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A Pilot Study Of The Use Of The Excebrane® Membrane In The Northern Alberta Renal Program Hemodialysis Population: A Biochemical And Quality Of Life Analysis

By

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DEDICATION

Firstly, thanks be to God in whom all things are done. "Being confident of this, that He who began a good work in you will carry it on to completion until the day of Jesus Christ." (Philippians 1:6). Without God's immense mercy, none of this would have been possible. May it reflect His glory and give Him praise in its reading.

This thesis is dedicated to my wife Joan. You have sustained me in times of sorrow, lifted me in times of darkness, gently reminded me of reality in times of arrogance, and encouraged me when I needed it the most. Thank you for allowing me to do this and by taking on the role of nurturing the family when I was working on this. I could not ask for a better friend, a better partner, nor a better equal to share my life with.

ABSTRACT

Death from cardiovascular disease is the most common form of death in the hemodialysis population, and antioxidant activity may be a factor in the cardiovascular complications of these subjects. The purpose of this study was to compare the performance of the Excebrane® and the F80S membranes in a two arm crossover study. Subjects were randomly assigned to the Excebrane® or F80S dialyzer for three months before being randomly switched to the other dialyzer for another three months. Study parameters included antioxidant activity and standard dialysis bloodwork. Thirty three chronic hemodialysis subjects were enrolled. Dialysis adequacy remained generally stable in all periods of the study, and there appeared to be no effect of either membrane on standard biochemical parameters, antioxidant measures, or lipid profiling. This randomized controlled trial demonstrates that the Excebrane® dialyzer is not more bioactive compared to the F80S membrane, and does not add any benefit as an antioxidant.

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Nullum Gratuitum Prandium.

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LIST OF SYMBOLS & ABBREVIATIONS

3-Nitrotyrosine	3NT
8-Hydroxy 2'-deoxyguanosine	8-OHdG
Alanine Aminotransferases	ALT
Calculated Total Antioxidant Status	cTAS
Cardiovascular Disease	CVD
Confidence Interval	C.I.
C-Reactive Protein	CRP
End Stage Renal Disease	ESRD
Enzyme-Linked Immunosorbent Assay	ELISA
F2 Isoprostanes	F2I
Glutathione Peroxidase	GPX
Hemodialysis	HD
High density Lipoprotein	HDL
Interleukin-6	IL-6
Left Ventricular Hypertrophy	LVH
Low Density Lipoprotein	LDL
Low Density Lipoprotein Malondialdehyde	LDL-MDA
Messenger Ribonucleic Acid	mRNA
Myeloperoxidase	MPO
National Kidney Foundation Dialysis Outcomes Quality Initiative	NKF-DOQI
Oxidized Low Density Lipoprotein	ox-LDL
Parathyroid Hormone Level	PTH
Percent Reduction of Urea	PRU
Phosphorus Level	PO4
Plasma Hydroxyl Radical Producing Activity	OHPA
Polyamid	PA
Polymethylmethacrylate	PMMA
Polymorphonuclear	PBL
Polysulfone	PS
Reactive Oxygen Species	ROS
Recommended Daily Allowance	RDA
Red Blood Cell	RBC
Repeated Measures Analysis of Variance	ANOVA
Serum Creatinine	SCr
Standard Deviation	SD
Superoxide Anion Radical Producing Ability	SOPA
Superoxide Anion Radical Scavenging Ability	SSA

Superoxide Dismutase	SOD
Thiobarbituric acid reactants	TBARS
Total Antioxidant Capacity	TAC
Total Antioxidant Status	TAS
Tumour Necrosis Factor-α	TNF-α
White Blood Cell Count	WBC
β2 Microglobulin	β2M

CHAPTER 1. INTRODUCTION

1.0 Statement of the problem

The worldwide incidence of End Stage Renal Disease (ESRD) continues to increase in all countries. The United States of America and Japan lead all other countries in incidence rate per million (311 and 234 respectively, 1998) (U.S. Renal Data System, 2001). While Canada's ESRD incidence rate per million lags farther behind at 142.4 in 1999, this rate is still an increase of 73% since 1990. This incidence rate places Canada third, followed by New Zealand, the Netherlands, Australia, and Poland. Due to better long-term medical management, the prevalence rate amongst countries has also increased. Japan has the highest prevalence of ESRD at 1,465 followed by the US (approximately 1200), then a mix of several countries including Germany, Italy, Sweden, France, and Canada. Canada's ESRD prevalence rate of 600 is approximately the same as this mix of other countries. This rate is followed by the Netherlands, Czechoslovakia, Australia, and Israel, whose rates all fall between 400 and 600. The prevalence rate experienced by Japan compared to the US is substantially higher, and is felt to be due to longer patient survival, and a difference in background death rates. It is estimated that the ESRD population will double by 2010 (U.S. Renal Data System, 2001). The increase in incident and prevalence subjects raises the

concern that ESRD will consume an ever-increasing portion of health care budgets.

The most common cause of death in the hemodialysis population is cardiovascular death (U.S. Renal Data System, 2001), with the cardiovascular incidence increasing with age. Cardiovascular disease is a multifaceted issue, with one of the proposed causes being oxidative stress (Sies, 1997). Vitamin E (α-tocopherol) has antioxidant properties and a number of studies have examined its role in oxidative stress. As chronic hemodialysis subjects take a multitude of medications, one possible mechanism to deliver antioxidative therapy is to deliver it during the dialysis session.

The Excebrane® membrane series (Terumo Medical Corporation) is a hollow fibre vitamin E-modified cellulose membrane. It is felt that the vitamin E coating may show beneficial effects in terms of cardiovascular complications, biocompatibility and antioxidant activity in the hemodialysis population, while retaining the same clearances as standard cellulose membrane.

1.1 Research Question

The purpose of the Excebrane® trial is to assess the effectiveness of the Clirans EE series membrane (CL*EE18NL, Excebrane®) against the F80S membrane in terms of efficiency, in vivo clearance, biocompatibility,

antioxidant activity, and lipid profiling. The primary objective is to show the antioxidant effect of the Excebrane® membrane compared to the F80S membrane as measured by antioxidant marker activity. The use of cardiovascular outcomes as an endpoint would require the enrollment of a large number of subjects for an extended period, and so antioxidant effect was selected as a surrogate marker of potential cardiovascular events. The secondary objectives include: to compare the biocompatibility of the membrane using c-reactive protein and homocysteine; to compare the β2 microglobulin (β2M) levels at three and six months; to compare the standard hemodialysis bloodwork drawn monthly; and to compare the patient perceived quality of life using the Short Form 36, the Visual Analog Scale, and CHOICE questionnaires.

CHAPTER 2. REVIEW OF THE LITERATURE

To identify background information, a broad search strategy was used. Using OVID, different medical literature systems ([All EBM Reviews – Cochrane Central Register of Controlled Trials, ACP Journal Club, Database of Abstracts of Reviews of Effects, and CCTR], PREMEDLINE, and MEDLINE from 1966 to July 2003 were queried. The following keywords were used in different combinations: cardiovascular disease, hemodialysis, renal dialysis, vitamin E, kidney failure, alpha-tocopherol, and Excebrane®. Using a Snowball sampling methodology (van Meter, 1990), additional publications were identified from review papers and references of the selected papers. In addition, major authors in this topic area were identified and other relevant publications by those authors were searched. In order to find citations that were not identified through the text word and MeSH searches, author searches were done for the following authors: Boaz M, Galli F, Loughrey CM and Ronco C. These authors were included in an author search because they were identified as having written at least one article specifically about antioxidation in hemodialysis subjects, or the Excebrane® membrane in the hemodialysis population. A study was considered relevant if it examined cardiovascular disease in hemodialysis, vitamin E and its role in antioxidation, the role of vitamin E and hemodialysis, and the Excebrane® dialyzer.

The Google, MSN, and Copernic Search engines were used to search

the internet for information about vitamin E, hemodialysis, and/or the Excebrane® membrane. Key hemodialysis websites were identified and searched: United States Renal Data Systems, Canadian Institute for Health Information, and The Nephron Information Center. Key journals were hand searched five years prior to July 2002. The above strategy yielded 71 citations that were relevant to the research question, and are used in this thesis to provide background information.

Of the papers that were relevant to the thesis research question, eight studies specifically dealt with the use of a vitamin E bonded membrane in hemodialysis (Buoncristiani et al., 1997; Galli et al., 1999; Tarng et al., 2000; Bonnefont-Rousselot et al., 2000, Girndt et al., 2000; Dhondt et al., 2000; Shimazu et al., 2001; Mydlik et al., 2001; Mune et al., 1999). The findings from these studies are summarized in Section 2.4. The main factors identified in the literature search were the epidemiology of cardiovascular disease in the hemodialysis population, basic laboratory estimates of antioxidation, the role of vitamin E as an antioxidant, and the potential of the Excebrane® membrane in the hemodialysis population. Therefore, the literature review focused on these factors.

2.1 Cardiovascular Disease in Hemodialysis

Life expectancy in US hemodialysis subjects is clinically significantly shorter than that seen in the general United States population (U.S. Renal Data System, 2003; Anderson & DeTurk 2002). As shown in Table 1, the expectancy difference runs from approximately 30 years less at age 40-44, to 12 years less in the elderly.

Hemodialysis subjects have an age-adjusted mortality risk 3.5 - 4 times of that of the general population (Harnett et al, 1995). Part of this mortality risk is related to reported co-morbid conditions at the time of dialysis initiation. They include congestive heart failure (31.7%), ischemic heart disease (24.8%), myocardial infarction (8.7%), transient ischemic attacks/ cerebrovascular accidents (9.4%), peripheral vascular disease (13.7%), diabetes (primary and/or contributing) (45.3%) and diabetes requiring insulin use (22.5%). This high prevalence of cardiovascular diseases amongst incident dialysis subjects suggests that this disease predates the onset of ESRD. This is understandable given that the case mix of incident dialysis subjects has changed over time. The incident population is older than that of transplant subjects as expected (70-74 years of age at onset of hemodialysis versus 40-44 years of age at time of transplant). These subjects are living longer with their original disease states, and are now surviving long enough to present with End Stage Renal Disease (ESRD).

The older patient population and the co-morbidities translate into cardiovascular events once dialysis is started. As shown in Figure 1, nearly 50% of subjects experience a cardiovascular-related event in the first year of hemodialysis, such that the cardiovascular disease mortality rate is estimated to be 5 to 20 times that of the general population (Ritz & Kock, 1993). Hyperlipidemia as well as co-morbid disease states, such as diabetes, have been well implicated in cardiovascular related events, and cardiovascular related mortality and morbidity.

2.2 Vitamin E and Antioxidation

Oxidative stress is the proposed mechanism of damage in disease states such as cardiovascular disease, diabetes, age-related neurodegenerative diseases, cataracts, respiratory distress syndrome. It is the suspected mechanism in some autoimmune diseases such as rheumatoid arthritis (Sies, 1997; Halliwell, 1996a). Oxidative stress is also thought to be a participant in some of the co-morbid conditions associated with ESRD (Galli, Canestrari, & Buoncristiani, 1999; Halliwell, 1996b; Hasselwander et al., 1999; Huysmans et al, 1998; Sies, 1997; Loughrey et al. 1994a; Toborek et al., 1992; Halliwell, 1996a). These prooxidative co-morbid conditions include left ventricular hypertrophy (LVH), anemia, hyperparathyroidism, volume

overload, hypoalbuminemia, prothrombotic factors, hyperhomocysteinemia, uremia, dialysate composition, and bioincompatible dialyzers.

Lipoprotein modification is a major event associated with oxidative stress and together with reactive oxygen species (ROS) damage to endothelial cells is considered to be the earliest key event in the formation of atheromatous plaque (Loughrey et al., 1994b). Recurrent leukocyte stimulation with complement activation and cytokine secretion leads to chronic inflammation. Eventually, this is felt to cause a defective immune response to specific stimuli. The immune cell dysfunction may also contribute to the noxious effects of ROS and increased production of tumour necrosis factor-α (TNF- α) (Galli & Ronco, 2000). Contact between blood and the dialyzer membrane upregulates proinflammatory cytokine production in a two signal process: transcription of cytokine messenger ribonucleic acid (mRNA) secondary to membrane bioincompatibility, and the likelihood that endotoxin passes from contaminated dialysate to the membrane to cause a second signal. Therefore chronic activation by hemodialysis (HD) membranes leads to low density lipoprotein (LDL) modification, upregulation of surface molecules and chemotactic factors. These mediate adhesion to endothelium and migration of activated phagocytes through the endothelial wall. As well, the attack of ROS on red blood cell (RBC) membrane lipids can influence their lifespan and increase susceptibility to hemolysis (Therond et al., 2000;

Bonnefont-Rousselot et al., 2000; Nakatan, Takemoto & Tsuchida, 2003; Usberti et al., 2002; Hasselwander et al., 1999, Buoncristiani et al., 1997). The decreased lifespan of red blood cells is further potentiated by a decreased production of erythropoietin in subjects with chronic renal failure. Although direct cause-effect relationship has not been established, this repeated activation of immune cells during extracorporeal treatment may be a key aspect in the onset of long-term side effects and increased morbidity and mortality in hemodialysis subjects.

Vitamin E has been proposed as an antioxidant agent. Vitamin E is an essential liposoluable vitamin, with its most important active compound being α-tocopherol. Compared to the other isomers (β, γ, and δ-tocopherol), α-tocopherol is more abundant in food, and has the most potent biologic activity. Vegetable oils such as corn, soybean, wheat germ, and sunflower are the highest sources of tocopherol (Meydani, 1995; Sies, 1997). Twenty to forty percent of α-tocopherol is absorbed by the intestine into the lymph and venous circulation, and the remainder is excreted in feces. The recommended daily allowance (RDA) of vitamin E is ten mg for males, and eight mg for females. As vitamin E can be taken in both natural and synthetic forms, one mg of natural α-tocopherol is equivalent to 1.49 IU of vitamin E, whereas one mg of synthetic α-tocopherol is equivalent to one IU of vitamin E (Meydani, 1995). Specific vitamin E supplements contain 100 IU

or more, whereas the amount of vitamin E in multivitamins is 30 IU or less (Stampfer et al., 1993). Few side effects have been reported with the use of vitamin E (Stephens et al., 1996), even with supra-therapeutic doses of 3200 mg daily.

There is no known specific carrier for α-tocopherol. It is bound to lipoproteins, specifically LDL and high density lipoproteins (HDL) and stored in adipose tissue, liver, and muscle. Plasma concentrations of vitamin E are affected by lipid content of the blood. α-tocopherol is known to inhibit protein kinase C, a key element in cellular proliferation. Smooth muscle cells proliferate in the presence of decreased amounts of α-tocopherol, and this proliferation is thought to be a key factor in atherosclerosis. Although the specific mechanism for this effect on smooth muscle cells is unclear, it is felt that vitamin E inhibits platelet function by decreasing platelet pseudopodia formation, and protects phospholipid membranes from oxidative stress. High dose vitamin E has been associated with inhibiting pro-atherogenic events and may stabilize atherosclerotic plaques. It reduces the expression of selectin and adhesion molecules involved in the endothelial attachment of monocytes to endothelial cells. Supplementing with vitamin E may increase erythrocyte life span and increase the reticulocyte count in genetically transmitted hemoglobinopathies. A deficiency of vitamin E results in

increased hemolysis due to membrane fragility, and signs and symptoms of neuromuscular disease (specifically a lack of coordination and balance).

Vitamin E has been used in various prospective clinical trials. Generally, an inverse relationship between the plasma concentrations of vitamin E and cardiovascular disease (CVD) was found, as summarized in Table 2. It is important to note however, that a beneficial effect of vitamin E supplementation in cardiovascular death has not been found in all studies, demonstrating the need for properly powered, randomized controlled trials.

Rimm et al (Health Professionals Follow-up Study, 1993) studied a large population of US health professionals looking at vitamin E use and coronary heart disease. The study population included dentists, veterinarians, pharmacists, optometrists, osteopathic physicians, or podiatrists. As this trial was an observational cohort study, subjects were not randomized to vitamin E. 51,529 males, ages 40-75, have been followed since 1986. A subset of this study, 39,910 healthy (no cardiovascular or related condition) males who had greater than 800kcal and less than 4200kcal diet, were re-questioned on dietary habits, for a total 139,883 person-years of follow-up. No information could be provided on the specific forms of vitamin E supplementation, rather information was gleaned from participant food surveys. Subjects were followed until January 21, 1990 or death. A multivariate analysis logistic regression analysis was conducted

controlling for age, smoking status, body-mass index, total calories, alcohol consumption, reported hypertension, regular Aspirin® use, physical activity greater than 90 minutes per week, parental history of myocardial infarction before age 60, and profession. Mantel-Haenszel methods were used to derive relative risks, along with the Mantel extension test for linear trends.

Quintiles were chosen using median vitamin E intake, and that median was chosen as the "typical value" for each quintile. Age adjusted and multivariate relative risks of coronary disease in each quintile are displayed in Table 3. Men in the highest quintile group had an improvement in the relative risk or coronary disease after adjusting for age. This improvement remained after multivariate analysis.

It should be noted that the fourth and fifth quartiles are the only ones in all the models that have a consistent statistically significant improvement in relative risks when comparing the 95% confidence intervals. In this study therefore, vitamin E in amount of 25.2 IU/day or greater shows a protective benefit against coronary heart disease over the four years of study.

Stampfer et al (1993) repeated the methodology used by Rimm by using data from the Nurses' Health Study. This observational cohort study began in 1976 with 121,700 registered nurses in the United States. Stampfer studied the association between vitamin E intake and the incidence of major coronary disease events. A subset of 87,245 subjects who were free from

cardiovascular disease and cancer completed dietary questions and were followed for eight years. In similar fashion to Rimm et al, nurses were split into quintiles based on median intake of vitamin E, using the major endpoint of coronary disease (nonfatal myocardial infarction or death due to coronary disease).

As shown in Table 4, quintiles four and five demonstrated a statistically significant decrease in the relative risk of coronary heart disease. It should be noted that the median amounts of vitamin E needed for that change is less than that seen with Rimm et al, which is in keeping with the difference in RDA guidelines for men and women.

Knekt's study in 1994 looked for an association between dietary intake of antioxidants (vitamin E, C and carotene) and mortality from coronary disease using a Finnish cohort with a follow-up from 12 to 16 years. 5,133 adults were taken from a larger cohort of 62,440. These subjects completed dietary histories, and the amount of carotenoids and vitamin E in their diet were calculated from analyses of national foods. The results of the study are summarized in Table 5.

An inverse relationship was noted between dietary vitamin E intake and fatal coronary heart disease, with a statistically significant advantage conferred upon women. When vitamin E intake is broken into tertiles based

on the distribution of the total study population, this relationship is preserved as summarized in Table 6.

The CHAOS study (Mitchinson et al., 1999; Stephens et al., 1996) randomized subjects to placebo or 800 IU daily of vitamin E. After an interim analysis showing adequate levels of α-tocopherol in 546 subjects, subsequent subjects were randomized to 400 IU of vitamin E daily or placebo. 2002 subjects were followed between three to 981 days (median 510 days), and the primary outcome was non-fatal myocardial infarction or a combination of cardiovascular death with non-fatal myocardial infarction. Due to the large geographic area covered by the study, dedicated databases were installed in the coronary care units serving the region's population. Serum α-tocopherol levels were measured routinely during the study. 73.2% of all prescribed medication (α-tocopherol or placebo) was taken by study participants. The results are summarized in table 7.

Higher doses of vitamin E made a statistically and clinically significant difference in the primary endpoint. However, this reduction was primarily due to a reduction in nonfatal myocardial infarctions (77%), and that treatment effect became apparent only after 200 days. There were more cardiovascular deaths in the treatment group as compared to the placebo group. There is no physiological explanation for the increase in

cardiovascular deaths. The authors noted that the majority of the deaths were in the non-compliant treated subjects.

As previously mentioned, the inverse relationship between vitamin E intake and cardiac events is not apparent in all studies, nor is the relationship robust in all positive studies. In the GISSI-Prevenzione trial (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico, 1999) vitamin E use on its own did not lead to a difference between groups. It was only with the combined use of n-3 PUFAs (polyunsaturated fats, 1 gm daily) and vitamin E led to a reduction in the combined outcome of death, nonfatal myocardial infarction, and nonfatal stroke (p=0.03), whereas n-3 PUFA demonstrated a difference on its own.

The Heart Outcomes Prevention Evaluation (HOPE) study (Lonn et al., 2002; Hoogwerf & Young, 2000) randomly allocated subjects to daily treatment with 400 IU vitamin E or placebo and with 10 mg ramipril or placebo. Follow-up was for an average of 4.5 years. The primary study outcome was the composite of myocardial infarction, stroke, or cardiovascular death. Like the GISSI study, it also showed no effect of vitamin E (relative risk = 1.03, 95% CI 0.88-1.21; P = 0.70) on myocardial infarctions, stroke, or cardiovascular death over the 4.6 years of follow-up.

In summary, the evidence for the use of vitamin E to directly reduce cardiac outcomes is in doubt. Positive evidence for vitamin E was only seen

in trials with long follow-up (range 200 days to 16 years), large subject population (at least 2,000 subjects) and required a minimum of vitamin E 400 IU/day. Additionally, the majority of benefit was seen in the cohort studies, whereas only one of the randomized controlled trials was able to show a benefit. The remaining two randomized controlled trials, with a combined total of almost 15,000 patients, were unable to show a statistically significant improvement with the use of vitamin E alone despite follow-up time of at least 3.5 years. This confusion over the efficacy of vitamin E may reflect the type of subjects willing to be involved in clinical trials. Ascertainment bias was not an issue in these positive cohort studies as clinical hard endpoints were used (myocardial infarction, death, surgical procedure). However, cohort designs are weakened by selection bias and that in these studies medication compliance could not be assured. Selection bias can be removed by randomization, and clear medication compliance needs to be assured. Furthermore, cohort studies are limited by possible confounding. Specifically, subjects taking vitamin E may be more health conscious and therefore more likely to practice other disease prevention behaviours (Block et al., 1988). A clear cause-and-effect relationship for the use of vitamin E in cardiac outcomes therefore cannot be demonstrated

2.3 Proof for vitamin E in Hemodialysis

Primary and secondary prevention clinical trials have been completed assessing the usefulness of vitamin E in hemodialysis subjects. In particular, the SPACE (Secondary Prevention with Antioxidants of Cardiovascular disease in Endstage renal disease) trial was a randomized, double blind, placebo controlled, multicenter trial assessing the efficacy of 800 IU/day of vitamin E in subjects with established cardiovascular disease (Boaz et al., 2000). One hundred and ninety six subjects aged 40 to 75 years of age with a history of cardiovascular disease (defined as a history of myocardial infarction, ischemic stroke, angina pectoris, transient cerebral ischemia, or peripheral vascular disease), all dialyzing greater than 12 hours per week for greater than three months were enrolled in this trial. Subjects were randomized to 800 IU vitamin E or placebo daily, and were followed for approximately two years. All subjects had routine pre-dialysis blood pressure monitoring, serum malondialdehyde, intact parathyroid hormone levels, and KT/V (a measure of dialysis adequacy) (Daugirdas, 1993). Fifteen subjects in each group had serum vitamin E levels drawn every six months.

During the two year follow-up, subjects were assessed for the incidence of the composite endpoint (fatal or non-fatal myocardial infarction; ischemic stroke; peripheral vascular disease; and unstable angina). Secondary endpoints included cardiovascular disease and total mortality. The

SPACE study had 80% power to detect a relative risk of less than 0.6 in the occurrence of primary outcome variable over two year follow-up (assuming a 30% event rate over two years).

Subjects receiving vitamin E had a 54% reduction in primary endpoint risk (P=0.014), and the adjusted Cox regression model in smokers showed that survival from the composite CVD endpoint was greater in subjects who received vitamin E treatment (P=0.02). There was a non-statistically significant decrease in the total and fatal myocardial infarctions in vitamin E group. Plasma levels of vitamin E increased by 25% when 800 IU of vitamin E was taken daily (22.04±7.7 vs. 27.8±9.3, p=0.03).

One of the confounders in this study is the use of other antioxidants such as folate, vitamins B_6, B_{12}, and C, all of which are known to either lower homocysteine concentrations or have a synergistic effect with vitamin E. Furthermore, no data are presented on criteria for hypertension, nor was blood pressure data presented for each group. Data was not presented on treatment regimens (i.e. antihypertensives, antihyperlipidemics) and severity of disease between groups. There was no diet analysis, analysis for diabetic sub-groups, nor were there any recommendations on what subject's diets were to contain. Despite these shortcomings, this study raises the possibility that use of high dose vitamin E may play a role in preventing secondary complications in subjects with established cardiovascular disease.

The second study in this area was done by Islam et al. (2000). In this study, 16 hemodialysis subjects and 17 peritoneal dialysis subjects were given 12 weeks of 800 IU/day of α-tocopherol supplementation. Seventeen control subjects were matched for age, gender, race, and body mass index. Subjects were excluded if they had clinical evidence of cardiovascular disease, were a current smoker, were presently using vitamin supplementation or fish oil, hyperlipidemic drugs, prednisone, anticoagulant therapy, thyroxine, birth control pills, or were drinking more than 1 oz per day of alcohol. Hemodialysis subjects were dialyzed on high flux polysulfone membranes. Laboratory parameters at baseline and study completion consisted of plasma fatty acids, LDL isolation, lipoproteins, plasma and LDL- α-tocopherol levels. Not all analyses were performed on all subjects due to insufficient sampling.

Control subjects had a significantly lower plasma triglycerides and higher HDL-cholesterol levels at the beginning of the study; otherwise the groups were well matched. All three groups had a significant increase in plasma and LDL- α-tocopherol levels as shown in Table 8.

Noted in this study was a prolongation in the conjugated diene lag in all groups, and a lipid peroxide lag in the control and peritoneal dialysis groups. The lag phase corresponds inversely with the severity of clinical atherosclerosis; therefore, an increase in lag could prove beneficial.

Finally, a small sub-study from Italy looked at supplementation with vitamin E (Galli et al., 2001). Seven chronic hemodialysis subjects with low levels of vitamin E were given 800 mg/day over three weeks. No within-group changes were noted in any parameters (HDL, LDL, total cholesterol, triglycerides, or oxidation parameters [thiols, thiobarbituric acid reactants , nitric oxide production]) other than a statistically significant increase in plasma and lipid levels of vitamin E (plasma 15.9±3.64 vs. 34.3±5.9, $p < 0.001$; lipid 10±6.8 vs. 18.8±6.3, $p < 0.01$).

In summary, supplementation with 800 IU/day of vitamin E causes a statistically significant increase in plasma and lipid vitamin E levels in hemodialysis and peritoneal dialysis subjects. In the SPACE study, a beneficial effect was also seen in cardiovascular endpoints over two years. However, this effect seen in hemodialysis subjects is not consistent throughout all trials with relatively healthy volunteers (Mitchinson et al., 1999; Stephens et al., 1996; Meagher, 2003; Hoogwerf et al., 2000). While the SPACE study was the largest study and was well-designed, the role of vitamin E in hemodialysis subjects is not clear. This confused picture may be because subjects with high levels of oxidant stress or depletion of natural antioxidant defence systems may be the most likely to benefit from antioxidant therapy. However, the potential role for vitamin E exists and this study attempted to elucidate some of that role.

2.4 Excebrane® Dialysis Membrane

The Excebrane® membrane series is a hollow fiber vitamin E-modified cellulose membrane. A block polymer composed of hydrophilic polymer and a fluorocarbon resin is chemically fixed to the hydroxyl groups on the cellulose surface. The fluorocarbon resin constitutes a hydrophobic support for the binding of oleic alcohols, which hydrophobically bind vitamin E. The vitamin E coating is therefore on the blood-exposed surface. Buoncristiani et al (1997) carried out one of the original studies using the Excebrane® membrane. This pilot study used seven chronic hemodialysis subjects in an *in vivo* crossover design. Subjects dialyzed using a conventional dialyzer for five weeks, then crossed over to the Excebrane® membrane for five weeks. Blood was sampled at various time points (0, 15, 60 and 240 minutes) from both the arterial and venous ports at the end of each time period looking for total polymorphonuclear count, complement (C3a) activation, adhesion molecules (CD11b) representing neutrophil activation, and plasma myeloperoxidase (MPO) levels. High MPO levels are known to induce LDL oxidation, and are thought to be an early cause in the development of atherosclerosis. At the 15 minute time point, total reduction of neutrophils was ameliorated with the Excebrane® membrane compared to baseline and conventional dialysis (55.2± 7.2% of baseline vs. 22.5±9.8%, $p < 0.05$). C3a increases were significantly lower in the Excebrane® group at the 15

minute time point onward, along with the CD11b increases. As expected, venous samples had increased expression of C3a and CD11b confirming that dialysis membrane contact is a pro-inflammatory state regardless of dialyzer. However, the Excebrane® membrane had significantly less expression of C3a and CD11b compared to the control dialyzer, indicating that the total inflammatory state was reduced. An *ex vivo* study was then undertaken using healthy human blood from six individuals (three male, three female) in a closed dialysis circuit. Blood was sampled at various time points (0, 15, and 60 minutes) looking for the same markers (C3a, CD11b, and plasma MPO). C3a levels increased within 15 minutes of the start of the study, and again the Excebrane® dialyzer had significantly lower expression (453.1±194.7% vs. 892.9±327% control, $p < 0.05$) compared to control, along with lower expression of CD11b (92.9±25.8% vs. 141.2±20.6% control, $p < 0.05$) and MPO (104.9±17.4% vs. 217.4±54.8% control, $p < 0.05$). This effect was maximally different at the 60 minute time period. This study therefore demonstrates that the Excebrane® membrane has less neutropenia, lower complement C3a levels, fewer adhesion molecules on neutrophils (CD11b), and less oxidation as measured by MPO.

Mune et al (1999) carried out a larger study looking at oxidation byproducts. Fifty age and sex matched chronic hemodialysis subjects were randomized to receive dialysis with the Excebrane® membrane or the control

membrane (cellulose membrane). They were then followed every six months for the next two years. The outcome parameters were low density lipoprotein malondialdehyde (LDL-MDA, a byproduct of lipid oxidation), oxidized low density lipoprotein (ox-LDL, thought to contribute to atherosclerosis), and progression of aortic calcification as measured by computed tomography scan. Treatment with the Excebrane® membrane significantly decreased post-dialysis levels of LDL-MDA at 18 and 24 months. Reduction of ox-LDL was more modest with a significant difference seen between groups post-dialysis at six and 18 months, but not between pre and post dialysis results. Finally, there was a reduction in the percent increase in aortic calcification (7.5% Excebrane® vs. 14% control, $p < 0.02$) at 24 months. While modest, this study showed that the Excebrane® dialyzer showed a benefit in lipid oxidation and aortic calcification in a small group of Japanese subjects over a two year period.

Confirmation of the bioactivity of the Excebrane® membrane continued with Girndt (2000), looking at proinflammatory cytokine induction (interleukin-6 and interleukin-10) along with proliferation of polymorphonuclear (PBL) cells. Hemodialysis is known to cause of high production of IL-6, and soluble vitamin E inhibits production of IL-6 in a dose dependent manner. IL-6 levels were determined using a single cell cytokine detection method. With this method, cells capable of cytokine production are

selectively sequestered whereas the remaining cells in circulation do not produce proinflammatory cytokines. Therefore, an inverse relationship is established between IL-6 counts and bioincompatibility. Twenty one stable hemodialysis subjects were randomized in a crossover fashion to either the Excebrane® membrane or the polyamid (PA) dialyzer for four weeks. Subjects then crossed over to the other arm of the study and were followed for a further four weeks. Exclusion criteria included acute or chronic inflammatory state, infection, use of immunosuppressive drugs, or evidence of malignancy. Dialysis dose was followed with a double-pool KT/V, a measure of delivered dialysis dose. There were no differences in the baseline characteristics of either group, or dialysis dose delivered. Both groups had a statistically significant increase in PBL count compared to baseline ($p < 0.05$), with no significant difference between the groups, demonstrating that extracorporeal treatment results in lymphocyte activation. Predialysis levels of IL-6 did not differ between groups; however, use of the PA membrane resulted in a statistically significant decrease in the secretion of IL-6 in the first twenty minutes after initiating dialysis, demonstrating that the PA membrane is more bioincompatible compared to the Excebrane® membrane. There was no difference in IL-10 counts, demonstrating that the Excebrane® membrane does not inhibit proinflammatory cytokine production better than the PA membrane. In summary this study indicated that there may be less

proinflammatory cytokine production with the PA membrane, however this appeared to be true with IL-6 alone.

The potential role of the Excebrane® membrane on oxidative stress was further elucidated by Tarng et al (2000). 8-Hydroxy 2'-deoxyguanosine (8-OHdG) is one of the most abundant oxidative DNA products, and has been established as a novel marker for assessing oxidative damage in ROS-mediated diseases. Previously unpublished data from this group showed that 8-OHdG levels were highest in hemodialysis subjects, followed by subjects with advanced renal failure who had not started dialysis, followed by healthy controls. Furthermore, this group established that 8-OHdG is higher in subjects dialyzed with cellulose membrane (least biocompatible) followed by synthetic membranes.

This study was a subset of a larger prospective cross-sectional study in four dialysis facilities in Taipei. Of the 353 subjects in that study, 110 were chosen for this study. Stable hemodialysis subjects without evidence of inflammatory, malignant or infectious disease were chosen to dialyze on an Excebrane®, high flux PMMA (polymethylmethacrylate), high flux polysulfone (PS), or cellulose membrane for eight weeks. The PMMA, and PS membranes are considered biocompatible, and the vitamin E bonding of the Excebrane® membrane placed it in the biocompatible category. 8-OHdG, plasma ascorbate and α-tocopherol levels were measured at zero, four, and eight

weeks. The demographics in the four groups were similar, but the subjects on the cellulose membrane had higher levels of plasma α-tocopherol and 8-OHdG, and lower lipid-adjusted α-tocopherol levels. When calculating independent predictors of 8-OHdG content, the use of the Excebrane®, PMMA, and PS membrane membranes was statistically significant. Further stepwise multivariate analysis determined that this difference could not be accounted for by differences in baseline laboratory parameters. Change from the cellulose membrane to the Excebrane® membrane resulted in a 41% reduction ($p < 0.01$) in 8-OHdG, and a 42% increase in plasma α-tocopherol ($p < 0.01$). Conversely, switching from the Excebrane® membrane to the cellulose membrane caused a 66% increase ($p < 0.01$) in 8-OHdG, and a 41% decrease in plasma α-tocopherol ($p < 0.01$). The effect on 8-OHdG was larger than that seen with the PMMA and somewhat equivalent to that seen with the PS membranes. The effect on in plasma α-tocopherol was larger than that seen with either membrane. This study appears to demonstrates that use of the Excebrane® membrane over 8 weeks results in a reduction of oxidative products in similar fashion to more biocompatible membranes, and an increase in plasma α-tocopherol greater than that seen with more biocompatible membranes.

At the same time, a group in France was studying the use of the Excebrane® membrane in an open label format (Bonnefont-Rousselot et al.,

2000). Inclusion criteria for this study included: absence of diabetes, malignancy, hepatitis or HIV; no supplementation with vitamin A, E, ß-carotene, or selenium; and no transfusions within 2 months prior to study start. Laboratory measurements included plasma triglycerides, phospholipid and cholesterol, total lipids concentration, oxidative stress (plasma TBARS [thiobarbituric acid reactants], α-tocopherol, vitamin A, ß-carotene, selenium and others), oxidative status (total antioxidant status (TAS), and calculated TAS (cTAS), and copper induced oxidizability of plasma LDLs and HDLs.

Twelve stable hemodialysis subjects were chosen for this study, with differing baseline dialysis membranes. Subjects were dialyzed for three months on the Excebrane® membrane and bloodwork was taken pre- and post-dialysis at the beginning and end of the study. Subjects had elevated TBAR concentrations, confirming that hemodialysis subjects exist in a pro-oxidative environment. There was a significant increase in plasma α-tocopherol in pre- and post-dialysis samples (10% predialysis, $p < 0.01$; 15% increase postdialysis, $p < 0.05$), and remained significant after adjusting for plasma cholesterol (15% increase in vitamin E:cholesterol ratio pre-dialysis, $p < 0.05$; 21% increase post-dialysis, $p < 0.05$). Furthermore there was an increase in the postdialysis ß-carotene levels in both the unadjusted and lipid adjusted samples (26% increase unadjusted, $p < 0.05$; 22% increase lipid adjusted, $p < 0.05$). The authors noted that the increase in α-tocopherol

could not be explained by the Excebrane® membrane. Previous *in vitro* studies have confirmed that transfer of α-tocopherol to the blood compartment does not take place with the Excebrane® membrane. Rather, it is felt that the constitutive pool of α-tocopherol is spared by the engagement of oxygen free radicals *in situ* by the α-tocopherol on the membrane. The authors also noted a lag phase preceding conjugated diene formation in HDL ($p < 0.05$), favouring a lower oxidizability in HDLs as opposed to no change in LDL oxidizability. These authors previously noted that α-tocopherol enrichment of HDL increases their resistance to oxidation (Bonnefont-Rousselot et al., 1999). In summary, these data show that hemodialysis subjects have an oxidative stress status. It also shows that the use of the Excebrane® membrane appears to increase plasma α-tocopherol, reduces the TAS levels both before and during dialysis, and increases the HDL lag phase resulting in a lower oxidative situation for subjects.

Hemodialysis-induced leukopenia is felt to be part of the bioincompatibility picture seen in subjects clinically. In similar fashion to the work done by Buoncristiani (1997), Dhondt et al (2000) looked at CD11b, CD11c, and CD45 expression on granulocytes and CD14 expression on monocytes. Ten stable hemodialysis subjects using a low-flux polysulfone dialyzer were randomized to one week of dialysis with either the Excebrane® membrane followed by the PS membrane, or the PS membrane for one week

followed by the Excebrane® membrane. Comparative analysis was done on the bloodwork taken from the third session, at 0, 15, 60 and 180 minutes post dialysis. All subjects had a significantly reduced monocytes and granulocyte counts at 15 minutes postdialysis ($p < 0.01$, PS and Excebrane® membrane), which returned to basal levels by 60 minutes. The effect was more pronounced with the Excebrane® membrane ($p < 0.01$). There was an increase of CD11b expression with the Excebrane® membrane starting at 15 minutes which remained until 60 minutes ($p < 0.01$ 15 minutes, $p < 0.05$ 60 minutes), and expression of CD11b was greater with the Excebrane® membrane as opposed to the PS membrane ($p < 0.01$ 15 minutes, $p < 0.05$ 60 minutes). Increases also held true for CD11c, and CD45, however there was no statistically significant difference between the Excebrane® and PS membrane for CD11c. After stimulation with PMA in an *in vitro* environment, CD11c, and CD45 response was blunted with the Excebrane® membrane, and the CD14 response was blunted with both membranes, with the blunting more pronounced with the Excebrane® membrane as opposed to the PS membrane. In summary, the Excebrane® membrane was inferior to the PS membrane when looking at biocompatibility parameters.

Another measure of antioxidant activity is superoxide anion radical producing ability (SOPA), plasma hydroxyl radical producing activity (OHPA), and superoxide anion radical scavenging ability (SSA). Shimazu et al (2001)

took 11 stable hemodialysis subjects and randomized them to the Excebrane® membrane, or a conventional membrane for a period of six months. It is worth noting that the conventional membrane is not specified. Blood was taken at zero, one, two, three, and six months. By six months post-randomization, subjects on the Excebrane® membrane experienced a rise in SOPA (2.1±.86 vs. 3.98±1.2 μmol/L/5x10^6 cells, $p < 0.05$) over time approaching normal values, a rise in OHPA (460±27 conventional vs. 580±89 μmol/L Excebrane®, $p < 0.05$) approaching normal values, and a rise in MDA and oxidized LDL. However even though this study notes that subjects were treated for six months, the change in MDA and LDL did not become significant until nine months. This study shows that treatment with the Excebrane® membrane improved the oxidative status in hemodialysis subjects over time based on SOPA, OHPA, MDA, and oxidized LDL.

Finally, other antioxidant parameters were looked at in a small randomized three arm study (conventional dialyzer, conventional dialyzer plus 400 mg vitamin E daily, and Excebrane® membrane). Each arm was three weeks in length, and laboratory parameters taken at the end of each parameter included superoxide dismutase (SOD), erythrocyte antioxidant enzymes, glutathione peroxidase (GPX), total antioxidant capacity (TAC), MDA, vitamins A, E, and C. Eight stable hemodialysis subjects, receiving adequate hemodialysis (KT/V 1.55±0.2) were enrolled in this trial.

Use of oral vitamin E did not change plasma concentrations of TAC or MDA but did cause an increase in serum vitamin E levels ($p < 0.05$). Use of the Excebrane® membrane increased plasma TAC 20% from the beginning of the study period to the end, and also resulted in a 30% drop in MDA (TAC: 1.48±0.14 vs. 1.78±0.22, $p < 0.05$; MDA: 1.37±0.20 vs. 0.96±0.10, $p < 0.05$), but did not change serum vitamin E levels as expected. This study shows that use of the Excebrane® membrane results in a change in the antioxidative state of subjects, with a resultant increase in TAC, and a decrease in MDA.

As shown in Table 9, the identified studies using the Excebrane® membrane in chronic hemodialysis patients demonstrate a reduction in pro-inflammatory cytokines, and a reduction in oxidative stress markers. In contrast to Bonnefont-Rousselot et al. (2000) and Mydilik (2000), Mune et al. (1999) were unable to show an increase in vitamin E levels with the use of the Excebrane® membrane.

Table 1: Comparison of the life expectancy in two United States populations: general population and chronic hemodialysis population

Age Range	General Population (United States)	Hemodialysis population (United States)
40-44	38.8	7.4
50-54	29.8	5.3
60-64	21.5	3.9
70-74	14.3	2.8

Figure 1: Cause of death in two United States populations: general population and chronic hemodialysis population

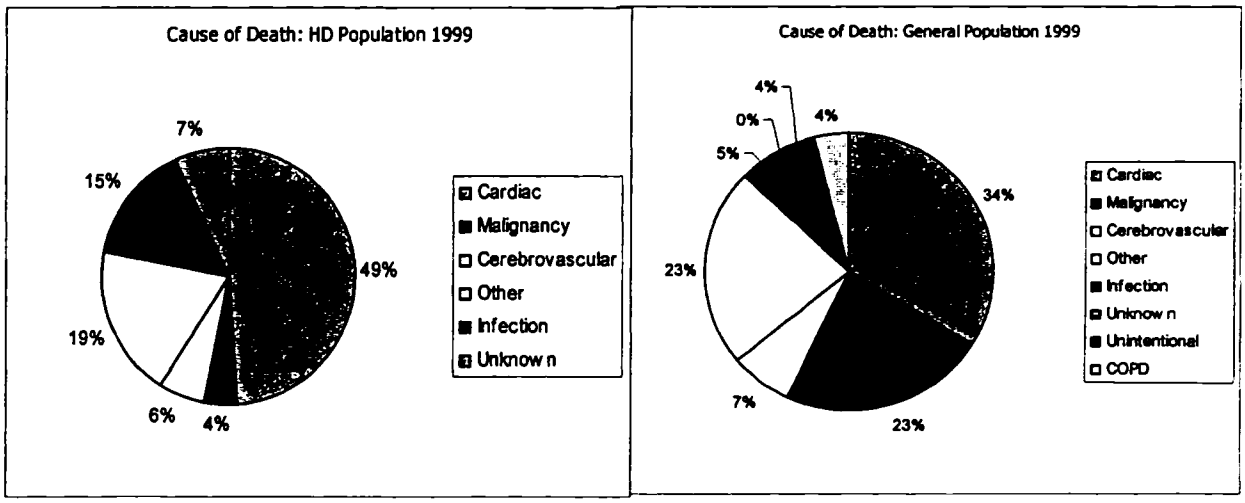

Table 2. Summary findings from cited studies of vitamin E usage in primary or secondary prevention of cardiac disease

Author, study sample	Study Type	Age	Sex	Endpoints	Results	Comment
Rimm, 1993. Vitamin E consumption and the risk of CHD in men (US) n=39,910	Prospective cohort, Health Professionals Follow-up Study	Mean age 53.6 years	100% male	Death, nonfatal MI[a], CABG[b], PTCA[c]	Multivariate vitamin E quintile (6.4, 8.5, 11.2, 25.2, 419 IU/day) risk: 1.0, 0.90, 0.82, 0.77*, 0.64*	4 year follow-up, No effect of carotene, Vitamin C
Stampfer, 1993. Vitamin E consumption and the risk of CD in women. (US) n=87,245	Prospective cohort, Nurses Health Study	Mean age 50.6 years	100% female	Death due to coronary disease, nonfatal MI	Age/Smoking adjusted vitamin E quintile (2.8, 4.2, 5.9, 17, 208 IU/day) risk: 1.0, 1.0, 1.15, 0.74†, 0.66†	8 year follow-up, increased effect seen with vitamin E supplementation as opposed to multivitamins.
Knekt, 1994. Antioxidant vitamin intake and coronary mortality in a longitudinal population study (Finland) n=5,133	Prospective cohort	Age range 3-69 years.	54% male	Death due to coronary disease	Age adjusted vitamin E intake in women 6.65 mg (alive) vs. 5.98 mg (dead), p=0.07	12-16 year follow-up, no difference for males

Stephens, 1996 Randomized controlled trial of vitamin E in subjects with coronary disease: CHAOS study. (UK) n=2,002	Double-blind, randomized controlled trial	Mean age 61.8 years	84% male	Nonfatal MI , death due to cardiovascular disease and nonfatal MI	Relative risk[d] 0.53 for vitamin E group for primary endpoint[‡]. Reduced rates of major cardiovascular events (Kaplan-Meier, log rank 0.015), nonfatal MI (p=0.0001)	800 IU vitamin E, 400 IU vitamin E, placebo. Median follow-up 510 days. Slight increase in cardiovascular deaths, and total deaths.
Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico, 1999 (GISSI-Prevenzione Trial) (Italy) n=11,324	Randomized controlled trial in MI survivors	51% less than or equal to 60 years	85% males	Combined endpoint (death, non-fatal myocardial infarction, and stroke)	No reduction in endpoints	Follow-up 3.5 years. n-3 polyunsaturated fatty acids, 300 mg vitamin E, control.

Lonn 2002 Effects of vitamin E on cardiovascular and microvascular outcomes in high-risk subjects with diabetes: results of the HOPE study and MICRO-HOPE substudy (Canada) n=3,654	Randomized controlled trial in diabetes. Substudy of HOPE trial	85% males	Combined endpoint (myocardial infarction, stroke, or CV[e] death.)	Neutral effect in endpoints.	Follow-up 4.5 years. Ramipril 10 mg or 400 IU vitamin E

a: myocardial infarction
b: coronary artery bypass grafting
c: percutaneous transluminal coronary angioplasty
d: Relative Risk
e: cardiovascular
*: p=0.003 for trend, Mantel Extension test for linear trends.
†: p= 0.001 for trend, Mantel Extension test for linear trends.
‡: p=0.005, Cox model

Table 3: Relative risk of coronary disease in men in the Health Professionals Follow-up Study (based on Rimm et al, 1993)

Variable	Quintile				
	1	2	3	4	5
Vitamin E intake (IU/day)	6.4	8.5	11.2	25.2	419
Age adjusted analysis[a] (95% CI)	1.0	0.88 (0.7-1.1)	0.77 (0.61-0.98)	0.74 (0.59-0.93)	0.59 0.47-0.75)
Multivariate analysis[b] (95% CI)	1.0	0.90 (0.71-1.14)	0.82 (0.64-1.07)	0.77 (0.60-0.98)	0.64 (0.49-0.83)
Multivariate analysis with antioxidants[c] (95% CI)	1.0	0.89 (0.7-1.14)	0.81 (0.62-1.05)	0.71 (0.54-0.92)	0.60 (0.44-0.81)

a: p=0.001 Test for trend
b: p=0.003 Test for trend
c: p=0.01 Test for trend

Table 4: Relative risk of coronary disease in women in the Nurses' Health Study (based on Stampfer et al, 1993)

Variable	Quintile				
	1	2	3	4	5
Vitamin E intake (IU/day)	2.8	4.2	5.9	17	208
Age adjusted relative risk[a] (95% CI)	1.0	0.90 (0.7-1.16)	1.00 (0.78-1.27)	0.68 (0.52-0.89)	0.59 (0.45-0.78)
Smoking and age adjusted relative risk[b] (95% CI)	1.0	1.00 (0.78-1.28)	1.15 (0.90-1.48)	0.74 (0.57-0.98)	0.66 (0.50-0.87)

a: p less than 0.0001 Test for trend
b: p less than 0.0001 Test for trend

Table 5: Relationship between dietary intake of vitamin E and coronary heart disease (based on Knekt, 1994)

Variable	Death Secondary to CHD	Alive	P Value
Males (n)	186	2,264	
Vitamin E (mg)	8.23	8.57	0.23
Females (n)	58	2,371	
Vitamin E (mg)	5.98	6.65	0.07

Table 6: Relative risk of coronary heart disease in Finnish males and females (based on Knekt, 1994)

Variable	Males			Females		
	1	2	3	1	2	3
Vitamin E intake (mg/day)	≤ 6.8	6.9-8.9	>8.9	≤ 5.3	5.4-7.1	>7.1
Age adjusted relative risk[a]	1.0	0.97	0.68	1.0	0.73	0.35
(95% CI)		(0.67-1.4)	(0.42-1.11)		(0.38-1.39)	(0.14-0.88)

a: p=0.01 for males, p < 0.01 for females

Table 7: Relative risk of a major cardiac event in subjects randomized to α-tocopherol or placebo (based on Stephens et al, 1996)

Variable	α-tocopherol n=1035	Placebo n=967
Age	61.8 ± 9.3	61.8 ± 8.9
α-tocopherol level (umol/L)	400mg 800mg	
	51.1 64.5	32.4
Relative risk of cardiovascular event [a]	0.53	1.00
(95% CI)	(0.34-0.83)	

a: p=0.005

Table 8: Vitamin E levels in hemodialysis, peritoneal dialysis and control patients taking 800 IU/day of vitamin E (based on Islam et al, 2000)

		Control (n=15)	Hemodialysis (n=15)	Peritoneal Dialysis (n=15)
Plasma vitamin E	Week 0	3.1±1.1	2.9±0.9	3.6±1.1
	Week 12	7.6±2.7*	7.3±2.9**	11.3±9.3**
LDL vitamin E	Week 0	21.4±10.1	17.9±11.2	21.8±10.5
	Week 12	41.6±22.4*	34.7±24.1*	51.2±35.6**

*:$p < 0.003$ week 0 vs. week 12

**:$p < 0.0001$ week 0 vs. week 12

Table 9. Summary findings from cited studies of the Excebrane® membranes in the hemodialysis population

Author, study sample	Study Type	Age	Sex	Endpoints	Results	Comment
Buoncristiani, 1997 Oxidative damage during hemodialysis using a vitamin-E-modified dialysis membrane N=7 and 6	*In vivo* and *ex vivo* biomarker study in chronic hemodialysis and healthy subjects.	Mean age 60 years, and 39 years	29% male, and 50% male	Total PMN[a], C3a, CD11b, MPO[b]	Reduction in pro-inflammatory markers throughout with the Excebrane® membrane	5 week treatment
Mune 1999 Effect of vitamin E of lipid metabolism and atherosclerosis in ESRD subjects n=50	Randomized controlled trial of the Excebrane® membrane in chronic hemodialysis subjects	Mean age 57 years	44% male	LDL-MDA[c], ox-LDL[d], aortic calcification (ACI)	Significant reduction in LDL-MDA ($p<0.01$), ox-LDL ($p<0.05$), and ACI($p<0.02$)	2 year treatment, no change in vitamin E levels, or lipid concentrations.
Girndt, 2000. Prospective crossover trial of the influence of vitamin E-coated dialyzer membranes on T-cell activation and cytokine induction n=21	Prospective crossover, randomized controlled trial in stable hemodialysis subjects.	Mean age 66 years	unk	PBL[e] proliferation, IL-6, IL-10	Significant increase in PBL in both groups ($p<0.05$), decreased IL-6 ($p<0.05$)	4 week treatment on each arm (8 weeks total), no change in IL-10

Tarng, 2000 Effect of vitamin E-bonded membrane on the 8-hydroxy 2'-deoxyguanosine level in leukocyte DNA of hemodialysis subjects n=110	Randomized controlled crossover trial in stable hemodialysis subjects- (subset of prospective cross-sectional trial)	Mean age 60 years.	40% male	8-OHdG[f] levels	41% reduction ($p<0.01$) in 8-OHdG, and a 42% increase in plasma α-tocopherol ($p<0.01$) with Excebrane® membrane. Similar results in 8-OHdG with PMMA and PS membranes.	8 week treatment
Bonnefont-Rousselot, 2000 Blood oxidative stress and lipoprotein oxidizability in haemodialysis subjects: effect of the use of a vitamin E-coated (Excebrane®) dialysis membrane n=12	Open label trial in stable hemodialysis subjects	Median age 50 years	66% male	TAS[g], cTAS[h], vitamin E, lipids, ß-carotene	Significant drop in pre/post dialysis TAS, cTAS in both membranes ($p < 0.02$), increase in pre/post dialysis vitamin E, vitamin E/cholesterol ratio, lipid-adjusted ß-carotene in Excebrane® group, higher ß-carotene, reduced HDL copper oxidizability (both)	3 month treatment, no change in LDL copper induced oxidizability (both groups)

Dhondt, 2000 Vitamin E-bonded cellulose membrane and hemodialysis bioincompatibility N=10	Randomized crossover study	Unk	Unk	Granulocytes, monocytes, CD11b, CD11c, CD45, CD14	Increase in CD11b ($p<0.05$), CD11c ($p<0.05$), CD45 ($p<0.01$), and decrease in CD14($p<0.05$) in Excebrane® membrane	1 week treatment, no washout period.
Shimazu 2001 Effects of a vitamin E-modified dialysis membrane on neutrophil superoxide anion radical production N=11	Randomized controlled trial	Mean age 47 years	55% male	MDA, ox-LDL, SOPA[i], OHPA[j], SSA[k]	Increase in SOPA ($p<0.05$), no change in SSA, increase in OHPA ($p<0.05$), decrease in MDA, ox-LDL. Decrease in SSA ($p<0.05$) with conventional membrane	6 month study

Mydlik 2001 A modified dialyzer with vitamin E and antioxidant defence parameters N=8	3 way randomized, crossover trial	Mean age 54 years	38% male	erythrocyte antioxidant enzymes, SOD and GPX, TAC, MDA, vitamins A, E, and C.	Increase of serum vitamin E (22%, $P<0.05$) with oral vitamin E, increase of TAC (20%, $p<0.05$), decrease of MDA (30%, $p<0.05$) in Excebrane® membrane	9 weeks in total (3 weeks each phase)

a: polymorphonuclear
b: plasma myeloperoxidase
c: low density lipoprotein malondialdehyde
d: oxidized low density lipoprotein
e: peripheral blood lymphocytes
f: 8-Hydroxy 2'-deoxyguanosine
g: total antioxidant status
h: calculated TAS
i: superoxide anion radical producing ability
j: hydroxyl radical producing activity
k: superoxide anion radical scavenging ability
l: superoxide dismutase
m: glutathione peroxidase

CHAPTER 3. METHODOLOGY

3.0 Study Design

The purpose of the Excebrane® trial was to assess the effectiveness of the Clirans EE series membrane (CL*EE18NL) compared to the F80S membrane in terms of efficiency, *in vivo* clearance, biocompatibility, antioxidant activity, and lipid profiling. This trial was a single centre, single-blind, open label, randomized, two way cross-over study. Two groups of chronic hemodialysis subjects were compared: dialysis with the Excebrane® membrane for three months followed by the F80S membrane for three months, or, dialysis with the F80S membrane for three months followed by the Excebrane® membrane for three months. A crossover design was chosen as crossover trials produce *within* participant comparisons, whereas parallel designs produce *between* participant comparisons. As each participant acts as his or her own control in crossover trials, they can produce statistically and clinically valid results with fewer participants than would be required with a parallel design (Jadad et al., 1996; Hazard, 2001). A crossover design should only be used under the following conditions:

- Chronic, incurable diseases (so that subjects who are cured by one or more of the interventions will not be eligible to enter subsequent periods of a crossover trial),
- Interventions should have rapid onset and short duration (as it minimizes the risk of drop out within each period and helps to keep the number of participants stable across periods), and

- The condition (or disease) is stable (if the disease is stable, the circumstances at the beginning of each period are more likely to be the same than if the disease is not stable).

This study design has been used in hemodialysis studies previously (Girndt et al., 2000; Mydlik, 2001).

3.1 Study Subjects

The source population included all stable chronic hemodialysis subjects aged 18 and older who were on hemodialysis between 20 June 2001 and 15 January 2002. The eligibility of each patient was determined at a pre-study screening visit, within two months prior to starting the study. The study was explained to each patient in detail. Subjects were informed about the study, had any questions answered, and had an information sheet to take away with them. Subjects were assured that they could withdraw from the study at any time for any reason and receive alternative conventional therapy as indicated, without prejudice. The medical records of all subjects in the Edmonton area who were receiving a minimum of 12 hours of hemodialysis weekly for a minimum of three months (n=300) were reviewed to identify subjects for this trial and to determine risk factors for hyperlipidemia, access function, and co-morbid conditions.

Inclusion Criteria

The inclusion criteria were:

- stable hemodialysis subjects, with the ability to dialyze using a steam sterilized dialyzer,
- subjects willing and able to participate in the full course of the study and from whom informed consent was obtained, and
- stable arterio-venous (AV) access as defined by no surgery in the past three months.

Exclusion Criteria

The exclusion criteria were:

- subjects with a history of alcohol or drug abuse, or signs of alcohol-induced organ damage,
- mental dysfunction or other factors limiting the ability to cooperate fully with the study,
- presently receiving immunosuppressive therapy,
- a hematocrit (Hct) less than 30 g/L or hemoglobin (Hgb) less than 100 g/L,
- known hypersensitivity to study dialyzer,
- presently receiving any experimental medication/device, including novel ethryocytosis stimulating protein (NESP),
- supplemental vitamin E use,
- known antioxidant use,
- inability to communicate in English.

3.2 Study Data

All study data were collected using an Excel spreadsheet (Microsoft Office 2000). The dataset supplied for this study was completely de-identified. All specific information regarding names, addresses, phone numbers, health information numbers and treating institutions were removed from the data prior to creating the dataset used in this study. Efficacy evaluations included the following parameters:

Medical History

This included weight; height; age; sex; the etiology of underlying renal disease leading to dialysis; the date of first dialysis; total duration and type of dialysis; a review of all prescription medications taken within the last seven days; and a review of monthly dialysis bloodwork in the two months preceding randomization.

Vascular Access Function and Survival

Vascular access function and survival was assessed at baseline, three, and six months after study entry. Vascular access function was monitored as suggested by the National Kidney Foundation Dialysis Outcomes Quality Initiative (NKF-DOQI) Clinical Practice Guidelines 7 (NKF-DOQI, 2004). These tests included access blood flows, recirculation studies (using transonic system), and/or dynamic venous pressure tests were performed monthly to ensure that there was no possible venous or arterial obstruction/stenosis. The results of these screening tests throughout the study were addressed

according to the clinical practice guidelines. Vascular access loss was defined as surgical revision of the current access, or the inability to use the current access for any further hemodialysis regardless of any possible revisions.

Patient Management

Hemodialysis adequacy, management of blood pressure, anemia, renal osteodystrophy and other chronic renal failure parameters were managed according to NKF-DOQI standards. The desired hemoglobin was 110-120 g/L. Adequate dialysis was considered a KT/V greater than 1.2.

Dietary Assessment

In order to exclude exogenous sources of vitamin E, subjects were counselled during the screening period by a registered dietician not to take any foods that contained antioxidants. Subjects were also counselled at baseline, three months and at the end of the study (six months) to ensure they were not changing their diet during the course of the study. If the diet was changed, these changes were to be captured in the dataset.

Laboratory Data

Routine laboratory data was recorded throughout the study as outlined in the protocol. The laboratory data was broken down into the following parameters:

- Hematology

Hemoglobin, hematocrit, erythrocyte count, total leukocytes plus differential count and platelets. Blood samples were collected midweek pre-dialysis.

- Plasma Chemistry

Electrolytes, parathyroid hormone, calcium, phosphate, glucose, fasting lipid profiles (total cholesterol, HDL, LDL, triglycerides), urea, creatinine, partial thromboplastin time, alanine aminotransferases (ALT), alkaline phosphatase, total bilirubin, albumin, Lp(a), homocysteine, C reactive protein, and β2M. Abnormal random glucose samples were repeated fasting (at least eight hours after the last meal). Blood samples not taken fasting were identified as such. Blood samples were collected midweek pre-dialysis.

Dialysis Monitoring

KT/V and percent reduction of urea (PRU) were collected from one mid week dialysis session each month as normally performed in this center.

Concomitant Medications

Patient management was not altered, other than removal during the screening period of any medication known to have anti-oxidant properties during the screening period. Anti-oxidant medications were prohibited during participation in the study however any concomitant medication known to have anti-oxidant properties used during the course of the study was recorded in the patient's study chart. If the medication was discontinued, or the dosage was changed, the date and the reason for the change was also recorded.

Quality of Life

The CHOICE questionnaire (Rubin et al. (1997) was used to measure the quality of life, along with the Short Form 36 and Visual Analog Scale. The Short Form 36 is a generic health status profile used to compare quality of life scores across disease states. It is comprised of eight scales: Physical Functioning, Role Physical, Bodily Pain, General Health Perception, Vitality, Social Functioning, Role Emotional, and Mental Health. The Physical Functioning scale measures physical limitations by asking about activities that require various amounts of endurance, strength, and flexibility. The Role Physical and Role Emotional scales ask subjects how much their activity or work is limited by physical or emotional problems, respectively. The BP scale evaluates the level of bodily pain and how much pain interferes with the patient's daily life. The General Health Perception scale measures how subjects rate and forecast their personal health generally. The Vitality scale measures energy level and fatigue. The Social Functioning scale asks subjects how much their physical or emotional problems interfere with their social activities with family, friends, neighbours, or groups. Finally, the Mental Health scale measures mental health status in terms of anxiety, depression, loss of behavioural or emotional control, and psychological well-being. Higher scores on the scales are associated with better health-related quality of life.

The Visual Analog Scale is a direct measure index score that is easy for subjects to utilize. It was chosen as one of the quality of life tools as it allows for easy comparison within groups in a study.

Finally, the CHOICE questionnaire was chosen as the third quality of life tool. Some domains are not included in the Short Form 36 that might be salient for subjects undergoing dialysis and may vary with treatment modality and dose. This includes eating behaviours (e.g., dietary restrictions are stricter on HD), sexual functioning, specific treatment side effects (e.g. peritonitis), or disease-related symptoms (e.g., nausea and vomiting). As well, while allowing for comparison between different disease groups, the Short Form 36 may have a floor effect in seriously ill populations, particularly in role functioning and physical functioning domains. The CHOICE questionnaire was designed to evaluate the effectiveness of alternative dialysis prescriptions, to complement the generic Short Form 36, to be sensitive to the effectiveness of alternative dialysis modalities and dosing regimens, and to be useful for longitudinal collection in routine practice. The CHOICE questionnaire addresses domains that may be sensitive to differences in dialysis modality and dose. It showed preliminary evidence for reliability and validity as a measure of health related quality of life in dialysis subjects (Wu et al., 2001; Rubin et al., 1997; Bass et al., 1999).

Adverse Events

All clinical adverse events encountered during the study were reported. An adverse event was defined as an unintended clinical occurrence or laboratory test result observed in a patient on the study, which was related in time but not necessarily caused by participation in the study. Clinically relevant laboratory abnormalities were identified from the laboratory data records. Each adverse event was recorded along with its date of occurrence and disappearance and whether the event was thought to be treatment related. An adverse event was ascribed to the dialyzer unless it could be explained by a clearly identified or suspected intercurrent condition/medication (e.g. flu epidemic, etc.). The reason for not attributing the adverse event to the dialyzer was given as a comment. Adverse events were graded on a three-point scale (mild, moderate, or severe), as follows:

- Mild: Discomfort noticed but no disruption of normal daily activity, Moderate: Discomfort sufficient to reduce or affect normal daily activity, or
- Severe: Incapacitating, with inability to work or perform normal daily activity, but not severe enough for being a serious adverse event.

The clinical status of the subjects was closely monitored by the treating physician, and adverse events were documented, evaluated and treated as necessary.

3.3 Specialized Testing

All blood samples were collected midweek pre-dialysis.

Vitamin E

A modification of the HPLC method of Taibi and Nicotra (2002) was utilized to determine plasma α-tocopherol concentration. Briefly, to 200 µL of patient plasma, 200 µL of acetonitrile-tetrahydrofuran (4:1) was added. Acetonitrile precipitated the protein while the tetrahydrofuran facilitated the extraction of the α-tocopherol. This was vortexed for 30 seconds then centrifuged at 11,000 g for 10 minutes. The clear supernatant was transferred to glass tubes and 500 µL of HPLC grade hexane was added. This was vortexed for 30 seconds and the centrifuged at 3000 g for 10 minutes. The resulting clear supernatant was transferred to clean dry glass tubes and evaporated to dryness under a nitrogen stream. This was reconstituted with 250 µL of mobile phase and 100 µL was injected onto the column.

The liquid chromatographic system consisted of a Waters 510 isocratic pump at a flow rate of 1.3 ml/min from mobile phase, methanol/water (12/44/44), by volume; a Supelco C18 reversed-phase column (15 cm long × 5 mm internal diameter) packed with octadecyl (C18) bonded to spherical silica (5 mm particle diameter and 100 Å pore diameter) for separation; and a one-channel Waters 481 ultraviolet-visible wavelength detector linked to a HP3320 integrator recorder.

α-tocopherol

After calibration and reconstitution of the extract with 250 μl of mobile phase, 100 μl was injected into the HPLC system. α-tocopherol was detected at 292 nm. The average retention time in minutes for α-tocopherol was six minutes. A standard curve was evaluated by linear regression analysis obtained by plotting peak–area against the concentrations of the external standards. All-trans-retinol, DL-α-tocopherol, were purchased from Sigma–Aldrich (St. Louis Mo). Hexane, methanol, acetonitrile and tetrahydrofurane were all HPLC grade purchased from Caledon Laboratories (Georgetown, Ontario).

Antioxidant assays

Plasma 3-nitrotyrosone (3NT), interleukin-6 (IL-6), and F2-isoprostanes (F2I) were all measured using commercially available ELISA kits. Original analysis planned for using an HPLC method for the detection of 3-nitrotyrosine. However, analysis of the samples demonstrated no 3-nitrotyrosine detectable down to 0.2 μg/mL possibly due to dialysis of the molecule. An increase in sensitivity to 2 ng/mL was obtained using an ELISA assay (Hycult Biotechnology), purchased through CEDARLANE Laboratories Limited (Hornby, Ontario). ELISA assays for plasma IL-6 and F2 isoprostanes both had a sensitivity of 10 pg/mL and were purchased from CLB Pelikine and Oxford Biomed, respectively. All spectrophotometric readings were

performed on a Bio-Tek EL-312 96-well plate reader (Fisher Scientific, Nepean, Ontario).

3.4 Sample Size Calculations

To detect a difference in F2-isoprostances between the treatments of 200 pg/mL with 80 percent power, based on a within subject standard deviation of 150 pg/mL and a two sided 5.0 percent significance level, 30 subjects were needed for this two treatment crossover study (Lim et al., 2002, Handelman et al., 2001; Ikizler et al., 2002).

3.5 Statistical Analysis

Data were collected by a study coordinator in MS Excel 2000. The database was prepared by first importing it into SPSS and then checking each field for missing or erroneous values. Statistical analysis was performed using SPSS Version 11.0.1. Analysis included all endpoints occurring between 20 June 2001 and 15 April 2002. All data was assessed for normal distribution and equal variances using the test of homogeneity of variances (Levine's test), and P-P plots. Probability plots are generally used to determine whether the distribution of a variable matches a given distribution. Any data variables that had a statistically significant difference in variances as expressed by Levene's Test of Equality were log transformed, as well as data variables that did not appear to fit the test distribution on the P-P plots. A

randomization variable was assigned to denote which group subjects were initially randomized to, and used to assess for the effect of randomization on outcomes. The between subjects factor for this analysis was randomisation (use of the F80 dialyzer in the first time period or use of the Excebrane® dialyzer in the first time period), and the within subjects factor was time (baseline, end of the F80S time period, and end of the Excebrane® time period).

A comparison between F80S and Excebrane® membranes was performed by paired samples t-test. As each subject is measured against themselves, the paired samples t-test avoids the variability due to 'between subject' differences resulting in a smaller error term, and is therefore more powerful than an independent samples t-test. The assumptions of independent paired differences, and normal distribution of the paired differences were met before using the paired samples t-test.

A within-group comparison among baselines and the values at end of the F80S and Excebrane® testing periods was performed by repeated measures analysis of variance (ANOVA). Using repeated measures ANOVA reduces the error terms and increases the power of the study, which in turn allows for fewer subjects. However, in order to use repeated measures ANOVA, the assumption of compound symmetry must be met. The first part of this assumption states that correlations across the measurements are the same, that is, that the correlation between Baseline and the F80S time

period, the F80S time period and the Excebrane® time period, and finally Baseline and the Excebrane® time period are the same. The second part of the assumption states that variances across the three time points are equal. When the assumption of compound symmetry was met, the univariate results were reported. Univariate results were utilized as they are more powerful than the multivariate results. The multivariate results do not treat the within subjects factor is not treated as an independent variable but rather as multiple independent variables (Hazard, 2001).

Mauchly's Test of Sphericity was therefore utilized to ensure that the assumption of compound symmetry was met. This approach was also confirmed by examining the epsilon values. When these values approach one, it indicates that multiplying the degrees of freedom by the epsilon value will not change any of the end results. When corrections are made using the epsilon value, a larger *F* test is required for significance, as the resulting degrees of freedom are smaller (Hazard, 2001). If the assumption of compound symmetry was not met, the Wilk's Lambda multivariate results were reported. Data is expressed as mean values ± SD, unless otherwise noted. A p value of 0.05 or less was considered statistically significant.

It is important to note that the purpose of this study was to study the effect of the Excebrane® membrane on antioxidant parameters in a chronic hemodialysis population as opposed to proving the efficacy of the Excebrane® membrane in preventing cardiovascular complications.

3.6 Research Ethics and Permissions

This study and the patient consent form were approved by The University of Alberta & Capital Health Authority Research Ethics Board using the International Conference on Harmonization Good Clinical Practice criteria. This protocol conforms to the Declaration of Helsinki and all participating personnel were instructed to act according to the principles therein. Permission to use the CHOICE questionnaire was granted by Dr. Neil Powe. Randomization using the pseudo-random number generator of Wichmann and Hill was granted by Dr. Jerry Dallal. Permission to use the Short Form 36 survey was granted by Dr. John Ware (President and CEO, QualityMetric Inc.)

CHAPTER 4. RESULTS

4.0 Demographics

Between 20 June 2001 and 15 January 2002, 48 subjects were approached for the study. 15 subjects did not participate for which various reasons were given for not participating: on the transplant waiting list, unable to give informed consent, and no desire to change dialyzer. 33 subjects signed an informed consent prior to enrollment in the study and were enrolled. Table 10 lists the characteristics of the enrolled subjects.

4.1 Paired t-test Results

These biochemical characteristics were then assessed using paired samples t-test between the F80S and Excebrane®. The mean difference between the F80S dialyzer and the Excebrane® dialyzer for the variables under consideration is shown in Table 11.

For example, the mean difference in KT/V between the F80S dialyzer and the Excebrane® dialyzer was 0.08 (95% C.I. -0.15, 0.30). While the level of urea postdialysis (mmol/L) is statistically significant by the paired samples t-test (95% C.I mean difference = -1.61 to -0.03), the values are not clinically significant. This is also verified by the fact that the PRU (percent reduction in urea), a more robust indicator of dialysis efficacy, is not significantly different between the two study groups. The F80 dialyzer has statistically significantly lower β2M predialysis levels, β2M postdialysis levels and an higher percentage decrease in β2M levels during dialysis compared to the Excebrane® dialyzer.

Finally, cholesterol is also statistically significant, but in similar fashion to postdialysis urea is not clinically significant.

4.2 Repeated Measures Analysis of Variance Results

Biochemical parameters were also analyzed using repeated measures ANOVA with each variable tested as a single main effect to compare the effect of the F80S and Excebrane® dialyzers to baseline measurements. When the dataset was analyzed the only statistically and clinically significant variable became beta 2 microglobulin (β2M), a middle level marker of dialysis efficacy (Table 12).

This analysis demonstrates that the F80 dialyzer is a more effective dialyzer as demonstrated by β2M predialysis levels (a marker of β2M build up in-between dialysis sessions), β2M postdialysis levels (a marker of dialysis efficacy during the dialysis session) and the percentage decrease in β2M levels during dialysis. However, for all other measures of dialysis efficacy, including KT/V which is a more robust measure of dialysis efficacy, no clinically significant difference could be found between the dialyzers. Post-dialysis urea (mmol/L) was statistically significant (6.92 ± 2.28 baseline, 5.82 ± 2.25 F80S, 6.64 ± 2.60 Excebrane®, p=0.04), but not clinically significant.

When the P-P plots of CRP pre and post dialysis at all time points were examined, they did not appear to fit to the test distribution. Therefore they were log transformed, re-plotted using P-P plots, and the repeated measures

ANOVA applied. The analysis of triglycerides also revealed unequal variances. Therefore, the triglyceride data were log-transformed prior to being analyzed. As shown in Table 13, neither log-CRP nor log-triglycerides were statistically significantly different at any time point.

4.3 Paired t-test Results: Antioxidant Parameters

All antioxidant and inflammatory parameters were analyzed with paired samples t-tests to examine if a clinical or statistical difference could be seen between the F80S and Excebrane® groups at the end of three months of treatment as shown in Table 14.

While there was a trend toward increased endogenous α-tocopherol with use of the Excebrane® dialyzer, as measured by serum vitamin E levels 10.62 μmol/L ± 9.11 μmol/L F80S, 12.48 μmol/L ± 7.76 μmol/L Excebrane®, p=0.42), there was no statistical significant difference. There was a trend to increased F2 Isoprostane (mean difference -0.04 ng/mL, p=0.40), 3 Nitrotyrosine (mean difference -4.69 ng/mL, p=0.30), and IL-6 (mean difference 0.39 pg/mL, p=0.801) levels with use of the Excebrane® dialyzer.

As there were no changes in vascular access function or survival, nor to any medications known to have antioxidant properties, these data were not further analyzed. There were no dialyzer-related clinically significant adverse events noted in this study.

Table 10. Baseline characteristics of study subjects

Parameter	n	Mean	SD[a]
Age (years)	33	59.78	14.60
Male/Female	33	25/8	--
Hemoglobin (g/dL)	33	117.39	17.33
Hematocrit (%)	33	35.0	5.0
White blood cell count (WBC) (x 10^6 /L)	33	6.68	1.82
Platelets (x 10^9 /L)	33	218.82	65.05
Serum creatinine (μmol/L)	33	837.90	288.46
Urea level predialysis (Urea Pre) (mmol/L)	33	20.95	7.12
Urea level postdialysis (Urea Post) (mmol/L)	32	6.92	2.28
Cholesterol (mmol/L)	31	4.19	0.99
Triglycerides (mmol/L)	31	1.79	1.08
HDL (mmol/L)	31	1.03	0.45
LDL (mmol/L)	29	2.56	0.90
Lp(a) (mg/dL)	32	0.24	0.28
C reactive protein level predialysis (CRP Pre) (μg/L)	32	22.99	44.94
C reactive protein level postdialysis (CRP Post) (μg/L)	33	23.20	45.28
Increase in C reactive protein level (%)	32	2.31	19.07
Homocysteine (μmol/L)	31	25.89	12.26
β2M level predialysis (β2M Pre) (μmol/L)	32	27.68	8.18
β2M level postdialysis (β2M Post) (μmol/L)	33	21.00	9.78
Change in β2M level postdialysis (%)	32	2.31	19.07
KT/V	32	1.34	0.35
Percent Reduction in Urea (PRU) (%)	32	65.43	13.89
Phosphorus level (PO4) (mmol/L)	33	1.91	0.68
Parathyroid hormone level (PTH) (ng/L)	18	19.24	23.39

a: standard deviation

Table 11. Mean difference between the F80 and Excebrane® dialyzers: biochemical parameters

Parameter	n	F80S (SD)	Excebrane® (SD)	Mean Difference	95% CI	P Value[a]
Hemoglobin (g/dL)	32	118.38 (14.82)	118.22 (17.71)	0.16	-8.20, 8.51	0.97
Hematocrit (%)	32	35.00 (46.00)	35.00 (5.00)	0.00	-2.00, 2.00	1.00
WBC (x 10^6 /L)	32	7.08 (2.21)	6.87 (1.96)	0.20	-0.72, 1.14	0.66
Platelets (x 10^9 /L)	32	217.44 (75.76)	221.22 (71.91)	-3.78	-22.67, 15.11	0.69
SCr (µmol/L)	32	778.22 (288.28)	846.22 (224.75)	-68.0	-150.57, 14.57	0.10
Urea Pre (mmol/L)	32	20.00 (6.22)	21.73 (6.90)	-1.73	-3.96, 0.50	0.12
Urea Post (mmol/L)	32	5.82 (2.25)	6.64 (2.60)	-0.82	-1.61, -0.03	0.04
Cholesterol (mmol/L)	27	3.90 (0.99)	4.26 (1.06)	-0.35	-0.59, -0.12	0.005
Triglycerides (mmol/L)	16	1.73 (0.90)	1.81 (1.20)	-0.08	-0.45, 0.29	0.65
HDL (mmol/L)	16	0.93 (0.33)	0.91 (0.33)	0.02	-0.23, 0.06	0.62
LDL (mmol/L)	15	2.45 (0.92)	2.43 (0.78)	0.02	-0.23, 0.27	0.88
Lp(a) (mg/dL)	29	1.88 (8.87)	0.28 (0.33)	1.60	-1.74, 4.95	0.33
CRP Pre (µg/L)	26	20.21 (36.23)	15.95 (23.31)	4.26	-13.00, 21.53	0.62
CRP Post (µg/L)	29	19.01 (32.53)	16.03 (120.37)	2.98	-10.98, 16.93	0.67
Increase in CRP (%)	26	5.01 (29.19)	14.82 (36.25)	-12.18	-31.52, 7.17	0.21
Homocysteine (µmol/L)	28	25.14 (10.32)	26.69 (14.18)	-1.55	-5.67, 2.56	0.45
β2M Pre (µmol/L)	27	24.09 (7.98)	30.17 (10.17)	-6.07	-8.62, -3.53	<0.0001
β2M Post (µmol/L)	29	11.61 (4.25)	27.14 (8.38)	-15.53	-17.98, -13.08	<0.0001
Decrease in β2M level postdialysis (%)	27	46.65 (31.55)	6.95 (30.24)	38.97	24.47, 53.47	<0.0001
KT/V	32	1.50 (0.51)	1.42 (0.40)	0.08	-0.15, 0.30	0.48
PRU (%)	32	69.37 (14.69)	69.33 (7.33)	0.04	-4.89, 4.97	0.99
PO4 (mmol/L)	32	1.69 (0.81)	1.85 (0.79)	-0.16	-0.53, 0.20	0.36
PTH (ng/L)	8	55.5 (29.5)	59.8 (30.5)	-4.30	-20.86, 12.26	0.56

a paired samples t-test

Table 12. Single main effect repeated measures ANOVA comparing baseline, F80S, and the Excebrane® dialyzers: biochemical analysis [a]

Parameter	n	Baseline Mean (SD)	F80 Mean (SD)	Excebrane® Mean (SD)	P value
Hemoglobin (g/dL)	32	117.39 (17.33)	118.38 (14.82)	118.22 (17.71)	0.97
Hematocrit (%)	32	35.00 (5.00)	35.00 (46.00)	35.00 (5.00)	1.00
WBC (x 10^6/L)	32	6.68 (1.82)	7.08 (2.21)	6.87 (1.96)	0.66
Platelets (x 10^9/L)	32	218.82 (65.05)	217.44 (75.76)	221.22 (71.91)	0.69
SCr (µmol/L) [b]	32	837.90 (288.46)	778.22 (288.28)	846.22 (224.75)	0.10
Urea Pre (mmol/L)	32	20.95 (7.12)	20.00 (6.22)	21.73 (6.90)	0.12
Urea Post (mmol/L)	32	6.92 (2.28)	5.82 (2.25)	6.64 (2.60)	0.04
Cholesterol (mmol/L)	27	4.19 (0.99)	3.90 (0.99)	4.26 (1.06)	0.005
Triglycerides (mmol/L) [c]	16	1.79 (1.08)	1.73 (0.90)	1.81 (1.20)	0.65
HDL (mmol/L)	16	1.03 (0.45)	0.93 (0.33)	0.91 (0.33)	0.62
LDL (mmol/L)	15	2.56 (0.90)	2.45 (0.92)	2.43 (0.78)	0.88
Lp(a) (mg/dL)	29	0.24 (0.28)	1.88 (8.87)	0.28 (0.33)	0.33
CRP Pre (µg/L)	26	22.99 (44.94)	20.21 (36.23)	15.95 (23.31)	0.62
CRP Post (µg/L)	29	23.20 (45.28)	19.01 (32.53)	16.03 (120.37)	0.67
Increase in CRP (%)[b]	26	2.31 (19.07)	5.01 (29.19)	14.82 (36.25)	0.36
Homocysteine (µmol/L)	28	25.89 (12.26)	25.14 (10.32)	26.69 (14.18)	0.45
β2M Pre (µmol/L)	27	27.68 (8.18)	24.09 (7.98)	30.17 (10.17)	<0.0001
β2M Post (µmol/L)	29	21.00 (9.78)	11.61 (4.25)	27.14 (8.38)	<0.0001
Decrease in β2M level postdialysis (%)	27	25.44 (20.07)	46.65 (31.55)	6.95 (30.24)	<0.0001
KT/V	32	1.34 (0.35)	1.50 (0.51)	1.42 (0.40)	0.48
PRU (%)	32	65.43 (13.89)	69.37 (14.69)	69.33 (7.33)	0.99
PO4 (mmol/L)	32	1.91 (0.68)	1.69 (0.81)	1.85 (0.79)	0.36

a Univariate GLM Repeated Measures

b Mauchly's test statistically significant, therefore multivariate (Wilk's Lambda) is reported
c Levene's test statistically significant

Table 13. Repeated measures ANOVA comparing baseline, F80S, and the Excebrane® dialyzers: transformed data

Parameter	N	Baseline ± SD	F80S ± SD	Excebrane® ± SD	P Value
Log-CRP predialysis	26	2.22 ± 1.27	2.22 ± 1.08	2.05 ± 1.12	0.65
Log-CRP postdialysis	29	2.17 ± 1.37	2.21 ± 1.13	2.15 ± 1.10	0.46
Log-Triglycerides	16	0.44 ± 0.53	0.37 ± 0.48	0.45 ± 0.57	0.86

Table 14. Mean difference between the F80 and Excebrane® dialyzers: antioxidant parameters

Parameter	n	F80S (SD)	Excebrane® (SD)	Mean Difference	95% CI	P Value[a]
F2 isoprostane level (ng/mL)	30	0.16 (0.16)	0.19 (0.19)	-0.04	-0.13, 0.06	0.40
Vitamin E level (µmol/L)	24	10.62 (9.11)	12.48 (7.76)	-1.86	-6.54, 2.83	0.42
3 nitrotyrosine level (ng/mL)	25	109.58 (20.87)	114.27 (23.55)	-4.69	-13.79, 4.40	0.30
IL-6 level (pg/mL)	10	2.83 (4.18)	3.21 (2.48)	-0.39	-3.79, 3.01	0.80

a paired samples t-test

CHAPTER 5. DISCUSSION

5.0 Summary of Findings and Implications

The purpose of this study was to compare the performance of the Excebrane® (Clirans CL*EENL, high flux, modified, vitamin E) and the F80S (polysulfone) membranes in a two arm crossover study. Dialysis adequacy remained stable in all periods of the study, and there was a non-statistically significant trend to increased levels of plasma vitamin E. This study, while not finding evidence of improved bioactivity (decrease in IL-6, c reactive protein, or total white blood cell count) with the Excebrane® dialyzer, indicates that the use of the Excebrane® dialyzer is generally as effective as the F80S membrane in terms of dialysis efficacy. This study also demonstrates that use of the Excebrane® membrane in chronic hemodialysis subjects does not afford any additional benefit as an antioxidant. As cardiovascular death remains the leading cause of death for chronic hemodialysis patients, the results of this study would indicate that further research should shift from the Excebrane® dialyzer to other known causes of cardiovascular death such as hypertension and cardiomyopathies.

5.1 Strengths

There are various strengths to this study. Firstly, this study looked at a number of antioxidation parameters, whereas previous randomized controlled trials with sufficient subject numbers either examined a singular

marker of oxidative stress (Tarng 2000), or inflammatory cytokine production as a surrogate of oxidative stress (Girndt 2000). The inclusion of homocysteine, plasma vitamin E, and 3 Nitrotyrosine allowed for further analysis into the potential of the Excebrane® membrane as a source of antioxidation.

This study also examined a number of inflammatory markers: IL-6, c reactive protein, and total white blood cell count (WBC) as a measure of bioactivity. This trial did not find any potential for the Excebrane® membrane to act as a more bioactive membrane compared to the F80S membrane, nor are subjects undergoing dialysis with the Excebrane® membrane afforded any benefit with regard to decreased inflammation.

This randomized crossover trial was designed with sufficient sample size. Previous studies with the Excebrane® membrane had sample sizes of less than 20 patients (Buoncristiani, 1997; Girndt, 2000; Dhondt, 2000; Shimazu, 2001; Mydlik, 2001) which calls into question the clinical relevance of statistically significant findings in those studies. Furthermore, the crossover design is preferable from both a fiscal and ethical point of view. As the number of subjects needed is significantly reduced compared to a parallel group design, it clearly decreases the cost of research. A crossover design also limits the number of subjects exposed to an experimental situation. Finally, a randomized controlled trial is the most robust method of examining

novel therapies in patient populations. Not all previous studies were randomized (Bonnefont-Rousselot, 2000).

5.2 Limitations

In both the paired samples t-test and the repeated measures ANOVA analysis, many of the laboratory parameters were not statistically significant due to a high standard deviation and a higher coefficient of variation seen in standardized clinical laboratory assays. There were technical difficulties with the baseline measurements of F2 isoprostanes, vitamin E, 3 nitrotyrosine, and IL-6. The inclusion of these baseline measurements, while unlikely to change the outcome of this study, would have allowed for the use of repeated measures ANOVA, a more robust statistical methodology. This study was appropriately powered for the primary study variable (F2-isoprostances), but caution should be used when interpreting the secondary variables, as they may not have had enough subjects. There was more variability seen in the F2-isoprostance data than that previously described in the literature (Lim et al., 2002, Handelman et al., 2001; Ikizler et al., 2002), and this may have had an effect of the ability of this study to find a statistically significant difference between the two dialysers. This has implications for if the true standard deviation of F2-isoprostane is as large as that seen in this study, future studies will need to have more subjects participating in order to find a significant difference.

It is not surprising that a three month trial of the Excebrane® dialyzer did not show a difference in vitamin E levels. In his 1999 paper, Mune *et al* found a significant reduction in LDL-MDA and oxidized LDL after using the Excebrane® dialyzer for a minimum of six months. However, Mune also noted that there were no significant changes in plasma vitamin E and lipid concentrations between the control group (cellulose membrane) and the Excebrane® group (Mune et al., 1999, Mune et al., 1999). In recently published data, Hara et al looked at oxidized LDL in four groups: ten healthy volunteers, 14 subjects with mild to moderate renal failure, seven peritoneal dialysis subjects and 47 hemodialysis subjects, eight of which were changed to the Excebrane® membrane (HD) for 12 months. Subjects were not randomized, and statistical testing was not explicitly stated. They found HD subjects had the highest levels of ox-LDL, and dialysis sessions increased ox-LDL levels. The use of the Excebrane® membrane did not affect pre-dialysis levels of ox-LDL, but reduced ox-LDL post-dialysis compared to baseline post-dialysis levels and had a positive effect on net change, which is more reflective of lack of pre dialysis change and positive post dialysis change (Hara T. et al., 2004).

This trial was only a single blind trial in that the principal investigator was not aware of patient randomization until after the study was completed. This meant that subjects, clinicians caring for the subjects, and the study coordinator were unblinded during the course of the trial. It was not feasible

to blind the dialyzers as the Excebrane® was a "wet pack" dialyzer (fluid filled at the time of packaging), necessitating a different dialysis setup from the F80S. However, it is unlikely that knowing what dialyzer subjects were on would have led to differing biochemical and antioxidation parameters.

Dietary data was not accurately measured during this study, and this lack of data introduced confounding into the study. This limitation was hopefully overcome as all subjects were counselled on exogenous sources of vitamin E and were instructed not to change their diet, and were prescribed the same multivitamin tablet, which did not have any vitamin E content. The dietary instructions were repeated to subjects on a regular basis by the study coordinator and a renal dietician throughout the course of the study.

5.3 Antioxidant Implications

Some changes cannot be explained by this study: an increase in F2 isoprostanes and an increase in plasma 3-nitrotyrosine. While statistically non-significant, this would need to be repeated in a larger multi-centre study to ascertain the true effect of the dialyzer.

5.4 Future Research

While there was a trend to an increase in plasma vitamin E, there is little potential for future research with the Excebrane® membrane. The manufacturer has withdrawn the Excebrane® membrane from the market due

to lack of efficacy as a bioactive membrane. The results of this study confirm this decision.

5.5 Conclusions

The results of this study suggest that while there may be a role for vitamin E as an antioxidant in hemodialysis subjects, the Excebrane® membrane while performing equally as well as the F80S did not add any benefit as an antioxidant nor as a bioactive membrane. Larger parallel group studies could examine whether increased contact time with the dialyzer would change any of the above measured parameters, as indicated by Mune (Mune et al., 1999) and Hara's (Nakamura et al., 2003; Hara T. et al., 2004) research, but as the membrane has been withdrawn by the manufacturer these studies will not be done.

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A PILOT STUDY OF THE USE OF THE EXCEBRANE MEMBRANE IN THE NORTHERN ALBERTA RENAL PROGRAM (NARP) HEMODIALYSIS POPULATION

These will be used to measure lipids and some markers of inflammation. The blood will be taken from the dialysis lines, and the total amount of study blood work will be about 70 ml (15 teaspoons).

You will also be asked to fill out a Quality of Life Survey at the start of the study and at 3 and 6 months. Total amount of time for all 3 surveys is 1 hour. You will also be required to fill out a 3 day food record at the start of the study and at 3 and 6 months. A registered dietician will instruct you on how to fill it out correctly.

Possible Side Effects:

The Excebrane is not expected to have any extra side effects from those of other dialysis membranes. You are already aware of the side effects of dialysis. Every reasonable precaution will be taken to ensure your safety during this study. If any knowledge gained from this or any other study becomes available which could influence your decision to continue in the study you or your legally acceptable representative will be promptly informed.

Possible Benefits:

The benefit of participating in this study is that the Excebrane membrane may be more effective in reducing lipids, and may be more compatible for your body (less inflammation). There are no guarantees you will directly benefit from this research; but the results may help others with this condition the future. No additional expenses will come about for participating in this study.

Compensation:

There will be no monetary costs to you for participation in this study. You will not be charged for the membrane or any research procedures. If you become ill or injured as a direct result of receiving the study medication when used according to the study plan, necessary medical treatment will be available at no additional cost to you. By signing this consent form, you are not releasing the investigator (s), institution (s) and/or sponsor (s) from their legal responsibilities.

Confidentiality:

Information concerning your participation in this study may be reviewed by the study sponsor (Terumo Inc.), the Therapeutic Products Programme (TPP) and/or the US Food and Drug Administration (FDA) in the presence of an investigator or a research nurse/coordinator. When copies of your records are forwarded to the sponsor, or if they are forwarded to the TPP or the FDA, they will be identified by a code number only. If the results of the trial are published, your identity will remain confidential.

Alternative Therapies:

<u>You do not have to be in this study.</u> You are free to withdraw from this study at any time, and your medical care will not be affected in any way. Being involved with this study does not affect your eligibility to be put on the transplant list, nor will it change your chances of getting transplanted.

APPENDIX 3: SOCIODEMOGRAPHIC QUESTIONNAIRE

Hosp Label

Employment, Sociodemographic Questionnaire

Date of Birth: _____ **Gender: M or F** **Race (optional):** _____

1. What is your current work activity of employment status? (Circle one)

(a) employed *full time*	1
(b) Employed part-time	
i) less than half of the time	2
ii) Half of the time	3
iii) Greater than half of the time	4
Type of work_____	
(c) Unemployed	5

2. The main reason you are not employed full time is: (Circle one)

Temporarily laid off	1
Looking for work	2
Personal choice	3
Unable to work because of health	4
Retired prematurely because of health	5
Retired prematurely for personal reasons	6
Retired at usual retirement age	7
You are a student: (i) full time	8
(ii) part time	9

4. What is your current marital status? (Circle one)

Married or common law	1
Widowed	2
Divorced	3
Separated	4
Never married	5

5. How many years of education have you completed in total?

_____Years

6. What is the highest degree you have obtained? (Circle one)

Never graduated from highschool	1
High school diploma	2
Trade school or technical college	3
University or college degree	4
Advanced degree (MA., MSc., PhD, MD, etc.)	5

7. What is your approximate income level? (Circle one)

<$12,000	1
$12,000 - $24,000	2
$24,001 - $40,000	3
$40,001 - $60,000	4
$60,001 or greater	5

8 Have you ever smoked cigarettes? (Circle one)

Yes No

(No means less than 20 packs or 400 cigarettes or 12 oz. of tobacco in a lifetime, or less than 1 cigarette a day for a year)

Do you currently smoke cigarettes? (Circle one)

Yes No

How old were you when you first started regular cigarette smoking? _____years old.

How many cigarettes do you smoke per day now? _____ cigs/day

On average, for the entire time you have smoked, how may cigarettes have you smoked per day? _____cigs/day