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Effects Of Dietary Intake Of Antioxidants And Omega-3 Fatty Acids On Free Radical Production In Children At Various Stages Of Chronic Kidney Disease

Linda Marie Sawyer
Wayne State University,

EFFECTS OF DIETARY INTAKE OF ANTIOXIDANTS AND OMEGA-3 FATTY ACIDS ON FREE RADICAL PRODUCTION IN CHILDREN AT VARIOUS STAGES OF CHRONIC KIDNEY DISEASE

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LINDA MARIE SAWYER

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DEDICATION

In loving memory of my parents, Pat and Jim Stockman

To my husband, Jim, and daughter, Lauren, for all their love and support throughout the program

To Dr. Jen for sharing her knowledge and for her steadfast dedication to teaching and research

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CHAPTER 1: INTRODUCTION

Background and Clinical Significance

Chronic kidney disease (CKD) is identified as a public health priority because the mortality rate due to CKD has risen by 82% over the past two decades (Lozano et al., 2012; Mroue, Xu, Zhu, Morris, & Ramamoorthy, 2016; Radhakrishnan et al., 2014). Cardiovascular (CV) morbidity and mortality are increased across all ages in patients with CKD (Flynn, 2012), including those on dialysis and post renal transplantation. Himmelfarb, Stenvinkel, Ikizler, and Hakim (2002) stated that "cardiac mortality for dialysis patients aged 45 years or younger is more than 100-fold greater than in the general population" p. 1524. Moreover, CV mortality in pediatric-onset renal failure is 1,000 times higher in comparison to the general population (Flynn, 2012) and accounts for 25% of deaths due to cardiovascular disease (CVD) in children with end-stage renal disease (ESRD) (Silverstein, Mitchell, LeBlanc, & Boudreaux, 2007). In children post renal transplantation, CV mortality is 100 times higher than their age-matched population, resulting in 40% of deaths due to cardiovascular disease (Kaidar et al., 2014) . Increased CV morbidity and mortality in patients on dialysis and post renal transplant underscore the importance of prevention through nutritional and life-style management strategies, as well as treatment options aimed at decreasing the risk of CVD in the pediatric CKD population.

According to the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), nutritional intervention is a treatment strategy to delay progression of CKD and reduce the risk of CVD (NIDDK, 2017). Delaying the progression of CKD will also reduce the risk of CVD and other co-morbidities in the pediatric CKD population. Determining

nutritional strategies for children with CKD may hold the key to delaying “disease progression” in the pediatric CKD population. This clinic-based, comparative cross-sectional study was designed to evaluate the cardioprotective effects of dietary intake of antioxidants and ω-3 fatty acids in children pre-dialysis, during treatment with dialysis (hemodialysis [HD] and peritoneal dialysis [PD]), and post renal transplantation.

Stages of Chronic Kidney Disease

Creatinine is a biomarker of kidney function. Glomerular filtration rate (GFR) is a measure of renal function in both children and adults. A quick way to estimated GFR is 0.413 x (height in cm/serum creatinine) (Schwartz et al., 2009). CKD occurs in five stages. Stages 1 to 4 indicate kidney damage, with a normal or high GFR in Stage 1, to a severely low GFR in Stage 4. The GFR for the stages of CKD are as follows: Stage 1: GFR ≥ 90 mL/min per 1.73 m^2; Stage 2: GFR 60-89 mL/min per 1.73 m^2; Stage 3: GFR 30-59 mL/min per 1.73 m^2; Stage 4: GFR 15-29 mL/min per 1.73 m^2; and finally, Stage 5 indicates kidney failure with a glomerular filtration rate below 15 mL/min per 1.73 m^2 and requires dialysis or renal transplantation (" Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). Since there is not a surrogate biomarker of kidney function in dialysis patients, Kt/V is used to measure the adequacy of dialysis (Elshamaa et al., 2009). K measures dialyzer clearance in milliliters per minute (mL/min), t stands for time, with Kt as a measure of clearance multiplied by time for completely clearing urea during one treatment; and finally, V is the volume of body water (NIDDK, 2009). To determine adequacy of HD, estimates of Kt/V for < 1.0, < 1.2 and < 1.4 were used as a comparison (Goldstein, Brem,

Warady, Fivush, & Frankenfield, 2006). Kt/V for adequacy of HD is at least 1.2 (NIDDK, 2009); whereas, adequacy of PD is at least 1.7 (KDOQI, 2006).

Co-morbidities of Chronic Kidney Disease

With an earlier age of onset of CKD, there is a greater risk of co-morbidities associated with the disease. Co-morbidities of CKD include malnutrition, growth retardation, joint pain, dental problems, hypertension, and CVD.

The pathogenesis of CVD in children with CKD is multifactorial. The pathogenesis includes a) commonly known risk factors, such as elevated blood pressure, lipid abnormalities, overweight/obesity, and deceased physical activity (Civilibal et al., 2007; Lilien, Schroder, & Koomans, 2005); b) renal failure (uremia) related factors, such as anemia, high parathyroid hormone, elevated phosphorus and calcium product, and volume overload (Civilibal et al., 2007; Lilien, Schroder, et al., 2005); and c) novel risk factors, such as systemic inflammation and oxidative stress (Elshamaa et al., 2009; Lilien, Schroder, et al., 2005).

Poor blood pressure control remains an underlying risk factor for CVD in nearly 70% of children on HD, PD, and post renal transplantation (Flynn, 2012). Hypertension remains a major concern and “clinically important risk factor” for CVD in children post renal transplantation (Kaidar et al., 2014). Increased sodium (Na) intake is a risk factor in the development of hypertension and preferences for consuming Na are developed during childhood (Appel et al., 2015; IOM, 2013). In the United States, the average Na intake is 3,400 mg/day (IOM, 2013) and 3,100 mg/day (Appel et al., 2015), in adults and children respectively. The recommended guidelines for Na intake in adults is 2,300 mg/day (IOM, 2013). According to the Institute of Medicine (IOM), the upper limit (UL) for

the dietary reference intakes (DRI) of Na in children is as follows: 1) 1-3 years of age: 1,500 mg/day, 2) 4-8 years of age: 1,900 mg/day, 3) 9-13 years of age: 2,200 mg/day, and 14-18 years of age: 2,300 mg/day (Appel et al., 2015; IOM, 2005b). Children and adults in the United States consume excess Na in the diet which is a contributing factor to the development of CVD in patients with CKD (Appel et al., 2015; IOM, 2013).

Moreover, increased intake of dietary Na increases albuminuria and lowers serum albumin levels (Hui et al., 2017). Albumin levels reflect protein status and affect growth. According to Hui et al., 2017, children with CKD experience growth failure due to reduced energy intake and protein wasting. The IOM's dietary reference intakes (DRI) of protein based on age are as follows: 1) 1-3 years of age: 1.05 g/kg/day or 13 g/day, 2) 4-8 years of age: 0.95 g/kg/day or 19 g/day, and 3) 9-13 years of age: 0.95 g/kg/day or 34 g/day (IOM, 2005a). The estimated average protein requirement for adolescents 14 -18 years of age is 0.73 g/kg/day and 0.71 g/kg/day, in boys and girls respectively (IOM, 2005a). According to the KDOQI, children with CKD require a daily protein intake at 100-140% (stage 3) and 100% (stages 4-5) of the DRI per ideal body weight ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). A high body mass index (BMI), indicating obesity, and a low BMI, reflecting growth failure, place children in ESRD at increased risk for mortality (Hui et al., 2017)

Accumulation of uremic toxins due to deficient renal clearance contributes to endothelial dysfunction leading to CVD in adults and children alike (Himmelfarb et al., 2002; Moradi, Sica, & Kalantar-Zadeh, 2013). Urea is a product of protein catabolism (Vartia, 2013) and uremic toxins contribute to "the progression of cardiovascular disease in patients with CKD" (Moradi et al., 2013, p. 136). In CKD, uremia also decreases red

blood cell survival contributing to anemia (Elshamaa et al., 2009) and anemia is a contributing factor for increased lipid peroxidation and CVD (Elshamaa et al., 2009).

In addition to the accumulation of uremic toxins, components of HD, such as the access device and filter membrane, promote inflammation and oxidative stress (Elshamaa et al., 2009; Himmelfarb et al., 2002; Lilien, Koomans, & Schroder, 2005). Components of PD, such as the dialysate, damage the endothelial structure and impairs endothelial function. (Covic, Goldsmith, Florea, Gusbeth-Tatomir, & Covic, 2004; Lilien, Schroder, et al., 2005). Moreover, other mediators, such as hypertension, anemia, and elevated high sensitivity C-reactive protein (hs-CRP) are cardiovascular risk factors in children with ESRD (Civilibal et al., 2007).

Oxidative Stress

Oxidative stress is a known risk factor for CV morbidity in patients with uremia. Free radical damage begins early in CKD progression, *well before* treatment with dialysis, and results in the formation of inflammation and atherosclerosis leading to CVD (Dounousi et al., 2006; Elshamaa et al., 2009; Schmidl & Labuza, 2000). Oxidative stress results from an imbalance between antioxidant activity and pro-oxidants leading to free radical damage (Himmelfarb et al., 2002; Schmidl & Labuza, 2000). Free radicals, such as hydroxyl radical (OH·), superoxide radical (O_2·-), nitric oxide radical (NO·), and lipid peroxyl radical (LOO·) *are unstable, damaged* molecules that react with other molecules in the body leading to cellular injury and disease (Schmidl & Labuza, 2000). Polyunsaturated fatty acids, due to their numerous double-bonds, are easily oxidized *in* vivo by free radicals and other reactive oxygen species (Daschner et al., 1996) Peroxidation of arachidonic acid (C20:4, ω-6) causes free-radical injury through

prostaglandin-like compounds called isoprostanes (ISOP) (Osorio, Ferreyra, Perez, Moreno, & Osuna, 2009). 8-Isoprostanes (8-ISOP) are the most reliable biomarkers of oxidative stress *in vivo* (Osorio et al., 2009) and are reliable for use in patients with uremia (Dounousi et al., 2006). Thiobarbituric Acid Reactive Substances (TBARS) reliably measures malondialdehyde (MDA), a water-soluble lipid peroxidation product of polyunsaturated fatty acids resulting from cellular injury and is another indicator of oxidative stress in uremia (Daschner et al., 1996).

A study of pediatric patients on HD (n = 10) and PD (n = 11) showed that elevated plasma MDA levels were due to oxidative damage from uremia rather than from the dialysis treatment itself (Daschner et al., 1996). Kotur-Stevuljevic et al. (2013) found that MDA, a marker of oxidative stress, was "markedly increased" and superoxide dismutase (SOD), a marker of oxidative defense, was "compromised" in 52 pediatric patients (n = 10 with CKD, n = 22 post renal transplant, and n = 20 on HD; compared to a control group of 36 healthy children). The authors also concluded that atherosclerosis begins early in children with CKD in response to oxidative stress and dyslipidemia (Kotur-Stevuljevic et al., 2013).

ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is used for measuring total antioxidant capacity *in vivo* by metmyoglobin in plasma and other body fluids (Cayman, 2016). The ABTS assay measures the ability of antioxidant systems, including detoxification enzymes, macromolecules (such as, albumin and ferritin) and small molecules (such as, β-carotene, ascorbic acid, α-tocopherol, and uric acid) to reduce oxidative stress and cellular damage (Cayman, 2016).

Dietary Antioxidants and Omega-3 Fatty Acids

Vitamin C, a well-known antioxidant, improves endothelial function (ED) and lessens the risk of CVD, especially in patients who are at increased risk for CVD (Ashor, Lara, Mathers, & Siervo, 2014) and is a cofactor with iron to boost red blood cell production and improve states of anemia. According to the Food and Nutrition Board, Institute of Medicine, National Academies, (www.nap.edu), the recommended dietary intake of vitamin C for children by age is as follows: 1–3 years: 15 mg, 4–8 years: 25 mg, 9–13 years: 45 mg, boys 14–18 years: 75 mg, and girls 14–18 years: 65 mg.

Intima media thickness (IMT) and brachial artery flow-mediated dilation (FMD) are measures of ED in CVD. In an experimental study that administered 250 mg/day of vitamin C for 1 month duration to 18 children with CKD (9 on HD, 4 on PD, 3 on pharmacological treatment, and 2 post renal transplant) and to a control group of 19 children without CKD, found that vitamin C significantly reduced the IMT in the experimental and control groups ($p < 0.05$) and increased the brachial artery FMD in both groups reaching statistical significance in the control group (Sabri, Tavana, Ahmadi, & Gheissari, 2015). Administration of 250 mg/day of intravenous (IV) vitamin C after HD sessions three times a week for 12 weeks was also found to significantly reduce uric acid and improve the lipid profile in children on HD (n=30) compared to the placebo group receiving IV saline (n=30) (El Mashad, ElSayed, & Nosair, 2016).

Vitamins C helps to control blood pressure. A meta-analysis of randomized controlled trials for the effects of vitamin C on blood pressure reported that vitamin C lowered both SBP and DBP in short-term trials (Juraschek, Guallar, Appel, & Miller, 2012). In addition to improving endothelial function, vitamin C helps to form the basement

membrane of blood vessels by Type IV collagen formation and functions as an antioxidant in free radical scavenging (May & Harrison, 2013).

Carotenoids, such as β-carotene, lycopene, and lutein, as well as other carotenoids, act as antioxidants and pro-oxidants in humans and animals (Fiedor & Burda, 2014). The fine balance between antioxidant and pro-oxidant activity is important to human health because loss of antioxidant activity "results in oxidative stress" contributing to chronic health conditions, such as CVD, cancer, neurological disorders, immune diseases, and visual impairment (Fiedor & Burda, 2014). Beta-carotene is a carotenoid and an antecedent to vitamin A (retinol). Retinol (ROH) and the transport protein for vitamin A, retinol-binding protein (RBP), accumulate when the kidneys are impaired and the glomerular filtration rate is reduced ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). Vitamin A is not cleared by dialysis. Fassinger, Imam, and Klurfeld (2010), studied two groups of children receiving dialysis (children < 12 years: HD, n = 8; PD, n = 19) and (children > 12 years: HD, n = 19; PD n = 29) and found that children in ESRD had elevated serum levels of ROH, RBP, and transthyretin (TTR). In children with CKD, supplementation with vitamin A is only indicated for individuals with very low dietary intake ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). The upper limit for supplementation of preformed vitamin A by age for children is as follows: 1–3 years: 2,000 IU, 4–8 years: 3,000 IU, 9–13 years: 5,667 IU, and 14–18 years: 9,333 IU (www.nap.edu).

Beta-carotene works synergistically as an antioxidant with vitamin E, mainly α-tocopherol, to trap free radicals (Fiedor & Burda, 2014). Vitamin E demonstrates the

ability to protect the heart and kidneys through anti-inflammatory and lipid-lowering activity (Daud et al., 2013; Fiedor & Burda, 2014). Studies on vitamin E in children with CKD were not found in the literature. A randomized, double-blind, placebo-controlled study by Daud et al. (2013) demonstrated that oral administration of vitamin E tochotrienol-rich fraction (TRF) in a dosage of 180 mg tocotrienols and 40 tocopherols twice daily improved lipid profiles at 12 and 16 weeks in elderly patients (age > 65 years) receiving HD.

Trace minerals, such as copper, selenium, and zinc are monitored in children with CKD ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). Trace minerals are monitored to assess for signs of deficiency or excess. Low serum copper and low serum zinc levels may result from poor nutrition or removal by dialysis; whereas, selenium is not removed by dialysis ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). Moreover, the amount of selenium in food depends on the soil and low serum selenium levels are noted in children with CKD ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). Selenium works with glutathione peroxidase and other enzymes to function as a free radical scavenger and antioxidant (Loo, 2009). The RDI for children for selenium, zinc, and copper are 15–55 μg/day, 2-11mg/day, and 200-900 μg/day respectively (Loo, 2009).

Omega-3 fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) are known to have anti-inflammatory and cardioprotective properties and have been studied in children and adults on dialysis (Daud et al., 2012; Goren, Stankiewicz, Goldstein, & Drukker, 1991; Zhu et al., 2014). Daud et al. (2012) conducted a

randomized, placebo-control pilot study of 63 patients, age 18 years and older (on HD for > 3 months) to determine the effects of protein and ω-3 supplementation on lipid profiles, nutritional parameters and inflammatory indices. The patients in the study received an ω-3 supplementation (total DHA 600 mg and total EPA 1800 mg; n= 31) or a placebo of olive oil along with protein supplementation three times a week during dialysis treatment (Daud et al., 2012). The findings of this study showed that ω-3 supplementation treatment had a favorable effect on the lipid profile and C-reactive protein levels (Daud et al., 2012). Plasma fatty acid composition was studied in children (6 – 18 years of age) to determine the effects of estimated intake of nutrients on the risk for CVD in post renal transplant patients (Aldamiz-Echevarria et al., 2004). Nutrient intake pattern of these children included: 1) high intake from oleic acid (C18:1), a monounsaturated fatty acid (MUFA) mainly derived from olive oil (mean 0.7 g/kg/day; 20% of energy intake from MUFAs/day), 2) lower intake of saturated fatty acids (13% of energy intake/day) compared to unsaturated fatty acids (26% of energy intake/day), and 3) 39% energy intake/day from total fat which was above the recommendation (30%) for children with renal disease (Aldamiz-Echevarria et al., 2004). Results showed that intake of nutrients from MUFAs and polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid lowered total cholesterol, LDL-C, and apolipoprotein A (Aldamiz-Echevarria et al., 2004). Consistent with previous literature, and as reported in this study, the authors suggest that *cis* fatty acid, oleic acid, may increase plasma HDL-C and recommend that total fat intake (10% total calorie intake/day *from each* saturated fat, MUFA and PUFA), as well as intake of linoleic acid be limited to 3-6 g/day to prevent desaturation competition with ω-3 fatty acids in children post renal transplantation (Aldamiz-Echevarria et al., 2004).

The effects of dietary intake of antioxidants and ω-3 fatty acids on biomarkers to measure oxidative stress (8-ISOP, TBARS, and ABTS) in children with CKD at various stages of treatment are unknown even though adequate intake of antioxidants and ω-3 fatty acids may benefit children with CKD. Similarly, the intakes of antioxidants and ω-3 fatty acids of these children are not documented. To the best of our knowledge, this was the first clinical study that is uniquely involved in researching the dietary effects of antioxidants and ω-3 fatty acids on "disease progressive oxidative stress" in children at various stages of CKD.

Overall Objective

The purpose of this study was to understand the relationship between dietary intake of antioxidants and ω-3 fatty acids on free radical injury and free radical scavenging in children with CKD so that complications of kidney failure, especially cardiovascular disease, may be avoided or delayed.

Specific Aims

Aim #1: To test the hypothesis that high dietary intake of antioxidants and ω-3 fatty acids would lower free radical damage, as measured by lipid peroxidation products (8-ISOP & TBARS) in children with chronic kidney disease pre-dialysis, during treatment with dialysis (HD and PD), and post renal transplantation.

Aim #2: To test the hypothesis that high dietary intake of antioxidants and ω-3 fatty acids would produce better free radical scavenging ability, as measured by ABTS levels in children with chronic kidney disease pre-dialysis, during treatment with dialysis (HD and PD), and post renal transplantation.

Aim #3: To test the hypothesis that children with CKD and high dietary intake of antioxidants and ω-3 fatty acids would have a better metabolic profile and bone health as compared to those with lower intake of these nutrients.

CHAPTER 2: METHODS

Design and Setting

This clinic-based, comparative cross-sectional study investigated children of both genders, 3 to 21 years of age, receiving treatment in the nephrology clinic pre-dialysis and post renal transplantation, as well as children on HD and PD treated in the dialysis units at Children's Hospital of Michigan (CHM), Specialty Center (Detroit, MI).

Treatment Groups

1. Pre-dialysis: CKD stages 2 to 4
2. Hemodialysis: CKD stage 5
3. Peritoneal Dialysis: CKD stage 5
4. Post Renal Transplantation

Inclusion Criteria

Inclusion criteria were: (1) children diagnosed with CKD receiving treatment pre-dialysis, during treatment with dialysis (HD and PD), and post renal transplantation at CHMs - Specialty Center; (2) age 3 to 25 years; (3) on dialysis for at least 3 months prior to entering the study, and (4) renal transplant patients on follow-up in the nephrology clinic and at least 3 months after surgery for recent transplant. The inclusion criterion for children on dialysis for at least 3 months is supported by previous research (Elshamaa et al., 2009; Tsai et al., 2010; Zimmerman et al., 2014).

Exclusion Criteria

Exclusion criteria included: (1) diseases of the liver and spleen, (2) human immunodeficiency virus (HIV) infection, (3) cancer, (4) hematological disorders, and (5) inflammatory disorders. Infectious diseases, inflammatory disorders, and cancer may

alter the results of biomarkers of oxidative stress and were therefore listed in the exclusion criteria for this study.

Participants

Participants in this study were children. Children's Hospital of Michigan is one of the first in the state to provide outpatient dialysis services to children. Children were recruited from the HD unit, PD unit, and nephrology clinic at the Children's Hospital of Michigan, Specialty Center. The HD unit has provided chronic dialysis services on average to 15 children at any given time point per year, with clinic visits scheduled three times a week, on Monday, Wednesday, and Friday. The PD unit also services, on average, 15 children at any given time point throughout the year; although, as determined by the exclusion criteria, children under 3 years of age were not eligible to participate in this study. Children on PD were seen during their monthly follow-up appointment in the clinic. The nephrology clinic services on average 60 to 70 pre-dialysis children and 20 to 40 kidney transplant children per year. In this study, we enrolled a total of 47 children: 15 children in the pre-dialysis treatment group, 11 children in the HD treatment group, 6 children in the PD treatment group, and 15 children in the post-transplant treatment group.

Institutional Review Board Approval, Informed Consent and Assent

Institutional Review Board approval was obtained from the Detroit Medical Center/Children's Hospital of Michigan and from Wayne State University. A pediatric nephrologist informed the children and their parents/caregivers about this study during their routine office visits, and if the children and parents/caregivers were interested, consents from the parents and assent, for children 13 years and older, were obtained by

the RN Principal Investigator or the Pediatric Nephrologist. Each participant was identified by a code number for data collection and analysis.

Data Collection

Demographic data (age, gender, race/ethnicity, financial status, household composition, modes of transportation) on each participant was collected at the start of the study. Demographic data was collected by the RN Principal Investigator by means of interview and data obtained from the child's electronic medical records (EMR).

Anthropometric measures - Both weight and height measures were carefully obtained by the clinic RNs since undernutrition and edema are complicating factors present in children with CKD ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). Age and gender specific body mass index (BMI) percentile and z-scores were then calculated or obtained from the child's EMR at the start of the study.

24-hour diet recall and prospective 3-day dietary records – The KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update recommends assessing nutritional intake by means of a prospective 3-day dietary records, as instructed by a skilled registered dietitian. The nutritional management of children with CKD was coordinated by the renal dietitian in collaboration with other members of the multidisciplinary pediatric nephrology team ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). As part of this team, the RN Principal Investigator was responsible for obtaining a 24-hour diet recall (identified by a code number) during a routine clinic visit and children and their parents/caregivers were asked to return, within one week, the prospective 3-day dietary records (identified by a

code number) in a self-addressed, stamped envelope addressed to the investigators in the Department of Nutrition & Food Science at Wayne State University. A five-dollar ($5.00) gift card was offered as an incentive for completing and returning the 3-day dietary records. During the dietary recall data collection, food models and measuring devices were provided to the child or parents/caregivers to assist them to identify the amount of each food consumed. A multi-passage method was used to collect the dietary recall data (Johnson, Driscoll, & Goran, 1996). In this procedure, during the “first pass”, the child or parents/caregivers were asked to write down everything the participant ate over the previous 24 hours (Johnson et al., 1996). The “second pass” clarified the types of food eaten at meals and snacks. Finally, the “third pass”, defined portion sizes of each food entered on the dietary records (Johnson et al., 1996). At the end of the dietary recall, 3-day dietary record forms were given to the child or parents/caregivers. In this form, spaces were provided to record food items consumed at breakfast, mid-morning snack, lunch, mid-afternoon snack, dinner and evening snack. Spaces were provided to record serving size, preparation methods, brand names or restaurant names. An instruction sheet was also provided to the child or parents/caregivers to remind them about the information that was needed to complete the food diary. The 3-day dietary records included two weekdays and one weekend day. Dietary intake as recorded in the dietary records/recall records was analyzed.

The database and software Food Processor® Nutrition Analysis by EHSA (Version 11.3.285, Salem, OR, USA) were used to analyze nutrient intakes. A “lightly active” physical activity level was used as a basis for analyzing nutrient intakes on all diet records. Only 13% of adolescent with CKD meet the recommended guidelines set by the American

Academy of Pediatrics for physical activity in children (Clark, Denburg, & Furth, 2016; Hui et al., 2017).

Routine laboratory testing – Blood samples were obtained during *routine* blood draws performed by a skilled dialysis nurse or phlebotomist according to hospital protocol. Standard laboratory methods for processing the specimens and measuring routine laboratory tests were analyzed by Detroit Medical Center laboratories. Routine laboratory testing (clinical measures) included serum levels of hemoglobin (Hgb), albumin, ferritin (Fe), sodium (Na), potassium (K), blood urea nitrogen (BUN), creatinine, calcium (Ca), phosphorus (P), magnesium (Mg), parathyroid hormone (PTH), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and TC/HDL-C.

Oxidative stress (8-ISOP, TBARS and ABTS) measurements – From routine *laboratory blood draws*, an additional 3 mL of blood were collected from each child in a heparinized vacutainer. The samples were labeled (identified by a code number) and transported in a cooler, on ice, to a laboratory in Department of Nutrition & Food Science at Wayne State University. All blood samples were centrifuged at 1,000 x g for 10 minutes at 4°C. Plasma was then separated and stored in small aliquots in a -80 °C freezer until assays were performed later.

ABTS Antioxidant Assay: Antioxidant Assay Kits from Cayman Chemical Company (Ann Arbor, Michigan, United States) were used to determine free radical scavenging ability. ABTS antioxidant assay has an intra-assay coefficient of variation of 3.4%, with an assay range of 0.044 to 0.330 mM Trolox standards (Cayman, 2016).

Assay procedures were implemented and data were analyzed according to Trolox standard curves per the instruction manual provided by Cayman Chemical Company.

8-ISOP Assay: 8-Isoprostane EIA Kits from Cayman Chemical Company (Ann Arbor, Michigan, United States) were used to determine lipid peroxidation (Yu & Navab, 2007). The measuring range for 8-Isoprostane ELISA is based on a dose concentration of 2 to 500 pg/mL. Samples were purified and stored at -80°C in the presence of 0.005% BHT (10 µl of 5 mg/ml solution in ethanol per 1 ml sample) according to pre-assay preparation procedures. Assay protocol and analysis were implemented per the instruction manual provided by Cayman Chemical Company. All samples were measured in one single assay.

TBARS Assay: TBARS Assay Kits from Cayman Chemical Company were used to determine lipid peroxidation (McDonald et al., 2004). This assay has an intra-assay variability of 5.5%, with an assay range of 0 to 50 µM MDA equivalents (Noguchi & Tanaka, 1995). All TBARS assay results in each of the treatment groups were within the range of 0 to 50 µM MDA equivalents. Pre-assay preparation, assay protocol, and analysis were implemented according to the instruction manual provided by Cayman Chemical Company.

Blood pressure measures - For accurate measures of systolic and diastolic blood pressure, an appropriate size blood pressure cuff was used on each child. Blood pressures were obtained from the arm using an electronic blood pressure machine while the child was seated. A skilled dialysis nurse or registered nurse in the clinic obtained and recorded the blood pressure readings in the EMR according to hospital protocol. Routine blood pressure readings, as recorded in the EMR, were used in this study.

Bone Health - To establish a bone health profile, we calculated an antioxidant index by first standardizing intakes of vitamin A, β-carotene, vitamins C, E and selenium. The z scores were added together to produce an index for antioxidant intake. In addition, z score of ω-3 fatty acids was added to the antioxidant index to produce another index representing antioxidants + ω-3 fatty acids intake. Then, these indices were divided equally into three parts to generate categorical variables for mean comparisons, 1 being the lowest one third of the index and 3 being the highest of the index.

Renal Metabolic Profile - To calculate renal metabolic profile, normal ranges of the following minerals were used: Na: 135-137 mEq/L; K: 3.5-5.3 mEq/L; Ca: 8.8-10.1 mg/dL; Mg: 1.5-2.6 mg/dL; P: 4.1-5.5 mg/dL. Metabolic wastes were considered normal when BUN levels were below 20 mg/dL and creatinine levels were below 0.7 mg/dL. For minerals, any values outside of the normal ranges were considered as unhealthy while any values above the metabolic wastes were treated as unhealthy. Since only a few children had serum mineral levels outside of the normal ranges, we created a composite score using z scores for BUN and creatinine only as an indicator of renal function (Renal profile 2). Renal profile 2 was an indicator of poor renal function in children in the non-dialysis group (pre-dialysis and post renal transplant). GFR was used as an indicator of adequate renal function also in children not on dialysis; whereas, Kt/V was used to measure adequacy of dialysis in children on HD and PD.

Data Analysis

Mean and standard deviation (SD) or standard error of mean (SEM) were calculated for each group and for each parameter measured. Statistical Package for Social Sciences (SPSS) software (Version 22, IBM, Armonk, NY, USA) was used for data

analysis. Statistical methods included correlation analysis, analysis of variance (ANOVA), and post-hoc t-tests. Dependent variables included 8-ISOP, TBARS, ABTS and other routine laboratory tests including serum levels of hemoglobin, albumin, blood urea nitrogen, ferritin, calcium, phosphorus, parathyroid hormone, and lipid profile, SBP, DBP, and BMI percentile. Correlation analysis determined the relationships between dietary intake of antioxidants and ω-3 fatty acids as well as other nutrients with 8-ISOP, TBARS, ABTS, routine laboratory results, SBP, DBP, and BMI percentile. ANOVA tests were conducted to determine differences among the four comparative groups or different stages of CKD. If a significant difference was identified among the groups, post-hoc t-tests were performed to identify the groups that contributed to the overall difference. The significance level was set at $p<0.05$.

CHAPTER 3: RESULTS

Demographic Data

There were 34 male (72.3%) and 13 female (27.7%) children recruited into this study. The age range of children in this study was from 3 to 21 years of age. The mean age was 12.2±5.2 years (Mean ± SD). The age range was divided into four categories for statistical analyses: younger than 6 years (n=10), 7 – 12 years (n=14), 12 – 18 years (n=14) and older than 18 years (n=9). Age by treatment distribution is shown in Table 1. Differences in age by treatment were not evident. Gender by treatment was as follows: 1) pre-dialysis: 73% males, 27% females; 2) HD: 64% males; 36% females; 3) PD: 50% males, 50% females; and 4) post-renal transplant: 87% males, 13% females. Racial and treatment distribution is shown in Table 2. Lower percentage of African American children were in the post-transplant group than that of the White or Hispanic groups, but the difference was not statistically significant (p=0.06).

Anthropometric Measures

No difference was noted in the BMI percentiles and z scores for the pre-dialysis, HD, PD, and post-transplant treatment groups (Table 3). There was no difference in the distribution of BMI categories in treatment groups (data not shown).

Clinical Measures

The systolic blood pressure (SBP) in each age group was within the normal recommended blood pressure range for age. Only the 6 – 10-year age group had significantly lower diastolic blood pressure (DBP) than recommended. Table 4 shows the comparison of blood pressure and recommended blood pressure ranges by age. Figure 1 shows that among the four groups of children, SBP readings were similar; whereas, DBP readings were higher among children on dialysis.

Table 5 shows hemoglobin (Hgb) levels according to age categories. All age groups had similar Hgb levels. No difference was noted in Hgb levels in children younger than 6 years of age and the normal level. Children ages 6-12 years, 12-18 years, and males older than 18 years of age had significantly lower Hgb levels than normal ($p<.001$). Females older than 18 years of age also had lower Hgb levels than normal ($p<.01$).

Table 6 depicted the routine clinic measures for the four treatment groups. Hemoglobin levels of the four groups were significantly different from each other. Children on PD had significantly lower Hgb levels than that of the non-dialysis group but similar to that of children on HD.

Among the four treatment groups, serum albumin levels were significantly lower in the PD group ($p<.001$) than the other three groups. Blood urea nitrogen (BUN) levels were higher in children on HD and PD ($p<.0001$) than the levels in pre-dialysis and post-transplant groups. BUN levels were lowest in the post-transplant group. A similar pattern was observed for serum creatinine levels but no difference in creatinine levels between the two non-dialysis groups was observed ($p<.0001$).

In the PD group, serum Fe levels were significantly higher than the other two groups ($p=.001$). Serum ferritin levels were not ordered in the post-transplant group.

Serum Na, K and Ca levels were not statistically different among the four treatment groups. Serum P were higher in the dialysis groups than that of pre-dialysis and post-transplant groups ($p=.001$). Serum Mg levels were also higher in the dialysis groups than the levels observed in the pre-dialysis and post-transplant groups. Magnesium in the post-transplant group was significantly lower than the pre-dialysis group ($p<.0001$). Parathyroid hormone (PTH) levels were higher in children on PD as compared to children

pre-dialysis and on treatment of HD (p=.005). Only one child in the post-transplant group had a PTH level measured.

Although TC, HDL-C and LDL-C levels were not statistically different among the four treatment groups, HD and post-transplant groups had lower TC/HDL-C levels than the pre-dialysis group (p=0.019). TG levels in the HD and post-transplant groups were similar and both were lower than that of the pre-dialysis and PD groups.

The post-transplant group had lower BUN and creatinine levels per renal profile 2 scores than in the pre-dialysis group (p<.001). The GFR was higher in the post-transplant group than in the pre-dialysis group (p<.0001). Among children on dialysis, the Kt/V was higher in the PD group than in the HD group (p=.001).

Biomarkers for Free Radical Injury and Free Radical Scavenging

Table 7 shows the comparisons of biomarkers for free radical injury (8-ISOP & TBARS) and free radical scavenging (ABTS) in the four treatment groups. Less free radical scavenging, as measured by ABTS (p=.015), was noted in the PD and post-transplant groups while the pre-dialysis, HD and PD groups had similar ABTS levels. No differences were noted among treatment groups for free radical injury, as measured by 8-ISOP and TBARS.

Relationships between Clinical Measures (Table 8)

All children: When all children were considered, SBP was significantly correlated with DBP. DBP was correlated with creatinine. Hemoglobin was positively correlated with albumin, but negatively with BUN, creatinine, Fe, P and PTH. Albumin was positively correlated with BUN and negatively correlated with creatinine, Fe, PTH, TC, TG, LDL-C and TC/HDL ratios. BUN showed positive relationships with creatinine, Mg, and P.

Creatinine was positively correlated with Mg, P, and LDL-C levels. Serum Fe showed positive relationship with Mg and PTH. Serum Na was positively correlated with serum LDL-C and TC/HDL levels. Serum K had positive relationships with Mg and TG. Serum Ca was positively correlated with Mg but negatively with TG. Serum Mg showed a positive correlation with P. Serum P was positively correlated with PTH. Serum TBARS was negatively correlated with serum Mg while ABTS levels were positively related to serum BUN, K and Ca. PTH levels showed a positive relationship with TG and a non-significant positive correlation with 8-ISOP.

Pre-dialysis: For the 15 pre-dialysis children, SBP was positively correlated with creatinine and negatively with serum Mg. SBP also showed positive relationships with DBP and serum Na but the correlations failed to reach statistical significance. DBP was inversely related to BUN. Hemoglobin levels showed negative correlation with serum P. Albumin was negatively correlated with serum Fe but positively with serum Ca. Serum Na showed positive relationships with creatinine and K. PTH and 8-ISOP were positively related but failed to reach statistical significance. Renal profile 2 was negatively correlated with serum Hgb but positively correlated with TBARS and 8-ISOP.

Dialysis: Seventeen children were either on HD or PD. SBP revealed inverse correlations with BUN and serum Fe. Hemoglobin levels were negatively correlated with serum Fe, PTH, and 8-ISOP. Albumin and serum Mg as well as ABTS were positively correlated but albumin and PTH were negatively correlated. BUN was positively related to TBARS. Serum Fe and Mg as well as 8-ISOP showed positive relationships. Serum Na was positively correlated with Mg but negatively with P. PTH and TG, TC/HDL-C and 8-ISOP were positively correlated. Kt/V was negatively correlated with SBP and creatinine

and showed a non-significant negative correlation with Hgb. Kt/V had positive correlations with TC and TG. KTt/V was positively related to PTH but failed to reach statistical significance.

Post-transplant: For the 15 post-transplant children, SBP was positively correlated with DBP, Hgb, albumin, creatinine, and Mg. The negative relationship between SBP and serum Ca missed statistical significance. DBP was positively correlated with creatinine and negatively with Ca. Albumin was positively related with Hgb and Mg levels but negatively with serum Fe levels. Serum Fe and PTH showed a positive relationship. Serum K was positively related to Mg and P, while serum P was positively correlated with TBARS.

Non-dialysis: Thirty children were not treated with dialysis (pre-dialysis and post-transplant groups). For this group of children, SBP was positively correlated with DBP and Hgb while DBP was negatively correlated with BUN. The relationships of Hgb was positive with albumin but negative with BUN and P. Serum albumin was inversely correlated with TC, LDL-C and TC/HDL-C. Serum BUN and K, P and ABTS showed significant positive relationships. Serum creatinine was positively related to serum Na, K, TC, TG, LDL-C and TC/HDL-C ratios. Serum Na was negatively correlated with Ca but positively correlated with TG, LDL-C and TC/HDL-C ratios, whereas serum Na and TC missed statistical significance. Serum Mg inversely correlated with HDL-C levels. ABTS levels were positively related to BUN, P and K but negatively although non-significantly with PTH. Renal Profile 2 was inversely correlated with DBP and Hgb, but positively correlated with K, TG, and ABTS. The GFR negatively correlated with BUN, serum Na, K, Mg, TG, TC/HDL-C and missed positive statistical significance with serum Ca.

Relationships between Oxidative Stress and Scavenger Biomarkers and Blood Lipid Profile (Table 9)

All children: In all 47 children, 8-ISOP positively correlated with TG and TC/HDL-C and negatively correlated with HDL-C. TBARS positively correlated with TC and LDL-C; whereas, the correlation with TG missed statistical significance. No relationship between ABTS and blood lipid levels were identified.

Dialysis: In 17 dialysis children, 8-ISOP positively correlated with TG and TC/HDL-C and negatively correlated with HDL-C, although missed statistical significance. ABTS negatively correlated with TG. ABTS showed negative relationships with TC and TC/HDL-C, although failed to reach statistical significance. TBARS was not related to any of the blood lipid parameters.

Non-dialysis: In 30 children not on dialysis, 8-ISOP positively correlated with TG and TC/HDL-C and negatively correlated with HDL-C. TBARS positively correlated with TC and LDL-C. No relationship between ABTS and blood lipid levels were observed.

Dietary Measures

Nutrient Intakes from 24-Hour Diet Recall

Table 10 shows nutrient intakes of the four groups based on 24-hour diet recall records. Only the nutrients that contribute to the bone health and kidney disease are presented. Compared to children in the pre-dialysis and post-transplant groups, children in the two dialysis groups had significantly lower total daily caloric intake (kcal/day); although, the energy intake of the PD and post-transplant groups were similar. Similar patterns were observed for calories and grams from total fat and saturated fat (SAT). No differences were observed among the four groups for total protein and carbohydrate

(CHO) intakes. No differences were observed in ω-3, and ω-6 fatty acids (FAs) intakes among the four groups.

Protein intakes (g/kg), either based on 24-hour diet recall or 3-day dietary records, were analyzed according to the age distribution used by the National Academy of Science (NAS, www.nap.edu). The data showed significantly different intakes among the four age groups. Younger children consumed more protein compared to older children but all age groups consumed significantly higher amount of protein as compared to the Recommended Daily Intake (RDI) for each age group as shown in Table 11.

Micronutrient intake for water soluble and fat soluble vitamins, as well as mineral and water intake did not significantly differ among the four treatment groups, except for calcium intake which was significantly lower in children on HD as compared to non-dialysis groups while children in the PD group had calcium intake that was not different from the other three groups (p=0.001). Sodium intakes were significantly lower among children on HD and PD than that of the other two groups (p=0.003). However, Na intakes were not different among the four age groups and not different from the NAS Na recommended intake either (Table 12).

Total antioxidant intake: Since we hypothesized that total antioxidant intake and ω-FAs intake would improve the metabolic profile in CKD children, we calculated total antioxidant intake, with or without added ω-FAs, for both 24-hour diet recall and 3-day dietary records. This was accomplished by standardizing intakes of vitamin A, β-carotene, vitamin C, vitamin E and selenium. The resulting z scores for these nutrients were summed together to produce composite scores for total antioxidants and total

antioxidant plus ω-3 FAs. The total antioxidant z scores and antioxidant plus ω-FAs z scores from 24-hour recall were not different among the four treatment groups.

Nutrient Intakes from 24-Hour Diet Recall as Percent Recommended

Table 13 shows the percent recommended nutrient intakes in all four groups based on 24-hour diet recall records. Based on the percent recommended, daily energy intake was lower in HD children although the difference failed to reach statistical significance (p = 0.061). Whereas, total SAT intake and calories from SAT were lower among children on HD and PD than that of pre-dialysis and post-transplant children (p=0.022 and p=0.022, respectively). However, intakes of ω-3 and ω-6 fatty acids as percent of recommendations were not different among the four groups, but all were less than 35% of the recommendations for ω-3 FA intake.

The micronutrient intake as percent of recommended for water soluble and fat soluble vitamins, as well as mineral and water intake did not significantly differ among the four treatment groups, except for Ca intake. The Ca intake as percent of recommended was significantly lower in children on HD than in pre-dialysis and post-transplant children but not different from the children on PD (p=0.003). The Ca intake as percent of recommendation by age for children on PD was different from the other three groups (data not shown).

Nutrient Intakes from Average of 3-Day Dietary Records

Table 14 shows the nutrient intakes of the four groups based on the average of 3-day dietary records. Different from the 24-hour diet recall records, energy (kcal) and calories from fat were similar among treatment groups; whereas, only the intake of total SAT and energy from SAT (kcal) differed among the treatment groups. As reflected in the

24-hour diet recall records, average of 3-day dietary records also showed that SAT calories (kcal) and SAT (grams) were lower in children on HD and PD as compared to the pre-dialysis children, but intakes of children on PD and post-transplant children were similar. No statistical differences were detected on analysis of the 3-day dietary records for monounsaturated fat (MUFA) intake and trans fat intake; while, the difference in polyunsaturated fat (PUFA) intake failed to reach significant difference (p=0.076).

Statistically significant findings on analysis of the 3-day dietary records showed that Ca intake (p= 0.003) and phosphorus intake (p=0.017) differed among treatment groups. Calcium intake, same as in the 24-hour dietary recall, was significantly lower in the HD groups than the other treatment groups. Phosphorus intake was also lower in the HD group compared to PD and post-transplant groups but were similar to the pre-dialysis group. Magnesium intake among the four groups of children missed statistical significance (p = 0.071). Average 3-day antioxidant plus ω-3 FA intake were significantly different among the four treatment groups. The PD group had significantly higher intake than the pre-dialysis and HD groups but not different from the post-transplant group. The average total antioxidant intake showed similar pattern and approached to be statistically significant.

Percent Recommended Nutrient Intakes from Average of 3-Day Dietary Records

Table 15 lists the percent recommended nutrient intakes of the four groups based on 3-day dietary records averages. Three-day averages for SAT, protein, MUFA, and PUFA intake varied significantly among treatment groups. PUFA intake and MUFA intake were higher among children on PD and similar among the other treatment groups. SAT fat intakes intake were lower in children on HD than that of children pre-dialysis but not

different from the other two groups. Calcium intake was lower in the children on HD although the difference failed to reach statistical significance.

Energy and macronutrient intakes from 24-hour diet recall and 3-day dietary records were also analyzed based on CKD stages 3 to 5 and post-transplant children. Figure 2 shows the energy, CHO, fat and protein intakes as percent of recommendations based on 24-hour diet recall (top panel) and 3-day dietary records (lower panel). Significant differences in energy and fat intakes were observed on the 24-hour diet recall records. Energy intake was similar among stages 3, 4 and post-transplant children but significantly lower in stage 5 children compared to stage 4 and post-transplant children. Fat intake was similarly higher in stage 4 and post-transplant children and low in stage 5. Carbohydrate intakes were lower and protein intakes were higher than the percent of recommendations but did not differ among the four groups. No difference was observed among CKD stages 3 to 5 and post-transplant children when 3-day dietary records were analyzed.

There were no differences in bone-health related nutrients: Ca, vitamin D, and P or in antioxidants: vitamins A, C, E and selenium based on the stages of CKD (Figure 3) or among the four treatment groups (data not shown). Vitamin D intakes of children in stages 4 and 5 were below 25% of the recommended daily intake. Daily Ca intake based on 24-hour recall and expressed as a percent of recommended intake showed a significant difference. Younger children (4-8 years) had significantly higher intake than children in the 9-13 year and older than the 14-year age groups (95±17%, 48±9% and 48±8%, respectively, $p=0.006$). The older children had Ca intake less than half of the recommended amount.

Relationship between dietary intake and clinical measures (Table 16)

All Children: Based on the 24-hour diet recall and 3-day dietary records, dietary total energy was negatively correlated with serum BUN, creatinine, Ca, Mg, and P. Dietary protein intake was reversely related with serum BUN, and Mg. Carbohydrate intake was inversely correlated with creatinine and serum P. Increase in total fat intake was associated with reductions in serum albumin, BUN, creatinine, and HDL-C, but with an increase in LDL-C. SAT intake was inversely correlated with DBP, BUN, creatinine, Ca, P, and HDL-C, and positively correlated with LDL-C. Intake of MUFA was positively associated with serum Fe and PTH but negatively with albumin. The positive correlation between MUFA and serum K failed to reach statistical significance. Increase in PUFA intake was positively correlated with PTH and LDL-C but inversely with albumin and serum Fe. Omega-3 FA intake was positively related to TC, TG, LDL-C and 8-ISOP while ω-6 intake was associated with SBP, TC, and LDL-C. Increase in trans fatty acid intake reduced serum Fe. Increase in dietary cholesterol intake increased SBP, Hgb, PTH but reduced serum Mg and ABTS. Vitamin C was positively correlated with serum Ca and vitamin D was negatively correlated with serum K. Vitamin E intake was positively correlated with serum Fe, Mg and PTH. Dietary Ca intake was inversely correlated with SBP, DBP, BUN, creatinine, Mg, P, and LDL-C. Dietary Na intake was negatively related to BUN, creatinine, Ca, and Mg, but positively correlated with serum Na. Intake of K reduced serum Mg while intake of Mg was positively associated with serum Fe. Dietary Se increased SBP and serum Fe.

Total antioxidant intake and antioxidant plus ω-3 FAs intake from both 24-hour dietary recall and 3-day dietary records showed positive relationship with serum Fe. No

relationship between antioxidant intake with or without adding ω-3 FAs and other blood parameters were observed, although total antioxidant intake (3-day dietary records) showed a negative trend with TBARS concentrations.

Dialysis: Higher caloric intake reduced serum K, TBARS, and increase adequacy of dialysis per Kt/V. Protein intake was inversely related to albumin and BUN but positively correlated with serum P. Increased CHO intake reduced creatinine, serum K and TBARS. Total fat intake was negatively associated with albumin and BUN but positively related to PTH. Higher SAT intake increased albumin. MUFA increased serum Fe and PTH. Intake of PUFA was negatively correlated albumin but positively with serum Fe, PTH, and Kt/V. Both ω-3 and ω-6 FAs were inversely related to serum Mg. Dietary cholesterol was inversely related to serum Mg but positively with serum P. Vitamin C intake reduced serum K and increased adequacy of dialysis per Kt/V, while vitamin E intake increased serum Fe and PTH. Increase in Ca intake reduced creatinine, but increased serum Fe and Kt/V. Increase in P intake positively correlated with serum P but negatively correlated with serum albumin levels. Intake of K reduced albumin and serum Mg. Increase in Mg intake reduced albumin but increased PTH. Dietary Se intake increase albumin and serum P but reduced BUN.

Total antioxidant intake in the dialysis children was positively related to serum Fe and Kt/V but negatively related to BUN. Increased intake of total antioxidant plus ω-3 FAs increased serum Fe and Kt/V. Intake of antioxidant plus ω-3 FAs also reduced SBP significantly, and BUN and creatinine non-significantly based on either set of dietary intake data.

Non-dialysis: Total energy intake was positively correlated with serum K but negatively with serum Ca. Increased protein intake reduced SBP but increased serum Fe. Higher CHO intake increased serum K but lowered TBARS. Total fat intake was reversely correlated with serum Ca. SAT intake was inversely related to albumin and serum Ca, although missed statistical significance with GFR. Omega-3 FA intake was positively associated with Hgb, TG and LDL-C while ω-6 FA intake increased TG, LDL-C and TC/HDL-C. Trans fatty acid intake increased DBP and reduced serum Fe. Total cholesterol intake elevated SBP and Hgb levels. Increased vitamin A intake was associated with higher SBP, creatinine and serum Fe. Dietary carotene intake was positively correlated with SBP, creatinine, serum Fe but negatively with serum P. Vitamin C intake increased SBP and serum Ca. Higher vitamin D intake lowered serum K while higher vitamin E intake increased serum Mg and P. Dietary Ca intake was negatively correlated with SBP, DBP, albumin, TC, LDL-C and TC/HDL-C levels. Dietary P and serum Fe were negatively correlated. Dietary Na intake was associated with higher SBP and serum Na and lower GFR. Dietary Se was positively correlated with Hgb.

Antioxidant intake was associated with SBP and creatinine and inversely related to TBARS and GFR, although both missed statistical significance. Total antioxidant plus ω-3 FAs was positively related to SBP. Adding ω-3 fatty acid intakes to the total antioxidant z-score improved Hgb levels.

Renal Metabolic Profile

The results for GFR in the non-dialysis group and Kt/V in the dialysis are compared with nutrient intakes as shown in Table 16. In the dialysis group, Kt/V correlated with an increase in caloric intake, PUFAs, vitamin C, Ca, antioxidants, and antioxidants plus ω-3 FA intake. In the non-dialysis group, dietary intakes of SAT, Na, and antioxidants showed negative trends with GFR. Dietary sodium intake correlated with serum Na levels and SPB and was inversely correlated with GFR.

Bone health-related nutrients based on antioxidants with or without ω-3 FAs

Intakes of bone-health-related nutrients (vitamin D, Ca, P, K, Mg and protein) were analyzed based on the categories of antioxidant and antioxidant plus ω-3 FA intakes. Antioxidant as well as antioxidant plus ω-3 FA were divided into three equal groups, the lowest one-third, the middle one-third, and the highest one-third. The results are shown in Table 17. Children with higher antioxidant intakes with or without added ω-3 FA intakes were also consuming more Ca, P, K, Mg and protein (total and per kilogram of body weight). Total antioxidant intake was also negatively correlated with blood TG levels but failed to be significant (r=-.37, p=0.08).

CHAPTER 4: DISCUSSION

To the best of our knowledge, this was the first clinical study that was uniquely involved in researching the dietary effects of antioxidants and ω-3 FAs on "disease progressive oxidative stress" in children at various stages of CKD. In addition to the dietary effects of antioxidant and ω-3 fatty acids, our study compared nutrient intakes with clinical measures at various stages of CKD. Since free radical damage begins well before treatment with dialysis, variations in the children pre-dialysis compared to the children on dialysis (HD and PD) and children post-transplant were examined.

Overall Objective

The purpose of this study was to understand the relationship between dietary intake of antioxidants and ω-3 fatty acids on free radical injury and free radical scavenging in children with CKD so that complications of kidney failure, especially cardiovascular disease, may be avoided or delayed.

Specific Aim #1

We tested the hypothesis that high dietary intake of antioxidants and ω-3 FAs would lower free radical damage, as measured by lipid peroxidation products (8-isoprostanes & TBARS) in children with chronic kidney disease pre-dialysis, during treatment with dialysis (HD and PD), and post renal transplantation.

Our findings showed protective effects of antioxidant plus ω-3 FAs in the dialysis and non-dialysis groups. In the non-dialysis group, adding ω-3 FAs to antioxidant intake intake improved Hgb levels. In children on dialysis, higher antioxidant intake with ω-3 FAs improved dialysis outcome, as indicated by higher Kt/V values, reduced SBP, and increased serum Fe levels. However, our findings of children in various stages of CKD

did not support our hypothesis that dietary intake of antioxidants and ω-3 FA intake would lower free radical damage, although there was a trend toward reducing free radical injury (TBARS).

Patients with CKD are known to have higher oxidative stress (Elshamaa et al, 2009; Lilien et al, 2005) due to dialysis products/process. Hence, higher antioxidant intake may be beneficial for these dialysis patients. One possible explanation for our negative result with 8-ISOP and slight correlation with TBARS is that oxidative products other than the ones used in this study may be more effective to test for free radical injury in patients with renal disease. Future studies to measure other oxidative products, such as advanced oxidation protein products (AOPP) or analyzing several oxidative stress products together may shed more light on the effects of antioxidant intake and oxidative stress status in children with CKD.

It should be noted that children on HD had lower intake of antioxidants and antioxidants plus ω-3 FAs while children on PD had the highest levels. We did not measure these children's appetite or general feeling of well-being, however children in the HD treatment group had lowest energy intake (24-hr recall) and the low antioxidant intakes may be a result of low food intake in these children. On the other hand, children in the PD group had similar energy intake as the post-transplant children but significantly higher antioxidant intake. Hence, quality of the diet of these children played a role in the amount of antioxidants consumed. Our results also showed that children with higher antioxidant intake also had significantly higher total protein intake as well as protein intake based on body weight, and these intakes were significantly higher than the recommendations. While increased antioxidant intake has health benefits for the CKD

children, increased protein intake above the amount required for optimal growth should be discouraged. These results demonstrated the importance of including a registered dietitian in the pediatric renal management team to educate the children and their caregivers regarding how to consume enough protein for growth but not too much to put an extra burden on the diseased or newly transplanted kidneys.

The current results showed the importance of antioxidant intake but also the need for further large-scale study to validate the current findings. Our study also revealed the relationships of antioxidant and ω-3 FAs intakes with other important nutrients in children with CKD. Results from previous studies in this area are mixed. A single-blind, randomized, placebo-controlled study of 22 adult renal transplant patients, receiving 6 grams/day of ω-3 FA supplementation orally for 6 months, actually found an increase in oxidative stress as measured by 8-ISOP levels (Ramezani, Nazemian, Shamsara, Koohrokhi, & Mohammadpour, 2011). Their study also found that ω-3 supplementation significantly reduced serum cholesterol levels in renal transplant children (Ramezani et al., 2011).

In our study, no relationship was found between dietary intake of ω-3 FAs and 8-ISOP levels; although, all four groups that we studied had similar ω-3 FAs intake and all four groups were below 35% of the recommended daily intake for ω-3 FAs. Omega-3 FAs intake correlated with TC, TG, and TC/HDL-C levels. As previously stated, ω-3 FAs supplementation was shown to benefit children on HD dialysis by having a favorable effect on lipid profiles and C-reactive protein levels (Daud et al., 2012). Our study was different from the study conducted by Daud et al. in that ω-3 FA supplements were not administered, and all children in our study had low ω-3 FAs intakes. Thus, it is possible

that ω-3 FAs supplement above the dietary level would benefit the CKD children in blood lipid levels. When the intake of ω-3 FAs is low, no such beneficial effects of ω-3 FAs could be observed.

In all children and in children not on dialysis, 8-ISOP had strong positive correlations with TC, TG and LDL-C and a negative correlation with HDL-C. Similarly, TBARS showed a positive correlation with TC and a weak correlation with TG. Even though the casual relationship between dyslipidemia and elevated oxidative stress products cannot be established in children with CKD, the mechanisms of this relationship between the two parameters deserves further investigation. Dyslipidemia is a common co-morbidity among children with CKD (Khurana & Silverstein, 2015). The administration of statins may be indicated to improve the lipid profiles and offset oxidative damage. A recent, three year randomized, placebo-controlled, double-blind study of 91 children with autosomal dominant polycystic kidney disease was conducted to determine the effects of pravastatin on plasma biomarkers for inflammation and oxidative stress (Klawitter et al., 2015). From findings of this study the authors concluded: “Pravastatin therapy diminished the increase of cyclooxygenase- and lipoxygenase- derived lipid mediators.” (Klawitter et al., 2015, p. 1543). Statins are the only lipid-lowering drug classification approved by the U.S. Food and Drug Administration (FDA) for use in children (Khurana & Silverstein, 2015), and our results support the use of statin drugs to lower the lipid levels in children with CKD. This lipid lowering may indirectly reduce oxidative stress in these children.

Moreover, cardioprotective and renal protective nutritional measures for children with CKD may include improving the balance between ω-3 fatty and ω-6 fatty acids. Higher intakes of ω-6 fatty acids may promote inflammation (Ehrlich, 2015). The ω-6 FA,

arachidonic acid, produces inflammatory cytokines (Efstratiadis, Konstantinou, Chytas, & Vergoulas, 2008; Noori et al., 2011). In our current study, higher intakes of both ω-6 and ω-3 FAs increased blood lipid levels but high intakes of ω-6 FAs also elevated systolic blood pressure. Hence, ω-6 FAs play a more important role in determining blood pressure than ω-3 FAs. The usual dietary intake of ω-6 fatty acids is 14 to 25 times higher than ω-3 FA intake (Ehrlich, 2015). A preferred ratio of ω-6 to ω-3 is 2:1 to 4:1 or lower (Ehrlich, 2015). In our current study, the ω-6 to ω-3 ratio was around 9:1 (range: 8-11 to 1) for both 24-hour recall and 3-day diary records. It is apparent that children with CKD need to reduce the intake of ω-6 fatty acids. Research conducted by Noori et al. (2011) included the collection of three-day diet diary and diary interviews from 145 hemodialysis patients for six months and were followed over 6 years. They reported that a higher mortality rate and inflammation (higher CRP levels) were related to higher ω-6 to ω-3 fatty acid ratio (Noori et al., 2011). Reducing intake of red meat, especially grain fed beef, safflower, sesame, soy, sunflower, and corn oil and increasing intake of fatty fish, including salmon, mackerel, and tuna will improve the balance of ω-3 fatty and ω-6 fatty acids (Ehrlich, 2015).

BUN levels correlated with TBARS in the pre-dialysis group. No studies were published showing a relationship between BUN and TBARS in CKD children, although a study of 51 dementia patients and 30 control patients showed a correlation between increased MDA levels and increased serum BUN levels in dementia patients (Chaturvedi, 2015). In order to design an effective dietary management plan for children with CKD, a well-controlled large-scale study is needed to investigate the relationship between BUN and blood TBARS levels.

Specific Aim #2

We tested the hypothesis that high dietary intake of antioxidants and ω-3 FAs would produce better radical scavenging ability, as measured by ABTS levels in children with chronic kidney disease pre-dialysis, during treatment with dialysis (HD and PD), and post renal transplantation.

While dietary intake of antioxidants and ω-3 FAs failed to demonstrate free radical scavenging ability as measured by ABTS levels, other clinical measures that had a strong positive correlation with ABTS included serum albumin (dialysis group), BUN (all children and non-dialysis group), K (all children and non-dialysis group), Ca (all children) and P (non-dialysis group) did show relationships. In addition to supporting growth, albumin has been shown to have antioxidant potential. Albumin bound bilirubin (Alb-BR) demonstrated free radical scavenging in the plasma and extravascular tissue but was less effective than the antioxidant vitamin C (Stocker, Glazer, & Ames, 1987). Results from our study demonstrated that for all the children together, lower intakes of total fat, MUFA and PUFA (from 3-day dietary records) may increase albumin levels, hence may indirectly elevate antioxidant potential. It should be noted that this dietary manipulation is also the dietary recommendation to reduce the risk of CVD, a co-morbidity of CKD. However, for children on dialysis, reducing protein, total fat, and PUFA intake while increasing the consumption of SAT may increase albumin levels and may have more favorable outcomes. For post-transplant children, reducing SAT intake should be encouraged. Thus, the recommendation for fat intake should be based on the stages/treatment of CKD. Reduced total fat and SAT intake also elevated serum Ca levels in post-transplant children, which in term may increase albumin levels and free radical

scavenging potential. For post-transplant patients, it has been reported that they tend to lose phosphorus (Sakhaee, 2010). Since a positive correlation between serum P and ABTS levels was observed in this group, increase intake of P in post-transplant children may be indicated, not only to improve serum P status, but also to improve the free radical scavenging. BUN and renal profile 2 demonstrated potential for free radical scavenging as measured by ABTS assays in non-dialysis children. Whether this positive relationship between renal profile 2 and ABTS was due to the fact that higher amounts of free radical scavenging was required when the renal waste products were high deserves further investigation.

Specific Aim #3

We tested the hypothesis that children with CKD and high dietary intake of antioxidants and ω-3 FAs would have a better metabolic profile and bone health as compared to those with lower intake of these nutrients. Our results supported this hypothesis.

Bone health, or strong bones and teeth, is supported by diet including vitamins, minerals, and hormones which will lessen the risk of osteoporosis and fractures in children with CKD. To determine bone health, we measured physiological parameters including serum parathyroid hormone, hemoglobin, and phosphorus levels. Bone health also included analysis of dietary intake of Ca, P, and vitamin D, although vitamin D3 supplementation and phosphate binders were not the focus of this study.

Growth retardation affects longitudinal bone growth at the epiphyseal growth plate and is common in children with CKD, stages 3 to 5 (Bao & Chen, 2016; Kuizon & Salusky,

1999). Growth retardation results from poor appetite, malnutrition, anemia, and hypoalbuminemia (Efstratiadis et al., 2008).

Clinical practice guidelines set forth by the KDOQI from the National Kidney Foundation, identified that growth is “adversely affected by renal osteodystrophy” ("KDOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update," 2009). Renal osteodystrophy is now termed chronic kidney disease – mineral and bone disorder (CKD-MBD) (Isakova et al., 2017). CKD-MBD is seen in both children and adults (Khouzam & Wesseling-Perry, 2017; Liu et al., 2015; Ray et al., 2017) and results in CKD-mediated vascular calcification and CVD (Hruska, Sugatani, Agapova, & Fang, 2017; Isakova et al., 2017; Khouzam & Wesseling-Perry, 2017).

According to Khouzam & Wesseling-Perry (2017), pediatric CVD begins before the development of secondary hyperparathyroidism and is related to impaired mineral and bone metabolism. Vitamin D along with PTH, from the parathyroid gland, and calcitonin hormone, from the thyroid gland, regulates Ca metabolism which is important for bone heath. Moreover, factors that inhibit Ca absorption include high P intake, vitamin D deficiency, lack of stomach acids, as well as phytates (from nuts, seeds and grains) and oxalates (from beet greens, spinach, and sweet potatoes) in the diet (Whitney & Rolfes, 2008). New KDOQI clinical practice guidelines recommend bone mineral density testing, joint assessment, and counseling regarding phosphate intake, including dietary measures and the administration of medications (Isakova et al., 2017).

From our findings in all children, there was a positive correlation with PTH and serum phosphorus levels. Normally, the PTH increases serum Ca levels by Ca reabsorption and P elimination via the kidneys (Whitney & Rolfes, 2008). Impaired kidney

function alters this mechanism resulting in elevated P levels in blood and induces secondary hyperparathyroidism. Figure 4 shows the pathogenesis of secondary hyperparathyroidism (http://www0.sun.ac.za/aotc/general/renal/rod.png).

In the current study, the PD group had significantly lower HGB levels than the pre-dialysis and post-transplant groups but not different from the HD group. The two dialysis groups also had significantly higher PTH levels. Furthermore, Hgb was also correlated negatively with PTH levels. These observed metabolic changes may result in impaired growth in children with CKD that were enrolled in this study.

The kidneys regulate erythropoietin which is responsible for the production of red blood cells in the body. With the progression of CKD, Hgb levels decrease leading to anemia as a result of decreased erythropoietin levels (Efstratiadis et al., 2008). Anemia is integral to the triad called the “cardio-renal anemia syndrome” (Efstratiadis et al., 2008). Anemia increases the risk of death by CVD in children with CKD (Efstratiadis et al., 2008). In our study, we found a strong reverse correlation between Hgb and serum PTH levels in all children and among children on dialysis. A previous study of 80 middle age and elderly adults with CKD found a weak reverse correlation between Hgb and serum PTH levels (Adhikary, Pokhrel, Yadava, Khadka, & Thakur, 2015). Approximately 70% of the body’s iron is stored in the Hgb (Gupta, 2014). Interestingly, our finding for serum PTH were positively correlated with serum Fe levels, and serum Fe was negatively correlation with Hgb. These findings implied that decreased erythropoietin levels in dialysis children resulted in reduced Hgb production and Fe not incorporated into Hgb resulting in elevated blood Fe levels.

Among all children and children on dialysis, we found a reverse correlation with PTH and serum albumin levels as well. As stated previously, serum albumin levels reflect protein status, affect overall growth, and have antioxidant potential. Moreover, Ca levels may also be affected by increased dietary Na intake even though Na intakes in this study were within the recommended amount. When Na intake is increased, both Na and Ca are excreted resulting in low blood Ca levels (Whitney & Rolfes, 2008). The loss of Ca affects skeletal growth and dental formation in children.

In our study of bone health nutrients (Ca, P, and vitamin D), we found that children with higher total antioxidant intakes (vitamin A, β-carotene, vitamins C, E and selenium) tended to have lower TG and TBARS concentrations. A recent study using NMR spectroscopy detected triglycerides in bone matrix (Mroue et al., 2016). This study identified that triglycerides in bones may contribute to bone fractures and other diseases (Mroue et al., 2016). No studies were found relating triglycerides to bone health in children yet. However, our results demonstrated the possibility that increase antioxidant intake had beneficial effects which may reduce TG deposition into the bones. Further large-scale studies are warranted to explore the relationship between blood TG and TG in bone matrix.

Adding ω-3 fatty acid intakes to the antioxidant index improved Hgb levels in children not on dialysis (pre-dialysis and post-transplant patients). As stated previously, anemia is a contributing factor for increased lipid peroxidation and increases the risk of death by CVD in children with CKD. Thus, prompt treatment of anemia in children with CKD and post renal transplantation is indicated to reduce free radical injury, delay disease progression, and reduce the risk of CVD.

Total energy and macronutrient intakes were analyzed according to recommendations for age and for differences among stages of CKD. Total energy and macronutrient intakes less than 75% and more than 200% of the recommendations for age were considered as inadequate. Energy, CHO, and fat intakes were all in the adequate intake range even though there was a significant difference in energy and fat intakes among children in different stages of CKD. Children on dialysis in stage 5 of CKD had significantly lower total energy (kcal/day) and fat intake per day. CHO intakes were lower and protein intakes were higher than the percent recommended but did not differ among the four groups. However, protein intakes of children post-transplant and in CKD stages 4 and 5 were higher than 200% of the recommendations. This high protein intake may add an extra burden on the kidneys (diseased or transplanted) and should be discouraged. Renal dietitians should emphasize more reduction of protein intake for these children with CKD.

Other studies in children with renal disease found similar findings. A nutritional assessment study of 30 children with CKD showed that actual energy and protein intakes were 86.5% and 127% of the recommendations, respectively (Apostolou, Printza, Karagiozoglou-Lampoudi, Dotis, & Papachristou, 2014). Another study found that caloric intake was less than 80% while protein intake was 150% of the RDA (Foreman et al., 1996).

Energy and protein requirements for children post-transplant should be the same as normal children (Rees & Shaw, 2007). Caution must be taken to prevent obesity in children post-transplantation since 13% of recipients may become obese due to an increased appetite secondary to steroid use (Rees & Shaw, 2007). In the current study,

40% (6/15) of the post-transplant children were overweight (2/15, 13.3%) or obese (4/15, 26.7%), higher than that reported by Rees and Shaw (2007). Therefore, a top priority for nutrition counseling in children post renal transplant should include instructions to consume a nutrient-dense diet to obtain adequate nutrients for growth but not excess energy to compromise weight.

In the pre-dialysis group, we found that worsening renal function was associated with lower Hgb levels and increased free radical injury (8-ISOP and TBARS). Total antioxidant intake plus ω-3 FAs was found to improve Hgb levels in non-dialysis group. From these findings, it is apparent that increased dietary antioxidant plus ω-3 FA intake helps to increase blood Hgb levels and may reduce free radical injury in children with CKD. TBARS also positively correlated with TC and LDL-C in the non-dialysis group.

Increased dietary intake of Na was shown to decreased kidney function (GFR) in the non-dialysis group. Dietary Na intake correlated with serum Na levels in the non-dialysis group; and serum Na levels nearly correlated with SBP in the pre-dialysis group. Dietary Na intake and serum Na levels should be closely monitored for all children, especially children pre-dialysis to help maintain kidney function, reduce blood pressure, and decrease the risk of CVD.

In children on dialysis, we found that vitamin C intake was shown to decrease serum K levels and vitamin E intake was shown improve serum Fe levels. Moreover, increased SBP and creatinine levels decreased adequacy of dialysis, while increased Hgb had a non-significant reduction in Kt/V. We also found that higher caloric intake, increase dietary intakes of Ca, vitamin C, PUFAs, antioxidants and antioxidants with ω3-FAs improved adequacy of dialysis, thus better renal functioning. The positive

relationships for PTH, TC, and TGs with adequacy of dialysis remains unclear. In all children and especially in children on dialysis, higher intakes of quality nutrients, such as foods higher in antioxidants (vitamins A, β-carotene, vitamins C, E and selenium) will help improve kidney function by decreasing BUN and creatinine levels, as well as improve the outcome of dialysis. Therefore, the importance of healthy nutrient intake cannot be over-emphasized. Children on dialysis are also more vulnerable to oxidative stress due to the iatrogenic effects of dialysis. Consequently, children and young adults on dialysis have a higher mortality from CVD than that the general population (Himmelfarb et al., 2002).

In our study, we found that the ω-6 to ω-3 FA ratio was 9:1 where ideally, the ratio should be 2:1 to 4:1 (Ehrlich, 2015). Moreover, we found that the dietary intake of ω-3 FAs was very low with children consuming only 35% of the recommended daily intake. However, this low intake of ω-3 FAs showed a positive correlation with TC, TG, and TC/HDL-C levels. Omega-3 FAs are reported to improve lipid profile levels. (Okreglicka, 2015). Furthermore, ω-3 FAs have renal protective and cardioprotective effects by reducing pro-inflammatory cytokines and leukocyte adhesion molecules (Okreglicka, 2015). It is not clear why the results from our study were not in agreement with previous findings and this deserves further investigation. It will be interesting to investigate if ω-3 FA supplements to raise blood ω-3 FA levels will improve the blood lipid profile in children with CKD.

Saturated fats and trans fatty acids when consumed in excess increase TC and LDL-C (Okreglicka, 2015). Trans fat intake in our study had a positive correlation with DBP, another contributing factor to the development of CVD in children with CKD. Since the kidneys play a major role in regulating blood pressure, reducing the intake of

processed foods is advisable for two reasons. First, processed foods are low in antioxidant nutrients, such as vitamin C and high in ω-6 FAs, saturated fats, and trans fatty acids. Omega-6 FAs, and trans fatty acids are known to cause systemic inflammation which is an underlying cause of cardiovascular disease (Calder, 2015; Noori et al., 2011; Okreglicka, 2015). Second, processed foods are high in sodium. Even though sodium intake among all age groups was within the recommended levels, a negative relationship between dietary Na intake and GFR was observed, indicating higher Na intake (even within recommended range) may impair kidney function in non-dialysis children. Furthermore, increased dietary sodium intake not only increases blood pressure but also causes the excretion of sodium and calcium (Whitney & Rolfes, 2008). Calcium loss affects bone growth, and when calcium is displaced from the bones, calcification in the vasculature of the heart contributes to CVD in children with ESRD (Chan, Lee, But, & Chau, 2013)

In our study, we found that saturated fat intake was higher in the pre-dialysis and post-transplant groups. Saturated fats also reduced albumin, Ca and the GFR in non-dialysis children whose SAT intake was higher than the recommended. Saturated fat intake also promotes inflammation and increases the risk of CVD in patient with CKD (Okreglicka, 2015). Thus, to preserve kidney function in pre-dialysis or post-transplant children, reducing SAT intake should be a priority goal for nutritional management.

The results from the current study demonstrated that higher antioxidant intake lowered TG levels. Reduced TG levels may decrease the risk of triglycerides being incorporated into the bone matrix and help prevent fractures in children with CKD. This finding warrants further investigation in pediatric CKD population. Lowering dietary Na

intake will also improve bone health. High dietary Na intake decreases Ca absorption which also increases the risk for fractures.

Specific antioxidant intakes that were found to be of interest in children on dialysis were vitamin C and vitamin E. Since high serum K levels are known to cause cardiac arrhythmias, we found that children on dialysis may benefit from higher intakes of vitamin C to reduce this risk. Moreover, vitamin C helps to form the basement membrane of blood vessels by Type IV collagen formation and functions as an antioxidant in free radical scavenging (May & Harrison, 2013). For these reasons, vitamin C was found to be cardioprotective in children on dialysis. Children enrolled in our study all had vitamin C intake above the amount recommended thus was not a nutrient of concern.

Another well-known antioxidant which offers cardioprotective effects is vitamin E. In addition to having anti-inflammatory and lipid lowering properties, vitamin E intake was shown to improve serum Fe levels in children on dialysis. Moreover, vitamin E supplementation may be indicated when hemoglobin levels are low and PTH levels are high. In addition, vitamin E may play a role in improving iron stores in hemoglobin (Jilani, Azam, Moiz, Mehboobali, & Perwaiz Iqbal, 2015) and should be investigated in children on dialysis. In the current study, pre-dialysis and PD groups had adequate vitamin E intake while HD and post-transplant groups had vitamin E intake less than 50% of the recommended amount. Although the difference in vitamin E intake among the four groups was not statistically significant, children on HD treatment and post-transplant may benefit from increased vitamin E intake.

In addition to antioxidants, our study showed that dietary Ca had health benefits in children with CKD, especially in children post-transplant. In addition to improving bone

health, increased dietary Ca intake also improved blood lipid profiles in children post-transplant. In children on dialysis, increased dietary Ca intake improved the adequacy of dialysis. In children not on dialysis, increased dietary Ca intake reduced systolic and diastolic blood pressure and improved blood lipid profiles. Reduction in dietary Na intake is another nutrition strategy to improve blood pressure and prevent the risk of CVD in children with CKD. These dietary management practices not only benefit adult renal patients but are also applicable to the pediatric CKD population.

Limitations

Valuable information was derived from this study which may help children with CKD in the future. Convenience sampling, at a single site clinic, limited our sample size, especially when considering the subgroups within the pediatric CKD population. The population for this study was selected because children with CKD are at extremely high risk for developing cardiovascular disease and antioxidants and ω-3 FAs intakes were hypothesized to decrease free radical injury and improve free radical scavenging. Moreover, determining the best nutritional strategies to help children with CKD delay or avoid the onset of cardiovascular disease is of utmost importance. For bone health, we did not collect data on the administration of supplements (vitamin D or calcium) and medications (phosphate-binders). This data should be collected in the future studies. Since this study only included children with CKD, the study results may not generalize to the population at large. Children's Hospital of Michigan – Specialty Center is in an urban setting. CHMs Specialty Center was selected because of the comprehensive services and quality medical care it provides to children with CKD. Urban centers tend to service individuals of lower socioeconomic status and educational level. Furthermore, following

instructions and relying on the accuracy of self-reporting information on dietary records are challenges. We attempted to control for these variables by offering thorough instructions, providing a handout on portion sizes, and giving the child and parents/caregivers measuring devices to aid in the accuracy of measuring and recording food intake. Despite the study limitations outlined, we believe our study has reliable data with important discoveries that will improve the health of children with CKD in the future.

Conclusion and Clinical Implications

Our study of children at various stages of CKD provides a physiological basis for the understanding of relationships between clinical measures, antioxidant, ω-3 FA and other nutrient intakes, and free radical activity. The relationships between clinical measures, nutrient intakes, and oxidative stress were explored.

Based on clinical measures, antioxidant intake, and free radical activity, we found several physiological relationships in children with CKD. We discovered that TGs may play a role in bone health and free radical activity. Moreover, we have shown that higher intakes of antioxidants (vitamin A, β-carotene, vitamins C, E & selenium) may help to improve kidney function and dialysis treatment outcomes.

Clinical measure that correlated with improved GFRs include lower serum Na, K, Mg, TG and TC/HDL-C. Since serum TC levels alone did not affect the GFR, the role of non-HDL particles were evaluated in relationship to kidney function. Remarkably, LDL-C did not show a relationship with impaired kidney function in any group; although, we did find that elevated serum TG levels reduced kidney function in children in the non-dialysis group. Recommendations for measures to lower serum TG levels and raise HLD-C levels

include limiting sugar intake and exercising daily. These measures will also improve blood pressure control.

Children on dialysis with higher albumin levels had better free radical scavenging ability. Better free radical scavenging ability was also associated with lower PTH levels and lower TG levels. These findings together demonstrated the importance of lowering blood TG levels in order to improve growth and bone health.

In children on dialysis, better caloric intake improved renal function. Thus, high quality, nutrient-dense caloric foods should be encouraged. Also, higher dietary protein intake was found to reduce serum albumin levels. Albumin is more than just a marker of nutritional status, albumin is becoming a biomarker of disease. Ischemia modified albumin is a novel biomarker for diseases associated with ischemia and oxidative stress, including renal failure, CVD and stroke (Kumar & Subramanian, 2016). Another association with albumin includes dietary intake of phosphorus. Dietary intake of P showed a positive relationship with serum P levels and a negative relationship with serum albumin levels. An elevated phosphorus and calcium product may not only contribute to the pathogenesis of CVD but may also be a physiological mediator affecting disease and protein status in children on dialysis. Therefore, dietary teaching should continue to emphasize the need for high Ca and low P containing foods. Children on dialysis should be encouraged to avoid foods with phosphorus additives, including soda beverages (sweetened and artificially sweetened), and processed foods. Sugar sweetened beverages (SSB) and dietary intake of P have been linked to obesity (Anderson, 2013; Bucher Della Torre, Keller, Laure Depeyre, & Kruseman, 2016), a finding we observed in this study. Moreover, SSB have been tied to hypertension (Malik, Akram, Shetty, Malik, & Yanchou Njike,

2014). Thus, children on dialysis and all children with CKD should reduce or avoid intake of soda beverages to help with weight and blood pressure control and slow the progression of renal disease.

Children on dialysis should also limit foods high in phosphorus, such as meat, dairy, and chocolate. In addition, a plant-based diet should be recommended to include whole grain foods, legumes, nut and seeds because the bioavailability of phosphorus is lower in plant than animal sources (NKF, 2017). Moreover, foods high in calcium and calcium-containing phosphate medications are a continued recommendation by the National Kidney Foundation.

In all children and in children in the non-dialysis group, free radical injury strongly correlated with poor lipid profiles. Whereas, dietary calcium intake improved blood pressure and lipid profiles. Thus, adequate dietary calcium intake should be encouraged. In children on dialysis, vitamin C was found to be cardioprotective by decreasing K levels and is well-known for its free radical scavenging abilities. Vitamin E, on the other hand, improved Fe levels in children on dialysis and improved Hgb levels in the pre-dialysis group. Therefore, adequate intakes of vitamins C and E should be strongly monitored and encouraged. The best food sources of vitamins C and E are fresh fruits and vegetables, vegetable oils, nuts and seeds.

Antioxidants showed a non-significant negative relationship with TBARS but not with other free radical activity or scavenging ability. Nevertheless, antioxidants did correlate with other clinical measures that may vary with free radical injury and free radical scavenging biomarkers. Children on dialysis with higher intake of antioxidant intakes with ω-3 FAs had better dialysis outcome and control of SBP.

It should be noted that in all groups of children, ω-3 FA intake was below 35% of the recommended daily intake and the ω-6 to ω-3 FA ratio was high at a ratio of 9:1. Therefore, the need to increase ω-3 FA intake is warranted in children with CKD to reduce the ω-6 to ω-3 FA ratio. Also, the negative relationship between dietary intake of PUFAs and serum albumin levels should be re-evaluated in children who are consuming at least 75% of the recommended daily intake of ω-3 FAs before any further conclusions may be drawn regarding protein and disease status and PUFA intake.

In all children, TC, TG, LDL-C and TC/HLD-C positively correlated with free radical injury. In children on dialysis, free radical scavenging ability was reversely related to TC, TG, TC/HDL-C. Thus, reducing dietary intake of cholesterol may decrease oxidative stress and the administration of statin drugs may be indicated for children with CKD.

Table 1: Age and treatment distribution of all children

		Pre-dialysis	Hemodialysis	Peritoneal Dialysis	Post Transplant	Total
Age	1	5 (50%)	0 (0%)	2 (20%)	3 (30%)	10 (21.3%)
	2	4 (20%)	4 (28.6%)	2 (14.3%)	4 (28.6%)	14 (29.8%)
	3	3 (21.4%)	5 (35.7%)	1 (7.1%)	5 (35.7%)	14 (29.8)
	4	3 (33.3%)	2 (22.2%)	1 (11.1%)	3 (33.3%)	9 (19.1%)
Total		15 (32.6)	11 (23.4%)	6 (12.8%)	15 (32.6)	

Ages: 1: younger than 6, n=10; 2: 7-12, n=14; 3: 12-18, n=14; 4: older than 18, n=9

Table 2: Racial distribution of the children, n (percent)

	Pre-dialysis	Hemodialysis	Peritoneal Dialysis	Post Transplant	Total
White	7 (41.2%)	1 (5.9%)	1 (5.9%)	8 (47.1%)	17 (36.2%)
AA	6 (25%)	10 (41.7%)	4 (16.7%)	4 (16.7%)	24 (51.1%)
Hispanic	0 (0%)	0 (0%)	1 (33.3%)	2 (66.7%)	3 (6.4%)
Asian/others	2 (66.7%)	0 (0%)	0 (0%)	1 (33.3%)	3 (6.4%)
Total	15 (31.9%)	11 (23.4%)	6 (12.8%)	15 (31.9%)	

Abbreviation: AA, African American

Table 3: BMI percentile, and Z-score (mean ± standard deviation) of the 4 treatment groups

	Pre-dialysis (n=15)	Hemodialysis (n=11)	Peritoneal Dialysis (n=6)	Post-transplant (n=15)	p
BMI percentile	75.8±24.5	53.8±41.2	59.6±29.5 (5)	67.1±28.0	ns
Z-score	1.04±1.1	.05±1.7	.31±.87	.62±1.0	ns

Abbreviations: BMI, body mass index; ns, not significant

Table 4: Comparison of blood pressure (mmHg, mean±SEM) and recommended blood pressure ranges by age

	3.0-5.9 y (n=6)	6.0-9.9 y (n=7)	10.0-11.9 y (n=10)	12.0-14.9 y (n=5)	> 15 y (n=18)	p
SBP	97.3±.3.9[a]	111.6±5.0[ab]	112.5±3.7[bc]	117.2±6.0[be]	127±3.5[de]	<.0001
Range	80-108	102-138	99-129	100-134	102-157	
Recommended Range	89-112	97-115	102-120	110-131	120	
DBP	57.0±5.9[a]	56.4±7.6[a]	69.5±10.9[c]	73.0±14.0[c]	66.6±2.0[c]	=.007
Range	50-68	46-66	47-87	62-92	46-79	
Recommended Range	46-72	57-76	61-80	64-83	80	

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure

Table 5: Hemoglobin (Hgb) levels (mg/dL) according to age categories (mean±SEM)

	< 6 years (n=10)	7-12 years (n=14)	12-18 years (n=14)	Male > 18 years (n=6)	Female > 18 y (n=3)	p
Hgb	11.2±.7	11.1±.5 [a]	11.9±.5 [a]	12.2±..6 [a]	11.3±.3[b]	=0.053
Normal Hgb	12.5	13.5	14.5	15.5	13.8	

a: significantly differently from normal Hgb value at p<.0001
b: significantly different from normal Hgb value at p<.01
Abbreviation: Hgb, hemoglobin

Table 6: Routine laboratory values for the 4 treatment groups (n) (mean±SEM)

	Pre-dialysis (n=15)	HD (n=11)	PD (n=6)	Post-transplant (n=15)	p
Hgb (g/dL)	12.3±1.8[ad]	11.2±1.8[ac]	9.4±1.2[bc]	12.7±2.1[ad]	=.003
Albumin (g/dL)	3.8±.4[a] (11)	3.7±.2[a] (10)	3.0±.5[b]	3.8±.3[a] (13)	<.0001
BUN (mg/dL)	33±15[a]	49±12[b]	54±15[b]	23±9[c]	<.0001
Creatinine (mg/dL)	2.0±1.3[a]	11.2±2.3[b]	8.3±2.5[c]	1.0±.4[a]	<.0001
Ferritin (ng/mL)	74±47[a] (11)	71±70[a] (10)	275±172[b] (4)	--	=.001
Sodium (mEq/L)	140±3	139±3	139±3	138±2	ns
Potassium (mEq/L)	4.4±.4	4.3±.6	4.1±.6	3.9±.7	ns
Calcium (mg/dL)	9.1±.6	9.7±1.1	9.4±1.0	9.1±.4	ns
Phosphorus (mg/dL)	4.3±1.0[a]	6.0±1.9[b] (10)	5.9±1.5[b]	3.9±.7[a]	=.001
Magnesium (mg/dL)	2.1±.3[a]	2.7±.2[b] (10)	2.5±.7[b]	1.7±.2[c]	<.0001
PTH (pmol/L)	79±39[a] (6)	314±310[a](10)	867±571[b](5)	145 (1)	=.005
TC (mg/dL)	231±50 (3)	163±8 (6)	184±22 (4)	171±16 (9)	ns
HDL-C (mg/dL)	40±.9 (2)	62±3.7 (5)	43	65±6 (5)	ns
LDL-C	128±99 (2)	--	--	82±12 (9)	ns

(mg/dL)					
TG (mg/dL)	275±58[a] (3)	83±12[bd] (7)	216 ± 43[a] (3)	117±21[cd] (9)	=.001
TC/HDL-C (mg/dL)	5.3±2.1[a] (2)	2.6±.2[b] (5)	3.6 (1)	2.8±.3[b] (9)	=.044
Renal profile 2 z score (BUN+creatinine)	-.75±.27	na	na	-1.58±.14	<.0001
GRF	36.5±3.8	na	na	68.0±4.4	<.0001
Kt/V	na	1.75±.09	2.65±.25	na	=.001

Abbreviations: HD, hemodialysis; PD, peritoneal dialysis; Hgb, hemoglobin; BUN, blood urea nitrogen, PTH, parathyroid hormone; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; Renal prof 2, z score (BUN + creatinine); GFR, glomerular filtration rate; Kt/V = adequacy of dialysis
--: No data
ns, not significant
na, not applicable

Table 7: Comparisons of biomarkers for free radical injury (8-ISOP & TBARS) and free radical scavenging (ABTS) in the 4 treatment groups (mean±SEM)

	Pre-dialysis (n=15)	Hemodialysis (n=11)	Peritoneal Dialysis (n=6)	Post-transplant (n=15)	p
8-ISOP (pg/mL)	419±296 (15)	157±65 (11)	450±424 (6)	440±326 (15)	=.069
TBARS MDA (μM)	14.2±5.6	13.6±3.7	13.3±6.2	17.2±5.3	
ABTS Trolox (mM)	0.06±0.05[a]	0.08±0.03[a]	0.04±0.04[ac]	0.03±0.04[bc]	=.015

Abbreviations: 8-ISOP, 8-Isoprostanes; TBARS, Thiobarbituric Acid Reactive Substances; ABTS, 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]

Table 8: Relationships between clinical measures of the children (Only significant correlations are shown)

	All Children (n=47)	Pre-dialysis (n=15)	Dialysis (n=17)	Post-transplant (n=15)	Non-dialysis[δ] (n=30)
SBP vs					
DBP	.54**	.53#	--	.71**	.63**
Hgb	--	--	--	.57*	.41*
Albumin	--	--	--	.69**	--
BUN	--	--	-.51*	--	--
Creatinine	--	.63*	--	.72**	--
Serum Fe	--	--	-.54*	--	--
Serum Na	--	.49#	--	--	--
Serum Ca	--	--	--	-.51#	--
Serum Mg	--	-.69**	--	.62*	--
Kt/V	na	na	-.49*	na	na
DBP vs					
BUN	--	-.68**	--	--	-.47**
Creatinine	.37*	--	--	.52*	--
Serum Ca	--	--	--	-.65**	--
Renal prof 2[f]	na	--	na	--	-.40*
Hgb vs					
Albumin	.57**	--	--	.66*	.56*
BUN	-.49**	--	--	--	-.55**
Creatinine	-.34*	--	--	--	--
Serum Fe	-.44*	--	-.53*	--	--
Serum P	-.43**	-.59*	--	--	-.41*
PTH	-.69**	--	-.73**	--	--
8-ISOP	--	--	-.48*	--	--
Renal prof 2	na	-.59*	na	--	-.48**
Kt/V	na	na	-.42#	na	na
Albumin vs					
BUN	.33*	--	--	--	--
Creatinine	-.33*	--	--	--	--
Serum Fe	-.57**	-.63**	--	-.64*	--
Serum Ca	--	.66*	--	--	--
Serum Mg	--	--	.51*	.78**	--
PTH	-.63**	--	-.59*	--	--
TC	-.70**	--	--	--	-.85*
TG	-.59*	--	--	--	--
LDL-C	-.84*	--	--	--	-.84*
TC/HDL	-.75**	--	--	--	-.85*
ABTS	--	--	.66**	--	--

BUN vs					
Creatinine	.59**	--	--	--	--
Serum K	--	--	--	--	.44*
Serum Mg	.52**	--	--	--	--
Serum P	.45**	--	--	--	.42*
TBARS	--	--	.64*	--	--
ABTS	.34*	--	--	--	.54**
Renal prof 2	na	.97**	na	.99**	.97**
GFR	na	--	na	--	-.60**
Creatinine vs					
Serum Na	--	.59*	--	--	.54**
Serum K	--	--	--	--	.44*
Serum Mg	.63**	--	--	--	--
Serum P	.54**	--	--	--	--
TC	--	--	--	--	.91**
TG	--	--	--	--	.89**
LDL-C	.95**	--	--	--	.95**
TC/HDL	--	--	--	--	.98**
Renal prof 2	na	.61*	na	.49#	.67**
GFR	na	--	na	--	-.80**
Kt/V	na	na	-.66*	na	na
Serum Fe vs					
Serum Mg	.48*	--	.60*	--	--
PTH	.62**	--	--	.63*	--
8-ISOP	--	--	.56*	--	--
Serum Na vs					
K	--	.52*	--	--	--
Serum Ca	--	--	--	--	-.39*
Serum Mg	--	--	.59*	--	--
Serum P	--	--	-.52*	--	--
TC	--	--	--	--	.75#
TG	--	--	--	--	.91**
LDL-C	.80*	--	--	--	.80*
TC/HDL	.79**	--	--	--	.90**
GFR	na	--	na	--	-.44*
Serum K vs					
Serum Mg	.36*	--	--	.56*	--
Serum P	--	--	--	.70**	--
TG	.58*	--	--	--	--
ABTS	.30*	--	--	--	.36*
Renal prof 2	na	--	na	--	.49**

GFR	na	--	na	--	-.43*
Serum Ca vs					
Mg	.36*	--	--	--	--
TG	-.68*	--	--	--	--
ABTS	.34*	--	--	--	--
GFR	na	--	na	--	.35#
Serum Mg vs					
P	.40**	--	--	--	--
HDL-C	--	--	--	--	-.81*
TBARS	-.31*	--	--	--	--
GFR	na	--	na	--	-.44*
Serum P vs					
PTH	.43*	--	--	--	--
TBARS	--	--	--	.60*	--
ABTS	--	--	--	--	.36*
Serum TC vs					
Kt/V	na	na	.66*	na	na
Serum TG vs					
Renal prof 2	na	--	na	--	.66*
GFR	na	--	na	--	-.74**
Kt/V	na	na	.89**	na	na
Serum HDL-C vs					
GFR	na	--	na	--	.71*
Serum TC/HDL vs					
GFR	na	--	na	--	-.75**
PTH vs					
TG	.76*	--	.93**	--	--
TC/HDL-C	--	--	.91**	--	--
8-ISOP	.42#	.79#	.56*	--	--
ABTS	--	--	--	--	-.72#
Kt/V	na	na	.45#	na	na
Renal prof 2 vs					
TG	na	--	na	--	.66*
8-ISOP	na	.53*	na	--	--
TBARS	na	.65**	na	--	--
ABTS	na	--	na	--	.49**

Statistical Significance: **: p<.01; *: p<.05; #:.10>p>.05; --: not significant
f: renal profile 2, composite score (sum of z scores of BUN and creatinine)
δ: pre-dialysis and post-transplant children
na, not applicable

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; Hgb, hemoglobin; BUN, blood urea nitrogen; Fe, ferritin; Na, sodium; K, potassium; Ca, calcium; Mg, magnesium; P, phosphorus; PTH, parathyroid hormone; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; 8-ISOP, 8-Isoprostanes; TBARS, Thiobarbituric Acid Reactive Substances; ABTS, 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]; GFR, glomerular filtration rate; Kt/V, adequacy of dialysis

Table 9: Relationships between blood lipid levels and oxidative and scavenger biomarkers

	All children (n=47)	Dialysis (n=17)	Non-dialysis (n=30)
8-ISOP vs			
TC	--	--	--
TG	.68**	.73*	.65*
HDL-C	-.59**	-.73#	-.58*
LDL-C	--	--	--
TC/HDL-C	.61**	.95**	.64*
TBARS vs			
TC	.63**	--	.72**
TG	.40#	--	--
HDL-C	--	--	--
LDL-C	.66*	--	.66*
TC/HDL-C	--	--	--
ABTS vs			
TC	--	-.56#	--
TG	--	-.62*	--
HDL-C	--	--	--
LDL-C	--	--	--
TC/HDL-C	--	-.77#	--

Statistical Significance: **: p<.01; *: p<.05; #: .10>p>.05; --: not significant

Abbreviations: TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; 8-ISOP, 8-Isoprostanes; TBARS, Thiobarbituric Acid Reactive Substances; ABTS, 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]

Table 10: Nutrient intakes of the 4 treatment groups based on 24-hour recall (n) (mean±SEM)

	Pre-dialysis (n=15)	Hemodialysis (n=11)	Peritoneal Dialysis (n=6)	Post-transplant (n=15)	Total (n=47)	p
Energy (kcal)	2009±172[a]	1262±146[b]	1411±172[bc]	1932±168[ac]	1733±97	=0.008
Protein (g)	70.2±5.5	52.7±6.9	57.7±9.4	75.0±9.4	66.0±4.1	ns
CHO (g)	246±24	162±31	202±38	229±26	215±15	ns
Fat (g)	81±9[a]	44±5[b]	42±12[b]	81±12[a]	68±6	=0.014
SAT (g)	23±3[a]	12±2[b]	8±2[b]	27±5[a]	20±2	=0.006
Fat %	35.7±2.4	33.1±3.1	27.0±5.5	36.7±3.7	34.3±1.7	ns
MUFA (g)	23±6	13±3	20±10	14±4	18±3	ns
PUFA (g)	11±3	8±1	9±3	8±2	9±1	ns
ω-3 FA (g)	0.6±0.2	0.5±0.1	0.5±0.2	0.5±0.2	0.5±0.1	ns
ω-6 FA (g)	5.0±1.3	5.4±1.1	4.7±2.0	4.5±1.7	4.9±0.8	ns
Chol (mg)	214±41	186±48	144±75	276±63	219±28	ns
Vit A (µg)	267±104	230±82	184±81	272±61	249±43	ns
Vit C (mg)	74±18	117±42	143±62	93±31	99±17	ns
Vit D (µg)	17.5±12.1	1.3±0.5 (10)	1.1±0.5	10.1±6.7	9.4±4.5 (46)	ns
Vit E (mg)	7.6±3.2 (14)	3.2±1.5	9.9±6.9	4.4±1.7	5.8±1.5 (46)	ns
Ca (mg)	869±133[a]	257±51[bc]	532±169[ac]	901±110[a]	694±71	=0.001
Na (mg)	3047±431[a]	1500±162[b]	1325±199[b]	3003±370[a]	2452±214	=0.003
Mg (mg)	152±25	77±13	141±26	149±38	132±16	ns
P (mg)	759±100	427±85	580±98	766±172	661±69	ns

K (mg)	1453±278	772±104	1222±292	1227±210	1192±122	ns
Iron (mg)	13±1.7	9±1.7	9±2.3	12±1.3	11±0.9	ns
Se (µg)	51±9.7	50±8.4	50±14.7	50±11.4	50±5.3	ns
Antioxidants[f]	-.14±.68	-.08±1.01	1.05±1.09	-.10±.60	.04±.39	ns
Antioxid+ω-3	-.06±.73	-.17±1.04	1.06±1.00	-.11±.81	.04±.44	ns

f: The sum of z scores of vitamin A, beta carotene, vitamin C, vitamin E and selenium

Abbreviations: CHO, carbohydrates; SAT, saturated fat; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω-3 FA, omega-3 fatty acids; ω-6 FA, omega-3 fatty acids; Chol, cholesterol; Vit, vitamin; Ca, calcium; Na, sodium; Mg, magnesium; P, phosphorus; K, potassium; Se, selenium; Antioxid+ω-3, antioxidants with omega-3 fatty acid; ns, not significant

Table 11: Protein intake (g/kg) based on age groups (mean±SEM) and comparison with RDI

	1 – 3 y	4 – 8 y	9 – 13 y	> 14 y	p
Ave protein intake (g/kg)	5.2 (n=1)	2.9±0.6[a]* (n=6)	1.6±0.1[b]** (n=6)	1.5±0.3[b]** (n=9)	=.03
Protein intake from recall		3.2±1.3[a]** (n=13)	1.4±0.8[b]* (n=13)	1.1±0.6[b]* (boys, n=14) 1.4±0.9* (girls, n=6)	<.001
RDI (g/kg)		0.95	0.95	0.73 (boys) 0.71 (girls)	

RDI: Reference Daily Intake
Numbers in the same row with different superscripts are significantly different from other age groups
*: significantly different from RDI at $p<0.05$
**: significantly different from RDI at $p<0.01$

Table 12: Sodium intake based on age distribution (mean±SEM) and comparison with recommendation by age

	1 – 3 years (n = 1)	4-8 years (n = 13)	9 – 13 years (n = 13)	> 14 years (n = 20)	p
Sodium intake (mg/day)	1066	2231±258	2307±491	2758±350	ns
Recommendation	1500	1900	2200	2300	

ns, not significant

Table 13: Percent recommended nutrient intakes of the four groups based on 24-hour recall (n) (mean±SEM)

	Pre-dialysis	Hemodialysis	Peritoneal Dialysis	Post-transplant	Total	p
	(n=15)	(n=11)	(n=6)	(n=15)	(n=47)	
Energy	101±11	62±8	92±14	101±12	91±6	0.061
Protein	206±29	146±29	254±65	242±44	209±20	ns
CHO	88.3±9	58.6±10	93.7±18	87.1±12	81.6±6	ns
Fat	136.4±21	67.5±11	93.8±31	138.0±24	115±12	ns
SAT	121±17[ac]	55±9[bd]	54±12[ad]	139±30[c]	103±12	0.022
MUFA	117±29	64±20	127±68	68±20	90±15	ns
PUFA	53±11	42±6	60±21	38±9	46±5	ns
ω-3 FA	27±8	25±4	35±14	25±9	27±4	ns
ω-6 FA	26±6	30±4	35±16	25±9	28±4	ns
Chol	90±20 (9)	73±20 (8)	63±36 (4)	103±31 (9)	86±13 (30)	ns
Vit A	40±13	36±11	31±13	48±13	40±6	ns
Vit C	196±52	230±71	377±127	218±78	234±37	ns
Vit D	117±81	8±3 (10)	7±3	67±44	63±30 (46)	ns
Vit E	101±47 (14)	36±21	146±118	43±21	72±22 (46)	ns
Ca	80±13[ac]	20±4[bd]	57±23[ade]	81±11[ce]	63.34±7	0.003
Na	142±19	65±6	66±11	139±17	113±10	ns
Mg	80±16	31±8	90±37	64±16	65±9	ns
P	97±13	54±12	79±20	102±25	86±10	ns
K	34±6	19±2	29±6	28±5	28±3	ns

Iron	137±18	87±23	100±38	123±13	116±10	ns
Se	134±27	120±22	149±52	118±24	127±14	ns

Abbreviations: CHO, carbohydrates; SAT, saturated fat; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω-3 FA, omega-3 fatty acids; ω-6 FA, omega-3 fatty acids; Chol, cholesterol; Vit, vitamin; Ca, calcium; Na, sodium; Mg, magnesium; P, phosphorus; K, potassium; Se, selenium; ns, not significant

Table 14: Nutrient intakes of the four groups based on 3-day dietary records average records (n) (mean±SEM)

	Pre-dialysis (n=9)	Hemodialysis (n=5)	Peritoneal Dialysis (n=3)	Post-transplant (n=5)	Total (n=22)	p
Energy (kcal)	1945±110	1536±176	1998±172	2222±398	1922±114	ns
Protein (g)	70±7	50±7	85±9	88±18	71±6	ns
CHO (g)	251±18	220±29	249±79	330±71	262±21	ns
Fat (g)	74±7	52±9	77±13	64±10	67±5	ns
SAT (g)	26±2.2[a]	16±3.4[bc]	16±4.0[bd]	20±2.2[acd]	21±1.6	=0.04
MUFA (g)	16.5±5.2	9.1±1.2	37.7±16.1	10.2±3.8	16.3±3.5	ns
PUFA (g)	6.8±1.8	8.5±0.7	14.7±1.4	6.2±2.4	8.1±1.1	ns
ω-3 (g)	0.4±0.1	0.4±0.1	1.9±1.2	0.8±0.6	0.7±0.2	ns
ω-6 (g)	3.6±0.5	3.6±0.7	6.9±3.5	3.4±1.2	4.0±0.6	ns
Cholest (mg)	196±29	151±45	191±110	304±120	209±34	ns
Vit A (µg)	719±234	253±200	551±282	378±148	512±118	ns
Vit C (mg)	98±22	96±42	202±112	142±57	122±23	ns
Vit D (µg)	5.9±4.0	3.4±1.5	1.9±0.6	12.2±5.9	6.2±2.2	ns
Vit E (mg)	6.7±3.1	1.9±0.7	18.4±12.9	5.6±2.4	6.9±2.3	ns
Ca (mg)	988±129[a]	305±45[b]	872±210[a]	936±247[a]	805±97	=.033
Na (mg)	2883±358	2227±520	1937±473	2826±445	2592±223	ns
Mg (mg)	130±16	70±27	222±12	176±63	139±19	ns
P (mg)	655±91[ab]	404±73[a]	1039±161[bc]	1044±223[c]	739±83	=.017
Pot (mg)	1381±148	820±178	1976±511	1668±429	1400±151	ns

Iron (mg)	14±2	18±10	21±3	16±4	16±2	ns
Se (µg)	49±7	35±10	76±4	70±30	54±8	ns
Antioxidants[f]	.28±1.02	-2.47±.41	3.08±.63	.12±1.36	.00±.62	ns
Antioxid+ω-3	.68±1.05[a]	-2.11±.38[a]	4.98±1.02[bc]	.96±1.88[ac]	.69±.74	=.036

f: The sum of z scores of vitamin A, beta carotene, vitamin C, vitamin E and selenium

Abbreviations: CHO, carbohydrates; SAT, saturated fat; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω-3 FA, omega-3 fatty acids; ω-6 FA, omega-3 fatty acids; Chol, cholesterol; Vit, vitamin; Ca, calcium; Mg, magnesium; P, phosphorus; K, potassium; Se, selenium; Na, sodium; Antioxid+ω-3, antioxidants with omega-3 fatty acids; ns, not significant

Table 15: Percent recommended nutrient intakes of the 4 treatment groups based on 3-day dietary records average records (n) (mean±SEM)

	Pre-dialysis (n=9)	Hemodialysis (n=5)	Peritoneal Dialysis (n=3)	Post-transplant (n=5)	Total (n=22)	p
Energy	100±11	70±10	134±8	116±25	102±9	ns
Protein	214±35[a]	126±21[a]	390±79[bc]	267±76[ac]	230±29	0.042
CHO	95±13	75±14	119±34	127±32	101±11	ns
Fat	124±19	74±10	168±36	108±23	115±12	ns
SAT	131±12[a]	68±11[bc]	107±23[ac]	105±19[ac]	107±9	0.042
MUFA	85±33[a]	38±6[a]	238±112[b]	46±15[a]	86±23	0.043
PUFA	39±13[a]	40±5[a]	100±15[b]	32±14[a]	46±8	0.035
ω-3 FA	21±4[a]	17±6[a]	122±79[b]	38±23[a]	38±13	0.053
ω-6 FA	22±5	17±2	50±25	20±8	24±4	ns
Chol	73±14 (5)	55±25 (3)	91±43 (2)	111±72 (3)	80±18 (13)	ns
Vit A	135±55	42±33	79±40	57±17	89±25	ns
Vit C	274±89	286±191	463±177	297±99	308±62	ns
Vit D	39±27	23±10	13±4	81±39	41±15	ns
Vit E	87±46	16±6	270±233	41±15	86±37	ns
Ca	93±13[a]	26±5[bc]	99±34[a]	80±25 [ac]	75±10	0.059
Na	135±14	104±26	92±13	132±24	121±10	ns
Mg	71±16	27±9	134±67	71±24	70±13	ns
P	98±19	49±14	142±24	111±34	96±13	ns
K	32±3	18±4	46±7	38±9	32±3	ns

Iron	157±30	208±127	181±46	164±36	173±31	ns
Se	128±23	80±18	219±81	158±55	136±20	ns

Abbreviations: CHO, carbohydrates; SAT, saturated fat; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; ω-3 FA, omega-3 fatty acids; ω-6 FA, omega-3 fatty acids; Chol, cholesterol; Vit, vitamin; Ca, calcium; Mg, magnesium; P, phosphorus; K, potassium; Se, selenium; Na, sodium; ns, not significant

Table 16: Relationship between dietary intake and clinical measures (24-h recall/3-day dietary records) (Only significant relationships are shown)

	All children (n=47)	Dialysis (n=17)	Non-dialysis (n=30)
Total energy vs			
BUN	-.29*/--	--/--	--/--
Creatinine	-.44**/--	--/--	--/--
Serum K	--/--	-.59*/--	.40*/--
Serum Ca	--/-.43*	--/--	--/-.62*
Serum Mg	-.31*/--	--/--	--/--
Serum P	--/-.46*	--/--	--/--
TBARs	--/--	--/-.86**	--/--
Kt/V	na	--/.65#	na
Protein intake vs			
SBP	--/--	--/--	--/-.58*
Albumin	--/--	--/-.86**	--/--
BUN	-.35*/--	-.59*/--	--/--
Serum Fe	--/--	--/--	--/.76*
Serum Mg	-.38**/--	--/--	--/--
Serum P	--/--	.55*/--	--/--
CHO intake vs			
Creatinine	-.29*/--	--/-.71*	--/--
Serum K	--/--	-.69**/--	.42*/--
Serum P	--/-.47*	--/--	--/--
TBARs	--/--	--/-.80*	-.42*/--
Total fat vs			
Albumin	--/-.52*	--/-.75*	--/--
BUN	-.30*/--	--/-.76*	--/--
Creatinine	-.41**/--	--/--	--/--
Serum Ca	--/--	--/--	--/-.76**
PTH	--/--	.62*/--	--/--
HDL-C	--/-.89*	--/--	--/--
LDL-C	--/.92*	--/--	--/--
SAT vs			
DBP	--/-.48*	--/--	--/--
Albumin	--/--	.57*/--	--/-.58*
BUN	-.31*/--	--/--	--/--
Creatinine	-.43**/--	--/--	--/--
Serum Ca	--/-.44*	--/--	--/-.63*
Serum p	-.35*/--	--/--	--/--
HDL-C	--/-.91*	--/--	--/--

LDL-C	--/.96**	--/--	--/--
GFR	na	na	--/-.49#
MUFA vs			
Albumin	--/-.48*	--/--	--/--
Serum Fe	--/.84**	.64*/.88**	--/--
Serum K	.26#/--	--/--	--/--
PTH	.55/.88**	.64*/.96**	--/--
PUFA vs			
Albumin	--/-.53*	--/-.83*	--/--
Serum Fe	--/-.58*	--/.81*	--/--
PTH	.51*/.68*	.58*/.81**	--/--
LDL-C	.71**/--	--/--	--/--
Kt/V	na	--/.79*	na
ω-3 FA vs			
HGB	--/--	--/--	--/.64*
Serum Mg	--/--	--/-.80*	--/--
TC	.77**/--	--/--	--/--
TG	.70**	--/--	.84*/--
LDL-C	.87*/.94**	--/--	.87*/--
8-ISOP	.33*/--	--/--	--/--
ω-6 FA vs			
SBP	.30*/--	--/--	--/--
Serum Mg	--/--	--/-.71*	--/--
TC	.55*/--	--/--	--/--
TG	--/--	--/--	.76*/--
LDL-C	.76*/.75**	--/--	.76*/--
TC/HDL-C	--/--	--/--	.85*/--
Trans FA vs			
DBP	--/--	--/--	--/.65*
Serum Fe	-.45*/--	--/--	-.84**/--
Dietary cholesterol vs			
SBP	.35*/--	--/--	.42*/.58*
HGB	--/.49*	--/--	--/.68**
Serum Mg	-.39**/--	-.54*/--	--/--
Serum P	--/--	.61/--	--/--
PTH	--/.78*	--/--	--/--
ABTS	--/-.45*	--/--	--/--
Vitamin A vs			
SBP	--/--	--/--	--/.58*
Creatinine	--/--	--/--	--/.66**
Serum Fe	--/--	--/--	.75**/--

Carotene vs			
SBP	--/--	--/--	--/.64*
Creatinine	--/--	--/--	--/.77**
Serum Fe	--/--	--/--	.65*/.96**
Serum P	--/--	--/--	--/-.57*
Vitamin C vs			
SBP	--/--	--/--	--/.64*
Serum K	--/--	-.60*/--	--/--
Serum Ca	.29*/--	--/--	.40*/--
Kt/V	na	--/.91**	na
Vitamin D vs			
Serum K	--/-.44*	--/--	--/-.62*
Vitamin E vs			
Serum Fe	.51*/.85**	.69**/.93**	--/--
Serum Mg	--/.44*	--/--	.43*/--
Serum P	--/--	--/--	.38*/--
PTH	.59**/.88**	.67**/.96**	--/--
Dietary Ca vs			
SBP	-.40**/-.53**	--/--	-.45/--
DBP	-.40**/-.53**	--/--	-.42*/--
Albumin	--/--	--/--	--/-.64*
BUN	-.35*/--	--/--	--/--
Creatinine	-.58**/-.51*	--/-.77*	--/--
Serum Fe	--/--	.59*/.86**	--/--
Serum Mg	--/-.31*	--/--	--/--
Serum P	-.31*/--	--/--	--/--
TC	--/--	--/--	-.85*/--
LDL-C	-.80*/--	--/--	-.80*/
TC/HDL-C	--/--	--/--	-.86*/--
Kt/V	na	--/.90**	na
Dietary P vs			
Albumin	--/--	--/-.72*	--/--
Serum Fe	--/--	--/--	--/-.56*
Serum P	--/--	.56*/--	--/--
Renal prof 2	na	na	--/--
Dietary Na vs			
SBP	--/--	--/--	.44*/--
BUN	-.37*/--	--/--	--/--
Creatinine	-.45**/--	--/--	--/--
Serum Na	.30*/.45*	--/--	--/.55*

Serum Ca	--/-.44*	--/--	--/--
Serum Mg	-.46**/-.52*	--/--	--/--
GFR	na	na	--/-.51#
Dietary K vs			
Albumin	--/--	--/-.72*	--/--
Serum Mg	--/-.45*	--/-.71*	--/--
Dietary Mg vs			
Albumin	--/--	--/-.90**	--/--
Serum Fe	--/.56*	--/--	--/--
PTH	--/--	.65**/--	--/--
Dietary Fe vs			
Serum P	--/--	--/--	--/-.56*
Dietary Se vs			
SBP	.30*/--	--/--	--/--
HGB	--/--	--/--	--/.61*
Albumin	--/--	--/.87**	--/--
BUN	--/--	-.56*/--	--/--
Serum Fe	--/.56*	--/--	--/--
Serum P	--/--	.67**/--	--/--
Antioxidants vs			
SBP	--/--	--/--	--/.76**
Fe	.51*/.73**	.64*/--	--/--
BUN	--/--	-.50*/--	--/--
Creatinine	--/--	--/--	--/.61*
TBARS	--/-.37#	--/--	-.35#/--
GFR	na	na	--/-.52#
Kt/V	na	--/.95**	na
Antioxidants+ω-3 FA vs			
SBP	--/--	-.71*/--	--/.75**
Hgb	--/--	--/--	--/.59*
Fe	.48*/.55*	.61*/.73*	--/--
BUN	--/--	-.47#/--	--/--
Creatinine	--/--	--/-.68#	--/--
Kt/V	na	--/.87**	na

*: p<.05
**: p<.01
#: 0.10>p>0.05
--: not significant
f: renal profile 2, composite score (sum of z scores of BUN and creatinine)
na, not applicable

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; Hgb, hemoglobin; BUN, blood urea nitrogen; Fe, ferritin; Na, sodium; K, potassium; Ca, calcium; Mg, magnesium; P, phosphorus; PTH, parathyroid hormone; 8-ISOP, 8-Isoprostanes; TBARS, Thiobarbituric Acid Reactive Substances; ABTS, 2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; Renal prof 2, z scores (BUN + creatinine); GFR, glomerular filtration rate; Kt/V = adequacy of dialysis; ω-3 FA, omega-3 fatty acids

Table 17: Bone health nutrients based on antioxidants (vitamins A, C, E and selenium) with or without ω-3 Fatty Acids

24-hr recall Antioxidants	Lowest 1/3	Middle 1/3	Highest 1/3	p
Vitamin D (μg)	4.5±4.2	13.5±11	9.3±6.6	ns
Ca (mg)	437±83[a]	763±113[b]	770±112[b]	=.046
P (mg)	321±73[a]	672±76[b]	970±148[c]	=<.001
K (mg)	646±160[a]	1195±156[b]	1717±243[b]	=.001
Mg (mg)	59±12[a]	120±17[a]	214±34[b]	<.001
Total protein (g)	49±5.4a	63±5.8a	85±7.6b	<.0001
Protein (g/kg)	0.94±0.12a	2.23±0.37b	2.30±0.31b	=.003
Antioxidants + ω-3 FAs	Lowest 1/3	Middle 1/3	Highest 1/3	
Vitamin D (μg)	4.9±4.5	18.7±11.6	2.5±0.8	ns
Ca (mg)	499±92	700±113	764±113	ns
P (mg)	261±56[a]	818±124[b]	837±106[b]	<.0001
K (mg)	520±103[a]	1381±230[b]	1588±177[b]	=.001
Mg (mg)	50±10[a]	138±21[b]	198±32[b]	<.0001
Total protein (g)	47±5.1[a]	68±7.2[b]	81±6.6[b]	=.004
Protein (g/kg)	1.18±0.24	2.05±0.35	2.19±0.31	=0.068
3-day records Antioxidants	Lowest 1/3	Middle 1/3	Highest 1/3	
Vitamin D (μg)	3.8±1.3	9.9±5.8	4.3±1.3	ns
Ca (mg)	493±118	957±164	944±176	=.085
P (mg)	510±95[a]	680±109[ac]	1034±167[bc]	=.028

K (mg)	1095±184	1295±191	1825±346	ns
Mg (mg)	86±20[a]	123±17[a]	211±42[b]	=.017
Total protein (g)	50±7.5	57±6.0	80±12.2	ns
Protein (mg/g)	1.02±0.20	1.94±0.35	2.35±0.53	ns
3-day records				
Antioxidants + ω-3 FAs	Same as above			

Abbreviations: Ca, calcium; P, phosphorus; K, Potassium; Mg, magnesium; ω-3 FAs, omega-3 fatty acids; kg, body weight in kilograms, ns, not significant

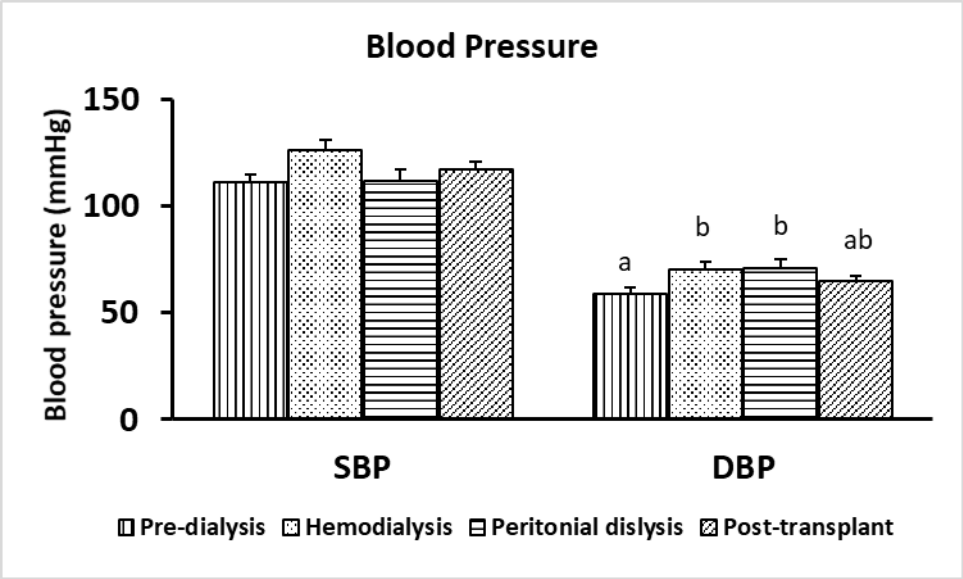


Figure 1: Systolic and diastolic blood pressure (mmHg) in four groups of children. Vertical bars with different letters are significantly different from each other

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure

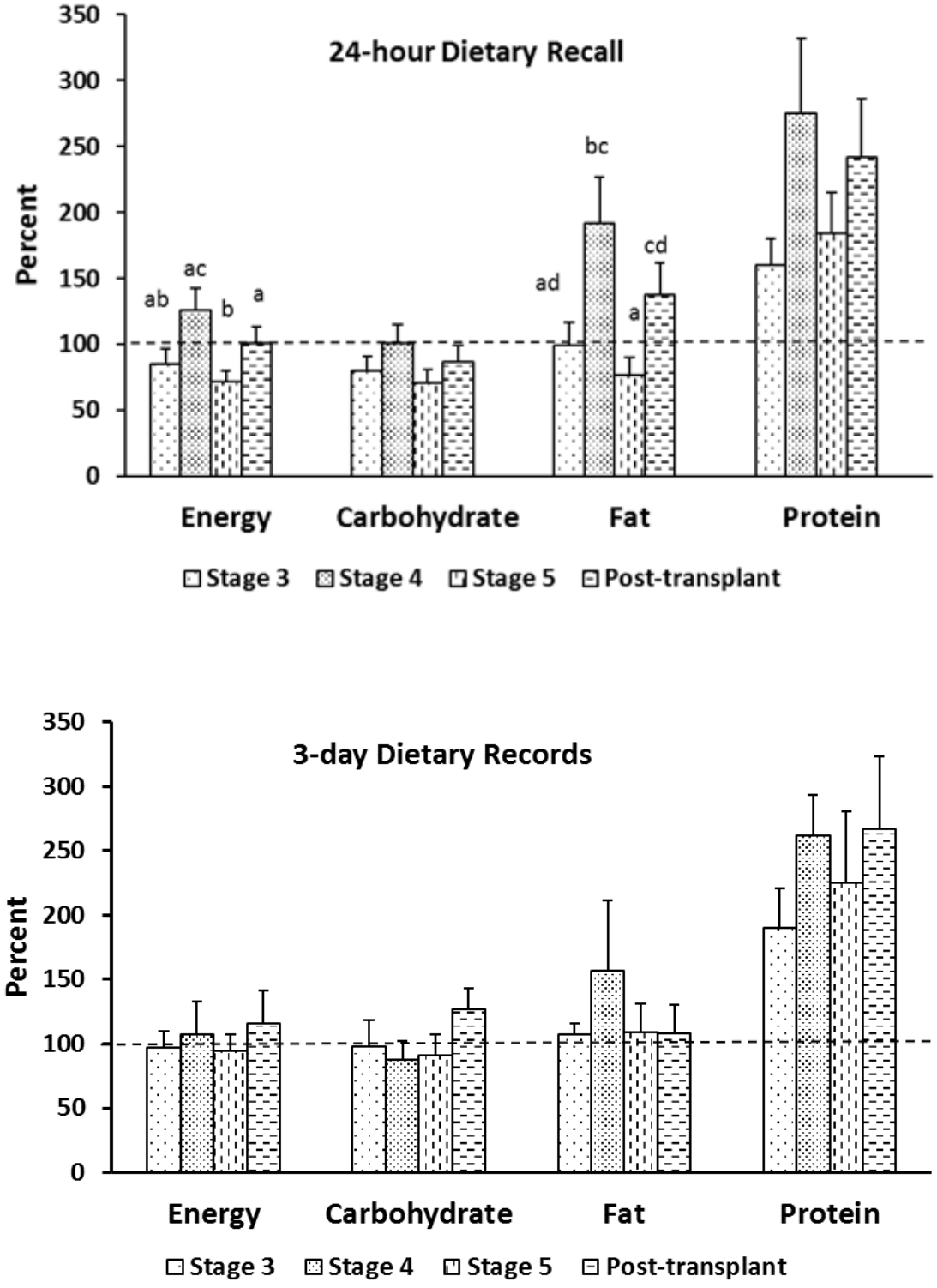
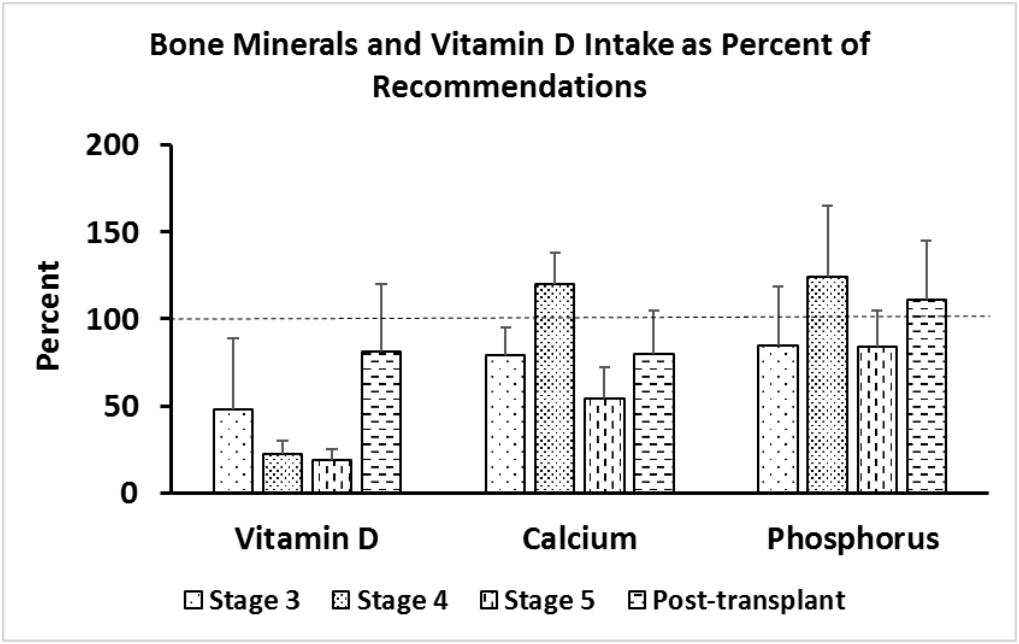


Figure 2: Energy and macronutrient intake as percent of recommendation at different stages of chronic kidney disease. Bars with different superscripts are significantly different from each other. Dash line represents 100% of recommmendations.



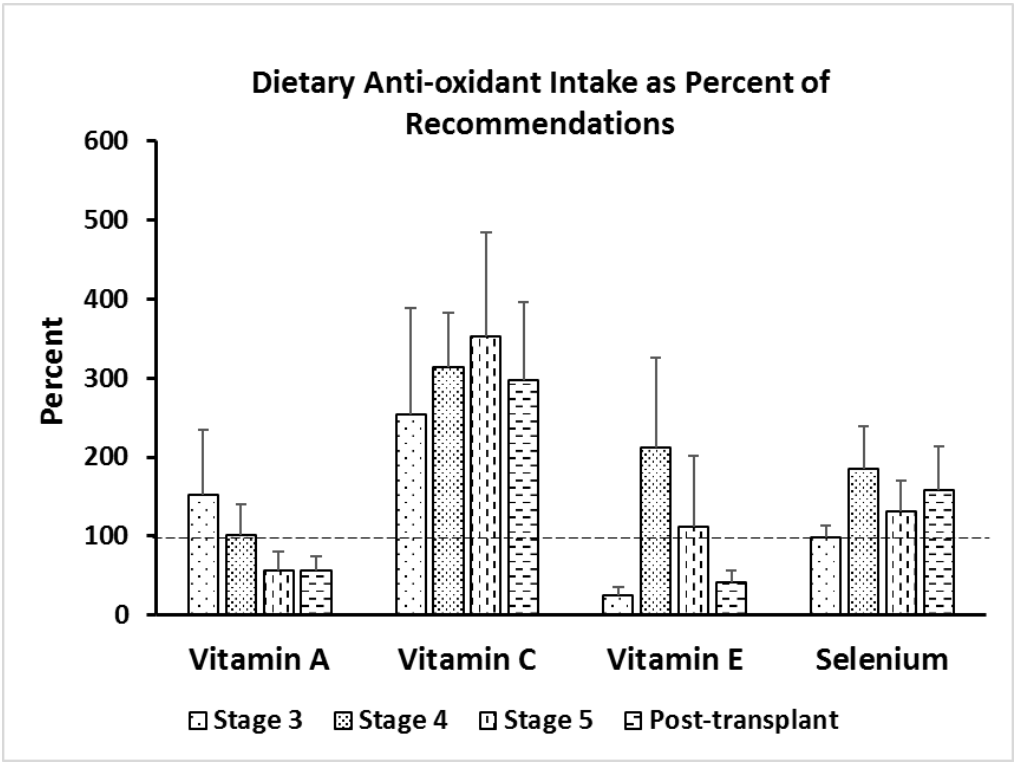


Figure 3: Intakes of nutrient related to bone health (top) and intakes of nutrient with anti-oxidant properties (bottom) at different stages of chronic kidney disease based on 3-day dietary records. Dash line represents 100% of recommendations.

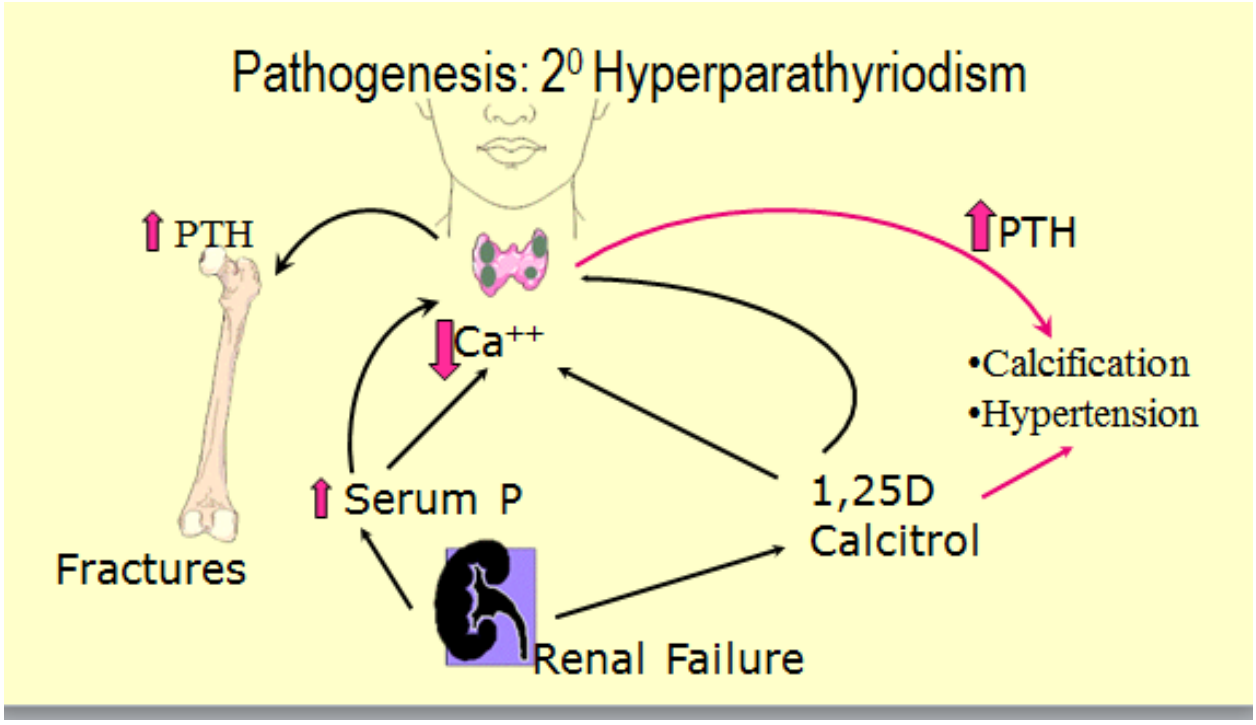


Figure 4. Pathogenesis of secondary hyperparathyroidism.
http://www0.sun.ac.za/aotc/general/renal/rod.png

Abbreviations: PTH, parathyroid hormone; Ca++, calcium; P, phosphorus

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ABSTRACT

EFFECTS OF DIETARY INTAKE OF ANTIOXIDANTS AND OMEGA-3 FATTY ACIDS ON FREE RADICAL PRODUCTION IN CHILDREN AT VARIOUS STAGES OF CHRONIC KIDNEY DISEASE

by

LINDA MARIE SAWYER

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Advisor: Dr. Kai-Lin Catherine Jen

Major: Nutrition & Food Science

Degree: Doctor of Philosophy

The purpose of this study was to understand the relationship between dietary intake of antioxidants and omega-3 fatty acids (ω-3 FAs) on free radical injury and free radical scavenging in children with chronic kidney disease (CKD) so that complications of kidney failure, especially cardiovascular disease, may be avoided or delayed. Children with CKD on dialysis and post renal transplantation are at an extremely high risk for cardiovascular morbidity and mortality. We hypothesized that children with CKD who have higher dietary intake of antioxidants and ω-3 FAs, would have lower free radical injury, as measured by 8-Isoprostanes (8-ISOP) and Thiobarbituric Acid Reactive Substances (TBARS) and higher free radical scavenging, as measured by ABTS (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) compared to children with CKD with lower intakes of these nutrients. Pre-dialysis was the baseline comparative group for this study.

Nutrient intake was assessed by means of a 24-hour diet recall obtained prior to a routine blood draw and the child or parents/caregivers were asked to complete and return 3-day dietary records by mail. Nutrient intakes were analyzed using the software Food Processor® Nutrition Analysis by EHSA. Forty-seven children with CKD, 3 to 21 years of age, 72.3% males were enrolled from the Detroit Medical Center (DMC) - Children's Hospital of Michigan (CHM) Specialty Center. The results showed that high blood lipid levels were correlated with oxidative stress biomarkers. Antioxidant intake was not correlated with oxidative stress, however antioxidant intake with ω-3 FAs increased blood hemoglobin and ferritin levels that may vary with free radical injury and free radical scavenging biomarkers. High cholesterol intake reduced ABTS levels. Children on dialysis with higher intake of antioxidants with ω-3 FAs had better dialysis outcome (Kt/V) and control of SBP. High triglyceride levels were shown to impair renal function and may affect bone health. Clinical implications include counseling by a renal dietitian about foods high in antioxidants, ω-3 FAs, and calcium to lower blood pressure, improve blood lipid levels and renal function, and indirectly reduce oxidative stress.

AUTOBIOGRAPHICAL STATEMENT

Licensure
Registered Nurse (RN), State of Michigan, Board of Nursing

Education
Ph.D., Liberal Arts & Sciences, Wayne State University (WSU), Major: Nutrition & Food Science, Minor: Kinesiology, Health & Sport Studies

Master of Science in Nursing (MSN), WSU, 1993, Major: Nursing, Parenting, and Families

Bachelor of Science in Nursing (BSN), Madonna College, 1985

Certification
Certified Nurse Educator (CNE), National League for Nursing, ID#: NLN390904

Awards
2006 NISOD Excellence Award in Teaching, University of Texas at Austin, National Institute for Staff and Organizational Development

Honors
Honor Society for Food Science – Wayne State University, Phi Tau Sigma Honorary Society

Honor Society of Nursing - Wayne State University, Sigma Theta Tau International & Lambda Chapters

Honor Society of Nursing - Madonna University, Sigma Theta Tau International, Kappa Iota Chapter

Presentations – Use of Technology to Enhance Student Learning
League for Innovation: Conference on Information Technology 2009, Detroit, MI, October 11, 2009

Trends in Occupational Studies: A Conference for Michigan Community College Educators, Grand Rapids, MI, October 2, 2008

Local Publications
A Parent's Guide to Newborn Intensive Care, Neonatal Intensive Care Unit, Henry Ford Hospital, Detroit, MI, June,1993

Global Education
Beijing, China – summer 2007, Central Michigan University, Educational Administration & Community Leadership, Course: EAD 750G, Professor: Dr. Pamela Eddy, Ph.D.