ABSTRACT

Effects of Eight Weeks of Curcumin and Boswellia Serrata Supplementation on Plasma Markers of Inflammation and Antioxidant Activity in Chronic Kidney Disease Patients

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The purpose of this study was to examine the effects of 8 weeks of curcumin and Bowellia serrata supplementation on changes in systemic inflammation and antioxidant activity in chronic kidney disease (CKD) patients. Sixteen CKD patients (56.0 ± 16.0 years, 171.4 ± 11.9 cm, 99.3 ± 20.2 kg) were randomized in a double blind fashion to ingest a daily supplement composed of 1340 mg of curcumin and Boswellia serrata (824 mg purified turmeric extract, 95% curcuminoids and 516 mg Boswellia serrata extract, 10% 3-acetyl-11-keto-beta-Boswellic acid) or 1340 mg of a roasted rice powder placebo. Patients provided fasting blood samples pre- and post-supplementation in order to assess changes in systemic inflammation and antioxidant activity. A 2 x 2 repeated measures MANCOVA with a probability level of 0.05 was used for the statistical analysis. No significant changes ($p > 0.05$) were observed for the indicators of inflammation, represented by plasma interleukin-6 and plasma tumor necrosis factor-alpha, as well as the indicator of antioxidant activity represented by plasma glutathione peroxidase. It appears that 8 weeks of curcumin and Boswellia serrata supplementation does not affect

inflammation levels and antioxidant activity in CKD patients. More research is needed to determine the impact that curcumin and Boswellia serrata supplementation have on changes in inflammation and antioxidant activity in diseased populations.

Effects of Eight Weeks of Curcumin and Boswellia Serrata Supplementation on Plasma Markers of Inflammation and Antioxidant Activity in Chronic Kidney Disease Patients

by

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A Dissertation

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DEDICATION

To

My husband, CPT Chad J. Moreillon (Retired)

Most will never know or understand the sacrifice you made for God and country. Every American who has not served his country is forever in debt to you for fighting for freedom. After all, freedom is not free.

To

Noel “Piercy” Pierce, Matt “Doc” Gremain, Ed Miller, and Russell “Alan” Harding

The Brothers in Arms who helped bring my husband home alive. I am forever in debt to you.

To

Army Ranger Sgt. Damien T. Ficek
January, 1 1978 – December 30, 2004

Who gave the ultimate sacrifice for his country. You are a testament to what a soldier is. May you rest in peace.

CHAPTER ONE

Introduction

According to the Kidney Disease Outcomes Quality Initiative (KDOQI), chronic kidney disease (CKD) is characterized by kidney damage or a decline in kidney function for 3 or more months (National Kidney Foundation, 2002). A decline in kidney function is measured by the glomerular filtration rate (GFR), representing the flow rate of filtered fluid through the kidney, and is often estimated (eGFR) using equations that take into account serum creatinine levels, gender, age, and race (Stevens, Coresh, Greene, & Levey, 2006), the most common being the Modification of Diet in Renal Disease Study equation and the Cockcroft-Gault equation. Decreased kidney function is defined as GFR < 60 mL/min/1.73m^2. Yet, kidney disease is also easily recognized by the presence of protein in the urine, usually albumin, and this is known as albuminuria.

CKD is divided into five stages. Stage 1 is characterized by normal eGFR ≥ 90 mL/min/1.73m^2 and constant albuminuria, while stage 2 is characterized by eGFR between 60 and 89 mL/min/1.73m^2. These are the early stages of CKD, as kidney function appears normal, often exhibiting no symptoms. Eventually, kidney function becomes impaired, and this is indicated in stages 3 (eGFR between 30 to 59 mL/min/1.73m^2) and 4 (eGFR between 15 and 29 mL/min/1.73m^2). Stage 5 is characterized by eGFR < 15 mL/min/1.73m^2), and is also known as end-stage renal disease (ESRD), with dialysis or transplantation necessary at some point to replace lost kidney function (National Kidney Foundation, 2002). Consequences of CKD include kidney failure and complications such as cardiovascular disease, anemia, bone disease,

and hyperlipidemia (Thomas, Kanso, & Sedor, 2008). Presently, the majority of those with CKD do not reach kidney failure and instead die early of cardiovascular disease (Weiner, 2007).

Inflammation, particularly when chronic, is known to play a major role in various diseased states, including cardiovascular disease, diabetes, obesity, cancer and CKD. In response to an injury or invasion, the body elicits an inflammatory response to remove sources of damage and promote the healing process. Components that regulate acute inflammation include complements, pro-coagulants, cytokines, and fibrinolytics. Acute inflammation is the initial response to injury and is characterized by the movement of leukocytes into the damaged tissue. Ultimately, this process will cease when macrophages have become inhibited (Silverstein, 2008). Chronic inflammation can be thought of as an inflammatory state that occurs over a lengthy period of time (months to years) in which the tissue is exposed to constant levels of damage, lacking a recovery period (Yilmaz, Carrero, Axelsson, Lindholm, & Stenvinkel, 2007). In addition to interstitial inflammation that occurs in the kidney in CKD (Eddy, 2005), chronic inflammation is also considered a problem in atherosclerotic cardiovascular disease, diabetes, and malnutrition (Papanicolaou & Vgontzas, 2000), all of which are major risk factors for the development and/or progression of CKD (U.S. Renal Data System, 2005). Inflammatory biomarkers that are present in patients with CKD include C-reactive protein (predictor of interleukin-6) (Stenvinkel et al., 2002), serum creatinine (Oberg et al., 2004; Shlipak et al., 2003), and cystatin C (Shlipak et al., 2005). Inflammation is not only associated with ESRD but also exists in mild to moderate CKD, and this contributes to morbidity and mortality. A number of established constituents of chronic

inflammation in CKD and ESRD include hypoalbuminemia/malnutrition, atherosclerosis, beta-glucans, L-fucose, esotoxins, acetate, silicone, and lipopolysaccharides (Horl, 2002). In addition, new factors have been identified that contribute to inflammation in CKD and ESRD, particularly advanced glycation end products (AGEs). AGEs play a role in diabetic complications such as vasculopathy, neuropathy, retinopathy, and vascular inflammation by acting upon vascular endothelial cells, resulting in inflammation and atherosclerosis (Hricik, Schulak, Sell, Fogarty, & Monnier, 1993). Glutathione peroxidase (GPx), an important endogenous antioxidant enzyme, has been shown to be decreased in CKD patients, thus, potentially reducing the ability to neutralize free radicals, further contributing to chronic inflammation (Alhamdani, 2005).

Cytokines are regulators of the immune system that act in such a way as to control the function of immune cells, seeking to re-establish homeostasis. When secreted, cytokines contribute to restoring both cellular and humoral immunity (Carrero, Yilmaz, Lindholm, & Stenvinkel, 2008) and are related to inflammation in CKD and ESRD. It has been demonstrated that plasma levels of pro-inflammatory cytokines are higher before and after the start of dialysis (Pereira et al., 1994). CKD patients also frequently have higher levels of serum interleukin-6 (IL-6) and soluble IL-2 receptor (sIL2R) compared to healthy control subjects (Costa et al., 2008). Elevated levels of certain pro-inflammatory cytokines such as IL-1, TNF-α, IL-6, and IL-13 are highly associated with an increased risk for mortality (Kimmel et al., 1998). Specifically, serum pro-inflammatory cytokines give rise to the production of adhesion molecules in capillary endothelial cells. These adhesion molecules attach to activated T cells (Hill, Lan, Nikolic-Paterson, & Atkins, 1994), eliciting the production of pro-fibrotic substances,

resulting in the advancement of CKD (Lebleu, Sugimoto, Miller, Gattone, & Kalluri, 2008). Inflammation will also cause alterations in serum levels of vascular molecules in CKD, including asymmetric dimethyl arginine, a substance that prevents the synthesis of nitric oxide and therefore vasodilation. Asymmetric dimethyl arginine is also increased in CKD patients (Kielstein et al., 1999), leading to higher levels of platelet aggregation and monocyte adhesion (Vallance, Leone, Calver, Collier, & Moncada, 1992). Inflammation decreases levels of serum fetuin-A, a molecule that prohibits calcifications in vivo. Calcifications commonly occur in atherosclerotic lesions, resulting in decreased vascular elasticity, often playing a large role in the incidence of atherosclerosis (Wexler et al., 1996). Consistently, decreased levels of fetuin-A can lead to the accumulation of vascular calcifications (Ketteler et al., 2003) and increased morbidity.

The continuous state of inflammation that exists in CKD contributes to comorbidities such as cardiovascular disease and protein-energy wasting (PEW), and each of these risk factors impact clinical outcomes. Therefore, it would be logical to consider anti-inflammatory treatment strategies to target inflammation in CKD patients. A number of pharmacological compounds have successfully demonstrated anti-inflammatory activity in CKD patients. In a randomized controlled trial, statins were utilized in a population of type 2 diabetics undergoing hemodialysis (Wanner et al., 2005), but no effect on mortality was observed. Aspirin was able to decrease cytokine levels of IL-8, IL-6, and TNF-α in pediatric hemodialysis patients (Goldstein, Leung, & Silverstein, 2006). CKD patients on angiotensin-converting enzyme (ACE) inhibitors displayed lower plasma levels of TNF-α, C-reactive protein (Stenvinkel et al., 1999), and adhesion molecules (Suliman, Qureshi, Heimburger, Lindholm, & Stenvinkel, 2006).

Sevelamer, a phosphate-binding drug used to treat hyperphosphatemia in chronic renal failure patients, is also showing promise in hemodialysis patients by affecting inflammatory markers and possibly demonstrating anti-atherogenic properties (Ferramosca et al., 2005).

The use of alternative and complementary therapies has gained recent interest in the scientific literature (Barnes, Powell-Griner, McFann, & Nahin, 2004; Eisenberg et al., 1998). These therapies consist of herbal and plant-based medicines that are thought to have fewer side effects than their conventional medicine counterparts. Two such novel anti-inflammatory compounds that are of particular interest are the polyphenol, curcumin and the pentacylic triterpene, Boswellia serrata. Curcumin, the active ingredient in the dietary spice turmeric, has been used in the Asian culture for over 2000 years in everything from cooking and medicine to cosmetics and fabric dying (Ammon & Wahl, 1991). Although curcumin has been utilized for thousands of years, only recently has the scientific community taken interest in its many beneficial properties. These proposed properties include anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic characteristics (Hatcher, Planalp, Cho, Torti, & Torti, 2008). Previous researchers using in vitro rodent models have shown that curcumin is able to demonstrate anti-inflammatory actions by preventing lipoxygenase (LO), and cyclo-oxygenase (COX) actions (T. S. Huang, Lee, & Lin, 1991), the formation of nitric oxide (Brouet & Ohshima, 1995), and the production of reactive oxygen species (ROS) (Joe & Lokesh, 1994).

LO and COX are involved in the arachidonic acid cascade, a signaling pathway involving the regulation of inflammation. Five-LO catalyzes the oxidation of arachidonic

acid into 5-hydroperoxyeicosatetraenoic acid (5-HETE) and eventually leukotrienes, while COX converts arachidonic acid into prostaglandin H_2 (Cook, Geisel, Halushka, & Reines, 1993). Nitric oxide is an endogenous vasodilator and among other things, is an inhibitor of leukocytes and other adhesion molecules to the endothelium (Rodenas, Mitjavila, & Carbonell, 1998). ROS are highly reactive molecules formed from the metabolism of oxygen and possess unpaired valence shell electrons. When cells are unable to protect themselves from overwhelming amounts of ROS, the result is oxidative stress, or an imbalance between the endogenous antioxdant system and reactive oxygen (Halliwell, 1991). Curcumin has also been shown to hinder pro-inflammatory cytokine production, particularly IL-8, IL-1α, TNF-α, monocyte inflammatory protein-1, and monocyte chemotactic protein-1 (Abe, Hashimoto, & Horie, 1999). Currently, curcumin is undergoing testing in human clinical trials and has been used to treat cancer in diseases such as multiple myeloma, pancreatic cancer, myelodysplastic syndromes, and colon cancer (Lao et al., 2006; Sharma et al., 2004).

The Boswellia species contains biologically active compounds known as pentacyclic triterpenes. The most appealing thus far of the Boswellia species has been Boswellia serrata. The first studies utilizing Boswellia serrata in rats illustrated medicinal properties (Kar & Menon, 1969; Menon & Kar, 1971). A study using osteoarthritic dogs taking 400 mg/10 kg body weight of Boswellia serrata daily for 6 weeks showed significant improvements in severity of joint disease (Reichling, Schmokel, Fitzi, Bucher, & Saller, 2004). In a placebo-controlled study of 42 patients, the use of Boswellia serrata caused a reduction in pain perception and disability score. Yet, radiological testing did not show any differences compared to baseline measures

(Kulkarni, Patki, Jog, Gandage, & Patwardhan, 1991). In a randomized, double blind placebo-controlled crossover study, patients taking Boswellia serrata extracts experienced less knee pain, greater knee flexion, and further walking distances, along with less swelling in the knee joint (Kimmatkar, Thawani, Hingorani, & Khiyani, 2003). Gupta et al (Gupta et al., 1997) compared the effects of Boswellia serrata (350 mg/3x/day) versus sulfasalazine (1g/3x/day), an anti-inflammatory agent used in inflammatory bowel disease, on total leukocytes and eosinophils for 6 weeks in patients experiencing ulcerative colitis. Both treatment (Boswellia serrata) and placebo (sulfasalazine) groups not only had favorable outcomes on the dependent variables, but remission rate in both groups was also similar, 82% of the treatment group versus 75% of the placebo group.

Inflammation in CKD is dependent on a number of factors including a build-up of inflammatory metabolic products, the development of AGEs, and the accumulation of pro-inflammatory cytokines and inflammatory markers, specifically IL-6 and C-reactive protein (Ikizler, 2008). Those patients suffering from ESRD undergoing hemodialysis are also exposed to a host of inflammatory processes occurring from the dialysis process itself (Caglar et al., 2002). Finally, the presence of comorbidities in those with CKD has been noted as another cause of inflammation, evidenced by the fact that the existence of infection is indicative of elevated C-reactive protein levels (Fine, 2002). As mentioned earlier, most CKD patients will die of cardiovascular disease rather than kidney failure. Inflammation is thought to contribute to the increased cardiovascular risk observed in CKD patients (Thomas et al., 2008). There are few studies that have assessed the efficacy of anti-inflammatory agents in the CKD population, particularly alternative

medicines (Biolo et al., 2002). Therefore, more research is needed on the effects these compounds may have on chronic inflammation occurring in CKD.

Statement of the Problem

Will ingestion of the dietary supplements curcumin and Boswellia serrata collectively affect inflammatory and antioxidant markers in CKD patients?

Purpose

The purpose of this study was to determine the impact of 8 weeks of curcumin and Boswellia serrata supplementation on plasma markers of inflammation (TNF-α, IL-6) and antioxidant activity (GPx) in CKD patients.

Hypothesis

Based on the examination of the available literature on supplementation of curcumin and Boswellia serrata compounds and CKD, the following hypotheses were proposed:

H_1: There will be a decrease in IL-6 when supplementing with curcumin and Boswellia serrata in CKD patients.

H_2: There will be a decrease in TNF-α when supplementing with curcumin and Boswellia serrata in CKD patients.

H_3: GPx levels in CKD patients will be below normal, healthy values at baseline.

H_4: There will be a negative relationship between baseline GPx levels in CKD patients and stage of disease.

H_5: There will be no change in antioxidant activity following 8 weeks of curcumin and Boswellia serrata supplementation in CKD patients as measured by the enzyme GPx.

Delimitations

This study was completed using the following guidelines:

1. Fifty CKD patients (≥ 18y) participated in this study.

2. All subjects were recruited from the Waco Family Health Center located in Waco, TX by flyers posted throughout the clinic and the Central Texas Nephrology Associates clinics.

3. Fasting venous blood samples, weight, blood pressure, heart rate, waist circumference, and quality of life were collected pre-supplementation and post-supplementation.

4. In a double-blind manner, all patients were randomized to receive either curcumin/Boswellia serrata or placebo. All supplements were prepared in capsule form by Life Extension Inc. and placed into non-marked, unidentifiable containers.

5. All supplements were ingested according to the supplementation protocol utilized in this investigation.

6. All participants in this study did not modify their nutritional intake in any manner during the course of the study.

7. Data collection was conducted at the Waco Family Health Center located in Waco, TX and all assays were conducted in the Exercise and Biochemical Nutrition Laboratory (EBNL) at Baylor University according to all policies and procedures.

Limitations

1. The number of participants that completed the study was limited to those volunteers that qualified to be in the study and follow the research guidelines.

2. The sensitivity of the technologies and protocols utilized to identify quantifiable changes in the criterion variables.

3. The daily schedules of each participant and the inherent circadian rhythms that exist for all humans as a result of slightly different testing times, stresses, etc.

Assumptions

1. Participants were fasted for 12 hours prior to reporting for blood work.
2. Patients had documented CKD as measured by serum creatinine levels.
3. Patients ingested their required dose supplement or placebo.
4. All participants maintained their regular dietary habits throughout the study.
5. All assay reagents and equipment used in the sample analyses were accurate and reliable in quantification of the criterion variables.

CHAPTER TWO

Review of Literature

Chronic Kidney Disease and Inflammation

Chronic kidney disease (CKD) is characterized by a loss of kidney function over time. As an individual progresses through the stages of CKD, kidney function will progressively become impaired, escalating from normal function with the presence of kidney damage (CKD 1-2), to reduced kidney function (CKD 3-4), and finally kidney failure (CKD 5) (National Kidney Foundation, 2002). A number of important risk factors have been identified that increase the risk and advancement of CKD, particularly diabetes, hypertension, old age, and African American ethnicity. Almost half (45%) of all kidney failure occurring secondary to other comorbidities/diseases is due to the presence of diabetes and approximately 20% can be attributed to chronic hypertension (U.S. Renal Data System, 2005). Both diabetes and hypertension are also established risk factors for cardiovascular disease, and can partially explain its rate of occurrence in CKD. Since the initial stages of CKD usually present no symptoms, secondary diseases such as cardiovascular disease and diabetes tend to emerge at the beginning of stage 3 (GFR < 60 mL/min/1.73 m^2). When kidney function ceases, requiring renal replacement and/or dialysis, typically cardiovascular disease, anemia, and bone disease ensue (Weiner, 2007). The major complications associated with CKD will be discussed briefly.

Anemia is said to occur when one or more of the main erythrocyte values is low, namely, hemoglobin concentration, hematocrit, or red blood cell count. While the World

Health Organization has specific hemoglobin cut-off points for the detection of anemia, the National Kidney Foundation uses its own set of guidelines that include a hemoglobin level < 13.5 g/dL for men and < 12.0 g/dL for women (A. Levin, 2006). Those with anemia-related CKD comprise approximately half of the CKD population. McClellan et al (McClellan et al., 2004) found that a strong association exists between progression of CKD and the prevalence of anemia, with 25% of CKD1, 50% of CKD2-4, and 75% of those with CKD5 on dialysis with anemia. The most significant cause of anemia in CKD is decreased production of erythropoietin, the hormone that regulates red blood cell production. This is usually the result of tubular atrophy, which produces tubulointerstitial fibrosis, impairment of renal erythropoietin capacity, and ultimately anemia (Ratcliffe, 1993). The adverse effects of anemia can lead to angina, left ventricular hypertrophy, and more advanced heart failure. This, in turn, leads to a decrease in kidney function, resulting in a malicious cycle that is known as the "cardiorenal anemia syndrome" (Besarab & Levin, 2000).

CKD patients also commonly incur bone and mineral disorders and this is evident from the structural changes that occur within the bone itself that accompany these disorders. Phosphate is eliminated almost entirely in the kidney and this is the location of 1-α-hydroxylation of vitamin D. Impaired production of 1,25 dihydroxy-vitamin D (from parenchymal scarring) leads to hyperphosphatemia in addition to decreased elimination of phosphate. The combination of these two processes results in the decrease of serum calcium levels, and the consequential release of additional parathyroid hormone (known as secondary hyperparathyroidism). This increases both calcium (via increased bone reabsorption and 1-α-hydroxylation of 25-hydroxy vitamin D) and phosphate levels.

The observed increase in phosphorus levels typically occurs in stage 3 of CKD (Gal-Moscovici & Sprague, 2007). These bone abnormalities, particularly hyperphosphatemia, increase mortality, as this condition increases susceptibility to cardiovascular disease (G. H. Lee, Benner, Regidor, & Kalantar-Zadeh, 2007).

The most serious risk factor that CKD patients face is the one associated with cardiovascular disease. It is now known that those with ESRD are 10 to 100 times more likely to die of heart disease compared to age- and sex-matched controls in the general population (Foley, Parfrey, & Sarnak, 1998). Although, the increased risk is not only present in those with ESRD, but occurs fairly early in the disease process. Another risk factor that contributes to heart disease is hypertension, and it has been shown that even in patients in stages 2-3 of CKD who concurrently have hypertension are at increased risk for new or continuous cardiovascular incidents (Muntner, He, Astor, Folsom, & Coresh, 2005). Diabetes at any stage of CKD is associated with unfavorable effects, as a lower risk of all-cause mortality and cardiovascular mortality is related to lower fasting plasma blood glucose levels and/or hemoglobin A1c levels in patients with even modest kidney damage (Tonelli et al., 2005). Left ventricular hypertrophy also contributes to increased cardiovascular risk in CKD patients, and two of the previously mentioned conditions, anemia and hypertension, are suggested to influence its progression (A. Levin, Singer, Thompson, Ross, & Lewis, 1996). CKD patients are also more susceptible to congestive heart failure. Bibbins-Domingo et al (Bibbins-Domingo et al., 2006) found that in a patient population of African and Caucasian Americans with CKD (all stages), those with a greater extent of kidney damage were more likely to incur congestive heart failure.

Another cardiovascular disease risk factor in CKD patients is dyslipidemia, and is caused by impaired function of the enzymes lipoprotein lipase and hepatic triglyceride lipase. This leads to a greater amount of lipoproteins in the systemic circulation and less absorption by other organs and peripheral tissue. There is also data from another study pointing to hyperparathyroidism and calcium buildup in the islet cells of the pancreas that may also play a role in dyslipidemia in CKD patients (Arnadottir & Nilsson-Ehle, 1995). Regardless of the cause, CKD patients with this abnormality are at increased risk of morbidity and mortality compared to the general population.

A final complication that CKD patients experience is alterations in metabolism. While macronutrient intake may be sufficient for energy needs, an altered metabolism leads to problems with energy production, and this may result in nutritional deficiencies. Those macronutrients and minerals that are most affected include protein, water, salt, potassium, and phosphorus (Appel, Blum, Chien, Kunis, & Appel, 1985).

Much of the impairment in CKD can be attributed to the cardiovascular disease risk factors previously mentioned. However, there are also other risk factors that are not necessarily considered "established" risk factors that also contribute to the distress of the disease. These risk factors tend to rise as kidney function becomes worse, and includes two risk factors already mentioned, anemia and dysregulation of calcium and phosphate metabolism, along with a third factor known as inflammation (Sarnak et al., 2003). Inflammation is a protective response that initiates the body's natural healing process. Without this process, damage may never be eradicated and the repair process may never begin. However, when inflammation becomes chronic, marked by constant damage to tissue, the period of regeneration and repair never occurs, resulting in continuous tissue

damage (Yilmaz et al., 2007). While there is no concrete definition on what constitutes "chronic," it has been noted that several diseases that are considered inflammatory take years to develop. For instance, studies have shown that low levels of inflammation in type 2 diabetes are found years or decades before diagnosis (Pickup, 2004; Thorand et al., 2005; Thorand et al., 2006). Similarly, the initial stages of atherosclerosis take place in the endothelium, creating an inflammatory reaction that may take decades to occur (Libby, 2002; Ross, 1999). Chronic inflammation has been shown to contribute to the progression of CKD. Common intermediaries of chronic inflammation in CKD include atherosclerosis, malnutrition, and hypoalbuminemia (Horl, 2002), as well as AGEs (Hricik et al., 1993). In addition, dietary iron has been shown to be pro-inflammatory in CKD patients, demonstrated by elevations in plasma and urine of the chemokine monocyte chemoattractant protein-1 from an intravenous dose of iron sucrose (Agarwal, 2006).

A number of studies have documented that inflammation levels are significantly increased in CKD patients 2-5 compared to healthy controls (Costa et al., 2008; Dervisoglu, Kir, Kalender, Caglayan, & Eraldemir, 2008; Kimmel et al., 1998; Oner-Iyidogan et al., 2009; Pawlak, Mysliwiec, & Pawlak, 2008; Porazko et al., 2009; Rastmanesh et al., 2008) and these levels only become more pronounced as kidney function fails (Dervisoglu et al., 2008; Oner-Iyidogan et al., 2009). Dervisoglu et al (Dervisoglu et al., 2008) reported that serum levels of IL-6 and TNF-α were significantly higher in hemodialysis patients compared to non-dialysis patients, with no difference between groups in serum C-reactive protein levels. In addition, serum levels of the calcification inhibitor fetuin-A were lower in dialysis patients versus non-dialysis

patients. When all chronic renal failure patients were analyzed together, there was an inverse relationship between serum fetuin-A levels and pro-inflammatory cytokines, indicating greater vascular calcification and thus greater all-cause mortality, while no correlations existed in the control group. Likewise, Oner-Iyidogen et al (Oner-Iyidogan et al., 2009) found higher plasma levels of TNF-α and IL-6 in dialysis (36 hemodialysis, 41 peritoneal dialysis) vs. non-dialysis CKD patients. TNF-α and IL-6 levels were also significantly increased in patients with moderate CKD vs. controls. Similar to the previous study (Dervisoglu et al., 2008), serum C-reactive protein levels were significantly higher in all CKD patients compared to controls with no difference between dialysis vs. non-dialysis groups. Another study also discovered lower levels of fetuin-A in CKD5 patients along with higher amounts of serum C-reactive protein and pro-inflammatory cytokines IL-6 and IL-18 compared to controls. Fetuin-A and IL-18 were also independent determinants of arterial wall stiffness (Porazko et al., 2009).

From the aforementioned studies, it is rather well established that inflammation is present in dialysis patients. However, few studies have examined the occurrence of inflammation in patients with mild and moderate forms of CKD. Pawlak et al (Pawlak et al., 2008) set out to answer this question and found that levels of circulating cytokines were significantly increased in hemodialysis patients and levels of IL-1 and TNF-α were independently associated with increased mortality risk compared to healthy controls. In addition, this was the only study that was able to include patients from all stages of CKD. Groups consisted of CKD stages 1 and 2 (group 1), CKD stage 3 (group 2), CKD stage 4 (group 3), and CKD stage 5 (group 4), and each group was analyzed individually compared to controls. Each group had significantly higher levels of IL-6 and TNF-α

compared to healthy controls. However, group 1 (stages 1 and 2 CKD) was almost entirely composed of stage 2 patients, with only two patients with CKD stage 1. Therefore, it is unknown if CKD1 patients have higher circulating cytokine levels compared to healthy individuals. Levels of vascular endothelial growth factor (VEGF), a regulator of blood vessel growth, endothelial integrity, and vascular permeability, were higher in patients in stage 3 of CKD than in healthy controls and increased significantly as renal failure became worse. VEGF levels were also associated with increased oxidative stress, as measured by total peroxide and Cu/Zn superoxide dismutase. These results demonstrate that growth factors that are associated with atherosclerosis, namely VEGF, and oxidative stress appear as early on as stage 3 of CKD. An additional study showed that in CKD3-4 patients, plasma levels of IL-6 and TNF-α were significantly elevated versus control subjects. There was also a significant negative correlation between IL-6 and GFR, but this was not apparent with TNF-α. In addition, suppressors of cytokine signaling (SOCS) molecules that are induced by cytokines that modulate the inflammatory response were increased in CKD monocytes and lymphocytes. Specifically, SOCS3 was increased in CKD monocytes and was significantly correlated with a progressive loss of renal function, as measured by eGFR and urea, while SOCS1 was increased in lymphocytes and was significantly correlated with TNF-α (Rastmanesh et al., 2008). These studies all demonstrate that the inflammatory process in CKD begins at least in the moderate stages of the disease and progressively becomes worse as kidney function declines.

IL-6 and TNF-α

Il-6, a 22 to 27 kDa molecule, is a pleiotropic cytokine that elicits both pro- and anti-inflammatory effects. The inflammatory cascade is initiated by IL-6 by way of the activation and proliferation of lymphocytes, B cell differentiation, engagement of leukocytes, and initiation of an acute phase protein response in the liver. IL-6 can be produced by the action of other cytokines, such as TNF-α or IL-1β, as well as bacterial endotoxins, exercise, and reactive oxygen species (ROS). IL-6 is able to exert its actions by utilizing a receptor system that is made up of a ligand binding subunit (IL-6R or gp80) and a signal transducing subunit (gp130). Following the formation of an IL-6 – IL-6R molecular complex, sequentially the signal transducing subunit gp 130 becomes dimerized (Taga et al., 1989). Specifically, it is the soluble form of both receptors that has been shown to direct the actions of IL-6. In regards to inflammation, sIL-6R plays a role in signaling that affects the movement of inflammation from the early to late phase response (Jones, Horiuchi, Topley, Yamamoto, & Fuller, 2001). IL-6 has the ability to exist systemically, while still exerting its actions at locations that are separate from its source, and because of this property, IL-6 may be regarded as an atherosclerotic and muscle wasting cytokine (Stenvinkel, Barany, Heimburger, Pecoits-Filho, & Lindholm, 2002).

IL-6 has been considered to be the most studied cytokine in CKD patients. Studies utilizing receiver operator curves (Honda et al., 2006) and multivariate modeling (Tripepi, Mallamaci, & Zoccali, 2005) reported that IL-6 was the best indicator of all-cause and cardiovascular mortality versus C-reactive protein, IL-1β, IL-18, TNF-α, intercellular adhesion molecule-1, and vascular cell adhesion molecule-1. An additional

study using receiver operator curves analysis was able to demonstrate that the individual value of IL-6 in predicting inflammatory effects compared to other inflammatory molecules was equal (Zoccali, Tripepi, & Mallamaci, 2006). While IL-6 appears to be a valuable marker in dialysis patients, it is unknown whether the same is true for non-dialysis patients.

There have been studies published that demonstrate a correlation between renal function and measures of serum inflammation that include C-reactive protein, IL-6, and TNF-α, pointing to the possibility that the kidney is involved in the removal of pro-inflammatory cytokines from the body. In one study using ESRD patients, serum creatinine levels were found to be indicative of plasma IL-6 levels (Bolton et al., 2001). The dialysis procedure itself can also contribute to increased IL-6 levels and this has been evidenced by two separate studies that demonstrated an increase in IL-6 plasma and mRNA expression in blood mononuclear cells (T. Takahashi, Kubota, Nakamura, Ebihara, & Koide, 2000) as well as hemodialysis-induced increases in systemic IL-6 production (Caglar et al., 2002). It has been suggested that these levels are increased even further from the disease process itself by the use of bioincompatible membranes and nonsterile dialysate (Stenvinkel & Alvestrand, 2002). In patients with ESRD, IL-6 is reportedly related to carotid atherogenesis (Stenvinkel, Heimburger, & Jogestrand, 2002), another major concern since CKD patients are at increased risk for cardiovascular disease.

TNF-α is a pro-inflammatory cytokine that has been referred to as the "master regulator" among the network of cytokines that offers immune protection from pathogens and other foreign entities. Yet in copious amounts TNF-α can lead to death. A 17 kDa

molecule, its cellular source is activated macrophages (Stenvinkel et al., 2005). TNF-α, along with its soluble receptor, is associated with kidney function in patients with various levels of CKD (Descamps-Latscha et al., 1995). TNF-α clearance is also influenced by impaired kidney function in the rat (Bemelmans, Gouma, & Buurman, 1993). This cytokine has played a role in catabolism by affecting malnutrition and anorexia in dialysis patients (Aguilera et al., 1998). Unlike IL-6, increased TNF-α levels do not have an established relationship with cardiovascular disease and mortality. Yet, one study did find that abnormally high levels of TNF-α were able to predict mortality in CKD patients on dialysis, after adjusting for age and serum albumin level (Kimmel et al., 1998). While TNF-α has more definitive associations with other diseases including coronary artery disease (affecting endothelial dysfunction) (Fichtlscherer et al., 2000), insulin resistance (increased expression in fat tissue) (Hotamisligil, Arner, Caro, Atkinson, & Spiegelman, 1995), systemic lupus erythematosus (link between hypertriglyceridemia and inflammation)(Svenungsson et al., 2003), and congestive heart failure (level of expression and severity) (Levine, Kalman, Mayer, Fillit, & Packer, 1990), there is a need to determine its role in CKD, particularly in those who are not yet on dialysis, as many of these diseases are also present in CKD patients, particularly coronary artery disease, insulin resistance, and hypertriglyceridemia. Since TNF-α is considered a prototypic member in the family of cytokines, its contribution to inflammation in CKD should not be underestimated. More research is needed in both dialysis and non-dialysis patients to determine the roles of IL-6 and TNF-α in the inflammatory process, especially how it relates to morbidity and mortality.

Glutathione Peroxidase in CKD

Reactive oxygen species (ROS) are potentially harmful molecules that contain unpaired valence shell electrons, forming as a result of the metabolism of oxygen (Meier, 2001). Under normal physiological conditions, these molecules are formed at a rate sufficient enough for the endogenous antioxidant defense system to protect itself against these compounds (Baynes & Thorpe, 1999). ROS are removed by way of specific enzymes and these include superoxide dismutases, catalases, glutathione peroxidases (Grignard, Morin, Vernet, & Drevet, 2005), and possibly selenoprotein P (Burk & Hill, 2005). Superoxide dismutase catalyzes the redox reaction in which the superoxide free radical (O_2^-) is dismutated to form oxygen (O_2) and hydrogen peroxide (H_2O_2). Hydrogen peroxide is then reduced to form water using either catalase or glutathione peroxidase, but more often than not, glutathione peroxidase is utilized, since catalase is often found in small amounts in tissues (Zachara, Gromadzinska, Wasowicz, & Zbrog, 2006).

Glutathione peroxidase (GPx) exists in blood in cellular and extracellular forms. The cellular or cytosolic form is found in red blood cells while the extracellular form is contained in plasma (Arthur & Beckett, 1994). Studies in humans and rabbits (Cohen, Chovaniec, Mistretta, & Baker, 1985; K. Takahashi & Cohen, 1986) report that the enzyme in red blood cells is different from that in plasma, not only structurally but functionally as well. Plasma GPx, which will be the focus of this review, is a tetrameric protein containing identical subunits of approximately 21.5 – 23.0 kDa, with four-gram atoms of selenium per mole of the enzyme (K. Takahashi, Avissar, Whitin, & Cohen, 1987). The proximal tubular cells of the kidney have been shown to be the main site for

production of human plasma GPx (Avissar et al., 1994). Additionally, it is produced in the liver, lung, heart, breast, intestine, brain, skeletal muscle, and placenta, followed by release into the extracellular fluid (Chu, Esworthy, Doroshow, Doan, & Liu, 1992).

Plasma GPx is important in kidney disease, with low levels likely contributing to the oxidative stress present in CKD. Studies on GPx levels of the red blood cell form of the enzyme have reported conflicting outcomes with authors reporting reduced (Richard et al., 1991; Roxborough, Mercer, McMaster, Maxwell, & Young, 1999; Zachara, Adamowicz, Trafikowska, Pilecki, & Manitius, 2000; Zachara, Trafikowska, Adamowicz, Nartowicz, & Manitius, 2001), elevated (Ceballos-Picot et al., 1996), or no difference (De Vega et al., 2002; Stachowska et al., 2005; Yoshimura et al., 1996) compared to healthy controls. In addition, levels of this enzyme were similar among the individual stages of CKD (Ceballos-Picot et al., 1996; Zachara et al., 2004; Zachara, Salak, Koterska, Manitius, & Wasowicz, 2004). Conversely, plasma GPx has consistently exhibited lower levels in CKD patients compared to controls (Ceballos-Picot et al., 1996; El-Far, Bakr, Farahat, & Abd El-Fattah, 2005; Moradi, Pahl, Elahimehr, & Vaziri, 2009; Zachara et al., 2004). Some researchers have even observed negative correlations between levels of plasma GPx and stage of the disease (Ceballos-Picot et al., 1996; Zachara, Salak et al., 2004), which has been attributed to the fact that the main site of production of this enzyme occurs in the kidney, and progressively reduced kidney function likely leads to decreased production of this enzyme.

Ceballos-Picot et al (Ceballos-Picot et al., 1996) observed decreases in plasma GPx levels in undialyzed CKD patients, and these levels became more pronounced as patients reached higher stages of the disease. A positive correlation was also found

between plasma GPx and creatinine clearance. Patients on hemodialysis had almost diminished levels of the enzyme compared to controls. Similarly, Zachara et al (Zachara et al., 2004) found lower plasma GPx levels in a group of CKD patients with varying stages of the disease. Enzyme levels were 37% lower in patients compared to healthy controls and decreased as patients reached progressively higher stages of the disease. In addition, a negative correlation was found between plasma GPx and creatinine levels. One month of selenium supplementation increased plasma GPx in all CKD patients by 15%, but this was mostly due to increases in the group with the mildest form of the disease, further suggesting that impairment of kidney function leads to decreased production of this enzyme. El-far et al (El-Far et al., 2005) also observed a negative correlation between plasma GPx and serum creatinine levels, further indicating that the progression of renal dysfunction is accompanied by a progressive drop in plasma GPx activity.

Studies have been unanimous demonstrating reduced plasma GPx activity in CKD patients compared to healthy controls. This impairment of antioxidant activity occurs early in CKD, gradually increases with its progression, and is virtually diminished once renal failure occurs. This impaired antioxidant activity, along with oxidative stress, contributes to the chronic inflammatory state in CKD, further contributing to morbidity and mortality.

Curcumin

Curcumin, the active ingredient in the spice turmeric, is a polyphenol compound obtained from the rhizome of the plant (Hatcher et al., 2008). Curcumin has been used for centuries in the ancient Indian medical system known as Ayurveda as well as in

Chinese traditional medicine (Ammon & Wahl, 1991). Curcumin is lipophilic and diffuses through cell membranes quickly (Jaruga et al., 1998). Its diketone group and two phenol rings serve as electron traps to inhibit hydrogen peroxide production and remove hydroxyl and superoxide radicals. The combination of two or more molecules of curcumin also has the ability to join together to chelate iron. Metabolites of curcumin that have been identified in human and rat intestine include curcumin glucuronide, curcumin sulfate, tetrahydrocurcumin, and hexahydrocurcumin (C. R. Ireson et al., 2002). Curcumin reportedly has a number of beneficial effects including anti-inflammatory, antioxidant, chemopreventive, and chemotherapeutic characteristics. However, it can also exhibit pro-oxidant properties under certain circumstances. It has been established that in addition to its role as a free radical scavenger and reducing agent it can also inflict damage upon DNA molecules when Cu or Fe ions are in its vicinity (Ahsan, Parveen, Khan, & Hadi, 1999). However, curcumin's active site and antioxidant mechanism are currently under debate. Most accounts report that a hydrogen atom transfer takes place but disparity occurs as to which group the hydrogen initiates from, the keto-enol or phenolic OH^- group (Jovanovic, Boone, Steenken, Trinoga, & Kaskey, 2001).

Study authors have reported that curcumin reduces both acute and chronic inflammation (Shishodia, Sethi, & Aggarwal, 2005). Most studies thus far have been done using in vitro and in vivo models. Research using in vitro models has demonstrated that curcumin's mechanism of action is related to its ability to affect the lipo-oxygenase (LO) and cyclo-oxygenase (COX) processes (Brouet & Ohshima, 1995; T. S. Huang et al., 1991; Lin & Shih, 1994; Sreejayan & Rao, 1997), and inhibit ROS (Joe & Lokesh, 1994). Formation of proinflammatory monocyte/marcophage-derived cytokines

including IL-8, monocyte inflammatory protein-1, monocyte chemotactic protein-1, IL-1β, and TNF-α have also been prevented by the use of curcumin (Abe et al., 1999).

Previous studies have demonstrated that curcumin is able to attenuate the inflammatory response as measured by proinflammatory cytokines in human macrophages and esophageal epithelial cells (Chan, 1995; Rafiee et al., 2009), rat models of nephrotoxicity (Kuhad, Pilkhwal, Sharma, Tirkey, & Chopra, 2007), acute liver damage (Reyes-Gordillo et al., 2007), hepatic fibrosis (Fu, Zheng, Lin, Ryerse, & Chen, 2008), renal failure (Ghosh et al., 2009), and in LPS-induced fever in rabbits (W. T. Huang, Niu, Chang, Lin, & Chang, 2008). However, there have been conflicting results on curcumin's effects regarding TNF-α. Most studies have observed decreases in TNF-α (Chan, 1995; Fu et al., 2008; Ghosh et al., 2009; W. T. Huang et al., 2008; Kuhad et al., 2007; Reyes-Gordillo et al., 2007) while only two studies have reported no effect (Banerjee, Tripathi, Srivastava, Puri, & Shukla, 2003; Literat et al., 2001) from curcumin. Using a cell culture model, Literat and colleagues (Literat et al., 2001) studied the effect of curcumin on lipopolysaccharide (LPS)-induced inflammation in preterm lung inflammatory cells. A dose of 20 μM of curcumin inhibited the protein expression of IL-1β and IL-8 expression, but did not inhibit the expression of TNF-α. It is important to note that TNF-α expression was reduced but did not reach statistical significance. Banerjee et al (Banerjee et al., 2003) determined the effect of curcumin and ibuprofen on chronic inflammation in a rat model of rheumatoid arthritis. Curcumin was able to reduce swelling and decrease IL-1β levels at a dose of 100 mg/kg/day over the course of 35 days, but had no effect on levels of TNF-α. However, the authors chose to observe the chronic phase of inflammation, measuring inflammatory mediators at days 21 and 35

of the study. Although TNF-α increased by three-folds on day 21, it was reduced to 88% by day 35. In a similar manner, IL-1β increased twice as much on day 21. However, by day 35 IL-1β levels had increased 10 fold, which were significantly brought down by curcumin. While IL-1β levels reached much higher levels over the course of the study compared to TNF-α, this still cannot explain the lack of effect by curcumin, since ibuprofen reduced the levels on day 21 and surprisingly, increased the levels on day 35. The authors suggested that although curcumin inhibited molecules mediated by TNF-α (S. Singh & Aggarwal, 1995), it did not directly affect its inhibition.

In addition, curcumin inhibits the nuclear factor kappa B (NFκB) signaling pathway by preventing the phosphorylation and degradation of its cytoplasmic inhibitor, inhibitor of kappa B (IκB) (S. Singh & Aggarwal, 1995). Chan et al (Chan, 1995) found that 5 μM of curcumin decreased TNF-α levels by 57% and IL-1 production by 69% in human mono mac 6 macrophage cells in vitro. At doses of 2.5 and 5 μM, curcumin inhibited activation of NFκB. Similar results were found in another study (Reyes-Gordillo et al., 2007) in which curcumin inactivated NFκB as well as the expression of proinflammatory cytokines TNF-α, IL-1β and IL-6, thereby preventing liver damage in an experimental rat model.

The literature is clear on curcumin's positive anti-inflammatory activity in various animal and in vitro models. It has been promising as a therapeutic agent in cancer development by inhibiting progression through obstruction of carcinogen activation and impeding malignant cell proliferation during advancement of carcinogenesis (Duvoix et al., 2005). While curcumin's effectiveness in human trials has only just begun in diseases that include multiple myeloma, pancreatic cancer, myelodysplastic syndromes,

and colon cancer (Lao et al., 2006; Sharma et al., 2004), it has yet to be used in CKD populations. A few animal studies have yielded positive results on curcumin's effectiveness activity against ischemia/reperfusion injury and renal failure, as well as experimental nephrotoxicity. Pretreatment with curcumin (200 mg/kg/day) for 7 days was able to improve urea and cystatin C levels yet was unable to affect creatinine from ischemia reperfusion injury in rats (Bayrak et al., 2008). Curcumin was able to increase serum GPx levels as well as prevent the depletion of kidney tissue superoxide dismutase, GPx, and catalase. In fact, renal sections from rats treated with curcumin demonstrated a reduction in histological features of renal injury that was described as more focal with mild tubular necrosis compared to untreated rats. A recent study by Ghosh et al (Ghosh et al., 2009) reported that curcumin was able to improve renal function in nephrectomized rats. In this study, curcumin was compared to enalapril, an ACEI, and ACEIs are known to improve experimental and human renal failure (Remuzzi, Perico, Macia, & Ruggenenti, 2005; Tsunenari et al., 2007). Curcumin and enalapril were equally successful in reducing proteinuria by 40-50% and in reducing blood urea nitrogen and creatinine compared to untreated rats. Animals treated with curcumin or enalapril demonstrated a reduction in macrophage influx by 53% and 43% respectively, while untreated rats demonstrated significant macrophage infiltration in the glomerulus and tubulointerstitium. In addition, curcumin and enalapril were able to significantly reduce IκBα degradation, leading to the translocation of a lower amount of p50 protein content into the nucleus compared to untreated animals, confirming curcumin's role as an NFκB inhibitor. Both curcumin and enalapril also elicited significantly lowered serum and mRNA content of TNF-α in kidney tissue. Likewise, Kuhad et al (Kuhad et al., 2007)

found that chronic curcumin use (2 days prior and 3 days after) dose-dependently prevented a rise in blood urea nitrogen and serum creatinine in a rat model of cisplatin-induced experimental nephrotoxicity. Curcumin was also able to dose-dependently improve creatinine and urea clearance as well as attenuate lipid peroxidation. In addition, curcumin was able to reduce inflammation as measured by reduced serum levels of TNF-α. Overwhelming evidence supports curcumin's role as an anti-inflammatory compound, but it is difficult to say how successful it will be when used in human trials. Initial human trials using curcumin have focused on its safety and pharmacokinetics. Research on curcumin's therapeutic properties are currently ongoing, with positive outcomes being reported in a portion of patients experiencing chronic anterior uveitis (Lal et al., 1999), idiopathic inflammatory orbital pseudo tumors (Lal, Kapoor, Agrawal, Asthana, & Srimal, 2000), post-operative inflammation (Satoskar, Shah, & Shenoy, 1986), and external cancer lesions (Kuttan, Sudheeran, & Josph, 1987). Clearly, more research needs to be done using this promising compound, particularly in more inflammatory disease states in which quality of life is poor.

Boswellia

Boswellia is composed of four main species that include Boswellia sacra, Boswellia carterii, Boswellia frereana, and Boswellia serrata. Boswellia species contain pentacyclic triterpenes, which possess a wide range of pharmacological activity. Boswellic acids can be found in either an α (geminal methyl groups at C-20) or β-configuration (vicinal methyl groups at C-19/C-20). The β-configuration species produce greater effects compared to the α-isomers. Those Boswellic acids that are considered to be of significant importance include β-Boswellic acid, 3-O-acetyl-β-Boswellic acid

(AβBA), 11-keto-β-Boswellic acid (KBA), and 3-O-acetyl-11-keto-β-Boswellic acid (AKBA). Boswellia serrata, also known as frankincense, is a tree that is grown in India, Northern Africa, and the Middle East. When the bark of this tree is removed, an oleo-resin is found, in which extracts of this resin have been used for centuries in Ayurvedic medicine, particularly for chronic inflammatory and joint diseases. Boswellia has exerted anti-inflammatory effects in a number of diseases including rheumatoid arthritis (G. B. Singh & Atal, 1986), osteoarthritis (Reichling et al., 2004), autoimmune encephalomyelitis (Wildfeuer et al., 1998), ileitis (Krieglstein et al., 2001), colitis (Anthoni et al., 2006), and hepatitis (Safayhi, Mack, & Ammon, 1991). It has shown therapeutic benefit for pain (Kulkarni et al., 1991), cancer (Janssen et al., 2000), hypercholesterolemia (Pandey, Singh, & Tripathi, 2005), and allergies (Gupta et al., 1998). In humans, Boswellia has been used in the treatment of rheumatoid arthritis, osteoarthritis, brain and malignant tumors, ulcerative colitis, Crohn's disease, and bronchial asthma (Poeckel & Werz, 2006).

In a similar manner to curcumin, in vitro studies have confirmed that Boswellic acids are able to suppress leukotriene formation by inhibiting the enzyme 5-LO. Leukotrienes are responsible for the effects of the inflammatory response and are present in conditions such as bronchial asthma, rheumatoid arthritis, psoriasis, and inflammatory bowel disease (Samuelsson, 1983). While it seems reasonable that 5-LO and the leukotrienes are the primary molecular targets of Boswellic acids and responsible for their anti-inflammatory actions, it is not entirely known if this is what actually occurs in vivo, and therefore responsible for the their effects on inflammation. Boswellic acids have also been shown to inhibit $[Ca^{2+}]_i$ (Li, Westwick, & Poll, 2002) and mitogen

activated protein kinase (MAPK) (Herlaar & Brown, 1999) signaling which have been shown to positively influence inflammatory leukocyte infiltration, as well as NFκB signaling, specifically by preventing activation of the IκB kinases (IKKs) (Syrovets, Buchele, Krauss, Laumonnier, & Simmet, 2005).

Conflicting data concerning Boswellia's effects on TNF-α have been reported (Cuaz-Perolin et al., 2008; Gayathri, Manjula, Vinaykumar, Lakshmi, & Balakrishnan, 2007). Gayathri et al (Gayathri et al., 2007) compared an isolated pure and crude extract of Boswellia serrata on LPS-induced inflammation on various cytokines, nitric oxide production, and MAPK signaling in human peripheral blood mononuclear cells (PBMCs) and mouse macrophages (RAW 264.7 cell line). Both the crude and pure extracts inhibited TNF-α and IL-1β 6 hours after stimulation with LPS. However, the crude extract was able to inhibit IL-6 while no effect was demonstrated in cells treated with the pure extract. The inhibition of all three pro-inflammatory cytokines likely occurred through inhibition of the MAPK signaling pathways, as the crude extract was able to inhibit the phosphorylation of all three MAPK pathways (ERK, JNK, p38). JNK and p38 MAPK have been shown to regulate gene expression of various cytokines involved in inflammation including TNF-α, IL-1, IL-6, and IL-8. Both extracts were also able to downregulate the T-helper 1 cytokines interferon and IL-12, while IL-10 and IL-4 were induced. In mouse macrophages, both extracts were successful in inhibiting LPS-induced nitric oxide production and this was attributed to a decrease in inducible nitric oxide synthase (iNOS) expression, the enzyme responsible for nitric oxide production. COX-2 mRNA expression was assessed and found to be only moderately inhibited by the crude extract with no effect observed with the pure compound, suggesting that the anti-

inflammatory effects of Boswellia serrata do not occur through COX-2. Overall, both extracts were able to suppress inflammation.

In contrast, Cuaz-Perolin et al (Cuaz-Perolin et al., 2008) were not able to find any effect on the expression of interferon-γ, IL-4, or TNF-α in blood isolated CD4, CD8, and natural killer T cells treated with acetyl-11-keto-β-boswellic acid (AKBA). There were also no changes in IL-6 and interferon-γ expression in natural killer T cells and CD4 lymphocytes isolated from liver treated with AKBA. Interestingly, there was no change found in untreated control cells, suggesting that levels of these cytokines were of insufficient magnitude to be inhibited in the first place. Cytokines that were found to be significantly lower in AKBA-treated animals included monocyte chemoattractant protein-1 and -3, IL-1α, macrophage inflammatory protein-2, lymphotactin, vascular endothelial growth factor (VEGF), and tissue factor. LPS was injected into mice deficient in apolipoprotein E, causing atherosclerotic lesions, but these lesions were reduced by ~50% when mice were treated with AKBA. To determine AKBA's mechanism of action, the authors examined the phosphorylation of IκB kinase (IKK) and IκBα and found that staining intensities of these parameters in atherosclerotic lesions from AKBA-treated animals were reduced compared to lesions from control mice, suggesting NF-κB inhibition by AKBA. In addition, low nuclear staining for the p65 protein was observed in lesions of AKBA compared to dark staining seen in the control group, suggesting greater nuclear localization in the control group. The authors also treated human and murine macrophages and PBMCs with LPS and found that AKBA treated cells yielded decreased phosphorylation of IκBα. While not the main outcome of the study, it was determined whether or not AKBA was able to affect cholesterol and triglyceride levels in

animals treated with LPS. AKBA was not able to affect lipids and the authors concluded that AKBA's effect on atherosclerotic lesions could not be explained by a decrease in lipid levels.

While Cuaz-Perolin et al (Cuaz-Perolin et al., 2008) were not able to find any hypolipidemic effects, Pandey et al (Pandey et al., 2005) were able to show that rats supplemented with Boswellia serrata and on an atherogenic diet demonstrated a 38-48% reduction in serum cholesterol levels compared to animals on an atherogenic diet alone. Even more profound, Boswellia serrata-supplemented rats showed a significant increase in high-density lipoprotein cholesterol (HDL-c) levels, whether they were on atherogenic or normal diets, suggesting a cardiovascular-protective effect of Bowellia serrata. While atherogenic diet animals demonstrated increases in body weight, this was attenuated in Boswellia serrata-treated atherogenic animals. The authors attributed this effect to the hypolipidemic property of Boswellia serrata. Histological examination of liver and kidney in atherogenic diet-treated animals showed changes in cellular architecture, fatty infiltration, inflammation, hepatic cell necrosis, and degeneration while this was prevented in the Boswellia serrata-treated animals. In line with Gayathri et al (Gayathri et al., 2007), this study also confirmed Boswellia serrata's role as a nitric oxide inhibitor, prohibiting its production in thioglycolate-activated macrophages, indicative of its role as an anti-inflammatory agent.

Kiela et al (Kiela et al., 2005) found quite different results in a mouse model of chemically-induced colitis. Using either 0.1 or 1% Boswellia serrata extract, not only were the extracts unable to improve pathological scores in two models of induced colitis, but animals treated with Boswellia had more symptoms and mucosal ulcerations, greater

multifocal transmural necrosis, and greater edema. Neither fraction of Boswellia was able to improve survival rate or prevent bodyweight loss. In addition, there was a larger bodyweight loss in animals fed Boswellia-supplemented diets. Contrary to what most studies have reported, four individual Boswellic acids (α-Boswellic acid, 3-acetyl-β-Boswellic acid, 11-keto-β-Boswellic acid, and 3-acetyl-11-keto-β-Boswellic acid) actually increased basal activity of the NF-κB-dependent promoter and three of the latter (50 μM) also increased IL-1β-stimulated activity of NF-κB in Caco-2 cells. The authors suggested that this may have occurred through oxidative stress, but it is not entirely known. In mice fed the higher dose (1%) of Boswellia serrata there was an enlargement in liver size that corresponded to a 48% increase in the liver to bodyweight ratio, with increased deposition of intracellular lipids. This was also confirmed in human HepG2 cells (50 μg/mL) representing lipid accumulation. These negative results may have been due to Boswellia's use in a different animal model.

Boswellia has been used as a topical application as an anti-tumor, anti-carcinogenic, anti-inflammatory, and anti-arthritic agent (M. T. Huang et al., 2000; S. Singh et al., 2008). Specifically in its use as a topical anti-inflammatory compound, Boswellic acids were able to dose-dependently inhibit acute paw edema in rats evoked by carrageenan injection. What is even more intriguing is that the author of this study compared results to a former study utilizing Boswellic acids in systemic application and results were nearly identical (S. Singh et al., 2008).

In human trials, Boswellia has been used primarily in diseases such as colitis (Gupta et al., 1997; Gupta et al., 2001; Madisch et al., 2007), osteoarthritis (Sengupta et al., 2008), and asthma (Gupta et al., 1998). All studies reported positive outcomes from

its use. Colitis patients showed improvements in abdominal pains and stools, remission, disappearance of physical symptoms, and restoration of histological parameters. Yet, all effects were not significantly different from the control group (Gupta et al., 1997; Gupta et al., 2001; Madisch et al., 2007). On the other hand, osteoarthritis patients experienced both clinical and significant improvements in pain and physical functioning (Sengupta et al., 2008). In the same manner, asthma patients saw improvements in remission and physical signs and symptoms (Gupta et al., 1998). While all studies were using Boswellia as a leukotriene synthesis inhibitor, no study measured this mechanism of action and/or any other inflammatory markers to determine if this indeed occurred. While there have been abundant studies in animal and cell culture models of inflammation, the effects of Boswellia supplementation in human clinical trials is lacking and more are needed to confirm how it exerts its biological effects.

The Boswellia species has demonstrated profound anti-inflammatory effects in various in vitro models but whether or not these actions carry over to human trials is currently unknown. In addition, the one study that reported negative outcomes in regards to Boswellia cannot be ignored and should prompt others using Boswellia in human studies to proceed with caution. There is usually sparse data on the ineffectiveness and/or negative side effects reported in the literature with alternative medicines and many times these compounds may result in more harmful consequences than prescription medications, as they do not have the scientific backing to support their rationale. Therefore, more human trials need to be conducted that specifically address the safety and toxicity of Boswellia, in addition to its purported effects.

The anti-inflammatory effects of curcumin and Boswellia have mainly been tested in cell culture and animal models. While many positive results have been attained, the question remains, "Do these experimental results carry over to the human species?" Often times, they do not. While curcumin's effect on inflammation is still in its infancy stage in human trials, its chemopreventive and chemotherapeutic properties in cancer patients is being tested, demonstrating promising results (Kuttan et al., 1987; Lal et al., 1999; Lal et al., 2000; Satoskar et al., 1986). However, it has yet to be used in inflammatory diseases in human trials, including CKD. Boswellia, on the other hand, has been used in a number of human trials and shown some clinical benefit. However, when compared to customary treatment, it does not appear superior. In addition, human Boswellia trials have either been non-randomized, consisted of small sample sizes, or have not measured its mechanism of action. While Boswellia works as a leukotriene and LO inhibitor in animal and cell culture models, it is unknown if it is able to exert its effects similarly in humans. Its use in CKD patients will add to the literature base on its efficacy in humans and share incite on how it exerts its biological effects. While curcumin and Boswellia are available together in the over the counter herbal remedies, the combination of their efficacy has not been researched to date. Therefore, the use of these two compounds in CKD patients will contribute to its effectiveness as anti-inflammatory agents.

Summary

In conclusion, the process of prolonged, chronic inflammation that occurs in various disease states such as CKD contributes to mortality, morbidity, and reduced quality of life. Anti-inflammatory medications are helpful in ameliorating this process

but also produce both short-term and long-term side effects that may affect vital organs. Recent interest in the use of alternative and complementary medicine has led to scientific research on these potentially helpful compounds, mainly due to the supposed therapeutic effects that natural and herbal medicines contain. However, most of these products lack scientific support of their usefulness, mechanisms of action, and safety. Of greater concern is the fact that these remedies are readily available as over-the-counter therapies in health food, supermarket, and online stores. Virtually anyone has access to these products.

CKD is characterized by a progressive loss of kidney function and brings with it complications that include but are not limited to anemia, bone disease, uremic malnutrition, hyperlipidemia, hypertension, diabetes, and cardiovascular disease. A multidisciplinary approach is necessary to manage CKD and slow the progression of kidney failure. There is a need to determine treatment strategies that will more effectively help manage this disease, slow its progression, and decrease mortality and morbidity. Curcumin and Boswellia serrata are two anti-inflammatory compounds that may be beneficial in CKD. Considering the side effects that many prescription medications have, future research should focus on determining the effectiveness of complementary medicines in inflammatory diseases such as CKD, as well as its effect on quality of life. Currently, these two compounds have not been used exclusively together. Due to their positive individual effects, it would be important to determine if curcumin and Boswellia serrata work synergistically. If so, their use may transform treatment strategies of clinicians looking to improve patient outcomes.

CHAPTER THREE

Methods

This study was carried out in order to determine if ingesting the combination supplement of curcumin and Boswellia serrata for 8 weeks would decrease levels of inflammation and increase levels of antioxidant activity in CKD patients. Therefore, the following hypotheses were made:

H_1: There will be a decrease in IL-6 when supplementing with curcumin and Boswellia serrata in CKD patients.

H_2: There will be a decrease in TNF-α when supplementing with curcumin and Boswellia serrata in CKD patients.

H_3: GPx levels in CKD patients will be below normal, healthy values at baseline.

H_4: There will be a negative relationship between baseline GPx levels in CKD patients and stage of disease.

H_5: There will be no change in antioxidant activity following 8 weeks of curcumin and Boswellia serrata supplementation in CKD patients as measured by the enzyme GPx.

Subjects

Fifty CKD patients (CKD stages 1-5) > 18 years of age were allowed to volunteer to participate in this proposed study and were recruited from the Central Texas area. All participants were cleared for participation by passing a mandatory medical screening by their physicians. Exclusion criteria for this study included the following: individuals currently on a nutritional supplement, use of a nutritional supplement within the past 3 months, allergy to roasted rice powder, current illness requiring hospitalization, life expectancy $\leq$ 3 months, malabsorption syndromes, pregnant, or any change in body

weight (±10 lbs.) over the previous 6 months. All eligible participants were asked to provide oral and informed written consent based on university-approved documents. Approval was granted by the Institutional Review Board for Human Subjects of Baylor University. Additionally, all experimental procedures involved in the study were conformed to the ethical consideration of the Helsinki Code.

Study Site

Data collection was conducted at the Waco Family Health Center located in Waco, TX and all assays were conducted in the Exercise and Biochemical Nutrition Laboratory (EBNL) in the Department of Health, Human Performance, and Recreation (HHPR) at Baylor University according to all policies and procedures.

Overview of Study Design

Table 1 shows the general research design protocol that was administered in this study. The independent variables were the treatment condition (2 levels, the nutritional supplement curcumin/Boswellia serrata composed of 824 mg purified turmeric extract, 95% curcuminoids and 516 mg Boswellia serrata extract, 10% 3-acetyl-11-keto-beta-Boswellic acid (AKBA), and the placebo composed of roasted rice powder, 1340 mg, both provided by Life Extension, Inc.), and number of data sampling times during the course of the study (2 levels). Dependent variables included the inflammatory markers IL-6 and TNF-α, and the antioxidant enzyme GPx.

Familiarization Session

Participants expressing interest in participating in this study were interviewed on the phone and/or in person with their primary care physician to determine whether they

appeared to qualify to participate in this study. Participants believed to meet eligibility criteria were invited to attend an entry/familiarization session. Once reporting to the clinic, participants completed a medical history questionnaire and underwent a general physical examination by a physician to determine whether they met eligibility criteria. Participants meeting entry criteria were familiarized to the study protocol by way of a verbal and written explanation outlining the study design. At the conclusion of the entry/familiarization session, each participant was given an appointment time to begin the study.

Experimental Protocol

Prior to the study commencing, a full physical and cardiovascular examination was performed by a physician. The participants' diets were not standardized and subjects were asked not to change their dietary habits during the course of the study. In a double-blind, placebo-controlled manner, participants were randomly chosen to receive either curcumin/Boswellia serrata or placebo (roasted rice powder). A randomization list was generated a priori using a random number generator that allocated subjects into treatment and placebo groups. Participants were asked to fill out the Godin Leisure-Time Exercise Questionnaire at each testing session in order to record any and all physical activity they performed during the study. The questionnaire included a list of activities and exercises, intensities at which they were performed, and frequency of these activities. Participants were required to attend two testing sessions. At each testing session, participants underwent an assessment that determined weight, height, heart rate, blood pressure, waist and hip circumferences, and quality of life, in addition to filling out the Godin Leisure-Time Exercise Questionnaire. During the two testing sessions, patients donated

approximately 50 milliliters of blood on a 12-h fast for later analysis of plasma inflammatory and antioxidant markers. At the initial testing session, participants were given a two-month supply of either curcumin/Boswellia serrata or placebo. Subjects ingested two capsules/day for 56 days. Following the 56 days of supplementation, subjects returned to the clinic for a final testing session and returned their empty pill bottles. Supplementation compliance was monitored by having subjects return their empty pill bottles at the end of 8 weeks of supplementation. The standard practice of pill counting was used to assess compliance. It has been recommended by some study authors that the standard for compliance should be between 80-100% (Jasti, Siega-Riz, Cogswell, Hartzema, & Bentley, 2005; J. Y. Lee et al., 1996). Therefore, patients that consumed ≥80% of the issued supplements in this study were considered compliant. If patients were not compliant (<80%) they remained in the study but their compliance percentage was calculated and noted. In addition, sample over-recruitment took place in order to account for patient drop out.

Venous Blood Sampling

After observing a 12 h fast, venous blood samples were obtained from a suitable vein in the antecubital region into four 6mL heparin tubes using a standard Vacutainer apparatus and standardized to the same time of day for each sample (each subject had his/her blood drawn at approximately the same time of day for all testing sessions). Blood samples were allowed to stand at room temperature for 10 min and then centrifuged. The plasma was removed and frozen at -20˚C for later analysis. Blood samples were obtained before beginning the supplementation period (pre-supplementation) and after 8 weeks of supplementation (post-supplementation).

Using enzyme-linked immunoabsorbent assays (ELISA), systemic levels of inflammation and antioxidant activity were assessed for plasma levels of IL-6, TNF-α, and GPx. These assays were completed to evaluate the potential anti-inflammatory and antioxidant effects of curcumin and Boswellia serrata supplementation.

Standard Quality of Life (SF-36)

Participants were asked to complete the SF-36 Quality of Life (QOL) inventory at each testing session to determine if any changes in quality of life took place throughout the study. The Medical Outcomes Survey (MOS) SF-36 is a questionnaire that covers 40 concepts related to health and was developed and used in the Medical Outcomes Survey. The SF-36 is a generic instrument which measures health-related quality of life (HRQOL) by assessing 8 different scales: physical functioning (10 items), role limitations caused by physical health problems (4 items), bodily pain (2 items), general health perceptions (6 items), energy/fatigue (4 items), social function (2 items), role limitation caused by emotional problems (3 items), and emotional well-being (5 items). Higher scores represent better HRQOL. The 8 dimensions have shown reliability through the use of two separate approaches, internal consistency and test-retest methods. Criterion for adequate reliability is considered 0.70 when doing group comparisons, and the majority of the published reliability statistics surpass this minimum value (Manocchia et al., 1998). The same reliability statistics have also been demonstrated in 24 patient groups that varied across socio-demographic attributes and diseases/conditions (McHorney, Ware, Lu, & Sherbourne, 1994; J. E. Ware, Snow, Kosinski, & Gandek, 1993; J. E. Ware, Kosinski, & Keller, 1994). For underserved populations, reliability has

been shown to be lower, yet reliability statistics have repeatedly out performed the minimum requirements for group comparisons (J. E. Ware Jr & Gandek, 1998).

Due to the extensive use of the SF-36 instrument, studies indicate that the survey has demonstrated content, concurrent, construct, criterion, and predictive validity (J. E. Ware Jr & Gandek, 1998). When compared to other commonly utilized generic health surveys (J. E. Ware et al., 1993) the SF-36 comprises 8 of the most commonly represented health concepts. With the exception of general health, the scales used in the SF-36 are able to account for two-thirds of the reliable variance in individual assessments of present health status in the United Kingdom, United States, and Sweden (J. E. Ware Jr, Keller, Gandek, Brazier, & Sullivan, 1995). The SF-36 was created to replicate the longer MOS constructs (measures) and has achieved approximately 80-90% empirical validity in studies concerning physical and mental health "criteria" (McHorney, Ware, Rogers, Raczek, & Lu, 1992). Particularly, the scales that have illustrated the most valid mental health measures in cross-sectional and longitudinal studies utilizing known-groups validity include Mental Health, Role-Emotional, and Social Functioning, as well as the Mental Health Summary. The most valid physical domains include Physical Functioning, Role-Physical, and Bodily Pain scales, in addition to the Physical Health Summary. In the preliminary known-groups validation of the SF-36, accepted standards also comprised clinical symptoms of diagnosis and degree of depression, heart disease, and other conditions, and this is evidenced in peer-reviewed publications and in the two user's manuals (Kravitz et al., 1992; McHorney, Ware, & Raczek, 1993; J. E. Ware et al., 1993; J. E. Ware

et al., 1994; J. E. Ware Jr et al., 1995). The SF-36 has also been recognized as accurately identifying disease burden, with the most commonly researched conditions being arthritis, back pain, depression, diabetes, and hypertension (Manocchia et al., 1998).

Supplementation Protocol

All participants were randomly assigned to one of two groups, placebo (roasted rice powder) or curcumin/Boswellia serrata (Life Extension, Fort Lauderdale, FL) in a double-blind manner. All subjects were instructed to take their daily dose of supplement according to the manufacturer's recommended guidelines. Briefly, participants in both supplementation groups ingested two capsules each day, which corresponded to a daily dose of 1340 mg in the placebo group and 1340 mg in the curcumin/Boswellia serrata group (824 mg purified turmeric extract, 95% curcuminoids and 516 mg Boswellia.serrata extract, 10% AKBA) throughout the 8-week supplementation period. All supplements were completely blinded by an independent third party prior to any data collection. Throughout the supplementation period, participants were asked not to change their dietary habits.

Reported Side Effects from Supplements

Participants were asked to immediately report any adverse events/medical problems they experienced throughout the course of the study. After conclusion of the study, participants were asked to indicate how the supplement was tolerated and if any adverse events were noted as a result of supplementation. All clinically relevant side effects were immediately reported to the physician for appropriate follow-up.

Medical Monitoring

Interested participants were invited to familiarization sessions. During this time, participants signed consent forms and completed medical history information. Participants then underwent a mandatory medical exam by a physician to determine whether the subject met entry criteria to participate in the study. This exam included evaluating the medical history questionnaire and performing a general physical examination by a physician. Based on this examination, participants were assessed for their risk of cardiovascular disease and then a recommendation was made on whether the participant met entry criteria, and was therefore able to participate in the study. Trained, non-physician exercise specialists certified in CPR, as well as a physician, supervised participants undergoing testing and assessments. A telephone was in the clinic in case of any emergencies, and there was no less than two researchers working with each subject during testing sessions. In the event of any unlikely emergency one researcher was able to check for vital signs and begin any necessary interventions while the other researcher was able to alert the physician who was present. Instructions for emergencies were posted in the clinic in the event that any other research investigators were available for assistance. Participants were informed to report any unexpected problems or adverse events they may have encountered during the course of the study to their physicians. If deemed necessary, the participant was referred to Ronald Wilson, MD or Jackson Griggs, MD for medical follow-up. Dr. Wilson is a Nephrologist and Dr. Griggs is a Family Medicine Physician. They agreed to provide medical support and consultation for this study. Dr. Wilson or Dr. Griggs evaluated any complaints and made a recommendation on whether any medical treatment was needed and/or whether the participant was able to

continue in the study. If Dr. Wilson or Dr. Griggs felt medical follow-up was necessary, the participant was referred to obtain medical treatment from their personal physician. New findings and/or medical referrals of unexpected problems and/or adverse events were documented, placed in the participants research file, and reported to the Baylor IRB committee.

Methods

Body Mass

Total body mass was determined using a self-calibrating scale accurate to ± 0.02 kg. Other than general instructions, special skills were not required to measure body mass.

Blood Pressure and Heart Rate Assessment

Blood pressure was assessed using an automatic blood pressure and heart rate monitor.

Blood Samples

Participants donated approximately 50 mL of fasting venous blood during each testing session into four heparin vacutainer tubes. Blood was drawn by inserting a needle into the antecubital vein using standard phlebotomy procedures by study personnel trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services. Study personnel wore personal protective clothing (gloves, lab coats, etc.) when handling blood samples. Subjects were seated in a phlebotomy chair. A tourniquet was applied high on the brachium (upper arm) and was

tight enough to visibly indent the skin, but not cause the patient discomfort. The entry site was thoroughly cleaned with an alcohol prep pad and allowed to dry. The participant was instructed to lower his/her arm and make a fist several times in order to maximize venous engorgement. The appropriate vein was selected. If a suitable vein was difficult to identify, the pads of the first and second fingers were used to "slap" the veins gently to help dilate them. Alternately, if needed, the arm was covered with a warm, moist compress to help with peripheral vasodilatation. If, after a meticulous search, no suitable veins were found, then the tourniquet was released from above the elbow and placed around the forearm to search in the distal forearm, wrist and hand. If still no suitable veins were found, then the other arm was checked, taking extreme care to stay away from arteries, which are pulsatile. Once a suitable vein was found and blood was drawn, the tourniquet was released. Gentle pressure was applied over the vein with a gauze pad, just proximal to the entry site to prevent blood flow. The needle was removed and disposed in an appropriate sharps container. Blood samples were then centrifuged into plasma samples, transferred into labeled plasma storage containers, and stored at -20˚C for later analysis.

Plasma Analyses

Using enzyme-linked immunoabsorbent assays (ELISA), plasma samples were used to assess cytokine levels representative of changes in inflammation and antioxidant levels indicative of protection from oxidative stress as a result of the supplementation protocol using a Wallac Victor-1420 microplate reader (Perkin-Elmer Life Sciences, Boston, MA). All assays were performed in duplicate using the manufacturer recommended wavelength against a known standard curve depending on the

specifications of the protocol. Inflammation was assessed by measuring plasma levels of IL-6 and TNF-α. Antioxidant activity was assessed by measuring plasma levels of GPx. All dependent variables were assessed using commercially available ELISA kits from Cayman Chemical (Ann Arbor MI).

Briefly, IL-6 and TNF-α were measured using separate immunometric (sandwich) ELISA kits. For IL-6, each well of the plate was coated with a monoclonal antibody specific for IL-6 (IL-6 capture antibody) binding any IL-6 introduced into the well. An acetylcholinesterase:Fab' Conjugate, which binds to a different epitope on the IL-6 molecule, was also added to the well. Once IL-6 was added to the well, the two antibodies formed a "sandwich" by binding on opposite sides of the molecule. The "sandwiches" were immobilized on the plate so that the excess reagents were washed away. The concentration of the analyte was then determined by measuring the enzymatic activity of the acetylcholinesterase by adding Ellman's Reagent to each well, which contains the substrate for acetylcholinesterase. The product of the acetylcholinesterase-catalyzed reaction has a distinct yellow color, which absorbs strongly at 412 nm. The intensity of this color was determined spectrophotometrically, is directly proportional to the amount of bound Conjugate, which therefore was directly proportional to the concentration of IL-6.

One hundred μL of sample was added per well and all samples were analyzed in duplicate. Then, 100 μL of human IL-6 acetylcholinesterase:Fab' Conjugate was added to all wells except two blank wells. The plate was then covered with plastic film and incubated overnight at 4˚C. After incubation, the wells were emptied and rinsed five times with wash buffer. Two hundred μL of Ellman's Reagent were added to each well.

The plate was covered with plastic film and an orbital shaker was used to allow the plate to develop in the dark for a few hours. After this time period, the bottom of the plate was wiped with a clean tissue to remove fingerprints, dirt, etc. and the plate cover was removed. The plate was read at a wavelength between 405 and 420 nm. Once the concentrations of the samples were determined, the average absorbance of the blank wells were subtracted from the absorbance of the remaining wells in the plate. The average absorbance for each standard and sample was then calculated. A standard curve was plotted using the plot absorbance vs. concentration for the standards. A best-fit line was then constructed through the points and used to calculate the IL-6 concentration of the samples. IL-6 concentrations were calculated by subtracting the y-intercept from the absorbency of each sample, and dividing this quantity by the slope. This number was multiplied by the dilution to get the IL-6 concentration. According to the kit insert, the intra- and inter-assay coefficients of variation for IL-6 are approximately 7.9% and 27.8%. TNF-α was analyzed in the same manner as IL-6, with the exception of using a monoclonal antibody specific for TNF-α (TNF-α capture antibody), coated to each well of the plate. According to the kit insert, the intra- and inter-assay coefficients of variation for TNF-α are approximately 9.2% and 9.0%.

For the plasma GPx assay, 120 μL of assay buffer and 50 μL of co-substrate mixture were added to two background wells. One-hundred μL of assay buffer, 50 μL of co-substrate mixture, and 20 μL of diluted GPx (using sample buffer, 50 mM Tris-HCL) were added to two rows of control wells. Twenty μL of sample was added to each remaining well. To each sample well, 100 μL of assay buffer and 50 μL of co-substrate mixture were added. Reactions were initiated by adding 20 μL of cumene hydroperoxide

to all wells. The plate was then shaken for several seconds in order to mix the solution. The absorbance was read once every minute at 340 nm using a plate reader to obtain a minimum of five time points. The change in absorbance/minute was calculated by selecting two points on the linear portion of the curve. Then, the change in absorbance was determined during that time. The rate of absorbance per minute for the background and control wells was then calculated and subtracted from the rate of the sample wells. GPx activity was calculated by dividing the change in absorbance per minute by 0.00373 μM^{-1} [nicotinamide adenine dinucleotide phosphate (NADPH) extinction coefficient] x 0.19 mL/0.02 mL x sample dilution = nmol/min/mL. According to the kit insert, the intra-assay coefficient of variation was 5.7% and the inter-assay coefficient of variation was 7.2%.

Statistical Analysis

Plasma samples will be analyzed by a 2 x 2 [Group (placebo or curcumin/Boswellia) x Time Point (pre-supplementation and post-supplementation)] multiple analysis of covariance (MANCOVA) with repeated measures on time point to determine differences between criterion variables. Activity level will be used as a covariate to determine if amount of exercise affects inflammation levels. Significant between-group differences will then be determined using a repeated measures MANOVA Post Hoc Test. All statistical procedures will be performed using SPSS 17.0 software and an α level of 0.05 will be adopted throughout.

Table 1. *Overview of Research Design*

Fam session	Week 0	Week 8
Familiarization session	SF36	SF36
Informed consent	Fasting blood samples	Fasting blood samples
Demographics	Side effect form	Side effect form
Medical history	Exercise questionnaire	Exercise questionnaire
General exam Determination of height, weight, BP, HR, & waist and hip circumferences	Randomized, double-blind assignment	Determination of height, weight, BP, HR, & waist and hip circumferences

CHAPTER FOUR

Results

Demographics

Twenty-three CKD patients signed informed consent documents to participate in this study. For personal reasons, two patients decided not to participate in the study after signing the informed consent documents and therefore never began the study. Two patients dropped from the study prematurely due to side effects of dizziness, fainting, and nausea; however, both patients were taking placebo. A third patient dropped due to a diagnosis of colon cancer while participating in the study. One patient died four weeks into the study from complications due to congestive heart failure, which was not related to participation in the study. One patient participated in the entire study but was excluded from the analysis due to the use of the strong prescription anti-inflammatory drug methotrexate. Sixteen CKD patients (56.0 ± 16.0 years, 171.4 ± 11.9 cm, 99.3 ± 20.2 kg) finished the study for a completion rate of 69.6%. Four patients were in stage 2 and 12 patients were in stage 3 of CKD. There were no significant differences between groups (CB = curcumin/Boswellia serrata, P = placebo) in regards to GFR ($p = 0.805$), blood urea nitrogen (BUN, $p = 0.084$), creatinine ($p = 0.062$), and albumin ($p = 0.288$) levels at baseline. However, there were significant differences between groups for body mass index (BMI) ($p = 0.011$), waist circumference ($p = 0.034$), and hip circumference ($p = 0.024$), with a trend toward a significant difference for height ($p = 0.052$). The placebo group had a higher BMI (40.3 ± 9.5 vs. 29.5 ± 5.2), greater waist circumference (120.4 ±

12.9 vs. 101.8 ± 16.1) and hip circumference (49.8 ± 6.5 vs. 42.3 ± 4.9) compared to the treatment group (see Table 2). All patients that completed the study were able to tolerate the supplementation protocol for 8 weeks. Table 2 provides demographics and markers of kidney function at baseline.

Table 2. *Demographics and Baseline Markers of Kidney Function*

Variable	Treatment	Placebo	p-value
Age (yrs)	53.3 ± 18.0	59.4 ± 13.4	0.468
Height (cm)	176.4 ± 10.2	164.9 ± 11.4	0.052
Weight (kg)	92.6 ± 21.8	107.9 ± 15.3	0.137
BMI (kg/m^2)	29.5 ± 5.2	40.3 ± 9.5	0.011
SBP (mmHg)	140.4 ± 15.7	142.7 ± 28.5	0.841
DBP (mmHg)	75.6 ± 13.3	72.1 ± 10.6	0.588
HR (bpm)	68.1 ± 13.5	75.6 ± 9.5	0.236
Waist (cm)	101.8 ± 16.1	120.4 ± 12.9	0.034
Hip (cm)	107.5 ± 12.6	126.6 ± 16.5	0.024
GFR ($mL/min/1.73m^2$)	51.9 ± 12.6	53.9 ± 18.7	0.805
BUN (mg/dL)	20.6 ± 4.2	27.3 ± 9.8	0.084
Creatinine (mg/dL)	1.7 ± 0.4	1.3 ± 0.4	0.062
Albumin (g/dL)	3.7 ± 0.4	3.5 ± 0.4	0.288
TLAS	8.3 ± 15.0	0.0 ± 0.0	0.166

Note. Data are presented as mean ± SD.
TLAS = Total Leisure Activity Score.

Supplement Compliance

Throughout the 8-week supplementation protocol, patients were called weekly in order to confirm they were ingesting their supplement, as well as to note any side effects they were experiencing. Two patients reported missing several doses due to unexpected hospital stays but continued to take their supplements upon returning home. Pill compliance throughout the 8 weeks ranged from 50.0-100.0%, with an average compliance of 84.6%. One patient was only 50% compliant. This patient claimed that she never takes the entire amount of pills she is prescribed. The other reason for patients not being compliant was forgetfulness.

Adverse Events

All patients completed side effect questionnaires at each of two testing sessions. Patients were asked to report both the frequency and severity of any side effects such as dizziness, headache, racing heart rate, heart palpitations, shortness of breath, nervousness, blurred vision, or nausea. The 8-week supplementation protocol was well tolerated with only minor side effects reported. One patient experienced nausea, which is a reported side effect in the literature (Sengupta et al., 2008; Pari, Tewas, & Eckel, 2008). However, three patients experienced increased urination, which has not been reported as a side effect of either curcumin or Boswellia serrata.

SF-36 Quality of Life

All patients were asked to fill out the SF-36 Quality of Life surveys. However, only 9 of the 16 patients completed the questionnaire due to the length of time required to

complete the survey as well as personal time constraints. Therefore, this data is not presented.

Godin Leisure-Time Exercise Questionnaire

The Godin Leisure-Time Exercise Questionnaire (Godin & Shephard, 1985) was used to measure physical activity levels throughout the 8-week study. Exercise was divided into three intensities: strenuous (heart beats rapidly), moderate (not exhausting), and mild (minimal effort). Scores were calculated by multiplying the frequencies of the 3 intensities of exercise by the suggested metabolic equivalents needed to perform the exercise. Scores for each intensity were added together for a total leisure activity score (TLAS). Results from this questionnaire were used as a covariate to determine if physical activity levels affected inflammation levels.

Plasma Markers of Inflammation and Antioxidant Activity

Repeated measures MANCOVA revealed no significant difference between groups for any of the dependent variables ($p = 0.732$). In addition, activity levels from the TLAS did not significantly affect inflammatory or antioxidant levels in this study ($p = 0.987$). Analysis of the data revealed no significant within subjects time effect ($p = 0.312$), time x activity level interaction ($p = 0.685$), or time x group interaction ($p = 0.760$) (See Table 3). There were no significant differences between groups for baseline values of either IL-6 ($p = 0.844$) or TNF-α ($p = 0.881$), but there was a significant difference between groups for baseline values of GPx ($p = 0.040$).

Table 3. *Repeated Measures MANCOVA Results*

Variable	p-value
Group	0.732
TLAS (covariate)	0.987
Time	0.312
Time x TLAS	0.760
Time x Group	0.678

Univariate Results

Although a repeated measures MANCOVA showed no significant results, the univariate analyses are listed in order to provide completeness to the dissertation.

IL-6

Plasma IL-6 was determined at two time points throughout the study (pre-supplementation and post-supplementation) in order to assess systemic inflammation. Figure 1 presents the raw data that were observed in both groups throughout the supplementation protocol. Univariate tests revealed no significant group ($p = 0.426$) or time ($p = 0.545$) effects, time x activity level interaction ($p = 0.944$) or time x group interaction ($p = 0.259$). Effect size between groups for IL-6, reported as partial eta squared was 0.049. In addition, delta values (Post-supplementation – Pre-Supplementation), as shown in figure 2, demonstrated no significant difference between groups ($p = 0.259$). Hypothesis 1, which states that there will be a decrease in IL-6 when supplementing with curcumin and Boswellia serrata in CKD patients, is therefore rejected. These results are presented in Table 4.

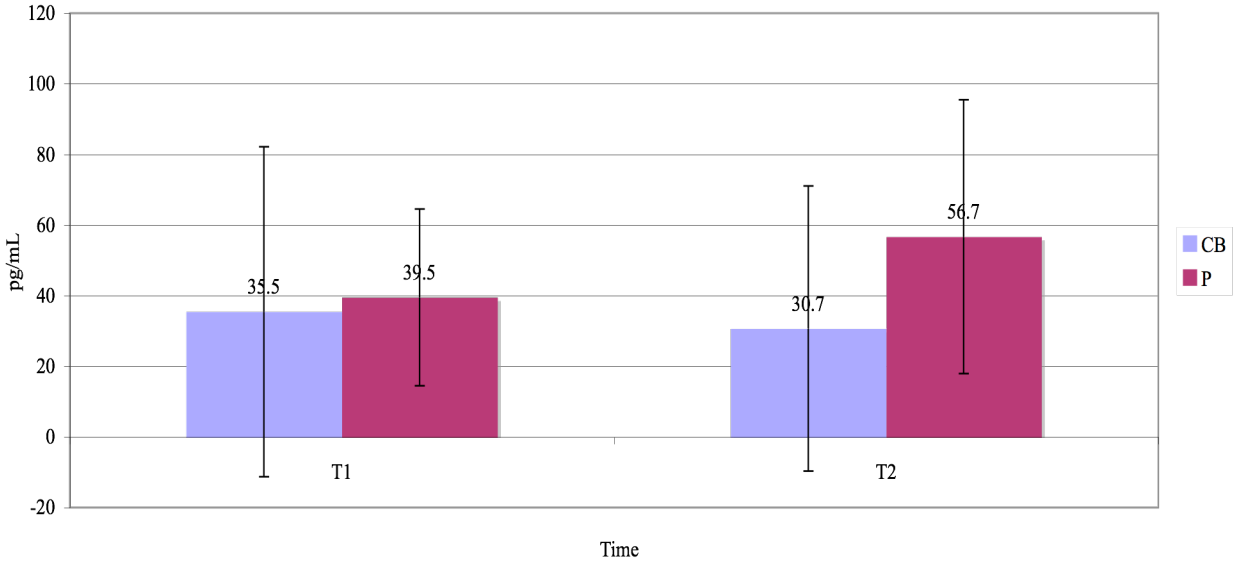

Figure 1. Data for IL-6 (pg/mL) represented as means ± SD for all time points

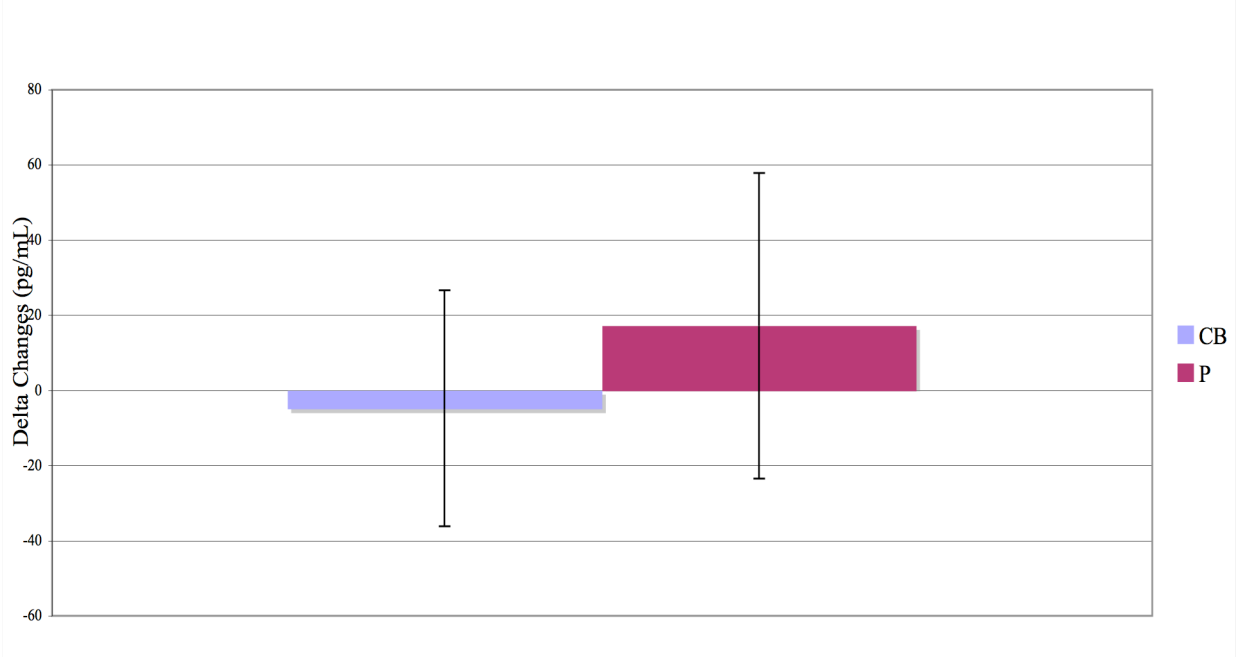

Figure 2. Delta Values for IL-6 (pg/mL)

Table 4. *IL-6 Results*

Variable	p-value
Group	0.426
Time	0.545
Time x TLAS	0.944
Time x Group	0.259
Delta Value	0.259
Effect Size (Group)	0.049

TNF-α

Plasma levels of TNF-α were determined at two time points throughout the study (pre-supplementation and post-supplementation) as an additional marker of systemic inflammation. Figure 3 represents the values that were observed in both groups throughout the study. Univariate tests revealed no significant group ($p = 0.945$) or time effects ($p = 0.237$). In addition, there were no time x activity level ($p = 0.275$) or time x group interactions ($p = 0.757$). Effect size between groups for TNF-α, reported as partial eta squared, was 0.000. Delta values, as shown in figure 4, also demonstrated no significant difference between groups ($p = 0.757$). Hypothesis 2, which states that there will be a decrease in TNF-α when supplementing with curcumin and Boswellia serrata, is therefore rejected. These results are presented in Table 5.

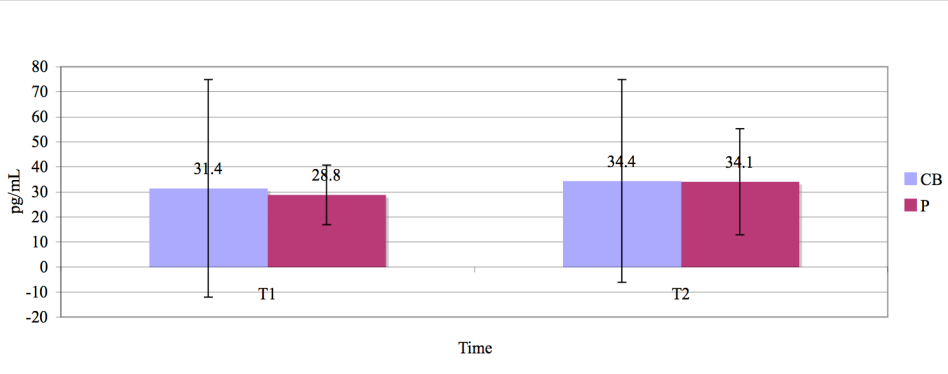

Figure 3. Data for TNF-alpha (pg/mL) represented as means ± SD for all time points

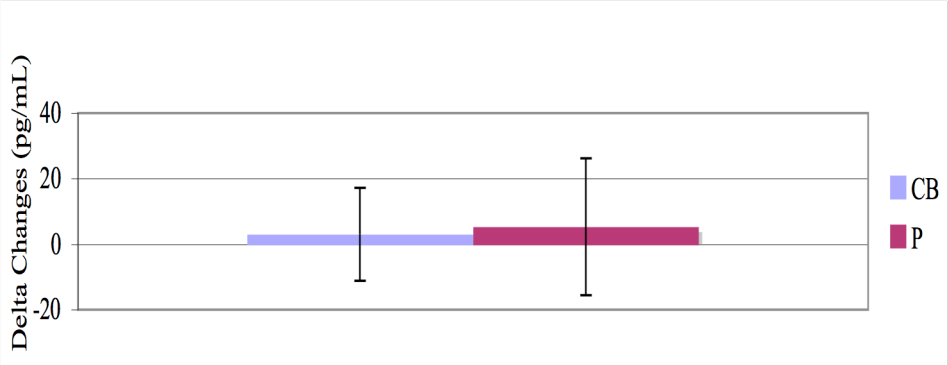

Figure 4. Delta Values for TNF-alpha (pg/mL)

Table 5. *TNF-α Results*

Variable	p-value
Group	0.945
Time	0.237
Time x TLAS	0.275
Time x Group	0.757
Delta Value	0.757
Effect Size (Group)	0.000

Glutathione Peroxidase

Plasma enzyme activity of glutathione peroxidase (GPx) was measured at two time points throughout the study (pre-supplementation and post-supplementation) in order to assess changes in antioxidant capacity. Baseline values of GPx were significantly different between groups (CB = 4.9 ± 3.0 vs. P = 11.1 ± 7.6 nmol/min/mL, p = 0.040). Univariate tests revealed no significant group (p = 0.595) or time effects (p = 0.207), time x activity level interaction (p = 0.703), or time x group interaction (p = 0.537). Effect size between groups for GPx, reported as partial eta squared, was 0.022. Figure 5 shows values observed in all patients throughout the study. In addition, delta values, as shown in figure 6, demonstrated no significant difference between groups (p = 0.022). Hypothesis 3 states that GPx levels in CKD patients will be below normal, healthy values. Due to the low baseline levels of GPx observed in this study, we therefore fail to reject hypothesis 3. When baseline values of GPx were compared by stage, results revealed no significant differences (p = 0.124) between stages 2 and 3 of CKD. These results are presented in figure 7. Hypothesis 4, which states there will be a negative relationship between plasma levels of GPx and stage of disease, is therefore rejected. Hypothesis 5 states there will be no change in antioxidant activity following 8 weeks of curcumin and Boswellia serrata supplementation. Therefore, we fail to reject hypothesis 5. These results are presented in Table 6.

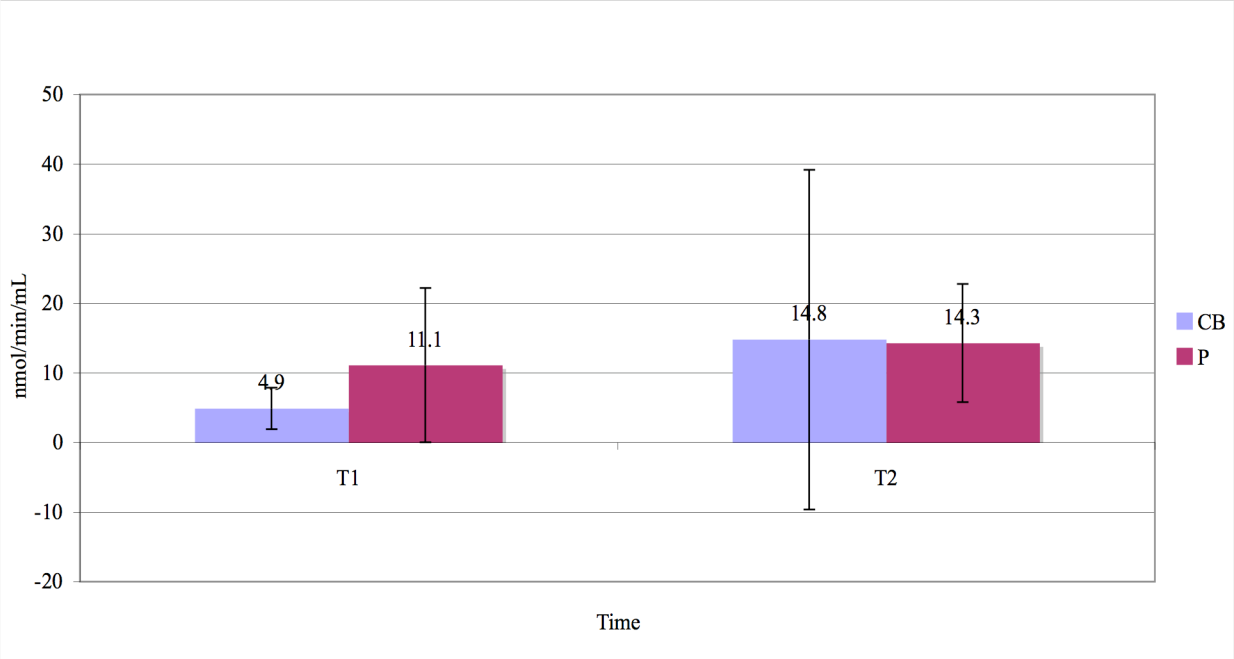

Figure 5. Data for GPx (nmol/min/mL) represented as means ± SD for all time points

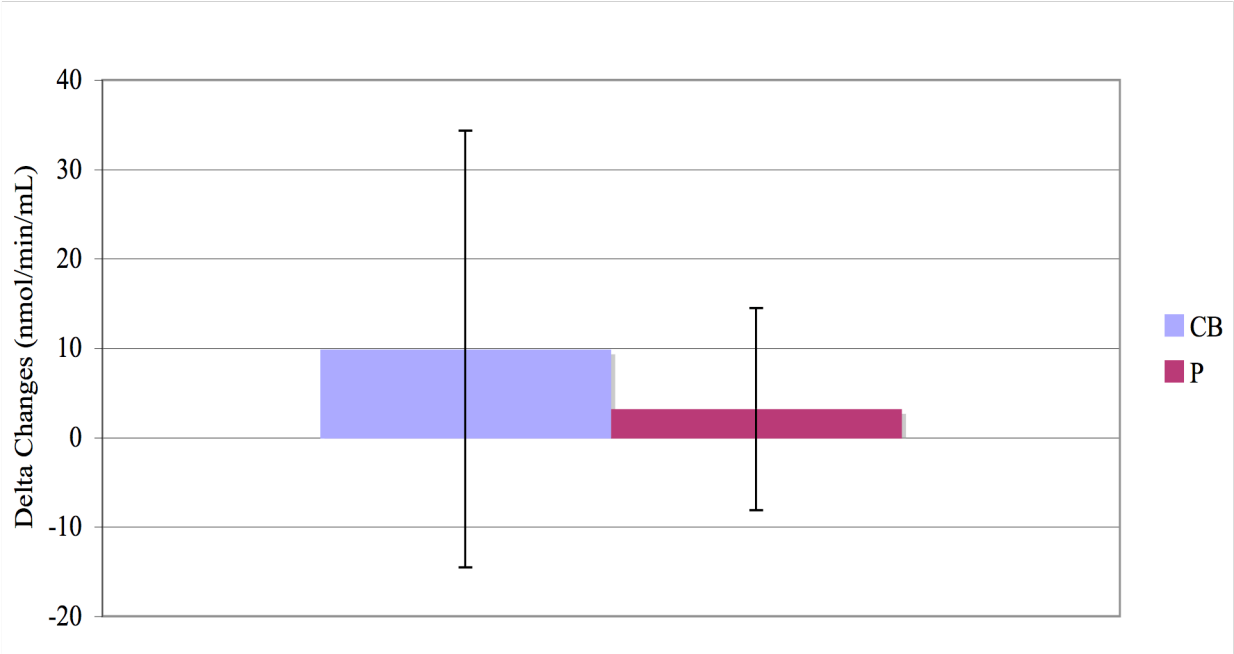

Figure 6. Delta Values for GPx (nmol/min/mL)

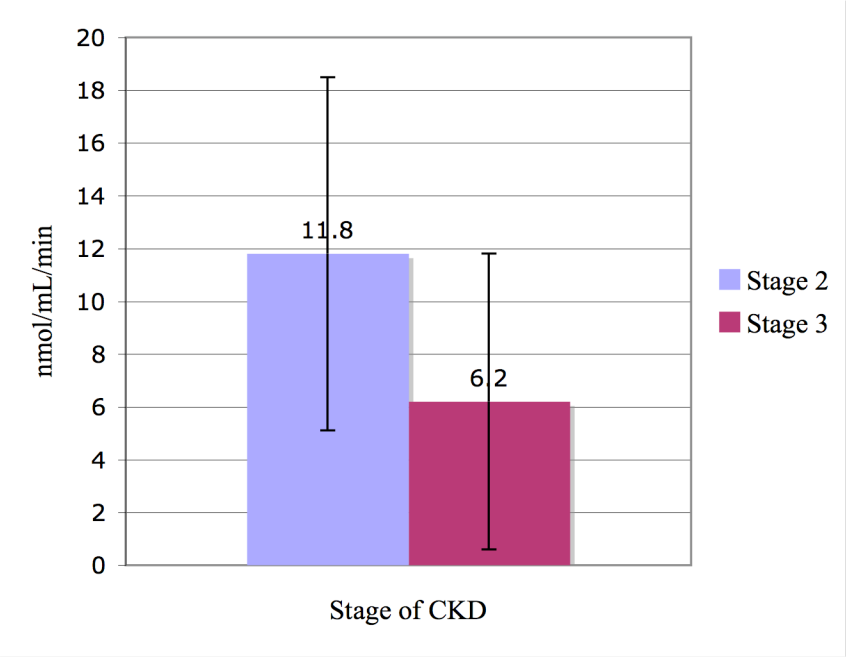

Figure 7. Baseline Plasma Levels of GPx by Stage

Table 6. *GPx Results*

Variable	p-value
Group	0.595
Time	0.207
Time x TLAS	0.703
Time x Group	0.537
Delta Value	0.537
Effect Size (Group)	0.022

CHAPTER FIVE

Discussion

Patients with CKD are often underdiagnosed, which leads to numerous complications. Although treatment is available, it is many times sought too late when the progression of the disease has already become severe. As shown in this study, many patients with kidney disease have diabetes and/or hypertension, the two leading causes of CKD. Since diabetes and hypertension are also risk factors for cardiovascular disease, it is no surprise that most patients will die of this rather than kidney failure. By the time patients are diagnosed with CKD, the presence of the associated complications makes it difficult to treat. The co-occurrence of so many diseases and lack of treatment early paints a bleak picture for many of these patients. Because of the combination of these factors, it is important to keep in mind the health status of the patients in the current study. Most individuals exhibited high levels of inflammation and low antioxidant capacity. Therefore, this study examined the effects of 8 weeks of curcumin and Boswellia serrata supplementation on changes in systemic markers of inflammation and antioxidant activity in a population of CKD patients. It was hypothesized that 8 weeks of supplementation would be associated with changes in systemic markers of inflammation, specifically IL-6 and TNF-α, compared to placebo. Furthermore, it was hypothesized that antioxidant activity in this population would be below normal, healthy levels, and that supplementation would not lead to changes in antioxidant activity, as measured by the antioxidant enzyme, (GPx). Results from this study indicate that eight weeks of curcumin and Boswellia serrata supplementation had no effect on plasma levels of IL-6

or TNF-α. Therefore, the hypotheses associated with these two markers of inflammation were rejected. Additionally, GPx levels in the current study of CKD patients were below normal, healthy values. Also, the treatment group did not exhibit any significant changes in plasma levels of GPx throughout the eight-week supplementation period. Therefore, we fail to reject these two hypotheses associated with antioxidant activity. Results from this study suggest that curcumin and Boswellia serrata supplementation had no effect on the markers of interest after an 8-week supplementation period.

Evidence of Inflammation in CKD

It has been well documented that CKD is a condition characterized by decreased kidney function, as evidenced by increased levels of serum creatinine and either an estimated or measured glomerular filtration rate (GFR) below normal (≤ 60 mL/min/1.73m^2) (Graves, 2008; Thomas et al., 2008; Weiner, 2007). The most common equation used to determine eGFR, and therefore stage of CKD, is the Modification of Diet in Renal Disease Study equation. This equation, which was used in the current study, is comprised of various factors that affect levels of serum creatinine, including age, sex, gender, and race (Levey et al., 1999; Thomas et al., 2008; Weiner, 2007). Among the 16 patients that participated in this study, 4 patients were classified as stage 2 and 12 patients were classified as stage 3. CKD2 is considered one of the early stages of kidney disease, and although it is marked by kidney damage, patients are usually asymptomatic. CKD3 is considered a moderate form of the disease in which symptoms tend to present themselves and damage to the kidney becomes progressively worse. A normal GFR is ≥ 90 mL/min/1.73m^2 (National Kidney Foundation, 2002). The average GFR in this study was 52.8 mL/min/1.73m^2. This value is not only below normal, but also indicates the

time at which most symptoms begin to manifest themselves, including an increase in inflammation levels.

Inflammation levels in the current study were assessed by measuring plasma concentrations of IL-6 and TNF-α. It is hard to compare the levels of inflammation found in this study to previous studies due to the fact that there are multiple stages of CKD, and it is difficult to carry out a study in which only one stage of CKD is represented. Thus, it is common for studies to include patients from one or more stages, as this was the case for the current study as well. Of all the patients we had access to, only those in stages 2 and 3 decided to enroll and complete the study in its entirety. An additional challenge in comparing the current results is that some studies do not report CKD stage and instead group CKD patients into one of two categories: those on dialysis (CKD5) and those non-dialyzed, which by definition could include patients in stages 1 through 5, as there are some CKD5 patients who have not reached kidney failure. When patients are classified as only dialysis or non-dialysis patients, it is not possible to determine which stages of CKD are represented in a particular study. Other clinicians or study authors instead prefer to use creatinine clearance as entry criteria into a research study rather than stage of CKD. Creatinine clearance utilizes serum creatinine and a timed urine specimen to determine GFR. This has been reported to take a great deal of time and is commonly carried out incorrectly due to timing errors and urine collection (Graves, 2008).

The current study was comprised of CKD patients in stages 2 and 3. Pre-supplementation plasma levels of IL-6 in the treatment and placebo groups were 35.5 and 39.5 pg/mL, respectively. Previous studies documenting inflammation in non-dialyzed

CKD patients have reported lower values of IL-6 than what was found in the present study (Dervisoglu et al., 2008; Oner-Iyidogan et al., 2009; Pawlak et al., 2008; Rastmanesh et al., 2008). Two of these studies included CKD patients with more compromised kidney function than patients in our study (Dervisoglu et al., 2008; Rastmanesh et al., 2008). Dervisoglu et al (Dervisoglu et al., 2008) grouped all non-dialyzed CKD patients together and reported serum creatinine levels instead of stage of CKD (Dervisoglu et al., 2008), which was reportedly 2.6 mg/dL, considerably higher than the levels found in our study (CB = 1.7 mg/dL, P = 1.3 mg/dL). The second study included CKD patients in stages 3 and 4, grouping all patients together into one analysis (Rastmanesh et al., 2008). Although both of the previous studies included patients in higher stages of CKD, plasma concentrations of IL-6 were approximately 5-6x lower compared to our results (Dervisoglu et al., 2008; Rastmanesh et al., 2008). Finally, two studies were able to include subjects in all stages of CKD (Oner-Iyidogan et al., 2009; Pawlak et al., 2008). Even so, IL-6 concentrations in both studies were still lower compared to our study, even in patients in stage 5 of CKD (Oner-Iyidogan et al., 2009; Pawlak et al., 2008). One explanation for the increased IL-6 levels in the present study could be explained by differences in exclusion criteria. The exclusion criteria in all the previous studies mentioned was extremely rigid compared to our own. For example, some of the studies excluded CKD patients with other comorbidities such as diabetes (Dervisoglu et al., 2008; Rastmanesh et al., 2008), autoimmune diseases (Dervisoglu et al., 2008; Pawlak et al., 2008), or other conditions known to affect cytokine levels (Dervisoglu et al., 2008), as well as those on lipid-lowering therapy, non-steroidal anti-inflammatory drugs, or immunosuppressive therapy (Pawlak et al., 2008; Rastmanesh et

al., 2008). The present study included CKD patients that had diabetes, those on lipid-lowering therapy, as well as patients that had other co-morbidities that are often associated with higher levels of inflammation such as coronary artery disease (Libby, 2002; Pearson et al., 2003; Vogiatzi, Tousoulis, & Stefanadis, 2009), osteoarthritis (Pelletier, Martel-Pelletier, & Abramson, 2001; Spector et al., 1997), congestive heart failure (Torre-Amione, 2005), and obesity (Fontana, Eagon, Trujillo, Scherer, & Klein, 2007; Pou et al., 2007; Straczkowski et al., 2002). Specifically, the presence of diabetes in our patient population is worth noting. Fourteen of 16, or 88%, of patients in the present study had diabetes in addition to CKD (13 with type 2 and 1 patient with type 1). This is not surprising, as diabetic nephropathy is responsible for most cases of CKD worldwide. Therefore, it would be difficult to recruit patients without diabetes. It is also not a new concept that patients with diabetes in addition to CKD show evidence of increased inflammatory markers including C-reactive protein, serum amyloid A, fibrinogen, and IL-6 (Dalla Vestra et al., 2005). Dalla Vestra et al (Dalla Vestra et al., 2005) were able to demonstrate that a number of acute phase inflammatory markers, including IL-6, are higher in diabetic patients with overt nephropathy compared to diabetic patients with normal albumin excretion rates (no evidence of kidney disease), supporting evidence that there is a link between diabetic nephropathy and inflammation. In a similar manner, Choudhary et al (Choudhary & Ahlawat, 2008) found a significant association between urinary albumin excretion and inflammatory markers in patients with type II diabetes at early stages of nephropathy. Specifically, IL-6 and hs-C-reactive protein were higher in patients with albuminuria (i.e. a type of proteinuria which is evidence of kidney disease) than those with normal albumin levels or microalbuminuria.

In addition, levels of IL-6 and hs-C-reactive protein significantly decreased in all three groups of diabetic patients that were treated with varying combinations of diet therapy, oral hypoglycemic drugs, and/or insulin, supporting the hypothesis that glycemic control contributes to a decrease in inflammatory markers. Thus, it would be expected that poor glycemic control would lead to increases in inflammation. Although it is unknown if patients in our study had sufficient glycemic control, this could potentially explain the increased levels of IL-6. Further evidence for a relationship between concentrations of IL-6 and diabetic nephropathy is the fact that IL-6 appears to be locally expressed in the kidney. A study using high resolution in situ hybridization found that IL-6 mRNA is expressed by glomerular resident cells and interstitial cells in renal tissue of patients with diabetic nephropathy and may be involved in tissue injury in these patients (Suzuki et al., 1995).

Pre-supplementation plasma concentrations of TNF-α were 31.4 and 28.8 pg/mL in the treatment and placebo groups, respectively. In a similar manner to IL-6, the majority of previous studies report lower plasma concentrations of TNF-α (Dervisoglu et al., 2008; Oner-Iyidogan et al., 2009; Rastmanesh et al., 2008). Again, due to our liberal inclusion criteria compared to previous studies, it would be expected that levels of TNF-α would be higher. However, one study reported TNF-α values that were approximately 3-4x higher than our own, even in patients with mild CKD (stages 1 and 2) (Pawlak et al., 2008). This is surprising, since the same study also reported concentrations of IL-6 that were lower than what we observed. Intuitively, it would be expected that both cytokine levels in our study would be higher, considering the presence of comorbidities in addition to CKD. However, this was not the case. IL-6 was lower, but TNF-α was much higher

than the concentrations observed in our study, even in patients in stages 1 and 2. The association between TNF-α and impaired renal function is well documented (Descamps-Latscha et al., 1995; Nakanishi et al., 1994), but it appears that most of the evidence linking TNF-α with CKD has been demonstrated in end-stage renal disease (ESRD) patients (Kimmel et al., 1998; Stenvinkel et al., 1999). Its role in mild to moderate stages of CKD has yet to be elucidated, and is therefore unlikely to explain the differences observed in these two studies. In addition to CKD, TNF-α is also associated with lipid metabolism, coagulation, insulin resistance, and endothelial dysfunction (Stenvinkel et al., 2005). One study demonstrated a strong association between TNF-α and endothelial dysfunction in patients with coronary artery disease (Fichtlscherer et al., 2000) while another study showed that TNF-α decreases endothelium-dependent relaxation in vivo (Wang, Ba, & Chaudry, 1994), and is capable of downregulating endothelial nitric oxide synthase mRNA by decreasing its half-life (Yoshizumi, Perrella, Burnett, & Lee, 1993). In the study by Pawlak et al. (2008), 24 patients, or 44%, of the study population had been diagnosed with cardiovascular disease in addition to CKD (Pawlak et al., 2008), while only 6 of our patients, or 38%, had cardiovascular disease. Perhaps the greater presence of cardiovascular disease in the previous study could be the factor causing such high levels of TNF-α compared to our own. However, IL-6 is also considered a proatherogenic cytokine and is considered to have a stronger association with cardiovascular disease and mortality compared to TNF-α (Stenvinkel et al., 2005). For instance, recombinant IL-6 injections enlarged lesions in atherosclerotic-prone mice. The authors concluded that IL-6 may not only be a consequence but a contributor to lesion development in those susceptible to this disease process (Huber, Sakkinen, Conze,

Hardin, & Tracy, 1999). In addition, IL-6 has been shown to induce production of C-reactive protein (Heinrich, Castell, & Andus, 1990), and this biomarker is a well-known indicator of cardiovascular disease (Venugopal, Devaraj, Yuhanna, Shaul, & Jialal, 2002). Yet, TNF-α is considered to have a weak assocation with C-reactive protein (Stenvinkel et al., 2005). Considering that levels of IL-6 in our study were higher compared to Pawlak et al., the presence of cardiovascular disease may only be a partial explanation for the discrepancies observed with TNF-α. TNF-α's role in the pathogenesis of insulin resistance has been also demonstrated in various patient populations such as obese patients (Kern, Ranganathan, Li, Wood, & Ranganathan, 2001) as well as in experimental essential hypertension (Togashi, Ura, Higashiura, Murakami, & Shimamoto, 2000). The use of thiazolidinediones has been shown to counteract TNF-α, resulting in increased insulin sensitivity (Solomon, Usdan, & Palazzolo, 2001). Most of our patients had diabetes and were on medications to improve insulin sensitivity, and this also may have contributed to lower TNF-α levels. A third explanation for the disparities observed in TNF-α could be the use of lipid-lowering therapy. Pawlak et al specifically excluded patients on lipid-lowering therapy while we did not. Patients at the clinic we recruited from (Family Health Center) presented with multiple co-morbidities in addition to CKD. Because of this, it was nearly impossible to recruit any CKD patients that were not on any medication, specifically lipid-lowering therapy. Fifteen of our patients, or 94%, were on lipid-lowering medication, most notably statins. While statins are known to treat dyslipidemia, they also exhibit anti-inflammatory properties (Ridker, Rifai, Pfeffer, Sacks, & Braunwald, 1999; Zhao & Zhang, 2003). One study has shown that statins effectively decreased levels of TNF-α but not IL-6 in a group of CKD

patients. Goicoechea et al (Goicoechea et al., 2006) examined the effect of atorvastatin on inflammatory markers in CKD patients in stages 2, 3, and 4. The group taking atorvastatin showed significant reductions in C-reactive protein, IL-1β and TNF-α, but not IL-6. While the study authors were able to show a significant correlation between C-reactive protein and IL-6, there was no significant reduction in plasma IL-6. The authors concluded that other cytokines may contribute to C-reactive protein regulation, or IL-6 concentrations may have a greater diurnal variability and shorter half-life than C-reactive protein, explaining the lack of change observed in IL-6. This may be an additional explanation as to why our patients, who were on statins, had lower levels of TNF-α but not IL-6 compared to Pawlak et al's (Pawlak et al., 2008) patients. Finally, although plasma cytokine measurements aid in determining the presence of a chronic inflammatory state, they do not take into account the fact that cytokines are frequently influenced by their inhibitors and other cytokines. Since cytokines do not act in isolation, a change in one cytokine will lead to changes in several others (Stenvinkel et al., 2005). Therefore, there may have been other cytokines not measured in our study or the study by Pawlak et al. that could have affected levels of TNF-α, therefore explaining the differences that were observed.

Evidence of Impaired Antioxidant Activity

In line with previous research, our data indicate that plasma concentrations of glutathione peroxidase (GPx) in CKD patients are lower than normal, healthy values (CB = 4.9 ± 3.0 vs. P = 11.1 ± 7.6 nmol/min/mL)(Ceballos-Picot et al., 1996; El-Far et al., 2005; Moradi et al., 2009; Zachara et al., 2004). GPx is one of several enzymes in the mammalian species involved in the endogenous antioxidant system that neutralizes free

radicals or reactive oxygen species (ROS). Along with superoxide dismutases (SODs), catalases (CATs), and selenoprotein P, GPx scavenges free radicals by regulating their rate of movement through a number of scavenging reactions (Baynes & Thorpe, 1999; Galli, Canestrari, & Buoncristiani, 1999) (Grignard et al., 2005). Under normal physiological conditions, there is a balance between an organism's antioxidant system, and oxidant activity (Baynes & Thorpe, 1999; Galli et al., 1999). However, when there is an imbalance, such as occurs when there is an increased level of free radical production, or when antioxidant levels are decreased, the result is increased oxidative stress. This scenario has been shown to occur in a number of diseases that include diabetes, atherosclerosis, and CKD (Marx, 1987). Of the two forms of GPx found in blood, it is the extracellular form, or plasma GPx (Arthur & Beckett, 1994) that appears to have clinical benefit in CKD patients, as the production of GPx mainly occurs in the proximal tubular cells of the kidney (Avissar et al., 1994). One of the first studies to document the relationship between plasma GPx concentrations and renal failure was carried out by Ceballos-Picot et al (Ceballos-Picot et al., 1996). The authors demonstrated that plasma GPx activity in non-dialyzed, chronic renal failure patients decreased as the disease became progressively worse, becoming almost completely abolished in hemodialysis patients (75% of GPx levels in control group). In the non-dialyzed group, patients with severe CKD had approximately 50% of the plasma GPx concentrations observed in the control group. Other studies in non-dialyzed CKD patients have reported similar findings, with plasma GPx concentrations reported to be 37% (Zachara et al., 2004) and 62% (El-Far et al., 2005), of healthy control levels. In addition, both studies also confirmed the inverse relationship between plasma GPx levels and severity of kidney

disease (El-Far et al., 2005; Zachara et al., 2004). In the present study we were unable to recruit patients with all stages of CKD. Rather, we were able to include patients in both mild (CKD2) and moderate (CKD3) stages of CKD, with a majority of patients in stage 3. Patients in stage 2 did have slightly higher baseline levels of GPx compared to those in stage 3 (11.78 vs. 6.24 nmol/mL/min), however this difference was not significant. This study also demonstrates that, at the very least, one of the enzymatic components of the antioxidant system in CKD is severely imbalanced, even in mild and moderate stages of the disease. This lack of defense against oxidative stress would explain the high incidence of hypercholesterolemia and hyperlipidemia observed in the patients in the present study, likely due to increased lipid peroxidation, and therefore a higher risk of cardiovascular disease. In CKD patients undergoing hemodialysis, other contributors to the antioxidant defense system that have also shown to be impaired are superoxide dismustase, catalase, and plasma sulphydryl (Dursun et al., 2008).

While we did not have a control group composed of healthy volunteers to compare plasma GPx concentrations to, levels of the enzyme were lower than what has been reported in healthy individuals (Ceballos-Picot et al., 1996; El-Far et al., 2005; Moradi et al., 2009). Surprisingly, however, plasma GPx levels observed in our study were lower than what has been reported for kidney patients in mild and moderate stages of the disease. In fact, our levels were lower, even compared to what has been observed in HD patients (Ceballos-Picot et al., 1996; Moradi et al., 2009). The extremely low GPx values that we observed may be representative of the impaired antioxidant mechanisms that have also been suggested in diabetes, especially since so many of our patients were diabetic. It is unknown whether oxidative stress is part of the cause, or rather a

consequence of tissue damage in diabetes (Baynes & Thorpe, 1999; Oberley, 1988). Type 2 diabetes is thought to be multi-factorial in nature, partially due to insulin resistance and partially due to β cell dysfunction. In terms of β cell dysfunction, excess glucose in the cell overloads the normal pathways that metabolize glucose, leading to the production of reactive oxygen species (Robertson, 2004), and inadvertently, chronic oxidative stress. Interestingly, the pancreatic β cell contains the lowest amount of endogenous antioxidant defense mechanisms in the form of superoxide dismutase-1, superoxide dismutase-2, catalase, and GPx compared to any other tissue (Tiedge, Lortz, Drinkgern, & Lenzen, 1997). Conversely, the islet cell contains ample amounts of γ-glutamylcysteine ligase mRNA, the rate-limiting enzyme in glutathione synthesis. Over time, excess cellular glucose reduces expression of γ-glutamylcysteine ligase, and this is associated with decreased glutathione synthesis (Catherwood et al., 2002; Lu, Bao, Huang, Sarthy, & Kannan, 1999). As for insulin resistance, this can be partially explained by excess adiposity. In humans, adipose tissue correlates with systemic oxidative stress, and prolonged amounts of reactive oxygen species decrease insulin sensitivity (Furukawa et al., 2004). Furukawa et al (Furukawa et al., 2004) found that hydrogen peroxide production only increased in white adipose tissue of obese mice, while tissues of the liver, skeletal muscle, and aorta were not affected. The authors concluded that white adipose tissue was the only site of increased oxidative stress because mRNA expression levels of NADPH oxidase (nicotinamide dinucleotide phosphate oxidase, a generator of oxidative stress) increased, and mRNA levels of antioxidant enzymes decreased, namely superoxide dismutase, catalase, and GPx. Also, total superoxide dismutase and GPx activities were significantly lower in white adipose

tissue of the obese mice compared to the control mice. In the present study, BMI levels in the treatment and placebo groups were 29.5 and 40.3, and waist measurements were 101.8 cm and 120.4 cm. While these are not direct measures of adiposity, it indicates that the subjects in the present study were likely overweight or obese. The comorbidity of diabetes, as well as excess adiposity along with CKD, could in and of itself explain the extremely low GPx levels observed in the present study.

Inflammation and Curcumin/Boswellia Serrata Supplementation

There has recently been a strong push in the scientific community to investigate the effects of complementary medicine that elicits biological actions with little or no side effects compared to their prescription counterparts. Two of these compounds are curcumin and Boswellia serrata. Curcumin is a polyphenol and the active ingredient in the spice turmeric. It has been used for centuries in China and India (Ammon & Wahl, 1991). Although the use of curcumin in the past has been coined a "folk remedy," research has now demonstrated that it has widespread properties as an anti-inflammatory, antioxidant, anti-cancer, and chemotherapeutic agent (Hatcher et al., 2008). The Boswellia species are a rich source of pentacyclic triterpenes, compounds with a conglomeration of bioactive properties. Of the Boswellia species, it is Boswellia serrata that contains the most potent source (Poeckel & Werz, 2006). Boswellia serrata has also been called 'frankincense', referring to the lipophilic fraction of its gum resin. It has been used in Indian medicine, mainly as a topical treatment for various joint disorders and diseases. Early studies using Boswellia serrata have shown that it exhibits analgesic effects, (Kar & Menon, 1969; Menon & Kar, 1971) and today it has been used in both human and animal research for the treatment of inflammation in various diseases, as well

as for cancer, hyperlipidemia, hypercholesterolemia, and allergies (Poeckel & Werz, 2006). In regards to its anti-inflammatory properties, curcumin has been used almost entirely in animal and cell culture studies showing much promise (Chan, 1995; Fu et al., 2008; Ghosh et al., 2009; Kuhad et al., 2007; Rafiee et al., 2009). Presently, it has not been used in a human trial of CKD patients. Boswellia serrata, on the otherhand, has been researched more extensively in humans, mainly for the treatment of colitis (Madisch et al., 2007) and osteoarthritis (Kimmatkar et al., 2003; Sengupta et al., 2008), but there have been no published reports on its use in kidney disease in animals or humans. Findings from the current study suggest that the combination of curcumin and Boswellia serrata supplementation does not significantly affect inflammation levels as measured by plasma concentrations of IL-6 and TNF-α in mild to moderate CKD patients after 8 weeks of therapy. This was the first study to use curcumin in a human trial of CKD and the first to use Boswellia serrata in CKD patients. To the author's knowledge, there are no studies that have used curcumin and Boswellia serrata in combination exclusively. Two studies did utilize these two compounds together for the treatment of juvenile Crohn's disease (Slonim, Grovit, & Bulone, 2009) and osteoarthritis (Kulkarni et al., 1991), with positive results. However, other ingredients were used in combination with these herbs, specifically other anti-inflammatory compounds. Therefore, it is unknown which of the therapies contributed most to the outcome variables of interest. The findings in the present study do not support curcumin's anti-inflammatory effects observed in experimental models of renal failure (Ghosh et al., 2009), nephrotoxicity (Kuhad et al., 2007), liver injury (Fu et al., 2008), esophageal inflammation (Rafiee et al., 2009), and LPS-induced inflammation in human macrophages (Chan, 1995). Human

trials utilizing Boswellia serrrata have shown a number of positive outcomes, but these results should be taken with caution. First, these studies were carried out using the gold standard for research design: double blinded and placebo-controlled. Secondly, the majority of dependent variables that were measured were assessed using self-report questionnaires, rather than physiological measurements. For instance, two studies evaluated the efficacy, safety, and tolerability of Boswellia serrata in osteoarthritis patients (Kimmatkar et al., 2003; Sengupta et al., 2008). Based on self-report, the authors noted improvements in pain, functional ability, swelling, knee flexion, and stiffness. However, in the first study (Kimmatkar et al., 2003), radiological measurements demonstrated that statistically there was no change between Boswellia serrata and placebo. The second study shows a bit more promise, as the authors did find that levels of a cartilage degrading enzyme matrix known as metalloproteinase-3 were significantly lower in the groups taking Boswellia serrata (Sengupta et al., 2008). Even more importantly, the authors compared low and high doses of the extract and found that patients taking the higher dose had significantly reduced levels of the enzyme compared to patients taking the low dose (Sengupta et al., 2008). Madisch et al (Madisch et al., 2007) reported a significant difference in clinical remission in collagenous colitis patients taking Boswellia serrata. However, remission was based on self-reported stool frequency. Histological examinations showed no difference between groups after 6 weeks of therapy (Madisch et al., 2007).

There are a few explanations as to why the present study was not able to show a decrease in inflammatory markers. As mentioned earlier, the majority of patients in our study had comorbidities in addition to CKD, namely diabetes and hypertension. In

addition, 15, or 94% of our patients were also diagnosed with dyslipidemia. The co-occurrence of diabetes, hypertension, and dyslipidemia are also criteria for metabolic syndrome, and it is evident that most of our patients fell into this category. Every patient with dyslipidemia was taking lipid-lowering medication, and as mentioned earlier, a majority of patients were prescribed a statin drug. Statins, or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, have become the standard of care for the treatment of atherosclerosis. The use of statins are backed up by a number of large, clinical trials that have not only demonstrated their use in the prevention of coronary artery disease, but also in reductions in mortality (Randomized trial of cholesterol lowering in 4444 patients with coronary heart disease: The scandinavian simvastatin survival study (4S).1994; Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. the long-term intervention with pravastatin in ischaemic disease (LIPID) study group.1998; Heart Protection Study Collaborative Group, 2002). Statins have even been reported to decrease acute coronary events in individuals with average cholesterol levels (Downs et al., 1998; Sacks et al., 1996; Sever et al., 2003). Mechanistically, statins block the enzyme HMG-CoA reductase, which catalyzes the rate-limiting step in cholesterol production in the liver. The result is reduced cholesterol synthesis and release of lipoproteins from the liver, as well as a decrease in the LDL receptor and subsequent removal of lipoproteins comprised of apolipoprotein E and B (Stein, Devaraj, Balis, Adams-Huet, & Jialal, 2001). However, the benefits of statins go far beyond their lipid-lowering benefits, affecting other cellular pathways, especially inflammatory processes. They are able to reduce levels of inflammatory cytokines, chemokines, adhesion

molecules, and C-reactive protein. It appears that the ability of statins to effectively reduce lipids could explain their anti-inflammatory effects, especially since LDL-c is so toxic, contributing to the inflammatory process inside the endothelial lining (Pentikainen, Oorni, Ala-Korpela, & Kovanen, 2000; Yla-Herttuala, 1999). However, research is ongoing to determine if the anti-inflammatory effects of statins are independent of their lipid-lowering abilities. Statin use is widespread in CKD patients because they are at increased risk for cardiovascular disease (Patient mortality and survival. United States Renal Data System.1998; Tonelli et al., 2001), but few studies in non-dialysis CKD patients have determined their role in the inflammation process. The studies that have been published show promising results both from in vivo studies in humans and in vitro studies in cell culture, specifically in regards to inflammatory cytokines and the inflammatory molecule C-reactive protein (Goicoechea et al., 2006; Mantuano et al., 2007; Panichi et al., 2006). Goicoechea et al (Goicoechea et al., 2006) found that atorvastatin significantly decreased levels of C-reactive protein, TNF-α, and IL-1β with no changes in IL-6, while Panichi et al (Panichi et al., 2006) found that simvastatin significantly reduced levels of C-reactive protein and IL-6. It is unknown why the disparities exist between these two studies in regards to IL-6. Perhaps, the type of statin plays a role as to which inflammatory markers are affected. In line with previous research in non-dialysis patients (Dervisoglu et al., 2008; Oner-Iyidogan et al., 2009; Rastmanesh et al., 2008), these two studies utilized CKD patients with much lower levels of inflammation compared to what was observed in our patients (Goicoechea et al., 2006; Panichi et al., 2006). This is another reminder of the number of comorbidities that we encountered in this CKD population. Since our patients had been prescribed statins prior

to participating in our study, there is no way to know what inflammation levels would have been had they not been on this medication. Therefore, it is possible that inflammation levels would have been higher if the patients in our study were not taking statins. The duration of these two studies is also worth noting, since these studies were carried out for six months (Goicoechea et al., 2006; Panichi et al., 2006), indicating that effects from these compounds may take longer than 8 weeks to show results. Finally, a majority of our patients were taking aspirin or other non-steroidal anti-inflammatory drugs (NSAIDS), most likely for the prevention of coronary events. Since NSAIDS operate in the same manner as curcumin, by blocking the cyclo-oxygenase enzymes and subsequently prostaglandin synthesis, the use of aspirin may have inhibited curcumin's effectiveness.

Curcumin Supplementation and Antioxidant Activity

In addition to curcumin's anti-inflammatory properties, it also possesses antioxidant activity (Hatcher et al., 2008). There is evidence to show that curcumin is capable of increasing antioxidant activity, specifically levels of GPx, in animal studies (Bayrak et al., 2008; El-Agamy, 2010; Naik, Thakare, & Patil, 2010). Even more so, animal models of renal failure and nephrotoxicity show curcumin is able to attenuate the reduction seen in GPx (Bayrak et al., 2008; Cekmen et al., 2009; Farombi & Ekor, 2006; Venkatesan, Punithavathi, & Arumugam, 2000). Since it has been consistently reported that plasma GPx concentrations in CKD patients are below normal levels, even in non-dialyzed patients, it was a secondary purpose of our study to determine if curcumin had the ability to affect antioxidant activity in our patient population. While results from the present study do not statistically coincide with previous animal research, it may show a

trend toward statistical significance, that curcumin could potentially increase plasma levels of GPx in mild to moderate stages of GPx. Our results indicate that curcumin increased levels of plasma GPx to a greater extent when compared to placebo (CB = +9.9 nmol/min/mL vs P = +3.2 nmol/min.mL), albeit non-significantly. One limitation to our study is the small sample size (N = 16), and the possibility of it being underpowered to detect significant differences. In addition, the present study was only carried out for 8 weeks while effects regarding curcumin's antioxidant properties may take longer to appear. This warrants further research into the use of curcumin as an antioxidant in populations with decreased levels of endogenous antioxidant activity, specifically GPx.

In addition to curcumin, other antioxidant compounds have been investigated to determine their role in oxidative stress in CKD. L-carnitine (Sener, Paskaloglu, Satiroglu et al., 2004) and melatonin (Sener, Paskaloglu, Toklu et al., 2004) have been shown to ameliorate the reductions in GPx observed in rat models of chronic renal failure, but the majority of research in human trials has been devoted to selenium (Bellisola, Perona, Galassini, Moschini, & Guidi, 1993; Saint-Georges et al., 1989; Zachara et al., 2000; Zachara et al., 2004; Zachara et al., 2009). Premier studies on GPx concentrations in renal patients initially reported that reduced levels of this enzyme were attributed to selenium deficiency (Richard et al., 1991; Saint-Georges et al., 1989). Selenium is attached to GPx, aiding in its role as a free radical scavenger, catalyzing the reduction of hydrogen peroxide and other hydroperoxides to alcohol and water (Burk & Hill, 2005). While conflicting reports exist on selenium levels in CKD patients, a majority of the studies indicate that levels are reduced compared to healthy controls (Ceballos-Picot et al., 1996; Dworkin, Weseley, Rosenthal, Schwartz, & Weiss, 1987; Foote, Hinks, &

Lloyd, 1987; Girelli et al., 1993; Richard et al., 1991; Yoshimura et al., 1996; Zachara et al., 2000; Zachara et al., 2001). Yet, unlike plasma GPx levels, selenium deficiency is not present to the same extent in CKD patients, with levels in non-dialyzed patients in all stages of the disease (stage 1 to ESRD) reportedly anywhere from 12.5% to 44.1% lower than healthy control subjects (Ceballos-Picot et al., 1996; Girelli et al., 1993; Richard et al., 1991; Zachara et al., 2000). In addition, selenium levels do not appear to decrease in a linear fashion with increases in severity of the disease, as do levels of plasma GPx. There are conflicting results on whether selenium supplementation increases GPx levels in CKD patients on hemodialysis, although a majority of the data appears to show that it does not affect the enzyme (Saint-Georges et al., 1989; Zachara et al., 2000; Zachara et al., 2004; Zachara et al., 2009). One study did report increases in GPx from selenium supplementation in hemodialysis patients, but these levels still remained below values in the control group (Saint-Georges et al., 1989). However, selenium supplementation may show some benefit for individuals in more moderate stages of the disease (Zachara et al., 2004). Zachara et al (Zachara et al., 2004) found that 200 μg of selenium supplementation for three months significantly increased plasma GPx levels in CKD patients. While this study also included patients on dialysis, the authors concluded that GPx levels increased only in the patients who were in the early stages of the disease. In the patients with more compromised kidney function, there were only slight increases in the enzyme that statistically did not differ to their baseline values. These studies support the finding that reduced plasma GPx levels in CKD patients are more likely due to a lack of normal kidney function, specifically decreased nephron mass, and the fact that the

kidney is the main site for production of this enzyme (Avissar et al., 1994; Ceballos-Picot et al., 1996).

It appears that there is much research to be done in the area of antioxidant supplementation in CKD patients, especially those who have mild and moderate forms of the disease. Although statistically significant findings were not found in the present study, a greater sample size as well as a longer duration of supplementation may show clinical benefit to this population in the future.

Godin Leisure-Time Exercise Questionnaire

Physical activity was measured using the Godin Leisure-Time exercise questionnaire. This self-reported questionnaire asks subjects to report the average number of times per week they engage in various forms of exercise for more than 15 minutes during their free time. Exercise is divided into three categories: strenuous (heart beats rapidly), moderate (not exhausting), and mild (minimal effort). Results from this study indicate that our population of CKD patients performed little to no exercise on most days of the week, likely due to their multiple comorbidities. In addition, the amount of activity performed did not change during the course of this study. Therefore, physical activity did not play a role in the inflammatory and antioxidant variables measured.

Bioavailability and Dosing

Currently, there is no known optimal dose of curcumin in humans. Since testing is still in its infancy stages, it is likely that more studies will be assessing this in the near future. Curcumin is quickly metabolized, poorly absorbed, and has limited bioavailability (Cheng et al., 2001; C. Ireson et al., 2001; Sharma et al., 2004). In a phase

I trial of cancer patients, patients were given up 8g/day for three months, with no observed side effects. However, the maximum dose only yielded a serum concentration of 1.77 μM (Cheng et al., 2001). In another study, in which cancer patients were given up to 3.6 g of curcumin daily for four months, only half of the patients taking the high dose had detectable levels in plasma. Curcumin was not measurable in the plasma of patients on lower doses (Sharma et al., 2004). Because of its poor bioavailability, scientists are looking into combining curcumin with other substances that may increase it. One such compound is the alkaloid piperine, which comes from black pepper and long pepper (Shoba et al., 1998).

Boswellia serrata appears to have the same problem as curcumin of poor bioavailability. The Boswellia extract used in this study, Boswellia serrata or 3-acetyl-11-keto-beta-Boswellic acid (AKBA), is reportedly the most potent of all the pentacyclic triterpenes. Kruger et al (Kruger et al., 2008) were not able to detect any metabolites in vivo of the Boswellia acid AKBA, which is customary of the metabolism of many of the Boswellic acids. The authors noted that the acetyl group at position 3 was the reason for the limited metabolism. Similar to curcumin, AKBA had low systemic availability, and to the authors' surprise it was not due to hepatic metabolism, but rather may be due to its lack of absorption. Overall, it seems that more research is also needed in the metabolism of Boswellic acids, particularly Boswellia serrata or AKBA. In terms of dosing, only one review suggested taking 300-400 mg of a standardized extract containing 60% Boswellic acids, three times daily (Boswellia serrata.2008). However, it is unknown what this dose was based upon.

In the present study, curcumin and Boswellia serrata were taken together as a combination supplement. This was the first study to use these two compounds together exclusively. The present study was carried out for 8 weeks and taken at a daily dose corresponding to the manufacturer's instructions as follows: curcumin/Boswellia serrata composed of 824 mg purified turmeric extract, 95% curcuminoids and 516 mg Boswellia serrata extract, 10% 3-acetyl-11-keto-beta-Boswellic acid (AKBA). If the authors (Boswellia serrata.2008) who suggested that a dose of 300-400 mg three times daily indeed are correct, then our percentage of Boswellic acids would have been approximately 50% of the recommendation. Overall, the use of these herbal compounds is still relatively new, especially in CKD. Future research should focus on better methods of bioavailability and perhaps longer duration of supplementation.

Conclusion

Results from the current study indicate that 8 weeks of curcumin and Boswellia serrata supplementation do not significantly affect the plasma inflammatory markers IL-6 and TNF-α, as well as the antioxidant marker GPx, in CKD patients in stages 2 and 3. However, there were several limitations that were encountered. Although our patients had mild and moderate forms of kidney disease, they had other significant comorbidities, namely diabetes and hypertension. In addition, most if not all of our patients likely had metabolic syndrome. Any one of these factors would increase the risk for cardiovascular disease, but the accumulation of these factors makes the risk that much more apparent. In other words, this was a very sick, diseased population, and because of their comorbidities, they were taking many medications. Of these medications, most were prescribed at least one drug with anti-inflammatory properties. This may be one of the

reasons we did not observe any effects from the supplementation protocol. In addition, it appears that taking curcumin and Boswellia serrata in combination do not enhance the effects of these other anti-inflammatory medications, at least when consumed for 8 weeks. With a possible trend seen in plasma levels of GPx, future research should examine the use of curcumin as an antioxidant in diseased populations with extensive oxidative stress, particularly in larger studies. Future research should also focus on the use of these and other potent herbs in a less diseased population, specifically in patients not taking other anti-inflammatory medications. Recently, curcumin's anti-inflammatory properties have been shown to be comparable to those of statins in patients with type 2 diabetes (Usharani, Mateen, Naidu, Raju, & Chandra, 2008). This is promising, as these herbal remedies have been shown to exhibit fewer side effects compared to their prescription counterparts. With low bioavailabilty reported in these two compounds, there is much room for improvement in terms of pharmacokinetics.

GLOSSARY

Antioxidants – Compounds that protect the body's cells against the effects of free radicals.

Boswellia species – A genus of trees with biologically active gum resins typically used in Indian ayurvedic medicine that exhibit anti-inflammatory and anti-cancer properties.

Boswellia serrata – The most potent herb of the Boswellia species.

Catalase – An enzyme that catalyzes the reaction in which hydrogen peroxide is decomposed to water and oxygen.

Chronic Kidney Disease (CKD) – A broad term that encompasses all conditions that damage the kidneys, decreasing their ability to function properly.

Chronic Renal Failure (CRF) – Another name for chronic kidney disease.

Creatinine – A breakdown product of creatine generated from muscle metabolism. An increase in creatinine in serum above normal values indicates poor clearance by the kidneys, and therefore kidney impairment.

Curcumin – The active ingredient in the dietary spice turmeric with a wide range of beneficial properties including anti-inflammatory, antioxidant, chemopreventive, and chemo-therapeutic activity, that has a long history of use in traditional medicines in China and India.

Cytokines – Small secreted proteins which mediate and regulate immunity, inflammation, and hematopoiesis that act by binding to specific membrane receptors, which signal the cell via second messengers to alter its behavior.

Diabetes Mellitus Type 2 – The most common type of diabetes in which either the body does not produce enough insulin or the body's cells are resistant to its effects.

End-Stage Renal Disease (ESRD) – The complete, or almost complete failure of the kidneys to function. Commonly referred to as stage 5 of CKD.

Free Radicals – Atoms or groups of atoms with an odd (unpaired) number of electrons that can be formed when oxygen interacts with certain molecules.

Glomerular Filtration Rate (GFR) – A test used to assess the function of the kidneys. Specifically, an estimate of how much blood passes through the glomeruli (filters in the kidney) each minute.

Glutathione Peroxidase (GPx) – An enzyme that catalyzes the reduction of hydroperoxides by reduced glutathione and functions to protect the cell from oxidative damage.

Inflammation – The process by which the body responds to injury or infection. While acute inflammation is a natural, adaptive part of the body's healing process, chronic inflammation is an important part of atherosclerosis, which can lead to cardiovascular disease.

Interleukin-6 (IL-6) – A type of cytokine made by leukocytes (white blood cells) and other cells in the body and is considered a marker of inflammation in many diseases.

Leukocytes – White blood cells whose function is to protect the body against microorganisms causing disease.

Macrophage – A type of white blood cell formed from differentiated monocytes that ingests foreign material, kills bacteria, and release substances that stimulate other cells of the immune system.

Monocyte – A type of white blood cell that helps remove dead or damaged tissues, destroys cancer cells, and regulates immunity against foreign substances.

Oxidative Stress – Occurs when the generation of reactive oxygen species in a system exceeds the system's ability to neutralize and eliminate them.

Reactive Oxygen Species (ROS) – A phrase used to describe a variety of molecules and free radicals derived from molecular oxygen.

Superoxide Dismutase – An enzyme that catalyzes the redox reaction in which the superoxide free radical (O2 -) is dismutated to form oxygen (O2) and hydrogen peroxide.

Tumor Necrosis Factor-α (TNF-α) – A pleiotropic inflammatory cytokine that is produced by several types of cells, but especially macrophages, which initiates a cascade of cytokines, increasing vascular permeability, thereby recruiting macrophages and neutrophils to the site of infection.

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