A COMPARATIVE STUDY OF BONE NUTRIENT LEVELS IN CHILDREN WITH AND WITHOUT AUTISM

A Thesis

Presented in Partial Fulfillment of the Requirements for

the Degree Master of Science in the Graduate

School of The Ohio State University

By

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ABSTRACT

As a growing developmental disorder in the United States, autism has several eating behaviors that may increase the risk for bone nutrient deficiencies in children. This study was a review study comparing dietary intakes of bone nutrients in autistic and typical children from two previous studies.

Data from both the RUPP (n = 113) and the NHANES studies (n = 7,693) were used to analyze intakes in autistic children and typical children, aged 4 – 13 years old, respectively. Calcium and magnesium levels were found to be lower in autistic children than typical children. Vitamin C intakes were high in the typical children, while low in the autistic children. Of bone nutrients examined in autistic children, vitamin D had the lowest AI percentage with the widest range (2.99% to 268.89%). These data justify the necessity for further studies examining the link between autism and deficient bone nutrient levels, specifically, vitamin D.
DEDICATIONS

To my family, friends, and dog, Brutus
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CHAPTER 1

INTRODUCTION

Background and Significance of the Problem

Autism is one of the fastest growing serious developmental disabilities in the United States. With every 1 in 150 children diagnosed with autism, this condition is more common than pediatric cancer, diabetes and AIDS combined.\(^1\) In addition, boys are four times more likely to have autism than girls.\(^1\) Autism is a part of a group of developmental disorders known as autism spectrum disorders (ASD) which includes: Asperger syndrome, Rett syndrome, childhood disintegrative disorder, and pervasive developmental disorder not otherwise specified (PDD-NOS).\(^2\) Typically, severe deficits in social interaction and communication, as well as stereotyped and repetitive behaviors are common in ASD and potentially last throughout a person’s lifetime.\(^1,2,3\) Because of the increase in autism cases, there has been a surge in research studies targeting children with ASD and as a result, a rise in public awareness during these years. However, the question of whether there really is a higher prevalence of children with autism, or if diagnosis criteria has simply changed throughout the years is unclear.\(^3,4\)
Not only is autism neurodevelopmental, but it is also an intellectual disability filled with many challenging behaviors such as unusual eating habits, rigid and repetitive routines, abnormal sleep patterns, temper tantrums, and aggression to self and to others.\(^6\,7\)

Many clinicians and parents of children with ASD continually report feeding difficulties and problem eating behaviors as some of the most challenging (behaviors) in these children. Because children with autism often present with feeding problems that many times inhibit their desire to eat certain foods or even eat at all, their diets may be inadequate to meet their nutrition needs possibly resulting in multiple deficiencies for the child. Various studies have been done regarding diet quality of autistic children and/or vitamin D and its relation to bone health, but little research has been found concerning the vitamins C, D and K, calcium, and magnesium present in the diets of children with autism. To our knowledge, no studies have examined levels of bone health vitamins in both children with autism and typical children, as a means of comparison.

Because of the risk for osteopenia, rickets and fractures with decreased vitamin D and bone health nutrients, practitioners working with autism patients should be well aware of vitamin levels to ensure that patients are receiving adequate amounts for bone formation, and to prepare for crucial adolescent bone development as they grow and develop.
Objectives

The primary objectives of this study were to:

1) Compare dietary data from the Research Units on Pediatric Psychopharmacotherapy (RUPP) study and the NHANES regarding nutrients involved in bone health in children

2) Examine the relationships between bone health and autism in children by identifying vitamins C, D and K, calcium and magnesium levels of children in the RUPP study

Research Questions

This study sought to discover whether children with autism have different vitamin D and other bone health nutrient intakes when compared to typical children. This study addressed the following:

1) How does vitamin D and other bone health nutrient levels in children with autism compare to levels in typical children?

2) Is there a relationship between levels of vitamin D and other bone health nutrients in autistic children from the RUPP study?

Research Approach

This study was conducted as a review of dietary intake data from two previously conducted studies: NHANES survey data from 1999-2006 and RUPP study conducted from 2005-2007.
List of Definitions

**Adequate Intake (AI):** Nutrient intake that appears to support good health though not firmly believed to be beneficial in all age groups of population; used when RDAs have not been established

**Anti-epileptic drugs (AED):** Group of medications used in the treatment of seizures, usually from epilepsy; used to suppress the excessive firing of neurons

**Autism Spectrum Disorder (ASD):** A group of spectrum disorders characterized by deficits in social interactions and communication, and repetitive or stereotyped behaviors; includes autism, Asperger’s Syndrome, and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS)

**Asperger’s Syndrome:** a mild form of autism spectrum disorder in which people have autism-like symptoms, though normal intelligence and verbal skills

**Bone cortical thickness:** A form of measurement of the compact bone that functions to provide movement, protect organs, and store minerals

**Dual Energy X-Ray Absorptiometry (DXA/DEXA):** A scanner that is used to measure bone mineral density; consists of two different X-ray beams that are used to measure soft tissue and bones of the patient

**Dietary Reference Intake (DRI):** System of nutrition guidelines composed of recommended dietary allowances (RDA), estimated average requirements (EAR), adequate intakes (AI), and tolerable upper limit intakes (UL); used in USA and Canada

**Estimated Average Requirements (EAR):** Amount of the nutrient that is thought to meet the requirements of 50% of healthy people in that age population
**Hypotonia:** low muscle tone; often involved in reduced muscle strength

**NHANES:** National Health and Nutrition Examination Survey; a cross-sectional study examining both physical examinations and interviews of the United States population

**Osteopenia:** lower than normal bone mineral density; often thought to be a precursor to osteoporosis

**Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS):** A milder form of autism spectrum disorders; refers to those persons with some, but not all, of the characteristics of autism

**Research Units on Pediatric Psychopharmacology (RUPP):** A network of researchers dedicated to multi-site clinical trials in children, particularly those affected with mental illness
List of Abbreviations

AED ......................... Antiepileptic drugs
AI ............................. Adequate Intake
ASD ......................... Autism Spectrum Disorder
CADI .......................... Computer-Assisted Dietary Interview
CDC ........................... Centers for Disease Control
DRI ........................... Dietary Reference Intake
DHA ........................... Docosahexaenoic Acid
EAR ........................... Estimated Average Requirements
FFQ ........................... Food Frequency Questionnaire
FNDDS ....................... Food and Nutrition Database for Dietary Studies
GERD ......................... Gastroesophageal Reflux Disease
GFCF .......................... Gluten-Free, Casein-Free
GI ............................. Gastrointestinal
MEC ........................... Mobile Examination Center
NCHS ......................... National Center for Health Statistics
NHANES ...................... National Health and Nutrition Examination Survey
PDD-NOS ...................... Pervasive Developmental Disorder-Not Otherwise Specified
RDA ........................... Recommended Dietary Allowance
RNI ........................... Reference Nutrient Intake
RUPP ......................... Research Units on Pediatric Psychopharmacology
USDA ......................... United States Department of Agriculture
CHAPTER 2

REVIEW OF LITERATURE

Introduction

Autism is a neurodevelopmental disorder characterized by deficits in both verbal and non-verbal communications, problems with social interactions, and stereotyped and/or repetitive behaviors.\(^8\) Discovered in 1943, Leo Kanner first described the syndrome that we now associate with ASD.\(^9\) Though there has been much research on autism spectrum disorders recently, the etiology still remains unclear and the disorder is poorly understood.\(^10\) However, it is known that autism has a large genetic component, although the exact genetic mechanisms are not currently known.\(^10,11\) Diagnosis is often made before a child is 3 years of age, since these children appear physically normal and early development may be unremarkable.\(^12,13\) A triad of impaired developmental markers is used for characterizing children within the autism spectrum disorder.\(^13\) These three markers consist of poor social interaction, impaired use of communication skills, and the absence of imagination in play.\(^13\) While all children diagnosed with autism may share the same areas of difficulty in the triad, they will all be affected in many different ways by this condition, all varying in different degrees of severity.\(^14,15\)
Often, the analogy of a jigsaw puzzle is used in describing ASD, in which some people have a few pieces of the puzzle, and others have many.\textsuperscript{12} Also, the jigsaw puzzle represents a symbol for autism because autism is a complex and puzzling.

In addition to deficits in socialization and communication, children with autism often present with ritualistic behavior and stereotypical behaviors that appear to be obsessive in nature such as clinging to certain routines or schedules.\textsuperscript{12,13} Often, these behaviors tend to carry over into their feeding and food behaviors leading to bizarre eating habits and restrictive or “picky” diets, causing feeding difficulties and much frustration for parents.\textsuperscript{12,13}

For many years, parents of children with autism spectrum disorders have reported troubles in getting their children to eat.\textsuperscript{16} It is generally accepted by many autism clinicians and the written literature that these children frequently have significant eating difficulties with highly restricted range of food choices.\textsuperscript{5,16} In a study by Cornish comparing feeding difficulties in both children with and without autism, 94% of parents with children with autism reported feeding difficulties compared to 59% of parents with normally developing children.\textsuperscript{13} Idiosyncratic food preferences and/or unusual feeding or presentation behaviors are not uncommon in children with autism.\textsuperscript{12,16} Diets limited in variety, aversions to textures and tastes (i.e. eating only crunchy or salty food items), and presentation behaviors (i.e. only drinking from baby bottles) are just a few of many feeding behaviors that present in these children.\textsuperscript{12,16} Problem eating behaviors can also limit the amount of opportunities for children with autism to participate in social
activities with peers and family members, in addition to causing significant stress for families.\textsuperscript{12,16} Eating behaviors are often considered in the process of diagnosing autism spectrum disorders.

Medical disorders or symptoms such as seizures, immune system deregulation, and gastrointestinal (GI) symptoms including diarrhea, gastroesophageal reflux disease (GERD), constipation, abdominal pain, and others are often associated with children with autism.\textsuperscript{10} Pica, the ingestion of non-food items, also has an increased prevalence in children with autism and could cause some of the above symptoms as well as interfere with nutrient absorption and digestion.\textsuperscript{17} Parents of autistic children with any feeding difficulties (including selectiveness, food refusal or oral motor delays) may closely monitor the certain foods that are exacerbating the symptoms in their children, as the children may decrease their desire for this food over time with symptom development.\textsuperscript{10} Because of the increased GI symptoms in these children, treatments focusing on the GI tract such as elimination diets (gluten- or casein-free diets) and use of supplements have been used to decrease frequency of symptoms or to better regulate GI function.\textsuperscript{10} These special diets, in combination with the child’s restricted diets, have been studied to examine the effects of gluten and casein withdrawal from the diet of ASD children.\textsuperscript{15,18}

\textit{Nutritional Deficiencies in Children with and without Autism}

Throughout the United States, nutritional deficiencies in the general population are widespread. Approximately 30\% of Americans have low vitamin C status, and an additional 15\% of the population is actually considered vitamin C deficient.\textsuperscript{18} Vitamin C is not the only vitamin that has been found to be in low or deficient levels in the
American public. Other vitamins and minerals such as calcium, iron, folic acid, magnesium, and vitamin D remain a significant problem in the United States. Inadequate nutrient intake may also be a problem in children with autism because of the restrictive eating patterns of children with autism. There seems to be mixed results on whether autistic children are truly nutrient deficient in their diets.

Some researchers suggest that there is not a difference in nutrient intakes between children with ASD and children without ASD. The most current study, a cross-sectional descriptive study by Lockner et al. investigated dietary intake and parents’ reported perceptions of food behaviors of 20 3- to 5-year-old children with ASD. Twenty typically developing children matched for age, sex and ethnicity were also studied as a case-control comparison. A 3-day food record was used to determine nutrient intake and the food records were adjusted for day-to-day variation to determine the usual intake distribution for the two groups. In this study, nutrient intake was similar for both groups of children with the majority of children consuming more than the recommended amounts for most nutrients. Lockner at al. found that children with ASD were more likely to consume a vitamin/mineral supplement than typically developing children. Results showed that while nutrient intakes were similar between participants, the nutrients least likely to be consumed (by both groups of children) in recommended amounts were vitamins A and E, fiber and calcium.

Raiten and Massaro conducted a study using diet records to compare the nutritional intake of 40 autistic children with 34 non-autistic age-matched control children over seven days. The parent or primary caregiver was responsible for keeping the diet record, and participants were asked to complete a questionnaire on nutrition and
health issues, attitudes and beliefs about nutrition, and nutrition knowledge. Results showed that the autistic group had a significantly greater intake of all nutrients studied including: protein, carbohydrates, niacin, thiamine, riboflavin, calcium, phosphorus, and iron. Primary caregivers/parents of autistic children reported a more positive belief in the relationship between diet and behavior, and also a more positive attitude about the importance of nutrition. A higher incidence of pica, food cravings and eating problems was also noted by the parents of children with autism.

Shearer et al.\textsuperscript{21} conducted a quantitative evaluation of the nutrient intakes of autistic children to determine if their diets were sufficient, and to compare their nutrient intakes to those of typical children. Nutrient and mineral intakes of calcium, zinc, magnesium, copper, lead and cadmium were evaluated from scalp hair samples 12 children with autism and 12 typical children serving as the control group. The comparisons between the two groups were made by examination of 3-day intake diaries from the study participants. Vitamin and mineral supplements were not recorded. Results of the study indicated that the only statistically significant mineral difference found was a lower cadmium level in the autistic children and the clinical significance of this finding was reported as unknown. Autistic children were found to have a lower calcium and riboflavin intake than the controls, but still remained $> 100\%$ of the recommended daily amount of all nutrients, including calcium and riboflavin.

While there are studies supporting the idea that children with ASD have the same nutrient profile as children without ASD, several other studies prove otherwise. Six studies showed that children with autism had lower nutrient levels than those of children
without ASD. Many studies that revealed nutrient deficiencies in autistic children found on average, much lower levels of vitamins A, C, D, and E, and all B vitamins (except choline) as well as zinc, magnesium, and selenium in this population of children.\textsuperscript{18}

Cannell\textsuperscript{22} reviewed multiple studies related to the etiology and epidemiology of autism in the last 20 years. Animal data in multiple studies showed that severe vitamin D deficiency during gestation causes dozens of proteins involved in adult brain development to be disrupted. A lack of pathological specimens from infants with autism prohibits the full elucidations of the similarities of animal and human pathology, thus it is hard to determine the effects of vitamin D deficiency on brain development in humans. However, it has been shown that severe gestational vitamin D deficiency in rats leads to rat pups with increased brain size and enlarged vesicles, abnormalities similar to those found in autistic children.

Lindsay et al.\textsuperscript{23} conducted a study focusing on the nutritional status and impact of risperidone on dietary status in 20 children with autism, who were participating in a randomized, placebo-controlled trial of risperidone for disruptive behaviors. Prior to beginning the double-blind phase of the study, quantitative Food Frequency Questionnaires (FFQ) were used to measure dietary intake of 20 of the 101 children participating in the RUPP risperidone study. Parents or primary caretakers were asked to complete the FFQs based on nutrient intake over the last month. During the double-blind phase of risperidone treatment, nine FFQs were available for those children receiving risperidone and eight FFQs were completed for children receiving placebo. Results of the study showed deficiencies in the baseline nutrients for autism subjects. The deficiencies were not representative of the group as a whole, but rather individual
participants in the study in which <80% of the DRI was met. Overall mean intakes were above Dietary Reference Intakes (DRIs). Intakes were low for calcium (9 of the 20 participants), pantothenic acid (6 of 20), vitamin D (5 of 20), and vitamin K (8 of 20). The calcium, vitamin D, and possibly vitamin K intakes may have corresponded with the low or no dairy intake by these children.23

Strambi et al.24 conducted a study comparing plasma and erythrocyte concentrations of magnesium in children with autism, children with ASD, girls with Rett syndrome, and healthy age-matched controls. Thirty-four patients ranging in age from 15 months to 11 years old who were admitted consecutively to the Child Neuropsychiatry Unit at Siena Hospital in Siena, Italy served as the subjects for the study along with 14 typical children to serve as the controls. Participant makeup included 12 autistic children (10 boys, 2 girls), 17 children with other autistic spectrum disorders (14 boys, 3 girls), 5 girls with classic Rett syndrome, and 14 typical children (7 boys, 7 girls) of the same age. Blood samples of 5 mL were taken from each child. Results showed that there were no differences in intracellular Mg between controls and other subjects; however, autistic children and children with other autism spectrum disorders had significantly lower plasma concentrations of Mg than normal subjects (p = 0.013 and p =0.02, respectively.

A randomized, double-blind, placebo-controlled pilot study conducted by Adams and Holloway18 examined the effects of a moderate dose multivitamin/mineral supplement in children with autism and also the levels of vitamin B<sub>6</sub>, vitamin C, and alpha lipoic acid in these children to determine if a multivitamin supplement would be helpful in reducing symptoms of autism. Prior to supplementation, researchers compared baseline data with typical children of the same age. Supplementation lasted three months
and participants were encouraged not to make any changes in psychiatric medications, nutritional supplements, diet, or behavioral therapies. Results indicated that no significant differences occurred between the children in the placebo and supplement groups for B$_6$ levels (54 and 56 µg/mL, respectively), though both group’s levels were well above the references range of 22-47 µg/mL for typical children ages 3-16. When these results were compared to typical children not taking vitamin supplements, the difference between the total autism group and the typical children was statistically significant ($p < .001$). The B$_6$ measurement includes pyridoxine, pyridoxal, pyridoxamine, and their phosphorylated forms. At the end of the study, vitamin C levels in the placebo group were significantly below average for typical children at 1.0 mg/100 mL. Interestingly, the supplemented group was also below average at 1.33 mg/100 mL even with the high dose of vitamin C, showing that high-dose supplementation was only partially helpful to children with autism in this study.

Cornish$^{15}$ conducted a study to determine whether gluten-free, casein-free diets placed children with autism at risk of nutrient deficiency and comparisons were made with ASD children not following the gluten and/or casein free diets. A questionnaire was sent to parents of children aged 3-16 years, diagnosed with ASD, and detailed dietary information and a 3-day food diary were collected in these patients. The sample size was small with 8 subjects using gluten/casein free diets and 29 subjects not following any diet. Results showed that nutrient intakes fell below the Lower Reference Nutrient Intake (LRNI) in 12 children (32%) for zinc, calcium, iron, vitamin A, vitamin B12 and riboflavin in the non-diet group and four children (50%) for zinc and calcium in the diet group. The diet group had higher levels of fruit and vegetable intake and lower intakes of
cereal, bread and potato than the non-diet subjects. The study concluded that the exclusion of gluten and/or casein did not adversely affect the nutrient intake of the diet children, but did not significantly improve it either.

In a similar study, Cornish\textsuperscript{13} investigated the dietary intake and behavior of children diagnosed within the autism spectrum. Parents of 17 autistic children aged 42-117 months were interviewed and asked to do a 3-day dietary recall for foods eaten by their child. The parents also filled out food frequency questionnaires related to food consumption patterns of their child to help assess nutrient intake. Results indicated that nutrient intakes were below the reference nutrient intake (RNI) levels for 53\% of the children in one or more of the following: vitamin C, iron, vitamin D, niacin, riboflavin, vitamin B\textsubscript{6}, calcium and zinc. A few children reported extremely low nutrient intakes with one child falling below the LRNI for iron and three children falling below the LRNI for vitamin D from dietary sources. Due to high milk consumption in 13 children, calcium and riboflavin intake raised to levels above 200\% of RNI levels. Findings also indicated that where some children would be deficient in any one nutrient, the quantities of foods chosen by the child as the preferred food were eaten in such large quantities in a continuous snacking pattern thereby making up the nutrient intakes from unusual sources such as calcium from bread or vitamin C from milk. These preferred foods along with fortified foods can be used to meet nutrient intake while working towards a more varied diet.
Common Dietary Supplements for Children with Autism

Other nutrient deficiency studies have been conducted in children with autism examining whether mega-doses of certain nutrients are beneficial in resolving or lessening the symptoms in this population. Supplementation of vitamin C has been of interest to many clinicians for its antioxidant role in the autistic child.25, 26 Vitamin B₆ and magnesium (given together), omega-3 fatty acids and melatonin are the most common mega-dose nutrients in children with autism.27, 31, 32, 34, 35

In a 30-week, double-blind, placebo-controlled trial with autistic children aged 6 to 19 years old, Dolske et al.26 supplemented the children with oral vitamin C by use of a crossover design. The design consisted of three 10-week time frames, where all subjects were supplemented with vitamin C for the first 10 weeks, and then in the last two phases of the study, half of the children were randomly assigned to placebo and then vitamin C supplementation, and then the remaining half were assigned to vitamin C supplementation and then placebo. Testing for typical symptoms and behaviors seen in autistic children was completed after each 10-week phase. Results showed improvements in social, affective, sensory and language behaviors in the group of children who went from placebo to vitamin C supplementation. The group of children who were supplemented first and then received placebo (in the last phase) had worsening of symptoms and behaviors (P = 0.02). Thus, this study concluded that vitamin C and its antioxidant qualities may have beneficial effects on lessening problem behaviors and autistic symptoms in children with ASD.

Nye and Brice27 conducted a systematic review to determine the efficacy of vitamin B₆ and magnesium for treating social, communication and behavioral responses
of children and adults with autism. All of the studies were required to meet rigorous
criteria for selection in the systematic review. Criteria for inclusion included studies in
which participants were already diagnosed with autism spectrum disorders and were
randomly allocated prior to intervention and in which outcomes were then compared to
either a placebo or non-treated group were also included in the review. Results from the
systematic review showed that 3 studies\textsuperscript{28, 29, 30} were identified as high quality. In all
three of the studies, no statistically significant differences or effects were found between
the treatment and control groups. The study concluded that no recommendations could
be advanced at this time regarding the use of B\textsubscript{6} and Mg as a treatment for autism.

Mousain-Bosc et al.\textsuperscript{31} conducted a study in 33 children with PDD syndrome to
study changes in clinical symptoms through vitamin B\textsubscript{6}-Mg supplementation. A group of
36 children without PDD syndrome served as the control, and all children were followed
for at least 6 months. All PDD children were given a B\textsubscript{6}-Mg supplement (6 mg/kg/d Mg, 0.6 mg/kg/d B\textsubscript{6}) and no other medical treatments were given to these children during the
study period. Intra-erythrocyte Mg\textsuperscript{2+}, serum Mg\textsuperscript{2+}, and blood ionized Ca\textsuperscript{2+} were
measured before and after the treatment. Results showed that while lab values of serum
Mg\textsuperscript{2+} and blood ionized Ca\textsuperscript{2+} did not statistically differ from the control children, intra-
erthrocyte Mg\textsuperscript{2+} were significantly lower in PDD children, even after treatment.
However, after the treatment, there was a statically significant increase in erythrocyte
Mg\textsuperscript{2+} in PDD children (Wilcoxon test \( p = 0.0198 \)). Supplementation improved
symptoms in 23 of 33 PDD children (\( p<0.0001 \)) with no adverse affects including
improvements in social interactions, communication, stereotyped restricted behaviors,
and abnormal/delayed functioning. Symptoms reappeared in the PDD children once the
B₆-Mg supplementation was stopped. The study attributed the behavioral improvement seen with the B₆-Mg supplementation in PDD children to an association with concomitant modifications of the erythrocyte Mg²⁺ values.

Amminger et al.³² conducted a randomized, placebo-controlled, 6-week pilot trial investigating the effects of 1.5g/d of omega-3 fatty acid supplementation in 13 children with autistic spectrum disorders ranging in age from 5 to 17 years old. The children had severe behavioral symptoms including severe tantrums, aggression, and self-injurious behaviors. The Aberrant Behavior Checklist (ABC) was used as the outcome measure at the end of the 6-week study. There was an observed advantage of omega-3 fatty acid supplementation compared with placebo for hyperactivity and stereotypy in the children with autism. Large effect sizes were seen in both hyperactivity and stereotypy. No adverse effects were elicited in either group.

Schultz et al.³³ conducted a case-control study to determine whether breastfeeding or the use of infant formula supplementation of docosahexaenoic acid (DHA) and arachidonic acids and is associated with autism. Data from an online parental study, the Autism Internet Research Survey, was collected from 861 children with autism and 123 control children. The study found that using infant formulas lacking added docosahexaenoic acid (DHA) and arachidonic acids was associated with a 4.41 increased odds of developing ASD as compared to exclusive breast feeding for more than six months in all cases, and when cases were limited to those children with regression in development, there was a 12.96 increased chance of developing ASD.

Wirojanan et al.³⁴ conducted a 4-week, randomized, double blind, placebo-controlled crossover design to determine the efficacy of melatonin treatment for sleep
problems in children with autism and fragile X syndrome (FXS). Twelve participants had been assessed for autism spectrum disorders and DNA tested for FXS. Eight participants of the 12 participants had a diagnosis of ASD (5 participants only had ASD and 3 participants had FXS with ASD). This study began following a 1-week baseline period. Placebo or 3 mg melatonin was given to participants for two weeks, and then they crossed over to for an additional two weeks. Researchers examined sleep variables consisting of sleep onset time, total night sleep duration, sleep latency and number of night awakenings (recorded by actigraphy). Results showed that 10 of 12 participants improved their sleep duration on melatonin compared with placebo (p = 0.012). Nine participants had earlier sleep onset and shorter sleep latency during treatment than placebo. The study concluded that the results of this study fully support the tolerability and efficacy of melatonin treatment on sleep problems of children with ASD and FXS.

In a randomized, placebo-controlled, double-blind, crossover study, Garstand and Wallis\textsuperscript{35} aimed to determine whether melatonin was an effective treatment for children with sleep problems in autism spectrum disorder (ASD). Eleven children between the ages of 4 and 16 years old, with a diagnosis of ASD and significant difficulties sleeping at night were enrolled in the study. Those children who had used melatonin before were excluded from the study. Participants were randomized at the start of the study regarding the sequence in which the two treatments, placebo or 5 mg melatonin, would be received. The children took each medication for 4 weeks with a washout period of one week in between the two treatments. Parents were asked to complete sleep pattern charts for the children at baseline, using the charts for 1 week before the treatments began, and continuing the charting until the end of the study. Each sleep chart included information
about sleep latency, total sleep time, night awakenings and morning awakenings. Results showed that 7 participants completed the study. A significant reduction in sleep latency and increased sleep time were found in the participants who were receiving melatonin. Although the study was small, the study concluded that melatonin does show effectiveness in children with sleep difficulties and ASD.

**Bone Health in Children with Autism**

In recent years, more studies have focused more on bone health and its involvement with autism. Hedinger et al.\(^{36}\) found that dairy-free and unconventional food preferences could put boys with autism and ASD at higher than normal risk for thinner, less dense bones when compared to a group of boys the same age who do not have autism. Researchers believe there are multiple factors that put these boys at higher risk for weak bone development. These include lack of exercise, a reluctance to eat a varied diet, lack of vitamin D, digestive problems, and diets that exclude casein, which are a popular diet among parents of children with autism. Casein is a protein found in milk and milk products which when excluded from the diet could be linked to the lack of vitamin D in children with autism as well.

In the Hedinger et al.\(^{36}\) study, researchers took a hand-wrist X-ray of the left hand of the boys with ASD or autism at the initial assessment for bone age. They assessed the cortical thickness of the second metacarpal on the hand-wrist X-rays and compared its development to a standardized reference based on a group of boys without autism. Researchers found that the bones of boys with autism were growing longer but were not thickening at a normal rate. At ages 5-6 years, the cortical bone thickness of the autistic
boys had significantly thinned out, and the difference only became more profound at ages 7-8. Those boys with autism on the casein-free diets also had the thinnest bones of all the boys. Their bone cortical thickness was about 19% thinner than typical boys of the same age, almost double the amount of the boys on unrestricted diets at 10.5% thinner than the typical boys.

Hedinger et al. found that the reported decrease in bone cortical thickness seemed to occur around age 6 years which could have resulted as a cumulative effect from chronically low calcium and vitamin D. As young children with GI issues and autism symptoms, they are commonly switched to a casein-free diet (basically dairy-free) to moderate their symptoms therefore eliminating the major dietary source of calcium and vitamin D in typical young children. Since dairy products, the source of casein, account for the bulk of calcium consumption in young children (~72%) and when fortified, are one of the best sources of vitamin D, they can become deficient in these nutrients unless replaced by supplements.

Results of this study showed that there may be a potential negative impact of gluten- and casein-free diets on bone development in children with autism. Although further research is still needed to confirm results of these studies, researchers encouraged parents and health care practitioners to be aware of the potential risk for decreased bone development with these exclusion diets in children with autism.

Many children with autism also have seizures, which puts the child at a significantly higher risk for feeding problems, GI dysfunction, and malnutrition. Because of the seizures, most of the children are put on antiepileptic drugs (AEDs), which may negatively impact nutritional status and quality of life. The nutritional
impacts are most often drug- or dose-dependent affecting the GI tract, hepatic or renal function, and/or bone density among the many others. The use of certain AEDs may deplete nutrients more than others so supplements may be recommended to the patients. Nine AEDs were included in this book chapter with the nutrients affected while taking the drug. The most commonly affected nutrients were calcium and vitamin D together, as well as in addition to other nutrients such as folate and/or carnitine. With long-term AED use in children, impaired osteoid synthesis and calcification, osteoporosis, fracture, and rickets have been reported. However, when given adequate amounts of calcium, bone density and serum calcium/phosphorus levels return to normal levels, even in children who have been on long-term AEDs.

**Vitamin D and its Relationship in Autistic Children**

Vitamin D is a fat-soluble vitamin and a steroid hormone that is naturally present in very few foods, added to others, and available as a dietary supplement. It can be obtained through diet or synthesized in the body when skin is exposed to ultraviolet (UV) radiation in sunlight. Because vitamin D from sun exposure, food, and supplements is biologically inert, it must undergo two hydroxylations to activate these physiological relevant forms of vitamin D in the body. The two forms of vitamin D are ergocalciferol (D$_2$) and cholecalciferol (D$_3$). Ergocalciferol is produced from UV irradiation of the plant steroid, ergosterol. This form is not produced in the human body, but is commonly sold commercially. However, another steroid, 5,7-cholestradienol, which is also known as 7-dehydrocholesterol is found in animals and humans and when exposed to UV light, cholecalciferol is produced. Both of these
forms can be obtained from the diet, though cholecalciferol is produced in humans and higher animals when the skin is exposed to sunlight.\textsuperscript{42, 43} The first hydroxylation then occurs in the liver where vitamin D is converted to form 25-hydroxycholecalciferol (25(OH)D\textsubscript{3} or calcidiol).\textsuperscript{40, 42} From the liver, 25-hydroxycholecalciferol is further hydroxylated in the kidneys into 1,25-dihydroxycholecalciferol, ((1,25(OH)\textsubscript{2}D\textsubscript{3}, or calcitriol), the main physiologically active form.\textsuperscript{40, 42, 43}

Throughout the years, vitamin D has been associated with healthy and strong bones.\textsuperscript{43} This association began many years ago when it was shown that rickets, a childhood disease characterized by improper bone development and bone softening, could be prevented by vitamin D, whether in the diet or by exposure to UV light.\textsuperscript{43} Adults deficient in vitamin D can have a similar bone softening effect known as osteomalacia, where bones are thin and brittle.\textsuperscript{40} In addition to bone development, vitamin D also plays an important role in brain development, cognitive and behavioral function and the suppression of autoimmunity.\textsuperscript{1} Currently, as many as 36\% of Americans are vitamin D deficient, and approximately 40\% of infants and toddlers have tested below the optimal blood threshold for vitamin D.\textsuperscript{44} With the recent increase in Recommended Dietary Allowance (RDA) for vitamin D from 200 IU to 400 IU, it is only likely that more Americans will be considered vitamin D deficient.\textsuperscript{45}

Autism, among other illnesses such as cancer, heart disease, and multiple sclerosis, has been associated with vitamin D deficiency.\textsuperscript{46} Dr. John Jacob Cannell, MD and Director of The Vitamin D Council, has been studying the effects of vitamin D since the late 90s and more recently, its relationship with autism. According to the “Vitamin D Theory of Autism” by Cannell, if vitamin D deficiency caused autism, all of the
unexplained questions would be answered. Due to decreased sun exposure in the last 20 years, vitamin D levels have been decreasing as rates of autism have been increasing.\textsuperscript{46} According to this theory, autism should be less common at latitudes closer to the equator.\textsuperscript{46} The CDC recently reported autism rates from 14 states, in which the state with the highest prevalence, New Jersey, was the second most northern state.\textsuperscript{4,46} Alabama was the most southern and least prevalence of autism of all 14 states.\textsuperscript{4,46} This theory speculates that the apparent sex difference in autism is due to the different effects that estrogen and testosterone have on vitamin D metabolism.\textsuperscript{46} Estrogen was found in multiple studies to have multiple enhancing effects, thought to protect the brain from vitamin D deficiencies, while testosterone exposed male brains to the same deficiencies.\textsuperscript{46} Among other characteristics of this theory are vitamin D deficiencies and: increased risk of infections; hypotonia, developmental motor delays, and decreased activity; the medication, Depakote; and seizures—all of which have been associated with children with autism.\textsuperscript{46}

**Other Nutrients Associated with Bone Health**

In addition to vitamin D, nutrients such as vitamins C and K, calcium, and magnesium are also involved in bone health. These nutrients play a vital role in conjunction with vitamin D in promoting and maintaining optimal bone health throughout life. Bone development is the most crucial beginning in childhood and continuing into young adulthood.\textsuperscript{47} During adolescence, bone formation is actually faster than bone loss, with approximately 50 percent of peak bone mass accrued during this
time. By age 18, about 90 percent of total bone mineral content is deposited and slowly continues to build until around age 30, where peak bone mass is reached. Generally, vitamin C is not known for its bone health qualities, but for its antioxidant status. Vitamin C, also known as ascorbate or ascorbic acid, is a water soluble vitamin and antioxidant that cannot be produced by the human body. Vitamin C is involved in the synthesis of carnitine, norepinephrine, and collagen as well as the tyrosine synthesis and catabolism. Vitamin C plays a role in bone health because of its ability to synthesize collagen, a structural protein found in skin, bones, tendons and cartilage. An adequate supply of vitamin C must be present for typical development and maintenance of bones, tendons and cartilage. Vitamin C also is involved in the formation of two amino acids, hydroxylysine and hydroxyproline by interacting with enzymes involved in conversion from lysine and proline to the amino acids stated previously. Vitamin C is used by the enzymes as a recycling agent to convert iron back from Fe$^{3+}$ to Fe$^{2+}$. Iron is also better absorbed when vitamin C is added to meals. Another role in bone health that vitamin C has is its relationship with calcium. Because of their acid and vitamin C content, consuming certain juices such as grapefruit or orange juice with added calcium or eating foods high in calcium is a good way to increase calcium absorption.

Vitamin K is an important nutrient known for many years to play a significant role in blood clotting. However, in the last decade, human studies have demonstrated that vitamin K can actually help improve bone health by not only increasing bone mineral density in osteoporotic people but also by reducing fracture rates. Further, the results of these studies showed that vitamins K and D work synergistically on bone density.
Perhaps, this is why emerging studies have found that vitamin K1, when given with vitamin D, may also benefit bone health. Increasing evidence has also shown that vitamin K positively affects calcium balance, a key mineral in bone metabolism.\textsuperscript{51} Among protecting against bone health and osteoporosis, vitamin K has been suggested to guard against atherosclerosis and hepatocarcinoma as well.\textsuperscript{51,52}

Calcium is the most prevalent mineral in the human body and is stored and used by the body for healthy bones and teeth, and proper function of the heart, muscles and nerves.\textsuperscript{53} About 99\% of total body calcium is found in bones and teeth.\textsuperscript{43} Because the body is unable to make calcium on its own, food is the preferred way of achieving proper calcium intake.\textsuperscript{53} Calcium is vital to building bone mass for reducing bone fractures and for support of lifelong physical activity.\textsuperscript{53,54} Because calcium is always being removed and replaced from our bones, it is important the bones are never depleted of this mineral.\textsuperscript{54} When more calcium is being removed than replaced, bones begin to get weak and may become brittle and break.\textsuperscript{54} Studies have found that when calcium is ingested with vitamin D, it is absorbed better in the intestines.\textsuperscript{54}

The fourth most common cation in the body, magnesium, is also involved in bone health.\textsuperscript{24} Magnesium has many functions, with which many overlap with those of calcium.\textsuperscript{24} It is involved in bone formation and is essential for brain development and maintenance, as well as functional well-being.\textsuperscript{24,55} Because Mg cannot be absorbed unless adequate levels of parathyroid hormone (PTH) and vitamin D are established, much oxidative damage can occur in the brain explaining the high rates of schizophrenia and autism in the winter and early spring months because of a low supply of vitamin D in the winter in some areas.\textsuperscript{55} Low levels of PTH and vitamin D contribute to low blood
levels of Mg, and when chronic levels of Mg occur, it can also lead to growth retardation and behavioral changes in patients.\textsuperscript{24}

Children with autism have been found to have lower levels of these nutrients. The deficiencies in these patients seem to explain many of their symptoms and put them at risk for many bone health issues in the future. Hedinger et al.\textsuperscript{36} found that the exclusion diets and selective eating patterns by these patients seem to put them at even higher risk for a vitamin deficiency of one of the bone health nutrients. Because of these restrictive eating habits and/or diets, there is a fair amount of research to date examining the nutritional deficiencies of children with autism. However, to our knowledge, there have not been any studies examining the link between bone health nutrients and the prevalence of children with autism and the deficiency of these vitamins.
CHAPTER 3

METHODOLOGY

*Overall Study Overview*

Autism is becoming increasingly more common each year in the United States. Because of the challenging eating behaviors involved in autism, their diets may be nutritionally inadequate to meet their needs, resulting in multiple deficiencies. Many studies have examined overall diet quality, but no studies, to our knowledge have been conducted comparing bone health nutrients in autistic children and typical children. This study was conducted as a review of dietary intake data from two previously conducted studies: NHANES survey data from 1999-2006 and RUPP study. The NHANES survey data was a nationwide survey of dietary intake patterns of typical children aged 4 to 13 years old. The RUPP study was a smaller study completed at three sites, examining dietary intake patterns of autistic children aged 4 to 13 years old. Use of the NHANES study was aimed to serve as a comparison to the RUPP study.
Purpose of the Study

The purpose of this study is to compare vitamin D and bone health nutrient intakes of children with autism to age and gender matched typical children. Nutrient intakes will be analyzed through dietary recalls and/or FFQs in both NHANES and RUPP data.

Objectives of the Study

Objectives for this study are:

1. Compare dietary data from the Research Units on Pediatric Psychopharmacotherapy (RUPP) study and the NHANES regarding bone health nutrients involved in bone health in children

2. Examine the relationships between bone health and autism in children by identifying vitamins C, D and K, calcium and magnesium nutrient intake levels of children in the RUPP study

Sources of Data

NHANES Overview

The National Health and Nutrition Examination Surveys\textsuperscript{56} (NHANES) is a series of cross-sectional, nutrition and health examination surveys conducted by the National Center for Health Statistics (NCHS) as part of the Centers for Disease Control and Prevention (CDC). These surveys are designed to assess the health and nutritional status of children and adults in the United States through interviews and physical examinations. NHANES has conducted these surveys since the early 1960s analyzing all cultures and disease states throughout America. In 1999, NHANES became a continuous survey, in
which data collection would occur annually without breaks between study periods allowing for steady and reliable data on less known prevalent conditions. Each year, approximately 5000 persons throughout the country are interviewed in their own home by a trained NHANES study team. Measurements are performed in specially designed NHANES mobile examination center (MEC) units. The survey and examination collect various components including but not limited to: demographic and socioeconomic information, dietary habits, lifestyle risk factors, physical examination, dental measurements, and biochemical assessments.

The overall goal of NHANES is to determine and manage a nationally representative sample of residents in the U.S. Sample selection is a multiple step process in which each geographic area known as “primary sampling units” (PSUs) are combined into strata and further, are divided into a series of neighborhoods. Households are then chosen randomly and interviewed for eligibility in the survey. NHANES approximates that this use of probability sampling represents 50,000 U.S. residents for each selected household participant. NHANES attempts to create a representative sample of whites, Mexicans, and African Americans by age, sex and income. For the hard-to-reach populations such as adolescents, the elderly, pregnant women, African Americans and Mexican Americans, oversampling was done to ensure a reliable representation of the US population.

NHANES survey results show the prevalence of certain chronic diseases and specific risk factors for diseases throughout different areas of the country. Many variables and factors can be compared and analyzed to determine possible correlations between a certain variable and disease state. National standards for many measurements
such as height, weight and blood pressure are based off the findings of NHANES data, which is further used to assist in health promotion and risk prevention. Health practitioners and researchers can use this information to identify any trends through the years, or even patterns of a certain region of the country, and work to educate patients on these health issues to prevent future healthcare problems.

**NHANES Data Collection**

Each household in the sample was mailed an introductory letter briefly describing the study and informing the participant that an NHANES interviewer would visit their home. The interviewer screened residents for the study and provided residents with a survey brochure containing information on the survey, the household interview, and the MEC examination. Before the household survey could take place, a signed consent form was obtained for each eligible individual. For eligible participants, a computer-assisted personal interviewing (CAPI) system was used to record data from the interview. The family questionnaire was completed by one adult family member in the household. Appointments for the MEC exam were assigned and a separate signed consent form was required for the MEC exam. For all children under the age of 18 years old participating in the sample, parental consent was required. Sample participants who refused to sign the consent form for either section were not allowed to participate in the survey study.

The complete health examination and interview was held in the MEC, which is composed of four trailers operating five days a week. A home examination occurred for a small amount of participants who were unable and/or unwilling to travel to the MEC.
Two four-hour sessions were scheduled each day. The full examination of each individual lasted approximately 3 ½ hours, but varied depending on age. These analyses used 24-hour dietary recall data and total body composition.

**NHANES Dietary Interview**

Public use data files regarding demographics and 24-hour dietary recalls were obtained from NCHS website and imported into SPSS (SPSS Inc., version 17.0, Chicago, IL) for preparation and analysis. Recoding was necessary for some variables to provide information related to the current study regarding demographics and dietary recall information.

The dietary intake interview was performed during the health examination in the MEC by professionally trained interviewers. Twenty-four hour dietary recalls conducted in English or Spanish were used to estimate the nutritional value of the foods consumed 24-hours prior to the dietary interview. Translators were present to conduct interviews for other languages. For children who were younger than 6 years old or other respondents unable to report data because of age or disability, a parent and/or guardian acted on behalf of them. The preferred proxy was the person who prepared the meals for the respondent. Children ages 6-11 years old were asked to recall foods eaten but were allowed assistance from an adult member in the household. Dietary intake data was obtained and input using a computer automated dietary intake system (CADI) to ensure consistency between data collection of participants.

The USDA Automated Multiple Pass (AMPV) instrument was used to obtain thorough descriptions of all foods and beverages. The multiple passes interview consisted first of a list of the foods and beverages consumed by the respondent in the 24-
hour period. Information was collected regarding the time on consumption, the occasion for eating, detailed food descriptions including food preparation, the amount, and where the food was obtained and eaten. The respondent then reviews the foods and beverages with the interviewer one last time to ensure that no foods were forgotten. Any food or beverage that was recalled during this time was added to the food record. A set of health-related questions were asked after the final review to collect information on the respondent’s usual intake, water consumption, salt use, and if any special diets are in place.

Data collected from the 24-hour recall interview were coded and linked to the USDA’s Food and Nutrition Database for Dietary Studies (FNDDS) where estimated dietary intakes were calculated. FNDDS is a database including descriptions of foods, nutrient values and common portion size weights. Dietary supplements were not included in the nutrient intake analysis.

Subjects

Approximately 41,474 total participants were interviewed across the eight survey years of 1999-2006. Children and adolescents with complete data including age (4-13 years old) and food intake data were included in this study. Because certain vitamins and/or nutrients were not tested the full eight years of the survey studies, the total participant numbers are varied between dietary components. These NHANES analyses included a population of 7,693 subjects for all but four dietary components where the population was 5,819 subjects. Nutritional information for total sugars, vitamin K, carotenoids, and folate data were not completed in the survey year 1999-2000. The 7,693
and 5,819 subjects are nationally-representative of 39.0 and 30.4 million US children and adolescents, respectively. Nutrients of concern in our study are vitamins C, D, and K, magnesium and calcium.

**RUPP Overview**

Research Units on Pediatric Psychopharmacotherapy (RUPP) is a network of research units devoted to clinical trials in children at multiple academic sites throughout the country. RUPP was established in the Department of Child Psychiatry at Columbia University and New York State Psychiatry Institute in October 1996 with a focus on efficacy and safety of psychotropic medications commonly used in children with anxiety, mood, and disruptive behavior disorders. The RUPP academic network sites include Columbia University, Johns Hopkins University, Pittsburgh University, Yale University, University of California-Los Angeles, Indiana University and The Ohio State University. Staff of the Division of Service and Intervention Research at the National Institute of Mental Health (NIMH) work collectively with the academic sites to support communications between research units, assist with specific protocols that may not be funded or sponsored by an industry, and to serve as the data management center for RUPP. Each of the RUPP sites has experts in child psychiatry, psychopharmacology, pediatrics, psychology, nutrition, and alternative research in children. Recent RUPP-supported multi-site studies include research in selective serotonin reuptake inhibitors (SSRIs) in children with anxiety disorders and risperidone for children with autism and behavioral disturbances among other studies.
**RUPP Subjects**

The sample population included participants at three recruiting sites: Indiana University, The Ohio State University, and Yale University. The institutional review boards of the clinical sites approved the study, and written informed consent was required of all parents or guardians of the participants. Participants were children ages 4 through 13 years old with a diagnosis of autism, Asperger’s Syndrome, or pervasive developmental disorder not otherwise specified (PDD-NOS). Baseline dietary data was collected for 124 participants; however, complete dietary data was only established for 113 participants in the RUPP study.

**RUPP Design**

The study design was a 24-week, controlled, parallel groups clinical trial involving 124 children, ages 4-13 years old, with a diagnosis of autism, Asperger’s Syndrome, or pervasive developmental disorder not otherwise specified (PDD-NOS). The diagnoses were accompanied by frequent tantrums, self injury, and aggression in the children. Children were randomized 2:1 to combination therapy involving risperidone treatment and parent training in behavior management or to medication alone. This study took place at three recruiting sites: Indiana University, The Ohio State University, and Yale University. Baseline dietary data before assignment to treatment groups was used for comparison with the NHANES dietary interview data.
Block Kids Food Frequency Questionnaire 2004 (Block Kids FFQ)

Block Kids Food Frequency Questionnaire (FFQ) 2004 was employed to collect the child’s nutrient intake data. The questionnaire included a list of 77 food items and was developed from the NHANES 1999-2002 dietary recall data. The nutrient database was derived from The USDA Nutrient Database for Dietary Studies, version 1.0.59 The Block Kids FFQ asks the frequency of certain foods that were consumed in the previous week and also assesses individual portion sizes of the foods. Pictures of food items were provided to ensure accuracy of quantification.59

Completed Block Kids FFQ surveys were analyzed by Block Dietary Data Systems in Berkeley, CA for nutrient intake. Dietary supplements were not included in the nutrient intake analysis. Secondary data analysis was completed at The Ohio State University in Columbus, OH. Dietary nutrient intake was compared to the RDA and DRI for children ages 4-13 years old. Nutrients of concern were vitamins C, D, and K, magnesium and calcium.

Data Preparation

To identify the percentage of children from the total population meeting current nutrient intake recommendations, nutrient intakes were compared to the Estimated Average Requirements (EAR) or Adequate Intake (AI) values, as established by the Dietary Reference Intakes (DRIs). The following equation was used for analysis of DRI recommendations met:

\[
\text{Nutrient intake} / \text{DRI recommendations} \times 100\%
\]
Individuals with a proportion of need greater than or equal to 100% (≥100%) were considered as “meeting or exceeding need.” Any individuals meeting less than 100% did not meet the DRI recommendations for the given nutrient.

**Data Analysis**

To compare differences in bone health nutrient intakes by children with and without autism, nutrient intake levels of vitamin C and K, magnesium, and calcium from the 1999-2006 NHANES mean data of typical children were compared with levels of the same nutrient levels for the children with autism in the RUPP study (Objective 1). Dietary data from the children in the RUPP study were be used to assess whether the levels of vitamins C, D, and K, calcium, and magnesium meet the RDA and AI levels for children of the same age and if any deficiencies in these bone health vitamins are present (Objective 2).

SPSS Complex Samples (SPSS Inc., version 17.0, Chicago, IL) was used to conduct analyses. This software allows for the correction for over-sampling of hard-to-read populations, which results in a nationally representative sample. SPSS Complex Samples is essential to provide adjusted population-based means that adjust for the oversampling of target populations, while also providing appropriate standard errors for statistical analysis that reflect the actual sample size.
CHAPTER 4

BONE NUTRIENT LEVELS IN CHILDREN WITH AND WITHOUT AUTISM

Structured Abstract

Autism is one of the fastest growing developmental disorders in the United States.\(^1\) The prevalence of autism and early diagnoses continue to rise as more information regarding symptoms and causes of autism spectrum disorders (ASD) are known. Because of the various challenging eating behaviors and gastrointestinal problems associated with autism, many autistic children may be at risk for multiple nutrition deficiencies, including bone health nutrients. Adequate intake of bone health nutrients is imperative for proper bone formation, growth, and development through adolescence.

Dietary intakes were used to compare vitamin D and other bone health nutrient intakes of autistic children to age and gender matched typical children. Data from the Block FFQ were used in the 2005-2007 RUPP (Research Units on Pediatric Psychopharmacology) study to analyze nutrient intakes in autistic children while the 24-hour dietary recalls from the 1999-2006 National Health and Nutrition Examination Surveys (NHANES) were used as a means for comparison of typical children for this study.
Mean calcium and magnesium levels were found to be lower in children with autism than typical children. Conversely, when looking at the number of children with likely adequate magnesium intakes (>100% DRI), there were a higher percentage of autistic children (73%) likely meeting the requirements for magnesium intake. Typical children reported slightly lower mean vitamin K levels than the levels of autistic children, although both groups were lower than the DRIs. Dietary intakes of vitamin C were very high in the typical children at 82.40 mg, while the mean level in autistic children was very low at 12.61 mg. Interestingly, in examining the breakdown of intake levels, 93% of autistic children met or exceeded 100% of the DRI for vitamin C. Vitamin D levels were not collected in the NHANES study so comparisons were not able to be made between autistic and typical children; however, children with autism reported very low mean levels of vitamin D (3.84 mg). Of the five bone nutrients examined in the autistic children, mean vitamin D had the lowest percentage of AI at 76.72 ± 4.79% [mean ± SEM] with a very wide range of intake from 2.99% to 268.89% of AI for vitamin D. It is critical that health care practitioners working with autistic children are aware of the importance of proper bone nutrient levels in their patients at early ages to prevent any osteopenia and/or fractures. This is especially crucial because of compromised nutrient intakes in many of these children due to texture issues or special diets such as the Gluten-free, Casein-free (GFCF) diets.
Introduction and Statement of Purpose

Autism is one of the fastest growing developmental disabilities in the United States, occurring in all racial, ethnic, and social groups.\(^1\) Autism is part of a group of disorders known as autism spectrum disorders, which are categorized by severe deficits in social interaction and communication, and also by stereotyped and repetitive behaviors.\(^1,2,3\) Gastrointestinal (GI) problems often lead to feeding difficulties and problem eating behaviors in autistic children, which can result in severely limited diets and aversions to tastes and textures in these children.\(^12,16\) Because of GI issues, elimination diets such as the gluten free, casein free (GFCF) diet, may be employed to decrease frequency of symptoms. There is some controversy over whether these special diets, in addition to the child’s own restricted diets, are nutritionally adequate for autistic children.

Multiple studies have been conducted examining the nutrient profiles of ASD children, with many (of the studies) using typical children as a means of comparison for the ASD children. The results seem to be mixed as to whether autistic children are truly nutrient deficient in their diets. Because autism is a spectrum disorder in which these children have differing degrees of severity of symptoms, some of the ASD children may be nutritionally compromised, while others may be nutritionally adequate, or even overnourished.\(^14,15\) Overnutrition can sometimes occur in ASD children when mega-doses of certain nutrients are taken by the child, usually from dietary supplements.

Current research has begun to focus more on vitamin D and its importance with strong bones as well as its role in reducing other chronic diseases.\(^60\) The relationship between bone health and autism has only recently become an item of concern.
Researchers believe that multiple factors put boys with autism at higher risk for weaker bone development and bones.\textsuperscript{36} Bone cortical thickness of the wrist has been shown to have less thickening in those boys with ASD when compared to a control group of boys without ASD.\textsuperscript{36} Differences in bone cortical thickness were also seen between ASD boys following casein-free diets and those on unrestricted diets, with ASD boys on casein-free diets having a bone cortical thickness much thinner than typical boys of same age.\textsuperscript{36} Since casein is found in dairy products, the elimination of casein also includes the exclusion of calcium, and possibly vitamin D (if fortified) from the young children’s diets, which could put the children at risk for deficiencies and thus, poor bone health.

Seizures have also become common amongst children with ASD, putting children at higher risk for malnutrition and feeding problems. Antiepileptic drugs (AEDs) are used in many children with ASD who suffer from recurrent seizures, and frequent use of AEDs can lower bone nutrient levels of calcium and vitamin D leading to osteoporosis, fractures, and/or rickets.\textsuperscript{39} However, when children with ASD taking AEDs (even long-term) are supplemented with these nutrients, serum levels will return to normal levels.\textsuperscript{39} Parents need to be aware of their children’s bone nutrient levels, if taking AEDs, to prevent bone weakening by ensuring that proper nutrient requirements of the child are met.

The goal of our study was to review dietary intake data from two studies: one study comprised of children with ASD and one study of typical children. Specifically, our review assessed the levels of certain bone nutrient levels, calcium, magnesium, and
vitamins C, D and K, and then compared these levels in children with ASD and typical children to further draw any conclusions on any relationships between bone health and autism.

Subjects

Subject data was obtained from two previously conducted studies: NHANES dietary data from 1999-2006 and RUPP study de-identified nutritional data from a clinical trial conducted from 2005-2007. In the eight survey years of the NHANES from 1999-2006, approximately 41,474 total participants were interviewed. Only children and adolescents with complete data including age (4 - 13 years old) and food intake data were included in the study. Not all vitamins and/or nutrients were tested the full eight years of the NHANES studies, accounting for some variation in total participant numbers for some nutritional intake. Of the five nutrients of concern in our study (vitamins C, D and K, magnesium, and calcium), vitamin C, magnesium and calcium were the only nutrients with data the entire six years with a population of 7,693 subjects. Vitamin K was not assessed in the survey year 1999-2000. Therefore, the population for vitamin K data over the six survey years was approximately 5,819 subjects. The 7,693 and 5,819 subjects were nationally representative of 39.0 and 30.4 million US children and adolescents, respectively. No nutritional data for vitamin D was collected in the NHANES from 1999-2006.

Subjects in the RUPP study were children ages 4 through 13 years old with a diagnosis of autism, Asperger’s Syndrome, or PDD-NOS. Calcium, magnesium, and vitamins C, D, and K were assessed in this study. Baseline dietary data were collected
for 124 participants, although complete data was only established for 113 participants in the RUPP study. Dietary data were considered complete if data for all four bone nutrients were present.

**Methods**

Dietary data from the 1999-2006 NHANES and the 2005-2007 RUPP study were used to compare the bone nutrient intakes of typical children with autistic children ages 4-13 years old. The NHANES surveys were designed to assess the health and nutritional status of children and adults throughout the United States through interviews and physical examinations by determining a nationally representative sample of whites, Mexicans, and African Americans by age, sex, and low income. For difficult to reach populations such as adolescents, the elderly, pregnant women, African Americans and Mexican Americans, oversampling was done to ensure adequate representation of the U.S. population. Twenty-four hour dietary recalls were performed during the dietary intake interviews as part of the health examination. Multiple pass interview formats were used to collect more detailed information about all dietary intakes. Additional information about the design and data collection of NHANES is available elsewhere.56

The RUPP study assessed baseline dietary data for all children with ASD by use of the Block Kids Food Frequency Questionnaire (FFQ) 2004. The questionnaire included 77 food items to measure the frequency of certain foods consumed in the previous week and also assessed individual portion sizes of the foods using the pictures provided for accurate quantification.
Data Preparation

Public use data files were obtained from NCHS website and imported into SPSS (SPSS Inc., version 17.0, Chicago, IL) for preparation and analysis of the NHANES. Some variables needed recoding to provide information related to the current study regarding demographics and dietary recall information.

In our secondary analysis for both NHANES and RUPP data, nutritional information was computed for the percentage of children who met the Estimated Average Requirements (EAR) and/or Adequate Intake (AI) recommendations, established by the Dietary Reference Intake (DRI) for the five bone nutrients examined in our analysis. The percentages of children ages 9 – 13 years old who did not reach the EAR or AI requirements were compared between children with and without autism. Dietary supplements were not included in the nutrient intake analysis.

Data Analysis

Comparisons in bone health nutrient intakes were examined in children with and without autism. Nutrient intake levels of vitamins C and K, magnesium, and calcium were assessed from the NHANES mean data of typical children and compared to levels of the same nutrient levels for the children with autism in the RUPP study. Dietary data from the children in the RUPP study was used to assess whether the levels of vitamins C, D and K, calcium, and magnesium met the RDA and AI levels for children of the same age and if any deficiencies in these bone health nutrients were present.

SPSS Complex Samples (SPSS Inc., version 17.0, Chicago, IL) was used to conduct analyses. This software allows for the correction for over-sampling of hard-to-read populations, which results in a nationally representative sample. SPSS Complex
Samples is essential to provide adjusted population-based means that adjust for the oversampling of target populations, while also providing appropriate standard errors for statistical analysis that reflect the actual sample size.

Results

Nutritional intakes related to bone health nutrients are described in Table 4.1 for both the NHANES and RUPP samples. The mean and the median were both presented because of the wide variation between nutrient intakes for outliers, thus affecting the mean but not the median in the data. Also, the standard error of the mean (SEM) and the standard deviation (SD) were both analyzed to express a better reflection of the mean based on the sample size and variance.

Mean levels of both calcium and magnesium were reported lower in children with autism spectrum disorder (ASD) than typical children, although mean levels for both nutrients were within the dietary reference intakes (DRIs) and/or recommended dietary intakes (RDAs) for their age groups. Calcium and magnesium intakes in the typical children had greater variability in standard deviation; however, children with autism varied quite a bit in calcium intakes, which could possibly correspond to some children following the GFCF diets. Vitamin C levels in the typical children were sufficiently above the DRI, but were very low in the autistic children, with mean levels only meeting about 50% of the DRI for Vitamin C. Vitamin D was not collected in the NHANES study so comparisons between the two studies for this nutrient could not be made. However, children with ASD reported very low mean intakes of vitamin D (3.84 mg), with varying approximate intake levels of 0.15 mg to 13.44 mg daily. Again, this may be possible because of those children following the GFCF exclusion diet. Vitamin K levels
for both groups were lower than the DRIs, with typical children having slightly lower mean levels than the ASD children (48.65 mcg vs. 52.99 mcg, respectively). A wider variation of intake was seen in the typical children with the minimum and maximum intake being reported at 0.80 and 2872.00 micrograms, respectively.

Table 4.2 shows the NHANES and RUPP study participants’ percentage of EAR and AI recommendation levels at baseline. In both studies, the mean percentages of magnesium (NHANES: 154.14%; RUPP: 169.71%) and Vitamin C (NHANES: 294.39%; RUPP: 572.58%) intakes exceeded the recommendation levels. Mean percentages of calcium were similar throughout the two groups, with NHANES and RUPP participants meeting 96.80% and 90.68% of the AI, respectively. In addition, vitamin K percentages were both below the recommended AI levels at 84.25% and 94.21% for the NHANES and RUPP children, respectively. Vitamin D levels were the lowest of all bone nutrients examined in the RUPP children, with a mean percentage of 76.72% meeting the AI. The percentage of recommendation levels for the RDA in both studies can be found in Table 4.3. For both NHANES and RUPP studies, mean percentages of both nutrients exceeded 100% of the RDA values.

In order to better explain participants’ nutrient intake, breakdown by study (typical children vs. autistic children) and age group (4-8 years old vs. 9-13 years old) was completed. Tables 4.4 and 4.5 show both the NHANES and RUPP study participants’ recommendation levels met (>100% of recommendation), or not met (<100% of recommendation) by unweighted sample size and weighted percentages, using the EAR/AI and RDA recommendations, respectively. In Table 4.4, overall data for most nutrients compared were fairly similar, with vitamin C being the main outlier between the
two studies. Total vitamin C levels tended to be higher in the RUPP study at 94% of EAR met, while 70% of the NHANES study met the EAR. Less than 50% of the AI for calcium was met in all children in both the NHANES (39%) and RUPP (33%) studies. Magnesium levels for both studies were 92% of the EAR in 4-8 years old children, but levels were lower in the 9-13 year old children with 55% and 39% of the EAR levels being met in the NHANES and RUPP studies, respectively. Calcium levels for 4-8 years old participants were low in both the NHANES (57% of AI met) and the RUPP (41% of AI met) studies, but were even lower in the 9-13 year old children. Calcium intake in the NHANES participants went from 57% to 22% of the AI met, and the RUPP study children dropped from 41% to 7%. Higher variability between the two age groups seemed to exist for all nutrient levels, with the exception of vitamin D (for which we could only derive data from the RUPP study). Vitamin K was very similar in both the NHANES and RUPP studies. Both 4-8 years old typical and autistic children reported low intake levels of vitamin K at 20% and 21%, respectively. Intakes increased slightly in children 9-13 years old, but still were not sufficient intakes at 24% and 36% of the AI. Figures 4.1 and 4.2 give a visual interpretation of the data from Table 4.4 to better understand the differences between the two study groups, and age groups. In Table 4.5, magnesium levels for both autistic and typical children were very similar, even by age group. At 4-8 years old, 85% of autistic children had slightly higher intake levels of magnesium, while 83% of typical children met the recommendations. For children 9-13 years old, recommendation levels met in the RUPP and NHANES study groups had fallen to 35% and 41%, respectively. Larger variations in nutrient intake occurred with vitamin C, as a larger amount of autistic children met recommendations levels in both 4-8
years old and 9-13 years old children (96% and 82%, respectively). Conversely, 79% and 55% of the typical children ages 4-8 years old and 9-13 years old met the recommendation levels, showing lower recommendation levels being met in 9-13 years old children.

Table 4.6 summarizes the individual bone nutrients at baseline for both the NHANES and RUPP studies, using AI or RDA values. Adequate calcium levels in both studies were similar with 57% and 41% in 4-8 year old NHANES and RUPP children, respectively. With the older children aged 9-13 years old, larger differences appeared between the two groups. Adequate calcium levels decreased in both groups with typical children dropping to 22%, and autistic children decreasing tremendously to only 7% likely meeting adequate levels. In the NHANES study, about 62% (n = 4413) and about 66% (n = 5303) of total children had adequate levels of magnesium and vitamin C, respectively. Twenty-four percent (n = 2103) had markedly inadequate levels of magnesium, and 27% (n = 467) of participants had inadequate intake of vitamin C. For the majority of participants in the RUPP study, intake of magnesium (n = 82 or 73%) and vitamin C (n = 105 or 93%) was adequate. Twenty-three (20%) participants had inadequate intake of magnesium and 5 (4%) had inadequate levels of vitamin C. In both studies, the total vitamin K intake was similar at 22% and 25% in the NHANES and RUPP studies, respectively. Vitamin K intakes in both typical and autistic children were similar at all ages, though improvements in likely adequate intakes in 9-13 year old children were more noticeable in the autistic children. Seventy-seven of 85 autistic children not meeting likely adequate levels consumed less than 80 percent of the DRI for vitamin K.
Overall, most children (both typical and autistic) had likely inadequate levels of vitamin K. Vitamin D levels were consistently poor in all age groups of autistic children with only 29% of all children meeting likely adequate levels of vitamin D intake. Of the remaining inadequate vitamin D intake, 69 of 80 autistic children fell below 80% of the DRI.

Discussion

The results of our review study provide insight into the bone nutrient intakes of both typical and autistic children. Data from our study showed about half of the bone nutrient averages as meeting the nutritional guidelines when grouped as a total for each study; however, when examining the individual breakdown of nutrient intakes, significant variability between children as well as age groups was found. The differences could possibly be attributed to limited diets and/or picky eating habits by the children. After reviewing bone health nutrient levels in children with autism, there does appear to be a relationship between lower levels of some bone health nutrients and autistic children, which offers the potential for ideas for future studies with bone health in this population.

In both children, less than 40% of participants met the AI for calcium. When calcium was examined by breakdown of age group levels, calcium had the highest level of variability with the 4-8 year olds having approximately two-thirds of AI met, and then a sudden drop-off occurred in the 9-13 year olds to less than 20% of the recommended levels. RUPP study children had extremely inadequate levels of calcium, as well as vitamins D and K, which was consistent with the findings from Lindsay et al.\textsuperscript{23}, though total calcium levels for NHANES children were inadequate, as well, which was similar to results from a previous study.\textsuperscript{19,23} Another study by Cornish\textsuperscript{15} also showed that calcium
levels were decreased in ASD children, regardless of whether children were following the GFCF diet, or non-GFCF diets.

Many of the levels of magnesium in children from the RUPP study were the same as NHANES children in 4-8 year olds, but lower than the levels in the NHANES children by 9-13 year olds, which is comparable to the lower levels of plasma Mg in autistic children when compared to typical subjects in a previous study. By contrast, when magnesium intake for both age groups were totaled, autistic children were slightly higher, which is likely due to the small sample size of the RUPP study.

A previous study by Cornish found 53% of ASD children were below reference nutrient intakes (RNI) for vitamins C and D, and calcium among a few other nutrients, and three ASD children were below the lower reference nutrient intake (LRNI) for vitamin D. Vitamin C levels in the RUPP study exceed levels present in Cornish’s study; however, levels of Vitamin D and calcium seem to be similar to those in the Cornish study with both nutrients below one-third of the dietary reference intakes (DRI) recommendations.

In a study by Lindsay et al. analyzing individual nutrient baseline intake in autistic children, results showed that the nutrients with the highest adequate levels were vitamin C and magnesium, which remained consistent with data in the RUPP study. Interestingly, mean levels of vitamin C were much lower than the AI for calcium; however, the majority (93%) of autistic children met adequate levels, which suggests that those children not meeting adequate levels had extremely low intakes of vitamin C. Calcium levels in the RUPP study were also very similar to the results found in the Lindsay et al. study. Forty-nine percent of autistic children had calcium levels less than
80% of the DRI, which was comparable to the 45% of autistic children (having levels less than 80% of DRI) in the Lindsay study.

There were several limitations to our review study related to secondary data analyses. For the NHANES study, our data was collected from 1999-2006. Nutritional information was recorded by use of a 24-hour dietary recall by the participants that represented the national population as a whole. While the interviewers for NHANES were professionally trained, many parents, children and adolescents may have experienced recall bias and/or response bias. For younger children, detailed descriptions of foods eaten including portion sizes may be harder to explain, especially if meals were consumed outside of the home (school, daycare, etc). Also, the 24-hour recall may have not reflected a typical intake for the subject, which would skew results.

Limitations for the RUPP study data included a fairly small sample size as well as any inaccuracies present with the Block Kids Food Frequency Questionnaire (FFQ) 2004. With use of the FFQ, seasonal variability could have occurred causing differences in usual nutrient intakes in the autistic children. The NHANES surveys account for seasonal variability by conducting their 24-hour dietary recalls throughout the year. FFQs also can be unreliable as many of the parents were reporting the frequency of foods consumed by their children, as well as the portion sizes of each of the food items consumed over the previous 7 days. Underreporting may have occurred too, as parents may not have known the precise amounts eaten by the child or perhaps did not want to admit how much was consumed by their child. In general though, parents of autistic children have a more positive belief about relationship between diet and behavior, and greater nutrition knowledge.20 Thus, it is possible that these parents may have been more
aware of the type and size of foods consumed, even in a 7-day period. Lastly, analyses were conducted using estimated average requirements (EAR), when present for the nutrients, which only looks at 50% of the average healthy children in the population. In other words, this measure may reflect intakes of the typical children accurately, but not for the autistic children as no measure of proper intakes for this population has been established.

Specifically, future studies identifying any link between bone health nutrients (vitamin D, in particular) and autism in children could utilize a combination of dietary intake measures such as FFQs, dietary recall, and/or food records for more accurate dietary intake and eating habits. To examine any link between vitamin D deficiencies and autism, bone mass and structure could be identified in the autistic children through use of a dual energy x-ray absorptiometry (DEXA) scan and then compared to dietary intake of vitamin D, and other bone nutrients.
<table>
<thead>
<tr>
<th>Bone Health Nutrients</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Standard Error of Mean</th>
<th>Standard Deviation</th>
<th>Median</th>
<th>Dietary Reference Index (DRI)</th>
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<tr>
<td><strong>Calcium (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>800 (1300)</td>
</tr>
<tr>
<td>Typical</td>
<td>0</td>
<td>5027.00</td>
<td>966.34</td>
<td>13.01</td>
<td>543.60</td>
<td>878.00</td>
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<tr>
<td>ASD</td>
<td>42.29</td>
<td>2447.62</td>
<td>802.61</td>
<td>39.26</td>
<td>417.33</td>
<td>719.40</td>
<td></td>
</tr>
<tr>
<td><strong>Magnesium (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>130 (240)*</td>
</tr>
<tr>
<td>Typical</td>
<td>0</td>
<td>1932.00</td>
<td>222.97</td>
<td>2.15</td>
<td>101.80</td>
<td>205.00</td>
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</tr>
<tr>
<td>ASD</td>
<td>44.60</td>
<td>612.07</td>
<td>209.56</td>
<td>8.69</td>
<td>92.39</td>
<td>193.99</td>
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<tr>
<td><strong>Vitamin C (mg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25(45)*</td>
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<td>945.80</td>
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<td>1.54</td>
<td>81.40</td>
<td>59.50</td>
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<tr>
<td>ASD</td>
<td>2.56</td>
<td>41.99</td>
<td>12.61</td>
<td>.59</td>
<td>6.28</td>
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<td><strong>Vitamin D (mcg)</strong></td>
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<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
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<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>ASD</td>
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<td>3.84</td>
<td>.24</td>
<td>2.54</td>
<td>3.41</td>
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<tr>
<td><strong>Vitamin K (mcg)</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>55(60)</td>
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<td>Typical</td>
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<td>2872.00</td>
<td>48.65</td>
<td>1.24</td>
<td>72.37</td>
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<tr>
<td>ASD</td>
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<td>387.47</td>
<td>52.99</td>
<td>5.48</td>
<td>58.20</td>
<td>31.34</td>
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</tr>
</tbody>
</table>

ASD$^a$ = Autism Spectrum Disorder

Note: Adequate Intake (AIs) for children ages 4-8 years and 9-13 years (the latter in parentheses)

*Recommended Dietary Allowances (RDAs) for children ages 4-8 years and 9-13 years (the latter in parentheses)

Table 4.1. NHANES and RUPP Study Subjects’ Nutritional Intake
<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
<th>Standard Error</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Standard Deviation</th>
</tr>
</thead>
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<tr>
<td><strong>NHANES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Calcium (mg)b</td>
<td>96.80</td>
<td>85.20</td>
<td>1.20</td>
<td>0</td>
<td>561.90</td>
<td>58.00</td>
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<tr>
<td>% of Magnesium (mg)a</td>
<td>154.14</td>
<td>139.50</td>
<td>1.55</td>
<td>0</td>
<td>966.00</td>
<td>78.40</td>
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<tr>
<td>% of Vitamin C (mg)a</td>
<td>294.39</td>
<td>198.20</td>
<td>6.30</td>
<td>0</td>
<td>4299.09</td>
<td>308.80</td>
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<tr>
<td>% of Vitamin K (mcg)b</td>
<td>84.25</td>
<td>58.37</td>
<td>1.24</td>
<td>1.33</td>
<td>4786.67</td>
<td>123.12</td>
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<tr>
<td><strong>RUPP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of Calcium (mg)b</td>
<td>90.68</td>
<td>80.18</td>
<td>4.85</td>
<td>5.29</td>
<td>305.95</td>
<td>51.52</td>
</tr>
<tr>
<td>% of Magnesium (mg)a</td>
<td>169.71</td>
<td>158.39</td>
<td>8.27</td>
<td>40.55</td>
<td>556.43</td>
<td>87.88</td>
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<tr>
<td>% of Vitamin C (mg)a</td>
<td>572.58</td>
<td>459.18</td>
<td>42.20</td>
<td>29.77</td>
<td>2182.82</td>
<td>448.66</td>
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<tr>
<td>% of Vitamin D (mcg)a</td>
<td>76.72</td>
<td>68.23</td>
<td>4.79</td>
<td>2.99</td>
<td>268.89</td>
<td>50.87</td>
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<tr>
<td>% of Vitamin K (mcg)b</td>
<td>94.21</td>
<td>56.98</td>
<td>9.85</td>
<td>14.55</td>
<td>704.49</td>
<td>104.72</td>
</tr>
</tbody>
</table>

EAR\textsuperscript{a} = Estimated Average Requirement  
AI\textsuperscript{b} = Adequate Intake  
Note: Vitamin D was not collected in NHANES 1999-2006; Vitamin K was only reported from 2001-2006

Table 4.2  NHANES and RUPP Study Subjects’ Percentage of Recommendation Levels (EAR\textsuperscript{a}, AI\textsuperscript{b})
<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Median</th>
<th>Standard Error</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Standard Deviation</th>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% of Magnesium (mg)</td>
<td>129.67</td>
<td>117.70</td>
<td>1.31</td>
<td>0</td>
<td>805.00</td>
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<tr>
<td>% of Vitamin C (mg)</td>
<td>257.68</td>
<td>173.80</td>
<td>5.54</td>
<td>0</td>
<td>3783.20</td>
<td>271.20</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>% of Magnesium (mg)</td>
<td>143.27</td>
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<td>7.01</td>
<td>34.31</td>
<td>470.82</td>
<td>74.56</td>
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<tr>
<td>% of Vitamin C (mg)</td>
<td>502.88</td>
<td>397.96</td>
<td>37.18</td>
<td>26.20</td>
<td>1920.88</td>
<td>395.18</td>
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</table>

RDA* = Recommended Dietary Allowance
Note: Vitamin D was not collected in NHANES 1999-2006; Vitamin K and Calcium were not computed for RDA.

Table 4.3 NHANES and RUPP Study Subjects’ Percentage of Recommendation Levels (RDA*)
<table>
<thead>
<tr>
<th>Nutrients</th>
<th>4-8 years old</th>
<th></th>
<th>9-13 years old</th>
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<th>Total</th>
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<tr>
<td></td>
<td>Not met</td>
<td>Met</td>
<td>Not met</td>
<td>Met</td>
<td>Not met</td>
<td>Met</td>
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<td>NHANES</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>1546 (43)</td>
<td>1827(57)</td>
<td>3446 (78)</td>
<td>874 (22)</td>
<td>4992 (61)</td>
<td>2701 (39)</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>302 (8)</td>
<td>3071(92)</td>
<td>2033 (45)</td>
<td>2287 (55)</td>
<td>2335 (27)</td>
<td>5358 (73)</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>545 (18)</td>
<td>2828 (82)</td>
<td>1553 (41)</td>
<td>2767 (59)</td>
<td>2098 (30)</td>
<td>5595 (70)</td>
</tr>
<tr>
<td>Vitamin K (mcg)</td>
<td>2101 (80)</td>
<td>499 (20)</td>
<td>2456 (76)</td>
<td>763 (24)</td>
<td>4557 (78)</td>
<td>1262 (22)</td>
</tr>
<tr>
<td>RUPP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>50 (59)</td>
<td>35 (41)</td>
<td>26 (93)</td>
<td>2 (7)</td>
<td>76 (67)</td>
<td>37 (33)</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>7 (8)</td>
<td>78 (92)</td>
<td>17 (61)</td>
<td>11 (39)</td>
<td>24 (21)</td>
<td>89 (79)</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>2(2)</td>
<td>83 (98)</td>
<td>5(18)</td>
<td>23 (82)</td>
<td>7 (6)</td>
<td>106 (94)</td>
</tr>
<tr>
<td>Vitamin K (mcg)</td>
<td>60 (71)</td>
<td>25 (29)</td>
<td>20 (71)</td>
<td>8 (29)</td>
<td>80 (71)</td>
<td>33 (29)</td>
</tr>
<tr>
<td></td>
<td>67 (79)</td>
<td>18 (21)</td>
<td>18 (64)</td>
<td>10 (36)</td>
<td>85 (75)</td>
<td>28 (25)</td>
</tr>
</tbody>
</table>

\[ AI^a = \text{Adequate Intake} \]
\[ EAR^b = \text{Estimated Average Requirement} \]

**Note:** Presented as unweighted sample size (weighted %)

Table 4.4 **NHANES and RUPP Study Subjects’ Recommendation Levels Met (AI\(^a\), EAR\(^b\))**
<table>
<thead>
<tr>
<th>Nutrients</th>
<th>NHANES*</th>
<th>RUPP</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4-8 years old</td>
<td>9-13 years old</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not met</td>
<td>Met</td>
<td>Not met</td>
</tr>
<tr>
<td><strong>Magnesium (mg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHANES* Magnesium (mg)</td>
<td>586 (17)</td>
<td>2787 (83)</td>
<td>2694 (59)</td>
</tr>
<tr>
<td>RUPP Magnesium (mg)</td>
<td>644 (21)</td>
<td>2729 (79)</td>
<td>1746 (45)</td>
</tr>
<tr>
<td><strong>Vitamin C (mg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHANES* Vitamin C (mg)</td>
<td>13 (15)</td>
<td>72 (85)</td>
<td>18 (64)</td>
</tr>
<tr>
<td>RUPP Vitamin C (mg)</td>
<td>3(4)</td>
<td>82(96)</td>
<td>5(18)</td>
</tr>
</tbody>
</table>

RDA\(^a\) = Recommended Dietary Allowance

Note: *Presented as unweighted sample size (weighted %)

Table 4.5  NHANES and RUPP Study Subjects’ Recommendation Levels Met (RDA\(^a\))
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Intake of Nutrients</th>
<th>NHANES Study</th>
<th>RUPP Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adequate (≥100% DRI)</td>
<td>Low (80-99% DRI)</td>
<td>Inadequate (&lt;80% DRI)</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>4-8 years old</td>
<td>2701 (39)</td>
<td>524 (15)</td>
</tr>
<tr>
<td></td>
<td>9-13 years old</td>
<td>874 (22)</td>
<td>598 (15)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>2701 (39)</td>
<td>1122 (15)</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>4-8 years old</td>
<td>2787 (83)</td>
<td>349 (10)</td>
</tr>
<tr>
<td></td>
<td>9-13 years old</td>
<td>1626 (41)</td>
<td>828 (17)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>4413 (62)</td>
<td>1177 (14)</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>4-8 years old</td>
<td>2729 (78)</td>
<td>175 (6)</td>
</tr>
<tr>
<td></td>
<td>9-13 years old</td>
<td>2574 (55)</td>
<td>292 (7)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5303 (66)</td>
<td>467 (7)</td>
</tr>
<tr>
<td>Vitamin D (mcg)</td>
<td>4-8 years old</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>9-13 years old</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Vitamin K (mcg)</td>
<td>4-8 years old</td>
<td>499 (20)</td>
<td>278 (10)</td>
</tr>
<tr>
<td></td>
<td>9-13 years old</td>
<td>763 (24)</td>
<td>307 (10)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1262 (22)</td>
<td>585 (10)</td>
</tr>
</tbody>
</table>

Note: Recommended Dietary Allowances (RDAs) in **bold** and Adequate Intake (AIs) in regular type
Presented as unweighted sample size (weighted %)

Table 4.6  Summary of Individual Nutritional Balance at Baseline for NHANES and RUPP Studies
(AI<sup>a</sup>, EAR<sup>b</sup>, RDA<sup>c</sup>)
Figure 4.1  NHANES Study Subjects’ Meeting EAR/AI Recommendations for Bone Health Nutrients by Age
Figure 4.2 RUPP Study Subjects’ Percent Meeting EAR/AI Recommendations for Bone Health Nutrients by Age
CHAPTER 5

CONCLUSIONS AND IMPLICATIONS

Conclusions

The data from our review study provides insight into the bone nutrient intakes of both typical and children with autism spectrum disorder (ASD) based on intake levels from both the NHANES and RUPP studies. Our data suggest deficient intakes of bone nutrients, specifically calcium, vitamins D and K, in many autistic children. Less than one-third of all autistic children met likely adequate dietary levels of these nutrients, and more than 60% of autistic children had intakes less than 80% of the DRIs for vitamins D and K signaling a possible risk for deficiency and poor bone health.

In comparison to typical children, autistic children tended to have much lower likely adequate intakes of calcium and vitamin K. Though comparisons with vitamin D intakes could not be made between the two groups because there was not vitamin D data collected in the NHANES study, it is reasonable to conclude that vitamin D intakes in most autistic children are likely inadequate. These findings suggest that further well-designed studies are needed to develop a clear link between autism and bone health nutrients, particularly vitamin D, as intakes of this nutrient by autistic children in our study were deficient.
Recommendations

As a result of these bone nutrient data from our review study, it can be concluded that there is a need for further studies examining different components possibly related to vitamin D deficiencies and autism in children. A combination of dietary intake measures such as FFQs, dietary recall, and/or food records should be used in any future research studies in autistic children to ensure accurate dietary intake and to display any unusual eating habits or patterns. Relationships and/or comparisons between genders should also be included in an ideal study as boys are more often diagnosed with autism than girls. Multiple dietary intake measures as well as gender differences should be used in combination with any of the components stated below for a more complete study.

Because the body has the ability to make vitamin D when exposed to sunlight, one area of concern related to the relationship between vitamin D and autism is regarding the amount of ultraviolet B (UVB) rays found from sunlight. An ideal research study would target different subjects from different UVB zones in the United States to examine the amount of serum vitamin D and the prevalence of autism in those areas, and throughout the United States. This could be done by zone coding when published for patient confidentiality. The synthesis of vitamin D is dependent on many factors, such as skin pigmentation, degree of latitude, season, the amount of cloud cover, and the amount of skin exposed. All of these would need to be considered in the research study. Hypothetically, the autistic children living in areas with more sunlight would have higher serum vitamin D levels than those in areas with less UVB rays.
Another component that should be included in a study would be the use of a dual energy x-ray absorptiometry (DEXA) scan to identify bone mass and structure of the autistic children. Dietary records and/or FFQs could be used to compare with vitamin D intakes, and other bone health nutrients, and any relationships between DEXA scores and vitamin D intakes could be assessed. To make further comparisons, DEXA scans and dietary intakes should be collected from typical children as well. To determine whether nutrient supplementation would be effective in the autistic children, bone mass would first have to be established as compromised in these children. For some children, other conditions may preclude the supplementation, such as chronic gastrointestinal (GI) issues.

Because many children with autism have seizures and are taking anti-epileptic drugs (AEDs), a study looking at the differences in vitamin D intakes between autistic children taking AEDs and those not taking the medication would be beneficial in showing any bone health differences possibly attributed to the AEDs. Typical children could be used as well for a control group comparison.

In the time frame of this review study, vitamin D data for the NHANES studies were not collected. This review study also could be conducted again in the future when the NHANES study includes vitamin D intakes for better comparisons of bone health nutrient intakes between autistic and typical children.
LIST OF REFERENCES


Accessed 2/10/09.
