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Association of Diet, Vitamin D Status, and Race/Ethnicity on Body Composition of Young Children (4 to 6 Years Old) Living in Southeastern United States

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Abstract

Background: While vitamin D is known to play a role in bone integrity during development, researchers are still investigating its role in body composition.

Objective: Determine the interaction of diet, vitamin D status, and ethnicity/race on body composition of 4 to 6 year old children.

Study design & methods: A longitudinal follow-up study of 140 children (41 Caucasian, 37 African American, and 62 Hispanic) aged 4-to-6-years whose mothers participated in a vitamin D supplementation trial during pregnancy was conducted. Bone mineral density (BMD), percent body fat, and bone mineral content (BMC) were evaluated by dual-energy x-ray absorptiometry (DXA). Vitamin D status (serum 25(OH)D concentration) was determined by radioimmunoassay. Diet was analyzed by validated Block Kids Food Frequency Questionnaire.

Results: Birth weight, age, and weight at visit 1 were positively associated with BMC (p=0.04, <0.001, and <0.001); age and body mass index (BMI) were positively associated with BMD ($p \leq 0.001$ and <0.001). Ethnicity/race, gender, and BMI were associated with percent body fat ($p \leq 0.001$). A subset of dietary variables was associated with percent body fat but not BMD or BMC. Percent body fat had negative association with 25(OH)D; however, no significant relationships were found between BMD or BMC and 25(OH)D concentration within the narrow range of values from this group of healthy children evaluated.

Conclusion: Based on ethnicity/race, body composition in childhood is differentially influenced by diet and vitamin D status of mother and child.

Keywords: Diet; Childhood; Bone mineral content; Bone mineral density; Vitamin D; Percent body fat; Body composition

Introduction

Well-balanced diet is critical for optimal growth and health of children [1]. Childhood obesity has increased significantly over the past few decades and is now a global health problem [2]. The consequence of unhealthy diet increases the risk of many non-communicable diseases in adulthood, including heart disease and diabetes. These studies also have shown that dietary decisions learned in childhood carry over into adulthood [3]. Many vitamins including vitamin D, minerals, and other dietary factors are necessary for development for healthy bone growth [4]. Specifically, vitamin D is essential for achieving higher bone mineral content (BMC), higher bone mineral density (BMD) and the highest bone mineral accretion in young children [4-7]. Vitamin D status in children is assessed by serum 25-hydroxyvitamin D (25(OH)D) concentrations. Important changes in body composition may be determined by one's dietary pattern, but also by ethnicity/race and maternal body mass index (BMI). To further understand the implications of these findings, current research seeks to elucidate not only which factors influence differences in BMD and body composition among children, but how these differences are realized across different ethnic/racial groups. For example, African Americans have lower 25(OH)D concentrations than white/Caucasians despite having higher BMD [8-9].

As there are few studies that address the effect of diet on BMC, BMD, and body composition of a diverse racial/ethnic group of Caucasian, African American, and Hispanic children, we sought to address this paucity of data. The objective of this study was to determine the effect of diet, vitamin D status, and ethnicity/race on the body composition of a cohort of healthy 4 to 6 year-old children. We hypothesized that dairy and calcium intake, as well vitamin D status, would influence percent body fat, BMD and BMC in these children to varying degrees, dependent upon ethnicity/race.

Materials and Methods

Subjects

A total of 140 children (41 Caucasian, 37 African American, and 62 Hispanic) participated in a longitudinal follow-up study at the Medical University of South Carolina in Charleston, South Carolina in the period November 2010 through December 2013. The subjects participating in this follow-up study sponsored by the Thrasher Research Fund were offspring of 350 women who had participated in a NICHD vitamin D supplementation trial during pregnancy (FDA IND # 66,346; ClinicalTrials.gov # NCT00292591) beginning at 12-to-16 weeks of gestation and continuing through delivery [10,11]. Women

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who participated in the NICHD trial provided their written informed consent. The follow-up study included 180 children, 140 of them ranging from 4-to-6 years of age. These children came to two separate visits over the course of two years (140 children for visit 1 and 80 for visit 2). Written informed consent was obtained from the parents/guardians on behalf of the children enrolled in this study. Approval for this study was obtained by the Institutional Review Board of the Medical University of South Carolina (HR #19641).

Dietary data

Each mother was asked to complete a validated Block Kids Food Frequency Questionnaire to obtain dietary intake information for her child. From the questionnaire, energy intake, dietary calcium, iron, saturated fat, monounsaturated fat, polyunsaturated fat, fiber, vitamin D, percent calories from fat, percent calories from protein, percent calories from carbohydrates, and servings of dairy per day were calculated (Block Kids 2-7, 2004, Nutrition Quest, Berkeley, CA).

Measurements

Whole body dual-energy x-ray absorptiometry (DXA) was performed using Hologic Discovery DXA at visit 1 between ages 4 and 6 years (Hologic, serial # 80345, Software version 12.7.3:3 Waltham, MA, USA) by a single board certified radiation technologist and a subsequent visit one year later in some of the children. Inter- and intra-assay coefficients of variation for the spine phantom were 1% or less. Lumbar spine, hip, and total body BMD (g/cm^2) were measured. Additionally, the DXA provided BMC (g) and body composition information including percent body fat for each child. A DVD was played to keep children still during the 15 minute scan.

A blood sample was obtained during the study visit to measure each child's serum 25(OH)D concentration. Because these children were offspring from a pregnancy study, maternal serum 25(OH)D at 16 weeks of pregnancy and one month prior to delivery were previously obtained and available for this analysis [10]. Blood samples were stored at -20°C until analysis. Total serum 25(OH)D concentration was measured in duplicate for each sample by radioimmunoassay (DiaSorin, Stillwater, MN) as previously described [11].

Statistics

Visit 1 characteristics for children and their mothers were compared by race/ethnicity using rank sum test for continuous variables and Fisher's exact test for categorical variables. Similar approaches were taken to compare children's visit 1 dietary characteristics by race/ethnicity.

The main outcomes of interest included children's BMD, BMC and percent body fat measured at visit 1. Spearman's rank correlation (r) was used to assess the associations between continuous variables and each of the three outcomes in each race/ethnicity. Variables with a p-value less than 0.1 were selected for further analysis. The effects of these selected variables adjusting for children's gender, age, and BMI on BMD, BMC, and percent body fat by race/ethnicity were examined by multivariable regression models. Normality and equal variance assumptions of regression models were examined using graphical methods. Data were analyzed using R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

Visit 1 characteristics of the 140 children are outlined in Table 1A. Of the 140 children who were evaluated at visit 1, 37(26%) were African American, 41(29%) were white/Caucasian and 62(44%) were Hispanic. A total of 80 children came for a follow-up visit 2 one year later. At visit 2, 17 (21%) children were African American, 21(26%) were white/Caucasian, and 42(52%) were Hispanic.

Maternal 25(OH)D concentration at visit 1 ($p<0.001$), maternal 25(OH)D concentration one month prior to delivery ($p=0.004$), and the child's 25(OH)D concentration at birth ($p<0.001$) varied by race/ethnicity. Specifically, African American children had similar 25(OH)D concentrations at birth compared to Hispanic children ($p=0.896$) but significantly lower 25(OH)D concentrations at birth compared to white/Caucasian children ($p=0.001$). At visit 1, African American mothers had significantly lower 25(OH)D concentrations (35.8 nmol/L) than to white/Caucasian mothers (76.5 nmol/L; $p<0.001$) and Hispanic mothers (53.8 nmol/L; $p<0.001$). At one month prior to delivery, African American mothers remained with lower 25(OH)D concentrations (71.5 nmol/L) compared to white/Caucasian mothers (109.8 nmol/L; $p=0.002$) and Hispanic mothers (91.5 nmol/L; $p=0.048$). African American, white/Caucasian, and Hispanic children also significantly differed in their BMI at visit 1 ($p<0.001$), birth weight ($p=0.011$), and maternal BMI at visit 1 ($p=0.002$) seen in Table 1A.

Variables	African Americans (n=37)	White/Caucasians (n=41)	Hispanics (n=62)	p-value
Child characteristics				
Child's age (years) at visit 1	4 (0.5, 4-6)	4 (0.7, 4-6)	4 (0.5, 4-6)	0.239
Child's BMI at visit 1	15.2(2.3, 12.7-25.9)	15.6(1.6, 12.1-21.0)	16.7(2.5, 13.7-26.9)	<0.001
Birthweight	3110(594, 1110-3950)	3328(576, 1445-4621)	3317(657, 948-4961)	0.011
Child's 25(OH)D (nmol/L) at birth	20.8(8.1, 3.6-45.3)	29.9(9.4, 12.7-47.8)	19.1(7.0, 5.5-42.3)	<0.001
Child's gender (n%)				0.155
Female	15(40.5%)	16 (39.0%)	35(56.5%)	
Male	22(59.5%)	25(61.0%)	27(43.5%)	
Maternal characteristics during pregnancy				
Maternal BMI at visit 1	31.3(7.4, 19.9-45.5)	25.0(3.9, 19.7-35.2)	26.2(5.8, 19.0-49.6)	0.002
Maternal 25(OH)D (nmol/L) at visit 1	35.8(11.8, 6.0-53.8)	76.5(24.8, 30.0-172.5)	53.8(16.3, 17.3-92.5)	<0.001
Maternal 25(OH)D one month prior to delivery (nmol/L)	71.5(45.0, 16.5-176.0)	109.8(33.3, 61.5-193)	91.5(36.0, 24.5-169.5)	0.004

[1]Data are median (SD, range) for continuous variables and n(%) for categorical variables.

[2]Rank sum test for continuous variables and Fisher exact test was used to test for categorical data (gender). Significant at p-value <0.05.

Table 1A: Characteristics of children ages 4-6 years old (n=140) by race/ethnicity [1,2].

Dietary data of the 140 children at visit 1 are outlined in Table 1B. African American, white/Caucasian, and Hispanic children did not differ significantly in calcium, saturated fat, monounsaturated fat, polyunsaturated fat, fiber, vitamin D, and daily dairy servings. There were significant differences, however, on the basis of race/ethnicity on the following measures: daily caloric consumption (p=0.022), iron intake (p=<0.001), percent calories from fat (p<0.001), percent calories from protein (p=0.005), and percent calories from carbohydrates (p<0.001).

African American, white/Caucasian, and Hispanic children did not differ significantly in body BMD (p=0.437) and body BMC (p=0.708), but there were significant differences in body fat percentage among the three groups (p<0.001). Notably, Hispanic children had higher body fat percentage compared to African American (p<0.001) and white/Caucasian children (p<0.001).

Total bone mineral content

Total body BMC data revealed no significant association among the groups at visit 1 (p=0.708). The mean body BMC (95% CI) at visit 1 was 645.1 g (615.0, 675.3), 636.4 g (609.2, 663.7), 630.8 g (612.3, 649.4) for white/Caucasian, African American and Hispanic children, respectively. Table 2A summarizes the association between each variable of interest and BMC at visit 1. Children's BMI at visit 1 significantly correlated with BMC in African American children (*r*=0.57, p<0.01), white/Caucasian children (*r*=0.61, p<0.01) and

	African Americans (n=37)			White/Caucasians (n=41)			Hispanics (n=62)			
	Median	Range		Median	Range		Median	Range		p value
Calories (kcal)	1666.0	749.0	3927.0	1507.0	927.7	2671.0	1776.0	979.0	2936.0	0.022
Calcium (mg/d)	909.1	337.2	1606.0	963.4	460.1	1510.0	1122.0	407.4	1676.0	0.053
Iron (mg/d)	11.2	4.5	29.7	11.8	6.3	17.7	16.0	6.8	26.2	<0.001
Saturated Fat (g/d)	22.7	8.3	55.5	21.6	7.6	40.7	23.0	10.3	38.4	0.77
Monounsaturated Fat (g/d)	21.9	8.7	56.9	20.3	9.1	44.6	21.3	10.3	38.1	0.816
Polyunsaturated Fat (g/d)	10.4	3.9	24.8	8.9	4.5	19.2	8.8	3.9	17.1	0.159
Fiber (g/d)	12.0	4.9	27.6	12.6	5.1	24.2	13.2	6.2	22.1	0.22
Vitamin D (IU/d)	204.9	38.7	399.0	209.2	22.0	392.1	248.3	31.3	420.7	0.12
Percent calories from fat	31.6	21.9	40.5	33.1	24.7	41.5	29.6	21.5	38.5	<0.001
Percent calories from protein	14.0	10.8	20.4	15.7	12.2	24.3	14.8	10.4	18.7	0.005
Percent calories from carbohydrates	55.8	47.0	67.8	54.7	43.2	62.6	57.2	45.7	70.3	<0.001
Daily dairy servings	2.3	0.5	4.7	2.4	1.0	4.4	2.9	0.5	4.8	0.076

Rank sum test for continuous variables and Fischer exact test for categorical variables. Significant at p-value <0.05 are shown in red.

Table 1B: Dietary characteristics of children ages 4-6 years old by race/ethnicity.

Variables	African American (n=37)			White/Caucasian (n=41)			Hispanic (n=62)		
	r	95% CI		*r*	95% CI		*r*	95% CI	
Maternal BMI at visit 1	0.38	0.07	0.63	0.07	0.29	0.45	0.02	0.24	0.29
Maternal 25(OH)D at visit 1	0.04	0.38	0.29	0.35	0.61	-0.04	0.12	0.14	0.34
Maternal 25(OH)D prior to delivery	0.06	0.26	0.37	0.24	0.56	0.10	0.04	0.24	0.32
Birthweight	0.11	0.23	0.42	0.22	0.06	0.45	0.23	0.01	0.45
Child's BMI at visit 1	0.57	0.29	0.75	0.61	0.40	0.76	0.43	0.19	0.64
Child's age at visit 1	0.52	0.19	0.76	0.53	0.27	0.73	0.48	0.24	0.66
Child's 25(OH)D at visit 1	0.16	0.15	0.46	0.07	0.42	0.27	0.01	0.27	0.26
Baby 25(OH)D at delivery	0.07	0.42	0.32	0.06	0.37	0.26	0.02	0.23	0.27
Calories (kcal)	0.17	0.13	0.46	0.06	0.26	0.37	0.01	0.24	0.28
Calcium(mg/d)	0.32	0.02	0.60	0.09	0.25	0.39	0.10	0.18	0.36
Iron(mg/d)	0.07	0.26	0.39	0.02	0.34	0.30	0.10	0.36	0.19
Saturated Fat(g/d)	0.15	0.17	0.44	0.05	0.34	0.27	0.03	0.22	0.30
Monounsaturated Fat (g/d)	0.10	0.21	0.38	0.06	0.27	0.39	0.04	0.28	0.22
Polyunsaturated Fat (g/d)	0.09	0.21	0.38	0.09	0.24	0.39	0.13	0.35	0.14
Fiber(g/d)	0.04	0.30	0.33	0.07	0.25	0.38	0.04	0.21	0.29
Vitamin D(IU/d)	0.27	0.07	0.59	0.08	0.24	0.39	0.03	0.23	0.28
Calories (%) from fat	0.08	0.40	0.27	0.21	0.52	0.12	0.09	0.34	0.16
Calories (%) from protein	0.08	0.28	0.40	0.04	0.23	0.33	0.16	0.06	0.38
Calories (%) from carbohydrates	0.04	0.32	0.38	0.25	0.11	0.55	0.01	0.24	0.24
Daily dairy servings	0.35	0.00	0.64	0.04	0.27	0.34	0.13	0.12	0.38

[1] Spearman's rank-order correlation

[2]Significant at p-value <0.1 are shown in red.

Table 2A: Associations between variables of interest and BMC in children by ethnicity/Race[1,2].

Hispanic children (*r*=0.43, p<0.01). These differences in correlation coefficient may be due to the differences in the number of patients in each racial/ethnic subgroup.

Children's age at visit 1 was a significant predictor of BMC in the African American children (*r*=0.52, p<0.01); white/Caucasian children (*r*=0.53, p<0.01); and Hispanic children (*r*=0.48, p<0.01). For African American children, in addition to children's age and BMI at visit 1, independent predictors of BMC also included maternal BMI at visit 1 (*r*=0.38, p=0.03) and daily dairy servings (*r*=0.35, p=0.04), with a trend with calcium (*r*=0.32, p=0.06). For white/Caucasian children, the level of BMC also was associated with maternal 25(OH)D concentration at visit 1 (*r*=0.35, p=0.03).

Multivariate regression analysis on BMC stratified by ethnicity/race showed differences among African American, white/Caucasian, and Hispanic children. African Americans showed no significant relationship of BMC to maternal BMI, children's BMI or age at visit 1; additionally, gender, daily dairy servings, or calcium intake at visit 1 were not associated with BMC (Table 2B). For white/Caucasian children, older age (p=0.002) and higher BMI (p<0.001) at visit 1 were significantly associated with higher BMC after adjusting for gender and maternal BMI at visit 1. For Hispanic children, older age (p<0.001) and higher BMI (p<0.001) at visit 1 also were significantly associated with higher BMC after adjusting gender and birth weight.

Total bone mineral density

Total body BMD data did not show any significant differences among the ethnic/racial groups (p=0.437). The mean body BMD (95% CI) was 0.62 g/cm^2 (0.60, 0.63), 0.63 g/cm^2 (0.61, 0.64), 0.62 g/cm^2 (0.60, 0.63) for Caucasian, African American and Hispanic children, respectively. Table 3A shows the unadjusted association between each variable of interest and BMD at visit 1. Children's BMI at visit 1 significantly correlated with BMD in African Americans (*r*=0.66, p=0.02), Caucasians (*r*=0.57, p<0.01) and Hispanics (*r*=0.46, p<0.01). Children's age at visit 1 was a significant predictor of BMD in African Americans (*r*=0.39, p=0.02), Caucasians (*r*=0.50, p<0.01) and Hispanics (*r*=0.36, p<0.01). For African Americans, maternal BMI at visit 1 was also a likely predictor of BMD (*r*=0.29, p=0.10). For Caucasians, maternal 25(OH)D at visit 1 was significantly associated with BMD (*r*=-0.34, p=0.03). No dietary variables were significantly associated with BMD at visit 1.

Multivariate analysis stratified by ethnicities/races and adjusting for maternal BMI, children's age at visit 1, and gender showed a positive association between African American children's BMI at visit 1 and BMD (p=0.001). Caucasian children's BMI (p<0.001) and age (p=0.01) at visit 1 were associated with increased BMD, adjusting for gender and maternal 25(OH)D at visit 1. Hispanic children's gender (p= 0.028), age (p<0.001), and BMI (p<0.001) were associated with increased BMD (Table 3B).

Percent body fat

There were significant changes in percent body fat by race/ethnicity at visit 1 (p<0.001). The mean percent body fat (95% CI) was 24.3% (22.6, 26.1) for white/Caucasians, 22.1% (20.7, 23.5) for African Americans, and 28.6% (27.1, 30.1) for Hispanics. Table 4A shows the unadjusted association between each variable of interest and percent body fat at visit 1. For African Americans, maternal 25(OH)D at 1 month prior to delivery (*r*=-0.29, p=0.08) and children's age at visit 1 (*r*=0.28, p=0.10) were shown to be possible predictors of percent body fat. For Caucasians, possible predictors of percent body fat included baby's 25(OH)D at delivery (*r*=0.32, p=0.05) and maternal 25(OH)D 1 month prior to delivery (*r*=0.38, p=0.02). Daily dairy servings were also on the borderline of significance (*r*=-0.29, p=0.07). For Hispanics, children's BMI at visit 1 significantly correlated with percent body fat (*r*=0.70, p<0.01). Maternal BMI (*r*=0.24, p=0.09) and saturated fat (r=-0.24, p=0.06) might also affect the percent body fat of Hispanic children.

In addition, multivariate regression analysis on percent body fat stratified by ethnicity/race (Table 4B) showed differences among the African American, white/Caucasian, and Hispanic children. For African Americans, maternal 25(OH)D at one month prior to delivery was on the borderline of significance (p=0.07), meaning higher maternal 25(OH)D one month prior to delivery appeared to correlate with lower percent body fat, after adjusting for the potential effect of gender, children's age and BMI at visit 1. In contrast, higher maternal 25(OH)D

African American	**Estimate**	**Std. Error**	**p-value**
Maternal BMI at visit 1	2.529	1.913	0.197
Children BMI at visit 1	11.131	6.069	0.078
Children age at visit 1	31.371	25.84	0.235
Gender	-19.681	27.261	0.477
Daily Dairy servings	50.073	41.999	0.244
Calcium	-0.098	0.126	0.443
White/Caucasian	**Estimate**	**Std. Error**	**p-value**
Maternal BMI at visit 1	-1.545	1.126	0.178
Children BMI at visit 1	26.422	6.304	<0.001
Children age at visit 1	61.516	18.245	0.002
Gender	21.041	21.651	0.338
Hispanic	**Estimate**	**Std. Error**	**p-value**
Children BMI at visit 1	15.239	2.966	<0.001
Children age at visit 1	71.697	13.068	<0.001
Birth weight	-0.003	0.012	0.801
Gender	5.178	14.109	0.715

Multivariate regression model adjusted for children's gender, age, and BMI.

Significant at p-value <0.05 are shown in red.

Table 2B: Results of the multiple regression analysis of variables of interest and BMC by ethnicity/race.

Variables	African Americans			Caucasians			Hispanics		
	r[1]	95% CI		*r*	95% CI		*r*	95% CI	
Maternal BMI at visit 1	0.29	-0.04	0.55	0.05	-0.35	0.42	0.03	-0.24	0.32
Maternal 25(OH)D at visit 1	-0.04	-0.38	0.30	-0.34	-0.61	0.01	0.12	-0.13	0.34
Maternal 25(OH)D prior to delivery	-0.09	-0.43	0.27	-0.24	-0.54	0.07	0.16	-0.12	0.46
Birthweight	0.10	-0.27	0.43	0.20	-0.08	0.45	0.21	-0.06	0.45
Child's BMI at visit 1	0.66	0.39	0.82	0.57	0.35	0.74	0.46	0.20	0.64
Child's age at visit 1	0.39	0.04	0.67	0.50	0.24	0.72	0.36	0.10	0.59
Child's 25(OH)D at visit 1	0.11	-0.19	0.45	-0.03	-0.38	0.33	0.05	-0.17	0.30
Baby 25(OH)D at delivery	-0.01	-0.38	0.35	-0.04	-0.38	0.30	0.13	-0.12	0.38
Calories (kcal)	0.08	-0.21	0.36	0.15	-0.19	0.46	-0.01	-0.26	0.24
Calcium(mg/d)	0.16	-0.17	0.45	0.21	-0.08	0.49	0.07	-0.20	0.32
Iron(mg/d)	-0.01	-0.33	0.30	-0.02	-0.33	0.34	-0.17	-0.41	0.09
Saturated Fat(g/d)	0.05	-0.24	0.34	0.06	-0.26	0.40	-0.02	-0.30	0.27
Monounsaturated Fat (g/d)	-0.03	-0.30	0.26	0.16	-0.19	0.48	-0.04	-0.30	0.23
Polyunsaturated Fat (g/d)	-0.06	-0.34	0.24	0.14	-0.18	0.47	-0.14	-0.40	0.13
Fiber(g/d)	-0.04	-0.36	0.27	0.13	-0.20	0.44	0.00	-0.25	0.24
Vitamin D(IU/d)	0.14	-0.21	0.45	0.24	-0.09	0.54	0.01	-0.26	0.26
Calories (%) from fat	-0.14	-0.46	0.21	-0.09	-0.41	0.26	-0.09	-0.34	0.15
Calories (%) from protein	-0.02	-0.35	0.30	0.08	-0.24	0.39	0.18	-0.06	0.42
Calories (%) from carbohydrates	0.11	-0.27	0.44	0.13	-0.22	0.45	-0.03	-0.27	0.20
Daily dairy servings	0.20	-0.17	0.52	0.15	-0.17	0.43	0.09	-0.18	0.33

[1]Spearman's rank-order correlation.

Significant at p-value <0.1 are shown in red.

Table 3A: The associations between variables of interest and BMD in different ethnicity/race.

African American	Estimate	Std. Error	p-value
Maternal BMI at visit 1	0.001	0.001	0.571
Children BMI at visit 1	0.014	0.004	0.001
Children age at visit 1	0.014	0.017	0.427
Gender	-0.006	0.017	0.75
White/Caucasian	**Estimate**	**Std. Error**	**p-value**
Children BMI at visit 1	0.016	0.004	<0.001
Children age at visit 1	0.031	0.012	0.01
Gender	0.022	0.014	0.118
Maternal 25(OH)D at visit 1	-0.001	0.001	0.237
Hispanic	**Estimate**	**Std. Error**	**p-value**
Children BMI at visit 1	0.012	0.002	<0.001
Children age at visit 1	0.039	0.009	<0.001
Gender	0.02	0.009	0.028

Multivariate regression model adjusted for children's gender, age, and BMI.

Significant at p-value <0.05 are shown in red.

Table 3B: Results of the multiple regression analysis of variables of interest and BMD by race/ethnicity

at 1 month prior to delivery was associated with higher percent body fat (p=0.024) in white/Caucasian children after controlling for the effects of other variables in the model. In multivariable analysis adjusting for other variables in the model, white/Caucasian children, gender, BMI and daily dairy servings at visit 1 were also significantly correlated with the child's percent body fat at visit 1. It is noteworthy that higher dairy intake was associated with lower percent body fat in white/Caucasians (p=0.025) after controlling the effects of other variables in the model. Hispanic children's percent body fat was significantly correlated with gender (p<0.001), and BMI (p<0.001) at the first visit adjusting the effects of other variables. There was a trend where maternal 25(OH) D at visit 1 was borderline significant (p=0.079), suggesting higher maternal 25(OH)D at visit 1 was associated with lower percent body fat at visit 1.

BMC, BMD and percent body fat at visit 2

After examining the cohort at visit 2, a significant ethnic/race difference in percent body fat was found (p<0.001). The mean percent body fat (95% CI) was 22.8% (20.3, 25.3) for Caucasians, 20.5% (18.2, 22.8) for African Americans, and 28.2% (26.3, 30.0) for Hispanics. Hispanic children had significantly higher percent body fat than Caucasian (p<0.001) and African American children (p<0.001).

Due to the smaller sample size at visit 2 (17 African American children, 21 Caucasian children, and 42 Hispanic children), visit 2 data from different ethnicity/races were combined in the multivariate regression analysis. The effects of (1) maternal 25(OH)D at visit 1, visit 2, pre-delivery; (2) children's 25(OH)D at visit 1, visit 2, delivery; and (3) dietary variables on BMC, BMD, and percent body fat were examined

Variables	African Americans			Caucasians			Hispanics		
	r	95% CI		*r*	95% CI		*r*	95% CI	
Maternal BMI at visit 1	0.23	-0.13	0.55	0.06	-0.35	0.4	0.24	-0.02	0.47
Maternal 25(OH)D at visit 1	0.18	-0.47	0.15	-0.17	-0.49	0.15	-0.14	-0.36	0.08
Maternal 25(OH)D prior to delivery	0.29	-0.57	0.04	0.38	0.04	0.64	0.06	-0.22	0.33
Birth weight	0.07	-0.42	0.29	-0.01	-0.36	0.32	0.13	-0.16	0.39
Child's BMI at visit 1	0.05	-0.32	0.41	0.12	-0.2	0.42	0.7	0.52	0.82
Child's age at visit 1	0.28	-0.1	0.59	-0.18	-0.51	0.16	0.01	-0.27	0.28
Child's 25(OH)D at visit 1	0.22	-0.55	0.18	0	-0.29	0.31	-0.12	-0.36	0.13
Baby 25(OH)D at delivery	0.03	-0.4	0.32	0.32	-0.06	0.6	-0.13	-0.36	0.13
Calories (kcal)	0.17	-0.18	0.51	0.01	-0.31	0.31	-0.17	-0.39	0.05
Calcium(mg/d)	0.06	-0.39	0.26	-0.22	-0.5	0.08	-0.14	-0.36	0.07
Iron(mg/d)	0.27	-0.07	0.57	0.07	-0.25	0.38	-0.18	-0.4	0.06
Saturated Fat(g/d)	0.07	-0.28	0.43	0.02	-0.28	0.33	-0.24	-0.43	-0.03
Monounsaturated Fat (g/d)	0.13	-0.22	0.48	0.08	-0.23	0.39	-0.2	-0.39	0.03
Polyunsaturated Fat (g/d)	0.22	-0.14	0.54	0.18	-0.17	0.49	-0.15	-0.38	0.09
Fiber(g/d)	0.23	-0.11	0.53	0.06	-0.26	0.39	0.04	-0.2	0.29
Vitamin D(IU/d)	0.05	-0.35	0.26	-0.14	-0.44	0.18	-0.17	-0.39	0.08
Calories (%) from fat	0.17	-0.51	0.19	0.14	-0.17	0.43	-0.12	-0.35	0.12
Calories (%) from protein	0.01	-0.31	0.31	-0.09	-0.42	0.22	0.11	-0.14	0.33
Calories (%) from carbohydrates	0.13	-0.23	0.46	-0.1	-0.39	0.21	0.07	-0.19	0.33
Daily dairy servings	0.14	-0.45	0.22	-0.29	-0.57	0.03	-0.14	-0.36	0.1

[1]Spearman's rank-order correlation.

[2]Significant at p-value <0.1 are shown in red.

Table 4A: The associations between variables of interest and percent body fat race/ethnicity[1,2].

African Americans	Estimate	Std. Error	p-value
Children BMI at visit 1	0.015	0.015	0.323
Children age at visit 1	0.078	0.067	0.247
Gender	-0.061	0.059	0.311
Maternal 25(OH)D prior to delivery	-0.003	0.002	0.07
White/Caucasians	**Estimate**	**Std. Error**	**p-value**
Children BMI at visit 1	1.331	0.347	0.001
Children age at visit 1	-0.602	1.047	0.57
Gender	-4.819	1.381	0.002
Daily dairy servings	-1.698	0.715	0.025
Baby 25(OH)D at delivery	0.1	0.076	0.201
Maternal 25(OH)D prior to delivery	0.119	0.05	0.024
Hispanics	**Estimate**	**Std. Error**	**p-value**
Gender	-4.335	0.928	<0.001
Children age at visit 1	0.121	0.812	0.883
Maternal 25(OH)D at visit 1	-0.178	0.099	0.079
Children BMI at visit 1	2.065	0.252	<0.001
Saturated fat	-0.153	0.086	0.081

Multivariate regression model adjusted for children's gender, age, and BMI.

Significant at p-value <0.05 are shown in red.

Table 4B: Results of the multiple regression analysis of variables of interest and percent body fat by race/ethnicity.

by including each of these variables in a multivariate regression model adjusting for ethnicity/race, BMC, or BMD, or percent body fat. None of these variables were associated with BMC at visit 2 after adjusting the effect of ethnicity/race and BMC at visit 1. Similar results were found for children's BMD at visit 2 after adjusting for ethnicity/race and BMD at visit 1. At visit 2, only maternal 25(OH)D at visit 1 was significantly associated with children's percent body fat. Specifically, higher maternal 25(OH)D at visit 1 was associated with lower percent body fat at visit 2 after adjusting for race/ethnicity and percent body fat at visit 1.

Discussion

Previous studies have described the relationship between BMD and the vitamin D status of children. This study identifies a relationship among diet, body composition, and ethnicity/race in a diverse group of children ages' 4-to 6-years-old that has not been previously described. Furthermore, this study is novel due to the fact of the equal distribution of three different ethnicity/races. Specifically, US Hispanic children were the majority of the cohort at both visit 1 and visit 2 compared to previous studies, which examined only African American and white/Caucasian children [8,9]. Vitamin D status was quantified and analyzed by total circulating 25(OH)D concentration, which has a half-life of 15-25 days and can originate exogenously in the skin as the precursor vitamin D (cholecalciferol) following exposure to UV light or by oral supplementation [10]. Consistent with previous studies [8,9], Caucasian children had the highest serum 25(OH)D concentrations followed by Hispanic and African American children. These data are consistent with that obtained in a study of 4-to- 8 year old girls [8]. Our population of 4-to-6 year old children did not have any statistically significant total body BMD differences, unlike the study of Bell et al. [9] in 7-to-12 year old children, which showed that African American children had significantly higher total body BMD than Caucasian children [9]. Contrary to the study of Caucasian children in the United Kingdom, however, our study found no association between maternal 25(OH)D one month prior to delivery and BMD of children [7]. These results may differ from previous studies because the mean maternal 25(OH)D level one month prior to delivery in this cohort was 100 nmol/L, which exceeds the minimally sufficient concentration of 50 nmol/L [12] associated with bone mineralization.

The main finding of this study was that age and BMI affect BMD and BMC in a diverse group of 4-to- 6 year old children but not serum 25(OH)D concentration; however, 25(OH)D concentration was shown to be associated with percent body fat. These BMC and BMD outcomes are consistent with a study of 8-to-9 year old children in Sweden [13]. Also, this latter result complements a study that reported obesity and serum 25(OH)D concentrations are inversely related [14]. Another important finding from this study, consistent with earlier studies, was while the white/Caucasian children's vitamin D status was higher (averaging 73 nmol/L) compared to African Americans and Hispanics (52.3 and 50, respectively), there were no statistically significant differences between the group's BMC and BMD. This finding is consistent with previous studies that showed there may not be a dose-dependent relationship between serum 25(OH)D concentrations and BMD/BMC, but rather a sufficiency level of at least 50 nmol/L [15,16].

In African Americans, while dietary calcium was positively associated with BMC but not BMD, daily dairy servings did not affect BMD or BMC. These results could be due to the high intake of dairy foods among the children (mean of 2.6), which surpasses the recommendation of two servings per day [17].

Neither maternal nor child serum 25(OH)D concentration was associated with BMD or BMC. This outcome may be due to the mother's 25(OH)D concentration one month prior to delivery (mean of 250 nmol/L), the child's 25(OH)D at birth (mean of 58.5 nmol/L), and the child's 25(OH)D (mean of 67.8 nmol/L) at the follow-up visit all being greater than the IOM's recommended minimum vitamin D status of 50 nmol/L in both adults and children [12,18].

While inherent bias may be a limitation of this study since the participating children were a subset of the original NICHD vitamin D supplementation trial during pregnancy, there was an even distribution of the 140 children among the ethnicity/races and of the mothers in the 400, 2000, and 4000IU D_3/day groups. We chose to focus on total circulating 25(OH)D concentration as a biomarker rather than a treatment since it is a better indicator of what the actual vitamin D status of the mother and fetus was during pregnancy, and then later, for the child, and is not affected by adherence to protocol. The study examined the diet of the child at the clinic visit but did not control for prior feeding history during early infancy (i.e., breastfeeding vs. formula-feeding), which may impact early bone development and later percent body fat and BMI [19]. Furthermore, each of the three-ethnic/racial groups represented had diverse diets that seemed to affect BMI in significant ways. In addition, physical activity maybe also a limitation since it was not assessed in this study. Outdoor physical activity may contribute to an improvement of vitamin D status, reduce risk of obesity, and influence body composition.

In conclusion, vitamin D status and diet appear to play a role in the body composition of young children. The data indicate that while diet was not the best predictor of percent body fat, maternal and child vitamin D status was associated with percent body fat differently in each ethnic/racial group. Additional follow-up studies of children as they progress through childhood are essential to determine the potential long-term effects of maternal and child vitamin D status, as well as diet, on the health status of children. Such considerations may be essential in preventing health disparities that manifest early in childhood.

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