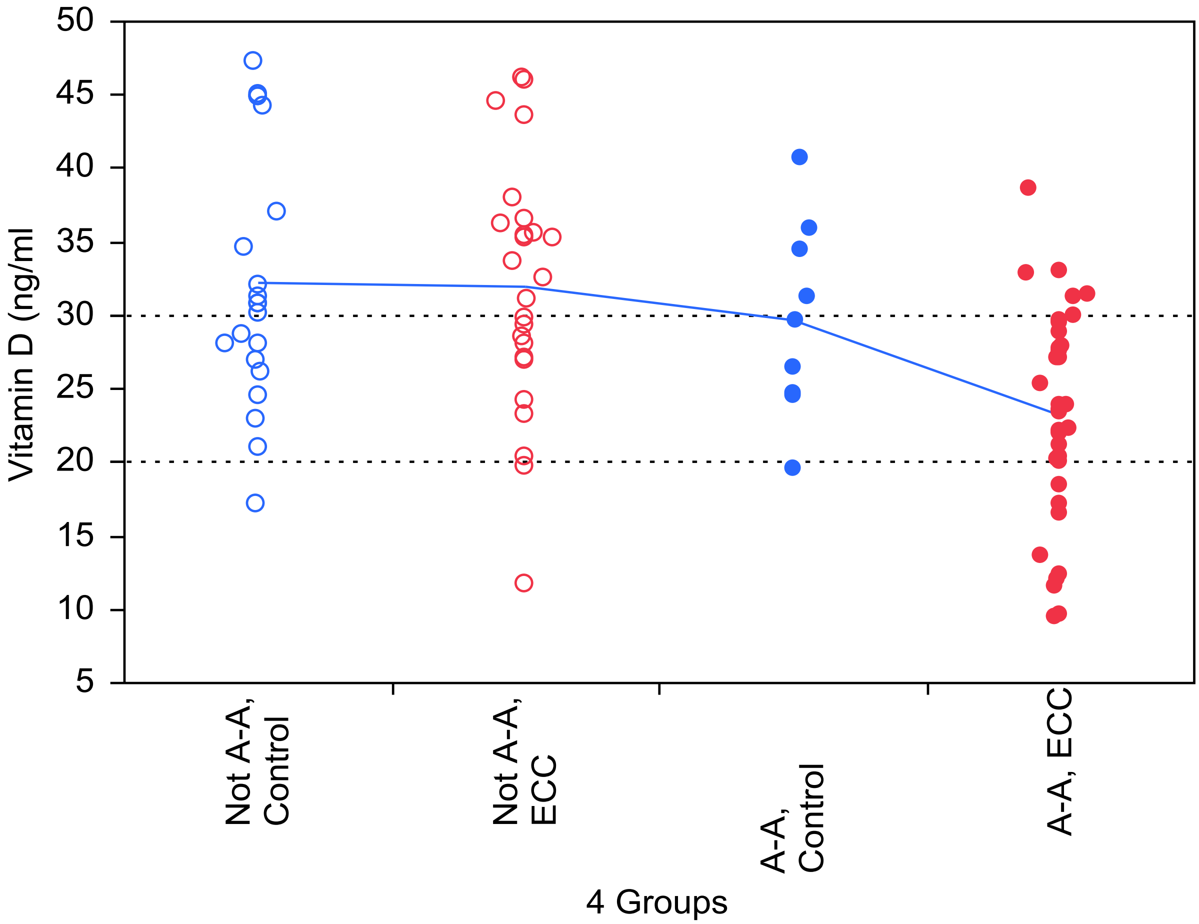

Figure 3. Mean Serum Vitamin D Level in 4 Groups

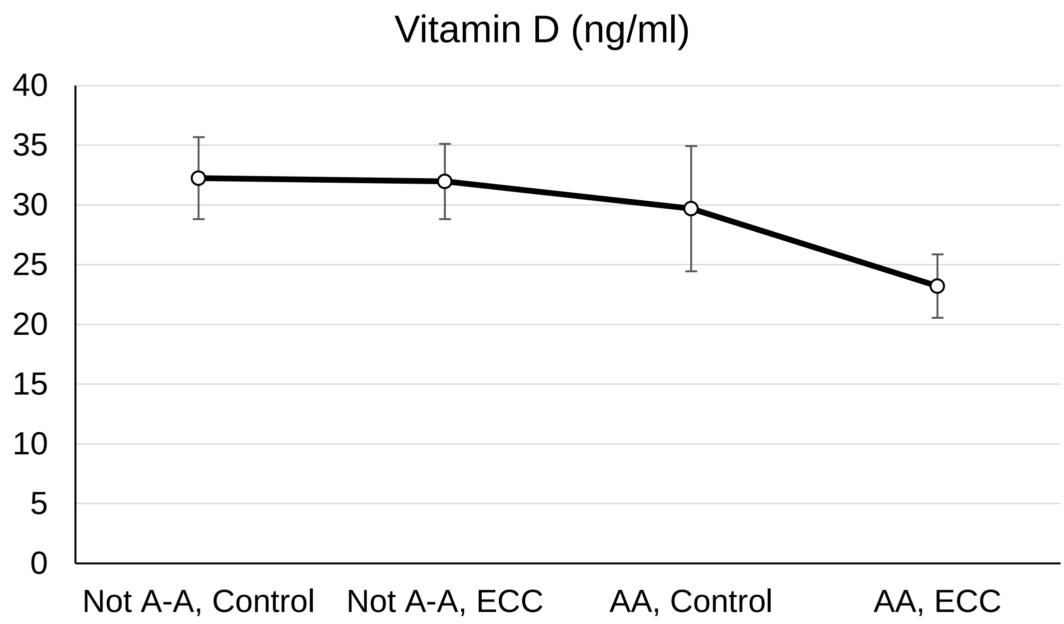

Figure 4. **Mean Serum Vitamin D Level of 4 Groups**

Appendix

Questionnaire for the Primary Caregiver

We are conducting a study about the role of Vitamin D deficiency in the development of dental cavities in children. Please *select the best answer* to the following questions.

These basic questions are about *your child's* age and background.		
How old is your child?	Age: _____	
What is your child's racial background? (check all that apply)	☐ White/Caucasian ☐ African American or Black ☐ Asian	☐ Native Hawaiian or Pacific Islander ☐ American Indian or Alaskan Native ☐ Other (please specify)____________

Please help us understand *your child's* medical history *(Select all that apply to your child)*	
☐ Respiratory disorder (asthma)	☐ Problems with eyes, ears, nose or throat
☐ Heart condition (murmur, arrhythmias)	☐ Genetic disorder/syndrome
☐ Blood disorder (Sickle cell anemia, hemophilia)	☐ Premature birth If so, how many weeks early? _______________ If so, were there any complications?___________
☐ Neurological disorder (seizures)	☐ Illness or infection as a newborn
☐ ADHD/ADD	☐ Other______________________
***Please explain any checked boxes:**	
Does your child take any medications or vitamin supplements? *If so please list them.*	Yes No Don't know *Medications*:_____________________________
How often does your child play outside during the day?	None At least 1hr/day 1-2hrs/day More than 2 hrs/day

The first few questions are about *your child's* teeth	***(Select one)***
1. Does your child have any cavities or fillings?	Yes No Don't know
2. Did your child's doctor or dentist prescribe fluoride drops or tablets?	Yes No Don't know
3. Is there fluoride in your drinking water at home?	Yes No Well Water
4. Does your child receive fluoride painted/put on their teeth from a health professional (doctor, dentist, nurse, hygienist, etc.)?	Yes No Don't know

Now we want to ask about *your child's* tooth care	***(Select one)***
5. How often does an adult brush your child's teeth with toothpaste?	☐Once a day ☐Twice a day ☐Three times a day ☐Never
6. Does your toothpaste have fluoride?	Yes No Don't Know
7. How often are your child's teeth flossed?	Daily Weekly Monthly Never

The following questions are about ***your child's*** dental care	***(Select one)***
8. How often do you take your child to the dentist?	☐ Never ☐ Only when in pain ☐ Yearly ☐ Twice a year
9. Is it very difficult to get your child to the doctor or dentist?	Yes No
10. Is your child's care covered by Medicaid or State Insurance?	Yes No Don't Know
11. Is your child covered by any health or dental insurance other than/or in addition to Medicaid or State Insurance?	Yes No Don't Know
12. Does your child participate in public assistance programs (ex: WIC)?	Yes No Don't Know

Next we ask about ***your child's*** eating habits	***(Select one)***
13. Does your child usually (throughout the day) drink from a bottle and/or sippy cup?	Yes No
14. How often does your child go to sleep while nursing or while drinking something other than water from a bottle/sippy cup?	☐Three or more times a day ☐One or two times a day ☐Weekly ☐Monthly ☐Never
15. After nighttime brushing, does your child eat or drink anything other than water before bed?	Yes No
16. How often do you give your child sugary snacks such as raisins, candy, cookies, cakes, or cereal between meals?	☐Three or more times a day ☐One or two times a day ☐Weekly ☐Monthly ☐Never
17. How often do you give your child sugary drinks such as regular soda, sweet tea, chocolate milk, strawberry milk or fruit juice between meals?	☐Three or more times a day ☐One or two times a day ☐Weekly ☐Monthly ☐Never
18. How often does your child typically drink tap water-including filtered water from the refrigerator?	Daily Weekly Monthly Never

Please continue to page 3 for questions about you (the caregiver).

Questions about ***you*** (the caregiver).

Please help us understand *your* pregnancy and delivery	
1. During your pregnancy, did you (the biological mother) take any medications or vitamin supplements? *If so please list them.*	Yes No *Medications during pregnancy*:__________________ ____
2. During your pregnancy, did you (the biological mother) receive Vitamin D supplementation?	Yes No *If yes*, how many months pregnant were you?________ *If yes*, when did you stop taking Vitamin D?_________
3. During your pregnancy, did you have any of the following illness? *Check all that apply.*	☐Gestational diabetes ☐ Preeclampsia ☐Iron Deficiency ☐Severe nausea/Vomiting ☐Other illness (infection, fever)
4. How old were you when you gave birth?	*Age*:_____
5. At what hospital did you deliver your child?	*Hospital*____
6. Were there any complications with delivering your child? *If so please list them.*	Yes No *Complications*:_________________________________
7. After delivery, did you breastfeed your child?	Yes No *If yes*, for how long did you breastfeed?____________ *If yes*, was your child prescribed Vit D supplements?__

Now we will ask about *your* teeth and *your* tooth care	***(Select one)***
8. Do you have cavities or fillings or have had teeth pulled in the last 2 years?	Yes No
9. Do you have any of your own natural teeth?	Yes No
10. How often do you brush your teeth?	☐Once a day ☐Twice a day ☐Three times a day ☐Never
11. Do you have dental insurance?	Yes No
12. How often do you get dental checkups?	☐ Never ☐ Only when in pain ☐ Yearly ☐ Twice a year

Now tell us a little bit about *you*…		
13. What is the highest level of education that you completed?	☐ Elementary and Middle School ☐ High School ☐ College ☐ Graduate school beyond college	
14. Is an adult in the child's household employed?	Yes	No
15. Which of the following categories best represents the combined income of all family members in your household for the past 12 months? *(select one)*	□ Less than $5,000 □ $5,000-$9,999 □ $10,000-$19,999 □ $20,000-$29,999 □ $30,000-$39,999	□ $40,000-$49,999 □ $50,000-$79,999 □ $80,000-$99,999 □ $100,000 or more □ Don't know

Thank you for answering these questions. This information will better help us to learn more about the relationship between vitamin D deficiency and children's dental health.

Vita

Susan Meinerz was born on January 7, 1987 in Racine, Wisconsin. She received her Bachelor of Arts in Biology with a second major in Spanish from the Marquette University in 2009. She pursed a doctor of dental surgery degrees at Marquette University School of Dentistry, which she completed in May 2013. Susan will complete her Pediatric Dentistry training at Virginia Commonwealth University in June 2016.