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ABSTRACT

THE RELATIONSHIP BETWEEN SERUM 25-HYDROXYVITAMIN D, VITAMIN D AND CALCIUM INTAKE, AND ADIPOSITY IN INFANTS

by
Carolyn W. Morris

Purpose: National prevalence of childhood overweight and obesity has plateaued in recent years, but rates remain high, with approximately 10% among children <2 years of age being classified as “high weight.” The relationship between adiposity and serum 25-hydroxyvitamin D [25(OH)D] status has been well-explored in older individuals, with inconsistent results. Furthermore, previous studies have suggested a relationship between adequate consumption of calcium and vitamin D and healthy weight status in older children and adults. However, in the infant population, there are few studies detailing the interaction between body composition and serum 25(OH)D or intake of calcium and vitamin D. Our study aims were to assess the association between serum 25(OH)D and body composition and to examine the association between adiposity and dietary intake of calcium and vitamin D in a sample of infants and toddlers. Methods: Our population included healthy male and female infants and toddlers from Pittsburgh, PA who participated in the “Practices Affecting Vitamin D Status in Pittsburgh Infants and Toddlers” study. Parents completed a Vitamin D and Sunlight Exposure Questionnaire, which assessed dietary intake of foods high in calcium and vitamin D as well as daily sunlight exposure (≥ 2 hours vs. > 2 hours). Anthropometric measures and bloodwork for serum 25(OH)D were obtained during at the time of the study visit. Weight-for-length (WFL) percentile status was determined using WHO growth standards (low weight <2.3 %ile, normal weight 2.3-97.7 %ile, high weight >97.7 %ile) and WFL z-scores were calculated. ANOVA was used to compare mean serum 25(OH)D and calcium and

vitamin D intake by WFL status. Chi square analysis was used to evaluate the relationship between serum 25(OH) D status (deficient = <12 ng/mL, insufficient = 12-20 ng/mL, sufficient >20 ng/mL), calcium intake status (sufficient = >700 mg), vitamin D intake status (sufficient = >400 IU) and WFL percentile status. Pearson's correlation coefficient was used to assess the strength and significance of associations between serum 25(OH)D, calcium and vitamin D intake and WFL z-score. The analysis was repeated after subdivision by race and sun exposure. Results: 125 infants and toddlers (9 to 24 months of age, 68% African American) participated in the study. Approximately 11% of the population had a high weight. Mean vitamin D intake (~600 IU/d) and median calcium intake (~1550 mg/d) exceeded recommendations. Prevalence of high weight was higher among children with adequate intake compared to those who consumed less than the recommendations (calcium: 41% vs. 36%, respectively; vitamin D: 45% vs. 29%, respectively). However, this difference was not statistically significant. Mean serum 25(OH)D level (37 ng/mL) was sufficient. When compared across WFL status, neither mean serum 25(OH)D nor mean intake of calcium and vitamin D varied significantly. No significant correlation was found between WFL and serum 25(OH)D for the cohort or any of the subgroups examined. Conclusions: Rates of infant overweight and obesity in our sample are similar in comparison with the national average. Our results do not support a relationship between calcium and vitamin D intake on weight status or an association between serum vitamin D and body composition in children of this age. Future studies are needed to re-examine these relationships in a larger group of children of more evenly distributed weight status.

THE RELATIONSHIP BETWEEN SERUM 25-HYDROXYVITAMIN D, VITAMIN D
AND CALCIUM INTAKE, AND ADIPOSITY IN INFANTS

A Thesis

By
Carolyn W. Morris

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1 John 4:7-12

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ABBREVIATIONS

1,25(OH) ₂ D	Calcitriol, 1,25-dihydroxyvitamin D
25(OH)D	Cholecalciferol, 25-hydroxyvitamin D
BMI	Body mass index
CCAAT	Calcium channel associated transcriptional regulator
C/EBP α	CCAAT-enhancer binding protein
D ₂	Ergocalciferol
D ₃	Cholecalciferol
DBP	Vitamin D binding protein (albumin)
DRI	Dietary Reference Intake
IOM	Institute of Medicine
AAP	American Academy of Pediatrics
NHANES	National Health and Nutrition Examination Surveys
PPAR γ	Peroxisome proliferator activated receptor γ
RDA	Recommended Daily Allowance
UL	Upper Intake Level
UVB	Ultraviolet B

CHAPTER I

INTRODUCTION

Topic and Problem

Despite the fact that obesity rates have plateaued in many sectors of the United States population, prevalence of overweight and obesity remains high among North Americans and poses a significant health concern. Ogden and colleagues (2012) analyzed the National Health and Nutrition Examination Surveys (NHANES) from 2009-2010 and determined that 16.9 % of children and adolescents were classified as obese during this time period, which did not represent a significant departure from figures derived from earlier NHANES reports (1). Although no change over time was reported for obesity in girls between 1999-2000 and 2009-2010, a significant increase in childhood obesity was noted among boys 2-19 years of age (1). In the infant sample used in the NHANES 2009-2010 report, 9.7% of children from birth to 2 years of age were determined to “high weight” based on weight-for-recumbent length percentile curve values ($> 95^{\text{th}}$ percentile = high weight) (1) on the Centers for Disease Control and Prevention (CDC) 2000 Growth Charts.¹

¹ For children <24 months of age, “high” and “low” weight, length, and weight-for-length are more correctly defined as >97.7 percentile and <2.3 percentile on the World Health Organization (WHO) growth charts. This classification is in accordance with recommendations from CDC, which advise use of WHO growth charts, rather than those issued by the CDC, to assess the weight and length of children <2 years. The former charts were designed using breast fed infants as a reference sample. From birth to 3 months, breast fed infants grow more rapidly than formula fed counterparts. At 3 months and above, however, this situation is reversed (2). According to the CDC, use of the recommended growth standards “identifies fewer infants (aged <12 months) as having high weight for length (5%–9%) than the CDC reference (9%–13%), [although] for children aged 18–23 months, the differences in high weight for length essentially disappear.”

Significance

Causes of the obesity epidemic are multifactorial and have been well-explored in other studies. Here, we are concerned with unhealthy weight in young children, particularly in regard to vitamin D intake and status. Research suggests that early, rapid weight gain (perhaps most importantly through 2 years of age) is predictive of overweight and obesity later in life (3). High prevalence of obesity in the United States holds interesting and important implications for vitamin D status. This paper explores two of these implications in greater depth. First, a significant body of research has documented an inverse relationship between excess fat and serum vitamin D concentration. Second, previous studies have also suggested a possible role for dietary vitamin D, along with dietary calcium, in protecting against obesity (3, 4). Although the relationship between adiposity and serum vitamin D concentrations has been studied extensively in adult populations and older children, this relationship is less well-studied in infants. Likewise, few prior studies have examined calcium and vitamin D intake and prevalence of overweight in this age group.

Purpose and Research Questions

Given the high prevalence of early overweight and obesity, as well as the recently reported decline in vitamin D status (6), analysis of the relationship between adiposity, serum 25(OH)D status, and calcium and vitamin D intake in young children is important. The specific aims of this study are to: 1) examine the association between adiposity and serum 25(OH)D concentration and 2) evaluate the relationship between vitamin D and calcium intake and adiposity in a sample of predominately African American infants

from Pittsburgh, Pennsylvania. We hypothesize that a negative association exists between serum 25(OH)D concentration and adiposity, as well as between calcium and vitamin D intake and adiposity.

CHAPTER II

REVIEW OF LITERATURE

Calcium and Vitamin D Intake in Infants and Toddlers

The Institute of Medicine's (IOM) current Recommended Daily Allowance (RDA) for calcium and vitamin D for children aged 1-3 years are 700 mg/day and 15 µg/day (600 IU), respectively (7). From birth to age 1, there are no established RDAs for any nutrient, although the American Academy of Pediatrics (AAP) recommends a total daily intake of 400 IU/day of vitamin D for infants, children, and adolescents. Supplementation is advised for infants who are exclusively breastfed, due to the low vitamin D content of breast milk (7, 8).

Nevertheless, other sources recommend maternal, rather than infant, supplementation in order to increase mothers' confidence in the benefit and adequacy of breastfeeding. In a systematic review, Thiele et al evaluated 3 articles in the literature that involved the supplementation of breastfeeding mothers with varying doses of vitamin D (10). Studies meeting their inclusion criteria were randomized controlled trials of longitudinal design. Vitamin D doses administered to lactating mothers ranged from 400-6,400 IU/day, and one study used a single dose of 60,000 IU/month (the 2010 DRI from the IOM is 400 IU/day for lactating women). The authors concluded that in all of the studies, maternal and infant increase in vitamin D serostatus were directly related to maternal supplementation, and no adverse effects were observed in either member of the mother-infant pair with any dose. In the 2 studies conducted in the United States, infants

were able to achieve “sufficient” vitamin D status of ≥ 20 ng/mL. Infants in the third study, conducted in the United Arab Emirates, were not able to achieve sufficient status, either with a daily dose of 2,000 or with the monthly dose of 60,000 IU. This discrepancy may be attributable to low maternal 25(OH)D during the pre-and post-natal stages, secondary to women’s minimal sun exposure. Therefore, in the United States, either maternal or infant supplementation in the appropriate amount would ostensibly ensure adequate vitamin D intake and sufficient 25(OH)D serostatus.

Tolerable upper intake levels (ULs) for calcium are 1,500 mg/day for infants from 6 months to 12 months of age and increase to 2,500 mg/day for the 1-3 year age range. Meanwhile, for vitamin D, ULs are 37.5 μ g/day (1,500 IU) for infants from 6-12 months and 62.5 μ g (2,500 IU) from 1-3 years (7). Bailey et al (2010) used NHANES 2003-2006 data to estimate total calcium and vitamin D consumption (from diet and supplements) for boys and girls aged 1-3 (11). They found that, in male children of this age, average total calcium intake was 1008 ± 28.3 mg/day, while total vitamin D intake was 9.1 ± 0.4 μ g/day (equivalent to 364 IU/day). Meanwhile, female children were reported to have slightly lower total average consumption of calcium at 977 ± 28 mg and vitamin D 8.4 ± 0.4 μ g (equivalent to 336 IU) on a daily basis.

Dietary sources of calcium are abundant and toddlers’ requirements should be met easily through consumption of dairy products and other calcium-containing foods. Nevertheless, dietary sources of vitamin D are extremely limited. Vitamin D is found in egg yolks, fortified milk and juices, irradiated mushrooms and fatty fish. For infants, breast milk and formula represent primary sources of these nutrients. Supplements are

available in either the ergocalciferol (D₂, plant-based) or cholecalciferol (D₃, animal-based) form.

Although previous research has demonstrated that adults' serum 25(OH)D levels respond better to supplementation with D₃, it does not necessarily follow that the same is true for infants, particularly in those <3 months of age (12). Gallo et al (2012) designed a study in which healthy infants of similar melanization were randomly assigned to receive either 10 µg/day (400 IU) vitamin D₂ or D₃ for 3 months. The researchers observed that more of the D₃-supplemented infants (96.2%) were able to achieve a serum 25(OH)D status of 50 nmol/L (20 ng/mL) than their D₂-supplemented counterparts (75.0%). However, they also noted that baseline 25(OH)D had actually been lower in the latter group. Taking this difference into account, increases in serum 25(OH)D were equivalent, and a mean improvement of 20 nmol/L 25(OH)D was observed during the study period. Preparation of vitamin D supplements also impacts infants' ability to absorb them. A study by Hollis and colleagues (1996) examined the effect of non-esterified D₂ and D₃ palmitate in two groups of infants (1 day old vs. >10 days old) (13). The vitamins were administered simultaneously in both groups in the amount of 0.7 mL/kg/body weight. In the former group, prior to peak absorption at 6-12 hours, circulating D₂ levels had reached 66.1±43.5 nmol/L (25.6±16.3 ng/mL), as opposed to the 26.4±19.7 nmol/L (10.3±4.0 ng/mL) circulating D₃ ($P < .0001$). More efficient absorption of non-esterified D₂ was also observed in the group of infants >10 days of age, although their absorption rate was 1.5 times more efficient than that of the younger group. Discrepancies in vitamin D absorption are explained by low bile salt production in neonates and low levels of

pancreatic enzymes, which renders unesterified vitamin D more bioavailable to infants during the first days of life.

Vitamin D Status in the U.S. Population

Vitamin D is perhaps best known for its role in calcium homeostasis and bone health. However, it has also been studied in relation to depression, immunity, gene expression, cardiovascular health, insulin secretion, and maintenance of healthy weight. Despite the metabolic importance of vitamin D, a statistically significant decrease in serum levels of this nutrient has been observed in recent decades. Average serum vitamin D status in 1988-1994 was 60.7 nmol/L (24 ng/mL), in contrast to the 2001-2006 mean of 55.2 nmol/L (22 ng/mL) (6). An especially noticeable decline in vitamin D status was reported among adolescents, with the percentage of adolescents with vitamin D levels <30 nmol/L (12 ng/mL) increasing from 5% in 1988-1994 to 12% in 2001-2006 ($P < 0.001$). No vitamin D status data for infants were reported for either time period. Likewise, little information exists for children age 2-5 and 6-11 years, as the 1988-1994 survey did not include these age groups in its consideration of vitamin D status (6). In 2001-2006, however, prevalence of 25(OH)D <30 nmol/L was approximately <1% and 3%, respectively, for these groups (6). Researchers attribute the decline in vitamin D status primarily to decreased time spent out-of-doors. Ultraviolet B (UVB) exposure is essential for the synthesis of endogenous vitamin D – in fact, sunlight exposure has been identified as the main determinant of vitamin D status (14). In addition to poor sun exposure, other risk factors for vitamin D deficiency include geographic location

(latitudes over 37°N translate to reduced vitamin D synthesis during non-summer months), and increased melanization of the skin (10).

In order to assess the interplay between various factors affecting serum vitamin D status, Rajakumar et al (2011) conducted a longitudinal study using a sample of 140 Caucasian and African American children (ages 6-12) in Pittsburgh, Pennsylvania (40.4° N) (15). The study protocol included a visit in the summer and winter. Data collected included BMI, dietary intake of calcium and vitamin D, sunlight exposure, skin color, serum vitamin D, and serum PTH levels. Based on serum vitamin D level, children were classified as being either vitamin D deficient, insufficient, or sufficient. The researchers found that calcium and vitamin D intake was equivalent between the two races.

Differences in sun exposure were noted, with a greater proportion of African American children having >2 daily hours of sun exposure (vs. ≥ 2 hours) and appeared less likely to use sunscreen than Caucasian children. Interestingly, no significant interracial differences were found in the proportion of children who were determined to be vitamin D deficient, insufficient, or sufficient in either the winter or summer season. Nevertheless, African American children were shown to have lower levels of vitamin D during both seasons (38.65 \pm 22.51 ng/mL vs. 45.40 \pm ng/mL for summer months, and 33.79 \pm 11.27 ng/mL vs. 35.82 \pm 10.84 ng/mL for winter months).

Metabolism of Vitamin D

Upon contact with UVB, 7-dehydrocholesterol (provitamin D), which is stored in the skin, is converted to precalciferol (previtamin D) (16). The exact location of 7-dehydrocholesterol depends on the age of the individual in question (17). In adults, the

majority (80%) of this pre-hormone is found in the dermis. Meanwhile, in infants, the compound is split fairly evenly between the dermis and the epidermis, due to the narrow width of each layer in the immature integument. Within the cells of the dermis (or epidermis, in the case of infants) previtamin D then undergoes a heat-induced isomerization to calciferol. This structural change allows the newly-formed compound to pass into the extracellular space. Vitamin D binding protein (DBP, albumin) subsequently “draws” 25(OH) into the blood vessels for transportation to the liver and kidney, where it will undergo hydroxylation first to cholecalciferol, then to active calcitriol (15, 16).

The enzyme that effects the hepatic conversion of calciferol to 25-hydroxyvitamin D is a cytochrome P450 hydroxylase (25-hydroxyvitamin D). Researchers postulate that, due to developing liver function, this this family of enzymes operates differently in very young infants than it does in older individuals. As a result, a C-3 epimer of 25(OH)D may be created in addition to 25(OH)D (18). Despite its structural similarities, the epimer does not play the same role as cholecalciferol, as it does not promote gene expression or calcium absorption to the same extent that 25(OH)D does. These metabolic differences are not believed to persist beyond the fourth month of life (11, 17). Furthermore, the immature kidney may also influence the absorption and transport of vitamin D by affecting the reabsorption of DBP in the nephron tubule (19, 20).

Vitamin D Storage

Body composition plays a role in determining circulating levels of vitamin D in the body. Because vitamin D is a lipophilic vitamin, it is stored in the adipose tissue.

Knowledge of the critical role of adipose tissue in vitamin D storage can be traced back to the late 1960s. In 1969, Rosenstreich and colleagues (21) presented research detailing an experiment in which a sample of laboratory rats were first maintained in a state of total vitamin D deprivation and subsequently divided into groups with each one receiving varying doses of radioactive C-ringer labeled vitamin D₃ (0.5 µg/day, 5µg/day, and 125 µg/day) for 14, 12, and 14 days, respectively. Following histological evaluation of all three groups, the authors found that, although 6% of the doses appeared to be taken up by non-adipose tissues initially, over time this percentage fell to 2-3% as vitamin D₃ administration was continued. In contrast, it was established that adipose tissue stored approximately 10% of the doses received throughout the time during which supplementation occurred.

In addition to supporting the role of adipose tissue in vitamin D storage, more recent research also raises concerns regarding the potential of large adipose stores to create a state of hypovitaminosis D in overweight or obese individuals through sequestration of this compound.

Illustrating the effects of this speculated sequestration, researchers at Emory University Hospital observed a sample of 20 female patients with baseline BMI over 35 kg/m² (Stage II obesity or above) over either 6 or 24 months following gastric bypass Roux-en-Y procedures (22). At 1 month post-surgery, mean BMI was 42.8±0.9, in contrast to subjects' mean baseline BMI of 47.5±0.9 ($P<0.001$). In addition to this decrease in BMI, the researchers noted significant increases in vitamin D status, from a baseline 25(OH)D level of 54.8±5.4 nmol/L to 65.7±6.7 nmol/L ($P<0.05$). Elevation in serum vitamin D was rapidly counteracted by the malabsorptive state induced by the

Roux-en-Y procedure, as evidenced by the fact that, at 6 months, a statistically significant decrease in serum vitamin D concentration was observed. Nevertheless, these results indicate that vitamin D is released from fat stores into the blood as the loss of adipose tissues continues. Multiple sources have reported that insufficient serum vitamin D status in overweight and obese individuals is associated with a number of related pathologies, including insulin resistance, hypertension, and unhealthy lipid profiles.

Association between Serum Vitamin D and Adiposity

A number of studies have documented the impact of BMI on vitamin D status in children from an array of ethnicities, geographical regions, and seasons during which serum samples were collected. While most of these studies show an inverse relationship between the two factors of interest, not all research supports this association.

Lagunova and colleagues (2011) conducted a study using a homogenous sample of 102 overweight and obese children and adolescents (mean age 14.5 ± 3.3 years) in a Norwegian weight management clinic (latitude 59°N) (23). Researchers obtained data on subjects' body composition using bioelectric impedance analysis. During their data analysis, no significant differences were identified between males and females. Mean serum concentrations of 25(OH)D (73 nmol/L, or 28.4 ng/mL, for the entire sample) were highest in the summer, peaking approximately at 80 nmol/L (31.1 ng/L). Serum 1,25(OH)₂D was also measured – highest levels of this compound were found in the spring. Increasing age was associated with decreasing serum 25(OH)D. Illustrating this fact, 72% of the subjects <13 years were discovered to have 25(OH)D ≥ 75 nmol/L (30

ng/mL), while only 42% of subjects ≥ 13 years met this mark. BMI increases, as well as increases in other anthropometric variables, were accompanied by decrease in 25(OH)D serostatus. 1,25(OH)₂D was not observed to have the same relationship with body composition-related parameters. Furthermore, study subjects in whom a vitamin D deficiency² was established were more likely to be obese and have elevated BMI than counterparts without D deficiency. In 17 of the 18 subjects with 25(OH)D < 50 nmol/L (20 ng/mL), BMI values were over the 95th percentile. The authors noted, however, that weaknesses of this study included lack of information on dietary intake of vitamin D and sun exposure.

Rodríguez-Rodríguez et al (2010) conducted a wintertime study of 102 Madrid (latitude 40°N) children. The authors reported a high prevalence of overweight and obesity in Spain (26.3% of individuals from 2-24 years of age are classified as overweight or obese) (24). Subjects had mean age 10.9 ± 1.00 years, mean BMI 19.5 ± 3.7 , and average vitamin D intake of 2.83 ± 3.17 $\mu\text{g}/\text{day}$ (113.2 ± 126.8 IU/day). A variety of anthropometric measurements (skinfold thickness at the biceps and triceps, and tricipital region, as well as waist and hip circumference, lean mass, and percent body mass using DEXA) were used to determine the relationship between regional fat distribution and vitamin D status. Subjects' food intake was recorded, weighed, and compared with calculations of expected energy expenditure. Information on time dedicated to physical activity, which was assumed to reflect sun exposure, was also collected. Children were divided into vitamin D sufficient and insufficient groups.³ Statistical analysis revealed negligible differences between the two groups in terms of sun

² Low vitamin D status was < 75 nmol/L (30 ng/mL), while deficiency was < 50 nmol/L (20 ng/mL).

³ Here, insufficient levels were defined as 25(OH)D < 70 nmol/L (27 ng/mL), whereas adequate D status was ≥ 70 nmol/L (27 ng/mL).

exposure and vitamin D intake. In contrast, weight and BMI were each found to have an independent, significant effect on serum vitamin D levels. Children who were over the 50th percentile of weight-for-age (OR 16.4, $P=0.002$), waist circumference (OR 5.1, $P=0.029$), skinfold thickness at the biceps (OR 2.7, $P=0.033$), and waist-height ratio (OR 5.3, $P=0.025$) were all at significantly greater risk of vitamin D deficiency, highlighting the possible importance of excess visceral and subcutaneous fat in the sequestration of vitamin D.

A study conducted by Alemzadeh et al. (2011) also supports an inverse relationship between adiposity and vitamin D status in children. The researchers assessed a racially heterogeneous group of 127 obese children (mean age 13.0 ± 3.0 years and mean BMI 37.1 ± 8.5) in Milwaukee, Wisconsin (latitude 43°N) to determine vitamin D status and its relationship to various other anthropometric and biochemical parameters (25). Vitamin D status was categorized into levels designated as hypovitaminosis D (which included the separate categories of insufficiency and deficiency) and vitamin D sufficiency.⁴ The anthropometric variables examined were comprehensive, including BMI and various measures of fat and lean mass, while biochemical markers measurements included insulin. BMI was significantly ($P<0.02$) greater in the group of children with hypovitaminosis D, and fat mass:fat-free mass ratio ($P<0.001$) in the 94 children that were determined to have hypovitaminosis D (<75 nmol/L) in comparison to their 33 vitamin D –sufficient peers.

Using another heterogeneous sample of 263 low-socioeconomic status children and adolescents (aged 9-14 years) in Somerville, Massachusetts (latitude 42°N), Sack

⁴ For this study, hypovitaminosis D was defined as serum 25(OH)D <75 nmol/L (30 ng/mL), whereas sufficiency was ≥ 75 nmol/L, and insufficiency and deficiency were said to be 50-74 (20-29 ng/mL) and <50 nmol/L (<20 ng/mL), respectively.

et al. (2011) studied the relationship between BMI z-score, vitamin D status, lipid profile, blood pressure, inflammatory biomarkers, and impaired glucose tolerance during the winter season, using a cross-sectional design (26). Study aims were to assess the relationship between 25(OH)D serostatus and obesity, as well as to identify the association between 25(OH)D serostatus and the “cardiometabolic risk factors” identified above, regardless of children’s weight status. Forty-five percent of this sample was determined to be obese. The researchers failed to find a significant association between body composition and vitamin D status, vitamin D deficiency and undesirable blood lipid profile, or 25(OH)D and biochemical markers of inflammatory states. One exception was the positive association between 25(OH)D concentrations and C-reactive protein (CRP), with $P < 0.05$. Researchers recognized that this overall lack of significance could be linked to the widespread prevalence of low serum 25(OH)D across the entire study sample, as 75% of children were determined to have vitamin D deficiency, and only 9 children were found to have serum vitamin D ≥ 75 nmol/L (30 ng/mL).⁵

Besides addressing the effect of excess fat on serum 25(OH)D levels, some studies have also described the impact of low 25(OH)D status on the development of overweight and obesity. In Bogotá, Colombia (latitude 4°N), Gilbert-Diamond et al (2010) studied the longitudinal effect of vitamin deficiency on various adiposity-related parameters in a population of 479 low- and middle-class primary school students (27). Children’s mean age at baseline was 8.9 ± 1.6 years, overweight prevalence was 11%, and mean serum 25(OH)D concentration was 73.2 ± 19.8 nmol/L. The study was approximately 3 years in duration. Over this time, a variety of anthropometric variables –

⁵ In its definition of vitamin D deficiency and non-deficiency, “deficiency” is designated as < 50 nmol/L and “non-deficiency” as ≥ 50 nmol/L.

BMI, subscapular:triceps skinfold thickness ratio (“truncal adiposity”), and waist circumference (“central adiposity”) – were tracked and examined in association with 25(OH)D serum levels. The children’s 25(OH)D levels were determined to be sufficient, insufficient (46.4%), or deficient (10.2%).⁶ Some maternal measurements (BMI and weight) were gathered, as well, in order to investigate potential confounding effects. According to the authors’ analyses, vitamin D deficiency at baseline was positively associated with a greater increase in subscapular:triceps skinfold ratio ($P=0.003$, P for trend=0.01) and waist circumference ($P=0.03$, P for trend=0.05). However, the reported mean yearly increases for these parameters seem to be fairly small (0.03 cm for skinfold thickness and 0.8 cm for waist circumference). These differences may lack clinical application and significance, especially considering the necessity of growth.

In contrast to the substantial body of literature available for older populations (children, adolescents, and adults), relatively little research addresses the effect of body composition on vitamin D status in infants. The existing literature reveals a lack of consensus in regard to the relationship of interest. One study by Arnberg and colleagues (2011), is from Denmark (latitude 56°N), where vitamin D supplementation is recommended for children under the age of 2 in the amount of 10 µg/day (400 IU/day) (28). The study sample ($n=255$) was comprised of 9 month-old infants of Caucasian ethnicity, with the exception of 11 children of mixed-race origin. Mean 25(OH)D serostatus was 77.2 ± 22.7 nmol/L (31 ± 8.8 ng/mL). This study found significant negative associations between serum vitamin D and the infants’ cholesterol levels (including HDL cholesterol), as well as measures of body composition (BMI and waist circumference)

⁶ Gilbert-Diamond and colleagues classify “sufficiency” as ≥ 75 nmol/L (30 ng/mL), “insufficiency” as 50-75nmol/L (20-30 nmol/mL) and deficiency as < 50 nmol/L (< 20 nmol/L).

after adjusting for gender, mothers' smoking habits, and season. These results indicate that, similarly to adult and child populations, an inverse relationship exists between adiposity and serum 25(OH)D status in infants.

A cross-sectional study by Gordon et al (2008) does not completely corroborate Arnberg's findings (29). This group studied a sample of 380 Boston infants and toddlers who ranged in age from 8 to 24 months. The children's racial heritages were predominately African American (61.3%) and Latino (28.9%). Adiposity was estimated using BMI, which is not generally recommended for use in populations under 2 years of age. Vitamin D status was classified as suboptimal (40%), deficient (12.1%), or severely deficient (1.9%).⁷ In toddlers, but not in infants, the authors were able to identify a significant inverse association between serum 25(OH)D and BMI in multivariable analysis. Therefore, due to discrepancies among infant studies, additional research is required to explore the relationship of adiposity and 25(OH)D serostatus in greater detail.

Association between Vitamin D and Calcium Intake and Adiposity

According to several murine studies, vitamin D and its metabolites have contradictory roles in relationship to overweight and obesity, depending on the maturity of the adipocyte. In mesenchymal cells which have yet to differentiate to a specific tissue type, 1,25 dihydroxyvitamin D, 1, 25(OH)₂D, acts in several ways to inhibit adipogenesis. First, it prevents the expression of genes coding for nuclear receptor proteins associated with maturation to full-fledged adipocyte status, namely, peroxisome proliferator-activated receptor γ (PPAR γ) and CCAAT-enhancer binding protein (C/EBP α) (4,30).

⁷ In this study, suboptimal levels were < 30 ng/mL, deficient levels were <20 ng/mL, and severely deficient concentrations were < 8 ng/mL.

In addition, 1,25(OH)₂D appears to have a synergistic relationship with the vitamin D receptor (VDR) protein (4). In the absence of this metabolite, VDR levels tend to drop as cell differentiation progresses, but when accompanied by sufficiently high levels of 1,25(OH)₂D, VDR is “stabilized,” enabling VDR to continue to suppress PPAR γ (4). VDR-1,25(OH)₂D binding is not required for VDR to perform this function at earlier stages of cell differentiation (4). Furthermore, it has been determined that PPAR γ must interact with a specific, as-of-yet undetermined ligand in order to induce adipocyte differentiation, and exposure to 1,25(OH)₂D hampers the formation of that ligand (30).

In mature adipocytes, the mechanism of action of 1,25(OH)₂D appears to be quite different. In a calcium-deficient state, additional 1,25(OH)₂D is formed and promotes the entry of calcium into adipocytes (31). In these cells, intracellular calcium facilitates the anti-lipolytic and pro-lipogenic functions of the agouti polymer (formed by the expression of the agouti gene). Researchers also found that elevated intra-adipocyte calcium is promoted by the agouti gene independently of 1,25(OH)₂D (5). These insights, derived from *in vitro* experimentation, point to the importance of adequate dietary calcium (to maintain calcium homeostasis and prevent the excess formation of 1,25(OH)₂D), as well as sufficient dietary vitamin D (to assist in calcium absorption) in preventing excessive agouti activity and undesirably high levels of intracellular calcium.

Some researchers have questioned this proposed mechanism of action for calcium and vitamin D, especially when applied to humans. Astrup (32) reminds us that dairy products are the most common source of dietary calcium. They contain a variety of compounds which could be responsible for the observed benefit on weight management (bioactive peptides, etc.). He also cites the low contribution of endogenous adipose

synthesis to total fat stores. Alternatively, high consumption of dietary calcium could be associated with overall improved diet quality and lifestyle.

Bortolotti et al (2008) conducted a crossover trial with 10 human subjects randomized to receive either 800mg calcium (or placebo) or 300 mg slow-release caffeine (or placebo) for 4 weeks (33). After this point, blood and adipose tissue samples were collected to assess free fatty acid circulation and changes in the expression of fat oxidation-related genes. Supplementation with calcium did not appear to induce any significant changes in resting energy expenditure, fat oxidation, or the level of free fatty acids in the blood. The researchers did not observe alterations in the expression of pertinent genes. Consequently, calcium may not be the primary impetus behind dairy's perceived benefit on weight. The possibility also exists that the regulation of lipolysis and adipogenesis by calcium and vitamin D may be different in animals (mice, specifically) than in humans. It is accepted, however, that calcium and fat impede one another's digestion through the formation of soaps and subsequent excretion in the feces (34). Taking these findings and caveats into account, a few recent studies which suggest a beneficial of dietary calcium and vitamin D are discussed below.

We located one study that used a sample of adult subjects to examine the relationship between vitamin D intake, calcium intake, and excess adiposity directly through measurement of adipocyte size. In a cross-sectional study conducted by Caron-Jobin and colleagues (2011), the total fat and lean mass of 43 predominately Caucasian women (mean age 47.2 years mean BMI of 27.2 ± 4.6) was determined using dual energy x-ray absorptiometry (DEXA) (35). Women were determined to be of pre-menopausal (39.5%), perimenopausal (39.5%) or post-menopausal (19%) status. The majority of the

patients (all but three) did not take vitamin D supplements or multivitamins. Food frequency data were collected to assess typical intakes of energy, dairy, calcium, and vitamin D (35). Subsequently, adipose tissue samples were extracted during gynecologic surgery. The diameters of individual adipocytes in subcutaneous and omental⁸ fat storage depots were then determined. The researchers' work revealed that higher levels of dairy product consumption (2 or more servings per day), as well as higher levels of calcium and vitamin D intake (of which dairy sources provided 59 and 52% of the total dietary amount, respectively) have a significant, moderate, negative correlation with the size of omental adipocytes for women in this sample ($r=-0.55$, $P<0.005$), although a similar correlation was not found for calcium intake (35). In a multiple regression model which included vitamin D consumption, calcium intake, BMI, visceral adipose tissue area, total body fat mass, season, and amount of physical activity, dietary vitamin D independently explained 9.6% of the variance found among adipocyte size in the location specified (35). A significant inverse relationship was also noted between serum 25(OH)D status and omental adipocyte diameter, body weight, BMI, waist circumference, and total body fat mass. Neither intakes of the foods and nutrients of interest, nor serum 25(OH)D status, were meaningfully associated with subcutaneous adipose size, however (35). This study suggests a role of dietary vitamin D in the processes of lipogenesis (and thus adipocyte growth) in an *in vivo* context, a relationship that has also been explored less directly in other studies.

A study by Tidwell and colleagues (2011) recruited a sample of 100 African American women aged 18-40 (mean age 26 ± 6.4 years) with average BMI of 29.8 ± 6.9 to examine the relationship between fat mass and calcium, vitamin D, and total energy

⁸ The omentum is a layer of fatty tissue which serves as protection for the visceral organs.

consumption (36). The population from which the subjects were drawn (African Americans living in Mississippi), has a very elevated prevalence of overweight and obesity (75.1%). Subjects were asked to keep a single 24-hour food diary from a representative day. The subjects' BMI was calculated and DEXA was used to assess total body fat (36). When using independent *t*-tests to compare mean dietary calcium and vitamin D in women with less or greater than the sample's median body fat percent (37.9%), researchers found a significant difference between the two groups ($P < 0.001$ in both cases). Women in the $< 37.9\%$ group consumed an average of 911.5 ± 208.3 mg of calcium per day, in comparison to the 528.6 ± 146.0 mg consumed in the $> 37.9\%$ body fat group. Similarly, the former group had a daily mean vitamin D intake of 5.0 ± 0.8 μg (200.0 ± 32.0 IU) in contrast to the mean intake of 3.8 ± 0.9 μg (152.0 ± 36.0 IU) in the latter group. Furthermore, across the entire sample, there was a significant inverse relationship of moderate strength between calcium and vitamin D consumption and body fat, after fat, carbohydrate, and protein intakes had been accounted for as partial correlation coefficients (for calcium, $r = -0.666$, $P < 0.001$, and for vitamin D, $r = -0.460$, $P < 0.001$) (36). This study either points to a directly benefit of calcium on weight maintenance, or healthier overall dietary habits of women who consumed greater amounts of calcium and vitamin D. Significant relationships of weak to moderate strength were discovered not only between calcium, vitamin D, total energy consumption and percent body fat, but also between this parameter and folate.

Alemzadeh et al (2008) compared vitamin D deficient ($n = 41$, < 50 nmol/L) and insufficient ($n = 53$, $50 - 74.9$ nmol/L) subjects and discovered that BMI ($P < 0.001$), and the ratio of fat mass to fat-free mass ($P < 0.001$), were significantly higher in the former

group, whereas vitamin D intake was significantly higher in the latter group of children (270.9 ± 149.3 vs. 210.9 ± 106.1 IU/d, $P < 0.0001$) (25). Finally, Skinner et al (2003) conducted a longitudinal study in order to evaluate changes in body fat measurements and calcium intake in a group of middle and upper socioeconomic class Caucasian children from 2 months of age through 8 years (37). Parents completed dietary interviews with nutrition professionals during the first two years of their children's lives and subsequently were trained to complete periodic two-day food records independently. One key finding from this study was that dietary calcium, along with intake of polyunsaturated fats, were inversely related to adiposity. It was also established that "longitudinal dietary calcium" accounted for just under 10% of the variability in subject adiposity.

These studies support a negative relationship between vitamin D intake, calcium intake, and obesity in adults, adolescents, and children. To our knowledge, the association between calcium and vitamin D intake and infants' adiposity has not been evaluated. Infants who are breastfed and do not receive supplementation are at risk for vitamin D deficiency, given that vitamin D content of breast milk is only 25 IU of vitamin D/L (9). After weaning is complete, calcium and vitamin D nutrition depends on the adequacy of the diet. Infants in the present study may be at particular risk for vitamin D deficiency owing to increased melanization in the African American population and the high latitude of the city (Pittsburgh, PA) from which the study sample was drawn.

CHAPTER III

METHODOLOGY

Participants

The study sample was comprised of infants and toddlers aged 8 to 24 months. Subjects were recruited from a population of children who received medical care at the Primary Care Center of Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center (UPMC). Children with health conditions or who were taking medications that would impact the absorption or metabolism of calcium or vitamin D were not eligible for participation.

Research Design

The present study is an observational cross-sectional study. It is a secondary analysis of an original study titled, "Practices Affecting Vitamin D Status in Pittsburgh Infants and Toddlers," which was conducted at UPMC and received approval from the University of Pittsburgh Institutional Review Board (IRB). The original study was designed to determine 25(OH)D serum status in 8-24 month-old infants in Pittsburgh, to determine the effect of feeding method, sun exposure, and vitamin supplementation on vitamin D status in this same sample, and to assess compliance with the AAP's current vitamin D supplementation recommendations. Parents of participating infants and toddlers signed a consent form and received a token financial reimbursement for their participation in the study. Demographic data including date of birth, gender, and race, as

well as anthropometric data (length in cm and weight in kg) were gathered from study participants' medical records. For the present study, infants' and toddlers' weight and length measures were plotted on gender specific weight-for-length growth charts and weight-for-length status was determined. Previous studies examining body composition and its relationship to vitamin D in children < 2 years have used BMI-for-age as an indicator of adiposity. However, the Centers for Disease Control and Prevention (CDC) do not endorse the use of BMI for infants and toddlers at this time.

The Vitamin D and Sunlight Exposure Questionnaire (SEQ) was completed by parents or guardians, who reported the subjects' race, multivitamin or vitamin D supplementation practices, and any recommendation received from a physician to supplement breastfeeding infants with vitamin D. Feeding practices, including consumption of breast milk or formula, were recorded, as was daily or weekly consumption of the following products determined to be significant sources of vitamin D, calcium, and iron: supplements, dairy products and alternatives, fortified orange juice, nutritional supplement drinks, toddler formula, baby cereal, conventional cereals, protein bars, breakfast bars, and baby food. Data collected on dietary intake were analyzed and quantified on Food Processor software by ESHA Research (Salem, OR). Information on sun exposure and sunscreen use was acquired through this survey, as well, detailing time spent out-of-doors, skin exposure to sunlight, sunblock use, and travel to sunny locations.

Statistical Methods

Data was analyzed using Statistical Package for the Social Sciences, (SPSS) Version 20.0 for Windows (SPSS Inc., Chicago, IL). Weight-for-length percentiles and

Z-score calculations were carried out with WHO Anthro 2005 (Department of Nutrition, WHO, Geneva, Switzerland). Sample characteristics, including age, weight, length, weight-for-length percentile and z-score, vitamin D intake (IU), calcium intake (mg), serum 25(OH)D concentration, and sun exposure, were described using frequency analysis. Measures of central tendency included means (for normally distributed data), or medians (for non-normal data whose distribution could not be altered with log transformation). Comparisons between genders were made using the independent samples *t*-test and the Mann-Whitney U test for normally- and non-normally-distributed data, respectively. Frequency of serum 25(OH)D sufficiency (≥ 20 ng/mL), insufficiency (12 to < 20 ng/mL), and deficiency (< 12 ng/mL), based on Institute of Medicine recommendations (7), was assessed. Frequency of low weight, normal weight, and high weight was determined (underweight = < 2.3 percentile, normal weight = 2.3 to 97.7 percentile, and high weight > 97.7 percentile), as well as low or adequate intakes of calcium and vitamin D in reference to guidelines from the AAP and the Institute of Medicine.

Analysis of variance was used to evaluate serum vitamin D level and vitamin D and calcium intake by weight-for-length status. Calcium intake was log-transformed for this purpose. Chi-square analysis was used to evaluate the association between the serum vitamin D status (sufficient, insufficient, deficient), as well as vitamin D and calcium intake (above or below recommended daily intake) and weight-for-length percentile status.

For assessment of the correlation between serum 25(OH)D status and weight-for-length z-score, the Pearson correlation was ascertained. The association between vitamin

D and calcium intake and the weight-for-length z-score was assessed by the same method. A correlation matrix was generated in SPSS to display correlation coefficients for each of the variable pairs of interest while subdividing by race and sunlight exposure. If the degree of correlation was sufficiently high, we planned to use a multiple regression model to ascertain the impact of weight-for-length on vitamin D status while adjusting for age, gender, ethnicity, sunlight exposure, and sunblock use. Significance was defined as $P < 0.05$.

CHAPTER IV

RESULTS

Sample Characteristics

Demographic characteristics for the sample population are shown in Tables 1 through 4. The median age of the sample was 13 months (range 9-24 months) with the majority of the participants being African American (65%) and male (54.4%) (Table 1). Thirteen children (10%) were reportedly taking a multivitamin supplement, 1 child took calcium supplements, and 6 children were reportedly taking vitamin D supplements. Approximately 27% of infants (n=34) were receiving infant formula. Among these, mean formula consumption was 18 oz/day. In contrast, only 5.6% of children were receiving breast milk at the time of the study, and all these participants were >1 year of age.

Median weight-for-length percentile fell within a healthy range at 78.9 (Table 2). After subdivision by gender, the median weight-for-length percentile was slightly greater in boys than girls, but was not significantly different. Neither gender group was determined to have an unhealthy median weight-for-length status. Comparisons of other anthropometric, serum 25(OH)D and sun exposure variables by gender did not reveal statistically significant differences. Although the boys' and girls' mean weights were within normal range, a high percentage of male infants were determinant to have high weight-for-length (13.2%). Several female infants also had unhealthy weight status, with 8.8% having high weight-for-length (Table 3).

Serum 25(OH)D data were available for 108 of the 125 subjects (Table 4). The children were found to have a mean serum 25(OH)D of 37.0 ± 8.7 ng/mL, which

exceeds the IOM minimum designated level for sufficient vitamin D status (>20 ng/mL). We observed that very few children had deficient (1.6%) or insufficient (0.8%) vitamin D status, while 84.1% of the total was determined to have sufficient status. When comparing 25(OH)D status between children with normal vs. high weight-for-length percentile, we found lower vitamin D status in the latter group (34.07 ± 8.32 vs. 37.4 ± 8.76 ng/mL). However, this difference was not statistically significant ($P=0.183$).

Table 1: Sample Demographic Characteristics

Variable	N (%)
Gender	
Boys	68 (54.4)
Girls	57 (45.6)
Race	
White/Caucasian	14 (11.2)
Asian American	2 (1.6)
Black/African American	85(68)
More than one race	23 (18.4)
Missing	1 (.53)

Mean daily intake of vitamin was 599.7 ± 330 IU, which exceeds the amount recommended by the AAP for infants, children, and adolescents of 400 IU/day (Table 5). This reported intake of vitamin D consumption, if accurate, satisfies the new recommendations from the IOM (600 IU/day for children from 1-3 years of age). Median reported calcium intakes in boys and girls in our population were elevated far beyond the RDA for children from 1-3, at 1552 mg/day and approximate the UL for children 6-12 months for this nutrient (1500 IU/day). The percentages of children in our population who met vitamin D and calcium recommendations are shown in Table 6.

Table 2: Sample Anthropometric Characteristics and Vitamin D Status

Characteristic	Total Subjects (N = 125)	Boys (N = 68)	Girls (N = 57)	Significance
Weight (kg) ^a	10.6 ± 1.6	10.83 ± 1.5	10.38 ± 1.64	<i>P</i> = 0.102
Height (cm) ^b	76.5 (73.7, 80.0)	77.0 (73.8, 80.0)	76.2 (73.7, 79.8)	<i>P</i> = 0.599
Weight-for-length percentile ^b	78.9 (52.5, 92.7)	82.1 (60.0, 94.7)	77.5 (44.4, 90.4)	<i>P</i> = 0.200
Weight-for-length z-score ^a	0.84 ± 1.0	0.91 ± 1.00	0.75 ± 1.02	<i>P</i> = 0.381
Sunlight Exposure ^d				
< 2 hours	65	35	30	---
> 2 hours	54	28	26	
Serum 25(OH)D concentration ^{a, c} (ng/L)	37.0 ± 8.7	37.9 ± 8.8	36.0 ± 8.6	<i>P</i> = 0.257

a. Mean ± SD values listed, independent *t*-test used to compare groups

b. Median (25%, 75%) values listed, Mann-Whitney U test used to compare groups

c. 17 subjects had missing serum 25(OH)D data

d. Counts, rather than medians or means, are provided for this nominal categorical variable; data available for 119 subjects

Table 3: Sample Weight-for-Length Percentile Status

	N	Low Weight (<2.3 percentile) N (%)	Normal Weight (2.3-97.7 percentile) N (%)	High Weight (>97.7 percentile) N (%)
Total	125	0 (0)	111(88.1)	14 (11.1)
Boys	68	0 (0)	59 (86.6)	9 (13.2)
Girls	57	0 (0)	52 (91.2)	5 (8.8)

Table 4: Sample Serum Vitamin D Status

	N	Deficient (<12 ng/mL) N (%)	Insufficient (12-20 ng/mL) N (%)	Sufficient (≥ 20 ng/mL) N (%)
Total	108	2 (1.6)	1 (.8)	105 (84.1)
Boys		0 (0)	1(1.5)	53 (77.9)
Girls		2 (3.5)	0 (0)	53 (93.0)

Table 5: Sample Mean and Median Nutrient Intakes

Nutrient	Total	Boys	Girls	Significance
Calcium ^a (mg/day)	1552.9 (1083.8, 2446.5)	1552.9 (1081.2, 2299.5)	1582.9 (1090.7, 2494.8)	P=0.835
Vitamin D ^b (IU/day)	599.7 \pm 330.0	610.6 \pm 343.4	586.8 \pm 314.6	P=0.692

a. Median values shown, Mann-Whitney U test used to compare groups

b. Mean values \pm SD shown, independent *t*-test used to compare groups

Table 6: Number of Participants Who Met Nutrient Intake Recommendations^a

	Recommendation Met/Exceeded N (%)	Recommendation Not Met N (%)
Calcium		
Total	109 (86.5)	14 (11.1)
Boys	59 (86.8)	8 (11.8)
Girls	50 (87.7)	6 (10.5)
Vitamin D		
Total	89 (70.6)	34 (27.0)
Boys	50 (73.7)	17 (25.0)
Girls	39 (68.4)	17 (29.8)

a. Calcium 700 mg/d (IOM); Vitamin D 400 IU/d (AAP), for children 1-3 years of age

Relationship between Adiposity and Serum 25(OH)D Concentration

No significant correlation was found between serum 25(OH)D or vitamin D intake and weight-for-height z-score in the population (Figures 1 and 2) or by gender (data not shown).

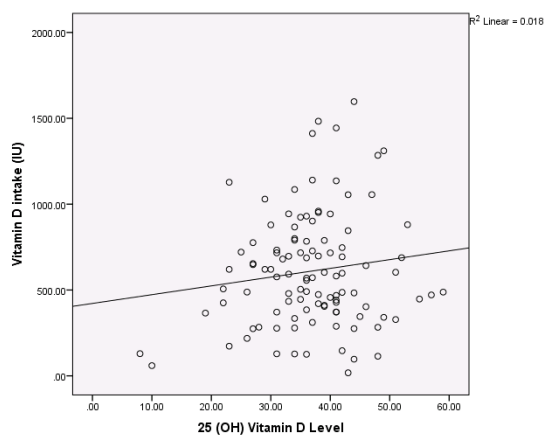


Figure 1: Bivariate Analysis to Examine Correlation Between Serum 25(OH)D Level and Vitamin D Intake

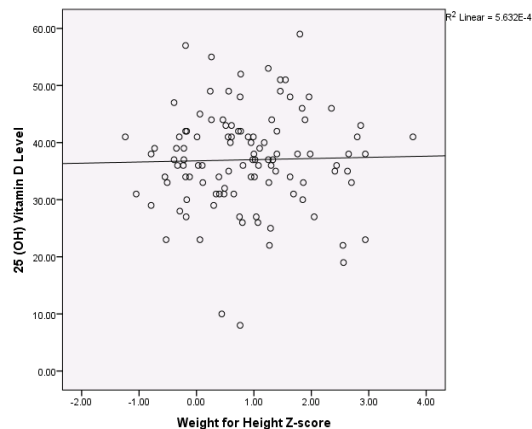


Figure 2: Bivariate Analysis to Examine Correlation Between Weight-for-Height Z-Score and Serum 25(OH)D Status

After subdivision by race and sun exposure, results continued to indicate a lack of correlation between adiposity and serum 25(OH)D. Consequently, regression analysis

was not conducted.

Relationship between Vitamin D and Calcium Intake and Adiposity

When compared across weight-for-length percentile status (low weight, normal weight, or high weight), neither mean serum 25(OH)D levels ($P=0.183$), nor mean intake of calcium ($P=0.150$) and vitamin D ($P=0.377$) varied significantly. Furthermore, a higher percentage of high weight infants and toddlers had adequate intake calcium when compared with normal weight children (calcium: 100% vs.85.6%, respectively; vitamin D: 85.7% vs. 69.4%). However, these differences were not statistically significant ($P=0.314$ and $P=0.431$ for calcium and vitamin D, respectively).

CHAPTER V

DISCUSSION AND CONCLUSIONS

Conclusions

Multivitamin, vitamin D, and calcium supplement use was very low in our population, and mean serum 25(OH) D was more than adequate when compared to IOM recommendations. The rate of high weight in our study sample was slightly elevated in comparison to national statistics for children <2 years of age. (11.2 vs. 9.7%).⁹ Total intake of calcium and vitamin D were high in many subjects, which may be an indication of overall high food intake. No correlation was found between vitamin D intake and 25(OH)D serostatus, or between 25(OH)D serostatus and weight-for-length z-score. Children were determined to have sufficient mean 25(OH)D serum status regardless of weight-for-length category. Average serostatus in the high weight group was lower than in normal weight children, although this difference was not significant.

Implications

We discovered that, in the present study, there was no significant correlation between serum 25(OH)D and vitamin D consumption. This contradicts the results of previous research (24), which reports a positive association between these two variables. We suspect an effect of recall bias on our dietary intake data. Also contrary to reports from prior investigations, we did not find a significant correlation between serum

⁹ Note that the national estimates cited here could have actually over-estimated prevalence of high weight in children <2 years of age due to use of the CDC 2000 Growth Charts, which define high weight for this age group as >95th percentile. Had the recommended WHO standards been used to assess the prevalence of high weight in infants and toddlers, the discrepancy between our results and the national rates may have been greater than it appears to be here (2).

25(OH)D and measures of adiposity (weight-for-length z-score). In a study of infants of approximately 9 months of age, Arnberg (2011) and colleagues found that BMI z-scores were inversely related to serum 25(OH)D (28). These results are consistent with studies on older children, which generally agree that serum 25(OH)D levels decrease with increasing measures of adiposity, as lipophilic vitamin D becomes sequestered in fat tissue. Early research indicates that adipose tissue is the primary site of vitamin D storage (21); therefore, this finding is logical. We located a few discrepancies in the literature, however. First, in study of schoolchildren, Sacke and colleagues did not find any relationship between the variables of interest (26). (As previously mentioned though, this finding was attributed, at least in part, to widespread 25(OH)D deficiency in the study sample.) Gordon and colleagues (2008) found a significant negative relationship between measures of BMI and vitamin D in toddlers (although not in infants), with each 1-unit increase in BMI decreasing vitamin D status by 2.7 ng/mL (29). Thus, there is not universal agreement on the relationship between adiposity and serum levels of vitamin D. Further demonstrating the complexity of this association in very young children, Josefson et al (2013) recently identified a direct relationship between infant adiposity and serum 25(OH)D in a sample of 48 neonates (20). Researchers suspect that the differences in vitamin D metabolism in infants are responsible for this novel finding. In addition, women with higher 25(OH)D serostatus are likely to give birth to infants with a higher percent of fat mass, as well as higher serum 25(OH)D. As all of the infants in the present study were ≥ 9 months of age, however, we would have expected our results to align more closely with those obtained in Arnberg's or Gordon's study. Elevated prevalence of high weight may have confounded our results.

A number of previous studies document an inverse relationship between adequacy of subjects' intake of calcium, vitamin D, or both nutrients, and excess adiposity. Tidwell et al (2011) reported an inverse correlation between intake of calcium and vitamin D with fat mass in adults (36). Similarly, in vitamin D sufficient children, Alemzadeh (2008) found a significantly higher intake of vitamin D, as well as a higher ratio of fat-free to fat mass than in vitamin D deficient children (25). However, as this study did not report vitamin D intake as a covariate in the regression analyses that they conducted, it is difficult to ascertain the exact effect of this variable on fat mass, although we assume that its influence was not significant.

In our work, we did not find a significant relationship between weight-for-length (adiposity) and intake of calcium and vitamin D. In this regard, our results corroborate those reported in the previously-cited article by Sackeck and colleagues (26), who did not find a relationship between subject's vitamin D intake and their BMI z-scores. Ongoing study of the roles of micronutrients in healthy weight maintenance and weight loss is important, as restriction of macronutrients (energy) below the RDA is generally not an acceptable measure in children (37). More complete understanding of the role of calcium and vitamin D in weight management would be helpful, particularly as NHANES data shows that many children in the 1-3 age range do not meet RDAs for either calcium or vitamin D (11).

Limitations and Suggestions for Further Research

The limitations of our study relate to the types of data collected and the methods of data collection used. The original study was cross-sectional in design. For this reason,

we were not able to track the variables of interest over time. Other potentially useful data absent from this study include infants' gestational age at birth, size for gestational age at birth, additional weight and height data points, maternal weight, smoking status, and serum 25(OH)D concentration, socioeconomic status, duration of breastfeeding in the mother-infant dyad and a complete food frequency questionnaire for the infant. These variables could be incorporated into future primary care center-based studies.

Due to the fact that the original study did not include the variables listed in the previous paragraph, it is not possible for us to assess whether any of them might have affected children's weight status in this sample. For example, in regard to maternal vitamin D status, after adjusting for confounders, Crozier and colleagues (2012) reported an effect of this variable on infant weight birth weight and weight at 6 years of age (14). Infants born to mothers with 25(OH)D serostatus of 75 nmol/L (30 ng/mL) had 10% greater fat mass at time of parturition than counterparts with maternal serum 25(OH)D < 50 nmol/L (20 ng/mL). These high vitamin D levels translated into 6% lower fat mass at age 6 (in comparison with mothers with low vitamin D status). In the present study, though, no data on maternal 25(OH)D status was collected.

Research has also examined additional factors affecting infant weight childhood weight trends. Reilly et al (2005) used a cohort study design to identify a set of variables in infancy that are significantly linked to obesity risk at age 7 (38). Variables included perinatal factors (including maternal smoking), one or both of the child's parents being obese, lack of physical activity, and curtailed sleeping hours. A weak association between poor diet at age 3 and obesity at age 7 was also noted. Feeding practices during infancy were not found to have a significant effect on obesity risk. No information on

smoking was provided in the original study, however.

Finally, it is important to acknowledge that our study population was drawn from relatively low-income families in the Pittsburgh area, many of whom were likely enrolled in Special Supplemental Nutrition Program for Women, Infants, and Children. In a 2012 study, Shim et al documented the high prevalence of “short breastfeeding duration” and “early introduction of solids” in the WIC population when compared to non-WIC recipients ($P < 0.0001$) (39). In addition, although Reilly did not find a significant effect of feeding practices on obesity risks, other studies document a “modest” protective effect of breastfeeding against high weight, related to duration and status as the infant’s sole food source through 6 months of age (40). Thus, many different factors affect infants’ and children’s weight status. At this time, it is not possible to identify which, if any, of the above-mentioned variables might have impacted the infants and toddlers in our study population.

Although we used weight-for-length percentile status and z-scores to approximate adiposity, our results may be improved by using more direct measures of adiposity, such as waist circumference or skinfolds (26, 27), dual X-ray absorptiometry (37), or bioelectrical impedance analysis (25) to evaluate subject’s body composition. Crozier, however, notes complications in using DEXA with infants due to restlessness and relatively low bone mineral density (14). Also in regard to anthropometric measurements, we noted a high percentage of overweight and obese children. Although the figures that we obtained are not completely implausible, we acknowledge that the possibility of measurement error always exists, particularly when assessing infants’ recumbent lengths.

Furthermore, we believe that information on dietary intake may have been skewed by parents' perception of serving sizes (i.e. what constitutes a cup, an ounce, etc.) and difficulty recalling the requested information. Future studies of this kind could assign a nutrition professional to conduct dietary interviews with parents. Food models and measuring instruments could serve as a helpful a reference (37). Alternatively, parents could be given the option of completing surveys at home, although this provision might increase difficulties with follow-up. With weighted food records and the comparison of reported food intake with expected energy expenditure (40), though, we would likely improve the accuracy of the data collection process.

In addition to the adaptations mentioned above, future research in this area would benefit from longitudinal study design with larger sample sizes with more evenly-distributed weight-for-length and vitamin D status. In conclusion, rates of infant overweight and obesity in our sample surpass the national average. Our results do not support a relationship between calcium and vitamin D intake on weight status or an association between serum vitamin D and body composition in infants and young children.

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APPENDIX A

VITAMIN D & SUNLIGHT EXPOSURE QUESTIONNAIRE

Subject ID: _____ Subject Initials: _____ Interview Date: _____

1. Date of Birth: _____

2. What is your child's age: _____

3. Height (cm): _____

4. Weight (kg): _____

5. Ethnic Group: Is your child Hispanic or Latino?

- Yes No Unknown/Declined to answer

6. Race: what do you consider your child's race to be?

- American Indian/ Alaskan Native Asian American
 Native Hawaiian/ Pacific Islander Black or African American
 White or Caucasian More than one race

7. Does your child take a multivitamin? Yes No

If yes,
Specific brand (s):

How often does he/she take the Multivitamin?

- Daily Not Daily
How many times per month: _____

8. Does your child take a calcium supplement? Yes No

If yes,
Specific brand (s):

How often does he/she take the Calcium supplement?

- Daily Not Daily
How many times per month: _____

9. Does your child take a vitamin D supplement? Yes No

If yes,

Specific brand (s): _____

How often does he/she take the Vitamin D supplement?

Daily

Not Daily

How many times per month: _____

If no, why not?

Never received a recommendation to do so

Too much hassle

Too expensive

Child doesn't like them

Other _____

10. Does your child take Cod Liver Oil? Yes No

If yes,

Specify how much per day: _____

Specific brand (s) : _____

11. On average, how many glasses (8 ounce/glass) of milk does your child drink per day?

12. Does your child drink an infant formula like Enfamil®, Similac®, or Goodstart®?

Yes

No

If yes,

How many ounces per day:

Specify brand(s): _____

13. Is your infant/child currently being breastfed or does your infant/child receive breastmilk?

Yes

No

If yes and the mother is using a breast pump,

How many ounces of breastmilk does your child drink per day? _____

If yes and the mother is nursing,

How many times per day is the infant/child nursing? _____

14. Besides milk, does your child drink/eat other dairy foods that may have been fortified with vitamin D?

If yes,

How many glasses (8ounce/glass) of Soymilk or Lactaid milk or Chocolate milk does your child drink per day?

How many servings of cheese (1 ounce or 1 slice/serving) does your child eat per day?..... _____

How many servings (1 cup/serving) of yogurt does your child eat per day?

15. Does your child drink vitamin D-fortified orange juice (for example: Minute Maid® Home Squeezed Style + Calcium and Vitamin D, Simply Orange® Orange Juice Calcium and Vitamin D)?

If yes,

How many glasses (8 ounce/glass) of vitamin D fortified orange juice does your child drink per day?

16. On average, how many times per month does your child eat the following foods? (please check the appropriate box on each line):

		None (0)	1x/ Month	2x/ month	3x/ month	4x/ month	More than 4 times/month
14a	Baked/fried fish						
14b	Lox (cured salmon)						
14c	Herring						
14d	Salmon						
14e	White fish						
14f	Sardines						
14g	Mackerel						
14h	Dried Mushrooms						

15. Does your child drink a nutrition supplement like Ensure®, PediaSure® or Carnation® Instant Breakfast? Yes No

If yes,

How many servings (8 ounces or 1 package/serving) per day: _____

Specify brand(s): _____

16. Does your child eat breakfast cereal? Yes No

If yes,

How many bowls (~2 cups/bowl) per week: _____

Specify brand(s): _____

17. Does your child eat breakfast bars or protein bars? Yes No

If yes,

How many servings (1 bar/serving) per week: _____

Specify brand(s): _____

18. Does your child drink a toddler formula like Enfagrow™ Premium™ Toddler or Gerber® Good Start® 2 formula? Yes

No

If yes,

How many ounces per day: _____

Specify brand(s): _____

19. Does your child eat infant cereal like Gerber®? Yes No

If yes,

How many ounces per day: _____

Specify brand(s): _____

20. Does your child eat jarred baby food? Yes No

If yes,

How many ounces per day: _____

Specify brand(s): _____

21. On average in the summer how many hours per day does your child spend outside in the sun each day?

2 hours or less

More than 2 hours

If more than 2 hours, how many hours: _____

22. When your child spends time outside, which of the following body parts are usually exposed?

22a.	Face	<input type="checkbox"/> Yes	<input type="checkbox"/> No
22b.	Hands	<input type="checkbox"/> Yes	<input type="checkbox"/> No
22c.	Arms	<input type="checkbox"/> Yes	<input type="checkbox"/> No
22d.	Legs	<input type="checkbox"/> Yes	<input type="checkbox"/> No

23. Do you apply sunscreen on your child when he or she goes outside? Yes No

If yes,

23a. What brand (s) do you use? _____

23b. What SPF (Sun Protection Factor) do you use? _____

23c. How often do you use sunscreen on your child?
 Often Sometimes Seldom

24. Did your child travel to a sunny location (like Florida, Texas, etc)? Yes No

If yes,

24a. Where did your child visit: _____

24b. When last did your child travel: _____ year _____ month _____

24c. How many days did your child spend in the sunny location: _____