DIET AND LIFESTYLE INTERVENTIONS TO IMPROVE CO-MORBID CONDITIONS OF CHRONIC KIDNEY DISEASE

BY

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DISSERTATION

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ABSTRACT

Chronic kidney disease is a progressive inflammatory disorder affecting approximately 15% of US adults, and the prevalence is increasing rapidly. Advanced chronic kidney disease requiring hemodialysis is associated with multiple co-morbid conditions that greatly reduce physical function and quality of life, including muscle wasting, bone disorders, and cardiovascular disease. Protein-energy malnutrition is especially common for reasons including poor nutrient intake, amino acid losses during dialysis, and elevated intradialytic catabolism; these factors promote loss of lean mass and declines in physical function. Low physical function and adverse changes in body composition accelerate development of other co-morbid conditions, highlighting the cycle of disease and disability characteristic of this population.

Numerous pharmacological therapies are commonly used in an effort to reduce the incidence or severity of chronic kidney disease co-morbidities, but these treatments are associated with high costs and significant side effects. Furthermore, the complexity of chronic kidney disease suggests multiple therapeutic approaches may be beneficial in this population. Intradialytic protein supplementation and exercise training during dialysis are two lifestyle interventions that have been suggested as potential methods to mitigate the cycle of disease and disability. Studies have shown that both parenteral and oral intradialytic supplementation improve protein homeostasis, increase serum albumin and prealbumin levels, and have anabolic effects on skeletal muscle. However, the effect of intradialytic protein on functional disease outcomes in this population is not known. Similarly, numerous studies have demonstrated that intradialytic exercise training has beneficial effects on physical function and quality of life, but surprisingly few studies have examined its effect on other clinical outcomes, particularly cardiovascular disease.

The goal of this research was to examine the relationships between the comorbid conditions associated with advanced chronic kidney disease, and determine the efficacy of intradialytic protein supplementation and exercise training as therapeutic approaches. This goal was accomplished through a series of studies both in animal models and also in clinical populations. In a mouse model of renal insufficiency, a combination of soy protein and exercising improved bone microarchitecture and a main effect of soy protein consumption was observed for improvements plasma urea as an indicator of renal function; results from this study an others prompted consideration of these effects in a clinical population.

In a cross-sectional analysis of sixty hemodialysis patients, we found multiple aspects of chronic kidney disease to be interrelated, supporting the idea of the cycle of disease and disability characteristic of these patients. This study was notable for its comprehensive inclusion of functional outcome variables associated with hemodialysis treatment in an effort to characterize relationships among these factors, and possibly provide information on how best to intervene to improve health outcomes in this extremely sick population. For the first clinical intervention study, seventeen hemodialysis patients completed a four month intradialytic cycling program; exercising during dialysis improved physical functioning and improved cardiovascular disease risk as measured by serum alkaline phosphatase and epicardial fat thickness.

Protein intake during dialysis, either soy or whey protein, attenuated inflammation associated with a single dialysis session and reduced the acute phase protein response after a six month supplementation program. Long-term protein intake also improved physical functioning and reduced circulating alkaline phosphatase levels, similar to the findings after four months of intradialytic cycling. Taken together, these data suggest modest benefits of intradialytic exercise and protein supplementation on functional outcomes in this critically ill population. Future directions include investigating the combined effects of intradialytic protein and exercise in maintenance dialysis patients, as the complexity of the disease suggests multiple therapeutic strategies may be necessary to improve health outcomes and quality of life for this population.

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TABLE OF CONTENTS

CHAPTER 1

STATEMENT OF PROBLEM & SIGNIFICANCE

The number of dialysis patients in the United States is expected to double in the next 30 years. At this stage of Chronic Kidney Disease (CKD), a person must undergo dialysis and remain on it for life in the absence of a kidney transplant. After reaching CKD Stage 5, an individual is more likely to die from cardiovascular disease (CVD) than kidney failure, and the rates of CVD are extremely high in this population. The increase in CVD risk seems to be due in part to an increase in mineral deposits in the arteries that lead to stiffer arteries and contribute to plaque formation and development. In addition, dialysis patients are extremely prone to a bone condition known as Chronic Kidney Disease Mineral Bone Disorder (CKD-MBD). This disorder represents a large increase in fracture risk for this population compared to healthy adults. The increase in mineral in the arteries and the decrease in mineral from the bone seem to be related, and may be caused by the impaired ability of the kidneys to regulate minerals in the body. Both the cardiovascular and bone complications in this population add to the decreased quality of life and functioning experienced by dialysis patients.

Many of the drug treatments currently prescribed to these individuals will treat one condition at the expense of the other, leading to additional soft tissue calcification or unwanted loss of mineral from the bone. Furthermore, treatment of dialysis patients accounts for a disproportionately large amount of the Medicare budget. Current therapies are very costly and new approaches are needed that could potentially address the conditions associated with CKD and dialysis treatment.

From a public policy perspective, intradialytic oral protein supplementation represents a low-cost, easy to administer treatment strategy that could potentially prevent CVD and bone disorders in dialysis patients. In addition, many dialysis patients experience protein malnutrition for a variety of reasons including acute inflammation and catabolism associated with the dialysis treatment procedure, making protein supplementation an appropriate target treatment for this patient group. In fact, the National Kidney Foundation recommends an increase in protein intake for these individuals to 1.2 g protein/kg of body weight compared to the 0.8 g protein/kg body weight recommended to the general adult population. This level of protein was determined from

small nitrogen balance and metabolic studies, and the effect of this recommendation on co-morbid disease outcomes in this population is unknown.

Additionally, the importance of a physically active lifestyle for physical and psychological health in the general population as well as in dialysis patients is well established. In spite of the many known benefits of physical activity, surveys have shown that < 40% of nephrologists regularly counsel their patients on physical activity participation, and < 5% provide written materials on physical activity to their patients. Not surprisingly, physical activity levels are extremely low in dialysis patients and the benefits of intradialytic exercise training on functional outcomes in unclear.

Owing to the large number of complications with this population, very little nutritional or exercise training research has been done, although it is desperately needed for these patients. It is the goal of this research to study safe, effective, and reasonable diet and lifestyle modifications for patients undergoing dialysis to reduce the development of co-morbid diseases and improve their quality of life.

CHAPTER 2

LITERATURE REVIEW

Chronic Kidney Disease: Overview

Chronic kidney disease (CKD) is a progressive inflammatory disorder that affects approximately 13% of adults in the U.S, and the prevalence is increasing rapidly[1]. The diagnosis of kidney disease falls into five stages depending on the glomerular filtration rate with CKD Stage 5 representing a GFR < 15 mL/min/1.73m (Table 1, below). This advanced stage of kidney disease, defined as kidney failure treated with dialysis or transplantation, increased in incidence by 43% in the United States in the decade following 1991[2]. Currently 26 million Americans suffer from CKD, and with diabetes and hypertension as the two leading causes of CKD, millions more are at high risk for developing kidney damage. Advanced CKD is associated with a variety of metabolic disturbances that increase morbidity and mortality including increases in serum creatinine, blood urea nitrogen, urine protein, parathyroid hormone and decreases in hemoglobin, serum albumin, and disturbances of calcium, phosphorus, and potassium. In addition, protein malnutrition, muscle wasting, bone disorders, and cardiovascular complications are especially common, and these co-morbidities greatly reduce physical function and quality of life in dialysis patients. Furthermore, 2/3 of patients die within 5 years of initiation of long-term dialysis treatment, mostly of cardiovascular disease (CVD)[3], and survival has not increased substantially in the past two decades[4] despite improvements in dialysis therapy. These data clearly indicate that new therapeutic approaches are needed to address the many co-morbid conditions associated with advanced kidney disease.

Table 2.1: Stages of Chronic Kidney Disease[a]

Stage	Description	GFR (mL/min/1.73 m^2)
1	Kidney damage with normal or high GFR	**≥ 90**
2	Kidney damage with mildly decreased GFR	**60-89**
3	Moderately decreased GFR	**30-59**
4	Severely decreased GFR	**15-29**
5	Kidney Failure requiring dialysis or transplant	**<15 (or dialysis)**

[a]*=adapted from National Kidney Foundation Guidelines, 2002©*

Standard of Care for Nutrition and Dialysis

Patients undergoing maintenance hemodialysis require a comprehensive nutritional standard of care plan. The goals of the nutritional management of CKD at this stage (Stage 5) include preserving protein and nutritional status, minimizing complications and symptoms associated with CKD, and maintaining blood chemistries within recommended ranges. Nutritional management should follow the Nutrition Care Process developed by the American Dietetic Association. The important components of a care plan include management of mineral intake due to deranged mineral metabolism, limiting of protein intake, managing anemia, controlling acid-base balance, and preventing malnutrition, specifically protein-energy malnutrition. The most commonly used clinical indicator of nutritional status in hemodialysis patients is serum albumin levels, as hypoalbuminemia is highly predictive of mortality in hemodialysis patients[5]. Serum prealbumin and creatinine are also routine biochemical markers of nutrition status, and along with anthropometric measurements, diet records, and other subjective measures, allow for assessment and management of protein and energy nutritional status in this population. Albumin levels below the reference range determined by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative of 3.5-5.0 mg/dL indicate additional nutrition support may be required; patient education and dietary counseling represent the first steps in correcting nutritional deficiencies, however, other methods including tube feeding, oral supplementation, or intravenous feeding may be required.

Patients are instructed to restrict their intake of fluid, phosphorus, potassium, and sodium to prevent fluid retention as well as the development of comorbid conditions including bone disorders and CVD. The current recommendation to prevent protein-energy malnutrition is 1.2 grams per kilogram body weight of protein, with 50% of the protein being of high biological value. Overall caloric recommendations for this population are 35 kcalories per kilogram body weight per day for individuals under the age of 60, and 30-35 kilocalories per kilogram body weight per day for individuals over age 60. The increase in protein and energy requirements accounts for the increase in energy expenditure associated with the dialysis procedure, and is intended to prevent protein-energy malnutrition in these patients. Due to the restrictive nature of this diet, frequent dietary counseling and patient education sessions are recommended for hemodialysis patients to prevent development of CKD co-morbidities.

Protein Malnutrition and Physical Functioning in CKD

Protein-energy malnutrition is very common in dialysis patients, with the incidence rate ranging from 25% to 75% in different studies[6-9]. There are a variety of reasons for this, including poor nutrient intake, physical illnesses affecting gastrointestinal function, protein losses during dialysis, and elevated whole body and skeletal muscle protein catabolism that occurs primarily during dialysis (reviewed in[10]). Protein malnutrition is associated with a loss of lean mass and declines in physical function in hemodialysis patients. These functional declines reduce physical activity levels, which exacerbates the development of co-morbidities like CVD and bone disorders. This cycle of disease and disability greatly reduces the quality of life (QOL) and increases mortality in dialysis patients (reviewed in[11, 12]). To mitigate these problems, the National Kidney Foundation recently recommended an increase in protein requirement to 1.2 g/kg/day for hemodialysis patients in comparison to the 0.8 g/kg/day recommended for healthy adults; this represents a recommended intake double the amount recommended for earlier stages of CKD to preserve renal function at 0.6 g/kg/day. The increase in protein recommendation applies immediately after initiation of renal replacement therapy, and patients often have difficulty switching to a recommended high-protein diet from months or years on a low-protein diet during earlier stages of CKD. The recommendation of 1.2 g/kg/day was based on several small prospective nutritional-metabolic studies indicating this intake level is necessary to ensure neutral or positive nitrogen balance in most dialysis patients[10] but the effect of this recommendation on functional disease outcomes is unclear. Scott et al recently found in a non-controlled study that three months of intradialytic supplementation with Nepro, a renal nutrition supplement (containing 425 kilocalories, 19.1 grams of protein, 39.4 grams of carbohydrates, and 22.7 grams of fat per 8 fluid ounce serving) improved one parameter of QOL as assessed by the Kidney Disease Quality of Life-Short Form[13]. However, no studies have assessed whether increasing protein intake improves lean mass, muscle strength, physical function, or other clinical endpoints.

Cardiovascular Disease: Leading Cause of Death in CKD Patients

CVD is the leading cause of death in CKD patients, and the risk increases as CKD becomes more severe. Cardiovascular events are 10 to 30 times greater in patients with renal failure than in age- and sex-matched subjects in the general population[14], and CVD is responsible

for greater than 50% of premature deaths in dialysis patients[15] and this increased risk cannot be explained by traditional CVD risk factors including elevated cholesterol, age, gender, and hypertension[16]. A primary reason for this increased CVD risk is the excessive vascular calcification (VC) in dialysis patients[17-19]. Calcification is part of a remodeling of the vascular wall in CVD that leads to deleterious functional outcomes, including increases in arterial wall intima-media thickness (IMT) and stiffness, endothelial dysfunction, changes in atherosclerotic plaque stability, and a variety of clinical end points, including left ventricular hypertrophy (LVH)[20, 21], myocardial infarction (MI)[22-24], poor surgical outcomes[25], and CVD mortality[22, 26, 27]. VC is rare in individuals with normal renal function under 40-50 years of age, but clinically significant levels develop before the age of 30 in many dialysis patients[28, 29] and tend to progress rapidly[30-34]. For example, Stompor et al. found that median coronary artery calcium (CAC) scores increased 4-fold after just 1 year in dialysis patients[34]. In general, dialysis patients have a 2 to 5 fold increase in CAC levels compared to age-matched subjects with angiographically proven CVD[33].

Abnormal Mineral Metabolism in CKD: Vascular Calcification and Bone Disorders

CKD also is associated with a complex metabolic bone remodeling disorder known as chronic kidney disease-mineral and bone disease (CKD-MBD)[35]. In normal individuals, serum calcium and phosphorus levels are tightly controlled, but this control is disrupted in CKD patients due to a variety of factors. Diseased kidneys have a decreased capacity to produce the activated vitamin D, which leads to a malabsorption of calcium from the intestine that stimulates the parathyroid glands to release parathyroid hormone (PTH). PTH release is also stimulated by reduced phosphorus excretion from damaged kidneys. This elevation in PTH (secondary hyperparathyroidism) stimulates the resorption of calcium from bone, leading to low bone density and strength, as well as to ectopic mineral deposition[36].

Renal bone disease can present as either high- or low-turnover bone disease. Hyperparathyroidism in CKD is normally associated with high-turnover bone disease. However, over-suppression of PTH caused by a variety of factors, including high calcium intake (through diet or calcium-based phosphate binders), excessive vitamin D therapy, diabetes, and aging[37] can result in slow bone turnover. Both forms of renal bone disease result in low bone density and

reduced bone strength, and hemodialysis patients over 50 experience a 4-fold increase in relative risk of fracture compared to age-matched controls[38].

Relationship between Vascular and Bone Disorders in CKD

Many CKD patients have low BMD and excessive VC. Historically, these have been considered independent disorders, but there is emerging evidence that the loss of mineral from bone and calcification of the vasculature are mechanistically linked. Once thought to be a passive precipitation of mineral, VC is now recognized as an active, regulated process with many properties similar to bone formation[39-41]. In response to a variety of stimuli, vascular smooth muscle cells (VSMC) are stimulated to differentiate into an osteoblast-like phenotype[41] capable of producing a bone-like matrix and mineralizing in the presence of calcium and phosphorus[42]. This process appears to be regulated by proteins secreted from VSMC and endothelial cells that either promote or inhibit calcium and phosphate precipitation and the formation of hydroxyapatite crystals[43]. Dialysis patients have abnormal circulating levels of several mineral regulatory proteins that may promote mineral loss from bone and its deposition in the vasculature, including fetuin-A, matrix Gla protein, osteoprotegerin, and bone morphogenetic protein-2a[44-46]. In particular, circulating levels of fetuin-A, an inhibitor of ectopic calcification[47], are reduced in CKD patients, and this has been correlated with excessive VC in this population[45]. The expression profile of these proteins and their subsequent effect in the vasculature and bone may be mediated in part by traditional CVD risk factors[48], including elevated glucose levels[49], high density lipoprotein-cholesterol (HDL-C)[50], inflammatory variables[51], and oxidized low-denisty lipoproteins (LDL)[52, 53]**,** as well as novel risk factors in CKD patients such as hyperphosphatemia[54] and malnutrition[55]. Therefore, factors which modify traditional CVD risk factors or improve nutritional status could alter the expression of these mineral regulating proteins, thereby influencing the rate and extent of mineral deposition in the vasculature, or mineral loss from bone.

Chronic Inflammation: Cause and Consequence of CKD Co-morbidities

CKD is a chronic inflammatory condition, as reflected in dialysis patients by elevated circulating levels of acute phase proteins such as c-reactive protein (CRP), and pro-inflammatory cytokines such as interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-a)[56-59]. This

excessive inflammation is believed to be both a cause and consequence of many of the co-morbidities associated with CKD, including protein malnutrition and muscle wasting, CVD, and bone disorders[56]. Inflammation is believed to promote malnutrition and muscle wasting through a variety of mechanisms, including increased skeletal muscle protein catabolism, cytokine-mediated hyper-metabolism, suppression of nutrient intake, and disruption of the growth hormone and insulin-like growth factor-1 (IGF-1) axis, which may prevent skeletal muscle anabolism[56, 60, 61].

It is well-established that inflammation plays a significant role in the development and progression of atherosclerotic CVD in non-uremic populations[62]. Recent evidence also suggests that inflammatory mediators, including oxidative stress, CRP and pro-inflammatory cytokines promote VC[63] and are associated with cardiovascular complications in CKD patients[56, 64-67]. For example, TNF-α can induce mineralization of calcifying vascular cells *in vitro*[68], and co-culture of these cells with macrophages, a primary source of proinflammatory cytokines, also increases vascular cell mineralization[69]. In humans, elevated CRP has been associated with increased VC in studies in both normal[70] and uremic populations[29]. This may be due in part to inflammation-induced reductions in fetuin-A[71], an inhibitor of VC.

Inflammation also is implicated in the development of renal bone disease[56, 72, 73]. For example, IL-6, which is expressed by both inflammatory cells and osteoclasts, has been shown to promote bone resorption in patients with renal osteodystrophy[73], and also has been implicated in high bone turnover in multiple myeloma[74] and estrogen deficiency[75]. Furthermore, bone loss in rheumatoid arthritis results from the release of metalloproteinases and the pro-inflammatory cytokines interleukin-1 (IL-1) and TNF-a[76]. Elevated CRP levels also are associated with low BMD in both normal[77] and uremic[72] populations. Taken together, these findings suggest that the chronic inflammation associated with advanced CKD promotes the development and progression of many of the co-morbidities that reduce the QOL and increase mortality rates in this population.

Current Approaches to Treat Vascular and Bone Disorders in CKD Are Ineffective

Numerous pharmacological therapies and nutritional approaches are commonly used in an effort to reduce the incidence or severity of CKD co-morbidities. To prevent protein malnutrition and muscle wasting, oral, enteral, and parenteral nutrition, anabolic steroids,

experimental growth factors, and anti-inflammatory medications[78] are often prescribed. While many of these treatments have significant benefits, malnutrition and wasting in CKD remains a significant problem, indicating that alternative treatment strategies are clearly needed. Vitamin D analogs and phosphate binders are often prescribed to prevent hyperparathyroidism that promotes bone disorders in CKD patients, but both may have significant adverse side effects on the vasculature. Both vitamin D[79, 80] and calcium-based phosphate binders[20, 28] have been shown to increase VC in rodents and humans, possibly by increasing the calcium-phosphate product (Ca X P), a well-established VC risk factor[81]. Recently developed non-calcium binders (e.g., Sevalemer) that do not increase the Ca *x* P product have been shown to inhibit the progression of VC in some studies[31, 82] but have not prevented significant VC accumulation in others[83]. There also are numerous concerns regarding costs[84-87] and side effects associated with Sevalemer, including low tolerance due to gastrointestinal distress[85, 88, 89] worsening of metabolic acidosis[90], hyperkalemia[91], and a variety of drug-to-drug interactions[92-94]. For these reasons, the efficacy of Vitamin D therapy and phosphate binders to prevent hyperparathyroidism and its clinical manifestations in dialysis patients is still in question[95] and alternative therapies are clearly needed.

Oral Intradialytic Protein Supplementation: Little is Known

Multiple strategies have been used to increase protein intake in dialysis patients, but little is known about the efficacy of these different approaches on clinical outcomes. Most dialysis patients receive nutritional counseling to increase dietary protein intake at home, but this approach has generally been unsuccessful in restoring nutrient intake to recommended levels, primarily due to low compliance[96, 97]. To improve compliance, intradialytic parenteral or oral supplementation has been recommended, as this allows intake to be closely monitored. Furthermore, intradialytic supplementation may be particularly efficacious in improving protein balance since it is provided during the time when protein catabolism is at its peak[10, 98, 99]**.** Studies have shown that both parenteral[100-102] and oral[96, 103, 104] intradialytic supplementation improve protein homeostasis, increase serum albumin and prealbumin levels, and have anabolic effects on skeletal muscle. However, oral supplementation is much more practical than parenteral due to high costs and restrictions on the use of parenteral nutrition by Medicare and other insurance providers. Despite this, no studies have examined if oral intradialytic protein supplementation

has a significant effect on muscle wasting and physical function, or if it inhibits the inflammation associated with malnutrition and muscle catabolism during dialysis.

Because protein malnutrition in dialysis patients promotes inflammation, it has been suggested that improving nutritional status may help prevent inflammation[60]. If so, this could have a beneficial effect on many CKD co-morbidities influenced by inflammation, particularly CVD[56, 72]. Indeed, a low protein diet increases VC in uremic rats[105], and malnutrition in CKD is associated with low circulating levels of the VC inhibitor fetuin-A[55], which is known to be reduced by chronic inflammation[46]. This suggests that protein supplementation could potentially inhibit VC in uremic/dialysis patients by reducing inflammation. Importantly, Shinaberger et al. recently found that both low protein intake and a reduction in protein intake over time are associated with increased risk of all-cause and CVD mortality in dialysis patients[106]. However, protein intake in this study was assessed using normalized protein nitrogen appearance (nPNA), a surrogate marker of protein intake whose precision has been questioned[106], and no studies have directly examined the efficacy of oral protein supplementation in dialysis patients on VC or other cardiovascular abnormalities. Another recent study reported that time on dialysis was associated with improvements in nutritional status and a trend towards a reduction in inflammatory variables over a three year period[107]. The authors concluded that time on dialysis was not necessarily associated with a decline in nutritional status or increase in inflammation; however, they failed to mention in the methods section that patients routinely received an intradialytic meal containing 25-30 grams of protein. This study suggests that routine intradialytic protein intake may attenuate the decline in nutritional status and increased inflammatory state associated with long-term dialysis treatment; however, this study lacked a control group and failed to provide information on subject compliance for this intradialytic meal and more well-controlled studies are clearly needed.

Protein supplementation also may have beneficial effects on bone health by modifying insulin-like growth factor-1 levels or activity. Though circulating levels of IGF-1 are typically normal in dialysis patients, its bioavailability is often reduced due to an excess of high affinity IGF binding proteins[108]. Both animal and human studies have shown that dietary protein intake positively influences bone formation in non-uremic populations by increasing the production and action of IGF-1[109]. However, no studies have examined the effects of protein supplementation on IGF-1 levels or bone health in dialysis patients.

Potential Role for Soy in Improving Cardiovascular Disease Risk and Measures of Bone Mineral Density

A non-pharmacological approach that has shown promise in some studies in improving risk factors for both CVD and bone disorders is the consumption of soy protein rich in isoflavones. Studies in animal models clearly demonstrate benefits of soy protein and/or isoflavones in reducing atherosclerosis[110] and increasing bone mineral density (BMD)[111, 112]. As for CVD, soy isoflavones may reduce long-term CVD risk by several mechanisms; studies have shown that soy supplementation induced beneficial blood lipid changes[113, 114] and exerts regulatory effects on blood pressure. In addition to the mechanisms listed above, soy protein supplementation may have effects on cardiovascular disease that are specific to CKD. For example, soy supplementation has been associated with reduced oxidized-LDL in hemodialysis patients[115], reduced urinary albumin excretion and LDL/HDL cholesterol ratio[116] in patients with diabetic nephropathy, and preservation of renal function in moderate kidney disease[116, 117]. In addition, studies have shown that soy/isoflavone supplementation impacts traditional CVD risk factors including an inverse association with plasma CRP[118] in CKD and an increase in osteoprotegerin[119]. Osteoprotegerin (OPG), a mineral regulatory protein, inhibits bone resorption by binding the OPG receptor on osteoclasts and also appears to inhibit calcification in the vasculature.

However, many studies have shown no effect of soy supplementation[120-122] on CVD risk and a recently published review indicates that the benefits of soy in humans may be more modest than suggested by the animal studies[123]. Recently, much attention has been given to the fact that the effects of soy protein intake seem to dependent on the biotransformation of isoflavones by gut bacteria. Isoflavones can be converted to metabolites in the gut, some of which have estrogenic properties while others do not. Specifically, daidzein is converted to the estrogenic equol by gut bacteria, which binds to the estrogen receptor β with high affinity[124]. Studies have shown that people who produce equol shown an enhanced response to soy protein feeding, suggesting that equol may be more metabolically active than the isoflavones or other metabolites. However, studies estimate that only about 11-30% of the population can produce equol from gut bacteria[125], which may explain why soy supplementation studies show little to no effect on outcomes of interest. Furthermore, most animal species are equol-producers, which may explain why the benefits of soy are more clearly demonstrated in animal models. However,

soy isolate supplementation has not been looked at in the context of vascular calcification and the related bone mineral disorders, and this represents a novel, promising, and appropriate therapeutic target based on the potential of soy to modify risk factors for these diseases.

Differences Between Whey and Soy Protein

Dialysis patients clearly have increased protein needs due to the acute catabolic effects of the dialysis procedure, increased energy expenditure during dialysis treatment, decreased appetite, and a variety of other reasons[10]. Both whey and soy proteins are high quality protein sources with similar digestibility and absorption kinetics[126]. However, differences in the efficacy of protein supplementation with regards to physical functioning, CVD, and bone disorders differ between plant and animal proteins. Whey protein contains a higher amount of essential amino acids compared to plant proteins, including a higher ratio of the branch-chain amino acids leucine, isoleucine, and valine[127] Leucine concentrations are especially important during a highly catabolic condition, as high amounts of leucine have been shown to potently stimulate muscle synthesis and inhibit protein breakdown in skeletal muscle and liver (reviewed in[128]). Protein supplementation, regardless of the protein source, during dialysis treatment will increase substrate availability, but whey protein may have the greatest impact on the ratio of protein synthesis to turnover leading to greater protection of lean body mass in patients with protein malnutrition. Conversely, Candow et al showed soy protein to be as effective as whey protein for promoting increase in lean body mass after resistance exercise, and soy protein may stimulate muscle accretion by increasing the amino acid pool[129].

While soy protein has been widely discussed for its purported CVD-lowering effects including reductions in plasma lipids, the role of whey protein in CVD risk reduction is less clear. However, studies have shown that whey peptides exert anti-hypertensive properties[130], anti-inflammatory effects in mouse model of dermatitis[131], and anti-oxidant properties in a model of experimental brain injury[132]. These data suggest that during conditions of high inflammation and oxidative stress, such as kidney failure, whey protein supplementation may impact CVD outcomes through modifications of related risk factors. However, few studies exist regarding the effects of whey protein on CVD in humans and it is unclear from these studies if the reduction in risk was due to whey-specific properties or protein intake in general.

Similarly, soy protein has been given more attention in relation to bone disorders due to soy isoflavones and other estrogenic components. However, whey protein, or increased protein intake in general, may protect against bone loss associated with a variety of conditions. Chen at al demonstrated that whey protein increased bone density in intact and ovariectomized rapidly growing female rats, but that a greater increase was seen in the animals receiving soy protein containing isoflavones[133]. Furthermore, milk basic protein (MBP), a component of whey, can promote proliferation and differentiation of osteoblasts both in vitro and in vivo (reviewed in[134]) and may inhibit bone resportion. Other studies have shown no effect of whey protein[135] on bone density and the relationship between whey protein intake and bone health remains unclear. Furthermore, the differences between soy and whey protein intake have not been considered in the context of malnourished dialysis patients, and more information is needed.

Intradialytic endurance exercise for improvement of functional outcomes

In recent years, numerous studies have demonstrated that intradialytic exercise training has beneficial effects on physical function and QOL, but surprisingly few studies have examined its effect on other clinical outcomes, particularly CVD. Intradialytic endurance exercise has been shown to help maintain muscle strength and joint structure and function, improve balance and gait speed, reduce the risk of falling, and help maintain independent living status (reviewed in[136]). Very few studies have examined the efficacy of intradialytic endurance exercise training on either CVD *risk factors* or functional CVD outcomes, and most of those were very small and/or did not include a control group (reviewed in[137]). For example, Deligiannis et al. demonstrated that 6 months of supervised endurance exercise training improved left ventricular systolic function[138], *and* Mustata et al[139] showed that 3 months of endurance exercise reduced arterial stiffness, though there was no control group in this study. Importantly, there have been no *randomized,* controlled clinical trials in hemodialysis patients to study the effects of exercise training on either *traditional CVD risk factors or functional CVD outcomes* such as arterial stiffness, IMT, LVH, or VC[136].

There are a variety of mechanisms by which exercise training may inhibit CVD outcomes in hemodialysis patients. First, it is well established that exercise training in non-dialysis populations improves traditional risk factors for atherosclerosis such as hypertension, plasma lipids, glucose and inflammatory variables[140]. Many of these same factors may increase the

development of VC and arterial stiffness[34, 52, 53, 141-143], so it is reasonable to assume that exercise training may also reduce VC and arterial stiffness by improving the CVD risk factor profile. Exercise training also has been shown to improve phosphate removal during dialysis[144], indicating a novel mechanism by which exercise training may inhibit VC, and thus, arterial stiffness, in hemodialysis patients.

Physical activity levels and quality of life in dialysis patients.

The importance of a physically active lifestyle for physical and psychological health in the general population[145] as well as in dialysis patients[136] is well established. In spite of the many known benefits of physical activity, surveys have shown that < 40% of nephrologists regularly counsel their patients on physical activity participation, and < 5% provide written materials on physical activity to their patients[146]. Not surprisingly, physical activity levels are extremely low in dialysis patients – data from the Renal Exercise Demonstration Project found that ~ 60% of hemodialysis patients participate in *no* physical activity beyond basic activities of daily living[147]. More importantly, accelerometer data indicates that average physical activity levels in dialysis patients are markedly lower than activity levels in *sedentary* healthy controls[148]. Educating nephrologists and their patients as to the benefits of increasing physical activity levels in this population would appear to be an important health goal.

With chronic conditions such as renal failure, functional limitations, disability, and co-morbidity increase, often resulting in compromised physical, emotional, and psychological well-being and QOL. The increasing number of adults with CKD and other chronic disorders has caused public policy to be directed at ways to maintain the independence, societal worth, and physical and mental well-being of this group. In essence, we have moved from simply trying to add quantity to life towards adding quality to those years of life. There is increasing evidence to suggest that physical activity interventions may represent an effective behavioral strategy for not only attenuating functional decline and reducing risk of disease and disability[149-151] but also for enhancing psychological well-being and QOL across the lifespan[152, 153]. Similar benefits on psychological well-being and QOL have been noted with intradialytic exercise in CKD patients[147, 154, 155] ; however there is a lack of large-scale, interdisciplinary, randomized, controlled trials targeting such outcomes[156]. Such research is needed to conclusively demonstrate the clinical importance of intradialytic exercise, which may influence current

standard clinical practice among nephrologists and, as such, improve the health and quality of life of this vulnerable cohort.

CHAPTER 3

INTRADIALYTIC EXERCISE TRAINING AND CHRONIC KIDNEY DISEASE CO-MORBIDITIES[a]

Introduction

Patients with chronic kidney disease (CKD) receiving maintenance hemodialysis treatment suffer from a variety of co-morbid diseases, many of which may be mechanistically linked. Muscle catabolism and wasting is especially common, and these lead to reduced muscle strength, declines in physical function, and low levels of physical activity[136]. Physical inactivity exacerbates these functional declines, and also promotes cardiovascular disease (CVD). This cycle of disease and disability greatly reduces the quality of life (QOL) and increases mortality rates in hemodialysis patients.

CKD is an chronic inflammatory condition, as reflected by elevated circulating levels of acute phase proteins such as C-Reactive Protein (CRP), and pro-inflammatory cytokines such as interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)[60]. Excessive oxidative stress associated with uremia is believed to play a critical role in the development of the chronic inflammation in CKD patients[157]. Oxidative stress contributes to inflammation in part by activating the nuclear transcription factor NF-κB, which mediates the expression of pro-inflammatory cytokines associated with immune and inflammatory responses[158]. Inflammation and oxidative stress both play a critical role in atherosclerosis development in CKD patients[60, 64], and also are related to the pathogenesis of functional CVD outcomes, including arterial stiffness, increases in arterial wall intima-media thickness (IMT), left ventricular hypertrophy (LVH), and declines in cardiac function[20, 21]. Inflammation also reduces circulating levels of fetuin-A, a reverse acute phase protein that acts as a systemic inhibitor of vascular calcification and arterial stiffness[159]. As a result of these abnormalities, cardiovascular events are 10 to 30 times greater in hemodialysis patients than in age- and sex-matched subjects in the general population[14].

[a]This chapter contains material previous published as follows: *Wilund KR, Tomayko EJ, Wu PT, Ryong Chung H, Vallurupalli S, Lakshminarayanan B, Fernhall B. Intradialytic exercise training reduces oxidative stress and epicardial fat: a pilot study. Nephrol Dial Transplant. Aug 2010;25(8):2695-2701. Used with permission of Oxford University Press.*

Numerous studies have highlighted the importance of adipose tissue in relation to the inflammatory burden in CVD, describing the expression and secretion of both pro-inflammatory and protective factors, collectively termed adipocytokines[160, 161]. More recently, research has begun to focus on the unique role of epicardial adipose tissue, a visceral fat depot surrounding the heart, on CVD risk. Like other white adipose tissue, epicardial fat functions as a lipid-storing depot, as an endocrine organ secreting hormones, and as an inflammatory tissue secreting cytokines. The close proximity of epicardial fat to the adventitia of the coronary arteries and the underlying myocardium suggests the possibility that it could play an especially important role in the pathogenesis of CVD[162]. Indeed, recent studies have shown that the expression and secretion of pro-inflammatory cytokines (TNF-α, IL-1B, and IL-6) are increased in epicardial fat, relative to subcutaneous fat, in coronary artery disease patients[163]. This suggests that therapies that decrease the amount of epicardial fat may reduce CVD risk.

It is well established that endurance exercise training helps reduce fat mass, but there is significant controversy regarding whether or not it promotes a reduction in visceral fat over other fat depots[164]. Recently, epicardial fat was shown to be reduced in young, healthy, obese men after 12 weeks of endurance exercise training (jogging)[165]. However, functional limitations and other factors may limit the intensity at which many hemodialysis patients can exercise[136], and it is not known if similar benefits would be realized with moderate intensity intradialytic cycling. While numerous studies have shown that intradialytic exercise improves physical function and quality of life in hemodialysis patients[136, 137, 147, 166], surprisingly little is known about the effects of endurance exercise training on CVD risk in this population[136]. As a result, the objective of this study was to evaluate the efficacy of intradialytic endurance exercise training (cycling) on factors related to the excessive CVD risk in hemodialysis patients, specifically, markers of systemic inflammation (CRP, and Interleukin (IL)-6, and fetuin-A), oxidative stress (lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) assay), cardiac structure and function and epicardial fat levels (ultrasound). We hypothesized intradialytic exercise training would favorably modify cardiovascular disease risk factors similar to the effects seen in healthy populations, and four months of training would improve our primary outcomes, specifically cardiac structure (IMT, LVM, epicardial fat thickness) and function (cardiac output and ejection fraction). However, we expect the beneficial effects of exercise in this population to

be more modest due to contributions of CKD-related comorbidities including elevated inflammation and oxidative stress.

Methods

Subjects. Seventeen patients on maintenance hemodialysis (9 females, 8 males) were recruited from the Champaign-Urbana Dialysis Clinic (Champaign, IL). Patients were screened for eligibility by administering a health and medical history questionnaire. All participants gave written informed consent and this study was approved by the University of Illinois Institutional Review Board. Inclusion/exclusion criteria for patients included the following: 1) age = 30-70 years; 2) nonsmoking; 3) BMI < 35 kg/m^2; 4) no orthopedic problems that prevented cycling during dialysis; 5) no chronic obstructive pulmonary disease (COPD), coronary heart failure (CHF), or cardiovascular surgery (e.g., coronary bypass, valve replacement, or angioplasty) in the past 6 months; 6) medical clearance from a primary care physician; 7) no participation in intradialytic exercise training for 6 months prior to recruitment in the study.

Study Design. Following recruitment, screening, and baseline testing, eligible subjects were randomly assigned to one of two groups: 1) Usual care/control (CON; n=9); 2) intradialytic exercise training (EX; n=8).

Exercise Training Intervention. Subjects in the EX group underwent a 4-month intradialytic endurance exercise training program. This program consisted of cycling 3 days per week on specialized cycle ergometers (Champ-Cycle™; Champion Manufacturing, Inc. Elkhart, IN) placed in front of each subject's dialysis chair. Subjects started the training program by cycling at a tolerable pace for 5 minutes during their first exercise session. The duration of exercise increased by 5 to 10 minutes per session, depending on each subject's individual tolerance, until they were able to cycle continuously for a total of 45 minutes per session at a rating of perceived exertion (RPE) of 12-14 ("somewhat hard"[167]). The subjects reached this level of exercise in 1.8± 0.6 weeks. Subjects maintained this duration and intensity of work for the remainder of the 4 month intervention. All exercise sessions were attended by study staff to encourage the subjects and monitor their response to exercise (e.g., heart rate and blood pressure). Compliance with the exercise protocol was measured as the percentage of exercise sessions successfully

completed. To count as complete, a minimum of 75% of the goal exercise time for that session had to be performed. Subjects in the CON group were not given access to cycle ergometers during their dialysis sessions.

Clinical Testing and Measurements. At baseline and immediately following the 4 month intervention (final testing), all patients underwent a series of tests described below to evaluate the effects of the intradialytic exercise program on our primary outcomes. All testing sessions were conducted on a "non-dialysis day", 18-24 hours following a dialysis treatment. All testing was analyzed by study personnel blinded to the subject's group assignment.

Incremental Shuttle Walk Test. Physical performance was measured by distance walked during an incremental shuttle walk test (ISWT). The ISWT is a progressive test in which patients walk back and forth continuously over a 10 meter course. The walking speed is paced by a series of beeps that signal when the subject should have completed the 10 meter walk. The pace is progressively increased so that the walking speed at the end of each successive minute is $\geq$ to: 1.12, 1.54, 1.88, 2.26, 2.64, 3.02, 3.4, 3.78 miles per hour. The test was terminated when the subject was unable to complete the 10m course before the subsequent beep. The ISWTs were performed on non-dialysis days, 18 to 24 hours after a previous dialysis session, and post-intervention evaluations were performed at least 36-48 hours after any previous exercise bout.

Blood Chemistry. Blood was collected from patients in a non-fasted state from their dialysis lines during regularly scheduled (monthly) blood collection times at the clinic, a minimum of 48-72 hours after any scheduled exercise bout. Blood collections were performed by trained technicians, and blood was collected from the dialysis line immediately after initiating dialysis treatment according to the standard protocol of the clinic. Plasma was collected from blood samples by centrifugation, aliquoted, and stored at -80^{o}C until analyzed. To control for variation between runs, baseline and final test samples from each subject were analyzed simultaneously. Plasma total cholesterol levels were measured using a commercial enzymatic kit (Wako Inc., Richmond, VA). CRP, IL-6 and Fetuin-A were measured in triplicate using commercially available enzyme-linked immunosorbet assay (ELISA) kits (hsCRP ELISA #1668Z, Diagnostic Automation Inc., CA; Quantikine HS Human IL-6 #HS600B, R&D Systems, MN; Human

Fetuin-A ELISA #RD1815, BioVendor, NC). Serum lipid peroxidation, a marker of oxidative stress, was measured by the thiobarbituric acid reactive substances (TBARS) assay, as described[168]. In brief, serum samples were diluted with PBS and incubated with or without 100mM of 2.2'-azobis, 2-amidinopropane hydrochloride (AAPH), a free radical generator, for 2 h at 37^{o}C. Lipid peroxidation was calculated by subtracting values obtained in the presence and absence of AAPH.

Serum potassium, phosphate, calcium, alkaline phosphatase (ALP), calcium-phosphorus product (Ca x P product), blood urea nitrogen (BUN) and albumin were measured using an autoanalyzer (Olympus, Inc.) by Spectra Labs (Rockleigh, N.J.).

Blood Pressure. Brachial blood pressure was measured using an automatic digital blood pressure monitor (Omron IntelliSense HEM-907XL, IL). Subjects were seated for 10 min prior to the first reading. Two measurements, 2 min apart, were performed. If these two measurements were within 10% of each other, the average of the two measurements was taken as the final recorded blood pressure. If not, a third measure was taken, and the two closest measures were averaged.

Echocardiography. Echocardiography was performed using a multifrequency (1.5-4.25 MHz) transthoracic transducer (Acuson Sequoia C512, Mountain View, CA) to assess parameters related to cardiac structure and function. To minimize the effect of variations in fluid volume in hemodialysis patients, studies were performed 18-24 after a hemodialysis session. Left ventricular mass (LVM) was measured by M-mode echocardiography to determine left ventricular mass, as described previously [169]. LVM index was measured according to the formula LVM index = LVM/body surface area. Relative wall thickness was measured as RWT = 2×(PWTd/LVEDD), where PWTd is the posterior wall thickness at end-diastole and LVEDD is the LV dimension at end-diastole. Myocardial performance index (MPI) reflects both systolic and diastolic function of the heart and was measured as MCOT-LVET/LVET where MCOT is the mitral valve closure to opening time and LVET is the left ventricular ejection time. Left atrial volume was calculated using the biplane area – length method at end ventricular systole with precaution taken to avoid foreshortening. The left atrial volume was indexed to body surface area to derive left atrial volume index.

The thickness of the epicardial fat layer also was measured by echocardiogram as previously described[170]. In brief, standard parasternal and apical views were obtained with subjects in the left lateral decubitus position. Epicardial fat was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of pericardium and its thickness was measured perpendicularly on the free wall of the right ventricle at end systole in three cardiac cycles [170]. Maximum epicardial fat thickness was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aorta annulus. The average value of 3 cardiac cycles for each echocardiographic view was considered as the epicardial fat thickness.

Statistical Analysis. All statistical analyses were performed using SPSS software and significance was based on a two-tailed alpha value of 0.05. Distribution statistics for the residuals were calculated to determine whether assumptions of normality were met (i.e., skewness and kurtosis < 2.0). Repeated measures Analysis of Variance (ANOVA) (Group X Time) was used to assess group differences in our major outcomes. Main effects were only considered when interactions were not significant, as a significant interaction indicates that the effect of one independent variable depends on the value of the other**.** When significant interactions were detected, paired sample t-tests were conducted to determine if values between time points differed significantly within each activity group. Correlation analysis was used to identify relationships between selected variables of interest.

Results

Subject characteristics at baseline and final testing are shown in **Table 3.1**. The etiology of each patient's renal failure and the presence of underlying CVD are described in **Table 3.2**. A total of 17 patients were recruited for the study, 9 in CON and 8 in EX. One subject in EX withdrew due to a hip fracture that was unrelated to the exercise intervention, one subject in CON withdrew due to moving out of area, and a second subject in CON did not complete the baseline shuttle walk test due to a scheduling error.

At baseline, the two groups did not differ significantly regarding age, body weight, height, body mass index (BMI), months on dialysis, frequency of dialysis sessions, blood pressure, physical performance, cardiac function, or hematological variables (**Table 3.1**). Due to the small

sample size, the mean baseline values varied widely among individuals; however, the differences between treatment groups were not significant due to the large variation present within each group. There were no interactive or main effects of activity group and time on BMI, systolic, or diastolic blood pressure (**Table 3.1**).

There was a significant interaction ($p < 0.05$) between activity group and time for serum TBARS and alkaline phosphatase activity, as they were reduced by 38% and 27%, respectively, in EX, but did not change in CON (**Table 3.1**).

There was a significant interaction between activity group and time for ISWT performance, as the distance walked during this test increased by 15 % in EX ($p = 0.03$), but did not change in CON (**Figure 3.1**).

There were no interactive or main effects of activity group and time on MPI, LVM index, relative wall thickness or LA volume index. However, there was a significant interaction between time and activity group for epicardial fat thickness, as it was significantly reduced in EX (-9.8%, $p = 0.03$), but did not change in CON (0.03%, $p = 0.96$) (**Figure 3.2**). Furthermore, when data from both groups were combined, the change in performance on the ISWT was inversely correlated to the change in epicardial fat ($r = -0.66$, $p = 0.01$) (**Figure 3.3**), and the change in serum alkaline phosphatase level ($r = - 0.60$, $p = 0.02$).

Discussion

The primary findings in this paper were that 4 months of intradialytic exercise training at a moderate intensity increased physical performance, and reduced serum TBARS, serum alkaline phosphatase, and the thickness of the epicardial fat layer. Furthermore, the change in epicardial fat levels was inversely correlated to the change in physical performance. To the best of our knowledge, this is the first time that intradialytic exercise has been shown to reduce levels of these CVD risk factors in the context of CKD, including the novel risk factor of epicardial fat thickness. These benefits occurred in response to a rather modest amount of exercise: intradialytic cycling 3 days per week for 45 minutes at a moderate intensity, an exercise dose that is easily achievable by most dialysis patients.

We also believe this is the first time that the ISWT has been used to measure changes in physical performance in dialysis patients in response to intradialytic exercise. Shuttle walk tests are appropriate to assess function in older and diseased people since functional limitations often

prevent these individuals from achieving standard criteria of more objective tests (e.g., VO_2max testing) [136]. The average change in distance walked during the ISWT in the EX group was 45 ± 16m, which appears to be marginally less than what has been reported following rehabilitation programs in COPD patients and other populations, but is still in the range of what is thought to be clinically significant[171].

Epicardial fat is a highly inflammatory fat depot surrounding the heart that recently has been shown to be a marker for the presence and severity of CVD[163, 172]. The close proximity of epicardial fat to the adventitia of the coronary arteries and the underlying myocardium suggests the possibility that it could play an especially important role in the development of CVD[160, 162]. Evidence indicates that epicardial fat may locally modulate cardiovascular morphology and function. Iacobellis et al. showed that epicardial fat is positively correlated with abdominal visceral adiposity[173], atherosclerosis[174], and cardio-metabolic risk[170, 175]. As a result, interventions that reduce the extent of epicardial fat may have clinical benefit. Recently, Kim et al[165] showed that 12 weeks of running at a moderate intensity reduced epicardial fat thickness in middle-aged obese men by 8.6%, which was similar to the 9.8% reduction in epicardial fat we found in the current study. However, the study by Kim et al did not include a non-exercising control group. Our study confirms and extends the findings of Kim et al by showing that moderate intensity exercise training significantly reduces epicardial fat compared to a non-exercising control group. Importantly, the reduction in epicardial fat was not correlated with weight change, suggesting that epicardial fat may be a very responsive adipose tissue that can reduced by relatively modest amounts of exercise in the absence of weight loss.

Previous studies in patients undergoing coronary artery bypass grafting have shown that epicardial fat is highly correlated with inflammation[163, 176], and coronary artery disease patients have significantly higher levels of inflammatory cytokines (IL-1b, IL-6, and TNFα) released from epicardial fat than that from subcutaneous fat [163, 177]. Despite this, we did not see changes in any marker of systemic inflammation in response to the change in epicardial fat, including traditional inflammatory markers such as CRP and IL-6, or markers of inflammation especially important in CKD patients, including albumin and fetuin-A. This is somewhat surprising, given that many, though not all, previous studies have shown that exercise training reduces systemic markers of inflammation[178, 179]. However, plasma concentrations of circulating inflammatory cytokines may not be correlated to inflammation in tissues[163], so it is possible that the reductions

in epicardial fat affected artery wall inflammation, but this was not captured by measuring systemic levels of inflammatory markers. Furthermore, the small sample size for this study may have limited the statistical power to detect changes in these variables.

Our data are consistent with the cross-sectional study of Hung et al. [180], which showed no evidence for a correlation between TNF-a and physical activity levels in hemodialysis patients. Castaneda et al. reported that 12-weeks of *resistance* exercise training reduced CRP and IL-6 levels in patients with moderate chronic kidney disease [181], but we are unaware of any studies that have demonstrated an anti-inflammatory effect of *endurance* exercise training in hemodialysis patients. We also hypothesized that the levels of fetuin-A would increase after the exercise intervention, based in part on our previous work showing that fetuin-A was positively correlated with VO_{2max} in older men [182]. Fetuin-A is a reverse acute phase protein which is reduced with inflammation, and a systemic inhibitor of vascular calcification. However, fetuin-A levels did not change in either group in this study, possibly because there was no change in other markers of inflammation. Taken together, these data suggests that the modest anti-inflammatory effects of exercise training seen in other populations may be insufficient to have significant effects on inflammatory variables in dialysis patients, a population with excessive inflammatory stresses.

Excessive oxidative stress is believed to be partly responsible for the increased inflammation and CVD burden in hemodialysis patients [157, 183, 184]. The increased oxidative stress in hemodialysis patients is mainly attributed to the retention of oxidized solute by the loss of kidney function[157]. Growing evidence indicates that endurance exercise training results in reducing O_2^- production and up-regulating antioxidant enzymes activity [136]. Our finding of decreased TBARS levels in the exercise group suggests a reduction in the burden of oxidative stress due to an increase in serum antioxidant activity, which may help reduce CVD risk in hemodialysis patients. However, TBARS was measured in non-fasted samples, and the potential anti-oxidant effect of exercise needs to be confirmed with more robust markers of oxidative stress.

The 27% reduction in serum ALP in EX (**Table 3.1**) may be clinically significant, as serum ALP levels are have been inversely associated with bone mineral density in hemodialysis patients[185], and positively associated with coronary artery calcification (CAC)[186], a complex disorder associated with abnormal mineral metabolism[39]. In healthy populations, exercise

training has been shown to increase serum ALP levels[187]. By contrast, our data coincides with data from Yurtkuran et al[188] indicating that physical activity *reduces* ALP levels in hemodialysis patients. This discrepancy may be due to abnormalities in mineral metabolism in the hemodialysis population. Regardless, the reduction in ALP suggests a potential mechanism by which intradialytic exercise improves CVD risk and reduces bone disorders in dialysis patients.

Elevated serum phosphate levels also are believed to contribute to vascular calcification. Vaithilingam et al. previously showed that 1 week of intradialytic cycling reduced serum phosphate levels, possibly by improving the perfusion of skeletal muscle during dialysis[144]. However, we did not find changes in phosphorous levels after our 4-month exercise intervention, and are uncertain of the reasons for our discrepant findings.

There were several limitations to our study. First, our sample size was relatively small, which may have limited our ability to detect differences in our primary outcomes due to low statistical power. The limited sample size also did not allow us to control for many factors that may have impacted the results, including diabetes status, medications, gender, and race. Furthermore, the 4 month intervention may have been too short to improve other CVD risk factors, including markers of inflammation, and metrics related to cardiovascular and renal function. Additional research with larger study populations and longer interventions will be needed to more thoroughly assess the cardiovascular benefits associated with intradialytic exercise training. We also cannot rule out the possibility that the improvements seen in the EX group may be partially attributed to the increased individual attention and social interaction they received from the research staff while exercising, despite our best attempts to socialize equally with patients in the CON group. Finally, we collected medication lists at baseline only, so any changes in medications (e.g., addition of immunosuppressive medications) that occurred after this time may have affected our results.

In summary, we demonstrated four months of intradialytic endurance exercise improved physical performance, and reduced one marker of serum oxidative stress (TBARS), alkaline phosphatase, and epicardial fat levels. These data suggest potential mechanisms by which intradialytic exercise training may reduce CVD risk; including a novel CVD risk factor in this population (epicardial fat thickness). Furthermore, this study adds to the considerable evidence providing support for the adoption of intradialytic exercise as a standard component of care for hemodialysis patients.

Tables

Table 3.1 Subject Characteristics at Baseline and Final Testing.

	CONTROL		EXERCISE	
	Baseline	**Final**	**Baseline**	**Final**
Age (yr)	59.0±4.9		60.8±3.2	
Gender	5f, 3 m		4f, 3m	
Time on Dialysis (months)	44.6±12.2		63.3±8.7	
Dialysis prescription (h/wk)	9.9±0.9		10.0±1.1	
Diabetic (%)	50%		43%	
BMI (kg/m^2)	29.0±2.0	28.3±1.8	30.1±2.4	30.3±2.5
SBP (mmHg)	128.6±10.1	153.0±17.2	150.0±2.3	147.1±14.9
DBP (mmHg)	74.6±5.4	85.7±7.7	74.4±3.2	77.3±8.7
Serum Parameters				
Cholesterol (mg/dL)	146.7±14.6	136.7±13.1	175.6±23.1	164.5±23.6
Albumin (g/dL)	3.9±0.14	3.9±0.15	3.8±0.09	3.8±0.06
Potassium (mEq/L)	4.6±0.16	4.9±0.25	4.8±0.25	4.9±0.30
Calcium (mg/dL)	9.1±0.26	8.86±0.25	9.1±0.36	8.8±0.59
Phosphorous (mg/dL)	6.3±0.73	5.9±0.5	5.2±0.39	6.5±0.76
Ca x P product	58.7±7.47	52.8±5.06	47.6±4.25	60.1±6.84
ALP (U/L) $^{++}$	102.0±12.6	116.8±14.99	110.5±14.77	87.4±11.34*
BUN/Creatinine ratio	6.9±0.83	6.6±0.85	6.4±0.52	6.7±0.46
CRP (mg/L)	5.2±0.78	4.9±0.69	6.2±0.22	6.0±0.67
IL-6 (pg/mL)	2.9±0.93	2.5±0.44	2.2±0.71	1.8±0.66
Fetuin-A (ng/mL)	18.2±2.7	19.2±3.26	19.8±1.01	17.6±0.60
TBARS (μmol/L) $^{++}$	7.2±.0.7	6.9±1.31	9.5±1.55	5.9±1.05*
Hematocrit	36.8±1.9	38.5±0.5	35.1±1.6	38.7±1.1
Cardiac Measures				
LV MI (g/m^2)	172.3±32.62	154.4±25.83	144.0±40.99	127.4±18.17
Relative Wall Thickness	0.68±0.04	0.63±0.03	0.66±0.08	0.62±0.08
LA VI	35.5±7.26	27.2±6.70	35.37.24	27.9±5.52
MPI	0.41±0.04	0.41±0.05	0.37±0.07	0.29±0.03

Data are reported by means ± S.E.M. $^{++}p<0.05$ for an interaction effect between time and physical activity group. *p< 0.05 compared to baseline measure within a physical activity group. Ca x P Product = Calcium phosphorus product; CRP= C-reactive protein; IL-6 = Interleukin 6; TBARS = Thiobarbituric acid reactive substances; PON = Paraoxonase; ALP = Alkaline Phosphatase; SBP and DBP = systolic and diastolic blood pressure, respectively; LV MI = left ventricular mass index; LA VI = left atrial volume index; MPI = myocardial performance index. h/wk = hours per week undergoing dialysis treatment.

Table 3.2: Etiology of CKD and Underlying Cardiovascular Disease in Study Patients

Etiology of CKD	CON (n=8)	EX (n=7)
Unspecified Hypertensive Renal Disease	3	3
Diabetes with Renal Manifestations Type 2	1	1
Focal Glomerulosclerosis	1	
Membranous Nephropathy		1
Diabetes Type 2 without Complication		1
Scleroderma	1	
Amylodisis		1
Etiology Uncertain	2	
Cardiac Disease		
Congestive Heart Failure	2	2
Myocardial Infarction	3	
Enlarged Heart	1	
Heart Murmur		1
No reported cardiac disease	2	3

Figures

Figure 3.1

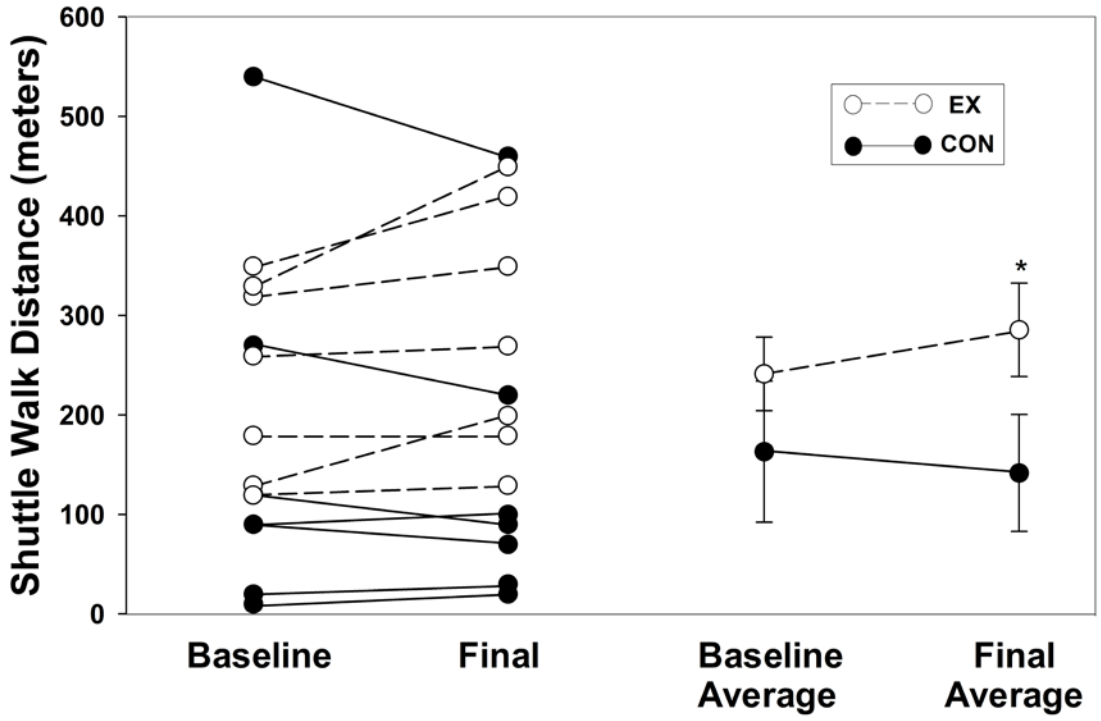

Figure 3.1. Performance on shuttle walk test. Patients in both the EX and CON groups had physical performance measured at baseline and final testing using an incremental shuttle walk test. Changes in the distance walked during the shuttle walk test for each individual are shown on the left, with group averages ± S.E.M. on the right. Performance on the test increased from baseline to final testing in the EX group ($p < 0.05$), but did not change in the CON group.

Figure 3.2

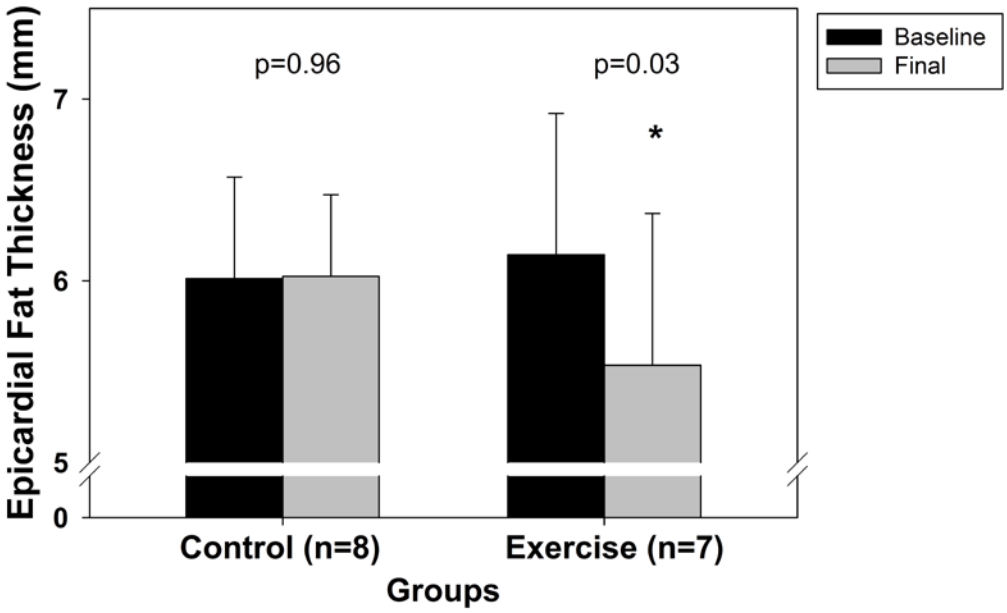

Figure 3.2. Epicardial fat thickness. The thickness of the epicardial fat layer was measured by echocardiography at baseline and final testing in all patients. There was a significant reduction in epicardial fat thickness (-9.8%) in the EX group, but not in CON. Values are expressed as means ± S.E.M. *$p<0.05$.

Figure 3.3

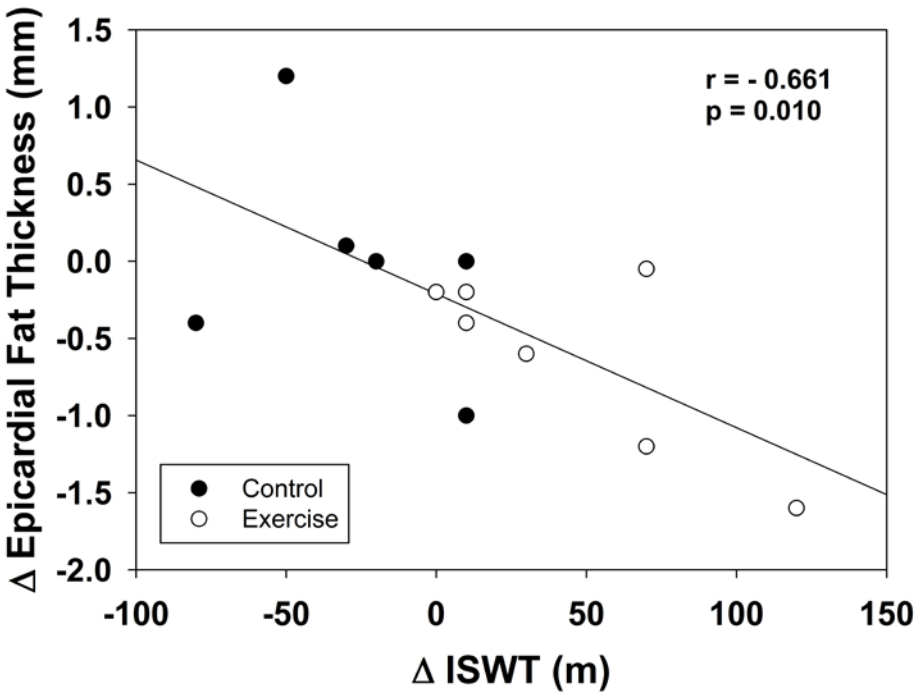

Figure 3.3. Correlation between the change (Δ) in performance on ISWT and the change (Δ) in epicardial fat thickness. When data from both groups (EX and CON) are combined, the Δ ISWT was significantly correlated with Δ epicardial fat thickness (r = -0.66, p=0.01).

CHAPTER 4

PROTEIN INTAKE AND EXERCISE TRAINING IN A MOUSE MODEL OF RENAL INSUFFICIENCY[a]

Introduction

Chronic Kidney Disease (CKD) patients have significantly elevated rates of osteoporosis[189] along with a specific metabolic bone remodeling disorder known as chronic kidney disease-mineral bone disease (CKD-MBD) [35] leading to increased fracture risk, mortality, and morbidity[190]. Elevated parathyroid hormone, common to CKD patients, stimulates resorption of calcium from bone, leading to low bone density and strength, as well as to ectopic mineral deposition [36].

The abnormal mineral metabolism is not confined to the bone, but also affects the vasculature. Cardiovascular disease (CVD) is the leading cause of death in CKD patients, and the risk may be partially explained by excessive vascular calcification (VC) in CKD patients[17-19]. Historically, loss of mineral from the bone and vascular calcification have been considered independent disorders, but there is emerging evidence that they are mechanistically linked; VC is now recognized as an active, regulated process with many properties similar to bone formation[39-41].

Most strategies aimed at inhibiting the progression of VC and bone disorders in CKD have focused on pharmaceutical interventions known to modify traditional CVD risk factors or improve bone density. However, many of these treatments have substantial side effects and are very expensive, indicating the need for additional therapies. A non-pharmacological approach that has shown promise in improving risk factors for both CVD and bone disorders is the consumption of soy protein rich in isoflavones. Studies in animal models clearly demonstrate benefits of soy protein and/or isoflavones in reducing atherosclerosis[110] and increasing bone mineral density (BMD) [111, 112]. As for CVD, soy isoflavones may reduce long-term risk in

humans by improving blood lipids [113, 114] and blood pressure. However, many studies have shown no effect of soy supplementation[120-122] on CVD risk and/or bone health in humans, and a recently published review indicates that the benefits of soy in humans may be more modest than suggested by the animal studies[123]. In addition, it is not known if these effects will persist in the context of CKD.

The benefits of exercise on cardiovascular risk and bone health have been well established in healthy populations, but few studies have looked at the effect of weight-bearing endurance exercise training for individuals with CKD, particularly in CKD stages 3-4 representing moderate to severe renal impairment. Exercise training in populations with normal kidney function improves traditional risk factors for atherosclerosis such as hypertension, plasma lipids, glucose and inflammatory variables[140]. Many of these same factors may increase the development of VC, so it is reasonable to assume that exercise training may also reduce VC by improving the CVD risk factor profile. Few studies have considered the effects of exercise training on bone health in individuals with CKD, and this is the first study to consider the relationship between exercise, bone, and vascular health in a model of CKD.

Due to the complex pathogenesis of CKD, it has been suggested that multiple therapeutic interventions will be necessary and should be used simultaneously to reduce co-morbidities in this population[191]. Previous animal studies have demonstrated prevention of bone loss and reduction of cardiovascular risk with a combination of soy isoflavones and exercise[192, 193], but this approach has not been examined in a model of kidney disease. Therefore, the purpose of this study was to evaluate the effectiveness of a soy protein diet and exercise training, alone and in combination, on vascular and bone measures in a mouse model of moderate to severe renal insufficiency. Apo $E^{-/-}$ mice were chosen because they rapidly develop atherosclerosis when placed on a high fat diet due to disordered lipid metabolism, allowing for a study of the relationship between CVD and CKD in this model of uremia-induced accelerated atherosclerosis[194]. Furthermore, Nikolov et al have demonstrated disordered bone metabolism in this model[195] which allows for the inclusion of this co-morbid condition in our intervention study. We hypothesized both exercise and consumption of a soy protein diet would attenuate development of atherosclerotic lesions and bone disorders, and that the effects of the combined treatments would be additive.

Materials and Methods

Animals. Sixty female apolipoprotein $E^{-/-}$ (B6.129-*Apoe*^{tm1Unc}/J, #002052) were obtained from The Jackson Laboratories (Bar Harbor, Maine) at six weeks of age and 18-20 grams. The mice were individually housed in plastic cages in a temperature controlled facility and maintained on a 12:12 hour light/dark cycle. Animals were provided ad libitum access to standard rodent chow and water. Animals were adapted to diet and environmental conditions for two weeks before initiation of the study protocol. All experiments and protocols were approved by the Illinois Animal Care and Use Committee at the University of Illinois, Urbana-Champaign.

Surgical Creation of Uremia. At 8 weeks of age all mice underwent a two-step surgical procedure to induce uremia. Animals were anesthetized using a combination of oxygen and 1-3% isoflurane. For the first procedure, the right kidney was approached through a 2cm long lumbar incision and was exposed by fine dissection of the surrounding tissues. The anterior and posterior poles of the kidney were resected, leaving the middle segment of the kidney intact. At 10 weeks of age, following a two week recovery, a total nephrectomy of the left kidney was performed by ligation of the renal artery with a 5-0 silk suture followed by excision of the kidney. Establishment of the surgical model was determined by plasma urea concentrations between 12 and 25 mM (normal mouse plasma urea $\leq$ 8 mmol/L). At 12 weeks of age, after an additional two weeks recovery, animals with urea levels > 12 mmol/L were randomized into one of the following four groups for the 16 week intervention: casein diet, sedentary (Cas/Sed, n=16); soy protein diet, sedentary (Soy/Sed, n=18); casein diet, exercise-trained (Cas/Ex, n=14); soy protein diet, exercise-trained (Soy/Ex, n=12). The different number of animals in each group reflects the number that survived the high-intensity surgery until the completion of the 16 week intervention.

Diet and Exercise Protocol. The compositions of the experimental diets are outlined in **Table 4.1**. Both diets were purified diets that derived 15% of kilocalories from fat and differed in the protein source. The protein content in the diet was chosen based on the level of protein provided in standard rodent chow. The soy protein diet (TD.06653 Harlan Teklad, Madison, WI) contained 20.0% w/w soy protein isolate and provided 250 mg of isoflavones/kg diet while the control diet (TD.06650 Harlan Teklad, Madison, WI) contained 20.0% w/w casein and contained

no isoflavones. The diets were specially formulated by Harlan Teklad for this study to be approximately matched for sulfur amino acids, available phosphorus, calcium, sodium, potassium, magnesium, iron, and choline. The animals received standard chow until 12 weeks of age (2 weeks after second surgery) at which time they were randomized to a purified experimental diet.

The exercise protocol consisted of running on a motorized treadmill (Jog-a-Dog, Toledo, Ohio) 5 days per week for 45 minutes/day during the intervention period. The mice were acclimated to the treadmill exercise such that by the second week of training they ran at 15 m/min. This corresponds to 60-75% VO_2max in C57BL/6J mice[196], which represents "moderate-intensity" exercise in these animals[197-199]. Negative reinforcement was not used but rather gentle prodding with a blunt instrument was employed to encourage the mice to exercise. Sedentary mice were not provided access to the treadmill.

Serum assays. Fasting blood samples were drawn from the retroorbital vein on two occasions: prior to the start of the dietary intervention and prior to euthanasia. Plasma and serum was collected by centrifugation, aliquoted into microfuge tubes, and stored at -80°C until analyzed. Plasma urea (BioAssay Systems, Hayward, CA) and total cholesterol (Infinity Incorporated, Melbourne, Australia) were measured enzymatically at both time points.

Quantification of Aortic Calcium and Atherosclerotic Lesions. Following the intervention period, each mouse was sacrificed by CO_2 asphyxiation. The heart, including the proximal aorta, was removed, washed in phosphate-buffered saline to remove the blood, placed in freezing medium (OCT, Fischer Scientific, Pittsburg, PA), and stored at -80°C until sectioning. Serial sections of heart tissue measuring a thickness of 8μm from the start of the aortic sinus to the ascending aorta were sliced, mounted on glass slides (Fischer Scientific, Pittsburg, PA), and frozen at -20^0C, as described by Daugherty et al[200].

Calcium in the cryosections was identified by Alizarin red at three specific anatomical regions of the proximal aorta each separated by approximately 200 μm , which coincide with the start of the aortic sinus, the orifices of the coronary arteries, and the start of the ascending aorta. For Alizarin Red staining, slides containing the cryosections were rinsed in 70% ethanol, placed in Alizarin red stain for up to 5 minutes, and rinsed in distilled water twice. Quantification of

calcium staining was graded on a scale of 0-4 by blinded investigators. 0 = no staining, 1 = weak, non-discrete staining, 2 = light, discrete staining, 3 = intense staining or multiple areas of light staining 4 = multiple, intense areas staining[201, 202]. Scores were determined by averaging the scores of four blinded investigators.

Additional slides containing cryosections of the same regions of the proximal aorta were stained for neutral lipids using Oil Red O to detect and quantify intimal atherosclerotic lesions at the same sites. For Oil Red O staining, slides containing the cryosections were rinsed with 60% isopropyl alcohol for 5 min, blotted, and then stained with filtered Oil Red O for 10 minutes and blotted once more. Slides were rinsed again with 60% isopropyl alcohol for 2 minutes, blotted, rinsed with distilled water, blotted, and then stained with hematoxylin for 10 seconds. The quantification was done using image analysis software (Microsoft Photoshop) and expressed as total area of the aorta covered with lipid-filled lesions.

Measurement of Bone Microarchitecture by Micro-Computed Tomography (μCT). The right femur was removed from each animal after sacrifice, cleaned of surrounding tissue, and stored in ethanol at -20°C. High resolution images of the femur were acquired using a desktop microtomographic imaging system (μCT40; Scanco Medical AG, Basserdorf, Switzerland). Each tissue sample was scanned at 45 keV with an isotropic voxel size of 6 μm, and the resulting two-dimensional cross-sectional images were shown in gray scale. Scanning began in the mid-epiphysis and extended proximally for 3.6 mm (600 CT slices/specimen). The scans resulted in reconstructed 3-D data sets with the μCT Evaluation Program. Trabecular bone was determined by specifying regions of interest with the provided software program. Using these regions of interest, the bone volume, trabecular volume and composition were calculated by the program using non-destructive three-dimensional reconstruction as described[203].

Statistical Analysis. All statistical tests were conducted using SPSS software with two-tailed significance set at $\alpha = .05$. Plasma variables, atherosclerotic lesions, and bone outcomes were assessed using a general linear model univariate two-way ANOVA with diet (casein or soy) and activity (sedentary or exercise) as between-subjects factors. In any analysis, if significant interactions were observed, variables were analyzed with post-hoc Tukey's test. Main effects were only considered when interactions were not significant, as a significant interaction indicates

that the effect of one independent variable depends on the value of the other. The ranked calcium scores were analyzed using the Kruskal-Wallis test for nonparametric data. Data are presented as mean ± SEM unless otherwise noted.

Results

Body weight and plasma variables. Body weight and plasma variable values can be found in **Table 4.2**. There were no interactive or main effects on change in body weight from baseline to final measurement. There was no treatment effect on change in plasma cholesterol, although all groups had a decrease in measured plasma cholesterol from baseline to the end of the intervention period. There was no interactive effect on plasma urea, but there was a significant diet main effect for the change in plasma urea values ($F_{1,49}$=0.614; p=0.013), with greater reduction of plasma urea in the Soy/Sed and Soy/Ex groups compared to Cas/Sed and Cas/Ex.

Atherosclerotic Lesion Area and Aortic Calcium. There was no significant effect of diet or exercise on atherosclerotic lesion area at the position in the proximal aorta corresponding to the cusps of the aortic valves or at the branch point of the coronary arteries (**Figure 4.1a**). At the position of the proximal aorta corresponding to the start of the ascending aorta, there was a significant interaction effect ($F_{1,54}$=4.945; p<0.05) with the Cas/Ex animals tending to have the greatest lesion area at this site. Nonparametric analysis of ranked calcium scores revealed no significant differences between groups, although the mean rank for the control group was the highest at each of the three aortic sections described above (**Figure 4.1b**).

Bone Microarchitecture. There was a significant interaction effect of diet and activity ($F_{1,51}$=4.006; p=0.05) on total volume (TV) but not for log-transformed bone volume. However, there were significant main effects of diet ($F_{1,50}$=4.086; p<0.05) and exercise ($F_{1,50}$=9.007; p=0.004) for log-transformed bone volume (BV) resulting in an overall main effects of diet ($F_{1,50}$=8.596; p<0.01) and exercise ($F_{1,50}$=10.070; p<0.01) on the total volume to bone volume ratio (BV/TV), or relative bone volume (**Figure 4.2a**). The Soy/Ex group showed a 61% increase in relative bone volume compared to the Cas/Sed animals. There were no interactive effects on measures related to bone architecture, but there were several main effects of our treatments. This included a significant main effect of diet ($F_{1,50}$=13.112; p=0.001) and activity

($F_{1,50}$=11.325; p=0.002) on trabecular number, with the Soy/Ex group tending to have the greatest number of trabeculae compared to Cas/Sed (**Figure 4.2b**). There were also significant main effects of diet ($F_{1,50}$=9.990; p=0.003) and activity ($F_{1,50}$=5.873; p=0.02) on trabecular separation (**Figure 4.2c**). Again, the Soy/Ex group tended to have less separation compared to Cas/Sed. There were also significant main effects of diet ($F_{1,51}$=9.950; p=0.003) and exercise ($F_{1,51}$=4.149; p<0.05) on the log transformation of trabecular connective density (**Figure 4.2d**), with a trend for an interaction effect (p=0.089). In a manner similar to trabecular number and separation, the highest connective density was in the Soy/Ex animals compared to Cas/Sed. There were no effects of treatment on trabecular thickness (0.0375±0.001, 0.0482±0.001, 0.0515±0.001, 0.0492±0.001 for Cas/Sed, Soy/Sed, Cas/Ex and Soy/Ex, respectively).

Discussion

The primary findings in this study were that both a soy protein diet and exercise training significantly increased relative bone volume and improved bone microarchitecture in a mouse model of disordered lipid metabolism and surgically-induced renal insufficiency . The effect tended to be greatest when these interventions were administered in combination. Although the interaction effect was not significant for any of the bone measures (with the exception of total volume as a component of relative bone volume, or BV/TV), the Soy/Ex combination group tended to have more favorable bone variables when compared to the other treatment groups. Furthermore, we found a main effect of diet on the change in plasma urea levels during the intervention period, with animals on the soy diet having lower plasma urea levels. Finally, neither soy nor exercise had much effect on the vasculature. There were no group differences in aortic calcium in any of the 3 proximal aorta sections, and no interactive or main treatment effects in atherosclerosis in 2 of the 3 proximal aorta sections. Surprisingly, we observed an interaction effect of diet and exercise on atherosclerotic lesion area in the section of the proximal aorta corresponding to the start of the ascending aorta, with the exercise only animals having the greatest lesion area. However, this effect was ameliorated with the combination treatment. These data suggest that a combination of a soy-rich diet and endurance exercise training may be beneficial for protection of bone health and preservation of renal function in individuals with CKD, although the effects in the vasculature remain unclear.

Observational studies have linked high soy protein consumption with lower osteoporotic fracture risk, but the treatment effect of soy protein on bone density and microstructure remains controversial in healthy populations, and even less is known about the effects of soy protein on bone health in the context of CKD. Intake of soy protein has been shown to improve bone mineral density while others have shown no effect on a variety of bone parameters, and much attention has been focused on the phytoestrogens contained in soy protein. Furthermore, recent research suggests that the efficacy of soy protein on bone depends on the ability of the body to convert the soy isoflavone daidzein to equol, a potent estrogenic metabolite. Only 20-35% of the population have been reported to have the ability to metabolize daidzein to equol via intestinal microflora[204], and this may partially explain the differential responses to soy protein interventions in the context of bone health; the effectiveness of equol production in the context of kidney disease is not known. In addition, mice produce equol prolifically when fed diets containing soy protein[205], and may explain why the efficacy of soy protein is more modest in human studies. In this study, we found a significant main effect of diet not only on relative bone volume, but also on bone microstructure including trabecular number, thickness, and density.

Furthermore, we also found a main effect of activity on the same parameters, with a trend toward a greater effect in the combined soy plus exercise group. Several studies have found a synergistic effect of phytoestrogens and exercise on improving bone parameters in ovariectomized mice, rats, and premenopausal women (reviewed in [206]). Exercise is known to stimulate the estrogen receptor on bone, and this action may contribute to the enhanced bone microarchitecture seen in the soy and exercise combination group. Oh and colleagues have suggested soy isoflavone supplementation protects against exercise-induced oxidative stress[193], which may explain why the benefits demonstrated in this study were more pronounced in the combination group compared to exercise training alone. While many of these animal studies used subcutaneous injections of soy isoflavones in doses ranging from 0.4-6.4 mg/day, we have demonstrated beneficial effects of intact soy protein on bone health in our uremic mouse model using 250 mg of isoflavones/kg diet (per manufacturing specifications, Harlan Teklad, Madison, WI) with an effective dose of about 0.625 mg of isoflavones per day. We believe the distinction between soy protein use and isoflavone supplementation to be important, as studies have suggested that the benefits of soy may be due to other components of the intact protein in addition to the phytoestrogenic effects of soy isoflavones.

Most studies have looked at the effects of soy protein on bone loss in women, targeting the stages before and after menopause. However, the metabolic and hormonal conditions and the subsequent consequences on bone may be different for an individual with CKD compared to healthy pre- or post-menopausal woman and the benefits of soy in pre- and post-menopausal women may not translate to this population. Specifically, CKD patients can experience both high- and low-turnover bone disease in response to the metabolic changes induced by declining kidney function. To date, no study has looked at the efficacy of soy protein for bone parameters in the context of CKD, either with animal models or in human populations. Furthermore, soy protein may represent a valid therapy in this population for treating CKD associated bone conditions, as studies have shown that soy proteins do not effect glomerular filtration rates or post-prandial renal blood flow in the same way that animal proteins do after a high protein meal[207]. Therefore, with soy protein consumption, CKD patients are able to get the benefits of protein without the negative effects on the kidneys that have been reported for animal proteins.

In addition to the mechanisms listed above, soy protein supplementation may have effects on CVD that are specific to CKD. For example, soy supplementation has been associated with reduced oxidized-LDL in hemodialysis patients[115], reduced urinary albumin excretion and LDL/HDL cholesterol ratio[116] in patients with diabetic nephropathy, and preservation of renal function in moderate kidney disease[116, 117]. In this study we measured plasma urea as a measure of the kidney's ability to metabolize nitrogenous waste; this ability is impaired with renal insufficiency and the consequent buildup of urea and other toxins may contribute to the common co-morbidities of CKD. We found a main effect of diet on change in plasma urea levels measured at baseline and prior to sacrifice, suggesting that soy protein may be improving uremic conditions. Protein restriction as a means to preserve renal function has been widely practiced for many years, but the efficacy of this treatment has recently been called into question[208]. As individuals with CKD progress to renal failure, the incidence of protein wasting sharply increases[9] and is associated with low quality of life, low physical functioning, and even mortality[12]. The high prevalence of protein malnutrition in CKD patients may be due in part to the clinical recommendations to restrict protein during moderate to severe renal impairment. As this study suggests, the addition of soy protein in moderate CKD may not have any detrimental effects on kidney function and has the potential to slow or prevent the occurrence of protein wasting in later stages of CKD.

We did not see any improvements in the vasculature, either in atherosclerotic lesion area or aortic calcium score, for any of the treatment groups compared to the control animals. In fact, we found an interaction effect of diet treatment group and exercise training on atherosclerotic lesions at the site of the proximal aorta corresponding to the ascending aorta, with the exercise-trained animals having significantly higher lesion area compared to the other groups. Combining soy protein diet plus exercise seemed to ameliorate this effect, but the lesion areas in the combination group were not significantly different from the control sedentary animals. Exercise training has been shown to reduce CVD risk in individuals with normal kidney function and to improve physical function in CKD, but virtually no studies to date have looked at exercise training on CVD outcomes in CKD patients, and these studies have focused on patients with renal failure (reviewed in [209]). Phan et al showed that pharmacological treatment in an identical animal model slowed the progression of uremia-associated atherosclerosis[210], while the same group found a reduction in atherosclerosis with the antioxidant n-acetylcysteine[211], highlighting the high burden of oxidative stress in CKD. However, we did not see such an effect with dietary or exercise intervention on aortic atherosclerosis or calcification, and this may be due to the severity of the surgical procedure used to create this model of renal insufficiency. It is possible that the deficiency of apoE combined with the high stress of the 5/6 nephrectomy was too severe to warrant endurance exercise training as a therapeutic means to reduce uremia-associated CVD with this particular model. Studies on the beneficial effects of exercise on cardiovascular risk in humans with varying degrees of kidney function are clearly needed.

There were several limitations to this study. First, we feel that the severity of the surgery with this model may have prevented the dietary and exercise intervention from having any effect in the vasculature. As these are primarily preventative measures, this may provide a rationale to begin such treatments before the development of severe renal impairment. Additionally, it is not known whether the same effects on the vasculature or bone would be seen in male mice, accounting for the estrogenic properties of soy isoflavones. As mentioned earlier, the efficacy of soy protein may depend of the ability to metabolize the isoflavone daidzein to equol; all mice are equol producers while only a percentage of humans possess this capability. Therefore, it is unclear if these results would be seen in humans with CKD. Food intake was not measured with these animals, so we are not able to determine the effect of food intake on the results of this study. Finally, apo E-/- animals have been shown to have an increase in bone mass compared to

wild type mice; renal insufficiency in this model decreases bone volume. It is not known based on this current study if the increase in bone volume would translate to improved fracture risk, or if it represents a pathological disorder of bone remodeling; furthermore, without indices of bone formation and resorption, we are unable to make these determinations.

In summary, we found beneficial effects of soy protein and exercise on properties of bone and plasma urea in mice with surgically induced renal impairment showing a trend toward a stronger effect with a combination approach. Further research involving individuals with CKD is needed to test the efficacy and practicality of these lifestyle interventions on preventing the decline in cardiovascular and bone health associated with CKD and perhaps the preservation of residual renal function in early stages of the disease.

Tables

Table 4.1: Composition of Study Diets

Selected Components	**Casein Diet HT TD.06650**	**Soy Diet HT TD.06653**
Isolated Soy Protein[1]	0.0 g/kg	200.0 g/kg
Casein	200.0 g/kg	0.0 g/kg
Sucrose	150.0 g/kg	150 g/kg
Corn Starch	371.8 g/kg	368.8 g/kg
Maltodextrin	120.0 g/kg	120 g/kg
Cellulose	50.0 g/kg	50.0 g/kg
Soybean Oil[2]	60.0 g/kg	60.0 g/kg
Mineral Mix AIN-93G	35.0 g/kg	35.0 g/kg
Vitamin Mix AIN-93G	10.0 g/kg	10.0 g/kg

[1]Contains 250 mg/kg isoflavones (per manufacturer's specifications)
[2] Soybean oil does not provide any isoflavones.
HT= Harlan Teklad

Table 4.2: Body weight, plasma cholesterol, and plasma urea at baseline and final measurement for all treatment groups[1].

	Cas/Sed (n=16)	Soy/Sed (n=18)	Cas/Ex (n=14)	Soy/Ex (n=12)
Body Weight:Baseline (g)	17.5±0.8	17.2±0.7	16.9±1.1	17.8±0.8
Body Weight:Final (g)	21.3±0.7	20.2±0.9	19.2±1.6	21.0±1.1
Body Weight: Delta	3.8±0.8	3.0±0.8	2.3±1.1	3.2±1.0
Cholesterol:Baseline (mg/dL)	409.9±25	484.4±25	466.5±22	429.1±15
Cholesterol:Final (mg/dL)	328.2±14	407.5±18	378.4±19	385.3±12
Cholesterol:Delta	-79±25	-78±33	-105±17	-44±15
Urea:Baseline (mmol/L)	16.3±0.9	15.9±1.0	15.9±0.7	17.0±0.6
Urea:Final (mmol/L)	17.0±0.7	14.1±0.6	15.6±0.5	15.3±0.7
Urea:Delta	0.71±0.9	-1.78±0.8*	-0.26±0.5	-0.73±0.5*

[1] Values are presented as mean ± SEM. * $p<0.05$ for diet main effect. Cas/Sed=casien diet, sedentary; Soy/Sed=soy diet, sedentary; Cas/Ex=casein diet, exercise trained; Soy/Ex=soy diet, exercise trained

Figures

Figure 4.1

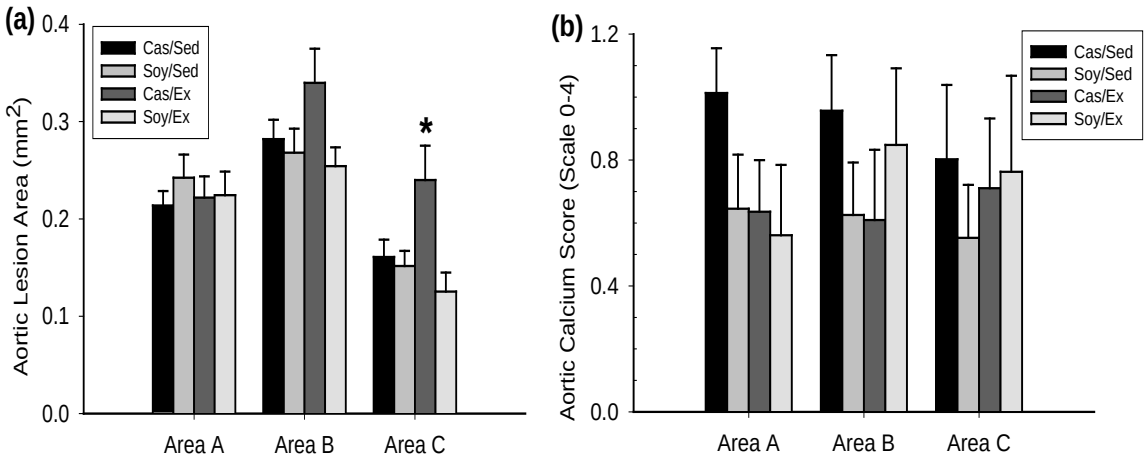

Figure 4.1: Atherosclerotic lesions and aortic calcium in sections corresponding to the cusp of the aortic valves (Area A), the branch point of the coronary arteries (Area B), and the ascending aorta (Area C). There was a significant interaction between experimental diet treatment and exercise training on atherosclerotic lesions at the site corresponding to the ascending aorta (Area C) with significantly higher lesion area in Cas/Ex (a). There was no significant interactive or main effects of diet or activity on aortic calcium score (b). * $p<0.05$ for an interaction effect at this site. Cas/Sed=casien diet, sedentary (n=16); Soy/Sed=soy diet, sedentary (n=18); Cas/Ex=casein diet, exercise trained (n=14); Soy/Ex=soy diet, exercise trained (n=12).

Figure 4.2

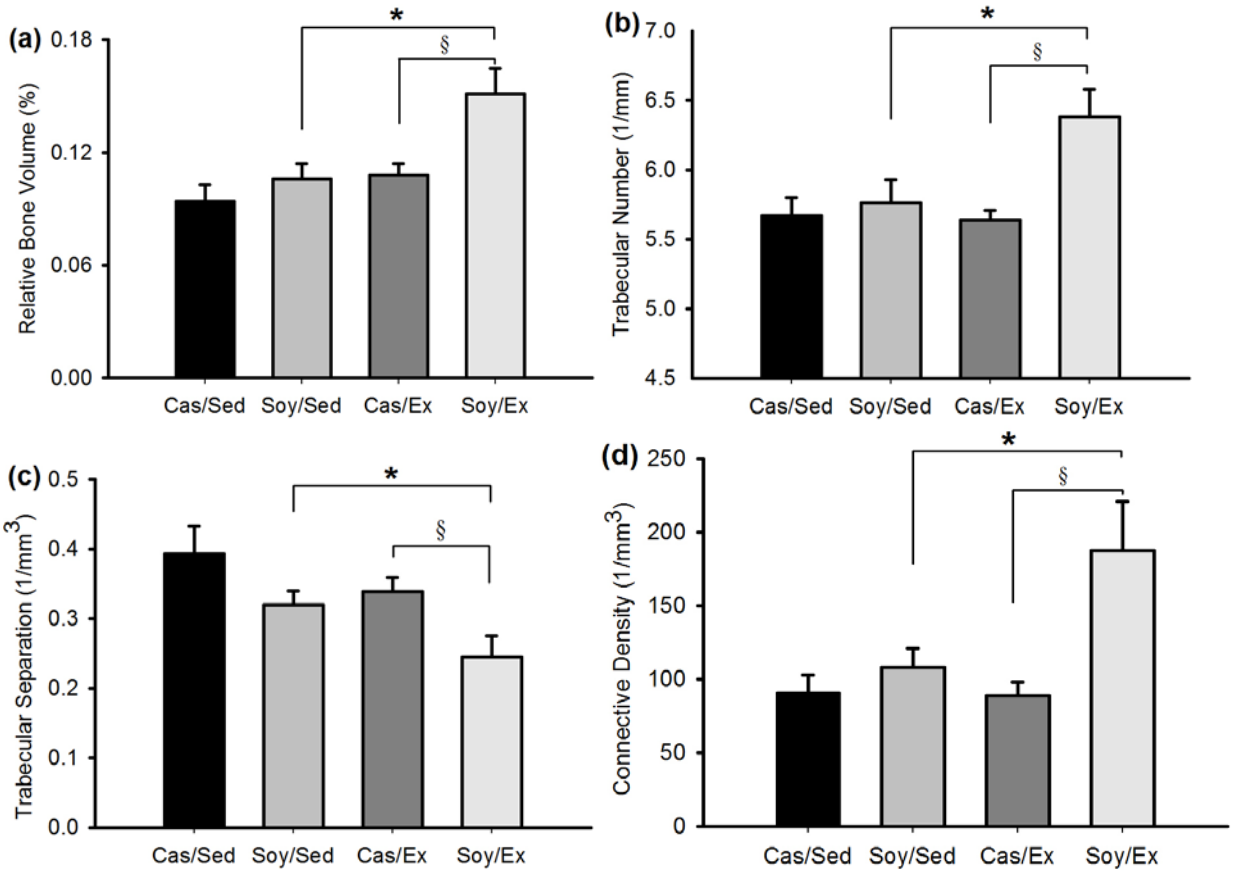

Figure 4.2: Relative bone volume and bone microstructure in response to dietary and activity intervention. There were no interactive effects for diet and activity on any variable measured. However, there was significant main effects of soy protein diet (* $p<0.05$) and exercise training (§ $p<0.05$) on relative bone volume (a), trabecular number (b), trabecular separation (c), and connective density (d). Cas/Sed=casien diet, sedentary (n=16); Soy/Sed=soy diet, sedentary (n=18); Cas/Ex=casein diet, exercise trained (n=14); Soy/Ex=soy diet, exercise trained (n=12).

CHAPTER 5

FACTORS RELATED TO CHRONIC KIDNEY DISEASE CO-MORBIDITIES

Introduction

Chronic kidney disease (CKD) patients receiving hemodialysis treatment (CKD stage 5) suffer from a variety of co-morbid diseases, many of which may be mechanistically linked and are influenced by a chronic inflammatory state. Cardiovascular disease (CVD) is the leading cause of death in individuals with CKD and cardiovascular events are 10 to 30 times greater in CKD than in age- and sex-matched subjects in the general population[14]. Traditional CVD risk factors, including elevated cholesterol, hypertension, age, and gender, do not fully explain the elevated CVD mortality, and it may be partially attributed to novel cardiovascular risk factors, including excessive inflammation and oxidative stress and abnormalities in mineral metabolism that contribute to increases in arterial stiffness, intima-media thickness, and vascular calcification.

Dialysis patients also have significantly elevated rates of osteoporosis[189] along with a specific metabolic bone remodeling disorder known as chronic kidney disease-mineral bone disease (CKD-MBD) [35] leading to increased fracture risk, mortality, and morbidity[190]. Furthermore, there is emerging evidence that the loss of mineral from bone and the stiffening of the vasculature are mechanistically linked. In response to a variety of stimuli, vascular smooth muscle cells (VSMC) are stimulated to differentiate into an osteoblast-like phenotype[41] capable of producing a bone-like matrix and mineralizing in the presence of calcium and phosphorus, thereby increasing the stiffness of the vessel. This process appears to be regulated by proteins, including fetuin-a and alkaline phosphatase (ALP), secreted from VSMC and endothelial cells that either promote or inhibit calcium and phosphate precipitation and the formation of hydroxyapatite crystals[43]. The expression of these proteins in vascular cells and bone may be mediated in part by inflammation[51] and malnutrition[55]

In fact, malnutrition-inflammation complex is very common in dialysis patients for reasons including poor nutrient intake, protein losses during dialysis, and elevated whole body and skeletal muscle protein catabolism that occurs during dialysis (reviewed in[10]). Protein malnutrition is associated with a loss of lean mass and functional declines in hemodialysis

patients[212] that reduce physical activity levels and exacerbates co-morbidities including CVD and bone disorders.

Beyond the role of decreased physical activity levels, it is well-established that inflammation contributes directly to the development and progression of other CKD co-morbidities. Evidence suggests inflammatory mediators are associated with cardiovascular complications in CKD patients[64], and recently research has begun to focus on the impact of epicardial adipose tissue, a visceral fat depot surrounding the heart, on CVD risk due to its role as a cytokine-secreting tissue. Inflammation is also is implicated in the development of renal bone disease[56, 72, 73]. Interleukin-6 (IL-6), expressed by both inflammatory cells and osteoclasts, has been shown to promote bone resorption in patients with renal osteodystrophy[73], and also has been implicated in high bone turnover in multiple myeloma[74] and estrogen deficiency[75]. Taken together, these findings suggest that the chronic inflammation associated with advanced CKD promotes the development and progression of many of the co-morbidities that reduce the QOL and increase mortality rates in this population.

Patients receiving hemodialysis therapy are at greater risk for a large number of these co-morbities compared to age- and sex- matched controls; this study aims to determine predictors of these important functional outcomes from a comprehensive set of variables including measures of cardiovascular risk, bone health, body composition, markers of inflammation, clinically relevant plasma variables, functional fitness, muscle strength and quality of life in hemodialysis patients. We hypothesize many of these factors are interrelated and also strongly influenced by inflammation.

Materials and Methods

Subject Recruitment, Screening, and Selection. Individuals with CKD receiving hemodialysis treatment at the Champaign-Urbana Dialysis Center in Champaign, IL and the Oak Park Dialysis Clinic in Oak Park, IL were recruited at their respective clinics. Patients that met the following criteria were enrolled in the study: **1)** Subjects must receive hemodialysis treatment at least 3 days per week. **2)** Subjects must be $\geq$ 30 years of age. 3**)** Subjects greater than 400lbs were excluded due to weight limitations on the dual x-ray absorptiometry (DXA) table. 4) Patients with congestive heart failure or chronic obstructive pulmonary disorder were excluded 5**)** Subjects must have been receiving dialysis treatment for $\geq$ 3 months as disruption in metabolic

factors is related to duration of dialysis treatment. A Health and Medical History Questionnaire was administered during the screening, and informed consent was obtained from each participant. All research protocols were conducted with the approval of the University of Illinois Institutional Review Board.

Sixty dialysis patients completed testing and were included in this cross-sectional analysis. With the exception of the blood chemistries, all testing and measurement were performed 18-24 hours after the most recent dialysis session on a non-treatment day. Blood collections were not performed on Mondays or Tuesdays to control for the extra day between treatments that occurs during the weekend. For example, blood would not be drawn for a Monday/Wednesday/Friday scheduled patient on Monday as they would not have dialyzed since Friday (two day lapse). For a Tuesday/Thursday/Saturday scheduled patient, blood would not be drawn during their treatment on Tuesday for the same reason. All testing was done on identical equipment at both sites; all blood chemistries and data analysis were performed in Champaign, IL.

Blood Chemistry. Non-fasted blood samples were drawn from patients at the dialysis clinic immediately after initiation of dialysis treatment during each participant's regularly scheduled treatment. Plasma was collected by centrifugation and divided into 1ml aliquots and stored at -80^{o}C until analyzed. Circulating levels of inflammatory markers interleukin-6 and polymorphonuclear elastase protease inhibitor complex were measured using commercially available ELISA kits (R&D Biosystems, Minneapolis, MN; EMD Chemicals Darmstadt, Germany). In addition, blood collected at the dialysis clinic during normally scheduled blood draws was assessed for standard clinical lab parameters (plasma albumin, phosphorus, calcium, etc) by a Spectra Laboratories, a renal specific laboratory service provider (Rockleigh, NJ).

Carotid Artery Compliance. A combination of ultrasound imaging of the common carotid artery with simultaneous arterial tonometry of the contralateral carotid artery (for estimation of carotid blood pressure) was used to determine carotid arterial compliance. Carotid ultrasound images were obtained from the common carotid artery, 1-2 cm proximal to the carotid bifurcation using a 7-13 MHz linear array transducer with a sampling rate of 1000 Hz. B-Mode and M-mode images were obtained and displayed simultaneously and automated wall tracking

software was used to detect changes in lumen size as described above**.** The electronic calipers were applied to the arterial wall using the B-mode image and wall tracking was conducted using the M-mode image in real time. Changes in lumen size between systole and diastole were recorded for 12 seconds and an ensemble average beat were constructed from which measurements of arterial compliance and stiffness were calculated. Arterial compliance (AC) and the beta stiffness index (β) were calculated together with lumen size in systole and diastole using the calculations shown below:

-AC (arterial compliance: index of blood vessel compliance) = π (Ds ×Ds - Dd×Dd) / [4 (Ps – Pd)]
-β (stiffness parameter: index of arterial stiffness) = In (Ps / Pd) / [(Ds – Dd) / Dd]

Brachial blood pressure was obtained using standard methods following a 10 minute rest period. Pressures were corrected for hold down pressure.

Epicardial Fat Thickness. The thickness of the epicardial fat layer was measured by transthoracic two-dimensional guided echocardiogram, as previously described[170]. In brief, standard parasternal and apical views were obtained with subjects in the left lateral decubitus position. Epicardial fat was identified as the echo-free space between the outer wall of the myocardium and the visceral layer of pericardium and its thickness was measured perpendicularly on the free wall of the right ventricle at end systole in three cardiac cycles [170]. Maximum epicardial fat thickness was measured at the point on the free wall of the right ventricle along the midline of the ultrasound beam, perpendicular to the aorta annulus. The average value of 3 cardiac cycles for each echocardiographic view was considered as the epicardial fat thickness.

Bone Mineral Density and Body Composition. Bone mineral density (BMD) and whole-body soft tissue composition were measured by DXA using a Hologic QDR 4500A bone densitometer (software version 11.2, Bedford, Massachusetts) from scans of the whole body, lumbar spine and proximal femur. All scans were analyzed and quality-controlled by the same two technicians. Short and long-term accuracy of the densitometer was verified by scanning a manufacturer's

hydroxyapatite spine phantom of a known density. Precision for DXA measurements of interest are ~1.0 – 2.0% in our laboratory. Mineral free lean mass (MFLM) was calculated from DXA values as lean mass of selected compartment – bone mineral content of selected compartment (whole body, trunk, leg, etc).

Anthropometric Measures. Barefoot standing height was measured to the nearest 0.1 cm with a stadiometer and body weight was measured on a balance scale with shoes and superfluous outer garments removed. Waist circumference was measured as the minimum circumference between the top of the iliac crest and the distal end of the rib cage along the midaxillary line. All measurements were taken in duplicate and averaged.

Functional Fitness Testing. Subjects underwent the following functional fitness assessments 1) *Chair Stand Test* where subjects were asked to stand up from a seated position as many times as possible within 30 seconds 2) *Arm Curl Test* in which subjects were asked to complete as many arm curls as possible during 30 seconds using either a 5 pound dumbbell for females, an 8 pound dumbbell for males 3) *Chair Sit and Reach Test* that asked subjects to reach forward with both arms to try and touch their extended leg to assess flexibility, 4) *Back Scratch Test* where subjects tried to touch their middle fingers behind their back to assess upper body flexibility, 5) 8 *Foot Up-and-Go Test* in which subjects walked around a cone placed 8 feet away from their chair and back again during a timed trial, and 6) *Stair Test* in which subjects walked up and down a flight of stairs during a timed trial. In addition, Gait Speed was determined by averaging the triplicate of time needed to walk a 10-meter course at a normal, natural pace.

Shuttle Walk Test. Participants underwent a shuttle walk test to assess physical performance in which they walk back and forth continuously on a 10 meter course. The walking was paced by beeps which were programmed to have participants maintain each speed for one minute and then the pace was increased. The speeds increased so that in each successive minute the speeds were as follows: 1.12, 1.54, 1.88, 2.26, 2.64, 3.02, 3.4, 3.78, 4.16, 4.54, 4.92, and 5.3 miles per hour. The patient continued until they failed to achieve the distance to the end of the course by the beep and the total time was calculated.

Measures of physical performance such as this shuttle walk test are frequently used to assess function in older and diseased people instead of more objective measures of aerobic capacity such as VO_2max testing. This is due to functional limitations like muscle weakness and shortness of breath that prevent these individuals from achieving standard criteria used in assessment of these more objective tests[136]. This shuttle walk test is well established as a part of the guidelines for assessment of fitness in patients with chronic pulmonary disease[213], and is often preferred to the six minute walk test because it is paced, and therefore more objective.

Muscle strength. Bilateral quadriceps femoris and hamstring muscle strength were evaluated using isokinetic testing modes. Following dynamometer calibration, knee extension and flexion isokinetic muscle torque were evaluated at a speed of 60 degrees per second on a Biodex System 3 dynamometer. The axis of rotation of the machine was aligned with the lateral epicondyle of the femur and the calf pad was positioned halfway between the lateral malleolus of the fibula and lateral epicondyle of the femur, and securely attached to the subject using straps. Participants performed two sets of 6 repetitions, with a 3-minute rest between sets, and the best effort was used for analysis.

Quality of Life Assessment. Physical and mental health status was measured by the 12-Item Short Form Survey derived from the Medical Outcomes 36-item Short Form Survey[214]. Global QOL was measured by the Satisfaction with Life Scale[215, 216]. This measure is a cognitive judgment of one's life taken as a whole, and is usually thought to involve comparing one's life against some standard. These scales have been shown to be reliable and valid indices of components of subjective well-being. The scales used in this analysis included the Basic Lower Extremity Function Scale (BLEF) to assess self-reported function in the lower extremities, self-reported Mental Health Score (MHS), Total Disability Score (TDS) as a composite of several disability-related subscales, and Godin Weekly Leisure Activity Score (GWLAS) as a measure of leisure-time physical activity for study participants.

Diet assessment. Participants were given two 24- hour food recall interview with a trained researcher on consecutive days to account for variations in eating patterns associated with dialysis treatment. The interview was conducted using a modified version of the United States

Department of Agriculture 5-pass method: the first pass asked for the patients to recall everything they ate during the previous 24-hours, the second pass probed for foods that may have been forgotten including beverages, snacks, and condiments, the third pass included prompts for portion sizes and food amounts, the fourth pass asked for details about the food including brand names, and the fifth pass consisted of a final review of the record. The records were analyzed for macronutrient composition and other dietary variables using Nutritionist Pro Data Analysis Software (Stafford, TX).

Statistical Analysis. All data are presented as mean ± SEM with a significance level set at $p<0.05$. Data records were analyzed by t-test for differences between treatment and non-treatment days. Multiple linear regression analysis was used to determine predictors of functional outcomes of interest. For all linear regression analysis, gender, diabetes status, and smoking were coded as follows: male=1, female=2; diabetes=1, no diabetes=2; smoker=1, nonsmoker=2. All analyses were conducted using SPSS v.17 (Chicago, IL).

Results

Patient Characteristics. Sixty patients undergoing dialysis treatment enrolled in this study and completed testing protocols. Subject demographics can be found in **Table 5.1**. The percentage of male participants was 56.7%, the mean BMI was 32.1±1.0 (obese category), and the mean time on dialysis treatment was 59.8±6.4 months.

Plasma variables are listed in **Table 5.2**. Measured levels of plasma IL-6 were 8.42±1.2 pg/mL and did not differ between genders. Plasma phosphorus, intact parathyroid hormone, blood urea nitrogen (BUN) and creatinine levels were elevated in this patient group compared to desirable reference ranges for dialysis patients according to the National Kidney Foundation Chronic Kidney Disease Outcomes Quality Initiative (NKF KDOQI). While plasma iron and tranferrin saturation levels were within normal range, ferritin levels were extremely elevated in both males and females and total iron binding capacity was lower than recommended clinical values. Additionally, mean hemoglobin and hematocrit levels were low, suggesting anemia of renal insufficiency. Mean plasma albumin levels of 3.94±.04 indicate normal nutritional status.

Diet Intake. Twenty-four hour dietary recalls were analyzed to assess nutrient intake on both dialysis treatment and non-dialysis treatment days. Values for selected nutrients measured are reported in **Table 5.3**. On dialysis days, patients reported intake of 199 fewer total calories ($p<0.05$), 32.6 fewer grams of carbohydrate ($p<0.05$), 80.7 fewer mg of cholesterol ($p<0.05$), 2.9 fewer milligrams of iron ($p<0.05$), and 167 fewer mg of phosphorus. There were no differences between dialysis and non-dialysis day for fat grams, percent of calories from fat, protein grams, or protein grams per kilogram body weight, and percentage of calories from protein was 2.7% higher on dialysis treatment days ($p<0.05$). Mean protein intake per kilogram body weight was 0.77 on dialysis days and 0.80 on non-treatment days. Multiple linear regression analysis did not produce any significant predictors of protein or calorie intake when gender, age, weight, mental health score, and total disability score were included in the model.

Factors Related to Functional Outcomes. We used multiple linear regression analysis to examine variables related to fitness and strength, body composition, and CVD risk. Results from multiple linear regression analysis are displayed in **Table 5.4**.

Measures of fitness and strength: Variables included in the model to determine predictors of fitness and strength were gender, age, diabetes status, albumin, leg mineral-free lean mass, Basic Lower Extremity Function Scale (BLEF), and plasma IL-6 levels. Female gender, presence of diabetes, and lower reported BLEF scores predicted poorer performance on the Walk Test (shorter time) and Gait Speed (longer time). Gender was the only predictor of peak torque of extension as a measure of muscle strength, although the overall model was not significant for peak torque.

Measures of Bone Density: Gender significantly predicted whole body bone mineral density in a model that included gender, weight, age, plasma IL-6, plasma alkaline phosphatase (ALP), and daily average calcium intake. Gender, weight, and ALP predicted total hip BMD in the same model, with female gender, low weight, and high ALP associated with lower hip BMD.

Measures of Body Composition: Gender and weight were the only predictors of both whole body lean mass and whole body percent fat, with adjusted R^2 values of 0.784 and 0.728, respectively. Female gender and lower weight predicted lower lean mass and higher percent fat. The total model included gender, weight, age, diabetes status, IL-6, albumin, and average daily protein intake.

Measures of cardiovascular risk: Epicardial fat thickness was predicted by whole body percent fat and plasma IL-6 levels, although the overall model did not reach statistical significance ($p=0.076$). The full model included gender, age, smoking, diabetes, albumin, IL-6, Godin Weekly Leisure Activity Score, whole body % fat, and average saturated fatty acid intake (SFA). We also found gender, age, smoking, whole body BMD, SFA intake and intima-media thickness predicted arterial β-stiffness in the same model used for epicardial fat thickness plus IMT and plasma calcium phosphorus product. Epicardial fat thickness was also positively correlated with plasma polymorphonuclear elastase protease inhibitor complex, a novel marker of inflammation in dialysis patients ($r=0.494$, $p=<0.05$) (**Figure 5.1**).

Discussion

Through this cross-sectional analysis, we found multiple aspects of chronic kidney disease that are interrelated, supporting the idea of the cycle of disease and disability characteristic of hemodialysis patients. This study was notable for its comprehensive inclusion of functional outcomes variables associated with hemodialysis treatment in an effort to characterize relationships among these factors, and possibly provide information on how best to intervene to improve health outcomes in this extremely sick population. We were able to consider cardiovascular disease risk, bone health, functional fitness capacity, muscle strength, body composition, dietary intake, clinically significant plasma variables, and demographics in 60 patients undergoing regular hemodialysis therapy in Champaign, IL (n=45) and Oak Park, IL (n=15). Patient demographics indicate a very similar percentage of male patients and incidence of diabetes to the national and state averages for dialysis patients. Patients in this study were slightly younger than average dialysis patients, with a much higher percentage of African-Americans.

We found elevated plasma phosphorus, iPTH, and creatinine levels which is characteristic of dialysis patients. In addition, we were able to assess several measures of iron status in this analysis. Over 95% of our participants were receiving recombinant erythropoietin (EPO), which is produced by healthy kidneys to stimulate red blood cell production. Anemia of renal insufficiency from lack of EPO and true iron-deficiency anemia are very prevalent in dialysis patients; however, many also suffer from a distinct type of anemia termed anemia of chronic disease induced by chronically elevated levels of inflammation. We found high levels of

ferritin, an acute phase protein, along with low total iron binding capacity (TIBC) in the presence of normal iron and transferrin saturation, indicative of anemia of inflammation rather than true iron deficiency anemia. The disregulation of iron that occurs with anemia of chronic disease seems to be driven particularly by the cytokine IL-6[217]; in response to increasing IL-6 levels, the liver produces increased amounts of the hormone hepcidin which prevents the release of stored iron. The high levels of IL-6 along with elevated ferritin and low/normal TIBC in this study suggest inflammation may be contributing to anemia and poor nutrition status in these individuals. In fact, some researchers are questioning the usefulness of ferritin as a marker of iron stores, suggesting instead that moderate hyperferritinemia is indicative of inflammation, infection, and malnutrition rather than an indicator of iron status[218].

We found dialysis patients intake significantly fewer calories (~200kcal) on dialysis days compared to non-dialysis days, along with lower amounts of carbohydrates, cholesterol, and iron. The National Institutes of Health Hemodialysis Study Group reported significantly lower total energy intake as well as lower protein intake on dialysis days compared to non-treatment days in a cross-sectional analysis of nearly 2,000 dialysis patients in 15 clinical centers nationwide[219]. However, the average amount of protein reported as consumed in this study was 0.79 grams protein/kilogram body weight. Even though we did not find any differences between treatment and non-treatment days, this suggests these patients are getting far below the recommended protein intake of 1.2 grams/kilogram body weight.

Decreased carbohydrate intake in this group accounted for about 120 fewer calories on dialysis days, suggesting other macronutrients may have been under-consumed as well, although these levels were not significant. Voluntary suppression of nutrient intake, typically total calories and protein, is commonly reported in dialysis patients with about 1/3 of patients classified as having CKD related anorexia[220]. Although the exact mechanism of appetite suppression is unknown, several studies have suggested the uremia-induced accumulation of inflammatory cytokines[61, 221, 222] may play a role in the voluntary suppression of nutrient intake and loss of appetite associated with dialysis treatment. However, we did not find inflammation to be predictive of protein or calorie intake in this study and larger studies are needed to determine the relationship between these variables.

Using regression analysis, we found significant associations between several functional measures and other outcomes of interest. For the Walk Test and Gait Speed, we found gender

and diabetes status predicted performance on these assessments, along with the self-reported BLEF. Leg lean mass was not a significant predictor, although this may be due in part to the negative correlation between female gender and lower lean body mass. Studies have suggested that females have lower physical activity levels than their male dialysis counterparts[223], and that this normal daily physical activity is important for maintenance of normal walk speed and ability to walk[224]. Indeed, we found a negative correlation with gender and GWLAS, although this measure is not a comprehensive scale of physical activity so this variable was not included in the model. Simple t-test analysis of males versus females and diabetics versus non-diabetics showed that females performed worse on 4 of the 6 measures of functional fitness, 12 of the 19 quality of life measures, and all 4 measures of muscle strength compared to males, while diabetics performed worse on 4 of 6 functional measures, 14 of the 19 quality of life measures, and none of the muscle strength measures compared to non-diabetics.

Furthermore, female gender significantly predicted lower whole body bone mineral density, lower total hip bone mineral density, lower lean body mass, and higher percent whole body fat, even when other correlated factors were considered in the model. Although it was not possible to model all of these outcome variables, these data indicate gender, body composition, functional fitness, and quality of life are interrelated in this population, supporting the idea of a cycle of disease and disability in this population. Surprisingly, age was not a significant predictor in any of these outcomes suggesting that other factors such as gender-associated changes in hormones may be contributing to an increased risk of disease and disability in female dialysis patients. In addition to gender and weight, we identified increased alkaline phosphatase as a predictor of low hip bone mineral density; a recent study by Park and colleages[225] suggests hemodialysis modifies the traditional risk factors for low bone density found in the general population, and that identification of dialysis-specific factors related to renal osteodystrophy such as ALP are needed. Other studies suggest that ALP contributes to increase cardiovascular risk and mortality in this population[226, 227], and targeting the levels of ALP in this population may modify both bone health and cardiovascular disease risk simultaneously.

Although female gender predicted poorer outcome for fitness, body composition, and strength, we found female gender was inversely related to arterial stiffness, suggesting a possible protective effect on the vasculature. The detrimental effects of smoking and increasing age on arterial stiffening is well known, and indeed we found smoking and age to be predictors of

arterial stiffness along with gender, saturated fat intake, and intima-media thickness. We found that higher whole body bone density was predictive of higher arterial stiffness although the opposite has been demonstrated in other clinical populations[228]. Aoki and colleagues studied the relationship between vascular calcification, aortic stiffness, and bone mineral density in dialysis patients, reporting associations between bone density and calcification and stiffness and calcification, but neglected to report any relationship between stiffness and BMD in this population[229]. CKD-MBD can include both high- and low-turnover bone disease, and DXA measurements are limited to bone mineral content and density without a report of turnover. Therefore, the relationship between arterial stiffness and bone density as measured by DXA is unclear and warrants further investigation.

We also found that higher IL-6 levels predicted higher epicardial fat thickness; polymorphonuclear elastase-protease inhibitor complex (PMN-Elastase PI), a novel marker of inflammation in dialysis patients[230], also was positively correlated with epicardial fat thickness. However, this marker was only measured in a small subset of study participants and was therefore not included in the linear regression model. However, these data suggest that inflammation contributes to epicardial fat thickness in dialysis patients, as shown by a very recent study by Turkmen and colleagues reporting increased epicardial fat thickness as the presence and number of components of the malnutrition-inflammation, atherosclerosis/calcification syndrome increased in dialysis patients[231].

We were able to confirm several well-known predictors of CKD co-morbidities as well as identify some novel predictors of functional outcomes including ALP and IL-6. We also showed a positive association of another inflammatory marker, PMN elastase-PI complex, with epicardial fat thickness which suggests that the inflammation may be both cause and consequence of the co-morbid conditions of hemodialysis treatment. Understanding the complex relationship between cardiovascular complications, renal bone disorders, and factors that contribute to their development including muscle strength and body composition will enable the development of appropriate therapeutic targets to improve the health and quality of life of hemodialysis patients.

Tables

Table 5.1: Subject Characteristics

Demographics	Total (n=60)
Gender (Male)	56.7% (n=34)
Diabetes Status (Diabetic)	46.7% (n=28)
Smoking Status (Smoker)	31.7% (n=19)
Age (years)	53.91±1.6
BMI (kg/m^2)	32.09±1.0
Time on Dialysis (months)	59.8±6.4
Race/Ethnicity	71.7% African-American (n=43) 25% White (n=15) 3.3% Hispanic (n=2)
Etiology of Kidney Failure	
Hypertension	52% (n=31)
Diabetes with renal manifestations, Type 1	3% (n=2)
Diabetes with renal manifestations, Type 2	25% (n=15)
Polycystic Kidney Disease	5% (n=3)
Nephritis/Nephropathy	10% (n=6)
Unknown/Other	5% (n=3)
Total	100% (n=60)

When appropriate, data are presented as mean ±SEM.

Table 5.2: Mean values of reported plasma values compared to desirable reference ranges

Plasma Variables	Mean Values	Reference Range (NKF KDOQI)
Interleukin-6 (pg/mL)	8.4±1.2	Normal ~1.0
Bicarbonate (mEq/L)	25±0.4	22-29
Phosphorus (mg/dL)	6.0±0.2	2.6-4.5
ALP (U/L)	115±14 M, 94±8 F	40-129 M, 35-104 F
IntactPTH (pg/mL)	428±55	14-72
Potassium (mEq/L)	4.8±0.1	3.5-5.1
Calcium (mg/dL)	9.0±0.1	8.4-10.2
CaxP	54±2	0-54
Albumin (g/dL)	4.0±.04	3.5-5.2
TransSat (%)	38±2	22-55
Iron	85±5 M, 76±8 F	45-160 M, 30-160 F
TIBC (mcg/dL)	217±4	228-420
Ferritin (ng/mL)	894±92 M, 888±122 F	22-332 M, 10-291 F
Hemoglobin (g/dL)	13.1±1.4	14.0-18.0
Hematocrit (%)	36.3±0.6	42.0-52.0
BUN (mg/dL)	53±2	6-19
Creatinine (mg/dL)	10.2±0.4 M, 11.8±2.9 F	0.5-1.2 M, 0.4-1.1 F
BUN_Creat (calculated)	6.2±.40	*(no reference provided)*
BUNPost (mg/dL)	15±0.9	6-19
URR (calculated)	73±1.2	65-80

Data are presented as mean ± SEM. Sixty (60) patients were included in this analysis.
ALP=alkaline phosphatase, PTH=parathyroid hormone, CaxP=Calcium Phosphorus product, TransSat=transferrin saturation, TIBC=total iron binding capacity, BUN=blood urea nitrogen, BUN_Creat=blood urea nitrogen to creatinine ratio, URR=urea reduction ratio

Table 5.3: Comparison of selected nutrient intake on dialysis and non-dialysis days

	Mean Dialysis-Mean Non-Dialysis	p-value
Calcium (mg)	-100±67	0.14
Calories (kcal)	**-199±96**	**0.04**
Calories (kcal)/Body Weight (kg)	-2.0±1.1	0.07
Calories (kcal)/Lean Mass (g)	**-0.004±0.002**	**0.05**
Carbohydrate (g)	**-32.6±15.0**	**0.04**
Carbohydrate (%)	-3.02±2.8	0.29
Cholesterol (mg)	**-80.7±32.1**	**0.02**
Fat (g)	-6.41±5.5	0.25
Fat (%)	0.25±2.3	0.91
Iron (mg)	**-2.9±1.4**	**0.04**
Fiber (g)	-0.6±1.2	0.63
Sodium (mg)	-359±290	0.22
Phosphorus (mg)	-167±89	0.07
Protein (g)/Body Weight (kg)	-0.032±0.065	0.62
Protein (g)	-1.8±5.7	0.76
Protein (g)/Lean Mass (g)	-0.00004±0.000	0.70
Protein (%)	**2.73±1.2**	**0.03**
Vitamin A	-160±122	0.19
Vitamin C	-7.4±14.3	0.61

Data presented are changes in mean ± SEM. $p<0.05$ is considered significant.

Table 5.4: Multiple Linear Regression Analysis to identify predictors of functional outcomes

	Walk Test	R^2=0.485, p<0.001	
	t	p	B
Gender	-2.72	0.011	-102.4
Diabetes	2.45	0.021	69.3
BLEF	2.03	0.053	8.09
	Walk Speed	R^2=0.492, p<0.001	
	T	p	B
Gender	3.32	0.003	4.69
Diabetes	-2.13	0.043	-2.23
BLEF	-2.31	0.029	-0.35
	Peak Torque Extension		R^2=0.112, p=0.196
	t	p	B
Gender	-2.42	0.024	-54.0

Variables included in model: Gender, Age, Diabetes Status, Albumin, Leg Mineral-Free Lean Mass, Basic Lower Extremity Function scale, Plasma IL-6

	Whole Body BMD		R^2=0.443, p<0.05
	T	p	B
Gender	-3.12	0.009	-0.14
	Total Hip BMD		R^2=0.462, p<0.05
	T	P	B
Gender	-2.02	0.066	-0.147
Weight	2.96	0.012	0.005
ALP	-2.60	0.023	-0.001

Variables included in model: Gender, Weight, Age, Plasma IL-6, Plasma Alkaline Phosphatase, Average Daily Calcium Intake

Table 5.4, continued: Multiple Linear Regression Analysis to identify predictors of functional outcomes

	Whole Body Lean Mass		R^2=0.784, p<0.001
Variable	T	p	B
Gender	-5.54	<0.001	-13837
Weight	7.56	<0.001	341
	Whole Body % Fat		R^2=0.728, p<0.001
Variable	T	p	B
Gender	5.86	<0.001	14.7
Weight	7.17	<0.001	0.324

Variables included in model: Gender, Weight, Age, Diabetes, Plasma IL-6, Albumin, Average Daily Protein Intake

	Epicardial Fat Thickness		R^2=0.317, p=0.076
Variable	t	p	B
IL-6	2.17	0.047	0.344
WB % Fat	2.91	0.011	0.201
	Beta Stiffness		R^2=0.662, p<0.05
Variable	t	P	B
Gender	3.59	0.007	6.25
Age	2.88	0.02	0.149
Smoking	-2.83	0.022	-4.02
WbBMD	2.33	0.048	26.8
SFA Intake	2.33	0.048	0.148
IMT	2.82	0.023	0.234

Variables included in the model: Gender, Age, Smoking, Diabetes, Albumin, IL-6, Godin Weekly Leisure Activity Score, WB % Fat, SFA Intake, Calcium Phosphorus Product (Beta stiffness only), Intima-media thickness (Beta stiffness only)

Figures

Figure 5.1

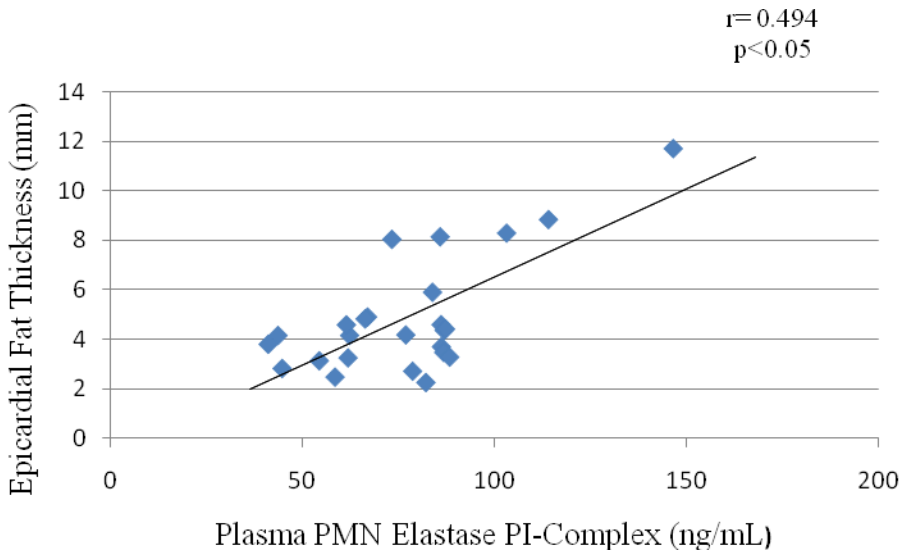

Figure 5.1: Association of Epicardial Fat Thickness and Plasma PMN Elastase PI-Complex (n=23). We found a significant positive association of epicardial fat thickness and levels of the inflammatory marker PMN Elastase PI-Complex ($r=0.494$, $p<0.05$).

CHAPTER 6

ACUTE EFFECTS OF INTRADIALYTIC PROTEIN SUPPLEMENTATION

Introduction

Chronic kidney disease (CKD) is a progressive inflammatory disorder that affects approximately 13% of adults in the U.S, and the prevalence is increasing rapidly[1]. Advanced CKD requiring dialysis treatment is associated with a variety of metabolic disturbances that increase morbidity and mortality; in addition, protein malnutrition, muscle wasting, bone disorders, and cardiovascular complications are especially common, and these co-morbidities greatly reduce physical function and quality of life in dialysis patients. Furthermore, 2/3 of patients die within 5 years of initiation of long-term dialysis treatment, mostly of cardiovascular disease (CVD)[3], and survival has not increased substantially in the past two decades[4]. New therapeutic approaches are needed to address the many co-morbid conditions associated with advanced kidney disease.

Although dialysis treatment procedures and protocols have improved over the years, mortality rates remain elevated in dialysis patients, and many believe protein-energy malnutrition and elevated inflammation are one of the primary reasons for this effect. Protein-energy malnutrition (PEM) is very common in dialysis patients, with the incidence rate ranging from 25% to 75% in different studies[6-9]. There are a variety of reasons for this, including poor nutrient intake, physical illnesses affecting gastrointestinal function, protein losses during dialysis, and elevated whole body and skeletal muscle protein catabolism that occurs primarily during dialysis[10]. Furthermore, PEM and inflammation tend to occur concurrently in dialysis patients, giving rise to the term malnutrition-inflammation complex or syndrome.

Inflammation may also be contributing to PEM by inducing appetite suppression, as high circulating levels of inflammatory cytokines has been shown to increase anorexia in dialysis patients[222]. This voluntary decrease in nutrient intake further exacerbates the malnutrition inflammation complex syndrome, leading to more inflammation and further declines in nutritional status. In particular, IL-6 seems particularly important as a marker and mediator of dialysis-associated inflammation. Tripepi and colleagues indicate IL-6, compared to several other inflammatory cytokines, adds the greatest predictive power with regards to all-cause and

cardiovascular mortality in the context of traditional and non-traditional risk factors for dialysis patients[232]. Furthermore, the dialysis procedure induces an acute increase of IL-6[233] during the course of a single treatment that persists into the post-dialysis phase, and other inflammatory markers may be transiently elevated in conjunction with dialysis treatment[230].

Anemia of chronic disease, often termed anemia of chronic inflammation, occurs in dialysis patients due to chronic immune activation, infection, and inflammation[234]. Individuals with advanced CKD very commonly suffer from another type of anemia called anemia of renal insufficiency due to the inability of the kidney to produce erythropoietin. Although the two types of anemia are often present concurrently and share some symptomatic indicators, they are considered to be distinct conditions with different eitologies[235]. Therefore, inflammation further exacerbates the declining nutritional state of patients who already experience a derangement of nutrient metabolism due to renal insufficiency.

Because protein malnutrition in dialysis patients promotes inflammation, it has been suggested that improving nutritional status may help halt this cycle of inflammation and decreased nutritional status[60]. If so, this could have a beneficial effect on many CKD co-morbidities influenced by inflammation, particularly CVD[56, 72]. In response to these issues, the National Kidney Foundation has recommended an increase in the protein requirement to 1.2 g/kg/day for hemodialysis patients in comparison to the 0.8 g/kg/day recommended for healthy adults and 0.6 g/kg/day recommended for earlier stages of CKD. This recommendation was based on several small prospective nutritional-metabolic studies indicating this intake level is necessary to ensure neutral or positive nitrogen balance in most dialysis patients[10]. However, most dialysis patients do not consume this recommended amount of protein, and a large multi-center study of dialysis patients showed protein and calorie intake to be significantly lower on dialysis treatment days compared to non-treatment days[219]. The combination of amino acids lost into the dialysate, suppressed nutrient intake on dialysis days, and the catabolic effects of dialysis treatment suggests that the time immediately prior to initiation of treatment would be most appropriate to administer a protein intervention to potentially offset the negative effects of dialysis.

The purpose of this study was to determine the effects of whey and soy protein supplementation on acute inflammation over the course of a dialysis treatment. While both protein sources will increase substrate availability, they differ in amino acid composition and

presence of other bioactive compounds such as soy isoflavones. We hypothesized protein supplementation, both WHEY and SOY, would attenuate the increase in inflammation (IL-6) associated with the dialysis procedure. Furthermore, we expected to see a greater attenuation in the SOY group due to bioactive components of soy protein.

Methods

Subject Recruitment, Screening, and Selection. Individuals with CKD receiving hemodialysis treatment at the Champaign-Urbana Dialysis Center in Champaign, IL (n=32) and the Oak Park Dialysis Clinic in Oak Park, IL (n=12) were recruited at their respective clinics. Patients that met the following criteria were enrolled in the study: **1)** Subjects must receive hemodialysis treatment at least 3 days per week. **2)** Subjects must be $\geq$ 30 years of age. 3**)** Subjects must not have congestive heart failure or chronic obstructive pulmonary disease 4**)** Subjects must have been receiving dialysis treatment for $\geq$ 3 months as disruption in metabolic factors is related to duration of dialysis treatment. A Health and Medical History Questionnaire was administered during the screening, and informed consent was obtained from each participant. After consent was obtained, participants were randomly assigned to one of three groups: whey protein (WHEY), soy protein (SOY), or placebo/control (CON). All research protocols were conducted with the approval of the University of Illinois Institutional Review Board. Information on participant recruitment, enrollment, and study completion is provided in **Figure 6.1**.

Blood Collection. The study protocol consisted of two blood draws per day on two separate days, one week apart (**Figure 6.2**). The first draw was taken immediately after the initiation of dialysis and then again 3 hours into dialysis treatment. On Day 1, participants did not receive a study beverage (control day) but had the two blood draws as described above. The first draw on Day 1 is referred to as "baseline". On Day 2, one week following Day 1, each participant received the study beverage to which they had been randomly assigned and consumed the beverage immediately prior to the initiation of dialysis treatment. After they consumed the beverage, blood was collected at the two time points described above. Therefore, each patient had one control day and one treatment day to allow for comparison of the treatment effect to their normal inflammatory response to dialysis treatment. Plasma was collected by centrifugation and divided into 1ml aliquots and stored at -80^{o}C until analyzed.

Blood collections were not performed on Mondays or Tuesdays to control for the extra day between treatments that occurs during the weekend. For example, blood would not be drawn for a Monday/Wednesday/Friday scheduled patient on a Monday as they would not have dialyzed since Friday (two day lapse). For a Tuesday/Thursday/Saturday scheduled patient, blood would not be drawn during their treatment on Tuesday for the same reason. All blood chemistries and data analysis were performed in Champaign, IL regardless of collection site.

Intervention: Protein Supplementation. Participants were given 30 grams of either a whey protein beverage, soy protein beverage, or placebo beverage immediately before dialysis treatment as described above. Components of the protein beverages are listed in **Table 6.1**. The whey beverage (True Protein, Inc., Oceanside, CA) contained 27 grams of cold-filtered whey protein isolate per 30 gram serving, with 110 calories per serving. The soy protein supplement (Solae, Gibson City, IL) contained 27 grams of soy protein isolate (Supro 670) per 32 gram serving, with 120 calories and 1.5 grams of fat. This soy protein isolate provided 40 milligrams of isoflavones per serving (12 mg daidzein, 22 mg genistein, 6 mg glycitein). Supplements were matched for protein amount, resulting in a slightly higher total gram amount of the soy protein supplement. The powders were mixed with a flavor pack containing natural/artificial flavors and colors, sucralose, and acesulfame k, and were prepared with 4-6 ounces of water for consumption. Both protein powders provided sodium, iron, phosphorus, calcium, and other nutrients that are of concern for dialysis patients; however, the levels provided in this beverage were lower than those in a commercially available renal-specific formulation at the same level of protein (Nepro, Abbott Nutrition, Columbus, OH). Patients in the CON group received 4-6 ounces of non-caloric Crystal Light prepared according to package directions (Kraft, Northfield, IL).

Blood Chemistry. Circulating levels of the inflammatory marker interleukin-6 were measured at all four study time points using commercially available ELISA kits (R&D Biosystems, Minneapolis, MN). In addition, blood collected at the dialysis clinic during normally scheduled blood draws was assessed for standard clinical lab parameters (plasma albumin, phosphorus, calcium, etc.) by a Spectra Laboratories, a renal specific laboratory service provider (Rockleigh,

NJ). Reported transferrin saturation is the ratio of serum iron and total iron-binding capacity, multiplied by 100.

Anthropometric Measures. Barefoot standing height was measured to the nearest 0.1 cm with a stadiometer and body weight was measured on a balance scale with shoes and superfluous outer garments removed. All measurements were taken in duplicate and averaged.

Statistical Analysis. Data presented are mean ± SEM unless otherwise noted, and significance was considered as $p<0.05$. Change in IL-6 levels was calculated using the following method: Δ Day 1 = Time Point 2 on Day 1 (3 hours into dialysis) *minus* Time Point 1 on Day 1 (start of dialysis); Δ Day 1 represents the change in IL-6 during dialysis with no study beverage. Δ Day 2 = Time Point 2 on Day 2 (3 hours into dialysis) *minus* Time Point 1 on Day 2 (start of dialysis/consumed study beverage); Δ Day 2 represents the change in IL-6 during dialysis with the study beverage.

Using the Δ values calculated above, Δ Day 2 *minus* Δ Day 1 represents the difference of the change in IL-6 during dialysis after consuming the study beverage *compared to* the difference in change of IL-6 during dialysis with no study beverage. The "Δ Day 2 *minus* Δ Day 1" variable was analyzed using ANOVA to compare means among the three treatment groups. Time point 1 and 3 on each day and Δ values for Day 1 and Day 2 were analyzed by paired samples t-test.

Distribution statistics were calculated to determine whether assumptions of normality were met (including skewness and kurtosis between -2 and 2). For some analyses, data from WHEY and SOY were combined to look at the effects of protein supplementation, regardless of source; this combined group assessing general protein intake is labeled PRO. Correlation analysis was used to identify relationships between selected variables of interest. Multiple linear regression analysis was used to determine predictors of intradialytic change in plasma IL-6 levels. For linear regression analysis gender and diabetes status were coded as follows: male=1, female=2; diabetes=1, no diabetes=2. All analyses were conducted using SPSS v.17 (Chicago, IL).

Results

Patient Characteristics. A total of 44 hemodialysis patients (WHEY, n=20; SOY, n=15; CON, n=9) completed this study. Patient characteristics are listed in **Table 6.2**. There were no significant group differences for age, weight, BMI, albumin levels, transferrin saturation, hemoglobin, hematocrit, or iron. According to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (KDOQI™), patients were in normal range for transferrin saturation (20-50%), albumin (3.5-5.5 g/dL), and iron (30-160 mcg/dL) and were slightly low compared to the reference range for hematocrit at 37-47% and hemoglobin at 12-16 g/dL.

Intradialytic Change in Plasma IL-6 Concentration (Three Group Analysis). Intradialytic changes in plasma IL-6 concentration are shown for WHEY (n=20), SOY (n=15), and CON (n=9) in **Figure 6.3**. All IL-6 values are expressed in ng/mL. On Day 1, plasma IL-6 increased by 2.94±1.2, 6.92±1.4, 3.00±1.2 in WHEY, SOY, and CON, respectively. The increase within each group was significant ($p<0.01$), but there were no between-group differences Δ Day 1. On Day 2, IL-6 increased 2.68±0.86, 3.45±1.4, 5.24±2.1 for WHEY, SOY and CON; the increase within each group was significant ($p<0.05$). The "Δ Day 2 *minus* Δ Day 1" mean values were -1.28±1.2, -3.47±1.6, and 2.24±1.6 for WHEY, SOY, and CON with a trend toward a significant time by treatment interaction ($p=0.065$). Comparison of within group differences between Δ Day 1 and Δ Day 2 showed SOY was significantly lower from Day 2 to Day 1 ($p<0.05$) while the differences within WHEY and CON between Δ Day 2 and Δ Day 1 were not significant.

Intradialytic Changes in Plasma IL-6 Concentration (Two Group Analysis). Intradialytic changes in plasma IL-6 concentrations are shown for PRO (n=35) and CON (n=9) in **Figure 6.3**. On Day 1, IL-6 increased by 5.23±0.94 in PRO and increased 3.01±1.22 in CON; the increase within each group was significant, but there were no between-group differences for change in IL-6 under the condition of no beverage on Day 1. On Day 2, PRO increased by 3.01±0.79 while CON increased by 5.24±2.1; again, the increase within each group was significant and there were no between group differences.

There was a significant time by treatment interaction for "Δ Day 2 *minus* Δ Day 1"; mean values were significantly lower in PRO (-2.22±0.98) compared to CON (2.23±1.6) ($p<0.05$), suggesting protein supplementation attenuates the intradialytic increase in plasma IL-6

by around 2 pg/mL. Although the "Δ Day 2 *minus* Δ Day 1" variable for the CON was positive (2.23±1.6), paired sample t-test analysis confirmed the within-group difference between Δ Day 1 and Δ Day 2 was not significantly different for CON between no beverage day and beverage day ($p=0.196$); paired sample t-test analysis of PRO for Δ Day 2-Δ Day 1 was significant ($p<0.05$), suggesting an effect of treatment on Day 2. Furthermore, ANOVA of the mean "Δ Day 2 *minus* Δ Day 1" as described above confirmed the significant time by treatment effect when mean differences in change were compared between CON and PRO.

Relationship of IL-6 to Albumin, Serum Iron, and Transferrin Saturation. We found a significant inverse relationship between plasma albumin levels and both baseline IL-6 concentration ($r=-0.451$, $p<0.01$) (**Figure 6.4**) and change in IL-6 on Day 1 ($=-0.484$, $p<0.01$) (**Figure 6.5**). There was a significant negative association between plasma transferrin saturation and both baseline IL-6 concentration ($r=-0.348$, $p<0.05$) (**Figure 6.6**) and change in IL-6 on Day 1 ($r=-0.378$, $p<0.05$) (**Figure 6.7**); serum iron was also inversely related to baseline IL-6 ($r=-.356$, $p<0.05$) and change in IL-6 on Day 1 ($r=-0.407$, $p<0.01$). However, only baseline IL-6 was predictive of change in IL-6 in a model that included gender, time on dialysis, diabetes status, albumin, and transferrin saturation (**Table 6.3**).

Discussion

We found that oral administration of 27 grams of protein immediately before dialysis treatment attenuates the increase in IL-6 associated with treatment, the first study to suggest the potential anti-inflammatory effects of intradialytic protein supplementation. In addition, baseline IL-6 and the intradialytic change in IL-6 were inversely related to circulating albumin, transferrin saturation, and iron.

CKD is a chronic inflammatory condition, as reflected in dialysis patients by elevated circulating levels of acute phase proteins such as CRP, and pro-inflammatory cytokines such as IL-6, and TNF-a[56-59]. The dialysis procedure also induces an acute inflammatory response[230, 233]; we were able to confirm the increase in IL-6 that occurs during dialysis treatment by showing an increase in IL-6 on Day 1 when no treatment was administered, although the increase may be due in part to hemoconcentration occurring during treatment. However, by measuring each person under the same dialysis conditions on both days, this effect was minimized and allowed

for a relative comparison of the effect of protein supplementation on inflammation during dialysis.

In a study by Caglar and colleagues, IL-6 levels in hemodialysis patients peaked during a 2-hour post dialysis phase[233], but we chose to collect blood at 3 hours into dialysis treatment in order to minimize the burden of the patient to remain in the clinic for several hours after completing dialysis treatment. While it is unclear what the effects of protein supplementation would be in this post-dialysis phase, we were able to demonstrate an effect of protein at the three hour time point. Furthermore, our study had a larger number of subjects (n=44) compared to the Caglar study (n=9) that may have allowed for earlier detection of the increase in IL-6 that occurs in conjunction with dialysis treatment.

We were able to show that intradialytic protein supplementation attenuates the increase in inflammation associated with the dialysis procedure. We compared the change in IL-6 over a dialysis session in patients on both a control day and a treatment day, allowing for better control over the variability of the inflammatory response in these patients. Although we were able to find an acute benefit, the beneficial long term effects of this acute reduction are unknown. Bossola et al reported time on dialysis was associated with improvements in nutritional status and a trend towards a reduction in inflammatory variables over a three year period[107], which seems contrary to current evidence considering the acute pro-inflammatory and catabolic effects associated with dialysis treatment compounded over time. The authors concluded time on dialysis was not necessarily associated with a decline in nutritional status or increase in inflammation; however, patients routinely received an intradialytic meal containing 25-30 grams of protein. Because this meal was given as part of routine care, they did not consider it to be part of the study protocol. This suggests routine intradialytic protein and/or calorie intake may be responsible for attenuating the decline in nutritional status and increased inflammatory state associated with long-term dialysis treatment. However, this study lacked a control group and failed to provide information on subject compliance for this intradialytic meal, and more well-controlled studies are clearly needed to see if the acute reduction shown in our current study would translate into long term beneficial outcomes. Furthermore, the effects of protein supplementation on functional outcomes related to inflammation in CKD patients, including cardiovascular risk and bone disease, warrant further investigation.

Although we demonstrated the attenuation of the increase in IL-6 in the protein combined protein group, we saw a trend for a time by treatment interaction when all three groups were considered ($p=0.065$). Although the overall time by treatment interaction was not significant, the within-group difference for SOY was significantly lower for Δ Day 2 compared to Δ Day 1 ($p<0.05$). Therefore, it may be possible that the source of the protein may be a factor in determining the intradialytic inflammatory response to protein supplementation. Fanti and colleagues demonstrated a marked inverse relationship between plasma CRP levels and the change in plasma isoflavone concentration measured before and after an 8-week supplementation with 25 grams of intradialytic soy protein[118]; they also noted a significant positive correlation between change in plasma isoflavone concentration and plasma albumin levels measured at the end of the study. A milk protein supplement was used as the control in this study, and the authors did not report on the relationship between milk protein intake and plasma albumin levels albumin, making it difficult to determine if the association of isoflavones and albumin was due to the protein component of the supplement or a function of the bioactive isoflavones found in soy. However, this study and others suggest a possible relationship between soy protein consumption and inflammation during dialysis treatment.

Protein supplementation during dialysis treatment, regardless of the protein source, will increase substrate availability, and this may help explain the modest effect of whey protein when the three groups were analyzed separately. Compared to soy protein, whey protein contains higher amounts of the branch-chain amino acids leucine, isoleucine, and valine. Leucine concentrations are especially important during a highly catabolic condition, and high amounts of leucine have been shown to potently stimulate muscle protein synthesis and inhibit protein breakdown in skeletal muscle and liver (reviewed in[128]). Therefore, intradialytic whey protein supplementation may potentially mediate the inflammatory state by reducing catabolism through increased availability of amino acids. Intradialytic protein supplementation may be particularly efficacious because it improves protein balance during the time when protein catabolism is at its peak[10, 98, 99]. Studies have shown that both parenteral[100-102] and oral[96, 103, 104] intradialytic supplementation improve protein homeostasis, increase serum albumin and prealbumin levels, and have anabolic effects on skeletal muscle, but the relationship of intradialytic protein supplementation and inflammation has not been previously demonstrated. While we did not

measure protein catabolism in this study, we hypothesize that substrate availability from protein administration may directly or indirectly decrease catabolism by reducing inflammation.

Although serum albumin levels have traditionally been used as a marker of nutritional status, evidence suggests that inflammatory cytokines partly determine the levels of albumin in dialysis patients[66, 236], making it difficult to separate the effects of inflammation and malnutrition on this negative acute phase protein. Indeed, we found albumin levels to be related to not only baseline IL-6 levels, but also the magnitude of increase in IL-6 during dialysis treatment. This suggests that presence of malnutrition, or increased inflammation, as indicated by albumin levels, is associated with the amount of increase in inflammation during dialysis treatment with more inflamed patients having a greater intradialytic inflammatory response.

We found the same negative relationship with both serum iron and transferrin saturation (ratio of serum iron to total iron binding capacity) with baseline IL-6 and change in IL-6. Because serum iron is used to calculate transferrin saturation, the data for serum iron are not shown. These markers are strongly influenced by inflammation, and the relationship with inflammation has caused their usefulness as markers of iron status to be questioned. Beerenhout and colleagues showed a positive association between serum ferritin and C-reactive protein levels and an inverse relationship of transferrin saturation in hemodialysis patients[237], while Nanami et al suggested inflammatory cytokines may be causing iron to be sequestered intracellularly due to changes in iron transporters in the cells induced by TNF-a[238]. Intracellular sequestering of iron decreases available serum iron and transferrin saturation and also induces increased production of ferritin, the primary intracellular iron storage protein. Both anemia of chronic disease, often termed anemia of inflammation, and iron deficiency anemia are characterized by low serum iron and low transferrin; however, while levels of ferritin >30ng/mL are highly predictive of iron deficiency anemia, anemia of inflammation has marked increases in ferritin in response to the large amount of iron being sequestered intraceullarly in a state of inflammation (reviewed in [235]). While we did not see any relationship between ferritin levels and IL-6 in this study, the mean value for ferritin was 949±91 ng/mL, suggesting the influence of inflammation. These data indicate serum iron, transferrin saturation, and ferritin are strongly related to the inflammatory state in hemodialysis patients and should be considered in that context.

There were several limitations to this study. Participants were enrolled in this acute study as part of a larger, longer intervention trial in which they were randomly assigned to four groups, two of which receive whey protein supplementation (one alone, one in combination with exercise training). This resulted in a larger number of participants randomly assigned to receive whey protein. Additionally, we need to confirm the possible anti-inflammatory effect of intradialytic protein with other measures of inflammation that were not included in this study, and the relationships between acute intradialytic protein supplementation and the inflammatory response and other factors such as oxidative stress need to be examined in a more comprehensive manner.

To our knowledge, this is the first study to suggest the benefits of intradialytic protein administration for attenuating the acute inflammatory response associated with the dialysis treatment procedure. Because patients typically dialyze three to four times per week for up to 5 hours per session, we believe the potential to mitigate the inflammatory and catabolic insult of repeated dialysis treatment has the potential to improve health outcomes over time. In particular, studies are needed to assess the effects of chronic intradialytic protein supplementation on CKD co-morbidities associated with inflammation including CVD, bone disorders, muscle wasting, anorexia, and overall quality of life. Intradialytic protein supplementation represents a low-cost, easy to administer therapeutic intervention that has the potential to improve health outcomes in hemodialysis patients.

Tables

Table 6.1: Composition of Whey Protein Isolate, Soy Protein Isolate, and Placebo Beverage[a]

Per Serving	Whey Isolate (30g) *True Protein, Oceanside, CA*	Soy Isolate (32g) *Solae, Gibson City, IL*	Crystal Light (2g) *Kraft, Northfield, IL*
Total Fat	0 g	0.9 g	0 g
Saturated Fat	0 g	0.3 g	0 g
Cholesterol	0 mg	0 mg	0 mg
Sodium	53 mg	373 mg	35 mg
Carbohydrates	0 g	0 g	0 mg
Protein	27 g	27 g	0 mg
Leucine	3.3g	2.1g	0 mg
Vitamin A	0 mg	<2%	0 mg
Vitamin C	0 mg	<2%	0 mg
Calcium	151 mg	23 mg	0 mg
Iron	0 mg	4.5 mg	0 mg
Phosphorus	72.6 mg	244 mg	0 mg
Potassium	194 mg	182 mg	0 mg
Isoflavones	0 mg	40 mg	0 mg

[a]Composition analysis was performed by a third-party laboratory at the University of Missouri

Table 6.2: Patient Characteristics and Selected Plasma and Serum Variables

Participant Demographics	WHEY (n=20)	SOY (n=15)	CON (n=9)	p-value
Gender	75.0%	53.3%	55.6%	
Age (years)	54.5±4.1	54.9±2.8	52.8±3.8	0.920
Weight (kg)	91.5±5.5	96.6±6.1	96.5±10.7	0.813
BMI (kg/m^2)	31.4±1.8	35.3±2.5	34.3±3.1	0.406
Smoking Status (yes)	22.2%	40%	26.7%	
Type II Diabetes (yes)	40%	33.3%	66.7%	
Plasma/Serum Variables				
Transferrin Saturation (%)	43.9±3.7	33.5±2.5	33.8±6.4	0.198
Albumin (g/dL)	3.98±0.07	3.97±0.10	4.00±0.11	0.983
Iron (mcg/dL)	99.2±9.2	77.8±6.2	81.4±16.4	0.263
Hemoglobin (g/dL)	15.5±3.9	11.7±0.32	11.6±0.44	0.611
Hematocrit (%)	35.7±0.88	36.0±0.91	35.6±1.4	0.968

Table 6.3: Factors related to intradialytic change in plasma IL-6

	Δ Day 1		R^2=0.356, p<0.002
Variable	T	p	B
Gender	0.707	0.486	1.05
Diabetes	-1.41	0.170	-2.21
Time on Dialysis	0.848	0.403	0.015
Baseline IL-6	2.90	0.007	0.273
Albumin	-1.04	0.307	-2.82
Trans Sat	-0.752	0.458	-0.114
Iron	0.535	0.596	0.034

Figures

Figure 6.1

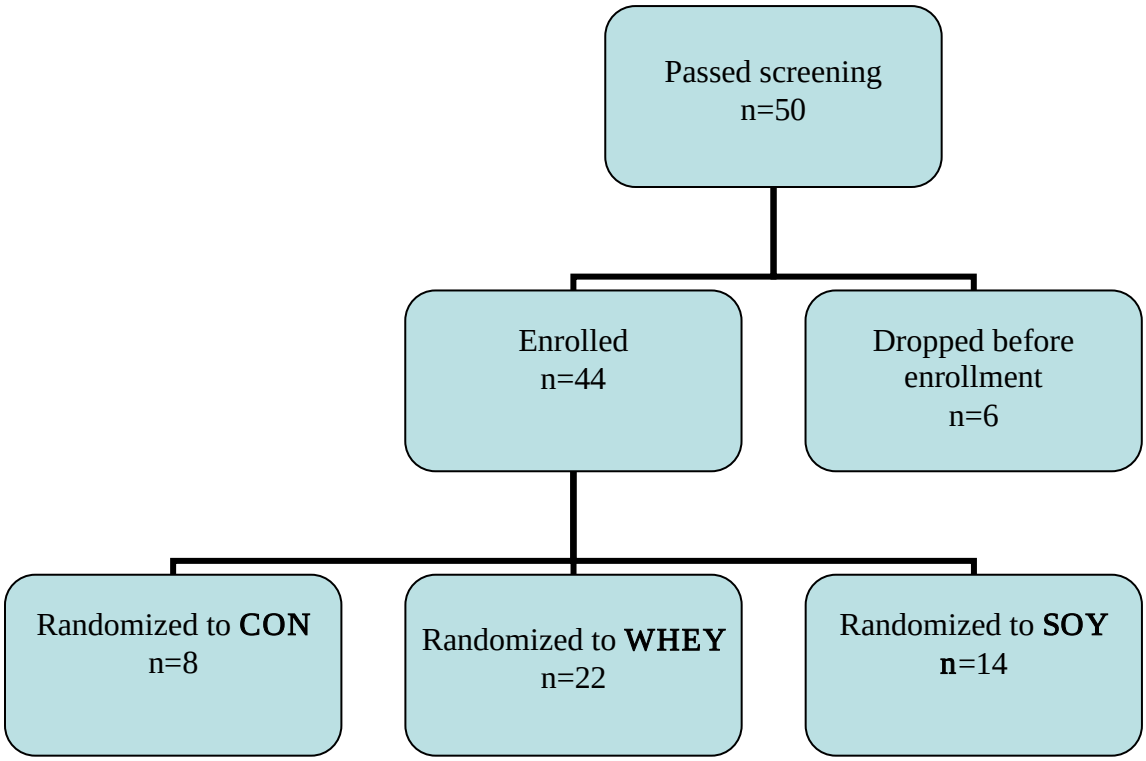

Figure 6.1: Recruitment, Enrollment, and Study Completion.

Figure 6.2

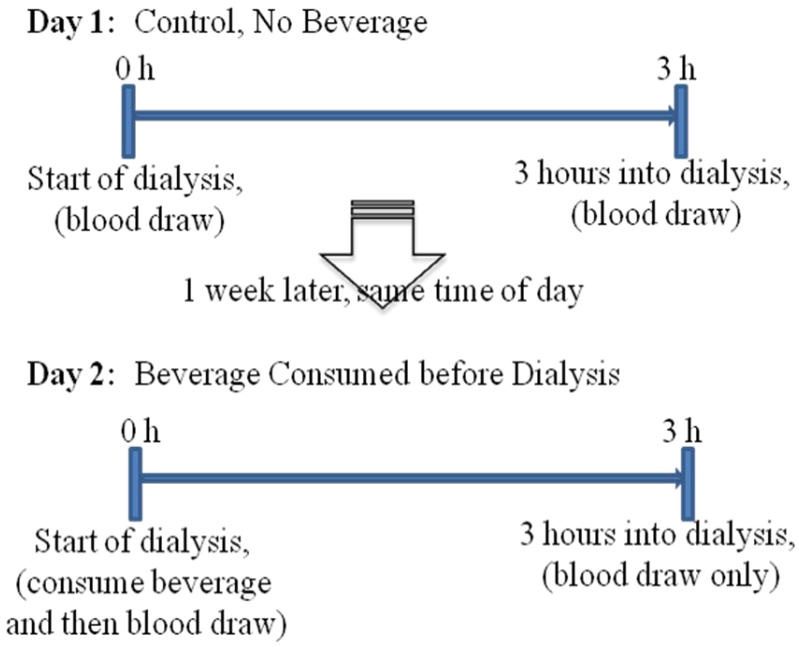

Figure 6.2: Study timeline. On Day 1, the first draw was taken immediately after the initiation of dialysis and then again 3 hours into dialysis treatment. On Day 2, one week following Day 1, each participant received the study beverage to which they had been randomly assigned and consumed the beverage immediately prior to the initiation of dialysis treatment after which blood was collected at the two time points described above.

Figure 6.3

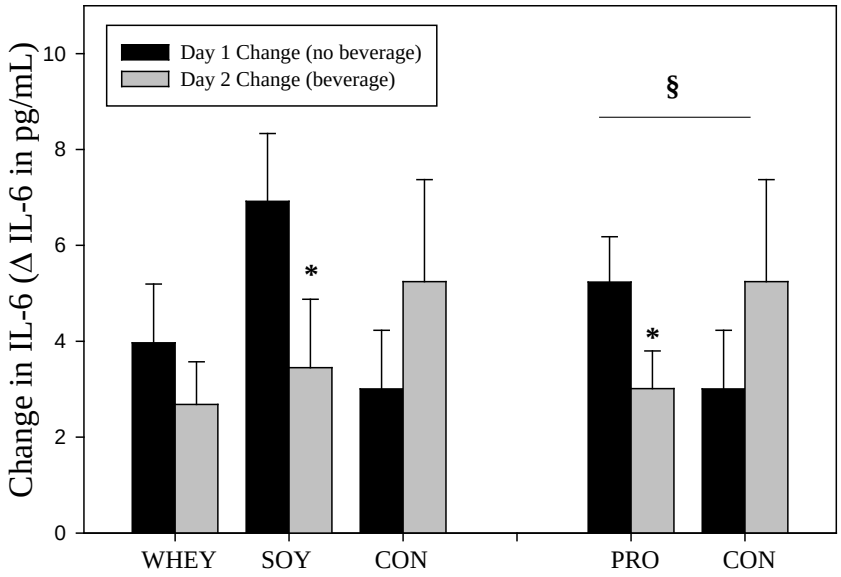

Figure 6.3: Comparison of change in IL-6 from Day 1 to Day 2 (Three Group and Two Group Analysis). A "*" indicates significantly lower increases in IL-6 on Day 2 compared to Day 1 for SOY ($p<0.05$) and combined PRO ($p<0.05$) analyzed by paired sample t-test for within-group changes. There was significant time by treatment effect for the Two Group analysis (§ indicates $p<0.05$ for interaction). Changes are presented as mean ± SEM.

Figure 6.4

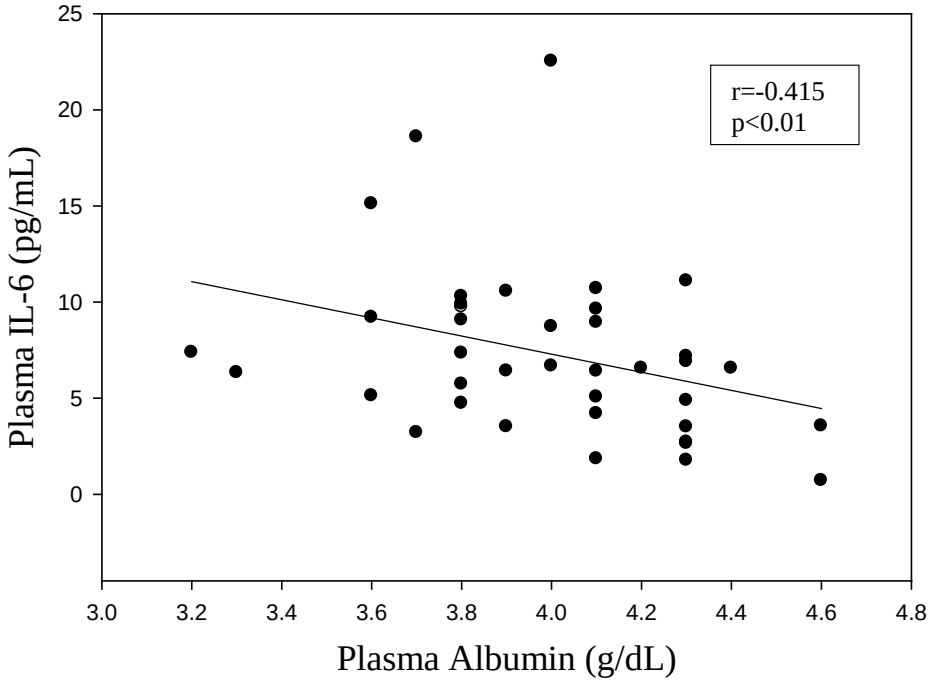

Figure 6.4: Correlation of plasma IL-6 at baseline with plasma albumin. Baseline IL-6 was inversely related to plasma albumin ($r=-0.415$, $p<0.01$).

Figure 6.5

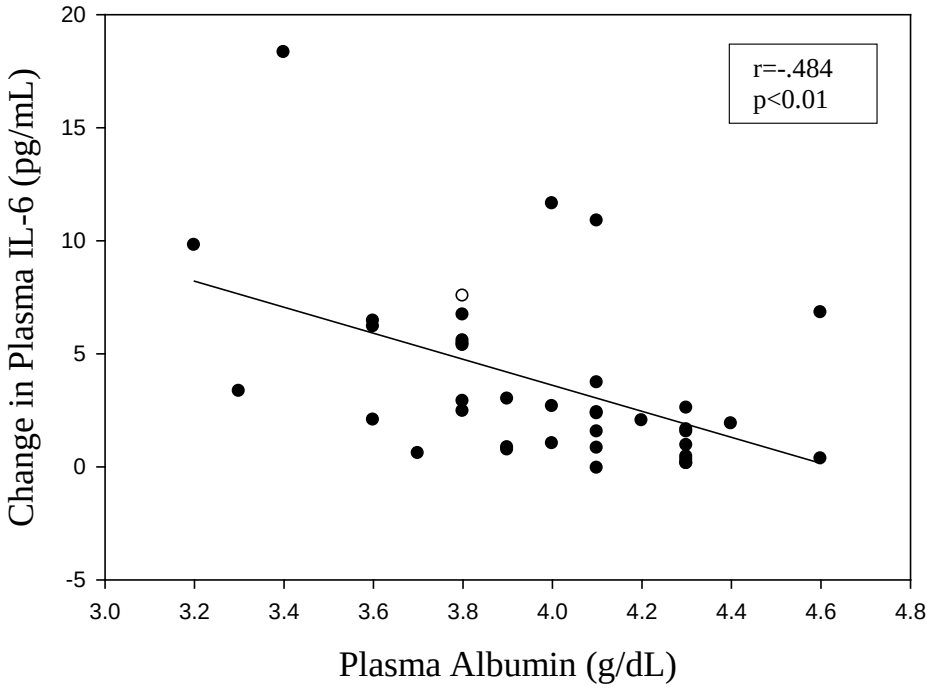

Figure 6.5: Correlation of change in plasma IL-6 on Day 1 with plasma albumin. The change in IL-6 from Time Point 1 to Time Point 3 on Day 1 was inversely related to plasma albumin ($r=-0.484$, $p<0.01$).

Figure 6.6

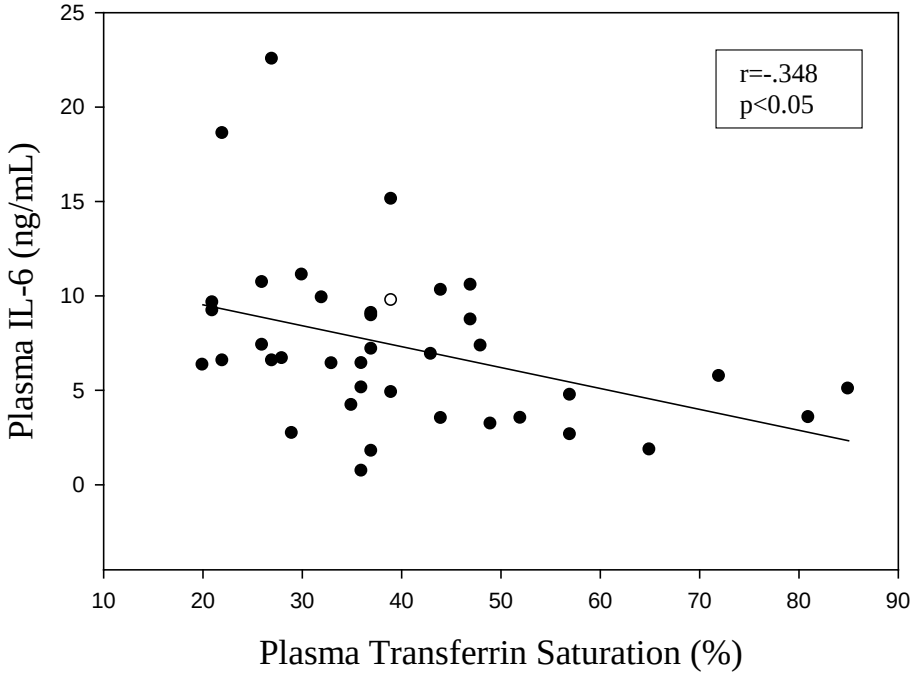

Figure 6.6: Correlation of plasma IL-6 at baseline with transferrin saturation. Baseline IL-6 was inversely related to transferrin saturation ($r=-0.348$, $p<0.05$).

Figure 6.7

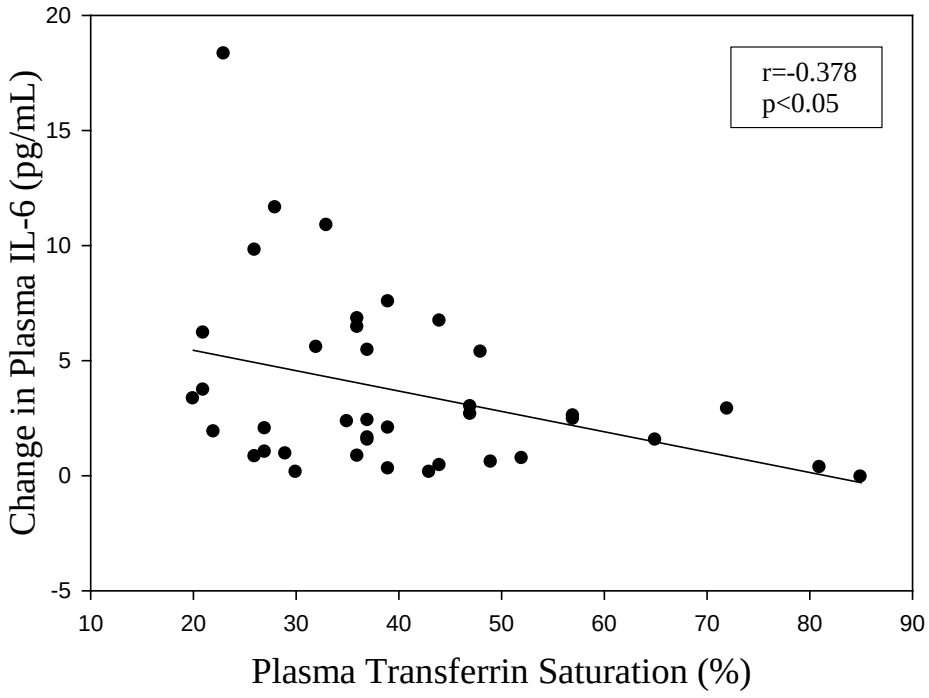

Figure 6.7: Correlation of change in plasma IL-6 on Day 1 with transferrin saturation. The change in IL-6 from Time Point 1 to Time Point 3 on Day 1 was inversely related to transferrin saturation ($r=-0.378$, $p<0.05$).

CHAPTER 7

LONG-TERM EFFECTS OF INTRADIALYTIC PROTEIN SUPPLEMENTATION

Introduction

Protein-energy malnutrition is very common in dialysis patients, with the incidence rate ranging from 25% to 75% in different studies[6-9]. There are a variety of reasons for this, including poor nutrient intake, physical illnesses affecting gastrointestinal function, protein losses during dialysis, and elevated whole body and skeletal muscle protein catabolism that occurs primarily during dialysis (reviewed in[10]). Protein malnutrition is associated with a loss of lean mass and declines in physical function in hemodialysis patients. These functional declines reduce physical activity levels, which exacerbates the development of co-morbidities like cardiovascular disease (CVD) and bone disorders. This cycle of disease and disability greatly reduces the quality of life (QOL) and increases mortality in dialysis patients[11, 12].

In response to these issues, the National Kidney Foundation has recommended an increase in the protein requirement to 1.2 g/kg/day for hemodialysis patients in comparison to the 0.8 g/kg/day recommended for healthy adults and 0.6 g/kg/day recommended for earlier stages of Chronic Kidney Disease (CKD). This recommendation was based on several small prospective nutritional-metabolic studies indicating this intake level is necessary to ensure neutral or positive nitrogen balance in most dialysis patients[10]. However, most dialysis patients do not consume this recommended amount of protein, and a large multi-center study of dialysis patients showed protein and calorie intake to be significantly lower on dialysis treatment days compared to non-treatment days[219].

The combination of amino acids lost into the dialysate, suppressed nutrient intake on dialysis days, and the catabolic effects of dialysis treatment suggests that the time immediately prior to initiation of treatment would be most appropriate to administer a protein intervention to potentially offset the negative effects of dialysis. Furthermore, intradialytic supplementation may be particularly efficacious in improving protein balance since it is provided during the time when protein catabolism is at its peak[10, 98, 99]. Leucine concentrations are especially important during a highly catabolic condition as high amounts of leucine have been shown to potently stimulate muscle protein synthesis and inhibit protein breakdown in skeletal muscle and liver[128]. Studies have shown that both parenteral[100-102] and oral[96, 103, 104] intradialytic supplementation

improve protein homeostasis, increase serum albumin and prealbumin levels, and have anabolic effects on skeletal muscle. However, oral supplementation is much more practical than parenteral due to high costs and restrictions on the use of parenteral nutrition by Medicare and other insurance providers. Furthermore, it is not known if these acute anabolic effects translate to long-term benefits on functional outcomes in this population.

The purpose of this study was to test the efficacy of six months of intradialytic protein supplementation, with either whey or soy protein, on CKD co-morbidities including measures of cardiovascular disease risk, bone health, measures of physical performance, body composition, QOL, and clinically relevant plasma markers. We hypothesized that protein supplementation, regardless of source, would improve functional outcomes by increasing substrate availability to attenuate protein catabolism and associated inflammation. For functional measures, we expected to see the greatest effect in whey protein supplementation compared to soy owing to the larger ratio of leucine, while we expect greater effects on bone parameters from soy protein due to the bioactive isoflavones.

Methods

Subject Recruitment, Screening, and Selection. Individuals with CKD receiving hemodialysis treatment at the Champaign-Urbana Dialysis Center in Champaign, IL and the Oak Park Dialysis Clinic in Oak Park, IL were recruited at their respective clinics. Patients that met the following criteria were enrolled in the study: 1) Subjects must receive hemodialysis treatment at least 3 days per week. 2) Subjects must be $\geq$ 30 years of age. 3) Subjects must not have congestive heart failure or chronic obstructive pulmonary disease 4) Subjects must have been receiving dialysis treatment for $\geq$ 3 months as disruption in metabolic factors is related to duration of dialysis treatment. 5) Subjects must weight <300 pounds due to weight limitations on the dual x-ray absorptiometry (DXA) table. A Health and Medical History Questionnaire was administered during the screening, and informed consent was obtained from each participant.

After consent was obtained, participants were randomly assigned to one of three groups: whey protein (WHEY, n=5), soy protein (SOY, n=6), or placebo/control (CON, n=7). All research protocols were conducted with the approval of the University of Illinois Institutional Review Board**.** Recruitment and retention information is provided in **Figure 7.1**.

Intervention Protocol: Protein Supplementation. Participants were given either a whey protein beverage, soy protein beverage, or placebo beverage immediately before every dialysis treatment for six months. Components of the protein beverages are listed in **Table 7.1**. The whey protein powder (True Protein, Inc., Oceanside, CA) contains 27 grams of cold-filtered whey protein isolate per 30 gram serving, with 110 calories per serving. The soy protein powder (Solae, Gibson City, IL) contains 27 grams of soy protein isolate (Supro 670) per 32 gram serving, with 120 calories and 1.5 grams of fat. This soy protein isolate provides 40 milligrams of isoflavones per serving (12 mg daidzein, 22 mg genistein, 6 mg glycitein). Supplements were matched for protein amount, resulting in a slightly higher total gram amount of the soy protein supplement. The powders were mixed with a flavor pack containing natural/artificial flavors and colors, sucralose, and acesulfame k, and were prepared with 4-6 ounces of water for consumption. Both protein powders provided sodium, iron, phosphorus, calcium, and other nutrients that are of concern for dialysis patients; however, the levels provided in this beverage were lower than those in a commercially available renal-specific formulation at the same level of protein (Nepro, Abbott Nutrition, Columbus, OH). Patients in the CON group received 4-6 ounces of non-caloric Crystal Light prepared according to package directions (Kraft, Northfield, IL). Compliance was tracked for each patient and a level of 75% compliance was established for remaining in the study.

Clinical Testing and Measurements. At baseline and immediately following the 6 month intervention (final testing), all patients underwent a series of tests described below to evaluate the effects of the intradialytic protein supplementation on our primary outcomes. All testing sessions, with the exception of blood collections were conducted on a non-dialysis day 18-24 hours following a dialysis treatment. All testing was analyzed by study personnel blinded to the subject's group assignment. Blood collections were not performed on Mondays or Tuesdays to control for the extra day between treatments that occurs during the weekend. For example, blood would not be drawn for a Monday/Wednesday/Friday scheduled patient on Monday as they would not have dialyzed since Friday (two day lapse). For a Tuesday/Thursday/Saturday scheduled patient, blood would not be drawn during their treatment on Tuesday for the same reason. All testing was done on identical equipment at both sites; all blood chemistries and data analysis were performed in Champaign, IL regardless of collection site.

Anthropometric Measures. Barefoot standing height was measured to the nearest 0.1 cm with a stadiometer and body weight was measured on a balance scale with shoes and superfluous outer garments removed. All measurements were taken in duplicate and averaged.

Blood Chemistry. Prior to the start of the intervention, and at monthly intervals until the final testing session (7 total time points), non-fasted blood was collected at the dialysis clinic during normally scheduled blood draws and assessed for standard clinical lab parameters (plasma albumin, phosphorus, calcium, etc) by Spectra Laboratories, a renal specific laboratory service provider (Rockleigh, NJ)..

Carotid Arterial Compliance. A combination of ultrasound imaging of the common carotid artery with simultaneous arterial tonometry of the contralateral carotid artery (for estimation of carotid blood pressure was used to determine carotid arterial compliance. Carotid ultrasound images were obtained from the common carotid artery, 1-2 cm proximal to the carotid bifurcation using a 7-13 MHz linear array transducer with a sampling rate of 1000 Hz. B-Mode and M-mode images were obtained and displayed simultaneously and automated wall tracking software was used to detect changes in lumen size as described above. The electronic calipers were applied to the arterial wall using the B-mode image and wall tracking was conducted using the M-mode image in real time. Changes in lumen size between systole and diastole were recorded for 12 seconds and an ensemble average beat were constructed from which measurements of arterial compliance and stiffness were calculated. Arterial compliance (AC) and the beta stiffness index (β) were calculated together with lumen size in systole and diastole using the calculations shown below:

-AC (arterial compliance: index of blood vessel compliance)= $\pi(Ds \times Ds - Dd \times Dd) / [4 (Ps - Pd)]$
-β (stiffness parameter: index of arterial stiffness) = $In (Ps / Pd) / [(Ds - Dd) / Dd]$

Brachial blood pressure was obtained using standard methods following a 10 minute rest period. Pressures were corrected for hold down pressure.

Bone Mineral Density and Body Composition. Bone mineral density (BMD) and whole-body soft tissue composition were measured by DXA using a Hologic QDR 4500A bone densitometer (software version 11.2, Bedford, Massachusetts) from scans of the whole body, lumbar spine and proximal femur. All scans were analyzed and quality-controlled by the same two technicians blinded to treatment status. Short and long-term accuracy of the densitometer was verified by scanning a manufacturer's hydroxyapatite spine phantom of a known density. Precision for DXA measurements of interest are ~1.0 – 2.0% in our laboratory. Mineral free lean mass (MFLM) was calculated from DXA values as lean mass of selected compartment – bone mineral content of selected compartment (whole body, trunk, leg, etc).

Shuttle Walk Test. Participants underwent a shuttle walk test to assess physical performance in which they walk back and forth continuously on a 10 meter course. The walking was paced by beeps which were programmed to have participants maintain each speed for one minute and then the pace was increased. The speeds increased so that in each successive minute the speeds were as follows: 1.12, 1.54, 1.88, 2.26, 2.64, 3.02, 3.4, 3.78, 4.16, 4.54, 4.92, and 5.3 miles per hour. The patient continued until they failed to achieve the distance to the end of the course by the beep and the total time was calculated.

Measures of physical performance such as this shuttle walk test are frequently used to assess function in older and diseased people instead of more objective measures of aerobic capacity such as VO_2max testing. This is due to functional limitations like muscle weakness and shortness of breath that prevent these individuals from achieving standard criteria used in assessment of these more objective tests[136]. This shuttle walk test is well established as a part of the guidelines for assessment of fitness in patients with chronic pulmonary disease[213], and is often preferred to the six minute walk test because it is paced, and therefore more objective.

Quality of Life Assessment. Physical and mental health status was measured by the 12-Item Short Form Survey derived from the Medical Outcomes 36-item Short Form Survey[214]. Global QOL was measured by the Satisfaction with Life Scale[215, 216]. This measure is a cognitive judgment of one's life taken as a whole, and is usually thought to involve comparing one's life against some standard. These scales have been shown to be reliable and valid indices of components of subjective well-being.

Diet assessment. Participants were given two 24- hour food recall interview with a trained researcher on consecutive days to account for variations in eating patterns associated with dialysis treatment. The interview was conducted using a modified version of the United States Department of Agriculture 5-pass method: the first pass asked for the patients to recall everything they ate during the previous 24-hours, the second pass probed for foods that may have been forgotten including beverages, snacks, and condiments, the third pass included prompts for portion sizes and food amounts, the fourth pass asked for details about the food including brand names, and the fifth pass consisted of a final review of the record. The records were analyzed for macronutrient composition and other dietary variables using Nutritionist Pro Data Analysis Software (Stafford, TX).

Statistical Analysis. All data are presented as mean ± SEM, unless otherwise indicated. Significance was considered when $p<0.05$. Generalized estimating equation (GEE) for longitudinal data was used to determine the effects of protein supplementation on monthly plasma variables. Plasma variables were collected each month from baseline to final testing for a total of 7 time points/person. The full model considers the interaction between time and treatment, controlling for gender, age, diabetes status, and smoking. The full model is as follows, where "Y" represents each plasma variable of interest, and "Y0" represents that particular variable at baseline to control for any baseline variations:

$$Y= \beta_0 + \beta_1 Y0 + \beta_2 \text{treatment} + \beta_3 \text{gender} + \beta_4 \text{age} + \beta_5 \text{diabetes} + \beta_6 \text{smoking} + \beta_7 \text{time} + \beta_8 \text{time*treatment}$$

For some analyses, data from WHEY and SOY were combined to look at the effects of protein supplementation, regardless of source; this combined group assessing general protein intake is labeled PRO. Repeated measures analysis of variance (ANOVA) was used to determine time by treatment interaction for functional outcomes. In the absence of significant interaction effect, Cohen's d was calculated for effect size. Correlation analysis was used to identify relationship between selected variables of interest. Differences in nutrient intake between baseline and final testing were determined using paired-sample t-test for each treatment group.

Results

Patient Characteristics. Subject characteristics at baseline are shown in **Table 7.2**, and the etiology of each patient's renal failure is described in **Table 7.3**. A total of 22 patients were recruited for this study, with 7 in WHEY, 8 in SOY, and 7 in CON. One subject passed away during the study (unrelated to study protocol), 1 subject dropped out to due gastrointestinal distress, 1 participant withdrew for unknown reasons, and one participant withdrew after receiving a kidney transplant during the intervention period. A total of 5 WHEY, 6 SOY, and 7 CON participants completed the 6-month intervention protocol. At baseline, the three groups did not differ significantly regarding age, body mass index (BMI), or any of clinical lab values. When protein groups were combined, there were also no significant differences between PRO and CON for any of the above factors. All groups were within normal range for plasma albumin levels, but had elevated iPTH, phosphorus, and ferritin, and depressed total iron binding capacity (TIBC) (**Table 7.2**). Levels of hematocrit and hemoglobin were marginally low in all groups compared to reference ranges suggested by the National Kidney Foundation Chronic Kidney Disease Outcomes Quality Initiative (NKF KDOQI).

Plasma Variables. Results of analysis of monthly plasma variables representing seven time points per patient from baseline to final testing are shown in **Table 7.4**. WHEY was associated with an increase of 0.051±0.07 mEq/L of bicarbonate/month ($p<0.01$), a decrease in phosphorus of 0.29±0.05 mg/dL per month ($p<0.05$), a decrease of ferritin/month of 25.6±8.1 ng/mL ($p<0.05$), a decrease of 0.15±0.02 mEg/L potassium/month ($p<0.05$), and an increase in TIBC/month of 1.1±0.7 mcg/dL ($p<0.05$). When protein groups were combined, PRO was associated with a decrease in alkaline phosphatase of 5.4±3.5 U/L ($p<0.05$). There were no significant time by treatment interactions for albumin, creatinine, hemoglobin, hematocrit, parathyroid hormone, blood urea nitrogen, calcium, iron, or transferrin saturation.

Functional Outcome Measures and Baseline and Final Testing. We did not find any significant time by treatment effects for our outcomes of cardiovascular disease risk, QOL, bone health, or body composition. Mean values at baseline and final testing are presented in **Table 7.5** along with calculated effect sizes (indicated by Cohen's d). We did not see any time by treatment effects for QOL (Total Disability Score, Satisfaction with Life Scale), bone measures

(Hip BMD, Whole Body BMD) or body composition (Whole Body MFLM , Whole Body % Fat). For central pulse wave velocity (PWV), our primary cardiovascular outcome, there were no statistically significant interaction effects. However, PRO had ~20% decrease in PWV (d=1.01) compared to an approximately 14% increase in PWV for CON (d=0.689).

Physical Performance. There was a significant time by treatment interaction effect for Shuttle Walk test; average walk time for WHEY significantly improved from baseline to final ($p<0.01$), time significantly declined for CON ($p<0.05$) and SOY, although the decline for SOY was not significant (**Figure 7.2**). When all groups were considered, change in Shuttle Walk time was positively correlated with TIBC measured at final testing ($r=0.594$, $p<0.05$) (**Figure 7.3**). There was a trend for correlation of final TIBC and final Shuttle Walk Time in all treatment groups, although this was not significant ($p=0.056$).

Diet Assessment. Protein intake per body weight was significantly higher on dialysis day at final testing compared to baseline (**Figure 7.4**); protein intake per body weight was not significantly different for non-dialysis day, but the average protein intake was significantly higher for PRO. There was no difference in protein intake for CON from baseline to final for dialysis or non-dialysis days. PRO had significantly higher intake of phosphorus and calcium on dialysis day at final compared to baseline ($p<0.05$). There were no differences for any of the selected nutrients for CON from baseline to final testing (**Table 7.6**) including grams of protein, carbohydrates, fat, and overall caloric intake.

Discussion

The primary findings in this paper were that six months of intradialytic whey protein supplementation improved physical performance as assessed by Shuttle Walk Test time, and the change in time was associated with total iron binding capacity (TIBC); whey protein supplementation also was associated with increased plasma TIBC, increased bicarbonate, and decreased phosphorus, potassium, and ferritin. When the protein groups were considered together, protein intake was associated with lower alkaline phosphatase (ALP). Furthermore, intradialytic protein supplementation increased protein intake on dialysis day and average protein intake from baseline to final testing, adjusted for body weight. Lastly, there was no significant

difference in pulse wave velocity in any group, but we saw a large effect size for reduction of pulse wave velocity in the PRO group (d=1.01) compared to an increase in PWV for CON (d=0.689). Taken together, these data suggest the benefits of intradialytic protein supplementation, particularly for whey protein; perhaps in part due to the larger ratio of branched-chain amino acids present in whey protein.

We were able to demonstrate that the intradialytic supplementation we provided significantly increased protein intake on dialysis day in both groups receiving protein, while there was no change in protein intake in the control group. Average intake reported in grams was not significantly increased in the PRO group from baseline to final as shown in **Table 7.6**, but when protein intake was normalized to body weight, the difference was statistically significant (**Figure 4**).

This increase in protein intake on dialysis day was accompanied by a significant increase in phosphorus and calcium intake due in part to the protein supplement we provided. Hyperphosphatemia independently predicts morbidity and mortality in dialysis patients[239] and both high phosphorus and calcium are associated with development of vascular calcification and other metabolic abnormalities[240]; for this reason patients are requested to restrict calcium and phosphorus, often taking the form of protein restriction, which further exacerbates the malnutrition-inflammation complex commonly seen in this population. However, despite the reported increase in dietary calcium and phosphorus, whey protein supplementation was associated with a significant decrease in plasma phosphorus of 0.288±0.06 mg/dL per month and no change was seen for either group in plasma calcium. This suggests that intradialytic protein supplementation, although a source of dietary phosphorus and calcium, did not detrimentally affect circulating levels of phosphorus and calcium in this study. Other studies have shown phosphorus intake does not , and other factors may be contributing more significantly to the regulation of these minerals in the blood.

Patients receiving whey protein performed significantly better on the Walk Test from baseline to final compared to SOY and CON, who performed worse on average at final testing. We have previously observed in our lab that gender and diabetes status are significant predictors of walk test performance; however, the improvement for WHEY remained significant after controlling for these two factors. Furthermore, performance on the Walk Test was associated with TIBC, suggesting a potential mechanism by which whey protein improves physical

performance. In fact, we also showed whey protein to be associated with an increase in TIBC of over the intervention period.

Low levels of TIBC and high ferritin levels, as seen in this study, suggest anemia of chronic disease, as the exact opposite would be observed in iron-deficiency anemia[235]. Anemia of chronic disease, often termed anemia of inflammation, results in derangement of traditional markers of iron metabolism. Ferritin, an acute phase protein, was well above desirable clinical ranges in all groups prior to this intervention, and intradialytic whey protein supplementation was associated with a 25.56±8.1 ng/mL decrease in ferritin levels per month of the study. Many regard elevated ferritin levels to be a marker of inflammation in dialysis patients[241], and this could potentially indicate an anti-inflammatory role for intradialytic protein supplementation. Because protein malnutrition in dialysis patients promotes inflammation, it has been suggested that improving nutritional status may help prevent inflammation[60], and could explain the relationship between intradialytic whey supplementation and reduced levels of the acute phase protein ferritin. Although the mechanism by which whey protein increases TIBC and decreases ferritin cannot be determined from the present study, improvement in iron metabolism and subsequent increase in red blood cell production could be partly responsible for the improvement in physical performance seen here.

Levels of alkaline phosphate (ALP) have been shown to be inversely associated with bone mineral density in hemodialysis patients[185], and positively associated with vascular calcification[186] leading to increased cardiovascular risk in this population. Furthermore, Regidor and colleagues reported elevated ALP to be an independent predictor of mortality in a 3-year cohort of over 70,000 dialysis patients, suggesting the clinical relevance of this marker[226] We found PRO to be associated with a significant decrease of 5.39±3.5 U/L ALP per month during this study intervention. We have previously demonstrated a reduction in ALP after 4-months of intradialytic exercise training[242], but the mechanism for intradialytic exercise or protein-induced reduction in ALP is unknown at this time.

There were several limitations to this study, most notably the small sample size. The CON group had a large proportion of men (6:1), was generally younger, heavier, and had fewer smokers. We have observed previously that gender is highly related to measures of fitness, strength, and cardiovascular disease risk in this population; also weight and gender predict bone mineral density and body composition, while smoking, age, and gender are all related to

cardiovascular disease outcomes in hemodialysis patients. Therefore, the large proportion of relatively younger, non-smoking, heavier men in the placebo group may have masked some potential differences in study outcomes due to the very small sample size. Larger, multi-center intervention trials are needed to expand on the findings of this current study. Additionally, we did not control for patient medications with this study.

Despite the small sample size, we were able to demonstrate significant effects of protein supplementation on functional outcomes and relevant clinical markers in hemodialysis patients. In summary, we found intradialytic whey protein supplementation was associated with improved physical performance and improvements in plasma variables related to CKD co-morbidities. We also found an association with lower ALP in patients receiving intradialytic protein, regardless of source. Furthermore, we did not see any significant changes in plasma calcium or calcium phosphorus product despite significant increases for dietary intake of phosphorus and calcium in both protein groups on dialysis treatment day; conversely, we demonstrated whey protein was significantly associated with a decrease in plasma phosphorus over the 6- month intervention period. In conclusion, intradialytic protein supplementation, particularly whey protein, induced modest favorable changes in functional outcomes and could represent a low-cost therapeutic treatment strategy for this critically ill population.

Tables

Table 7.1: Composition of Whey Protein Isolate, Soy Protein Isolate, and Placebo Beverage[a]

Per Serving	Whey Isolate (30g) *True Protein, Oceanside, CA*	Soy Isolate (32g) *Solae, Gibson City, IL*	Crystal Light (2g) *Kraft, Northfield, IL*
Total Fat	0 g	0.9 g	0 g
Saturated Fat	0 g	0.3 g	0 g
Cholesterol	0 mg	0 mg	0 mg
Sodium	48 mg	362 mg	35 mg
Carbohydrates	0 g	0 g	0 mg
Protein	27 g	27 g	0 mg
Leucine	2.9g	1.9g	0 mg
Vitamin A	0 mg	<2%	0 mg
Vitamin C	0 mg	<2%	0 mg
Calcium	151 mg	23 mg	0 mg
Iron	0 mg	4.5 mg	0 mg
Phosphorus	64.5 mg	237 mg	0 mg
Potassium	176 mg	176 mg	0 mg
Isoflavones	0 mg	40 mg	0 mg

[a]Composition analysis was performed by a third-party laboratory at the University of Missouri.

Table 7.2: Participant Characteristics at Baseline

	WHEY (n=5)	**SOY (n=6)**	**CON (n=7)**	**p-value**	**PRO (n=11)**	**p-value**
Gender	60.0%	50.0%	85.7%	0.415	54.5%	
Age (years)	57.6±8.5	54.0±8.5	49.5±2.1	0.701	55.6±5.8	0.442
Diabetic (yes)	40.0%	33.3%	57.1%	0.712	36.4%	
Smoker (yes)	60.0%	50.0%	28.6%	0.575	54.5%	
BMI (kg/m^2)	28.9±3.1	29.2±2.4	35.6±3.8	0.276	29.1±1.8	0.103
Albumin (g/dL)	4.04±0.11	3.93±0.06	3.99±0.08	0.698	3.98±0.06	0.969
iPTH (pg/mL)	458±536	945±668	427±157	0.720	762±421	0.601
Phosphorus (mg/dL)	6.58±0.89	5.88±0.60	6.51±0.78	0.781	6.20±0.50	0.727
Potassium (mEq/L)	4.80±0.28	4.70±0.43	4.84±0.31	0.956	4.74±0.25	0.813
TransSat (%)	31.4±5.1	34.0±3.1	34.0±3.5	0.872	32.8±2.8	0.793
TIBC (mcg/dL)	222±16	219±9	194±7	0.163	220±8	0.054
Ferritin (ng/mL)	1007±81	852±193	739±190	0.397	922±59	0.264
Iron (mcg/dL)	68.8±10.8	75.7±9.5	65.4±5.5	0.670	72.5±6.8	0.473
Hematocrit (%)	36.5±1.6	37.2±0.89	36.5±1.2	0.891	36.9±0.84	0.778
Hemoglobin (g/dL)	11.6±0.35	12.1±0.26	11.9±0.37	0.568	11.9±0.21	0.884

Data are presented as mean ± SEM. The first p-value column refers to differences among WHEY, SOY, and CON, and the second p-value refers to differences between PRO and CON.

Table 7.3: Etiology of Renal Failure for Participants

Etiology of Renal Failure	**WHEY**	**SOY**	**CON**	**PRO**
Hypertension	80% (4)	50% (3)	57.1% (4)	63.6% (7)
Diabetes w/ renal manifestations, Type 1		16.7% (1)		9.1% (1)
Diabetes w/ renal manifestations, Type 2		16.7% (1)	28.6% (2)	9.1% (1)
Polycystic Kidney Disease			14.3% (1)	
Nephritis/Nephropathy		16.7% (1)		9.1% (1)
Unknown/Other	20% (1)			9.1% (1)
Total	100% (5)	100% (6)	100% (7)	100% (11)

Table 7.4: GEE Modeling of Monthly Plasma Variables

	WHEY	p-value	**SOY**	p-value	**CON**	p-value
Bicarbonate (22-29mEq/L)	0.51±0.07	**<0.01**	-0.44±0.19	NS	-0.14±0.19	NS
Phosphorus (2.6-4.5 mg/dL)	-0.29±0.06	**<0.05**	0.007±0.08	NS	-0.02±0.05	NS
Ferritin (22-322 ng/mL)	-25.6±8.1	**<0.01**	15.4±4.5	NS	35.6±16.5	NS
Potassium (3.5-5.1 mEq/L)	-0.15±0.02	**<0.05**	0.02±0.01	NS	0.01±0.06	NS
TIBC (228-428 mcg/dL)	1.07±0.65	**<0.05**	-1.30±0.74	NS	-2.47±0.80	NS

Data are presented as mean ± SEM. P-values < 0.05 are considered significant. GEE Modeling included 7 time points/per measure for each patient, and p-value represents significant time by treatment interaction in a model that included time, gender, diabetes status, and smoking status.

Table 7.5: Functional Outcome Measures at Baseline and Final Testing

	PRO (n=11)			CON (n=7)		
	Baseline	Final	d	Baseline	Final	d
AI (%)	17.4±3.6	14.4±3.7	0.275	8.9±4.0	7.5±5.9	0.103
β Stiffness (U)	9.60±0.80	11.10±1.6	0.3926	7.41±0.80	8.52±1.5	0.3440
PWV (m/sec)	12.52±1.7	9.99±1.0	1.01	7.80±1.1	10.35±2.5	0.689
Total Disability Score	57.8±3.0	59.5±3.5	0.163	61.4±4.0	59.9±4.8	0.129
SWLS	20.1±2.2	20.8±2.7	0.087	18.0±3.5	19.1±3.1	0.131
Hip BMD (g/cm^2)	0.88±0.07	0.89±0.07	0.041	0.97±0.08	0.97±0.09	0.019
Wb BMD (g/cm^2)	1.06±0.05	1.07±0.04	0.063	1.13±0.07	1.14±0.07	0.031
Wb MFLM (kg)	54.50±3.8	54.6±3.8	0.003	67.0±7.1	66.8±6.2	0.015
Wb % Fat (%)	30.4±2.8	30.8±2.4	0.03	33.4±4.9	34.3±5.3	0.06

Data are expressed as mean ± SEM. Cohen's d represents effect size. AI=Augmentation Index, PWV=pulse wave velocity, SWLS=Satisfaction with Life Scale, BMD=Bone Mineral Density, Wb=Whole Body, MFLM=Mineral Free Lean Mass

Table 7.6: Comparison of Selected Nutrients on Dialysis and Non-Dialysis Days as assessed by 24-hour Dietary Recall , by Treatment Group

	Dialysis Day		Non-Dialysis Day		Average	
	Baseline	Final	Baseline	Final	Baseline	Final
PRO: Protein (g)	**59.9±7.0**	**81.2±6.7***	76.4±12.8	79.7±15.2	68.2±7.4	80.4±9.7
CON: Protein (g)	80.9±7.9	88.7±12.8	83.2±11.6	90.2±23.1	82.1±4.9	89.5±14.1
PRO: Carb (g)	153±15	163±34	173±21	178±30	163±14	170±23
CON: Carb (g)	188±38	157±35	177±30	190±43	183±31	173±32
PRO: Fat (g)	65.9±13.6	57.7±9.1	74.6±12.7	76.8±11.6	70.3±12.1	67.3±7.1
CON: Fat (g)	61.9±9.8	66.0±12.8	61.6±6.2	60.0±17.3	61.8±4.3	63.0±11.4
PRO: Calories (kcal)	1421±180	1591±192	1648±206	1708±266	1535±174	1649±150
CON: Calories (kcal)	1626±248	1582±276	1542±155	1645±395	1584±170	1613±261
PRO: Phos (mg)	**621±90**	**853±87***	910±195	1029±243	766±88	941±132
CON: Phos (mg)	704±122	1047±243	1016±271	1132±299	860±131	1090±220
PRO: Calcium (mg)	**456±81**	**673±65***	563±121	625±171	509±82	649±97
CON: Calcium (mg)	646±183	515±130	1073±305	482±122	860±170	499±88

Data are presented as mean ± SEM, with significance set at $p<0.05$ and signified by "*" as compared to baseline measurement within each group. No between-group comparisons were made for this analysis. Carb=carbohydrate, Phos=phosphorus

Figure 7.1

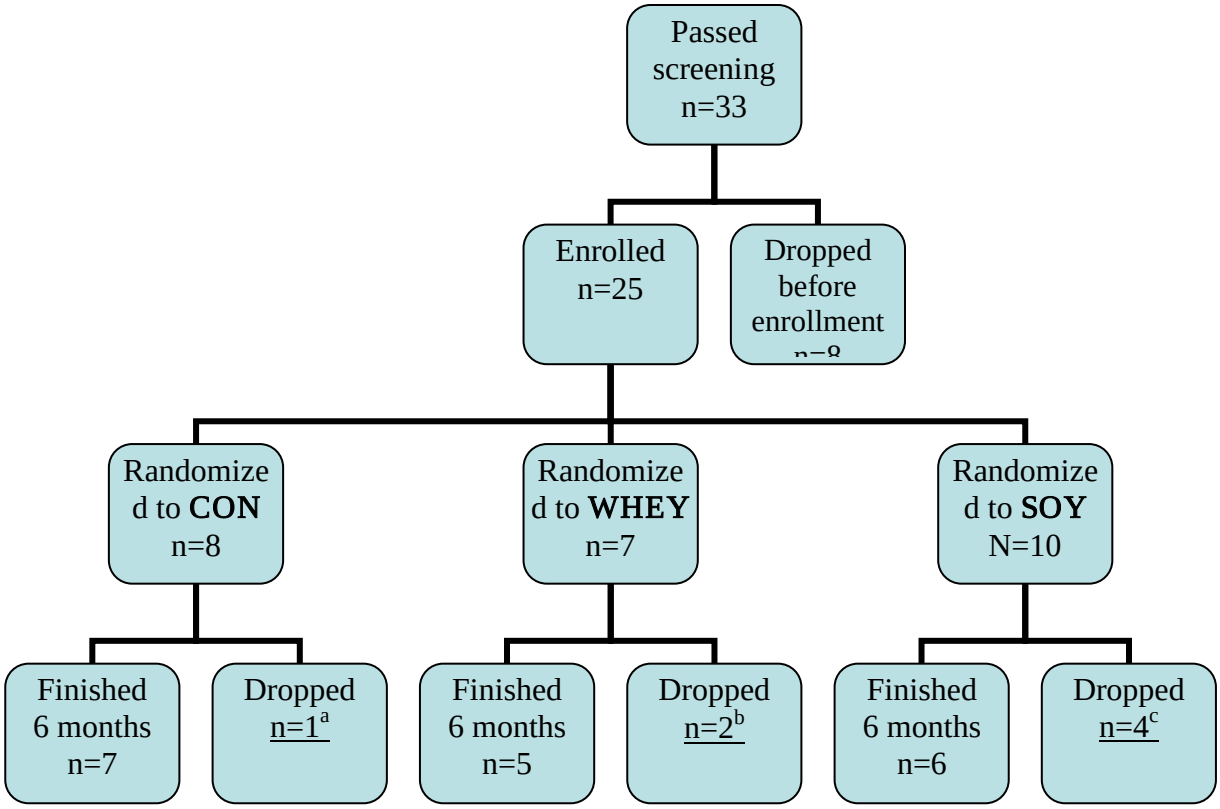

Figure 7.1: Recruitment and retention information for study participants. [a] n=1: Non-compliance Total CON n=1; [b] n=1: Non-compliance, n=1: Gastrointestinal Distress, Total WHEY n=2; [c] n=1: Non-compliance, n=1: No longer interested, n=1: Kidney transplant, n=1: Transferred clinics. Non-compliance was defined as consumption of study beverage less than 75% of scheduled dialysis sessions.

Figure 7.2

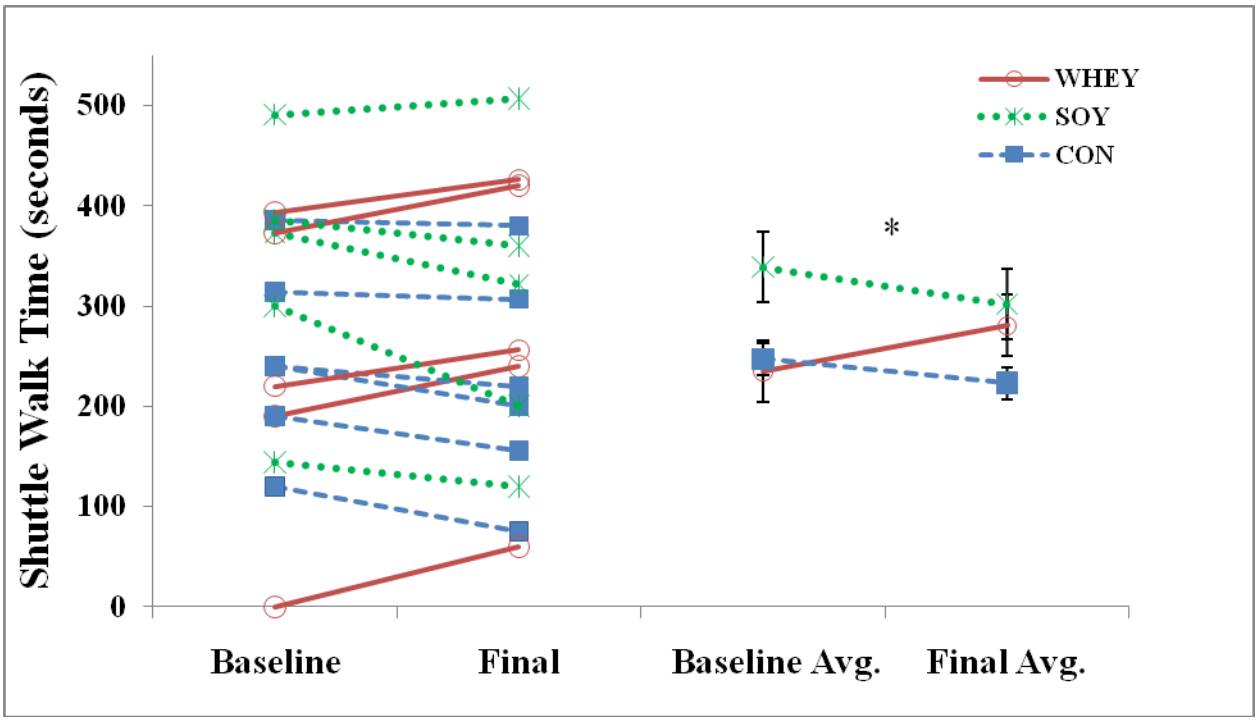

Figure 7.2: Performance on shuttle walk test. Patients in WHEY, SOY, and CON had physical performance measured at baseline and final testing using a shuttle walk test. Changes in the time walked during the shuttle walk test for each individual are shown on the left, with group averages ± SEM on the right. The "*" indicates a significant time by treatment interaction. Performance on the test increased from baseline to final testing in the WHEY group ($p < 0.01$), decreased in CON ($p<0.05$), and decreased in SOY, although this decrease was not significant ($p=0.124$).

Figure 7.3

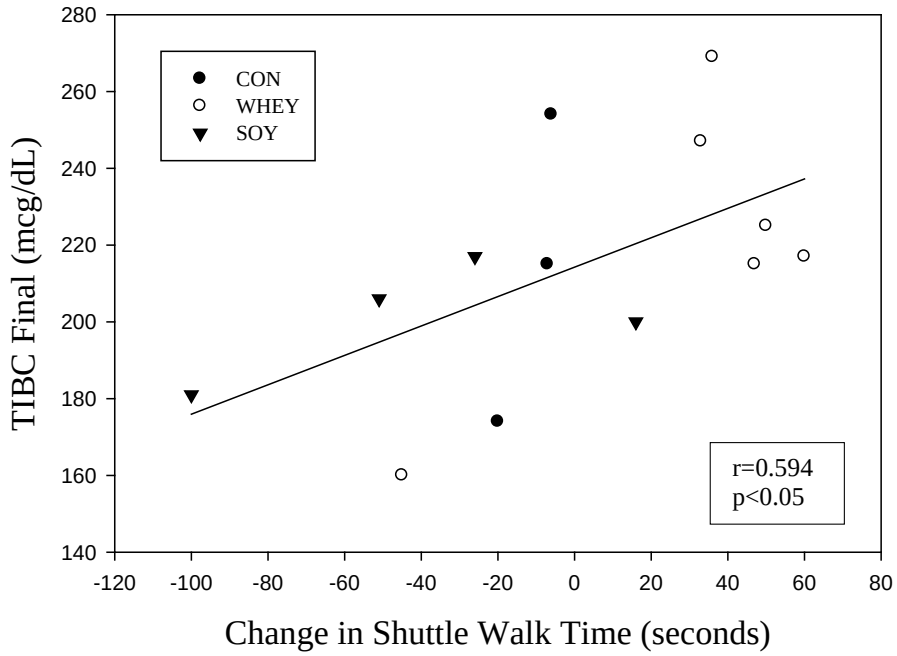

Figure 7.3: Correlation between Total Iron Binding Capacity (Final) and Change in Shuttle Walk Time. When all three groups were considered together, TIBC measured at final testing was positively associated with change in shuttle walk test ($r=0.594$, $p<0.05$).

Figure 7.4

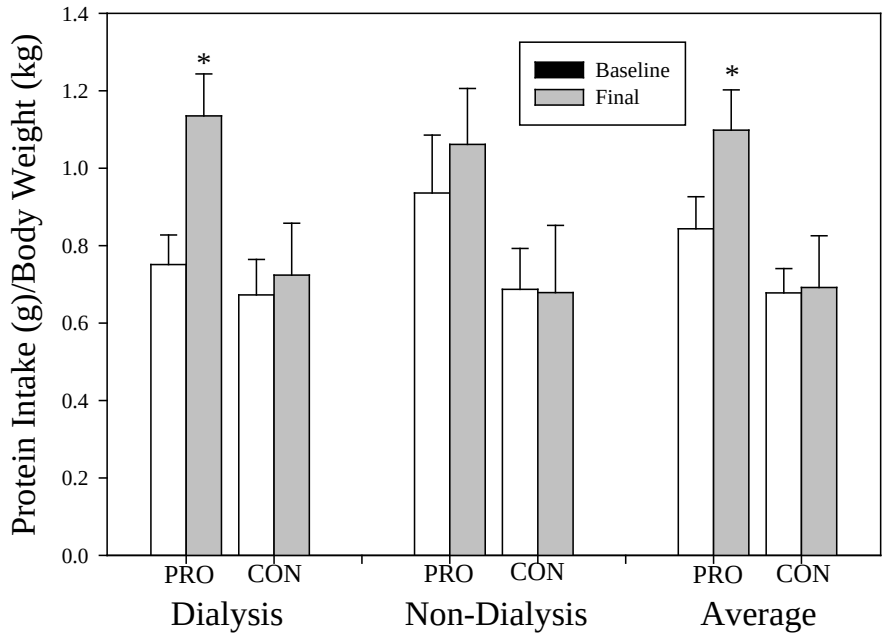

Figure 7.4: Comparison of protein intake on dialysis and non-dialysis days, by treatment group. In the PRO group, protein intake in grams per kilogram body weight was significantly increased on dialysis day from baseline to final testing, which contributed to a significant increase in average grams protein per kilogram body weight ($p<0.05$). There were no differences between baseline and final testing for CON on either day or on average.

CHAPTER 8

CONCLUSIONS

Chronic kidney disease patients on maintenance hemodialysis therapy suffer from a variety of co-morbid conditions that may be mechanistically linked. The high rates of these conditions, including cardiovascular disease, bone disease, and malnutrition, contribute to the low reported quality of life in this population. Results from this study indicate the safety and modest benefits of intradialytic exercise and protein supplementation on a variety of co-morbid conditions associated with dialysis.

Specifically, we found that a four-intradialytic training program:

- Improved physical performance as measured by shuttle walk test
- Reduced a marker of oxidative stress
- Reduced epicardial fat, cardiovascular disease (CVD) risk
- Improved circulating factors associated with CVD and bone health (alkaline phosphatase, ALP)

We provided evidence to support the effects of intradialytic protein supplementation for:

- Attenuating acute inflammation (IL-6) associated with dialysis treatment
- Improving physical performance as measured by shuttle walk test and gait speed (specific to whey protein)
- Improving circulating factors related to CKD co-morbidities (ALP, ferritin, total iron binding capacity, phosphorus, potassium)

In a mouse model of renal insufficiency, we found a benefit of combined exercise and soy protein intake on bone health parameters and plasma urea.

Taken together, these data suggest intradialytic exercise and protein supplementation to be safe and effective therapeutic interventions for this critically ill population. Furthermore, our lab is currently investigating the combined effects of protein and exercise during dialysis, as the complexity of the disease suggests multiple therapeutic strategies may be necessary to improve the health outcomes and quality of life for dialysis patients.

ABBREVIATIONS

AAPH	2-Amidinopropane Hydrochloride
AC	Arterial Compliance
AI	Augmentation Index
ALP	Alkaline Phosphatase
ANOVA	Analysis of Variance
ApoE -/-	Apolipoprotein E Knockout
BLEF	Basic Lower Extremity Function Scale
BMD	Bone Mineral Density
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
BUN_Creat	Blood Urea Nitrogen to Creatinine Ratio
BV	Bone Volume
BV/TV	Relative Bone Volume
Ca x P	Calcium phosphate product
CAC	Coronary Artery Calcium
Cas/Ex	Casein Diet, Exercise-Trained
Cas/Sed	Casein Diet, Sedentary
CHF	Coronary Heart Failure/Congestive Heart Failure
CKD	Chronic Kidney Disease
CKD-MBD	Chronic Kidney Disease Mineral and Bone Disorder
CON	Control
COPD	Chronic Obstructive Pulmonary Disorder
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DPB	Diastolic Blood Pressure
DXA	Dual X-Ray Absorptiometry
ELISA	Enzyme-linked Immunosorbet Assay
EPO	Erythropoetin
EX	Exercise
GEE	Generalized Estimating Equation
GWLAS	Godin Weekly Leisure Activity Score
HDL	High Density Lipoprotein
HDL-C	High Density Lipoprotein Cholesterol
IGF-1	Insulin-like Growth Factor -1
IL-1β	Interleukin-1 Beta
IL-6	Interleukin-6
IMT	Intima-Media Thickness
iPTH	Intact Parathyroid Hormone
ISWT	Incremental Shuttle Walk Test
KDOQI©	Kidney Disease Outcomes Quality Initiative
LA VI	Left Atrial Volume Index
LDL	Low Density Lipoprotein
LVEDD	Left Ventricular End Diastole Dimension
LVET	Left Ventricular Ejection Time

LVH	Left Ventricular Hypertrophy
LVM	Left Ventricular Mass
MBP	Milk Basic Protein
MCOT	Mitral Value Closure to Opening Time
MFLM	Mineral Free Lean Mass
MHS	Mental Health Score
MI	Myocardial Infarction
MPI	Myocardial Performance Index
NKF	National Kidney Foundation
Npna	Normalized Protein Appearance
OPG	Osteoprotegerin
PEM	Protein-Energy Malnutrition
PMN-Elastase PI	Polymorphonuclear Elastase-Protease Inhibitor Complex
PON	Paraoxonase
PRO	Protein
PTH	Parathyroid Hormone
PWTd	Posterior Wall Thickness
PWV	Pulse Wave Velocity
QOL	Quality of Life
RPE	Rating of Perceived Exertion
RWT	Relative Wall Thickness
SBP	Systolic Blood Pressure
SEM	Standard Error of Measurement
SFA	Saturated Fatty Acid Intake
SOY	Soy Protein
Soy/Ex	Soy Diet, Exercise-Trained
Soy/Sed	Soy Protein Diet, Sedentary
SWS	Satisfaction with Life Scale
TBARS	Thiobarbituric Acid Reactive Substances
TDS	Total Disability Score
TIBC	Total Iron Binding Capacity
TNF-α	Tumor Necrosis Factor Alpha
TransSat	Transferrin Saturation
TV	Total Volume
URR	Urea Reduction Ratio
VC	Vascular Calcification
VSMC	Vascular Smooth Muscle Cells
Wb	Whole Body
WHEY	Whey Protein
β	Beta Stiffness Index
Δ	Delta
μCT	Micro-Computed Tomography

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