THE EFFECT OF SOY PROTEIN ON SERUM LIPIDS IN KIDNEY TRANSPLANT PATIENTS RECEIVING RAPAMYCIN

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To the Dean of Graduate Studies and Research:

I am submitting herewith a thesis written by Elizabeth Hanawalt entitled "The Effect of Soy Protein on Serum Lipids in Kidney Transplant Patients Receiving Rapamycin." I have examined this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Nutrition.

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ABSTRACT

The Effect of Soy Protein on Serum Lipids
in Kidney Transplant Patients Receiving Rapamycin

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Rapamycin, a recently approved immunosuppressant, is widely used in patients undergoing a kidney transplant. A considerable drawback to the use of rapamycin is that it can cause an elevation in serum levels of triglycerides (TG) and cholesterol. A study was carried out to determine if a liquid formula containing soy protein, as opposed to one with casein, would lower serum lipids in kidney transplant patients. In a cross-over design, 10 subjects were given either a soy formula or a casein formula for 2 months and then the other formula for 2 months, with a 2 month washout period. The type of formula had no effect on serum levels of cholesterol, high-density lipoprotein, low-density lipoprotein, or TG. Thus, soy protein was not effective in lowering serum lipids in this population.

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CHAPTER 1

INTRODUCTION

Rapamycin is an experimental drug being used today as an immune suppressant in renal transplant patients. It has increased graft survival. However, there is one major drawback: it elevates triglyceride (TG) and cholesterol levels in some patients, causing rapamycin-induced hyperlipidemia, especially in patients who were hyperlipidemic before transplant. Even though all of the mechanisms are not known, Kahan and Hong (2000) state that the probable mechanism is that rapamycin interferes with the clearance of lipids from the blood by inhibiting apolipoprotein mediated lipolysis of low-density lipoproteins and/or by the disruption of the signal transduction by insulin or insulin-like growth factors. Some patients receiving rapamycin have had their medication discontinued when TG would not stay below 800 mg/dl. This level puts patients at an increased risk of developing pancreatitis. Any safe treatment to lower these lipid levels would be beneficial. Soy protein may be one of these treatments. The effect of soy protein on serum lipids has been extensively studied in animals and humans. Anderson et al. (1995) did a meta analysis of 36 studies, in which investigators had a variety of soy products, differing amounts of soy protein, different criteria for participant selection, and a variety of protocols. These investigators concluded that the substitution of soy protein for animal protein resulted in a significant decrease in serum total cholesterol, low-density lipoprotein cholesterol

(LDLc), and TGs without significantly altering high-density lipoprotein cholesterol (HDLc). Studies have also been conducted in Japan, where soy protein is a significant part of the diet, on the lipid lowering effects of soy isolates. These studies have demonstrated that total cholesterol decreased with increasing dietary intakes of soy product. Grundy and Abrams (1983) also found similar results in the US when comparing soy protein with casein. In a feeding study in which human subjects received either a casein or soy based liquid formula in men. These researchers concluded that soy protein can exert a lipid-lowering effect.

Anthony, Clarkson and Williams (1998) reviewed the mechanisms of the lipid lowering effect of soy protein and suggested that there are several mechanisms of action. One mechanism is that there is an enhancement of bile acid excretion. In this state, hepatic cholesterol metabolism shifts to provide cholesterol for enhanced bile acid synthesis. The net result is the removal of cholesterol from the blood. (Serum cholesterol is lowered despite there being a hepatic increase of hydroxymethyl glutaryl coenzyme A reductase when soy protein is used instead of casein). Consumption of soy protein is associated with increases in removal of LDL and very low-density lipoproteins by hepatocytes. Soy estrogens may contribute 60-70% of the hypocholesterolemic effect of soy protein. This decrease in serum cholesterol is mainly due to a decrease in the LDLc fraction. Soy protein contains arginine, which may also contribute to the protein's lipid- lowering effect. There are many medications that contribute to or exacerbate hyperlipoproteinemia. These include

diuretics and beta-blockers which are used in the treatment of hypertension. Donahoo et al.(1998) reviewed these drugs as well as steroids and drugs used in transplantation. These authors found that the effect of steroids depends on the drug dose and route of administration. Since transplant patients are on a multi-pharmaceutical treatment regimen, the elevation of TGs in particular can cause the need for the use of more medication so as to lower serum lipids, making the pharmaceutical regimen more complicated and increasing the possibility of drug-drug adverse interactions.

Since hyperlipidemia is a risk factor for long-term graft survival in patients undergoing a renal transplant, control of hyperlipidemia in these patients is highly desirable. Most patients are on many medications, often more than ten. Hyperlipidemia is often treated with more medication, which could adversely affect liver function. Often patients are given fish oil as a lipid lowering agent. However this treatment is not always well accepted thus compliance will be questionable, especially in large doses. Soy protein has become more widely used in the U.S. as more soy alternatives become available at the grocery store. Many of these are reasonably priced. This raises the possibility of using soy protein to control the severity of rapamycin-induced hyperlipidemia.

The purpose of this study is to determine whether soy protein will decrease serum lipid levels in renal transplant patients receiving rapamycin by giving patients an enteral product containing either soy protein or casein in a crossover study.

STATEMENT OF THE PROBLEM

Rapamycin-induced hyperlipidemia is a significant problem for transplant patients. Soy protein may be useful in ameliorating this condition.

In order to assess the effectiveness of soy protein in reducing cholesterol, TG, and LDLc in patients on the drug rapamycin, an enteral product containing 20 g soy protein per serving as well as an enteral product with 20 g casein was be provided daily to study subjects in a cross-over design.

NULL HYPOTHESIS

There will be no statistically significant difference in serum levels of cholesterol, TG, or LDLc when patients are given an enteral product containing either soy protein or casein.

CHAPTER II

REVIEW OF LITERATURE

Transplant Mortality

Renal transplants have become a viable option for people who are facing end stage renal disease. The alternative treatment for this stage of renal disease is either hemodialysis or peritoneal dialysis. Many people can not or will not tolerate dialysis. There are many diseases that result in renal failure. These include, but are not limited to, diabetes mellitus, polycystic kidney disease, high blood pressure and systemic lupus. There is concern over the high rates of morbidity and mortality of renal transplant patients from coronary heart disease (CHD) and cardiovascular disease (CVD). Raised serum cholesterol levels due to increased LDLc is the pattern most often observed in 60% of these patients. The cause is multifactorial, including age, pre-transplant hyperlipidemia, medications, and renal dysfunction. Forty percent of deaths post-transplant are attributable to cardiovascular disease in patients having a renal transplant. Increased levels of plasma total cholesterol (TC) and LDLc are primary risk factors for CHD/CVD (Bittar et al., 1990). It is possible that the pathogenesis of CVD is different in renal transplant recipients from that in the general population. If, for example, an infectious agent played a more important role in the pathogenesis of CVD in renal transplant patients, then standard risk factor modification may be less effective. The incidence of ischemic heart disease among all renal transplant recipients is 15.1% (Kasiske, 2000). Barbagallo et al. (1999) examined the effect of renal transplant on

carotid atherosclerosis. Fifty-seven renal transplant recipients and one hundred and thirteen non-transplant volunteers were enrolled in the study. Plaque formation and carotid lesions were more prominent in the transplant recipients than in the controls. The researchers concluded that carotid examination could be helpful in selecting transplant recipients at a higher risk of cardiovascular disease.

Heart Healthy Diet

At time of transplant, all patients are instructed on a low-fat (40g) heart healthy diet. Hines (2000) examined whether diet education has any impact on patient's serum lipid levels post-transplant. This author carried out a study that was performed between September 1994 to September 1997. The diet used was the Healthy Heart Nutrition Guidelines Step 1, which allows <30% of total calories form fat, <300mg cholesterol per day, and <10% of total calories from saturated fatty acids. Fasting/random serum total cholesterol, HDLc and LDLc were the values that were determined. The author concluded that serum lipids of renal transplant patients can be significantly decreased by a low-fat diet. The patient's dose of cyclosporine (CyA) and prednisone did not change significantly during the study. However, many patients still needed cholesterol-lowering medication to achieve desired cholesterol levels. Others have also studied the effect of a low fat diet on serum lipids in patients with a renal transplant. Lawrence et al. (1995) conducted a study on 38 British renal transplant patients for 12 months. The patients randomized into the experimental group were put on the equivalent of the Heart Nutrition Guidelines Step I diet, and the patients in the control group received no specific dietary

restrictions. Unlike Hines, Lawrence et al. found that the low fat diet had no significant effect on TC, HDLc or TG levels. The only decrease in LDLc in the experimental group occurred during the first month, thereafter LDLc values increased. Another study performed by Tonstad et al. (1995) enrolled 26 post-transplant recipients for a period of 12 weeks. Dietary goals for these patients were also to conform to the American Heart Association Step I diet. During the study period, no patients were treated for rejection and medications were not altered. After controlling for other variables, creatinine level was a significant determinant of mean LDLc levels and TG levels. The results of this study were not unlike Lawrence et al., i.e. the Step I diet failed to lower TC or LDLc levels. TG was only lowered in non-obese patients.

Rapamycin Pharmacokinetics

Rapamycin is a newly approved drug being used today as an immune suppressor in renal transplant patients. It was first used as an antifungal or antimicrobial agent in the mid-1970s. The liver metabolizes this drug in the cytochrome P450 system, producing more than 10 metabolites. Some of these metabolites have low immunosuppressive activity *in vitro*. The drug, which is more than 95% bound to red blood cells is widely distributed in the body. Rapamycin may exert its activity by prolonging the cell cycle, which selectively inhibits the synthesis of ribosomal proteins. This in turn inhibits the induction of mRNA for new ribosomal proteins. One characteristic feature of this drug is that it inhibits growth factor signaling for fibroblasts, endothelial cells, hepatocytes, and smooth muscle cells. This renders rapamycin as a good compound for the prevention of

chronic rejection because the drug has decreased the rejection rate from average 50 grafts per year, down to 12 for 1998 at the University of Texas Medical Center in Houston Texas. Rapamycin does not cause nephrotoxicity, neurotoxicity, or diabetogenicity, which often occurs in patients on CyA or tacrolimus, two drugs commonly used for transplant patients. However, there is one major drawback: the drug elevates TG and cholesterol levels in some patients (Brattstrom, 1998), especially in patients who were hyperlipidemic before transplant. For these patients who are hyperlipidemic before transplant, rapamycin is of particular concern. Some patients whose lipids are normal before transplant, do not experience rapamycin-induced hyperlipidemia. Most renal transplant patients are not on a monotherapy drug regimen as CyA and prednisone are often in combination with rapamycin. For some patients, the therapy has had to be discontinued when serum triglycerides exceeded a level of 800mg/dl, a level that puts patients at an increased risk of developing pancreatitis.

Brattstrom et al. (1998) studied 26 renal transplant recipients participating in 3 clinical trials evaluating the effect of oral rapamycin on the occurrence of acute rejection episodes in renal transplantation. In this study, rapamycin in combination with CyA was evaluated as well as using rapamycin alone. The study protocol stated that if the triglycerides exceeded 9 mmol/L (800mg/dl), the dose of rapamycin was to be reduced. If the serum triglycerides exceeded 18mmol/L (1600mg/dl), the drug was discontinued. The researchers observed that the rapamycin dose in 7 of 12 patients with hypertriglyceridemia had to be reduced and the drug had to be discontinued in one

patient. In this study, it was demonstrated that increased levels of serum triglycerides were dosed-dependent on the dose of rapamycin given and that this side effect occurred in 75% of these patients. Hypertriglyceridemia is also a known side effect of CyA, and the incidence is reported to be 20% to 40% in renal transplant recipients. When these two drugs are in combination for immunosuppresant therapy, this further increases the risk of hypertriglyceridemia. According to Kahan (2000) "although the mechanism of this action is uncertain, it appears that RAPA interferes with lipid clearance from the blood by inhibiting apolipoprotein action of lipolysis of low-density lipoproteins, and /or by disrupting signal transduction by insulin or insulin-like growth factors, thereby retarding the uptake of fatty acids." Rapamycin inhibits lipoprotein lipase which is insulin dependent and decreased activity may cause decreased catabolism of VLDL and chylomicrons resulting in hypertriglyceridemia. Rapamycin inhibits the release of lipoprotein lipase (LPL) from 3T3-L1 adipocytes from the microvessel endothelial cells. Kahan states that rapamycin prevents the release of the insulin-responsive factor from the endothelial cell, which is responsible for the LPL release from the adipocyte. If released, LPL is responsible for the hydrolysis of triglyceride from very low-density lipoproteins (VLDLs) and chylomicrons in serum , thereby promoting triglyceride breakdown and the subsequent tissue uptake of released fatty acids. Drugs normally used in transplant patients receiving rapamycin are atorvastatin and pravastatin. Atorvastatin is used when only cholesterol level is elevated, but there is no hypertriglyceridemia. Other drugs used to decrease elevated serum TG with some success includes gemfibrozil, simvastatin,

pravastatin, fluvastatin; fish oil has also shown to be effective. Unfortunately, the drugs can cause elevated levels of transaminases (indicating hepatic damage) and not all patients tolerate fish oil very well. These medications are HMG-CoA reductase inhibitors which lowers LDLc by reducing its synthesis and by promoting its degradation via up-regulation of LDL receptors.

Immunosuppressive Therapy

Quaschning et al.(1999) examined the effect of immunosuppressive therapy on hyperlipidemia in renal transplant patients. Two hundred and sixteen transplant recipients were enrolled in the study (81 female and 135 male). Total serum TG levels were elevated in all recipients, especially the females. TG-rich VLDL was pronounced in this population. Serum cholesterol was also found to be significantly increased in the female subjects. The drugs used by these recipients were prednisone, CyA, and azathioprine. The most significant increase in TGs was found in the recipients on triple therapy. Recipients whose CyA level were higher than 120 ng/ml, also had elevated serum TG levels. These researchers determined that post-transplant dyslipidemia is qualitatively and quantitatively dependent on gender, type of immunosuppressive drug used, and drug dose. Banyai-Faler et al.(1997) examined post transplant changes in the serum lipid levels of 76 patients, all of whom were on CyA drug therapy, with dosages adjusted to maintain blood trough levels of 80-120ng/ml. Some patients were also on prednisone. These patients were on the study for 18 months. During the study period, LDLc levels increased the entire time. The number of patients with increased LDLc

values (>142mg/dl) increased from 32% to 50%. All subjects had low HDLc (48 mg/dl) levels prior to transplant. The researchers concluded that 60% of renal transplant patients are affected by hyperlipidemia 18 months after transplantation. Kuster et al. (1994) also examined lipid levels in patients post-transplant. Thirty-five renal transplant recipients were included in the study. TGs were correlated with the CyA blood levels. The recipients' HDLc were significantly decreased by the end of the study. In conclusion these researchers stated that there is a positive correlation between absorptive efficacy for CyA and plasma TG and cholesterol. Schena et al. (2000) investigated whether renal transplantation would worsen, by itself, the atherogenic risk of uremic patients. Fifty renal transplant patients were recruited. All patients were taking triple immunosuppressive treatment, which included CyA, prednisone and azathioprine. The researchers found that after transplant, values for HDLc increased by 35.8% (from 38.8mg/dl to 52.7mg/dl) and LDLc by 21.1% (from 113mg/dl to 138mg/dl). TG levels did not change significantly. This increase in HDLc resulted in a lower LDL/HDL ratio in 68% of patients. These researchers concluded that immunosuppressive regimens in themselves do not increase the atherogenic risk related to lipoproteins in transplant patients significantly. They did find that genetic predisposition, diabetes mellitus, obesity, renal dysfunction with or without proteinuria or additional medications and infections significantly increase the lipid profile.

Drug induced hypertriglyceridemia was also examined by Radcliffe et al.(1997), using an animal model of this condition. The purpose of the study was to examine the

effect of partial replacement of casein with soy protein in rats, which were given diets having 13-cis retinoic acid, a drug known for increasing serum triglycerides in human subjects. The rats were separated into five groups, each on a different diet. One group (A) was fed a retinoid-free diet having casein as the sole source of protein. Group B was fed a 13-cis retinoic acid-containing diet with casein as the protein source. Groups C-E were fed 13-cis retinoic-acid containing diets in which soy protein isolate (which was isonitrogenous with casein) replaced 25, 50 and 100% respectively the formula for diet B. The rats were fed these diets for 14 days and then were anesthetized. Determination of serum cholesterol, TG was done. Rats fed diet B had the highest serum triglyceride level (5.41mmol/l, 209 mg/dl). This was significantly ($P<0.05$) higher than for group A (2.62mmol/l, 101mg/dl). Values for groups C-E, were 4.90 mmol/l,190 mg/dl; 4.04 mmol/l,156 mg/dl and 2.66 mmol/l,103 mg/dl respectively. The values for groups D and E were significantly ($P<0.05$) lower than that for group B. Not surprisingly, rats that were fed diet B had the most significant rise in triglycerides. The diet that had the most significant decrease in serum triglycerides was diet E, which had soy protein isolates as its sole protein. This study demonstrated that replacement of either 50 or 100% of casein with soy protein isolate significantly reduced the severity of retinoid-induced hypertriglyceridemia. This suggests that soy protein isolate may be used to control drug-induced hyperlipidemia in human subjects. There are many drugs that contribute to dyslipoproteinemia, as pointed out by Donahoo et al. (1998). These authors point out that many drugs used for hypertension such as diuretics, beta-blockers, alpha-blockers,

and ACE (angiotensin converting enzyme) inhibitors. They also reviewed steroid hormones and transplantation therapies, pointing out that while diuretics and beta-blockers adversely affect serum lipids, the peripherally acting alpha-blocking agents are lipid neutral. The effects of steroids that are used in transplantation were found to vary with the drug, dose and route of administration. The effect of medications on lipids is heightened when CyA is coupled with prednisone. Patients who are on this type of drug regimen often get placed on gemfibrozil, simvastatin, pravastatin or fluvastatin. Capone et al. (1999) conducted a study to see if these drugs affected serum lipid and CyA levels in renal transplant patients. This study was carried out on 31 renal transplant patients. All patients had high serum cholesterol of 200-500mg/dl and hypertriglyceridemia (>200mg/dl). Simvastatin reduced cholesterol 31.7% and TG 24.55%, and pravastatin reduced cholesterol 30.6% and TG 22.6% at the end of nine months. Neither drug affected either polyclonal or monoclonal CyA trough levels significantly. Evidently, 27% of plasma CyA is bound to lipoproteins, owing to the high lipid solubility. "If free drug is responsible for efficacy and/or toxicity of CyA, changes in lipoproteins may be clinically significant." The effect of gemfibrozil on CyA levels was investigated by Pisanti et al. (1998). Forty renal transplant patients were enrolled. The results showed that gemfibrozil did not alter CyA blood levels. In this study, gemfibrozil decreased TG by 38-48%. Fernandez-Miranda et al. (1998) conducted a comparative study examining lipoprotein abnormalities in liver and renal transplant patients. They concluded that the dose of CyA was found to be correlated with hyperlipidemia, especially with the LDLc

level. These authors also concluded that prednisone dose and kidney grafts were positively associated with increased post-transplant cholesterol levels. Vathsala et al. (1989) examined the effect of CyA/prednisone on lipid profiles. The study enrolled 356 patients who had renal transplants. There were three drug regimens, 1) Azathiopine(Aza)+Pred, 2) CyA+Pred and 3) CyA. The study examined these patients for a period of 36 months. Baseline values were determined for TG, cholesterol, blood urea nitrogen (BUN), creatinine, urine protein, and liver function tests. By the end of the sixth month, lipid abnormalities occurred in 70% of the patients in the first two drug regimens. The lipid abnormalities that occurred significantly were type IV lipoproteinemia in 60% of patients and type IIb/III in 31% of hyperlipidemic patients. Female patients displayed significant increases in cholesterol, but not TG. The researchers did find that hyperlipidemia did effect renal function. Serum creatinine values were increased in patients with hyperlipidemia as opposed to normolipidemic patients in the CyA/pred group. These researchers concluded that hyperlipidemia can be attributed to steroid-induced increases in hepatic cholesterol synthesis. There was a correlation between steroid dose and cholesterol level.

Lovastatin was examined by Kandus et al. (1998) for thirty-six months on 12 renal transplant patients. These researchers found that lovastatin decreased cholesterol by 31% and TG by 21% but did not alter HDL levels. The significant decrease in lipids occurred within the first three months of the study. Serum creatinine did not change significantly in 10 patients. The dose of drug was 20 mg per day, at this dose level there

were no signs of damage to the skeletal muscles and liver in the majority of renal transplant patients on CyA immunosuppression. The patients were examined and serum lipids were determined every three months. Potential damage to the liver and muscle were examined by alanine aminotransferase , aspartate aminotransferase, gammaglutamyl transpeptidase, creatinine kinase and serum myoglobin.

Wierzbicki et al. (1999) reviewed evidence for the involvement of hyperlipidemia and other cardiovascular disease risk factors in renal, cardiac, pancreatic and liver graft survival. These authors stated that some immunosuppressant cocktails cause clotting disorders and abnormalities in immune function. Thus, atheroma is increasingly being recognized as a major complication of transplantation. Factors contributing to atheroma in this patient population are insulin resistance, small dense LDL, lipoprotein a metabolism, and homocysteine metabolism. The author stated that insulin resistance, which is common in post-transplant patients, causes a shift in lipoprotein profiles with the formation of small dense LDLc and other atherogenic lipid subfractions. Steroids just exacerbate this fact by up-regulating acetyl CoA synthase, fatty acid synthesis and HMG-CoA reductase activity. CyA also inhibits cholesterol-26-hydroxylase, impairing bile acid formation and down regulating LDL receptor expression. Hypercholesterolemia is also associated with increased graft loss in renal transplant patients.

Hypercholesterolemia may be one of several alloantigen-independent risk factors. In many renal transplant patients, a proliferative and fibrotic intimal thickening of the graft's arteries is the hallmark of chronic rejection, closely resembles atherosclerosis. In

a retrospective study, Wissing et al. (2000) examined the effects of hypercholesterolemia on renal graft function in men and women. There were 442 patients who were enrolled in the study. The immunosuppressive therapy was CyA and prednisone. Patients also received OKT3, a monoclonal antibody prophylaxis. During this time period it was concluded that hypercholesterolemia was associated with significantly higher proteinuria irrespective of previous rejection. Patients whose cholesterol was <250mg/dl, had a graft loss of 24.3% as opposed to 50% in patients whose cholesterol was >250mg/dl at the end of 10 years. Patients whose cholesterol was <250mg/dl at the end of a year with one acute rejection episode, had better long term and overall immunological graft survival than patients whose cholesterol was >250mg/dl, similar to patients who had no acute rejection episode at all. These authors stated that immune-mediated injury caused by acute rejection could be enhanced and perpetuated by hypercholesteroemia. However the finding that hypercholesterolemia was an independent risk factor for overall and immunological graft loss was only found for male patients. It was suggested that there may be a protective effect of estrogen against graft loss in female patients due to the estrogen.

Phytoestrogens and Soy

Phytoestrogens are naturally occurring compounds found in many foods. These plant substances are structurally and functionally similar to 17 *B*-estradiol or that produce estrogenic effects. Phytoestrogens consist of a number of classes, including lignans, isoflavones, coumestans and resorcylic acid lactones. Isoflavones are generally restricted

to legumes, with the highest concentration found in soybeans and soy products. The most studied isoflavone is genistein, which is an inhibitor of tyrosine kinases, DNA topoisomerases I and II as well as ribosomal S6 kinase. Kapiotis et al. (1997) studied genistein's effect on LDLc oxidation prevention and protection of endothelial cells from damage by atherogenic LDLc. In this study, genistein and genistin,(isoflavones found in soy products) and other isoflavone derivatives were examined. Genistein was added to bovine and human endothelial cell systems. Genistein was able to overcome the LDL-oxidizing effect of the cells. They also examined whether genistein had to be present during LDL oxidation or if pretreatment could also have the same effect. The researchers' conclusion was that genistein was no only able to inhibit LDL oxidation in cell-free lipoprotein-oxidating system, but also in a cell-mediated lipoprotein-oxidating system. Genistin did not show these same positive results.

Hodgson et al. (1996) also examined the oxidation of LDL and the protective affect of isoflavonoids. Six males and six females were enrolled in the study. Blood samples were taken from the participants and exposed to copper (Cu++), which was the oxidation method used on the blood samples. Genistein, daidzein, equol and O-DMA, (O-desmethylangolensin) which is a metabolic product of daidzein metabolism in humans, were examined for their antioxidative effect. Equol and O-DMA are daidzein metabolites. The daidzein metabolites were more potent inhibitors of oxidation than their parent compound. Equol was approximately ten times more potent as an antioxidant than daidzein. The researchers concluded that flavonoids can inhibit lipid peroxidation in

vitro by acting primarily as free radical scavengers or as metal-chelating agents. Anthony (1998) also studied genistein and suggests that genistein has antioxidant properties, which reduce the size of LDLc particles and also help protect LDLc from being peroxidized. Others that are less studied are daidzein, daidzin, glycitein, and glycitin. The isoflavonoids are glycosides of genistein and daidzein. When ingested, these isoflavonoids undergo acidic and enzymatic hydrolysis and demethylation to yield the aglycones genistein and daidzein. These are then absorbed by the gut flora.

Soy products come from soybeans, which are native to Eastern Asia and are the major source of protein for millions of people in Asia. Nagata et al. (1998) did a cross-sectional study in which the researchers examined the relationship between soy products and serum cholesterol concentration in a community in Japan. Among the participants in the study, 1242 men and 3596 women, who attended the annual heath check-up program provided by Takayama Municipality between April an October 1992, were selected as the subjects of this study. Frequency questionnaires were used to estimate the amount of different soy products consumed by each participant. Some of the soy products consumed were tofu, miso, deep-fried tofu and bean curd fermented soybeans, soy milk, boiled soybeans, total soy products and total soy protein. In both men and women, after adjusting for menopausal status, serum total cholesterol concentrations decreased with increasing amounts of soy product consumed. Triglyceride levels were not determined. Jenkins et al. (2000) conducted a study in which some casein in the diet was replaced by a soy food product, which was cereal. Twenty-five hyperlipidemic subjects completed a

two 3-week ad libitum diet separated by a 2-week washout period in a randomized crossover design. The subjects were provided boxes of breakfast cereal supplement at the start of each treatment period. On the control diet, they were also given a measured amount of soy oil to be consumed daily with food. This was to make up for the calorie difference between the test and the control period. The supplements were identical in total fat (9.6g/d) and energy content (test, 376 kcal/day; and control 378kcal/d). Approximately 70% of the cereal was made with soy flour. The purpose of this study was to assess the effect of soy-based breakfast cereal on serum lipids and oxidized LDL. The authors analyzed conjugated dienes in the LDL fraction as a marker of oxidized LDL cholesterol. Total conjugated dienes in the LDL fraction were significantly reduced on the test compared with the control (P= .042). Therefore, this study demonstrated a positive effect of soy in reducing indices of LDL oxidation. Oxidized LDL is considered more damaging than normalized LDLc because they are more readily taken up by the macrophages of the scavenger system in the arterial wall and so contribute to plaque formation.

Wiseman et al. (2000) also studied the effect of soy on reducing lipid peroxidation in vivo and increased resistance of LDL to oxidation. Twenty-four adults participated in a randomized controlled crossover study. The participants made no changes in their diet or lifestyle other than to eat one burger daily during both of the 17-day study periods. Both types of burgers contained 15 g protein, 9 g fat, and 10 g carbohydrate. The high isoflavone burgers (HI) contained 21.2 mg daidzein and 34.8 mg genistein, whereas the

low isoflavone burgers (LI) contained 0.9 mg daidzein and 1.0 mg genistein. The researchers found that the lag time for LDL oxidation was significantly greater after the HI treatment than after the LI treatment. Isolated LDL showed increased oxidation resistance after the HI treatment, even though there was no significant difference in resistance of plasma to oxidation between the HI and LI treatments. Researchers concluded that there may be protective effect of the isoflavonoids against plasma oxidation that was masked by the protective effects of water-soluble antioxidants, such as ascorbate and urate, that were present in plasma but not in the isolated LDL system. There was an increase in HDL concentration in the HI treatment over the LI treatment. (HDL is preferentially oxidized in vivo before those in LDL). HDL also has an antioxidative effect, which is probably mediated by the esterase paraoxonase when associated with HDL in plasma (Wiseman, 2000). According to Tikkanen (1998) "The oxidative modification of low density lipoprotein (LDL) particles is considered to be a prerequisite for the uptake of LDLc by macrophages in the artery wall, an initial step in the formation of atheroma. This author also states that this leads to a rearrangement of fatty acid double bonds which produces the characteristic 234 nm absorption of conjugated dienes. The purpose of this study was to examine the oxidative resistance of LDLc in the presence of phytoestrogens. Six volunteers were enrolled in the study, 3 men and 3 women. Blood was drawn during the last 2 days of the 2-week baseline period, the last 2 days of the 2-week soy feeding period and the 12 days after discontinuation of soy intake. The volunteers were instructed to take 3 soy bars,

containing genistein (12 mg) and daidzein (7 mg), daily for 2 weeks. The researchers found no difference in the levels of cholesterol, TG, phospholipid, or protein in LDLc or in other lipoprotein fractions or whole plasma in any of the volunteers. However, after 2 weeks of soy supplementation, there was a significant prolongation of lag time of oxidation of LDLc in six individuals.

The consumption of soy protein (phytoestrogens), found in cereals, vegetables and medicinal plants seems to be highest in Japanese populations with levels up to 200 mg of phytoestrogens per day in the diet. This is significant considering that the average consumption of soy protein in Western countries is less than 5mg per day. Knight and Eden (1995). Yusuke et al. (2000) examined dietary intakes of Japanese women and the effect that this might have on plasma LDLc concentration. One-hundred and fifteen female subjects were enrolled. Each participant completed a 3-day dietary record during the study period. Total protein, albumin, total cholesterol, TG, HDLc, uric acid, creatinine, and hepatic indicators were determined. In examining the dietary diaries of the participants, the intake of flavonoids, which is one component that has a potentially protective effect, was attributable to vegetables (72.3%), fruits (15.6%), green tea (5.4%), potatoes (3.8%) and tofu (2.9%). Genistein made up most of the isoflavone intake (30.5 mg/d) and daidzein intake 16.6 mg/d).

Despite these large number of studies showing a beneficial effect of soy protein and isoflavones on serum lipids there are studies that do not show any beneficial effect of soy protein on lipids, there are studies that do not show any beneficial effect on lipids.

Simons et al. (2000) conducted a study on 20 postmenopausal women who had evidence of endothelial dysfunction. They were treated with 80 mg/day of isoflavones. Each participant took 2 soy tablets or 2 placebo tablets daily, with a washout period in between. Serum lipids did not change significantly between the periods; TC 5.52 mmol/l (489 mg/dl) placebo, 5.45 mmol/l (482 mg/dl) isoflavone; LDLc 3.69 mmol/l (142 mg/dl) placebo, 3.61 mmol/l (139 mg/dl) isoflavone; HDLc 1.34 mmol/l (52 mg/dl) placebo, 1.12 mmol/l (43 mg/dl) isoflavone; and TG 1.07 mmol/l (95 mg/dl) placebo, 1.12 mmol/l (99 mg/dl) isoflavone (Simmons, 2000).

Another feasible way of getting more soy in the diet is to take 20 g of soy protein once a day, in the form of commercially available soy drinks now on the market e.g., Revival Soy. The effect of soy protein on serum lipids in humans has been extensively studied. As mentioned previously, Anderson et al. (1995) did a meta analysis of 36 studies, in which a wide variety of soy products had been used. Also differing amounts of soy protein, as well as different criteria for participant selection, had been used and a variety of protocols. Despite these differences in experimental design, these authors noted that the substitution of soy protein for animal protein, in the form of casein, resulted in significantly decreased levels of serum total cholesterol, LDLc, and TG without significantly altering the level of HDLc. A metabolic study by Grundy and Abrams (1983) found similar results when these authors enrolled fourteen men for a month. The energy composition of the diet was 15% protein, 55% carbohydrate and 30% lard, with the diets being given in liquid form. There were two periods, one when casein

was used to provide dietary protein and the other when soy protein was used. These authors also found that in patients who were hypertriglyceridemic, the HDLc concentration with soy protein was unaffected but that the TG concentration was reduced significantly. Decreases in TG levels were not significant in normotriglyceridemic men. Also, their HDLc concentrations were unaffected. A study by Wong et al. (1998) examined two groups of men on the Step II American Heart Association cardiovascular diet, one with soy protein added to it and the other with casein added to it in liquid supplement form. The composition of the Step II diet is >30%fat kcals and intake of cholesterol under 200 mg per day. Researchers found that the ratio of LDLc to HDLc in both normocholesterolemic and hypercholesterolemic men were significantly lower in the soy protein phase of the study then when the participants were in the casein phase of the study.

There is some disagreement as to whether soy isoflavones are the active aniatherosclerotic agent or that soy peptide acting alone is the aniatherosclerotic agent Anthony et al. (1998). These authors conducted a study with cynomolgus monkeys. The purpose of this study was to examine the contributions of the soy protein amino acids and the soy isoflavones in reference to athrosclerosis. Three diets were used: 1) casein and lactalbumin, 2) soy protein isolate alone and 3) soy protein with isoflavones intact. The diet that resulted in the lowest levels of serum cholesterol was diet 3. LDLc and VLDLc fractions were highest in the #3 diet. Animals fed this diet had higher HDLc fractions than fed the other two diets. Teixeira et al. (2000) examined the effects of different levels

of soy protein on moderately hypercholesterolemic men. Eighty-one men were recruited for a 6 weeks study. Their cholesterol levels were between 220mg/dl and 297mg/dl. The four levels of soy: casein supplements were 50:0, 40:10, 30:20, 20:30 and 0:50. Blood was collected at baseline, 3 weeks and at 6 weeks for TC, HDL, non-HDL cholesterol (VLDLc + LDLc) and TG. At week 3, the diets having the two highest levels of soy 40 and 50g showed the largest decrease in non-HDLc concentrations. Reductions in TC concentration were significant at week 6 for the groups of 20, 30 and 50g. They concluded that even the lowest amount of soy has an effect of decreasing cholesterol over long term in moderately hypercholesterolemic men.

Female Subjects on Soy

Studies have also been conducted on just women, especially in the area of soy affecting menopause symptoms. Merz-Demlow et al. (2000) also studied the effect of soy isoflavones on plasma lipids in premenopausal women. Thirteen women were enrolled for approximately an eleven month study. Soy protein was provided in three isoflavone doses (control: 10.0 ± 1.1; low: 64.7 ± 9.4; and high: 128.7 ± 15.7 mg/d). Each subject consumed 3 soy protein intakes each for 3 menstrual cycles with a 2-3 week washout period between diets. The 3 soy protein diets were control, low isoflavone and high isoflavone. This was a randomized crossover study. This study showed that an intake of 129 mg isoflavones/day significantly lowered LDL cholesterol by 8-10% during the menopause stage. Unlike Merz-Demlow et al., Nestel et al, (1997) concluded that isoflavones failed to decrease serum lipids in women who were menopausal or

perimenopausal. This study was conducted for a period of 8 weeks. The investigators used a pure preparation of isoflavones and studied the effect on plasma lipid concentrations and other cardiovascular biomarkers. Each participant took 80 mg of isoflavone in the form of a pill daily. The researchers concluded that serum lipids were not changed significantly by isoflavones and the oxidizabilitiy of the LDLc was unchanged.

Soy and Cynomolgus Monkeys

Greaves et al. (1999) studied cynomolgus monkeys. The purpose of this study was to examine the effect of soy on intestinal cholesterol absorption and bile acid excretion in ovariectomized cynomolgus monkeys consuming a moderately high-fat and moderately high-cholesterol diet containing either 1) casein-lactalbumin as the protein source, 2) soy as the protein source 3) casein-lactalbumin as the protein source with the addition of a semipurified soy extract or 4) casein-lactalbumin as the protein source with the addition of conjugated equine estrogens. Adult female cynomolgus monkeys were fed treatment diets for 5 months. At the week 20, blood analysis showed that the soy group had lower plasma cholesterol and LDLc concentration compared to the three casein-lactalbumin protein groups. This may be because the soy group also had significantly lower percentage dietary cholesterol absorption compared to the other diets. The soy group also had higher HDLc concentrations than the other groups.

CHAPTER III

SUBJECTS AND METHODS

The study took 24 weeks. A total of ten subjects were randomly assigned to a casein-containing liquid formula and a soy liquid formula, both of which were provided by Rivival Soy, Winston-Salem, NC. Composition of the soy supplement is given in Table 1. Ten subjects completed the study. The participants were instructed to take the drink 2 hours before or after they took rapamycin, to lessen the chance of interaction. For the first 2 months, one group, group A (n=5), received 20 g of soy protein, which included 116 mg isoflavones in liquid form once a day, and the other group, group B (n=5), received the control product, which contained 20 g casein. During the next two months, which was the "wash-out" period, neither group was given these products. Next, the participants in the two groups had their treatment switched, so that group B got the soy protein formula and group A got the casein formula, (Appendix A). Blood samples were drawn monthly and used to determine serum lipid levels. Subjects gave written informed consent for participation in research as approved by the Human Subjects Review Board of Hermann Memorial Hospital and also from the Texas Woman's University Human Subjects Committee (Appendix B).

Subjects

Ten patients were originally enrolled from the kidney transplant clinic at Hermann Memorial Hospital in Houston Texas (seven males, three females). The study

Table 1

Composition of Soy Supplement

Composition of Chocolate Fructose Flavor	Value
Protein (g)	20
Fat (g)	2.7
Ash (g)	3.5
Carbohydrate (g)	36.5
Total isoflavones in all forms (mg/20g protein)	116
Total aglycones in all forms (mg/g protein)	68.4
Serving size (grams)	64
Daidzein (mg/g protein)	2.85
Genistein (mg/g protein)	2.22
Glycitein (mg/g protein)	0.74

Note: Total weight of product = 64 g

protocol is shown in Appendix A. One subject was allergic to soy and another subject dropped out because of GI discomfort from colitis. The underlying kidney disease was hypertension (n=1), diabetes (n=1), IgA nephropathy (n=1), glomerulonephritis (n=1), and unknown etiology (n=6). Clinical data on these patients are presented including pre and post data in Table 2. The average age of the study patients was 50.

None of the patients was on a calorie restriction, although all had been instructed on a 40g fat diet in the hospital at the time of their transplant. At the start of the study, all patients had to be living in the Houston metropolitan area, taking rapamycin, and be at least 6 months post transplant. Nine out of the ten patients at the start of the study had cholesterol values above 200 mg; however, none had familial hyperlipidemia. Two of the ten patients had type 2 diabetes mellitus (one patient had unknown etiology as cause for renal failure). All patients were on lipid lowering drugs. These drug regimens are listed in Appendix C. Values for patients'serum lipids (cholesterol and TGs) pre-study period are given in Table 3.

Procedures

A three-day food diary was kept during the midpoint of each eight-week period. The form used is presented in Appendix D. Weights for patients were retrieved from medical charts. Subjects recorded what was eaten and amounts. Compliance was assessed by questioning subjects weekly over the phone, about the number of packets used. The Food Processor computer program, version 7.3, was used to analyze these diet diaries for all nutrients. Triglyceride and cholesterol concentrations were measured by

standard enzymatic method by. HDL cholesterol was separated from plasma precipitating non-HDLc with use of dextran sulfate and magnesium chloride. LDLc was determined by using Friedewald's formula (LDL_c= Total cholesterol – HDL_c –$^{TG}/_5$). All analyses were done at Hermann Hospital, Houston, Texas. A paired, two-tailed, Student's t-test was used to compare the values obtained during the periods when subjects received either the casein or soy products. A value of $p<0.05$ was considered to be statistically significant.

Table 2

Clinical Characteristics

Subject	Age	Ht(cm)	Pre-Wt(kg)	Pre-Tx BMI	Post-Tx Wt(kg)	Post-Tx BMI
1*	58	170	90	32	92	32
2*	39	185	91	24	91	25
3*	69	175	64	20	59	17
4	61	147	55	25	55	25
5	35	157	56	23	66	25
6*	51	177	66	21	70	22
7	47	162	70	27	77	29
8*	41	183	71	21	80	24
9*	66	180	102	31	105	32
10*	34	178	90	21	92	23

Note: Ht= height, wt= weight,
BMI = Body Mass Index = $Wt(Kg)/Ht(m)^2$
*Indicates male
Subjects 1 and 2 are hispanic, others were caucasian..
There was no significant difference between pre and post wt/BMI.

Table 3

Pre-Study Lipid Levels

Serum Lipid	Concentration (mg/dl)
Cholesterol	242 ± 43
Triglyceride	192 ± 67

Note: Mean ± SD, n=10
*Pre-study values were only available for cholesterol and triglyceride only.

CHAPTER IV

RESULTS

Plasma Cholesterol

Individual pre-treatment serum lipid values for participants are given in Appendix F. Values for serum levels of cholesterol, HDLc, LDLc, and TG during the two treatment periods are given in Table 4. There were no statistically significant between-group differences for any of these parameters.

Intake of Energy Nutrients and Cholesterol

Data on the composition of the diets is shown in Appendix G for both periods. Mean daily macronutrient energy values for the subjects while consuming the casein containing drink were 2027 kcals, 238 g carbohydrate, 90 g protein, and 72 g fat. Mean daily energy and macronutrient values for the subjects while consuming the soy containing drink were 1744 kcals, 211 g carbohydrate, 96 g protein and 58 g fat, as shown in Table 5. There was no significance in macronutrient intake between study periods. When interviewed, most patients reported that they could taste the difference between the soy and casein, however, they viewed the difference not significant. The consumption percentage data of the supplement during both periods are shown in Appendix H. The mean percentage of soy packets consumed was 98.2%. The mean casein packets consumed were 99%, which is shown in Table 6.

Table 4

Values for Serum Lipids During Study Periods

Lipids	Soy	Washout	Casein
Cholesterol	255 ± 85 (198-417)	248 ± 37 (216-338)	251 ± 47 (202-335)
LDLc	53 ± 13 (38-75)	51 ± 9.9 (37-67)	53 ± 10 (39-73)
LDLc	153 ± 58 (109-303)	152 ± 24 (37-67)	154 ± 46 (110-242)
TG	246 ± 160 (88-650)	240 ± 88 (108-365)	221 ± 118 (101-482)

Note: HDLc=high density lipoprotein cholesterol, LDLc=low density lipoprotein cholesterol, TG=triglycerides. There were no significance differences found between groups. Values as mg/dl; mean ± SD, n=10.
Individual ranges are given in parentheses.

Table 5

Daily Intakes of Energy,Macronutrients, Cholesterol (Diet and Supplement)

Variable	Soy Period	Casein Period	Significance
Energy (kcal)	1744 ± 443 (1186-2914)	2026 ± 638 (957-2535)	NS
Carbohydrate (g)	211 ± 70 (121-355)	239 ± 57 (165-263)	NS
Protein (g)	96 ± 22 (56-128)	90 ± 33 (48-104)	NS
Fat (g)	58 ± 18 (60-101)	72 ± 33 (40-103)	NS
Cholesterol (mg)	230 ± 81 (133-267)	179 ± 73 (110-346)	NS

Note: Mean ± SD, n=10
Individual ranges are given in parentheses.

Table 6

Percentage of Reported as Consumed

Supplement	%Compliance
Casein Pacs	99 ± 1.4
Soy Pacs	98.2 ± 1.8

Note: Mean ± SD, n=10

The average fat intakes (as a percentage of total energy including supplement) were 32% and 30% during the casein and soy phases respectively.

Anthropometrics

Patients' values for both body weight and BMI at the beginning, and the end of the study for these two time points are shown in Table 2. There were no significant differences between the initial and final values (Table 7).

Table 7

Values for BMI and Body Weight

Variable	Pre-treatment ± SD	Post-treatment ± SD	Significance
BMI	24.8 ± 5.3	25.30 ± 5.6	NS
Weight (kg)	73.1 ± 15.8	75.00 ± 16.2	NS

Note: BMI = Body mass index = weight (kg)/height(m)2
Mean ± SD, n=10

CHAPTER V

DISCUSSION

The results of this study did support the null hypothesis, which was that there would not be a statistically significant difference in levels of serum cholesterol, TG or LDLc when patients were given an enteral product containing either soy protein or casein.

Because rapamycin-induced dyslipidemia is a significant problem, patients receiving this drug (usually in combination with other lipid-raising drugs such as CyA and prednisone) are given one or more lipid-lowering drugs. These include pravastatin (an HMG CoA reductase inhibitor) and gemfibrozil (a TG-lowering drug). All patients used in this study received pravastatin (5mg, 10mg, or 15mg per day) during either the soy or casein treatment periods and 6 patients received gemfibrozil (600mg per day in all cases). The present study investigated whether soy protein would decrease serum lipid values, rather than investigating the effect of soy protein per se on rapamycin-induced dyslipidemia. (In human subjects it would not be possible to determine the effect of soy protein per se, because denying treatment would not be ethical.) It would only be possible to determine the effect of soy protein per se on rapamycin-induced dyslipidemia using an animal model, i.e., rats treated with rapamycin. Thus far, there appears to be no other studies with human subjects that have investigated whether soy protein can

decrease serum lipids in addition to that brought about by the use of cholesterol lowering drugs such as the statins.

The possibility that the consumption of soy formula contributed to a lack of effect can be excluded, as compliance was extremely high for both formulas being 98.2 and 99% for soy formula and casein formula respectively shown by patient's records. One reason that soy formula may have had no statistically significant effect on serum lipids is that the amount of protein consumed was too low. Most of the studies in Anderson's meta-analysis used 25 g of soy protein or more per day. Several studies (Nestel, Simons) used 80 mg isoflavones and did not yield a significant decrease in serum lipids. Soy may have been effective in this study if two packets (40g soy protein) had been used. A future study could be done to investigate this possibility.

While some studies show a cholesterol lowering effect of soy protein (Wong et al. 1998; Anderson et al. 1995; Anthony et al. 1998; and Nagata et al. 1998) others have not (Grundy et al., 1983; Hodgson et al., 1996; and Nestel et al., 1997). The human studies above which found significant decreases in serum lipids in humans were seen in hypercholesterolemic subjects rather than in normocholesterolemic subjects. These inconsistent results may be partially attributed to differences in the source of soy protein. Many soy preparations were used such as soy flour, soy-protein concentrate, textured soy protein, isolated soy protein and soymilk. The question remains, would soy protein affect serum lipids in human subjects receiving rapamycin, if other sources of soy protein were used.

The current study utilized liquid formula supplements of known composition in addition to the participant's prescribed low-fat diet, 40 g per day. Both supplements contained 20g of protein. One consisted of isolated soy protein and the other contained casein, with the fat and carbohydrate composition of both formulas being identical. None of the subjects was compliant with the 40g fat diet for both study periods. It is possible that soy protein may be effective if patients are compliant with a 40g fat per day diet. (In this study fat intakes ranged from 31-142 g per day during the soy period and from 26-132 g per day during the casein period).

One drawback to the present study is the small number of participants who completed the study (n=10). Unfortunately, given the parameters of participation, i.e., being >6 months post transplant, living within Houston metropolitan area, and being prepared to be involved in a 6 month study the numbers of subjects available was limited.

Conclusion

In renal transplant patients, drug-induced hyperlipidemia is a significant problem, as the condition is associated with chronic organ failure (Bittar et. al., 1990),and increased risk of developing CAD. With severe hypertriglyceridemia (a serum value for TG of greater than 800mg/dl) there is the risk of developing pancreatitis. Increased serum levels of TG and cholesterol appear to be the most consistent predictor of chronic renal organ failure. It is not conclusive whether the association between chronic renal organ failure and hyperlipidemia may be a consequence rather than the cause of renal failure. Attempts to control hyperlipidemia include putting patients on a low fat diet.

However, this may be difficult for many patients to follow. Pharmacologic treatment has been the most commonly used way to lower serum lipids in this population, but since transplant recipients are on so many drugs, it would be beneficial if non pharmacologic approaches could be used to lower serum lipids. This could include the use of soy protein. The present study indicates that 20 g per day of soy protein would not be effective at decreasing serum lipids in this population. However a larger dose (40 g, for example, which could be achieved by using two packets per day rather than one of the product used) may do so. Because there was no effect of soy protein (vs casein) on serum lipids, the null hypothesis is not rejected.

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APPENDICES

APPENDIX A

STUDY DESIGN

SOY PROTEIN PROTOCOL

Twelve patients, who are on Rapamycin, will be enrolled in this study, which is designed to test the hypothesis that soy protein (which is rich in arginine, a triglyceride and cholesterol lowering amino acid) can reduce the severity of Rapamycin induced hypertriglyceridemia in renal transplant patients.. The criteria are that these patients are at least 6 months post renal transplant, on Rapamycin, a drug suppressing the immune system, and live in the Houston area. They will be randomized into two groups: Group A (n=6) receive the casein placebo, and Group B (n=6) receive the soy protein. Subjects will complete a 3-day food record at the beginning of the study and at the midpoint of each treatment period. The study will be 6 months long. At month 3, both groups will not be taking anything, which will constitute the "wash-out" period (see table below). At month 5, the patients in each group will be switched to the opposite group. Therefore, by the end of the study, all patients will have received both the soy protein and the casein formulas. A lipid profile will be drawn each month, along with the blood work done for the Rapamycin study. An analysis will be done on the food records by Food Processor. Two-factor ANOVA will be done to analyze the data.

Outline Of Study

1-2 MONTHS	3-4 MONTHS	5-6 MONTHS
GROUP A	WASHOUT	GROUP B
GROUP B	WASHOUT	GROUP A
FOOD RECORD	FOOD RECORD	FOOD RECORD

Appendix B

INFORMED CONSENT

I hereby consent to engage voluntarily in testing the soybean product drink, Revival at Hermann Hospital by Elizabeth Eschenburg. The purpose of this study is to test the effectiveness of soybean protein to lower the high serum cholesterol and triglyceride levels found as the result of taking the immunosuppression drug Rapamycin for the preservation of renal graft function.

The treatment will consist of ingesting soybean protein, dissolved in water, in a vanilla or chocolate drink every day, at no cost to the participant. The participant will mix up this drink at home for 9 months. Blood will be drawn monthly at Hermann Hospital at no cost and will be incorporated into the Rapamycin study blood draws whenever possible. A three-day diet history will be recorded at the beginning of the study, at 6 months of the study and again at the end of the study. I will be assigned randomly to either the control group or the experimental group. In both groups, the product will be in individual white packets.

There is some risk to me from this study. Participants who have an allergy to soy will not be eligible. There will be the inconvenience of the time spent at the hospital. The other risk is related to the blood draws, such as excessive bleeding, buising, nausea, and allergy to tape or latex gloves. Physician's Laboratories manufacture revival in the USA in a FDA-inspected and USDA-approved food laboratory.

The benefit of being in the experimental group for this study is that a safe and non-pharmaceutical product may successfully reduce cholesterol and triglyceride levels to a safe level.

My confidentiality will be preserved. My records are only available for study results. Each person will be known by medical record number, not proper name. The results of the study will be revealed to me at the termination of the study period.

My refusal to participate will be honored with out cost or penalty to me. And withdrawal from the study can happen whenever I deem necessary. The researcher will withdraw me if medical or psychological condition prevents me

from continuing in the study. This will be of no cost or penalty to me. I will not receive any money for this study, only the satisfaction of benefiting mankind.

The alternative treatment for high cholesterol and triglyceride levels consists of a low fat diet, fish oil and pharmaceutical agents, of which I may already be taking, and are not adequately reducing my serum levels.

In case of injury, contact Elizabeth Eschenburg RD, LD, CDE at (713) 704-4981, or John Radcliff RD, PhD at (713) 794-2371 for answers to pertinent questions about the research and research subject's rights, and any research related injury.

I have read the foregoing. I have had a chance to ask all questions and all questions have been answered to my satisfaction.

Participant's Signiture _____ Date: _____

Researcher's Signiture _____ Date: _____

Appendix C

PATIENT MEDICATION

Subject	Period 1 Medication	Period 1 Dosage	Period 2 Medication	Period 2 Dosage	Period 3 Medication	Period 3 Dosage
1	CSA	175/150	CSA	100/125	CSA	125/100
	Pred	12.5mg	Pred	10mg	Pred	7.5mg
	RAPA	2mg	RAPA	2mg	RAPA	2mg
	Prav	5mg	Prav	10mg	Prav	5mg
					Lopid	600mg
					Fish Oil	6000mg
2	CSA	75/75	CSA	75/50	CSA	75/50
	Pred	0	Pred	0	Pred	0
	RAPA	3mg	RAPA	3mg	RAPA	3mg
	Prav	10mg	Prav	20mg	Prav	20mg
3	CSA	150/150	CSA	125/125	CSA	100/175
	Pred	5mg	Pred	5mg	Pred	0
	RAPA	4mg	RAPA	4mg	RAPA	6mg
	Prav	5mg	Prav	5mg	Prav	5mg
	Lopid	600mg	Lopid	600mg	Lopid	600mg
4	CSA	75/50	CSA	50/50	CSA	50/50
	Pred	0	Pred	0	Pred	0
	RAPA	2mg	RAPA	3mg	RAPA	3mg
5	CSA	75/75	CSA	75/75	CSA	75/75
	Pred	0	Pred	0	Pred	0
	RAPA	5mg	RAPA	5mg	RAPA	5mg
	Lopid	600mg	Lopid	600mg	Lopid	600mg
6	CSA	0	CSA	0	CSA	0
	Pred	5mg	Pred	5mg	Pred	5mg
	RAPA	10mg	RAPA	9mg	RAPA	9mg
	Prav	10mg	Prav	15mg	Prav	20mg
	Lopid	600mg	Lopid	600mg		
7	CSA	100/75	CSA	75/75	CSA	75/75
	Pred	5mg	Pred	0	Pred	0
	RAPA	2mg	RAPA	3mg	RAPA	3mg
	Prav	10mg	Prav	15mg	Prav	15mg
8	CSA	75/50	CSA	50/50	CSA	125
	Pred	15mg	Pred	10mg	Pred	5mg
	RAPA	4mg	RAPA	5mg	RAPA	5mg
			Prav	5mg	Prav	5mg
			Lopid	600mg	Lopid	600mg
					Fish Oil	
9	CSA	125/100	CSA	100/100	CSA	75/50
	Pred	5mg	Pred	0	Pred	0
	RAPA	3mg	RAPA	4mg	RAPA	5mg
	Lopid	600mg	Lopid	600mg	Lopid	600mg
			Prav	10mg	Prav	10mg
10	CSA	100/75	CSA	100/75	CSA	100/75
	Pred	2.5mg	Pred	0	Pred	0
	RAPA	1mg	RAPA	1mg	RAPA	1mg
	Prav	5mg	Prav	5mg	Prav	5mg
	Fish Oil		Fish Oil		Lopid	600mg
					Fish Oil	

Appendix D

3 DAY DAILY FOOD INTAKE

Name:_____

Start Date:_____

	Type of Food	Amount
Breakfast		
Lunch		
Dinner		
Snacks		

DRINK ON ALL THREE DAYS: YES NO

Appendix E

Raw Data of Subject's Serum Lipids

Subject	Serum Lipids	Period	
		Soy	Casein
1	TC	227	249
	HDLc	47	45
	LDLc	128	130
	TG	262	371
2	TC	215	298
	HDLc	48	48
	LDLc	127	201
	TG	199	245
3	TC	222	285
	HDLc	38	39
	LDLc	144	204
	TG	200	211
4	TC	217	208
	HDLc	61	58
	LDLc	121	110
	TG	175	201
5	TC	198	226
	HDLc	39	40
	LDLc	129	153
	TG	152	163
6	TC	331	335
	HDLc	64	73
	LDLc	200	242
	TG	335	101
7	TC	233	214
	HDLc	54	53
	LDLc	124	129
	TG	273	158
8	TC	471	287
	HDLc	38	54
	LDLc	303	137
	TG	649	482
9	TC	223	202
	HDLc	62	55
	LDLc	143	118
	TG	88	144
10	TC	209	203
	HDLc	75	61
	LDLc	109	114
	TG	123	140

Appendix F

Individual Pre-Treatment Serum Lipid Levels

Participant	Cholesterol	Triglyceride
1	255	268
2	239	176
3	220	92
4	229	234
5	267	285
6	278	170
7	287	218
8	230	280
9	260	186
10	143	97

Appendix G

Diet Composition During Study Periods

Subject	Intake	Mean Formula Soy	Casein
1	Energy (kcals)	1872.00	1977.00
	Carbohydrate (g)	170.67	171.67
	Protein (g)	85.00	67.00
	Fat (g)	93.67	60.67
	Cholesterol (mg)	270	150
2	Energy (kcals)	1653.67	2222.67
	Carbohydrate (g)	187.00	233.00
	Protein (g)	60.00	98.33
	Fat (g)	77.67	103.00
	Cholesterol (mg)	323	346
3	Energy (kcals)	3247.67	3136.67
	Carbohydrate (g)	355.67	342.33
	Protein (g)	138.67	147.67
	Fat (g)	141.67	132.00
	Cholesterol (mg)	277	155
4	Energy (kcals)	1344.33	1904.33
	Carbohydrate (g)	214.33	299.33
	Protein (g)	57.67	77.00
	Fat (g)	31.33	44.67
	Cholesterol (mg)	203	147
5	Energy (kcals)	1873.00	1991.00
	Carbohydrate (g)	285.67	266.00
	Protein (g)	71.67	72.00
	Fat (g)	49.33	77.00
	Cholesterol (mg)	140	121

Subject	Intake	Mean Formula Soy	Casein
6	Energy (kcals)	1541.33	1557.67
	Carbohydrate (g)	158.00	165.00
	Protein (g)	76.33	78.33
	Fat (g)	67.67	65.67
	Cholesterol (mg)	189	235
7	Energy (kcals)	1186.67	1172.67
	Carbohydrate (g)	122.00	189.67
	Protein (g)	79.00	49.00
	Fat (g)	41.33	26.67
	Cholesterol (mg)	177	187
8	Energy (kcals)	1571.00	957.33
	Carbohydrate (g)	132.33	89.67
	Protein (g)	82.67	47.00
	Fat (g)	81.67	47.33
	Cholesterol (mg)	367	180
9	Energy (kcals)	1327.33	1937.67
	Carbohydrate (g)	139.67	228.33
	Protein (g)	57.00	89.67
	Fat (g)	60.00	76.67
	Cholesterol (mg)	263	136
10	Energy (kcals)	1527.67	2306.00
	Carbohydrate (g)	202.33	362.33
	Protein (g)	60.00	82.67
	Fat (g)	55.67	62.33
	Cholesterol (mg)	134	110

Appendix H

Consumption of Supplement Packets Per Period

Subject	Casein (%)	Soy (%)
1	100	98
2	100	97
3	98	98
4	100	95
5	100	100
6	98	100
7	98	98
8	96	96
9	100	100
10	100	100