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Nutritional Status In A Cohort Of Hemodialysis Patients Receiving Tocotrienol Supplementation

Rajeev Shahani
Wayne State University,

NUTRITIONAL STATUS IN A COHORT OF HEMODIALYSIS PATIENTS RECEIVING TOCOTRIENOL SUPPLEMENTATION

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RAJEEV SHAHANI

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CHAPTER 1: INTRODUCTION

The kidneys, located in the posterior wall of the body, perform many functions that the body relies upon to survive. Such processes include waste excretion, chemical balance (e.g. electrolyte and acid-base balance, Calcium-Phosphate metabolism), regulation of blood pressure, in addition to fluid balance [44]. The kidneys' complex structure contains a plethora of tissue layers and permeable membranes and vessels in a way where filtration, absorption, and secretion are some of the main objectives the kidneys are used for. Blood circulation through the kidneys as well as excretion of waste products from it through urine is essentially the paths that allow for the aforementioned functions to occur [44]. The anatomy of the kidneys (figure 1.0) include complex parts such as the nephrons and glomerulus within the cortex, as well as the medulla, pyramid, pelvis, and ureter that allows waste-carrying urine to flow on to the bladder to be excreted from the body [44]. The nephrons provide the structural and functional units of the kidneys where the aforementioned processes allow urine formation to occur [44]. Any disruption or abnormalities in any of these processes can show signs of damage or failure to the kidneys [44].

The circulation of blood flow through the kidneys is where the reabsorption, secretion, and excretion occurs [44]. Since these steps take place within the nephron via afferent and efferent arterioles, reabsorption of certain products (e.g. water, salts, sugars) can return to circulation within the body [44]. Secretion can occur to return certain components such as acids, minerals, urea, and byproducts of drugs in order to return to the renal tubule to be excreted in urine while other components reach circulation via the veins [44]. The glomerular capillaries are porous, allowing fluids that contain those byproducts mentioned previously (and proteins if there are underlying issues) [44]. The Bowman's Capsule that contains the glomeruli, essentially

carrying the glomerular filtrate, occurs at a rate (glomerular filtration rate) affected by blood pressure [44]. Membranes inside the capsule filters the entering plasma so that certain molecules such as water and solutes can pass through, whereas, larger molecules like proteins could problematic and cause for concern [44]. Surface area available for filtration is adjustable due to blood pressure changes [44]. Normal GFR is designated between 120-125 mL/min in normal, adult kidneys, and rates below that range could be problematic considering normal amounts of filtrate are not being processed [44].

Filtration in the remaining renal tubules (e.g. proximal, loop of Henle, distal) is responsible for secreting and reabsorbing certain contents in order to maintain a chemical balance within the blood affected by blood pressure [44]. This entails the reabsorption of molecules such as sodium ions, hydrogen ions, phosphates, bicarbonate, and water, as well as the secretion of urea, ammonia, and other toxic substances to be excreted in urine [44]. These two features allow the kidneys to help maintain fluid balance, electrolyte balance, control blood pH, eliminate nitrogenous wastes, as well as disposal of certain drugs and metabolites [44]. Keeping blood pH intact is necessary considering environmental conditions due to pre-existing conditions (e.g. diabetes, severe chloride depletion, lactic acid buildup) can either severely raise or lower those levels resulting in acidosis (low pH) and alkalosis (high pH) [44]. Waste management is especially important considering buildup of substances, such as urea, can affect kidney function in terms of a negative impact on GFR [44], [12].

Kidney failure is a progressive and debilitating disease that affects many Americans. Numerous factors negatively affect the kidneys' ability to function as they work to filter byproducts of multiple origins (e.g. metabolic wastes, drugs, fluid balance). The repeated abuses often overload the kidneys, reduce their efficacy, and may result in their failure altogether [1].

Certain conditions such as metabolic syndrome, hypertension, and diabetes, are recurrent with chronic kidney disease (CKD) with the latter being the most prevalent of causative factors [2]. Over time, the kidneys can stop functioning during CKD which can eventually lead to what is known as end stage renal disease (ESRD) [1]. Prevalence of ESRD has risen in the United States from 1980 until 2010. The adjusted rate of these prevalent cases has risen in 2010 to 1,763 per million populations, up 21 percent higher than 10 years prior (Figure 1.1).

Among the ESRD population, approximately 400,000 people within that group are seeking dialysis treatment, including 33% that are African American [2]. ESRD continues to be the highest for African Americans compared to other ethnicities [2], [3]. However, between mortality and survivability, certain factors show the African American dialysis patients have a greater survival rate than whites (e.g. body mass, lipid profiles) [2].

CKD is characterized by a decrease in kidney function over a given period of time [1]. The rate at which blood flows into the kidneys must be determined using the Modification of Diet in Renal Disease (MDRD) formula [4] resulting in assigning a numerical value. Again, this rate is known as the glomerular filtration rate (GFR), the rate at which blood flows into the vessels of the kidneys (glomeruli) through filters so that byproducts are separated and excreted out through urine [1]. The GFR levels (between 15 and 90 mL/min/1.72m^2) that define the stages of CKD are also seen in Figure 1.2 [5]. ESRD occurs after stage 5, when there is an irreversible loss of function and renal replacement therapy is required [1]. As this is examined further, reabsorption and secretion steps involving the filtrate through the glomeruli and tubules can drastically reduce or not occur at all due to failed kidneys [44]. Eventually, the inability to form urine would be a clear indication of problematic situation among the kidneys where waste products cannot be eliminated which are returned to the blood [44]. The overlaying effect would

contribute to a negative impact on homeostasis involving factors such as electrolyte balance, blood pH, and fluid balance, as aforementioned.

Aside from kidney transplantation, alternative forms of treatment modalities are peritoneal dialysis and hemodialysis. With peritoneal dialysis, the use of blood vessels in the abdominal lining, or peritoneum, fills in the kidneys aided by a dialysate [4]. This dialysate is essentially a cleansing fluid that aids in the filtration of waste products as fluid passes through the peritoneal space [4] that is delivered via a surgically implanted catheter inside the abdomen. Hemodialysis requires the use of a dialysis machine that functions as an artificial kidney, or dialyzer, to filter the metabolic waste products, salts, and fluids from the blood [6]. This requires access to the blood vessels by way of a minor surgical procedure via the patients arm in what appears to be two incisions: one for removal and cleansing of the blood and the other to return the cleaned blood back into the patient's system [4], [6].

Based on current research, causes of CKD and ESRD stem from multiple factors: drug use, hypertension, and diabetes. Drug use can lead to nephrotoxic effects on intra-glomerular blood flow, cell injury via cytotoxicity, and crystal induced obstruction of the vessels [7]. During hypertension, high blood pressure forces the heart to work harder over time, thereby damaging blood vessels throughout the body, including blood vessels within the kidneys [8]. Those inflicted with diabetes have higher levels of blood sugar that causes the kidney filters to become overloaded due to the extra work, eventually failing [8]. Early signs of diabetes induced kidney failure is detected when protein is found in urine [8].

ESRD has an impact on morbidity and mortality of the patients as evidenced by increasing risk of hospitalizations and long term complications, including cardiovascular disease, malnutrition, and chronic inflammation [9]. The risk for cardiac events in the ESRD population

that receive treatment is approximately 3.5 to 50 times higher than the general population [9]. The determinants of cardiovascular disease in this population include family history, hypertension, diabetes mellitus, dyslipidemia, and obesity [9].

Co-morbidities lead to an increased mortality risk in the general population, though the effects of these conditions react differently for those on chronic hemodialysis. Ikizler et al. noted that traditional risk factors towards mortality in the ESRD population appear to be disregarded when compared to the normal, healthy population. For example, low serum cholesterol usually associated with decreased risk of mortality in the general population is shown to be associated with higher rates of cardiovascular morbidity in the ESRD population [9]. High body mass synonymous with increased mortality in the general population has been shown to be protective in the ESRD population [9]. Researchers have been forced to explore nontraditional risk factors attributing to morbidity within the ESRD population. These factors which include anemia, disturbance in mineral metabolism, oxidative stress, chronic inflammation, and uremic malnutrition [9]. Chronic inflammation, oxidative stress, and malnutrition are especially important in the clinical outcomes of the ESRD population [9] since they can affect body composition. Interestingly, oxidative stress can increase inflammation within the body, which is caused by a buildup of toxins and decreased antioxidant intake stemming from malnutrition [9]. Inflammation can cause an increase in resting energy expenditure (REE) [10]. Gradually the body is forced to rely on its own protein stores to attenuate the energy deficit (net protein catabolism), thereby causing a breakdown of muscle/lean body mass [9], [11] (Figure 1.4). Since the body has low energy intake and increased energy expenditure, it would rely on its own stores to make up that deficit which forces the body to waste away, resulting in a net protein catabolic state known as Protein Energy Wasting (PEW) [10].

The process of inflammation has been found to be associated with vascular disease in the general and dialysis populations [12], [13]. It had been found that specific cytokines such as interleukins are known to induce anorexia or a decrease/suppression in appetite [9], [12]. Toxins such as advanced glycation end products (AGE), that result from glycation reactions during glomerular filtration, builds up and therefore lowers defenses against oxidative injury [12]. These AGE also activate mononuclear cells such as lymphocytes that triggers an inflammatory response [12], also inducing appetite suppression. It is possible appetite suppression occurs by a decrease in the hormones, leptin and ghrelin [11], that are responsible for controlling the patient's appetite. As a consequence, a decreased intake of energy and nutrients (including antioxidants) results [12], [13]. Cytokine and toxin buildup, coupled with a decrease in antioxidant intake, resulting in oxidative stress which further increases inflammation within the body [9].

Other sources of inflammation emanate from dialytic factors. Back filtration allows molecules such as endotoxins transported via dialysis fluid to end up in the blood due to high-flux dialyzers [14]. This triggers an immune response. Bio-incompatibility of dialysis membranes increases inflammatory factors within the dialysis patients through activation of mononuclear cells and white blood cells which are both adaptive responses the body has during an immune reaction, in addition to the activation of acute-phase responses (e.g. stimulation of cytokines, interleukins) [12].

Co-morbid conditions induce inflammation in ESRD patients. Infections are common among hemodialysis patients because they pose a risk due to impaired immunity or vascular access [12]. Sources of infections stem from septicemia in diabetic patients due to low albumin levels, imperfection among dialysis grafts, and overlooked skin lesions due to diabetic

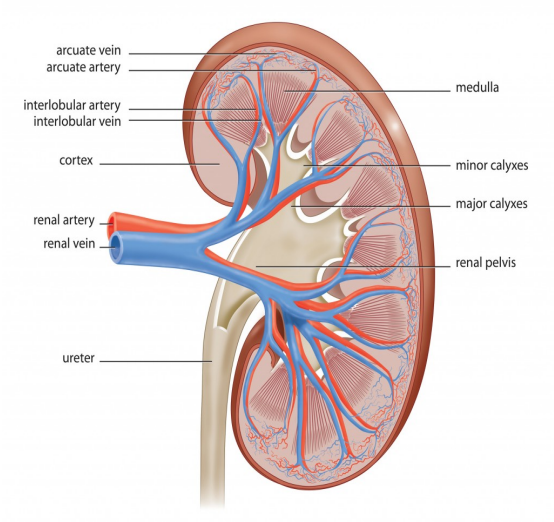
neuropathy of all which trigger an immune response [12] thereby increasing the rate of inflammation within the body[12], [13]. Moreover, uncontrolled blood glucose can give rise to non-enzymatic reactions of proteins (during the increase of AGE products) [12] can also trigger an immune response. During CVD, pro inflammatory cytokines are highly associated with this and can further increase oxidative stress in addition to inflammation within the body [12], [15].

Malnutrition is common in cases of CKD. Several researchers have reported on the prevalence of wasting among patients who have ESRD, especially those on hemodialysis [11]. Conversely, uremia is characterized by a buildup of waste products within the bloodstream [4]. Based on previous findings, this buildup of waste products stemming from ESRD complications is one of the main causative factors that primarily contribute to (uremic) malnutrition since it can affect nutrient intake due to inflammation. This wasting phenomenon had been defined as a consequence due to insufficiency of food intake with an increase in energy expenditure, resulting in a negative energy balance which ultimately causes the body to waste away [11].

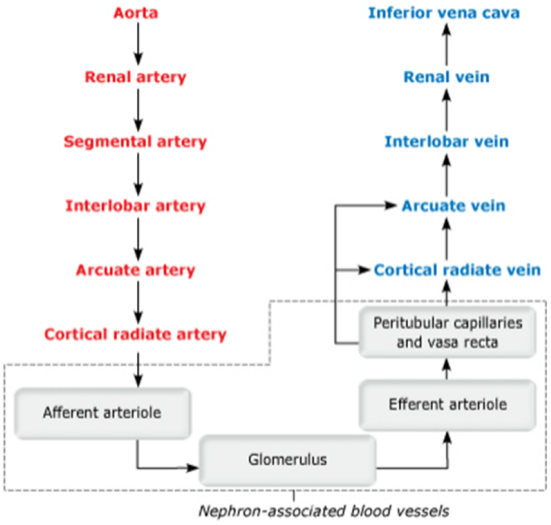
CVD is a clinical outcome that is common in ESRD patients due to the increase in mortality risk [15]. The ESRD patients are also at risk for hospitalization due to poor nutritional status and inflammation stemming from dialysis and co-morbid related issues [15]. Malnutrition and inflammation play a role in mortality since they impact nutritional and inflammatory markers that link CVD with ESRD. This may suggest that ESRD patients should be encouraged to control aforementioned complications in order to sustain health maintenance.

Figure 1.0: (a) Kidney anatomy; (b) Path of blood flow through renal blood vessels;

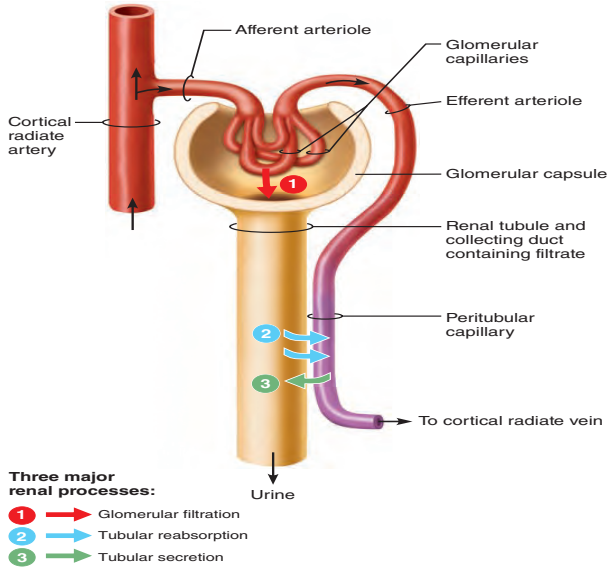
(c) Three major renal processes within the nephron



(a)



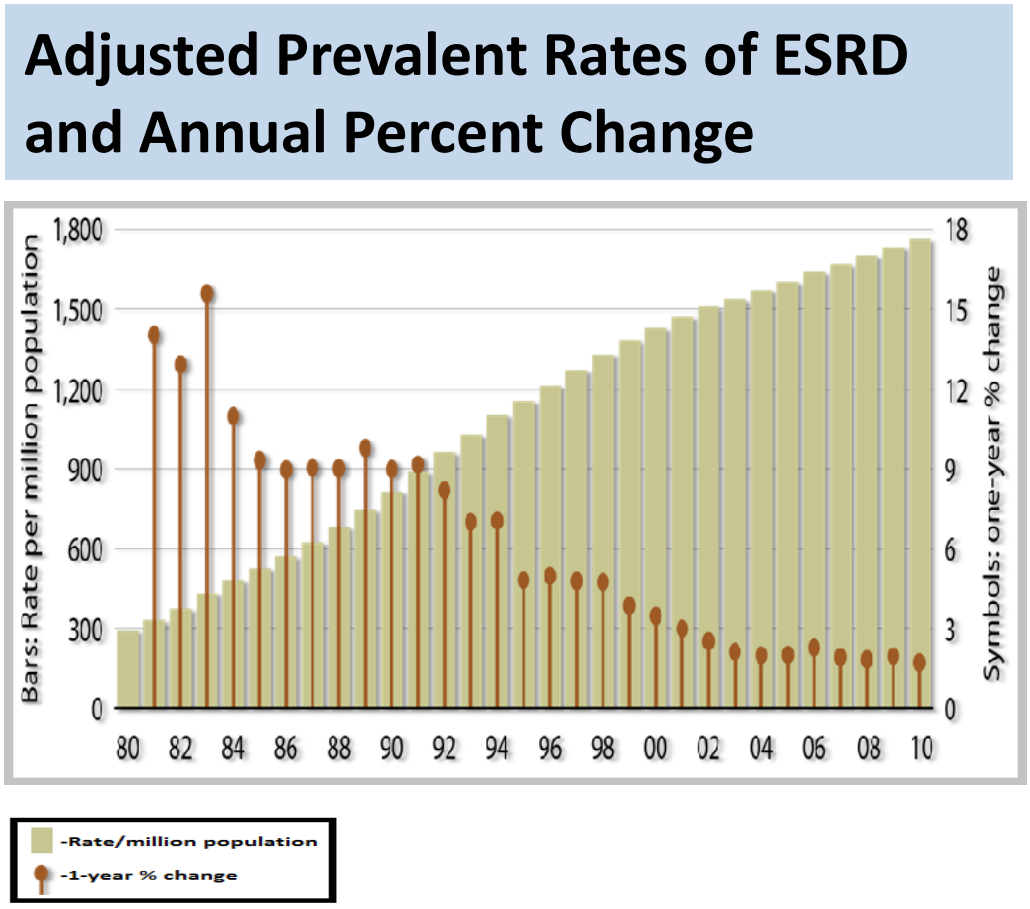
(b)



(c)

[Adapted from http://www.interactive-biology.com/3254/the-anatomy-of-the-kidney and Marieb *Human Anatomy and Physiology*, 9th Edition, 2012]

Figure 1.1: Adjusted Prevalent Rates of ESRD and the Annual Percent Change.



Note: The adjusted rate of prevalent cases of end-stage renal disease rose 2010 to 1,763 per million population, about 21 percent higher than that seen in 2000. The symbols represent the percent changed from the previous year's rate.

Figure 1.2: Stages of glomerular filtration rate (GFR)

Stage	GFR	Description
1	>90	Normal function, few abnormalities in urine
2	60-89	Mildly reduced function
3	30-59	Moderate reduction
4	15-29	Severe reduction
5	<15	Kidney failure, also known as End Stage Renal Disease (ESRD)

Note: units for GFR are expressed in ml/min/1.73 m^2; adapted from United States Renal Data System 2012

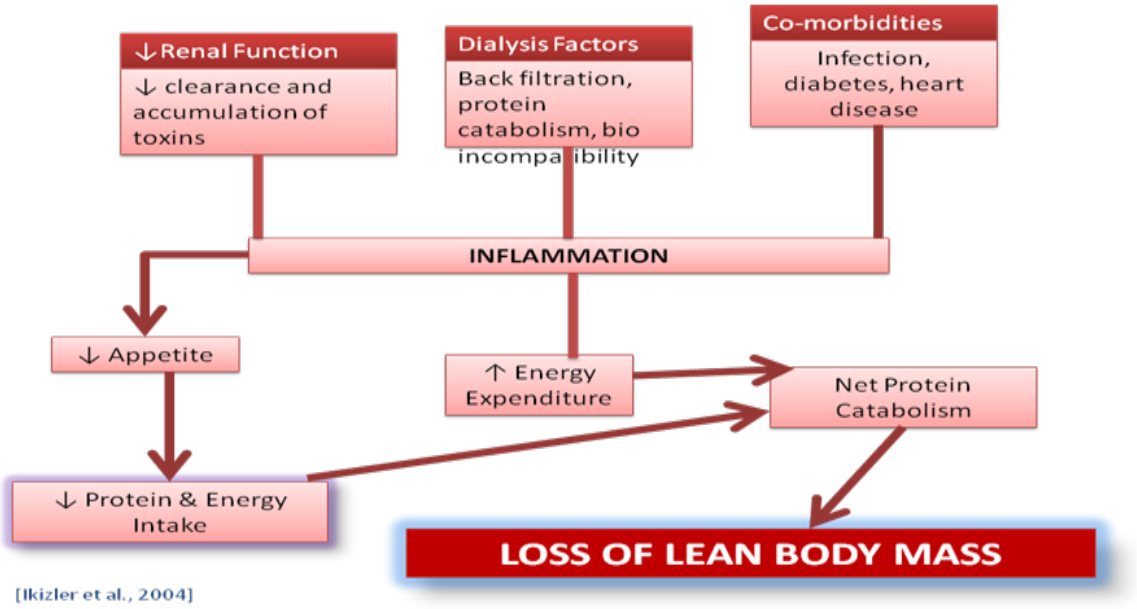
Figure 1.3: GFR categories in Chronic Kidney Disease

GFR category	GFR (ml/min/1.73 m2)	Terms
G1	>90	Normal or high
G2	60–89	Mildly decreased
G3a	45–59	Mildly to moderately decreased
G3b	30–44	Moderately to severely decreased
G4	15–29	Severely decreased
G5	<15	Kidney failure

Abbreviations: CKD, chronic kidney disease; GFR, glomerular filtration rate.

Note: Relative to young adult level. In the absence of evidence of kidney damage, neither GFR category G1 nor G2 fulfill the criteria for CKD. Adapted from KDOQI Guidelines, 2012: International Society of Nephrology

Figure 1.4: Causative factors and outcomes of kidney disease and co-morbidities.



CHAPTER 2: BACKGROUND AND RATIONALE OF THE STUDY

Renal disease is associated with a range of complex alterations to metabolic functions, which has an effect on physiological attributes [16]. These issues (e.g. diabetes, infections, toxin buildup) had been previously shown to be pro-inflammatory [9]. It was later noted that inflammation negatively affected the body which lead to malnutrition and eventually PEW from the combination of malnutrition and increased energy expenditure [9].

Methods of intervention are aimed at reducing mortality and morbidity stemming from CVD in the ESRD population. Previous research has established that causative factors subjected the body to inflammation and oxidative stress [9], [12]. Dietary intervention was thus aimed at combatting the complications that were attributed to inflammation and oxidative stress. These interventions explored anti-inflammatories and antioxidants as possible forms of diet therapy.

A study by Kaysen et al. [12] noted that chronic inflammation among ESRD population increases pro-inflammatory biomarkers (i.e. interleukins) that are known to induce anorexia (appetite loss) by suppression of nutrient intake. Thus an ESRD patient with inadequate nutrient intake is not consuming foods that have a high content of anti-inflammatories or antioxidants. The low nutrient intake is not enough to offset the damage caused by ESRD complications.

Both anti-inflammatories and antioxidants work by attenuating the buildup of markers responsible for oxidative stress and inflammation stemming from CVD and dialysis related factors. Under a high prevalence of oxidative stress stemming from an imbalance of an oxidant-to-antioxidant ratio, this increases the amount of oxidative damage [9]. Additionally, uremic conditions from toxin buildup in the blood also give rise to this imbalance [4]. Interestingly, serum albumin has anti-oxidant capabilities based on the fact that it is a free radical scavenger

and a toxic compound-binding agent, which are figures prominently in uremic environments [17]. This coincides with dialysis patients having a deficit in serum albumin [9]. In the presence of CVD, pro-inflammatory cytokines are secreted which are an indicatior of rising inflammation in ESRD patients [18] (responsible for decrease nutrient intake and the eventual PEW). The anti-inflammatory would thus be aimed at lowering the increase in cytokines [9], [18].

One possibility in the hemodialysis population involved administering Omega-3 supplements (due to their cardio-protective properties) [19]. Dietary sources of omega-3 PUFAs are anti-inflammatory and also offer benefits for improving lipid profiles, decreasing oxidative stress, and blood pressure [20]. Thus a combination of protein and Omega-3 supplementation was evaluated (against a placebo) for its ability to improve inflammation and nutritional status (the omega-3 as the anti-inflammatory and the protein supplement to attenuate the loss of protein stores). However, despite a marginal improvement in triglycerides, serum albumin levels were unaffected while no changes were observed in the normalized protein nitrogen appearance values and body mass index values [19].

More research is needed to combat complications associated with ESRD including malnutrition and PEW since they lead to increased mortality risk. Omega 3 and protein supplementation has been examined, however, the results are not conclusive [19]. Vitamin E exists in two forms that differ from each other chemically and in terms of their biological activity [21]. The two forms tocopherols (TP) and tocotrienols (T3), vary according to the characteristics of the side chain [21]. Most documented studies on vitamin E refer to TP, which is commonly used in research and sold commercially as supplements [21]. Tocotrienols are more potent than TP in terms of biological activity: antioxidant capabilities are more potent than TP and their anti-inflammatory effects were found to inhibit the secretion of biomarkers such as IL-6 [21], [22].

Tocotrienols are part of the vitamin E family [23], [24]. As shown in figure 2.1, they are molecularly similar to TP and contain a chromanol ring, a hydroxyl group that has the ability to donate a hydrogen atom [25] and a farnesyl tail, which is hydrophobic. The only difference between the two forms is the side chain on the T3 contains 3 double bonds at carbon number three, seven, and eleven, giving them a bent configuration [26]. T3 molecules have four isoforms: alpha, beta, gamma, and delta, which are determined by methyl group placement on the chromanol ring an vary in biological activity [23]. Tocotrienols circulate with the aid of a transfer protein [21], [27]. Though it has affinity for alpha-TP, they are still detected within plasma at very low concentrations [21], [27]. Postprandial studies of T3 reveal that in plasma delta-T3 peaked at approximately four hours and alpha/beta was at five hours after oral intake [28]. Size and mass of T3 are an average of 736.3 angstroms and 410.6 g/mol, considerably less than TP [25].

Tocotrienols are found in a vast array of foods, although the concentration is low compared to TP. Currently, majority of supplements containing vitamin E are primarily TP [24]. Tocotrienols are abundant in rice bran, wheat germ, oats, and palm fruit [24]. In fact, the oil extracted from palm oil has the highest concentration of T3 compared to other plant based sources [24]. Figure 2.2 summarizes the quantity of T3 listed from plant based sources [24].

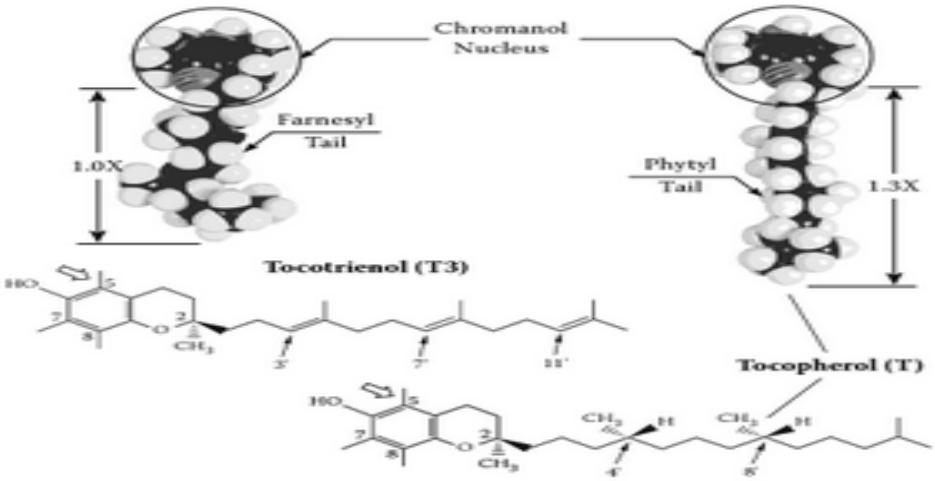
Limited studies have discussed the benefits of T3 [29]. Although T3 may not be abundant based on low transfer protein affinity, the concentration is small enough for beneficial effects [26]. Beneficial effects of T3 spans across a multitude of attributes, however, anti-oxidant and anti-inflammatory properties are most notable. The molecular structure of the T3, specifically the chromanol ring, contains a hydroxyl group that donates the hydrogen ion to scavenge reactive oxygen species and free radicals, a prominent effect of its anti-oxidant

capabilities [26]. T3 also induces other enzymes such as superoxide dismutase or glutathione peroxidase that takes up free radicals generated by environmental damage or oxidative stress [29].

Benefits of T3 are still under investigation. So far, T3 has been shown to be cardio-protective and anti-cancer. In some studies, T3 was detected among low density lipoproteins (LDL) while in transit and later found to prevent oxidation of those molecules [26]. Other cardio-protective attributes include T3's ability to inhibit HMG-CoA reductase while suppressing inflammation [29] thereby reducing the patient's LDL cholesterol. With regards to cancer, a rat study showed that mammary carcinogenisis was prevented by way of blocking chemically induced tumorigenesis of the mammary glands [26]. It noted that T3 suppressed cells' proliferation and perhaps induced apoptosis within the tumors [29].

The complications that stem from ESRD, dialytic factors, and co-morbidities subjects the body to inflammation, toxin buildup, and oxidative stress that ultimately leads to decreased nutrient intake, malnutrition and an eventual PEW [9]. It appears that T3 may be able to counteract the inflammation and oxidative stress based on its anti-inflammatory properties. An increase in pro-inflammatory cytokines may be inhibited, radicals produced during oxidative injury would be scavenged, and the oxidant-antioxidant ratio would improve to boost the overall antioxidant defense mechanisms. Therefore, an ESRD patient on hemodialysis would lower their mortality risk based on the stabilization or inhibition of complications associated with the aforementioned factors.

Figure 2.1: Molecule of Vitamin E



Methyl Group Position(s)	Tocotrienol*	Tocopherol*
5,7,8 - Trimethyl	alpha-T3 (58864-81-6)	alpha-T (59-02-9)
5,8 - Dimethyl	beta-T3 (490-23-3)	beta-T (16698-35--4)
7,8 - Dimethyl	gamma-T3 (14101-61-2)	gamma-T (54-28-4)
8 - Monomethyl	delta-T3 (25612-59-3)	delta-T (119-13-1)

*CAS number in parenthesis

Adapted from: National Center for Biotechnology Information at the National Institutes of Health.

Figure 2.2: Quantity of tocotrienols listed from plant based sources.

	Tocopherols (T1) milligrams per 1000 grams* / ppm				Tocotrienols (T3) milligrams per 1000 grams* / ppm				T3	T1 & T3
Sources	alpha	beta	gamma	delta	alpha	beta	gamma	delta	Total	Total
Palm Oil1	256	-	316	70	143	32	286	69	530	1172
Palm Oil2	279	-	61	-	274	-	398	69	741	1081
Palm Oil	152	-	-	-	205	-	439	94	738	890
Rice bran	324	18	53	-	116	-	349	-	465	860
Wheat Germ	1179	398	493	118	24	165	-	-	189	2377
Barley	350	50	50	-	670	120	120	-	910	1360
Oat	180	20	50	50	180	-	30	-	210	510
Coconut Oil	5	-	-	6	5	1	19	-	25	36
Palm kernel Oil	13	-	-	-	21	-	-	-	21	34
Soya bean Oil	101	-	593	264	-	-	-	-	-	958
Safflower Oil	387	-	387	-	-	-	-	-	-	774
Peanut Oil	130	-	216	21	-	-	-	-	-	367
Cocoa Butter	11	-	170	17	2	-	-	-	2	200
Olive Oil	51	-	-	-	-	-	-	-	-	51

1 HT Slover, Lipid 6;291 (1971)
2 KJ Whitte, et al. Analyst 92;423 (1967)

Adapted from www.tocotrienols.org

CHAPTER 3: OBJECTIVE

The primary objective of this study was to evaluate the nutritional status of the hemodialysis patients and to see if intervention with T3 supplements had any effect on their status. This was viewed based on probable change to the patients' anthropometric measurements and biochemical profiles. Complications of nutritional status such as PEW, the depletion of body mass, can be accounted for in BMI since that is a measure of lean body mass [9]. It had been established that reduced kidney function, dialytic factors, and co-morbidities raise inflammation which increases energy expenditure while depleting the body stores due to inadequate nutrient intake [9]. The effects of inflammation cause a decrease in appetite, thereby a decrease in nutrient intake resulting in a protein catabolic state of PEW since there was nothing to attenuate an increase in energy expenditure [9], [11].

The nutritional status of the patients on hemodialysis required a diet assessment so that the recall tracked the nutrient intake. Another value to the diet recall allowed the detection of T3 intake from foods consumed based on previous research. The supplementation of T3 was also taken into account so that the total intake was determined. Another aspect the research was analyzing the biochemical profile of serum albumin. Albumin's role is used to assess protein status, also known as visceral protein concentration [9]. Other biochemical profiles required to assess inflammatory status relies on the cytokine or interleukin concentrations [9].

There have been a limited number of studies that evaluate the effects of dietary intervention as seen in the Kuhlmann et al. that discussed anti-inflammatory diet/supplementation and reducing CVD risk. With respect to hemodialysis studies, we know that mortality risk is reflected in the presence of vascular disease [30] and an increase in PEW due to malnutrition and inflammation [10], ultimately encompassing CVD [9].

A connection had been established which states that PEW and mortality is reflected in serum albumin concentrations, as discussed by Jadeja et al. Diagnosis is often reflected in a comparison between nutritional requirements in CKD such as total caloric and protein requirement in accordance with the National Kidney Foundation's Kidney Dialysis Outcome Quality Initiative (KDOQI) guidelines (between 0.6-1.2 grams of protein/kg body weight depending on CKD stage and 30-35 kcal/kg body wt). There are no official recommendations on supplementation because the studies recommend the importance of protein and energy consumption while putting some emphasis on consumption of micronutrients such as antioxidants, vitamins, and certain minerals. The object is to attenuate the loss in protein stores due to renal disease and complications associated with dialytic factors co-morbidities.

Under the main outcome measures, the use of clinical profiles was required. This entailed a recording of nutrient data so that nutrient consumption was determined, in addition to tocotrienol consumption via food intake. Nutritional focused physical findings were also required such as anthropometric measurements of BMI and body weight that provided a visible outcome to nutritional problems. Finally, biochemical profiles were analyzed from blood samples which allowed the researchers to determine serum albumin to asses visceral protein concentrations (must be around 4 g/dL according to KDOQI guidelines) [9] which is a good predictor for mortality [3]. These are non-traditional factors whereas traditional risk factors for mortality tend to be opposite in ESRD patients (low serum cholesterol as a high risk factor for mortality) [9] and modifiable factors may not be applicable to the ESRD population compared to the normal population [9].

CHAPTER 4: MATERIALS AND METHODS

This project involved a collaborative effort of both Wayne State University Nutrition and Food Science and the Great Lakes Dialysis Center in Detroit, Michigan. The study's patients received routine hemodialysis treatment 4 days a week from the Great Lakes Dialysis Center.

The study conducted was parallel designed, randomized, and placebo controlled. The parallel design consisted of two groups within this dialysis participant study that were randomly given two different types of treatment. This clinical trial approach allowed the researchers to analyze and evaluate the outcomes of both types of treatment.

Under this study design, nutritional intervention of the groups were given either placebo or supplement capsules that were administered to the patients for dialysis and non-dialysis days. The key objective for the dialysis days was that it allowed the researchers to have a direct observation of treatment, making it easier to monitor the patients' compliance when taking the capsules. On the non-dialysis days, the patients were provided capsule organizers where each container had a compartment for each day of the week. These patients were grouped according to dialysis treatment: Sunday, Tuesday, Thursday, and Saturday; and Monday, Wednesday, Friday, and Sunday.

The supplements (Carotino, Johor Darul Takzim, Malaysia) were provided to both groups within the study population consisted of a placebo capsule and the tocotrienol rich fraction (TRF) capsule. The two groups were designated Placebo and TRF based on the supplements that were administered. The placebo capsule contents consisted of wheat germ oil that is 0.24 miligrams of tocotrienols and 0.44 milligrams of tocopherols. The TRF capsule contents consisted of Palm fruit oil that contained 90 milligrams of tocotrienols and 20 milligrams of tocopherols, for a total of 110 milligrams of vitamin E. To ensure compliance, the patients under directly observed

treatment were provided with two capsules during dialysis days three times a week. A weekly organizer was provided with capsules to be taken on non-dialysis days during the two main meals. The containers were returned each week and then refilled.

Another aspect of the study design was the 24-hour dietary recall performed by the registered dietitian. Each patient gave a recall at both baseline and then at week 16. The Nutritionist Pro software (First Databank, Chicago, IL) was used at the Wayne State University labs to complete the analysis after manually transcribing the hand-written recalls into the program. Estimations of portion size and quantity were recorded at the dialysis center and later transcribed by the Wayne State lab graduate research students. This kept track of the nutrient (macro and micro) and energy intake for all the participants. The total calories consumed during both time points, especially at week sixteen, gave an indication of whether or not the patients were consuming enough energy based on the recommended values. The macronutrients (i.e. fats, carbohydrates, protein) and the micronutrients (i.e. vitamins, minerals) were provided so that percentages of the diets were calculated in terms of composition. The data was then analyzed further utilizing statistical analysis software SPSS. It manipulated the data in order to determine food intake behavior based on group mean differences that used independent t-tests, chi-square tests for categorical data, and Pearson's correlation coefficients for correlation tests between two variables while significant P-values are $<.05$.

The 24-hour diet recall contained a very important aspect of the study since it was necessary that some foods consumed by the patients had naturally occurring tocotrienols. Previous research conducted by various institutions had determined the content of tocotrienols contained in processed foods. The tocotrienols are naturally found in oils, grains, and some animal products like eggs. The Wayne State nutrition labs pooled the data from the various

resources and created a database to determine the tocotrienol content of the foods [38-43]. Data was provided in milligrams of tocotrienols per kilogram (or 100 grams) of the food item. This was calculated according to the actual weight of the patients' food that determined the approximate amount of tocotrienols consumed.

Laboratory analysis of blood samples was performed for the patients at baseline and week 16 which obtained the biochemical profiles of both serum albumin and IL-6. Serum albumin was analyzed at an external laboratory via standard automated laboratory techniques (Bromocresol Green assay, Satellite Laboratory Services, Redwood City, CA). IL-6 was measured via the ELISA method per manufacturer protocol (Thermo Scientific, Cat No. EH2IL6). In addition to blood sample collection, medical professionals at the dialysis center recorded anthropometric measurements such as weight and BMI at the dialysis clinic.

In summary, the methods involved with the study for laboratory analysis at week zero and week sixteen were as follows: blood collection for serum albumin and IL-6 content that was analyzed for protein content and inflammatory markers; clinical profiles (i.e. body weight, BMI) that allowed the researchers to determine any fluctuations that may have indicated a decline (stemming from protein energy wasting); dietary analysis based on 24-hour diet recall was manually input into the Nutritionist Pro software and tocotrienols were calculated using external databases to analyze the nutrient intake (both macro and micro nutrients). Information on total calories, protein, and tocotrienols provided some insight into the possibility of malnutrition.

CHAPTER 5: RESULTS

Tables 5.1 through 5.10 depict the patients' clinical characteristics and nutrient intake data for baseline and week 16. The baseline values were recorded at the beginning of the study. At the end of the study, a comparison of the data between the two time points was made. The goal of the TRF supplements administered in comparison to a placebo was to observe a possible difference in the nutrient, clinical, and biochemical profiles of the patients' data.

Table 5.2 depicts the clinical characteristics of the study population. The BMI intervals, in accordance with the Center for Disease Control (CDC), are expressed in terms of kg body wt/m^2. The intervals are as follows: underweight (<18.9), normal (19-24.9), overweight (25-29.9), obese (30-34.9), very obese (35-39.9), and morbidly obese (40<). The average BMI for the study population was approximately 29.5 ± 8.1, which is overweight under CDC guidelines and an average of the placebo and TRF groups (28.7 ± 8.2 and 30.2 ± 8.1, respectively). Between the two groups, there was no difference throughout the range. In terms of distribution, majority of the patients were in the 19 to 29.9 BMI level. This distribution did not change at the end of the study, as the values were the same; majority of patients fell into the 19-24.9 and 25-29.9 ranges. Furthermore, there was no significance at either time point.

The next two measurements in table 5.2 were the biochemical profiles of serum albumin and IL-6. Serum albumin levels read at an overall average of 3.9 ± 0.3 g/dL. There was no difference between the placebo and TRF groups at both times. Moreover, nothing was significant after the t-tests were ran. The three distribution levels were determined by the SPSS software based on frequencies of levels for the patients. The KDOQI guidelines state that an approximate value of 4.0 g/dL represents a normal value for the dialysis population, and a significant drop indicates a decline in protein status further adding to the risk of mortality. It may appear that the

distribution started off with the majority of patients at 3.8 or less at week zero, but week 16 had most of them in the 3.9-4.1 range. The placebo group in week 16 had only three people move up into the 3.9-4.1 and 4.2 groups, however, no change in distribution. The remaining patients were removed from the study since denoted by the reduction in population from 40 to 36. Since the t-test proved no significance, again, these values did not change.

Biomarkers of inflammatory status, such as IL-6, can be viewed in table 5.2. At the beginning of the study, the average for the patients was at 6.9 ± 6.7 pg/mL. This translated into around 7.9 ± 7.9 pg/mL for the placebo and 5.8 ± 5.1 pg/mL for the TRF groups. There was no variation at baseline between the groups considering their averages were about 2 pg/mL apart and that there was no significant difference determined by the t-tests. At the end of the study, these values did not change and still had no significant difference between the groups.

The remaining tables, 5.3-5.10, provide information on nutrient intake and possible relationships among individual macro and micronutrients in addition to the biochemical and anthropometric profiles. In table 5.3, the average energy and macronutrient intake was provided for baseline and week 16 time points based on mean comparisons. As expected, baseline values should have no significant difference since nutrient data was acquired from the patients before the supplementation was administered. The average for all patients measured at 2013 ± 727 kcals per day. This accounts for 2097 ± 848 for the placebo and 1932 ± 585 for the TRF groups. However, there was no significant difference. This translates into a low kcal/kg body weight measurement at 25 ± 12 kcal/kg body wt for the average. Again, the two groups had no significant difference following the t-test even though the placebo group consumed approximately 27 ±14 kcal/kg body wt and the TRF consumed 23 ± 9. In addition, these averages are below the KDOQI recommendation of 35 kcal/kg body weight except for the

patients. Over all, these averages that fall below the recommendations suggest there is presence of under-nutrition in terms of total calories. At week 16, the average intake was approximately 1807 ± 481 kcal/day. However between the groups, there was no significant difference and overall no change in comparison to the baseline values.

In table 5.3, protein consumption was tracked but the values did not come up significant. Even though the baseline average of 94 ± 52 g which is 96 ± 68 g for the placebo and 91 ± 31 g for the TRF groups. With a p-value of 0.074, it had a tendency to be significant based on the p value that is greater than .05 but less than .1. Per the KDOQI guidelines (1.2 g/kg body wt), only the placebo group managed to reach that value at about 1.24 ± 0.77 g/kg body wt. This makes the overall consumption falls below to the amount of 1.16 ±.62 g/kg body wt when TRF is factored in as it only had 1.08 ± .42 g/kg body wt. A relatively large standard deviation like this may explain why the range of protein consumption per bodyweight varies so much. This pattern remained the same at week 16, no change in numbers and there was no significant difference between the groups. Overall consumption was 90 ± 51 g, which is 83 ± 33 g for placebo and 95 ± 62 g for TRF.

The next macronutrient on table 5.3, carbohydrates, had no significant difference between the two groups at both time points. Carbohydrate intake for all groups was evaluated at roughly 219 g ± 104 g (220 ± 103 g for placebo and 218 ± 107 g for TRF) at baseline and 202 ± 75 g (198 ± 65 g for placebo and 206 ± 83 g for TRF) at week 16.

Cholesterol intake, on table 5.3, was valued at an average of 431 ± 335 mg, placebo (448 ± 274 mg) and TRF (415 ± 388 mg) groups did not differ because there was no significant difference. There were unchanged values at week 16 indicated by an average of 397 ± 436 mg (353 ± 247 mg for placebo and 433 ± 544 mg for TRF) with no significance based on the t-tests.

Among the remaining lipids, total fat intake for the patients was 89 ± 48 g, which comprises of 99 ± 57 g for the placebo and 80 ± 36 g for the TRF groups, with a p value of 0.084. The difference between the groups had a tendency to be significant since the p-value is between .05 and .1. At week 16, total fat intake was at an average of 75 ± 30 g, &1 ± 24 g for the placebo and 79 ± 34 g for the TRF group, but no significant difference. Saturated fat intake fluctuated around 31 ± 26 g at baseline (36 ± 18 g for placebo and 29 ± 31 g for TRF), but was not significant. At week 16, there was no change since the average was 26 ± 23 g (21± 8 for placebo and 30 ± 30 g for TRF), but they had a tendency to be significant since the t-test's p value was 0.076. MUFAs, with a significance of .05, was detected at an intake of 31 g ± 20 overall, which is roughly 36 g ± 24 for the placebo and a lower intake of 27 g ±15 for the TRF group. Week 16 intake had an average of 24 ± 13 g overall, 23 ± 10 g for placebo, and 25 ± 15 g for TRF, which had a tendency to be significant (p-value = .067) but again no change. PUFA intake was the lowest intake for both groups with 18 ± 13 g overall, 19 ± 15 g for the placebo group, and 17 ± 11 for the TRF group, all with no significant difference. The week 16 values were at an average of 15 ± 12 g (15 ± 11 g for placebo and 16 ± 13 g for TRF) with no significant difference.

The next table, 5.4, presents the average intake for the remaining nutrients evaluated for the study population. Sodium intake at baseline was at an average of 3019 ± 1845 mg for all, which is 2961 ± 2000 mg for placebo and 2074 ± 1705 mg for TRF, but no significant difference. Week 16 sodium intake values does not show a change based on the average of 3352 ± 1628 mg for all, 3256 ± 1531 mg for placebo, and 3429 ± 1720 mg for TRF, with no significant difference. These values are above the recommended intake of 2 g per day [31].

The intake for potassium (see table 5.4) was also not significant. The baseline values are at an average of 2371 ± 2334 mg, 2295 ± 1115 mg for placebo, and 2371 ± 2333 mg for TRF, but no significant difference. For week 16, intake was at an average of 2015 ± 919 mg, 1962 ± 961 mg for placebo, and 2058 ± 894 mg for TRF, with no significant difference. Moreover, potassium intake was well within the recommended intake of 2-3 g/day [31].

Vitamin A intake, well above recommendations [31], was determined at in average intake of 7371 ± 19372 mg, which is 5524 ± 7415 mg for placebo and 9172 ± 26273 mg for TRF at baseline. The reason for high standard deviation is that one of the patients consumed a high amount of vitamin A by eating 16 ounces of liver in one day after reviewing their diet recall. Moreover, these differences were not significant. At week 16, average intake was 6015 ± 9645 mg, 6377 ± 12115 mg for placebo, and 5718 ± 7193 mg for TRF. Again, there was no significant difference.

Vitamin C intake (See Table 5.4), a water-soluble vitamin, is at risk for severe depletion during the dialysis process. It is recommended that there should be an intake 60-100 mg/day to attenuate this loss [31]. At baseline, the average intake was 99 ± 84 mg, which was about 90 ± 81 mg for placebo and 108 ± 86 mg for TRF. This had a tendency to be significant based on the fact that the p value (0.064) is between .05 and .1. At week 16, average intake was 90 ± 105 mg, 102 ± 112 mg for placebo, and 80 ± 98 mg for TRF with no significant difference.

Vitamin E-TP (see table 5.4) (tocopherol) intake had no significant difference at baseline or week 16. The average baseline intake was recorded at approximately 10 ± 10 mg, 9 ± 9 mg for placebo, and 10 ± 10 mg for TRF. At week 16, average intake was 6 ± 4 mg, 5 ± 3 mg for placebo, and 6 ± 5 mg for TRF.

The intake of Total T3s (table 5.4) at baseline was taken before the study began and was detected at an average of 2 ± 4 mg, 2 ± 4 mg for placebo, and 3 ± 4 mg for TRF with no significant difference. In week 16, interestingly, the values included supplement intake and were significant with a p-Value of <.0001. The average intake was 92 ± 91 mg, 2 ± 1 mg for placebo, 182 ± 3 mg for TRF. Dietary T3 intake at week 16 was at an average of 2 ± 2 mg, 2 ± 1 mg for placebo, and 2 ± 3 mg for TRF.

Another nutrient evaluated in the diet recalls was phosphorous (see table 5.4). Average baseline intake was 1173 ± 589 mg, 1159 ± 601 mg for placebo, and 1186 ± 585 mg for TRF, with no significant difference. At week 16, average intake was 996 ± 469 mg, 953 ± 431 mg for placebo, and 1031 ± 500 mg for TRF. These values appear to be at the recommended level [31].

The remaining nutrient intake, calcium, iron, and dietary fiber, are displayed on table 5.4 and neither one of them had any significant differences at baseline or week 16. Their relationships can also be viewed on tables 5.6-5.10 for any (significant) correlations. After analyzing both the mean values of the nutritional intakes for both time points, there appears to be little or no difference at all. This applies to the overall values as well as for the placebo and T3 groups.

Table 5.5 depicts the baseline and week 16 macronutrient correlation coefficients. This data would hold the behavior of certain macronutrient intake patterns in comparison with other macronutrients. The correlation coefficients that are displayed indicate that the relationship between the two nutrients is significant.

Total calories have positively correlated with all the macronutrients at baseline and continued their relationship with all except Saturated Fat and PUFA. Saturated Fat and PUFA

intake did not have any correlation with Total Calories because the relationship was not significant. Further examination of protein intake did show a (positive) correlation with all nutrients except saturated fat and PUFA (at week 16 only) because they were not significant. Carbohydrates only had positive correlation with total calories and protein at both baseline and week 16. Total fat intake positively correlated with all the nutrients displayed in table 5.5 except carbohydrates at baseline and week 16. Cholesterol also positively correlated at baseline and week 16 with all nutrients except carbohydrates and saturated fat, but only at baseline with PUFA. Saturated fat only positively correlated with Total Calories at baseline only, with Fat at baseline and week 16, but only with MUFA and PUFA at baseline. MUFA had positive correlation Total Calories, Protein, Fat, Cholesterol, and PUFA at baseline and week 16. MUFA did correlate with Saturated Fat only at baseline. PUFA correlated with Total calories, protein, Cholesterol, and Saturated Fat at baseline only, and with Fat and MUFA at both baseline and week 16.

Table 5.6 displays the baseline and week 16 micronutrient correlation coefficients between Total Calories, Sodium, Potassium, Vitamin A, Vitamin C, Calcium, Iron, Vitamin E (tocopherols), Phosphorous, Dietary fiber, dietary Vitamin E-T3, and Total T3 (dietary + supplement). Total T3 at baseline only consists of dietary sources of T3 because supplements were not administered when the first diet recall was recorded. According to this data, Total Calories positively correlated with Sodium, Potassium, Calcium, Iron, Vitamin E-TP, Phosphorous, and Dietary Fiber at baseline and week 16. Total calories also correlated with Vitamin C only at week 16. The remaining nutrients had no correlation with Total Calories since they were not significant. Sodium and Phosphorous had a positive correlation at baseline and week 16, but with Potassium, Calcium, Iron, and Dietary Fiber at week 16 only. Potassium

positively correlated with Vitamin C, Calcium, Iron, Phosphorous, and Dietary Fiber at baseline and week 16. Vitamin A positively correlated with Iron and Phosphorous at baseline and with Dietary Fiber at week 16. Vitamin C also had positive correlation Iron, Vitamin E-TP, Phosphorous, and Dietary Fiber at baseline only. Calcium had additional positive correlation with Iron, Phosphorous, and Dietary Fiber at both baseline and week 16. Iron positively correlated with Phosphorous and Dietary fiber at baseline and week 16, but only with Vitamin E-TP at baseline, in addition to the other relationships previously discussed. Vitamin E-TP also had positive correlation with Phosphorous at baseline and with Dietary Fiber at week 16. Phosphorous additionally had positive correlation with Dietary Fiber at baseline and week 16 and with Vitamin E-T3 at baseline only. Dietary Fiber, in addition to other nutrients, positively correlated with Vitamin E-T3 at baseline and week 16. Vitamin E-T3 did not have any further relationships with nutrients other than what had been mentioned previously because there was no significance. This applies to all the nutrients within Table 5.6, no presence of a correlation coefficient means that there was no significance between the two nutrient comparisons.

Table 5.7 contains the baseline and week 16 correlation coefficients between Total Calories and Protein with anthropometric and biochemical profiles. Based on the data provided on the nutrient intakes, anthropometric, and biochemical profiles, serum albumin did not have any interaction with any nutrient intake or biomarker. While further examining relationships IL-6 had with nutrients and biomarkers, there was a positive correlation only with dietary T3 which suggests IL-6 occurrence is higher with an increased intake of dietary T3. At the end of the study, IL-6 had no correlation with any nutrient or biomarker because they were not significant.

BMI had a positive relationship with protein intake, Triglyceride levels, and a negative relationship with HDL cholesterol. The latter two are in conjunction with previous literature on

the kidney disease population stating that high BMI is associated with higher triglycerides or lower HDL cholesterol [32]. At week 16, BMI continued to have a negative correlation with HDL cholesterol, agreeing with BMI-HDL relationship in previously established literature.

The following biomarkers were analyzed based on informational purposes to see if there is any conjunction with lipid profile data. Total Cholesterol (serum) only had positive correlation with Triglycerides at week 16, and LDL Cholesterol at both baseline and week 16. This claim is agreeable with previous literature [32]. Triglycerides negatively correlated with HDL cholesterol at baseline and week 16, in addition to, LDL cholesterol at week 16 only. HDL cholesterol also had a negative correlation with LDL cholesterol at week 16, which is already established in prior literature [32].

Table 5.8 depicts the interaction between macronutrients and biochemical profiles at baseline and week 16. Based on the data provided, there was no correlation between the nutrients and the biochemical profiles. However, there is an additional correlation test comparing Dietary Fiber with the other nutrient intakes and it was shown that it had positively correlated with Total Calories and Carbohydrates at baseline and week 16, but negatively correlated with (dietary) Cholesterol. The latter may suggest that those consuming fiber rich foods may consume less dietary cholesterol, or vice-versa.

Table 5.9 contains data on the baseline and week 16 interactions between Vitamin E, biochemical, and anthropometric correlations. The relationship between the biochemical and anthropometric profiles had already been discussed in table 5.7. Vitamin E-T3 and Total-T3 both had positive correlation with IL-6 at baseline.

The final table, 5.10, contained data that depicts the baseline and week 16 correlation between T3 and macronutrients. Correlation among the macronutrients had already been discussed in table 5.5. Between Vitamin E-T3 and the macronutrients, there was negative correlation with Cholesterol intake at baseline and positive correlation with Saturated Fat at week 16.

Table 5.1: Demographics and clinical characteristics of the study population.

Characteristics	***All (n=81)***	***Placebo (n=40)***	***TRF (n=41)***
Age	58 ± 12	58 ± 13	59 ± 12
Ethnicity			
African American		40 (100)	40 (97.6)
Other			1 (2.4)

Note: All values are presented as mean ± Standard Deviation except for gender and ethnicity.

Table 5.2: Baseline and week 16 anthropometric and biochemical profile data.

Characteristics	Baseline				Week 16			
	All (n=81)	**Placebo (n=40)**	**TRF (n=41)**	**P Value***	**All (n=71)**	**Placebo (n=34)**	**TRF (n=37)**	**P Value***
Body Mass Index[1] (%)	29.5 ± 8.1	28.7 ± 8.2	30.2 ± 8.1		29.5 ± 8.1	28.7 ± 8.2	30.2 ± 8.1	
<18.9		2 (5.1)	2 (5)			3 (7.9)	2 (5)	
19-24.9		11 (28.2)[a]	8(20)			8 (21.1)[a]	8(20)	
25-29.9		11 (28.2)	12 (30)			12 (31.6)	13 (32.5)	
30-34.9		8 (20.5)	6 (15)			6 (15.8)	6 (15)	
35-39.9		3 (7.7)	6(15)			5 (13.2)	4 (10)	
40<		4 (10.3)	6(15)			4 (10.5)	7 (17.5)	
Serum Albumin[2] (%)	3.9 ± 0.3	3.9 ± 0.3	3.9 ± 0.3		3.9 ± 0.3	3.9 ± 0.3	3.9 ± 0.3	
<3.8		16 (41.0)	18 (45.0)			7 (21.2)	15 (37.5)	
3.9-4.1		14 (35.9)	12 (30.0)			15 (45.5)	15 (37.5)	
4.2<		9 (23.1)	10(25.0)			11 (33.3)	10 (25)	
IL-6 (pg/mL)	6.9 ± 6.7	7.9 ± 7.9	5.8 ± 5.1		6.9 ± 6.7	7.9 ± 7.9	5.8 ± 5.1	

1. Values in accordance with the Center or Disease Control, kg body weight/(height in meters)^2
2. KDOQI guidelines state that serum albumin equal to or greater than the lower limit of normal range (approx. 4.0 g/dL for the Bromcresol Green Method) is the outcome goal

Note: all values are presented as mean ± standard deviation. P values derived using independent t-test for IL-6; BMI and serum albumin using χ^2 for categorical variables for each time point. *All non-significant unless denoted by a p-value otherwise. Overall there was no change in values from baseline to week 16. The distributions of BMI and Serum Albumin also remained the same due. It is suggested that caloric intake is the main cause do to the unlikelihood of increased physical activity. Additionally, there could be limitations to the study based on the fact that the 24-hour diet recalls were collected and evaluated at 2 time points and therefore unable to assess dietary activity between the 16 weeks. E.g.: the decline of 3 participants in 16 weeks from the 19-24.9 range could be attributed to patient disqualification since one is not accounted for if 2 patients experienced an increase in BMI.

Table 5.3: Baseline and week 16 macronutrient data.

	Baseline				Week 16			
	All (n=81)	**Placebo (n=40)**	**TRF (n=41)**	**P Value***	**All (n=71)**	**Placebo (n=34)**	**TRF (n=37)**	**P Value***
Energy (Kcal/day)	2013 ± 727	2097 ± 848	1932 ± 585		1807 ± 481	1746 ± 478	1856 ± 484	
Energy per body weight (Kcal/kg)	*25 ± 12*	*27 ± 14*	*23 ± 9*		*22 ± 7*	*22 ± 7*	*21 ± 7*	
Protein (g)	94 ± 52	96 ± 68	91 ± 31	*N.S. (0.074)*	90 ± 51	83 ± 33	95 ± 62	
Protein per body weigl	*1.16 ± 0.62*	*1.24 ± 0.77*	*1.08 ± 0.42*		*1.16 ± 0.62*	*1.24 ± 0.77*	*1.08 ± 0.42*	
Carbohydrates (g)	219 ± 104	220 ± 103	218 ± 107		202 ± 75	198 ± 65	206 ± 83	
Cholesterol (mg)	431 ± 335	448 ± 274	415 ± 388		397 ± 436	353 ± 247	433 ± 544	
Fat (g)	89 ± 48	99 ± 57	80 ± 36	N.S. (0.084)	75 ± 30	71 ± 24	79 ± 34	
SF (g)	*31 ± 26*	*34 ± 18*	*29 ± 31*		*26 ± 23*	*21 ± 8*	*30 ± 30*	*N.S. (0.076*
MUFA (g)	*31 ± 20*	*36 ± 24*	*27 ± 15*	*0.05*	*24 ± 13*	*23 ± 10*	*25 ± 15*	*N.S. (0.067*
PUFA (g)	*18 ± 13*	*19 ± 15*	*17 ± 11*		*15 ± 12*	*15 ± 11*	*15 ± 13*	

Note: All values are presented as mean ± standard deviation. P-values derived using independent t-tests for each time point. Protein intake at baseline along with saturated fat and MUFA at week 16, have a tendency to be significant. Normally this suggests that the p-Value is greater than 0.05 or less than 0.1.

* All non-significant unless denoted by a p-value otherwise.

Table 5.4: Baseline and week 16 micronutrient data.

	Baseline				Week 16			
	All (n=81)	**Placebo (n=40)**	**TRF (n=41)**	**P Value***	**All (n=71)**	**Placebo (n=34)**	**TRF (n=37)**	**P Value***
Sodium (mg)	3019 ± 1845	2961 ± 2000	3074 ± 1705		3352 ± 1628	3256 ± 1531	3429 ± 1720	
Potassium (mg)	2371 ± 2334	2295 ± 1115	2371 ± 2333		2015 ± 919	1962 ± 961	2058 ± 894	
Vitamin A (IU)	7371 ± 19372	5524 ± 7415	9172 ± 26273		6015 ± 9645	6377 ± 12115	5718 ± 7193	
Vitamin C (mg)	99 ± 84	90 ± 81	108 ± 86	N.S. (0.064)	90 ± 105	102 ± 112	80 ± 98	
Calcium (mg)	607 ± 507	540 ± 262	672 ± 662		493 ± 341	476 ± 324	507 ± 358	
Iron (mg)	14 ± 6	13 ± 5	14 ± 7		13 ± 7	12 ± 8	14 ± 7	
Vitamin E (mg) [1]	10 ± 10	9 ± 9	10 ± 10		6 ± 4	5 ± 3	6 ± 5	
Vitamin E-T3 (mg)	2 ± 4	2 ± 4	3 ± 4		2 ± 2	2 ± 1	2 ± 3	
Total T3 (mg) [2]	2 ± 4	2 ± 4	3 ± 4		92 ± 91	2 ± 1	182 ± 3	< .0001
Phosphorous (mg)	1173 ± 589	1159 ± 601	1186 ± 585		996 ± 469	953 ± 431	1031 ± 500	
Dietary Fiber (g)	13 ± 11	12 ± 7	15 ± 14		12 ± 8	12 ± 8	13 ± 7	

1. alpha-tocopherol
2. Combined tocotrienols from food + supplements for week 16 only.

Note: all values are presented as mean ± standard deviation. P-values derived using independent t-test between the two groups for each time point.

Vitamin C intake at baseline has a tendency to be significant.

* All non-significant unless denoted by a p-value

Table 5.5: Baseline and week 16 macronutrient correlation coefficients.

	Total Calories	Protein	Carbohydrates	Fat	Cholesterol	Saturated Fat	MUFA	PUFA
Total Calories		0.671	0.657	0.763	0.356	0.47	0.721	0.548
		0.597	***0.684***	***0.701***	***0.385***		***0.558***	
Protein			0.238	0.397	0.467		0.41	0.342
			0.306	***0.309***	***0.338***		***0.289***	
Carbohydrates								
Fat					0.381	0.553	0.945	0.793
					0.266	***0.255***	***0.743***	***0.537***
Cholesterol							0.362	0.277
							0.241	
Saturated Fat							0.434	0.544
MUFA								0.714
								0.37
PUFA								

Note: Week 16 values are bold face and italicized. E.g., total calories and protein is significant with a correlation coefficient value of 0.671. Only those with correlation values between the two variables are displayed. Those with blank fields are not significant and therefore have no correlative relationship.

Table 5.6: Baseline and week 16 micronutrient correlation coefficients.

	Total Calories	Sodium	Potassium	Vitamin A	Vitamin C	Calcium	Iron	Vitamin E-TP	Phosphorous	Dietary Fiber	Vitamin E-T3	Total T3
Total Calories		0.567	0.41			0.277	0.539	0.311	0.685	0.285		
		0.48	***0.533***		***0.394***	***0.468***	***0.283***	***0.245***	***0.648***	***0.357***		
Sodium									0.343			
			0.36			***0.377***	***0.393***		***0.426***	***0.385***		
Potassium					0.277	0.234	0.617		0.539	0.679		
					0.409	***0.368***	***0.357***		***0.61***	***0.463***		
Vitamin A							0.586		0.321			
										0.235		
Vitamin C							0.293	0.225	0.227	0.234		
Calcium							0.265		0.286	0.572		
							0.455		***0.597***	***0.382***		
Iron								0.247	0.652	0.495		
									0.361	***0.517***		
Vitamin E-TP									0.24			
										0.278		
Phosphorous										0.292	0.237	0.237
										0.448		
Dietary Fiber											0.229	0.229
											0.287	
Vitamin E-T3												
Total T3												

1. Vitamin E-T3 is dietary sourced T3
2. Total T3 is a combination of dietary sourced T3 and supplements.

Note: Week 16 are in bold face point and italicized. E.g., total calories and sodium is significant with a correlation coefficient value of 0.567. Only those with correlation values between the two variables are displayed. Those with blank fields are not significant and therefore have no correlative relationship.

Table 5.7: Baseline and week 16 anthropometric and biochemical profile correlation coefficients.

	Total Calories	Protein	Ser. Albumin	IL-6	BMI	Total Chol.	Triglycerides	HDL Chol.	LDL Chol.
Total Calories		0.671							
		0.597							
Protein					0.332				
Ser. Albumin									
IL-6									
BMI							0.331	-0.459	
								-0.336	
Total Chol.									0.889
							0.433		***0.923***
Triglycerides								-0.313	
								-0.272	***0.326***
HDL Chol.									
									-0.251
LDL Chol.									

Note: Week 16 values are in bold face point and italicized. E.g., total calories and protein is significant with a correlation coefficient value of 0.671. Only those with correlation values between the two variables are displayed. Those with blank fields are not significant and therefore have no correlative relationship. Other notable attributes: this suggests that BMI increases when triglycerides increase and decreases with HDL cholesterol increases. Triglycerides decrease with HDL cholesterol increase and HDL cholesterol increase entails an LDL cholesterol decrease due to cholesteryl ester transfer protein (CETP) activity. These statements are all in accordance that were established in previous findings.

Table 5.8: Baseline and week 16 macronutrient and biochemical profile correlation coefficients.

	Total Calories	Protein	Carbohydrates	Fat	Cholesterol	Saturated Fat	MUFA	PUFA	Dietary Fiber	Total Chol.	Triglycerides	HDL Chol.	LDL Chol.
Total Calories		0.671 ***0.597***	0.657 ***0.684***	0.763 ***0.701***	0.356 ***0.385***	0.47	0.721 ***0.558***	0.548	0.285 ***0.357***				
Protein			0.238 ***0.306***	0.397 ***0.309***	0.467 ***0.338***		0.41 ***0.289***	0.342					
Carbohydrates									0.61 ***0.345***				
Fat					0.381 ***0.266***	0.553 ***0.255***	0.945 ***0.743***	0.793 ***0.537***					
Cholesterol							0.362 ***0.241***	0.277	-0.284				
Saturated Fat							0.434	0.544					
MUFA								0.714 ***0.37***					
PUFA													
Dietary Fiber													
Total Chol.											***0.433***		0.889 ***0.923***
Triglycerides												-0.313 ***-0.272***	
HDL Chol.													***-0.251***
LDL Chol.													

Note: Week 16 values are in bold face point and italicized. E.g., total calories and protein is significant with a correlation coefficient value of 0.671. Only those with correlation values between the two variables are displayed. Those with blank fields are not significant and therefore have no correlative relationship.

Table 5.9: Baseline and week 16 Vitamin E, biochemical, and anthropometric correlation coefficients.

	Vitamin E-T3[1]	Total T3[2]	Ser. Alb.	IL-6	BMI	Total Chol.	Triglycerides	HDL Chol.	LDL Chol.
Vitamin E-T3[1]				0.238					
Total T3[2]				0.24					
Ser. Alb.									
IL-6									
BMI							0.331	-0.459	
								-0.336	
Total Chol.									0.889
							0.433		***0.923***
Triglycerides								-0.313	
								-0.272	***0.326***
HDL Chol.									
									-0.251
LDL Chol.									

1. Vitamin E-T3 is dietary sourced T3.
2. Total T3 is a combination of dietary sourced T3 and supplements.

Note: Week 16 values are in bold face point and italicized. E.g., total T3 and Vitamin E-T3 is significant with a correlation coefficient value of 1.000. Only those with correlation values between the two variables are displayed. Those with blank fields are not significant and therefore have no correlative relationship.

Table 5.10: Baseline and week 16 Vitamin E and macronutrient correlation coefficients.

	Vitamin E-T3[1]	Total T3[2]	Total Calories	Protein	Carbohydrates	Fat	Cholesterol	Saturated Fat	MUFA	PUFA
Vitamin E-T3[1]							-0.257			
								0.456		
Total T3[2]							-0.257			
Total Calories				0.671	0.657	0.763	0.356	0.47	0.721	0.548
				0.597	***0.684***	***0.701***	***0.385***		***0.558***	
Protein					0.238	0.397	0.467		0.41	0.342
					0.306	***0.309***	***0.338***		***0.289***	***0.044***
Carbohydrates										
Fat							0.381	0.553	0.945	0.793
							0.266	***0.255***	***0.743***	***0.537***
Cholesterol									0.362	0.277
									0.241	
Saturated Fat									0.434	0.544
MUFA										0.714
										0.37
PUFA										

1. Vitamin E-T3 is dietary sourced T3.
2. Total T3 is a combination of dietary sourced T3 and supplements.

Note: Week 16 values are in bold face point and italicized. Only those with correlation values between the two variables are displayed. Those with blank fields are not significant and therefore have no correlative relationship.

CHAPTER 6: DISCUSSION

The focus of this study is to analyze the nutritional status of patients with ESRD undergoing hemodialysis. Specifically, to study the effect of a prescribed vitamin E supplement on complications of ESRD of these patients. ESRD is the result of CKD after a gradual loss in kidney function as the rate at which blood flows through the glomeruli to filter out byproducts, falls to less than 15 mL/min/1.73m^2[1]. A buildup of waste products in the blood damages the blood vessels (also known as nephropathy). This causes the kidneys to fail, which makes hemodialysis as a form of renal replacement therapy necessary. Prevalence of ESRD has been on the rise since the 1980s according to the USRDS. ESRD afflicts a certain number of Americans, especially African Americans who constitute 33% of the 400,000 patients seeking dialysis treatment. ESRD continues to be the highest for African Americans compared to other ethnicities [2], [3]. However, between mortality and survivability, certain factors show the African American dialysis patients have a greater survival rate than Caucasian patients [1](e.g. body mass, lipid profiles) [2].

Based on current research, causes of CKD and ESRD stem from multiple factors. Drug use can affect intra-glomerular blood flow, lead to cell injury via cytotoxicity, and cause crystal induced obstruction of the vessels [7]. During hypertension, high blood pressure forces the heart to work harder over time, thereby damaging blood vessels throughout the body, including blood vessels within the kidneys [8]. Those inflicted with diabetes have higher levels of blood sugar that causes the kidney filters to become overloaded due to the extra work, eventually failing [8]. Early signs of diabetes induced kidney failure is detected by protein found in urine [8]. As a result, ESRD has an impact on morbidity and mortality of the patients as evidenced by increasing risk of hospitalizations and long term complications, including cardiovascular disease,

malnutrition, and chronic inflammation [9]. The risk for cardiac events is higher in this population, thereby contributing to their risk of mortality [9].

Researchers have been forced to explore nontraditional risk factors contributing to morbidity within the ESRD population. Ikizler et al. noted that traditional risk factors towards mortality in the ESRD population appear to be contrary to effects in the normal, healthy population. For example, low serum cholesterol is protective in the general population but opposite in the ESRD population, and high body mass which is normally a risk factor in the general population is protective for those with ESRD [9]. Other risk factors contributing to morbidity in ESRD patients include anemia, disturbance in mineral metabolism, oxidative stress, chronic inflammation, and uremic malnutrition [9]. Chronic inflammation, oxidative stress, and malnutrition are especially important in the clinical outcomes of the ESRD population [9] since they can affect body composition.

One of the major issues that is associated with dialytic and ESRD related conditions, is the amount of inflammation the body endures. Inflammation can stem from multiple origins, including vascular disease [12], [13], dialytic factors (e.g. incompatible dialyzer membranes, reduced kidney function), and co-morbidities (e.g. infections) [9]. Other sources of inflammation can result from uremic toxin buildup [4]. Interestingly, oxidative stress too can increase inflammation within the body, which is caused by a buildup of toxins and decreased antioxidant intake stemming from malnutrition [9]. It has been found that specific cytokines such as interleukins (IL-6) were found to be present during inflammation in addition to the accumulation of AGE products [9], [12]. This eventually lowers antioxidant defenses (e.g. serum albumin [9], [17]) against oxidative injury in addition to the suppression of appetite

(anorexia), which causes a decrease in nutrient intake (malnutrition), especially antioxidant intake [9], [13]. Additionally, inflammation has also has been found to increase REE [9].

Malnutrition and chronic inflammation have been found to be direct results of multifaceted effects of dialytic and co-morbid conditions. This ultimately leads to strong clinical outcomes and increased mortality risk [11], [12]. Ikizler et al. provided a systemic approach to the effects and root causes surrounding malnutrition and inflammation discussed in figure 1.4. These inflammatory responses to ESRD complications were found to have caused a reduction in appetite and further increased energy expenditure denoted by an increase in REE [9]. This becomes a net catabolic protein state in which the body is forced to rely on its own protein stores for energy, resulting in a loss of body mass, also known as PEW [11], [10].

CVD is a common clinical outcome in ESRD patients due to the increase in mortality risk [15]. The ESRD patients are also at risk for hospitalization due to poor nutritional status and inflammation stemming from dialysis and co-morbid related issues [15]. Malnutrition and inflammation play a role in mortality since they impact nutritional and inflammatory markers that link CVD with ESRD. This may suggest that ESRD patients should be encouraged to control aforementioned health complications in order to sustain health maintenance.

The objective of this project was to evaluate nutritional status of hemodialysis patients while undergoing an intervention of T3 supplements. The effects can be viewed through probable changes to the patient's anthropometric (BMI) and biochemical profiles (serum albumin, IL-6). The anti-inflammatory characteristic of T3 is a key component by way of attenuating the effects of damage brought forth by inflammation where it may combat the rise in inflammatory markers. Additionally, many improvements have been made on the oxidant-antioxidant ratio, attenuation to the loss of serum albumin, as well as the possible defense

towards prevention of inflammation. Many components of inflammation stem from decreased renal function, dialytic factors, and co-morbidities (i.e. diabetes, CVD, infections) [13], [12], [9]. Moreover, the properties of T3 could potentially attenuate the loss of albumin as an antioxidant defense mechanism, thereby restoring the oxidant-to-antioxidant ratio to a normal level. Additionally, the immune response is a form of an acute phase response that triggers the stimulation of cytokines and interleukins, which can reduce antioxidant levels [12], thereby inducing oxidative damage. Tocotrienols allow the hydroxyl group that donates a hydrogen ion to later scavenge reactive oxygen species and free radicals, which is one of its prominent anti-oxidant capabilities [26]. Another antioxidant property of T3 is the induction of other enzymes, such as superoxide dismutase or glutathione peroxidase, that take up free radicals generated by environmental damage or oxidative stress [29].

Records of dietary intake and blood samples were taken and evaluated at two time points: baseline (Week 0) and Week 16. Changes in biomarkers and nutritional status were observed. Nutritional status is based on both macro and micro nutrient intake and compared to the NKF's KDOQI guidelines.

As mentioned before, anthropometric measures were observed at the beginning and the end of the study. This allowed the researchers to see whether nutritional status had any impact on the measurements while T3 supplements were prescribed. It appears that no change was detected in the patients' BMI. There was no change in the average BMI values of both study groups (roughly 30 kg/m2, 29 kg/m^2 for placebo, and 30 kg/m^2 for TRF), as well as in the distribution (the majority of patients were in the 19-24.9 and 25-29.9 ranges) at both time points. Moreover, it appears that the decrease in patients from the second interval may be partly due to the reduction in patients from the study. One will also notice that the number of participants is

lower in week 16 compared to baseline. BMI did have a positive correlation with protein intake at week zero, considering the relationship between protein and body mass and how the body requires protein intake to build muscle. At the end of the study, there was no significance between BMI and protein, suggesting that there is no correlation at all. In other words, there was no change in the data and no way to determine if PEW among the patients is present due to the unchanged BMI values compared to nutrient (energy and protein) values at the end of the study.

There were additional results pertaining to BMI's relationships. During the correlation tests, BMI negatively correlated with HDL cholesterol (table 5.7) suggesting that high BMI entails low HDL cholesterol, which agrees with previously established literature [33]. Other notable observations, which agreed with current literature, were the positive correlation between BMI and triglycerides, positive correlation between total cholesterol and triglycerides, negative correlation between HDL cholesterol and triglycerides, and the positive correlation between LDL cholesterol and total cholesterol [33].

The biochemical profiles of serum albumin and IL-6 were observed to indicate possible changes in nutritional and inflammatory status [9], respectively. Any decrease in serum albumin or increase in IL-6 could raise mortality risk [5], [3], [9]. It is reported that no changes were detected in serum albumin or IL-6. Most patients remained in the <3.8 g/dL and 3.9-4.2 g/dL ranges at both time points. There was not any relationship between serum albumin and the individual nutrients when correlation tests were run. Moreover, IL-6 values were insignificant at an average of 6.9 pg/mL (7.9 pg/mL for placebo and 5.8 pg/mL for TRF). These values did not change at week 16 indicating no effect on inflammatory markers. There was some slight positive correlation with food-sourced T3, however, the study was to determine the effect of total T3 administered to the patients and therefore this was disregarded. At the end of the study there was

no significant change due to any of the nutrients. This may not be enough to state whether or not mortality risk has been affected in favor of lessening the risk or that there is a basis for IL-6 induced protein energy wasting [8].

One of the main points the study was trying to investigate was whether there was a presence of malnutrition detected in the nutrient intake of the patients. Interestingly, since the nutrient data was recorded at two time points starting at week zero and then week 16, the numbers should have indicated malnutrition based on the comparison with the United States Renal Data System (USRDS) data or other cohort hemodialysis studies.

The values for total calories consumed per kilogram of body weight were 27 kcal/kg body weight versus USRDS recommended 35 kcal/kg body weight. One can see that these values fell below the USRDS recommendations. This remained unchanged at the end of the study as well. It does not appear that the T3 supplements have had an effect on nutrient intake or appetite considering energy intake has remained the same at both time points. Moreover, the numbers may also appear lower; p-values were not significant, and therefore no significant difference existed between the caloric intakes between the two groups. These values that represent the study population are inadequate, meaning that population size or compliance may have played a factor in the t-test results.

After analyzing the protein intake, it appears that there was no change in intake over the course of the 16-week study. It started off approximately 18-19% of the meals, but remained the same (from an overall of 94 ± 52 g to 90 ± 51 g) at the end of the study. After analyzing the consumption patterns, it increased along with the consumption of other nutrients such as carbohydrates, fat, cholesterol and MUFA. There was no significant difference between the groups at both time points and the protein consumed by body weight also was unchanged. NKF

KDOQI guidelines recommend hemodialysis patients consume more than 1.2 g/kg body wt, which only the placebo group (1.24 ± 0.77 g/kg body wt) has at both time points. The patients in the TRF groups were consuming less than that recommended amount and are at risk for protein-calorie malnutrition and hypoalbuminemia [10]. It was recommended that HD patients consume protein that comprises ≥15% of the diet, which the study population appears to have achieved. However, the total energy intake does not reflect the individual protein intake. There may have been inaccuracies with the diet recall, issues with the population size, or non-significance of the values may have produced these results.

Carbohydrate was the nutrient that comprised 42-45% of the diet. As this was between baseline and week 16, not much difference between the. It should be about 50-60% of the diet [31], which the study population was not able to meet. This could also explain why the caloric density intake is below the KDOQI recommended 35 g/kg body weight. Average fiber intake was around 13 mg at baseline and week 16. Both groups did not meet the recommended intake of an estimated 20-25 mg [31]. As suggested by previous literature, the problem with a low fiber intake is that it can negatively affect lipid levels [31]. One interesting point that appears to stand out is the fact that baseline fiber intake decreased as cholesterol intake increased after analyzing the correlations in table 5.8. This suggests that the inverse relationship could be explained by the lipid lowering effect fiber has during consumption that is suggested in previous literature. However, cholesterol intake was higher than expectations thereby suggesting the opposite and setting the patients up for heart disease. The food choices many patients made were comprised of refined or simple carbohydrates instead of complex variety such as fiber.

Lipid intake in this study population reflects a diet that promotes an increased heart disease risk. Cholesterol intake of the patients exceeded the American Heart Association (AHA)

recommendation of <300 mg/day. This intake may subject the patients to hyperlipidemia according to the AHA, and given their current health state, the risk for mortality is greater. The high cholesterol intake is mainly due to the frequency of egg consumption as seen on the diet recalls. When it comes to Total Fat intake, the patients consumed a diet that is comprised of 36-40% of it. Moreover, Saturated fat comprised 14% of the diet and unsaturated fat was about 22% (MUFA-14%, PUFA-8%) at baseline. At week 16, the diets were 13% saturated fat and 19% unsaturated fat (MUFA-12%, PUFA-7%). Interestingly, MUFA and Saturated fat had a tendency to be significant since the p-value was between 0.05 and 0.1. The values may have a tendency to reflect the intake in both groups and a slight difference. However, there may have been factors that caused the statistical analysis test to come up non-significant (e.g. inaccurate diet recall), which is why certain nutrients that are not accounted for in the total caloric intake.

According to AHA, the study population did not meet their recommendations. The patients exceeded the recommended intakes for total fat and saturated fat [31] thereby increasing their risk for heart disease. This could be viewed in the correlation studies (see table 5.5) on how total fat and saturated fat positively correlated with Total Calories. This means that the intake of both increased while there was an increase of Total calories.

Along with macronutrient intake, micronutrients were tracked as some are held in high importance to the ESRD population (e.g. sodium, potassium, phosphorous, Vitamin A, Vitamin C). The majority of micronutrient consumption in the study did not change from baseline to week 16. Towards the end of the study, there was no significant difference between the groups.

When sodium intake was detected at averages around 3000 mg per day, it exceeded the recommendations of <2000 mg [31]. It appears to be cumbersome for the patients since sources of sodium are primarily dietary-based or found in dialysate [34]. Potassium intake remained at

around 2000 mg at baseline and week 16, therefore exhibiting no change. Both groups' values had no significant differences. When it was compared to other dialysis studies, it exceeds their recommended ranges as well [31]. Tracking its behavior with other nutrients, potassium increased with increases in Total Calories, sodium intake (week 16 only), vitamin C, calcium, iron, phosphorous, and fiber. Sodium intake increased along with the following nutrients (table 5.6): Total Calories and Phosphorous at both time points, and with Potassium, Calcium, Iron, and Fiber at week 16. Based on these observations, the foods frequently consumed contained these common nutrients. Since t-tests did not prove significance, it may explain why these micronutrient intakes are not consistent with caloric intake. Moreover, the results of other studies have shown high potassium intake associated with increased death or survival hazards [35]. It can be in part due to issues like hyperkalemia caused arrhythmia [31]. High sodium intake concurrent with hypertension in normal population is more problematic in the kidney disease population since CVD is associated with complications leading to greater mortality [34]. Based on these numbers, the study population managed to keep potassium intake under control. However, the validity of the results may be questionable considering the study limitations which is viewed in the t-tests.

Phosphorous intake is of high importance to the ESRD population. Patients are encouraged to consume low amounts through diet and take binders since it is also affects calcium levels within the body [36]. Other important attributes in the CKD population is the awareness of soft tissue calcification associated with CVD risk [36]. Baseline and week 16 intake was between 950-1200 mg. The t-tests proved that there was no significant difference between the groups at both time points. Phosphorous did correlate with the majority of micronutrients, including dietary-based T3 at baseline only. The reason for these numbers is that various foods

consumed by the patients contained large amounts of phosphorous (e.g. beef, chicken, processed foods, condensed soups). These foods have common nutrients such as phosphorous, which may be why it had correlated with the intake of other micronutrients. The intake of phosphorous have been above recommendations at baseline and below them at week 16 [31]. Because there was no significance, it would be difficult to make that observation on their diet behavior being affected by their state of health.

The vitamins that were analyzed hold relevancy to the dialysis population. Some of these micronutrients are fat soluble (e.g. Vitamin A, E) and water soluble (e.g. Vitamin C). The water soluble vitamins are easily depleted during dialysis [37]. The vitamins that had been analyzed are all antioxidants and knowing the intakes of each would provide insight into a patient's antioxidant status.

Average intake of Vitamin C at baseline and week 16 was about 80-110 mg, which happens to be within the recommended amount of 60-100 mg [31]. This may not be enough due to potential reduction in levels during the dialysis process [37], and therefore requires supplementation. Vitamin C is protective for heart disease because of its antioxidant attributes. Even though it may appear that Vitamin C had a tendency to be significant at baseline, it is important to note that the time of this diet recall was taken before supplementation and should not be different at all. Week 16 values had no significant difference between the groups meaning that the unchanged values carried over throughout the study.

Vitamin A is another form of anti-oxidant whose levels did not necessarily change in the 16 weeks. The average intake at baseline was around 7400 mg but there was no significant difference between the two groups. At week 16, even though intake was about 6000 mg, there still was no difference between the groups. One other interesting point is that one of the patients

consumed 16 ounces of liver, rich in vitamin A, which explains the unusually high standard deviation in the baseline TRF group. Based on this study population, it appears as shown in previous studies that excessive intake of vitamin A does not benefit patients since it has a pro-oxidant effect [31].

Some of these vitamins appear to surpass the recommendations, however, no significance may question the validity of their values due to the study's limitations and how they may not match with the values concurrent with a dialysis population. This may also explain, for example, why Vitamin C correlated with micronutrients at certain time points, or why Vitamin A correlated with few nutrients in question. Vitamin A's intakes were unusually high given that overall energy intake was low. Again, it would be difficult to argue this assumption considering these values were not significant.

The final vitamin, E, has been taken into account due to antioxidant capabilities. Both tocopherols and T3 were tracked. Tocopherol intake was around 10 mg at baseline and 6 mg at week 16. There were no significant differences at either time and the data tables also show that there was not any variation based on those t-test evaluations (which also applied to previous nutrient evaluations). Only baseline values have met recommended levels, however, there is not enough justification for the use of tocopherols in the prevention of coronary artery disease [31] according to the AHA. When the study population's T3 intake was analyzed, primarily dietary based at baseline, was marginal since they are between 2-3 mg with no significant difference. At week 16, one of the groups received supplementation in addition to dietary-based T3 and that is why there was a large variation of 2-182 mg, which may have explained the significance between the two groups. It is important to note that tocopherols and T3 share the same transport protein when in circulation in the body [26] for which tocopherols have a higher affinity.

Unfortunately, there was not enough evidence based on average intakes and non-significance to detect a change in the intake of other nutrients due to overlying causes as aforementioned. Also, the intake at week 16 is primarily sourced from supplements for the TRF group. Other notable inquiries in the results were that T3 and dietary cholesterol at baseline had a negative correlation, which means that T3 intake involved a decrease in cholesterol intake. This may be due to T3 rich foods being more prevalent in reference to cholesterol rich foods within the diet. T3 also increased with saturated fat at week 16, meaning that saturated fat-rich foods may also have either contained T3s or were eaten frequently with separate T3-containing foods. One interesting point would be the positive correlation between T3 intake and IL-6 at baseline only. It may be that the patients consuming T3-rich foods have a higher incidence of IL-6. Il-6 being a biochemical marker for inflammation should be decreased after supplementation, however, this did not happen based on no correlation with T3 intake at week 16. It may be easy to rule out T3's ineffectiveness, but the non-significance in baseline values for other nutrients may require more information or testing.

As one can observe here, the study population has less than required energy and protein intake, whereas the micronutrient intake varies. Some are below while certain micronutrients are above recommended levels. This may also explain why the study population's diet was not consistent with heart healthy as compared to similar dialysis studies [31]: fat and carbohydrate intake was not optimal. In addition to the macronutrients, sodium levels were high enough to put the patients in a hypertensive state. Basically, no discernible effect was detected in terms of nutrient intake after supplement treatment. Additionally, it could be due to the quality of analysis based on t-tests or correlations that make no detectable pattern. It would be difficult to identify signs of malnutrition since the week 16 data did not exhibit significant difference

between the two groups. Perhaps it is advisable to disregard the fact that T3 may have played a role in nutrient intake behavior since almost every nutrient average had no significant difference between the groups at the conclusion of the study.

Other notable issues that arose during the data analysis of the food intake were a possibility of inconsistencies with the actual diet recalls. It could be that the diet recalls may contain some inaccuracy since they are solely based on the patients' memory. Portion size may have been exaggerated or that estimation of servings during transcription into the Nutritionist Pro software may not be accurate. This could have had some influence on the patient's diet composition. Moreover, many foods recorded from patients were not found in the Nutritionist Pro's central database and alternatives were used in place of some food items.

The data that was collected on T3 intake through food was pooled from external resources and their accuracy is questionable. In other words, the data analysis run by SPSS that gave non-significant results could have been affected by the diet recall or transcription inaccuracy. This can affect the t-tests and correlations, leading to the conclusion that there was no change in nutrient data detected when T3 supplements were administered. Other notable inquiries would be study limitations. Diet recalls were limited to two times and only at the start and conclusion of the study.

One cannot conclude that T3s are ineffective against stabilizing the complications attributed to ESRD. One observation showed total calories correlated with some nutrients at week zero but less at week 16. This produces mixed results and would not be easy to provide any direct conclusion that T3s had any effect. A future study may want to test with an increased dose of T3 to compare with previous results and how nutritional behavior may react with a larger

dose so that optimal levels can be investigated. That, along with a larger population size, would entail a smaller margin of error.

As one would address the study's limitations, the logistical issues concerning data collection would require the researchers to take a more detailed account. Addressing issues with the effectiveness of a 24-hour diet recall could justify providing patients with their own food diaries so that they can record their meals immediately after consumption. This would ensure more accurate reporting of portion size. To address the discrepancy between energy and nutrient intake, it may be beneficial for patients to receive more extensive nutritional counseling, in order to ensure compliance with recommended intakes.

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ABSTRACT

NUTRITIONAL STATUS IN A COHORT OF HEMODIALYSIS PATIENTS RECEIVING TOCOTRIENOL SUPPLEMENTATION

by

RAJEEV SHAHANI

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Advisor: Dr. Pramod Khosla

Major: Nutrition and Food Science

Degree: Master of Science

Kidney disease is defined by a loss of kidney function over time [1]. It is expressed in terms of glomerular filtration rate at five stages calculated by the MDRD formula [5]. The fifth stage (<15 mL/min/1.73m^2) is designed as end stage renal disease requiring renal replacement therapy in the form of hemodialysis [5]. This requires the use of an artificial kidney (dialyzer) to filter out by-products from the blood for excretion [8]. The issue arises when toxin accumulation due to decreased renal function, dialytic factors such as incompatible dialyzer membranes reacting with blood, and co-morbid conditions such as infection or diabetes mellitus [3]. This increases inflammation within the body, leading to a negative nutrient/energy balance, resulting in a loss of body mass thereby increasing the mortality risk [10]. Tocotrienols (T3), a more potent and underutilized form of Vitamin E, are being administered to attenuate this increase in inflammation via supplementation in addition to food intake. A cohort of hemodialysis patients was analyzed in order to study this issue. The nutritional status was assessed utilizing three measures: examining nutrient intake profiles (while supplementation was taken into consideration), obtaining anthropometric findings of body mass index, and analyzing biochemical profiles of serum albumin and pro-inflammatory markers (i.e. IL-6). This data was

obtained via a randomized, double blind, placebo-controlled study of 81 dialysis patients. At two times over the course of 16 weeks, blood collection for serum albumin and inflammatory markers was analyzed and dietary intake was assessed using 24-hour diet recalls and Nutritionist Pro software. Statistical analyses of paired t-tests and correlation studies revealed no significant differences between the two groups later showing no noticeable effect of T3 supplementation. Reasoning may be multifactorial such as underestimation of food intake, transcription of diet recalls may not be truly representative, or T3 supplements may not have had an effect on the nutritional statuses. It may be beneficial for patients to receive more extensive nutritional counseling as opposed to nutritional supplementation to ensure compliancy with recommended intakes.