EFFECTS OF DIETARY POLYUNSATURATED FATTY ACIDS AND PROTEINS ON SERUM LIPID PROFILES AND RENAL FUNCTIONS OF UREMIC RATS

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF MASTER OF SCIENCE

IN THE GRADUATE SCHOOL OF THE

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF NUTRITION AND FOOD SCIENCES

BY

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MAY 1993

ACKNOWLEDGEMENT

The author wishes to express her sincere appreciation for the following individuals for their contributions in making this research possible:

To Dr. George Liepa, advisor and chairman of this project, for his advice, for his "stretchable" patience, and most of all for his cheerful and endless support,

To Dr. Mary Anne Gorman, statistician and committee member, for her patience and for her help in interpreting the statistical analysis,

To Dr. Annemarie Richmond, my "acting" mother, for her encouragement, for her constant support, and most of all for her warm-hearted friendship during tough situations,

To Dr. Ronald Smith, nephrologist, for his expert advice and also for his patience,

To Dr. Larry Forman, former committee member, for his suggestions and assistance,

To Dr. Betty Alford, committee member, for her constant support,

To Dr. Andie Hsueh, committee member, for her advice and support,

To Jessie Ashby, technician, for her constant support in laboratory matters, for her irreplaceable role during surgery, and especially for her friendship,

To Mr. James Harrison, surgeon, for his patience in teaching the author the surgical techniques, and for his encouragement and constant interest in this project,

To Mr. Pete Pederson, technician, for his assistance in fixing equipment,

To Carol Johnson and Sandy Graham, secretaries, for their cheerful assisstance in administrative matters,

To Texas Food and Fibers Commission, Dallas, Texas for funding this research,

To Jean Rim and Cecilia Sauter, friends, for their cheerful friendship and encouragement,

And for many other individuals that are too numerous too mention,

Finally, to my wonderful family:

Lukanto, my father, for his untiring support and comfort, for his inspiration in achieving my goal, and for his love,

Lusawanti, my mother, for her cheerful advice and patience, and most of all for her love,

Hendro, my brother, for always being there whenever I need him, for his expert guidance in computer matters, and for his constant support,

Santoso Budiman, friend, for his patience and understanding.

Abstract

Effects of Dietary Polyunsaturated Fatty Acids
and Proteins on Serum Lipid Profiles and
Renal Functions of Uremic Rats

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May 1993.

Sixty-three male Sprague-Dawley rats were partially nephrectomized and randomly assigned to one of the four experimental diets. All diets were isocaloric and contained the following ingredients: a) 24% Casein - 5% MaxEPA oil (MOCAS), b) 24% Casein - 5% Corn oil (COCAS), c) 24% Soy - 5% MaxEPA oil (MOSOY), and d) 24% Soy - 5% Corn oil (COSOY). Animals were maintained on their corresponding diets for 13 weeks. Blood pressures and serum blood profiles (total protein, albumin, creatinine, triglycerides and total, HDL and LDL cholesterol) were determined. No differences were noted among the groups in serum total protein, albumin, or creatinine, as well as the systolic blood pressure. Rats fed MO diets, regardless of the protein source, had lower serum triglyceride levels. Total and HDL cholesterol concentrations

were significantly lower in the MOCAS group when compared to the other three experimental groups. Feeding of the COCAS diet was nonsignificantly associated with high LDL concentrations. MaxEPA oil diets yielded significantly higher concentrations of serum EPA and DHA and significantly lower linoleic and AA concentrations when compared to CO diets. Rats that were maintained on MOSOY diets had the greatest degree of glomerular sclerosis. Urinary total protein concentrations were higher in the COSOY rats when compared to the other groups. Based on the results obtained, previous beneficial effects of MaxEPA oil and soy protein on serum lipid profiles and renal functions were not as apparent.

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CHAPTER I

INTRODUCTION

The kidneys are important organs that play an important role in maintaining homeostasis in the body. Any disruption to these organs can result in major clinical problems. Several studies have been done to investigate the effects of diet composition on the progression of renal disease.

It is now widely accepted that alterations in lipid metabolism can play a role in the progression of chronic renal disease (CRD). The deleterious effects of increasing dietary cholesterol and saturated fats have been demonstrated using animal models of progressive renal disease. Various animal studies have also indicated that high fat diets and hyperlipidemia may accentuate the progression of renal disease. However, studies with dietary omega-3 polyunsaturated fatty acids (PUFAs) indicate that this fatty acid family may provide a possible treatment for CRF patients since omega-3 fats are precursors of prostaglandins that may retard the loss of renal function. Administration of fish oil, a rich source of omega-3 fatty acids, has been shown to

result in a significant lowering of glomerular eicosanoid synthesis, which in turn, has reduced the sclerosis of the glomerulus (von Schacky et al., 1985; Clark et al, 1990; Wheeler et al., 1990). On the contrary, omega-6 PUFA consumption has been shown to induce the production of certain damaging eicosanoids.

Some studies have shown that the quality and the quantity of dietary proteins could also affect renal function. Hyperfiltration, as assessed by glomerular filtration rate and urinary protein excretion, has been reported to be associated with high dietary protein intake on a long term basis (Buzio et al, 1990; De Keijzer et al., 1990).

Studies with experimental animals have shown that protein hyperfiltration may lead to glomerulosclerosis through proliferation of mesangial cells. For many years, low protein diets have been used to prevent or slow down the development of uremic symptoms. Renal hyperfiltration and renal pressure have been shown to be reduced by the consumption of a low protein diet (Brenner et al, 1982, Maschio et al., 1982; De Keijzer et al, 1990).

Animal studies have also suggested that the source of the dietary protein may have an impact on the severity and progression of renal disease. Some animal proteins have occasionally been found to contribute to deleterious effects

on renal tissue. In contrast, plant protein may ameliorate uremic symptoms and could therefore increase the life span of nephrectomized rats (Williams et al, 1987; Iwasaki et al, 1988; Kontessis et al., 1990).

Recently, mesangial cells have been suggested to be a major site of kidney degeneration in patients with CRF. The accumulation of lipid in mesangial cells may play an important role in the progression of glomerulosclerosis (Schlondorff et al., 1987). Since mesangial cells have been observed to have a structure which is similar to smooth muscle cells, the effect of dietary components on these cells may be compared to changes seen in smooth muscle cells in coronary and peripheral arteries which have received dietary insults.

At this time, few studies regarding the effects of dietary protein and fat from different sources on the mesangial cells have been done. Any further investigation in this area would be beneficial for the treatment of renal disease patients.

Purpose of the Study

The purpose of this study was to determine whether certain combinations of different types of dietary fatty acids and proteins could ameliorate (or deteriorate) the clinical symptoms of uremic rats.

The specific objectives of this study were:

1. To determine the effects of combinations of two different types of dietary polyunsaturated fatty acids (omega-3 or omega-6 fatty acids) and dietary proteins (animal or plant origin) on the renal histopathology of uremic rats.
2. To determine the effects of dietary fat and protein manipulations on concentrations of nitrogenous compounds in the serum as well as in the urine of uremic rats.
3. To determine the serum amino acid and fatty acid profiles of uremic rats fed diets containing various combinations of plant and animal fats and proteins.

CHAPTER II

LITERATURE REVIEW

Significant proteinuria and hyperlipidemia are common complications in chronic renal disease (CRD) patients. Experimental studies have suggested that abnormal lipid metabolism may be a mediator in the modulation of progressive renal disease. Several factors that may affect the progression of renal failure, such as variations in dietary proteins and fats, have been identified (Adler and Kopple, 1983). A series of studies has been done to examine how dietary proteins and fats affect the structure and function of the kidney in both animals and human subjects with renal disease.

Dietary Fats

Polyunsaturated fatty acids (PUFA) have been suggested to be an important protective agent against coronary heart disease via their role in the reduction of serum cholesterol. Certain polyunsaturated fatty acids are also believed to be effective in preventing or slowing down a decline in renal function. Previous studies (Heifets et al., 1987) have shown

that omega-6 PUFAs could have positive effects on renal function.

Lipid Metabolism

Clinical studies have repeatedly shown beneficial effects of dietary omega-3 PUFA in preserving renal function in normal and hyperlipidemic subjects (Harris et al., 1983) and also in animal models (Wheeler et al., 1990, Ito et al, 1987, Ito et al., 1988).

Omega-3 fatty acids have been observed to be able to favorably influence lipid metabolism. Fish oil, which is rich in omega-3 PUFA, has been proposed to have both positive [lower triglyceride and very low density lipoprotein cholesterol (VLDL) and increase high density lipoprotein (HDL) cholesterol concentrations] and negative [higher total and low density lipoprotein (LDL) cholesterol concentrations] effects on lipid profiles (Illingworth et al., 1989; Sanders and Roshanai, 1983; Harris et al., 1983).

Polyunsaturated fatty acids of the omega-6 series more frequently reduce total, LDL and VLDL cholesterol. There are only a few reports of reduction of total triglycerides and they do not seem to affect HDL levels. Polyunsaturated fatty acids of the omega-3 series have been shown to: (a) have major suppressive effects on total plasma triglycerides, (b) produce some reduction of total cholesterol and have variable

effects on LDL cholesterol and (c) reduce VLDL cholesterol and increase HDL cholesterol.

Ito and co-workers (1987) also observed that serum triglycerides and cholesterol concentrations were lower in rats maintained on a 14% fish oil diet than those fed either 3% fish oil or 14% beef tallow diets. The levels of HDL cholesterol did not show a significant difference among the three groups, while the LDL cholesterol levels were lower in rats fed the 14% fish oil diet. All animals in the study were injected with adriamycin to induce nephrotic syndrome. Adriamycin nephropathy is a manifestation of direct glomerular toxicity with no immune involvement. In a later experiment using the same animal models, Ito et al. (1988) examined the effects of 3% and 14% fish oil diets on plasma lipids. Plasma triglyceride and cholesterol concentrations were markedly increased in all nephrotic rats, but the elevation was blunted by the fish oil feeding, with the greatest effect occuring in the rats fed the 14% fish oil. Wheeler et al (1990) also found that rats fed fish oil containing diets had significantly lower levels of plasma cholesterol and triglycerides.

Rolf et al. (1990) examined ten patients on chronic hemodialysis. They administered a salmon oil concentrate for 28 weeks and found that the total serum cholesterol and triglycerides levels decreased significantly (64% and 48%,

respectively). They also reported a significant increase in HDL cholesterol concentration. Mortensen et al. (1983) fed either 10 g of fish oil or 10 g of vegetable oil to healthy volunteers for 4 weeks. Each oil contained about 40% omega-3 and omega-6 PUFAs, respectively. They reported that plasma total lipids, triglycerides and VLDL concentrations were significantly decreased during the fish oil period. No changes were observed in the LDL or HDL fractions. In another study, Singer et al. (1985) observed a lipid lowering effect of mackerel and herring diets in patients with mild essential hypertension. Their results indicated that serum triglycerides, total cholesterol, LDL-cholesterol and lecithin cholesterol acyl transferase (LCAT) activity were significantly lowered after consumption of the mackerel diet. They also observed a significant increase in HDL cholesterol levels.

Eicosanoid Synthesis

Different types of eicosanoids and their metabolites can be formed from different types of fatty acids through the cyclooxygenase pathways (Lands, 1986). The most common PUFAs in the diet belong to the omega-3 and omega-6 series (Barcelli and Pollak, 1989). These two families of PUFAs are mutually competitive for the cyclooxygenase enzyme (Lands, 1986). A wide range of prostanoids (e.g. prostaglandins and

thromboxanes) are formed through the cyclooxygenase pathways, whereas, the leukotriene series is generated through the lipoxygenase system.

Linoleic acid (omega-6 fatty acid), which can be found in many vegetable oils, is a precursor of arachidonic acid. Arachidonic acid is a precursor of eicosanoids which can, in turn, be converted to dienoic eicosanoids by prostaglandin synthetase and the lipoxygenase systems. These different pathways are responsible for the difference in physiological properties between omega-3 and omega-6 PUFAs.

Eicosapentanoic acid (EPA), a polyunsaturated fat found in fish oil, competes with arachidonic acid as a substrate for cyclooxygenase (Culp et al., 1979). A diet rich in fish oil promotes the synthesis of trienoic eicosanoids, and interferes with formation of dienoic eicosanoids (Fischer et al., 1984). The balance between vasodilatory and vasoconstrictive eicosanoids may be shifted in favor of the former when EPA is the primary substrate for cyclooxygenase activity (Von Schacky et al., 1985).

Mesangial Cells and Progression of Renal Disease

The mesangium is a unique central-lobular structure in the glomerulus constituted in part by mesangial cells and the mesangial matrix. This part of the glomerulus has been shown to be uniquely permeable to large molecular proteins (Michael

et al., 1980). It has been suggested than the accumulation of lipid in the mesangial cells may enhance the development of focal glomerulosclerosis. These mesangial cells have a close resemblance to smooth muscle cells (Schlondorff, 1987). Lipoproteins, which function as carriers of lipids, have surface receptors in many cells, including smooth muscle cells and mesangial cells. Increased amounts of lipoproteins (low density and very low density) may stimulate collagen and proteoglycan production and lead to an increase in the "trapping" of lipids. This accumulation of lipids may cause the proliferation of mesangial cells and result in a thickening of the glomerular basement membrane and eventually to sclerosis of the glomerulus. Wight (1989) demonstrated that low density lipoproteins (LDL) can interact with proteoglycans and this interaction can delay the removal of these types of lipoproteins.

Recently, oxidized LDL has been proposed as an important pathogenic lipoprotein that participates in the development of atherosclerosis (Steinberg et al., 1989). In various studies, oxidized LDL has been shown to be cytotoxic to cultured mesangial cells and thus might contribute to glomerular damage (Wasserman et al., 1989; Wheeler et al., 1990; Keane et al., 1990). Although several studies have been done to examine the effects of altered lipoproteins on the progression of renal injury, the mechanism is still not

clear. Further investigation on how oxidized LDL affects the mesangial cells might be beneficial for kidney patients.

Fish Oil and Blood Pressures

Increases in both dietary omega-3 and omega-6 PUFAs have been suggested to lower blood pressure. However, dietary enrichment with omega-6 fatty acids has recently been reported to have a deleterious effect (Lands et al., 1986).

MaxEPA oil contains high levels of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). This type of fish oil has been found to significantly reduce systolic blood pressure in normal volunteers (Mortensen et al., 1983; Lorenz et al., 1983), hypertensives (Radack et al., 1991) and patients undergoing hemodialysis (Singer et al., 1985; Smith and Dunn, 1985). The blood pressure lowering effect of fish oil has also been observed in animal models (Karanja et al., 1989).

Knapp and FitzGerald (1989) examined the effects of both omega-3 and omega-6 PUFAs on blood pressure in men with mild essential hypertension. The subjects were randomly assigned to one of the four experimental groups. The supplements contained a daily dose of : 10 or 50 ml of MaxEPA oil, 50 ml of safflower oil, or a mixture of oils that is commonly found in the average American diet (contained approximately 15% polyunsaturated, 46% monounsaturated, and 39% saturated

fats). The study was carried out for a three month period. A significant reduction in both systolic and diastolic blood pressure was observed with the subjects supplemented with a high level of fish oil (50 ml per day). No significant difference in the blood pressure could be noticed in the other treatment groups. The safety of high dose fish oil supplementation is an important issues that still needs to be explored. Radack et al. (1991) conducted a randomized, controlled crossover study comparing how low doses of n-3 fatty acids and n-6 fatty acids affect blood pressure in subjects with mild hypertension. The subjects were given either 2.0 g/day of n-3 fatty acids or safflower oil (4.8 g/day of linoleic acid) for 12 weeks. They were then crossed over to the alternative encapsulated oil for another 12 weeks. Results showed that fish oil supplementation significantly reduced the mean diastolic blood pressure when compared to safflower oil supplementation. Rolf et al.(1990) also reported that there was a significant decrease in blood pressure when a salmon oil concentrate was given to patients on long-term hemodialysis.

Karanja et al. (1989) studied the effects of fish oil, corn oil, and butterfat diets on salt hypertensive rats (SHR). They found that fish oil consumption resulted in lower blood pressure when compared with butterfat and corn oil diets.

Dietary Fats and Kidney Disease

The effects of omega-3 PUFAs on renal function have been extensively explored, however, the mechanism behind these effect is still not understood (Izumi et al., 1986; Clark et al., 1990; Scharschmidt et al., 1987).

Izumi et al. (1986) studied two groups of five-sixth nephrectomized rats and two groups of sham-operated rats. One partially nephrectomized group and one sham-operated group received a high linoleic acid (HLA) diet containing 15% linoleic acid, whereas, the other two groups received a low linoleic acid (LLA) diet containing 0.28% linoleic acid. The rats were studied for 7 weeks during which time hypertension developed in the partially nephrectomized rats, as compared to the sham-operated rats. However, by the end of the study the blood pressure of the rats fed the HLA diet was lower than that of those fed the LLA diet. Differences were significant from week 4 to the end of the study. No differences in the urinary production of PGE2 and TXB2 were demonstrated between the two groups of partially nephrectomized rats. The clearance of creatinine, used as an index of GFR was lowered by partial nephrectomy, but was significantly higher in the HLA-fed rats than that in the LLA-fed rats; no differences in body weight were observed. In this experiment, it was not clear whether the differences in

blood pressure were associated with the differences in GFR. This study confirmed the findings that linoleic acid has a protective role in the renal function in this model.

Greenland Eskimos have a low death rate from coronary artery disease, which may relate to the high content of fish in their diet (Bang et al., 1980). An inverse relationship between fish consumption and mortality from coronary artery disease has also been observed from the Netherlands (Kromhout et al., 1985). This observation sparked the interest of many investigators to study the potential beneficial effects of fish or fish oil. The beneficial effect of dietary fish oil on the incidence of coronary artery disease has been attributed to the marine polyunsaturated fatty acid, eicosapentanoic acid (EPA). Consumption of this fish oil results in a relative anti-thrombotic state (Kromhout et al., 1985; Salmon and Terano, 1985; Von Schacky et al, 1985), because of a decrease in platelet aggregation which is induced by an alteration in the balance between prostacyclin and thromboxane. Despite the growing awareness of the beneficial effects of dietary fish on coronary artery disease, relatively little is known about this modification dietary fish oil intake impacts on renal prostaglandin synthesis. Trienoic prostaglandins have been identified in the urine of normal volunteers fed EPA (Fischer and Weber, 1984; Von Schacky et al., 1985; Salmon and Terano, 1985).

Von Schacky and coworkers (1985) found that humans consuming EPA excreted significant amounts of PGI3 while PGI2 production was unaltered. Furthermore, the excretion of the urinary metabolites of TXA2 was variably decreased and only small quantities of the metabolites of TXA3 appeared in the urine. These observations suggested that the renal production of vasodilators (PGI2 and PGI3) may exceed vasoconstrictor production (TXA2 and TXA3) during EPA feeding.

Clark et al. (1990) studied the efficacy of a low protein (6%) and PUFA containing diets in five-sixth nephrectomized female rats. The high PUFA diets contained 25% fat by weight, which was provided as fish or safflower oil (omega-3 and omega-6 fatty acids sources, respectively). All diets were started 1 week prior to surgery with control animals receiving regular animal chow throughout the 4 weeks study. The body weights remained comparable between groups. All of the experimental diets lowered urinary albumin excretion, but the fish oil diet had the greatest effect and this was statistically significant. Both fish oil and safflower oil diets have been shown to prevent the rise in IgG excretion. Clark et al. (1990) have extended their observations in the same model to study the effects of an isocaloric fish oil diet as compared to regular chow fed and sham-operated rats. These animals were studied for 20 weeks. The fish oil diet was shown to minimize reduction in renal clearance and

albuminuria caused by the partial renal ablation. The fish oil diet also diminished the glomerulosclerosis and glomerular fibrin deposition. Their data confirmed a protective effect for diets containing omega-3 PUFA in the renal ablation model, via the prevention of proteinuria and glomerulosclerosis, but without inducing a reduction in hyperfiltration. Other major effects were noted in blood pressure, lipids, coagulation and eicosanoid production.

The results reported by Scharschmidt et al. (1987), are at variance with the previously mentioned reports. They studied two groups of female five-sixth nephrectomized rats. The experimental diets were started 5 weeks after surgery and fed ad libitum for 12 weeks. The control diet was prepared with beef fat whereas the fish oil diet was prepared with menhaden oil. The rats fed fish oil had increased plasma creatinine, reduced hematocrit, and a shorter survival time as well as a reduced urinary excretion of PGE2 and TXB2. An additional rats were subjected to two-thirds nephrectomy and given the same dietary manipulations for 12 weeks. Fish oil-fed rats again did worse with more proteinuria, more glomerulosclerosis, and lower glomerular filtration rates. The authors suggested that the detrimental effects of fish oil in their study might be due to its effects on eicosanoid synthesis.

Diets rich in PUFAs have been used in studies with obese Zucker rats to evaluate PUFA effects on renal function and histology. Wheeler et al. (1990) studied uninephrectomized Zucker rats for 32 weeks. Rats were pair-fed diets containing 13 % beef tallow or fish oil. Rats fed the diet prepared with fish oil excreted less protein in their urine and showed a decreased degree of glomerulosclerosis than those fed beef tallow. The fish oil diet also significantly reduced the glomerular production of PGE2 and TXB2.

In a number of rat studies, an acute reduction in renal mass has been shown to prompt an increase in the rate of synthesis of PGE2, PGI2 and TXA2 in the remaining kidney tissues (Kirschenbaum et al., 1980; Stahl et al., 1986). Prevention of this increase in prostaglandin synthesis by pharmachological interference with cyclooxygenase activity has impaired compensatory increases in kidney mass and function in rats (Nath et al., 1986). These observations point to an important regulatory role of renal eicosanoids in the biological events which lead to renal adaptation to partial nephrectomy. However, it remains unclear whether enhanced prostaglandin metabolism promotes compensatory renal growth via a direct effect on cell processes or indirectly from increased work due to prostaglandin-dependent increases in glomerular filtration rate and renal blood flow.

The balance between vasodilatory (PGI) and vasoconstrictive (TXA) eicosanoids may be shifted in favor of the former when EPA is the primary substrate for cyclooxygenase activity (Von Schacky et al., 1985). Therefore, some authors have postulated that fish oil rich diet might exaggerate renal vasodilation following an acute reduction in renal mass and lead to a more pronounced increase in renal blood flow and glomerular filtration rate in the remaining nephrons. This possibility is indirectly supported by the observation that EPA feeding hastens the pace of glomerulosclerosis and renal failure in subtotally nephrectomized rats. This pattern may reflect the long-term consequence of early exaggeration of adaptive increases in glomerular pressure and flow (Scharschmidt et al., 1987).

The effect of dietary fish oil on compensatory renal growth has been studied previously . If dietary fish oil enhances renal function, then compensatory renal growth would be expected to be similarly enhanced according to the work hypertrophy theory (Brenner et al, 1982). However, several studies indicate that dienoic prostaglandins are direct regulators of cell growth in vitro (Taylor and Polgar, 1980; Habenicht et al., 1985).

Dietary Proteins

Only a few studies have been done to examine the effects of plant and animal protein on renal diseases. There is limited evidence that protein from plant origin may retard the progression of chronic nephropathy.

Protein Quality and Renal Disease

In their study, Iwasaki et al. (1988) replaced casein (animal protein) as the source of protein with soy protein (plant protein). They found that the rats fed the soy protein had longer survival times than the rats maintained on the casein diets. When lesions data were compared, the rats fed the casein diets showed a greater severity of chronic nephropathy than those fed the soy protein diets.

Williams et al. (1987) have previously studied the effects of different protein levels as well as different protein sources in experimental renal disease in models. Nephrectomized animals in the study were randomly assigned to one of four experimental groups. Group A received 24% casein, Group B received 12% casein, Group C was given 24% soya, and Group D was fed 12% soya. All diets contained similar amount of calories with identical amount of minerals and vitamins. At the end of their study, the animals maintained on soya diets revealed less renal tissue damage, improved life span, and fewer uremic symptoms when compared to animals fed casein

diets. The glomerular filtration rates and the effective renal plasma flow were also noticeably different between animals ingesting 24% casein and 24% soya. Blood urea nitrogen (BUN) concentrations were significantly higher in rats fed casein than those fed soya. The authors also proposed that the dietary amino acids might have an impact on the growth pattern of mesangial cells. Healthier mesangial tissues would result by diminishing the destructive effect of cationic amino acids (higher in animal proteins) and by increasing the positive effect of neutral amino acids which are higher in plant proteins.

Kontessis et al. (1990) studied the effects of different types of dietary regimens on renal and hormonal responses. Their study was divided into two parts: a chronic diet study and an acute protein load study. For their chronic diet study, they provided three different dietary regimens to a group of healthy individuals for a period of three weeks. These regimens included: a vegetable protein diet (VPD), an animal protein diet (APD), and an animal protein diet with fiber supplementation. Diets were presented to each subject in random order. The fractional clearance of albumin and IgG in animals fed the vegetable protein diet were reduced by 48% and 35%, respectively, when compared to the animal protein diet. The GFR and renal plasma flow were also significantly lower in animals fed the VPD than in those fed the APD. In

their acute study, both glomerular filtration rate and renal plasma flow increased significantly after a dietary meat load was provided. The authors suggested that hormonal responses to different types of proteins might be responsible for the different renal responses. Buzio et al. (1990) conducted two separate experiments that dealt with diet and kidney function. In the first experiment, healthy volunteers consumed a meat high-protein meal or a vegetable low-protein-meal. They found that the meat high protein diet contributed to a significant increase in GFR, total proteinuria, and albuminuria. In their second experiment, the subjects were given three protein rich meals which contained of about 80 g of protein in the form of cooked red meat, cheese, or soya. From this part of the study, the only significant differences between the groups were found in urea appearance and urea clearance which were lower and higher, respectively after the soya load.

Effect of Dietary Protein Intake on Renal Function

It has been demonstrated in numerous models of experimental renal injury that long-term dietary protein restriction reduces proteinuria and can ameliorate progressive structural damage (Hostetter et al., 1981; Diamond and Karnousky, 1987; Dworkin et al., 1984; Neugarten et al., 1991; Remuzzi et al., 1985; Wen et al., 1985).

Assessment of glomerular hemodynamics after institution of dietary protein restriction has been performed in several models of renal disease (Dworkin et al, 1984; Zatz et al., 1985; Dworkin and Feiner, 1986; Nath et al., 1986; Meyer et al., 1987). Glomerular capillary pressure is consistently reduced, however, the effects on the other determinants of glomerular filtration has varied with the timing and duration of protein restriction (Nath et al, 1986; Meyer et al., 1987).

In recent years, low protein diets have been repeatedly reported to prevent or slow down the progression of renal failure to end-stage renal disease in animals and also in humans (Brenner et al., 1982). A series of studies have been done to examine the effects of low protein diets on the progression of renal failure in humans. Maschio et al. (1982) studied the rates of progression of renal failure in three groups of patients. The patients were divided in groups based upon their initial serum creatinine values. Group 1 and Group 2 subjects were supplemented with 0.6 g/kg high quality protein, 1.0 to 1.5 g calcium, and approximately 650 mg phosphorus, while they were maintained on a 40 kcal/kg energy intake program. Group 3 served as controls and received an unrestricted diet. The control group diet contained 70 g protein, 900 mg phosphorus and 800 mg calcium. A significantly slower rate of renal failure was observed in

the protein restricted groups (Group 1 and Group 2) when compared to the control group.

Although a protein restricted diet has shown favorable effects on renal function, prolonged intake of a low protein diet may cause other problems. In a continuation of their study, Guarnieri et al. (1989) reported that there was a significant loss of muscle protein as well as a decrease in serum albumin and serum transferrin levels after an additional 5 years of dietary manipulation. This study suggested that the nutritional status of the patients tended to worsen after 5 or more years. It was not concluded from this study that the dietary protein restriction alone could cause protein wasting since energy intake was shown to be lower than prescribed. However, muscle wasting has been frequently reported as a response to protein restriction in CRF patients (Young et al, 1986; Schoenfeld et al, 1983; Wolfson et al., 1984).

Albuminuria has usually been associated with renal dysfunction. Nath and coworkers (1986) examined the effect of dietary protein restriction on renal function by measuring the glomerular filtration rate and the fractional clearances of albumin and IgG. Rats used in this study were made uremic by a two-thirds subtotal nephrectomy. The animals were then fed either a 6% or a 20% protein diet for three months.

Before the animals were given different levels of protein in their diets, the authors made sure that both groups exhibited similar levels of renal dysfunction. Urinary total protein concentrations in rats maintained on a 20% protein diet were significantly greater than in those fed a 6% protein diet. A significantly lower fractional clearance of albumin and IgG was also noticed in animals receiving a 6% protein diet. The authors (Rosenberg et al., 1987) suggested that the preservation of the size-selective properties of glomerular capillaries was the reason for why lower fractional clearances of albumin and IgG decreased when dietary protein intake was reduced from 2 to 0.55 g/kg/day for 11 days in patients with glomerular diseases.

In study by Olmer et al. (1989), the concentration of urinary albumin was lower in those patients with nephrotic syndrome who ate a low protein diet as compared to patients who ate a high protein diet. There were no significant differences in total protein or serum urea nitrogen concentrations of the two groups of patients. Moreover, the creatinine clearances significantly lower in the low protein diet patients when compared to the high protein diet subjects.

Chan et al. (1988) studied the effects of high protein intake on renal function and proteinuria in patients with glomerular disease. They indicated that the fractional

clearance of albumin and IgG declined while the GFR and RPF rate increased. Kaysen et al. (1986) examined the effect of high protein intake on GFR and the fractional clearance of albumin. They changed the protein levels in the diets of normal rats from 8.5% to 40%. A rise in GFR and an increase in the fractional clearance of albumin were observed.

Ando et al (1989) studied the effects of acute protein intake on renal function in human subjects. The feeding of a high protein meal consisting 1.2 to 1.5 g protein/kg body weight in the form of cooked beef resulted in a rise of both creatinine and PAH clearance and was also associated with an elevation in plasma glucagon levels. They suggested that the rise in the glucagon levels may indicate that glucagon mediates the augmentation of renal function.

Recently, De Keijzer and Provoost (1990) examined the effects of low (12%) and high (36%) protein diets on the renal function of Fawn Hooded rats. The high protein diet was found to raise the GFR and urinary protein excretion whereas systolic blood pressures were found to remain the same. They also reported that the low protein diets were associated with a prolonged period of altered renal function, limited proteinuria and an increased life span. The authors concluded that the low protein diet slowed down the development of renal failure but did not prevent it.

Alvestrand et al. (1983) treated 17 patients who had well-defined rates of creatinine clearance before beginning a regimen of 15-20 g of mixed-quality protein plus an EAA supplement which was provided as tablets (containing 1.8 to 2.8 g of nitrogen). The patients were followed for an average of 355 days. Progression of renal disease was apparent even though many patients had been consuming a diet which was restricted to 0.6 g protein/kg/day. Only 3 patients did not show a substantial slowing of the progression of their renal disease. Interim reports from an ongoing prospective, randomized trial by the same group have cast doubt on what influence diet exerts on these results and raises the possibility that better blood pressure control and closer follow up of the patients can slow the progression of renal disease (Bergstrom et al., 1989). Slowing the progression of renal disease appeared to be related to a very small, but significant reduction in diastolic blood pressure; loss of function was greater in patients with proteinuria. The relationship between blood pressure and renal disease appeared to exist at levels well within the range considered normal so more stringent blood pressure control than traditionally accepted could exert a favorable effect on renal function. Although 57 patients were initially enrolled in the study, only 5 subjects in the protein restricted group and 9 in the control group satisfied the requirements of the

study. The authors concluded that rates of disease progression before and after randomization did not differ, nor could an effect of dietary protein restriction on progression be discerned. The average intake of protein in the EAA-treated group was higher than prescribed and although significant, the difference from the intake of the unrestricted subjects (0.65 g/kg/day vs 0.86 g/kg/day) was not striking.

In summary, an EAA regimen can be effective in controlling the symptoms of CRF, but any benefit regarding the progression of disease remains uncertain. The major advantage of the EAA regimen over a conventional low-protein diet is the greater variety of food available, which might make the regimen more acceptable. It appears that the EAA diet has little or no advantage in terms of improved nitrogen conservation when compared with a conventional low-protein diet.

Very low protein diets supplemented with ketoacids also have been assessed. Barsotti and colleagues (1981) examined renal disease progression in 31 patients treated with a diet containing 0.5 g/kg of high quality protein and 600 mg of phosphorus/day; all showed a linear decline in creatinine clearance despite dietary protein restriction. Twelve subjects were then treated for 10 to 15 months with a diet containing approximately 0.2 g protein/kg/day, 300 mg/day of

phosphorus plus a ketoacid-amino acid mixture. Eleven of the 12 had a marked decrease in the loss of creatinine clearance; only one patient continued to lose renal function at the same rate. The same group reported their experience with this regimen in a larger number of patients whose renal insufficiency progressed despite therapy with a conventional low-protein unsupplemented diet. In patients who were compliant with their ketoacid regimen, the decline in creatinine clearance was halted. Patients who were less compliant (average urinary nitrogen appearance, 6.3 g N/day) continued to lose renal function.

Mitch et al. (1984) evaluated a regimen containing 20 to 30 g of protein supplemented with a mixture of the basic amino acid salts of ketoacids using 25 patients. Among 17 patients who demonstrated well-defined rates of disease progression, 10 (59%) exhibited a significantly slower rise in the serum creatinine level during long-term treatment before the serum creatinine reached 8 mg/dl whereas 6 had stable serum creatinine. This regimen had a more favorable outcome if it was initiated relatively early in the course of renal failure.

All these studies suggesting a beneficial effect of protein and/or phosphorus restriction are based on creatinine measurements, which can be unreliable estimates of progression of renal function. These results also have not

been adequately compared to ketoacid-based regimens, EAA regimens or with low protein, unsupplemented diets. Walser et al. (1987) evaluated 12 patients who were given a regimen containing 0.3 g protein/kg/day plus EAA; all showed a progressive decline in creatinine clearance. After the EAA supplements were changed to ketoacid supplements, renal failure in all patients whose serum creatinine level exceeded 7.5 mg/dl continued to progress. In contrast, 6 of 7 patients whose serum creatinine level was between 6.0 and 7.4 mg/dl at crossover had stable GFR values during the one to two year followup; one patient who was noncompliant had to go on dialysis.

In summary, despite many provocative observations, it is not established whether dietary protein and/or phosphorus restriction can slow down the progression of CRF of whether a ketoacid-based regimen confers a therapeutic advantage.

Rosenberg and coworkers (1987) measured several humoral factors in renal patients and reported that the mean values of plasma renin activity were elevated either in the supine of standing position when the subjects were on a high protein diet. Also, according to their study, the urinary excretion of prostaglandin E (PGE) and 6-keto prostaglandin F-1 alpha was significantly higher during the period of high protein intake. From these results, they suggested that the cause of

the elevated urinary prostaglandin excretion might be mediated by the rise of plasma renin activity.

Vanrenterghem et al. (1988) reported that the response of renal function parameters, such as creatinine, inulin and paraaminohippuric acid (PAH) clearance, to an acute oral protein load were attenuated by a pretreatment with indomethacin, which is a prostaglandin synthesis inhibitor. They also measured urinary PGE2 excretion and found that pretreatment with indomethacin partially inhibited the rise of urinary PGE2 after the acute protein load. They concluded that renal prostaglandins play a role in the renal hemodynamic response to protein loading.

In patients with chronic glomerular disease, fractional albumin and globulin excretion declined and glomerular size perselectivity improved following short-term dietary protein restriction of 11 to 14 days (Rosenberg et al., 1987). In these studies, reduction in proteinuria and improvement in permselectivity were evident prior to any possible morphologic amelioration. Similarly, when rats that had undergone subtotal nephrectomy three months earlier were subjected to a 14 day period of reduced protein intake, fractional clearances of albumin and globulin declined in the absence of improved glomerular morphology (Nath et al., 1986). These data suggest that dietary protein restriction can directly improve glomerular size permselectivity by a

mechanism independent of its protective effect on glomerular structure.

Neugarten et al. (1983) have previously demonstrated that long-term dietary protein restriction reduced proteinuria and ameliorated glomerular injury in patients with a mild form of nephrotic serum nephritis. These studies did not distinguish between functional versus structural changes as the cause of the reduced proteinuria. Neugarten et al. (1991) studied a more severe model of nephrotoxic serum nephritis in the uninephrectomized rats characterized by hypertension, heavy proteinuria and renal insuffiency. Protein restriction of three days duration promptly reduced proteinuria. Glomerular size permselectivity, impaired in nephritic rats maintained on a 24% protein diet, was partially restored by short-term dietary protein restriction in association with a decrease in the fraction of glomerular filtrate permeating the shunt pathway. Fractional clearance of protein declined by nearly 50% within three days after the institution of a low protein diet as compared to a complete remission of proteinuria following three months of dietary protein restriction (Neugarten et al., 1983).

At variance with Neugarten's findings, several studies have suggested that glomerular basement membrane pore structure and barrier function are not influenced by the manipulation of dietary protein content (Remuzzi et al, 1987;

Chan et al., 1988). Remuzzi et al. (1987) evaluated proteinuria and glomerular size selectivity in rats made nephrotic with adriamycin and fed 20% or 35% protein diets for 21 to 45 days. Nephrotic rats fed the 20% protein diet showed reduced GFR and elevated fractional clearances of large neutral dextrans. In those fed the higher protein diets, proteinuria increased further and the GFR rose. Fractional clearances of neutral dextrans were not influenced, however, by protein feeding. These investigators suggested that the increase in proteinuria following high protein feeding is merely a consequence of an increased filtered load of protein. However, since proteinuria increased an average of 71% in rats fed the high protein diets whereas GFR rose only 43% and serum protein concentration remained constant in the controls, this conclusion may not be justified.

CHAPTER III

EXPERIMENTAL DESIGN AND METHODOLOGY

Selection of Animal Model

Experimentally induced renal failure in animals has now been performed for more than a century. The most commonly used approach for the induction of chronic renal failure has been either ligation of the renal artery branches or surgical removal of renal parenchyma (partial nephrectomy).

The first partial nephrectomy in rats was performed in 1898 by Tuffier. In his procedure, he removed one kidney, and a portion of the other. He observed no changes in the volume of urea as well as in the water eliminated. A year later, Bradford (1899) used surgical excision to reduce the renal mass in dogs and studied various aspects of renal function. During the following years partial nephrectomies were performed on cats (Allen, 1925), rabbits (Anderson, 1924), and sheep and goats (Bainbridge, 1907). The use of these species in renal function studies has been criticized since their dietary habits and digestive mechanisms do not closely resemble those of man. Chanutin and Ferris (1932) developed a procedure that became known as the 5/6 nephrectomy in which

sufficient renal tissue destruction could be induced surgically in rats. This animal model was found to closely mimic chronic renal disease found in humans.

In 1966, Morrison published a detailed description of a 5/6 nephrectomy procedure in rats. Currently, a refined version of this procedure in which removal of the right kidney and surgical infarction of the two branches of the renal artery of the left kidney has been successfully developed. Animals that were subjected to this surgical method produced symptoms which were similar to those found in humans with chronic renal failure. Therefore, this procedure has been used widely in renal failure studies.

Experimental Design

The experimental design is outlined in Figure 1. Sixty-three (63) male Sprague-Dawley rats (Sasco Inc., Houston, Texas), weighing approximately 125-140 grams were used for this study. The animals were housed individually in metabolic cages at the Texas Woman's University animal facility (Denton, Texas). Approval for this study was granted by the Texas Woman's University Animal Care Committee (Appendix A). During the 4 day acclimation period, the animals were fed the Teklad rat diet and given free access to water. Following acclimation, sixty three animals were subjected to

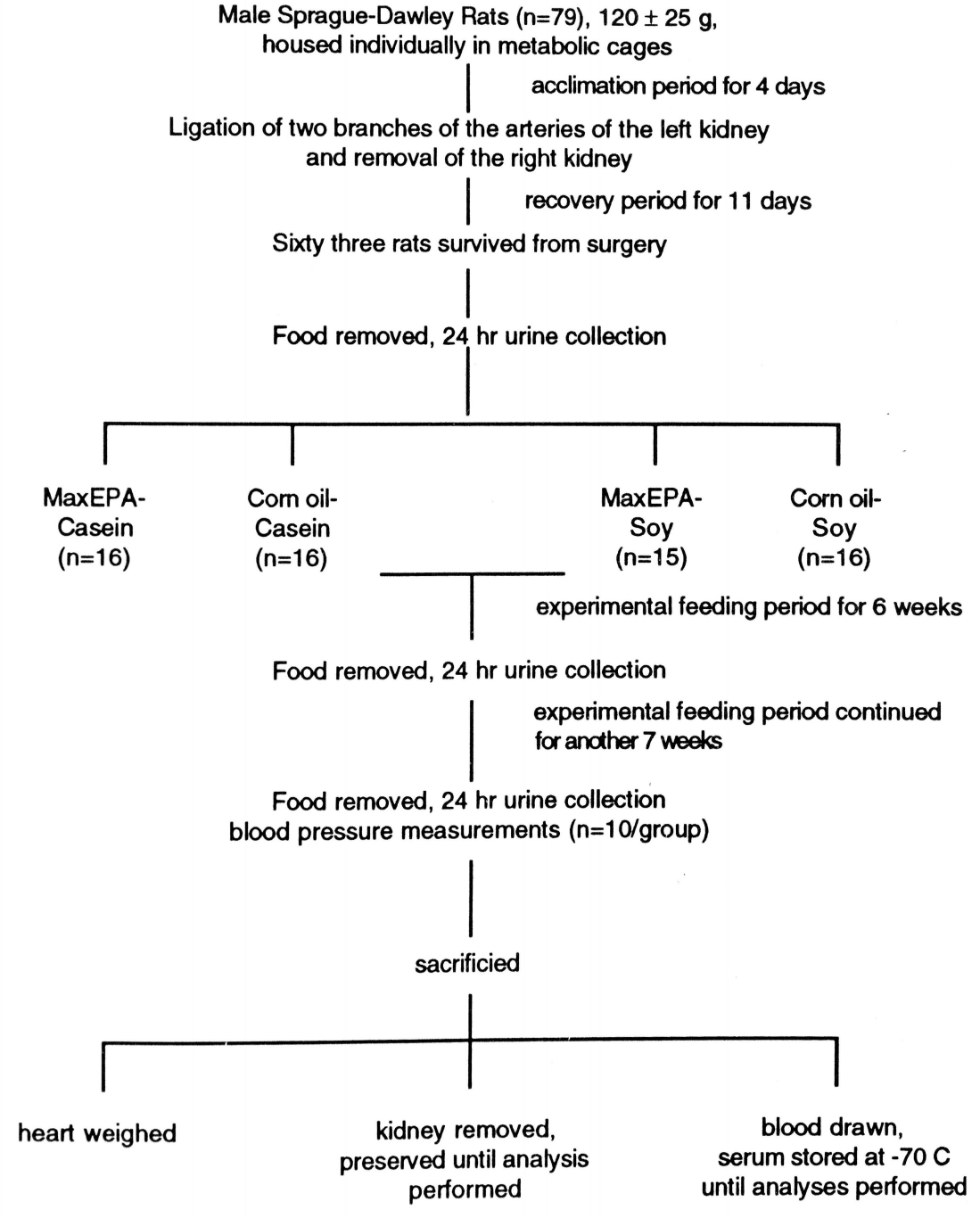

Figure 1. Experimental Design

partially nephrectomy by the removal of their right kidney and surgical infarction of their left kidney. This procedure is currently used at the University of Texas Southwestern Medical Center, Dallas, Texas (Appendix B). All animals were allowed to recover from surgery for 11 days during which time they received Teklad rat diet and water ad libitum. Those that survived from surgery were randomly assigned to one of the experimental diets. The diets contained the following main ingredients: a) 24% Casein - 5% MaxEPA oil (MOCAS), b) 24% Casein - 5% Corn oil (COCAS), c) 24% Soy - 5% MaxEPA oil (MOSOY), and d) 24% Soy - 5% Corn oil (COSOY). The experimental diets were isocaloric and isovitaminic (Table 1). The concentrations of fatty acids and amino acids in the diets are listed in Table 2 and 3, respectively. The experimental diets were based on the composition of the AIN-76A Semi-purified Rat Diet, however, they contained fat from different sources (either MaxEPA or corn oil) and also protein of different quality (either soy protein or casein). All experimental diets were formulated and pelleted by U.S. Biochemical Corporation, Cleveland, Ohio. Animals were fed their corresponding diets and provided water ad lib for 13 weeks. Body weights were measured weekly and food intake was determined every other day. During the last week of study period (week 13), blood pressures were taken from rats (n=10/group) using a tail cuff method (Appendix C).

TABLE 1

Chemical Composition of Experimental Diets[1]

Component	MaxEPA-Casein %	Corn oil-Casein %	MaxEPA-Soy %	Corn oil-Soy %
Moisture	8.3	7.5	8.3	6.8
Protein	23.6	22.7	23.1	22.8
Fat	4.7	4.7	4.4	4.8
Fiber	2.5	2.9	2.7	2.6
Ash	2.7	2.7	3.3	3.3
Sodium	0.12	0.12	0.42	0.42
Potassium	0.39	0.39	0.41	0.41
Calcium	0.46	0.46	0.54	0.52
Phosphorus	0.54	0.55	0.59	0.58
Magnesium	0.054	0.054	0.073	0.072
Carbohydrates	58.2	59.5	58.2	59.7
Calories per 100 g	370	371	365	373

[1]Analysis performed by Pope Testing Lab, Dallas, Texas.

TABLE 2

Fatty Acid Composition of Experimental Diets[1]

Fatty Acids	MaxEPA-Casein (% of TFA[2])	Corn oil-Casein (% of TFA)	MaxEPA-Soy (% of TFA)	Corn oil-Soy (% of TFA)
Lauric	0.1	<0.1	<0.1	<0.1
Myristic	12.7	0.1	8.8	<0.1
Myristoleic	0.6	0.1	0.5	<0.1
Palmitic	27.0	11.0	21.4	10.5
Palmitoleic	10.3	0.1	11.3	<0.1
Stearic	4.9	2.5	5.1	2.1
Oleic	13.7	26.6	16.7	27.0
Linoleic	4.9	58.1	5.1	59.2
Linolenic	0.8	1.4	<0.1	1.2
Arachidonic	0.7	<0.1	0.9	<0.1
Eicosapentanoic acid	16.0	<0.1	17.8	<0.1
Docosahexanoic acid	8.3	<0.1	12.4	<0.1

[1]Analysis performed by NET Midwest Inc., Chicago, IL.
[2]Total Fatty Acids

TABLE 3

Amino Acid Composition of Experimental Diets[1]

Amino Acid (mg/g diet)	Diet Group			
	MaxEPA-Casein	Corn oil-Casein	MaxEPA-Soy	Corn oil-Soy
Aspartic	17.71	16.79	21.57	26.83
Threonine	10.32	9.76	6.99	8.35
Serine	11.10	9.75	7.57	9.02
Glutamic	57.50	54.24	37.72	45.74
Glycine	4.66	4.40	7.93	9.71
Alanine	16.43	7.14	8.11	9.90
Valine	17.39	16.48	10.54	12.68
Cystine	0.75	0.81	1.56	1.88
Methionine	8.46	8.66	4.71	5.19
Isoleucine	13.55	13.01	9.74	11.75
Leucine	23.47	22.70	15.56	18.90
Tyrosine	10.35	9.83	5.44	6.63
Phenylalanine	12.59	11.96	9.92	12.11
Lysine	21.34	19.76	12.27	14.85
Histidine	6.74	6.27	4.35	5.10
Arginine	8.53	8.15	13.47	16.44
Proline	28.30	26.84	10.53	13.21

[1]Analysis performed by Genetic Screening and Counselling Services, Denton, Texas

Sample Collection for Analysis

Twenty four hour urine collections were performed three times for each animal: a) before the experimental feeding period (day 0), b) six weeks after the experimental feeding was initiated, and c) at the end of study period (after 13 weeks of experimental diet consumption) (Figure 1). During these urine collections animals were provided tap water ad libitum, however, food was removed to prevent the inclusion of the food into the urine samples. The urine samples were stored at -70°C for future analyses. At the end of study period, blood was drawn via cardiac puncture. Blood samples were then allowed to clot for about 15 minutes at room temperature and centrifuged at 4,000 rpm. Serum was obtained after centrifugation and stored at -70°C until biochemical analyses could be performed.

The hearts were discarded after being weighed. The remnant kidneys were removed, weighed, and preserved in formalin solution and sent to Southwestern Medical Center for histopathological analysis (Dallas, Texas).

Biochemical Analyses and Methodologies

Biochemical analyses are outlined in Figure 2. Serum samples were sent to Dallas Veterinary Laboratory (Dallas, Texas) for determination of total protein, albumin,

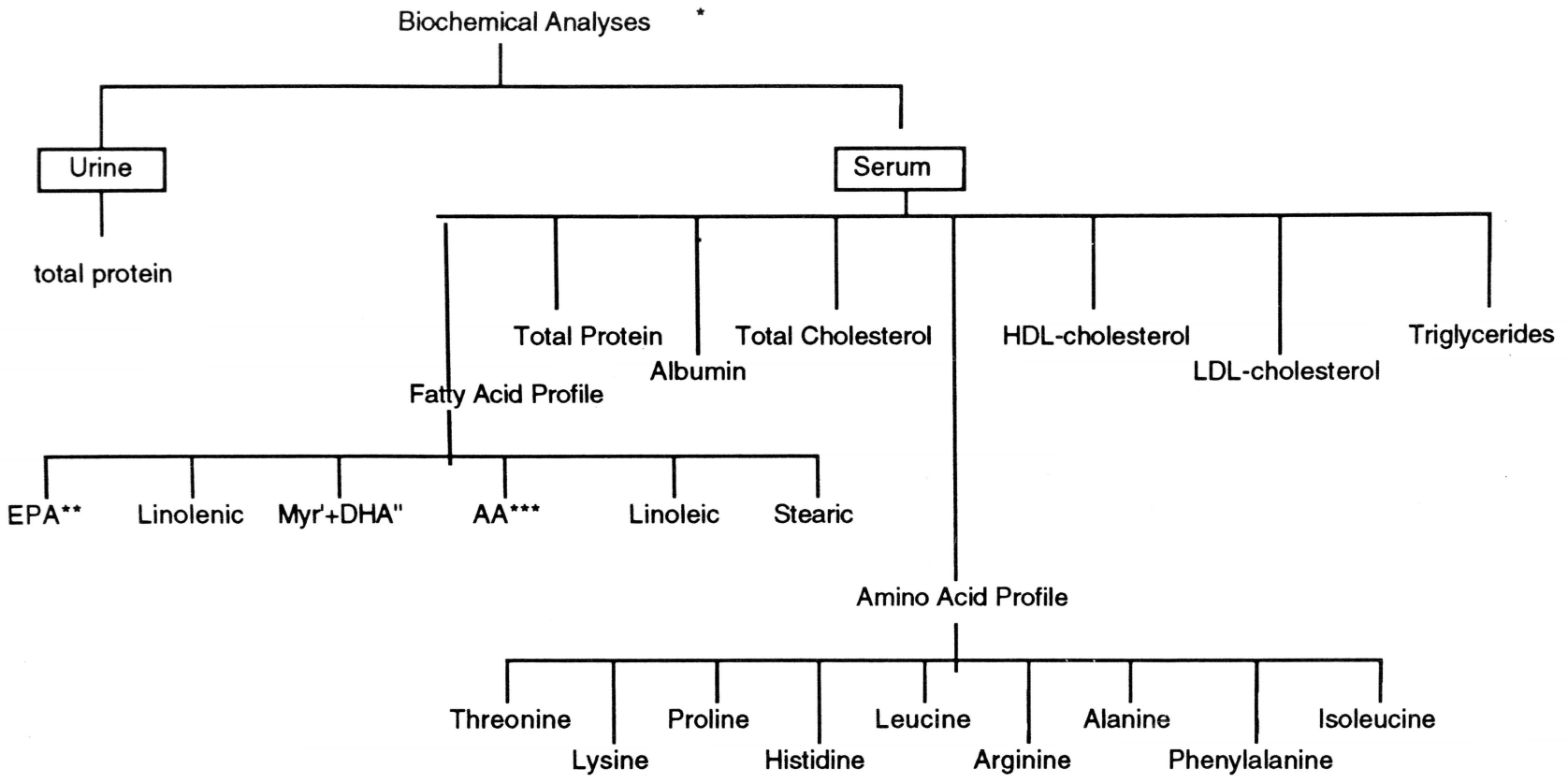

Figure 2. Biochemical Analyses Performed

* Only samples from animals that survived until the end of the study period were used
** Eicosapentanoic Acid
*** Arachidonic Acid
' Myristic
" Docohexanoic Acid

creatinine, triglyceride, and total, HDL-, and LDL-cholesterol concentrations. Amino acid profiles were determined using an Amino Acid Autoanalyzer (performed by Genetic Screening and Counseling Services, Denton, Texas). Serum fatty acid [eicosapentanoic acid (EPA), docosahexanoic acid (DHA)/myristic, linolenic acid (LNA), linoleic acid (LA), arachidonic acid (AA)] concentrations were determined using reversed-phase high performance liquid chromatography, according to the modified method of Miwa et al., 1986 (Appendix D) and was performed by this investigator.

Statistical Analysis

Data from animals that survived until the end of study period were analyzed using ANOVA to determine differences between groups. When significant differences were detected using ANOVA, a post hoc test was performed. Statistical analysis was performed using the Statistical Package for the Social Sciences Series X (SPSSX).

CHAPTER IV

RESULTS

The primary objective of this study was to determine the effects of dietary manipulation, especially the combinations of certain types of dietary polyunsaturated fatty acids and proteins of different quality, on the progression of renal lesions as well as on those which are biochemical parameters commonly associated with renal problems in nephrectomized rats.

The specific objective of this study was to observe the effects of the designed manipulations on certain serum constituents such as total protein, albumin, creatinine, triglycerides and total, HDL- and LDL-cholesterol. Serum amino acids (arginine, lysine, leucine, proline) and fatty acids (EPA, DHA/myristic, linolenic acid, linoleic acid, and arachidonic acid) concentrations were also measured. In addition, urinary total protein and blood pressures were also determined. Finally, a histopathological examination of remnant kidneys was performed to ascertain the effects of dietary fatty acids and proteins on renal cellular tissue damage.

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Body Weight and Food Intake of Nephrectomized Rats

Body weight was recorded weekly throughout the study period. The mean body weight of animals in each of the experimental groups after 13 weeks of feeding was 470.4 ± 12.6 g for the MOCAS group (n=13), 427.7 ± 14.5 g for the COCAS group (n=16), 439.3 ± 15.5 g for the MOSOY group (n=11), and 473.8 ± 9.3 g for the COSOY group (n=14). No significant differences were found in body weights. There was a slightly decrease in weight gain at the end of study period in all groups (Figure 3).

Data of food intake showed that the animals consumed similar amounts of food at the end of study period. There was a slightly increase in the food intake of the MOSOY group but it was not statistically significant when compared to the other groups (Figure 4).

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Total Protein and Albumin Concentrations

The MOSOY group exhibited the highest protein concentration (5.9 ± 0.1 g/dL) and the COSOY group exhibited the lowest serum total protein concentration (5.8 ± 0.1 g/dL). The serum total protein concentrations of the MOCAS and COCAS groups were 5.8 ± 0.1 g/dL and 5.8 ± 0.1 g/dL, respectively. No significant differences were observed

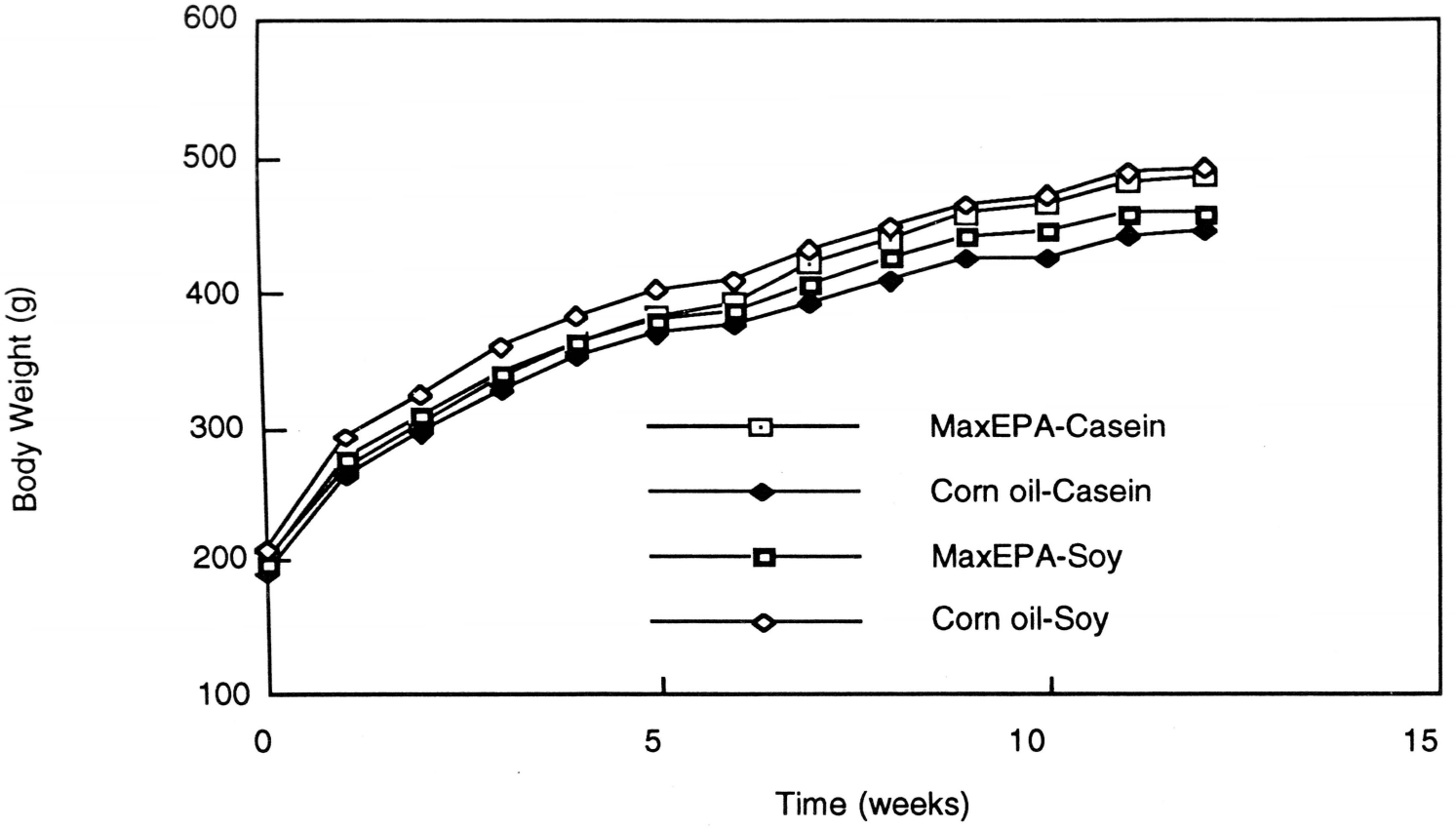

Figure 3. Effect of dietary polyunsaturated fatty acids and proteins on body weight* of nephrectomized rats.

* Values are means

**Study period was 13 weeks. Only data from the animals that survived until the end of the study period were included.

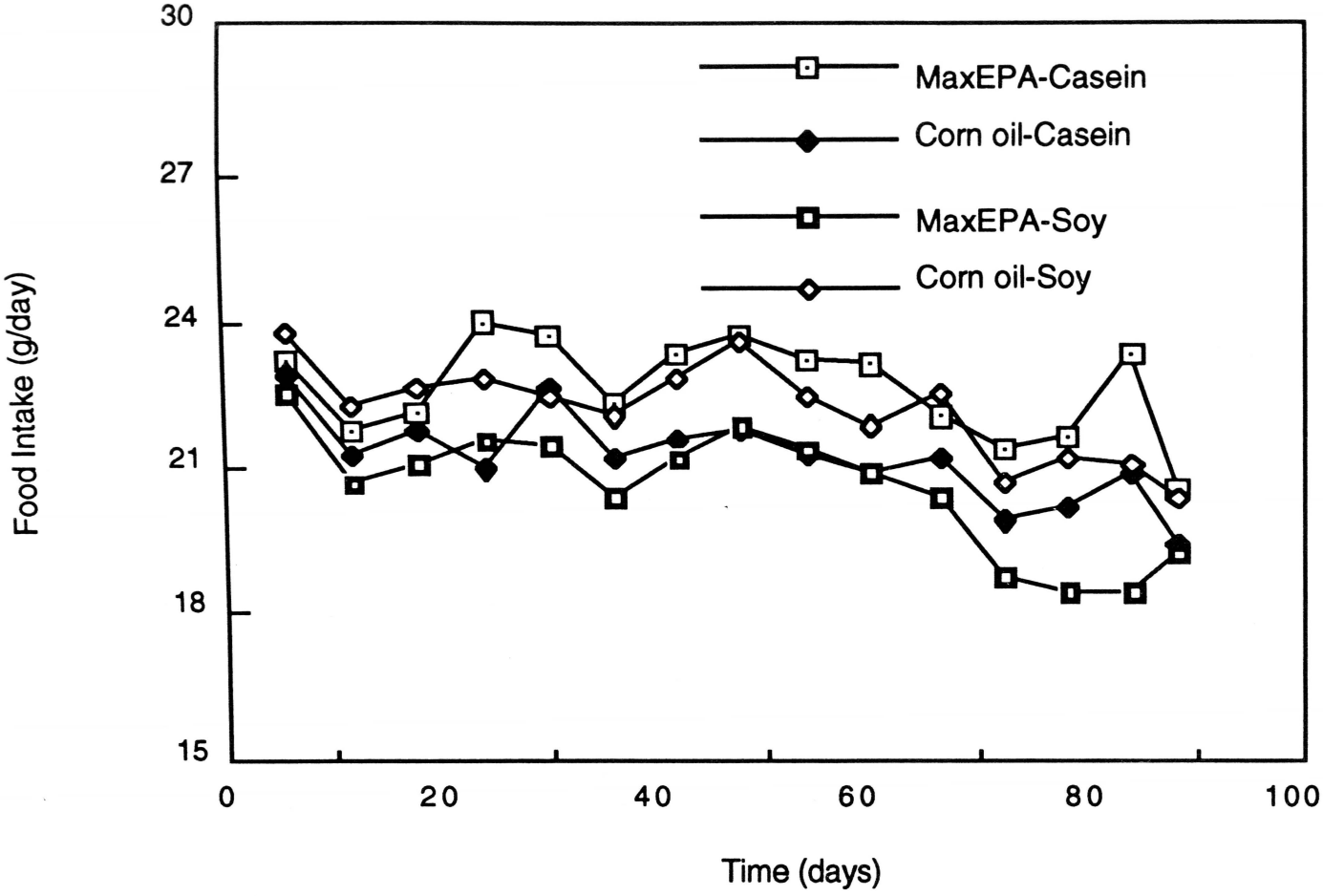

Figure 4. Effect of dietary polyunsaturated fatty acids and proteins on food intake* of nephrectomized rats.

* Values are means

($p<0.05$) in the serum total protein concentrations (Figure 5).

Determination of serum albumin concentrations showed that there were no significant differences ($p<0.05$) between groups. Rats fed the MOCAS diet exhibited slightly higher serum albumin concentrations when compared to the other experimental groups, however, the increase was not significant. The mean serum albumin concentrations of the MOCAS, COCAS, MOSOY, and COSOY diets were 1.6 ± 0.1 g/dL, 1.5 ± 0.0 g/dL, 1.5 ± 0.1 g/dL, and 1.5 ± 0.1 g/dl, respectively (Figure 6).

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Creatinine Concentrations

Rats fed the COSOY diet exhibited the lowest serum creatinine concentrations (0.9 ± 0.0 mg/dL), followed by the MOCAS group (1.2 ± 0.3 mg/dL), the COCAS group (1.3 ± 0.3 mg/dL) and the MOSOY group with the highest value (1.4 ± 0.4 mg/dL) (Figure 7). No significant differences in serum creatinine concentrations were detected between groups.

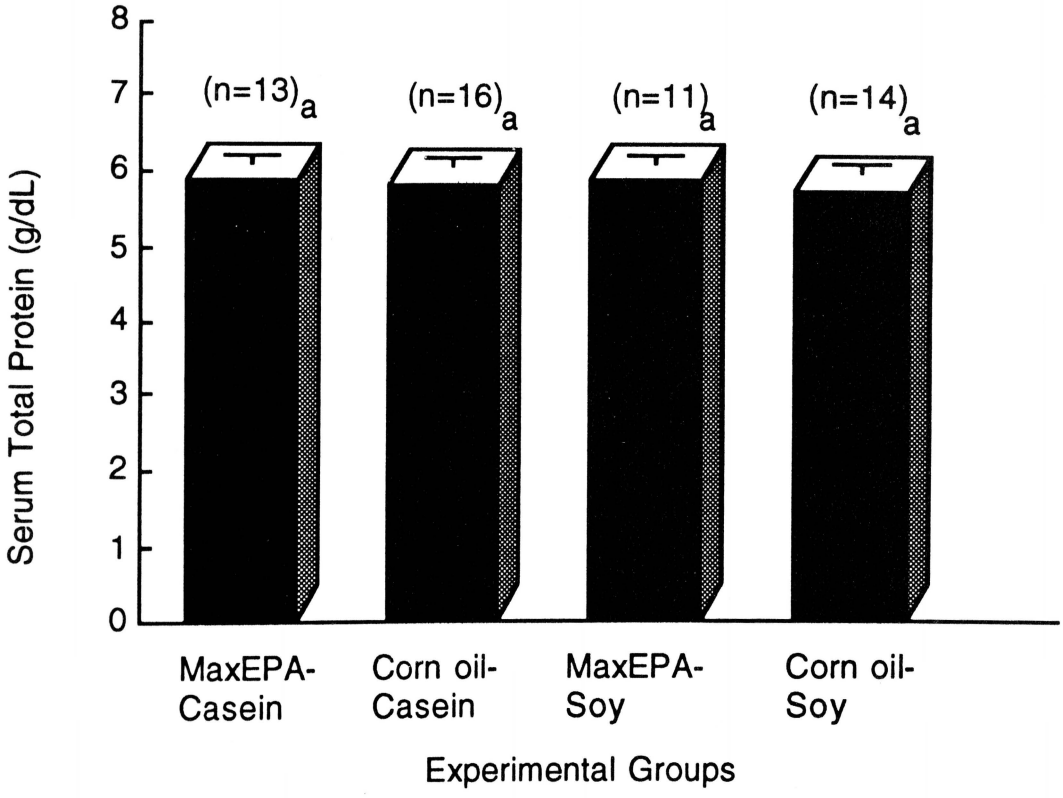

Figure 5. Effects of dietary polyunsaturated fatty acids and proteins on serum total protein concentrations*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences ($p<0.05$)

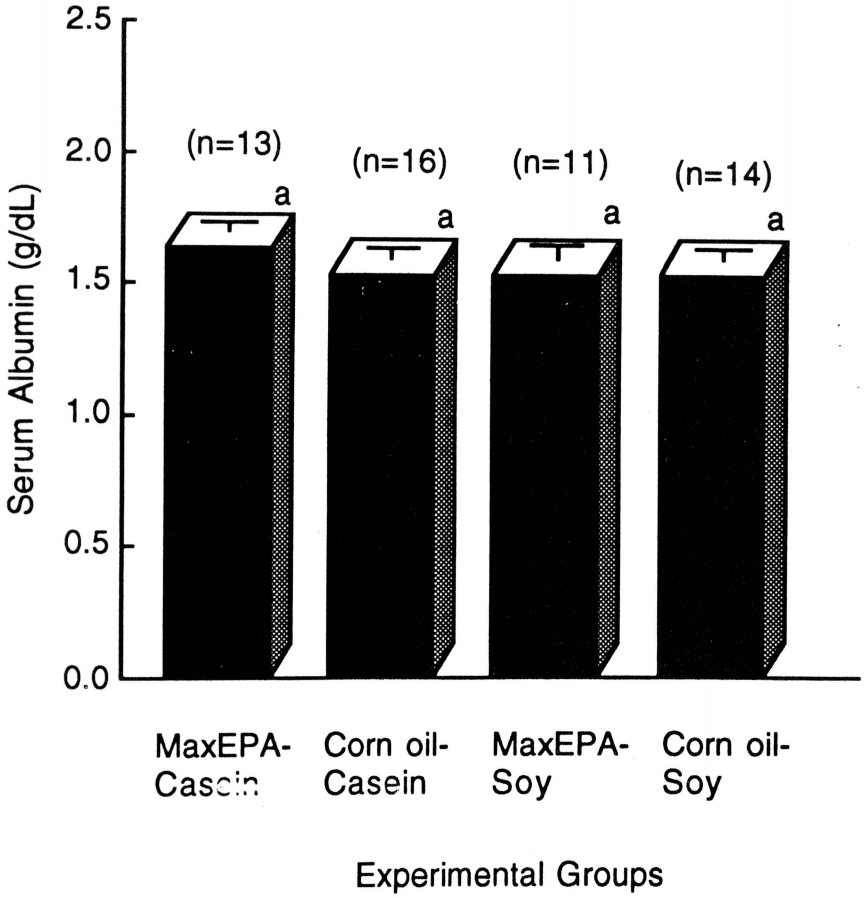

Figure 6. Effects of dietary polyunsaturated fatty acids and proteins on serum albumin concentrations*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences at $p<0.05$

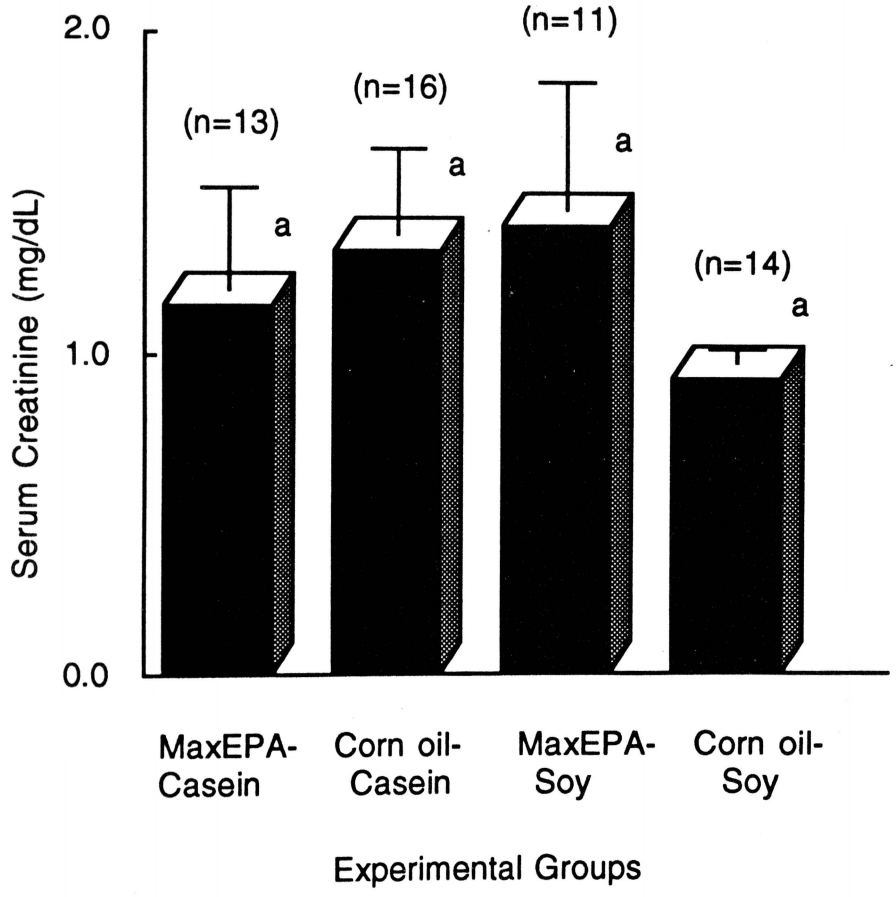

Figure 7. Effects of dietary polyunsaturated fatty acids and proteins on serum creatinine concentrations*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences at $p<0.05$

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Triglyceride Concentrations

Determination of serum triglyceride concentrations showed that the MO-fed rats had non-significantly lower serum triglyceride concentrations when compared to the CO-fed rats. Rats fed COCAS diet exhibited the highest serum triglyceride concentration (130.2 ± 17.7 mg/dL), followed by the COSOY group (112.4 ± 16.1 mg/dL), the MOSOY group (110.8 ± 23.7 mg/dL), and the MOCAS group (91.3 ± 14.9 mg/dL) (Figure 8).

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Total Cholesterol Concentrations

Figure 9 shows that CO-fed rats had higher serum total cholesterol concentrations when compared to those fed the MO diets. Casein lowered serum total cholesterol when fed concomitantly with MO. The combined casein and corn oil diets contributed to significantly higher serum total cholesterol concentrations than did the diets containing casein and MaxEPA oil (151.7 ± 16.0 mg/dL vs 81.2 ± 11.3 mg/dL). When fed concomitantly with soy protein, MaxEPA oil exerted a cholesterol-lowering effect as compared to Corn oil feeding (116.3 ± 25.2 mg/dL vs 139.6 ± 12.5 mg/dL).

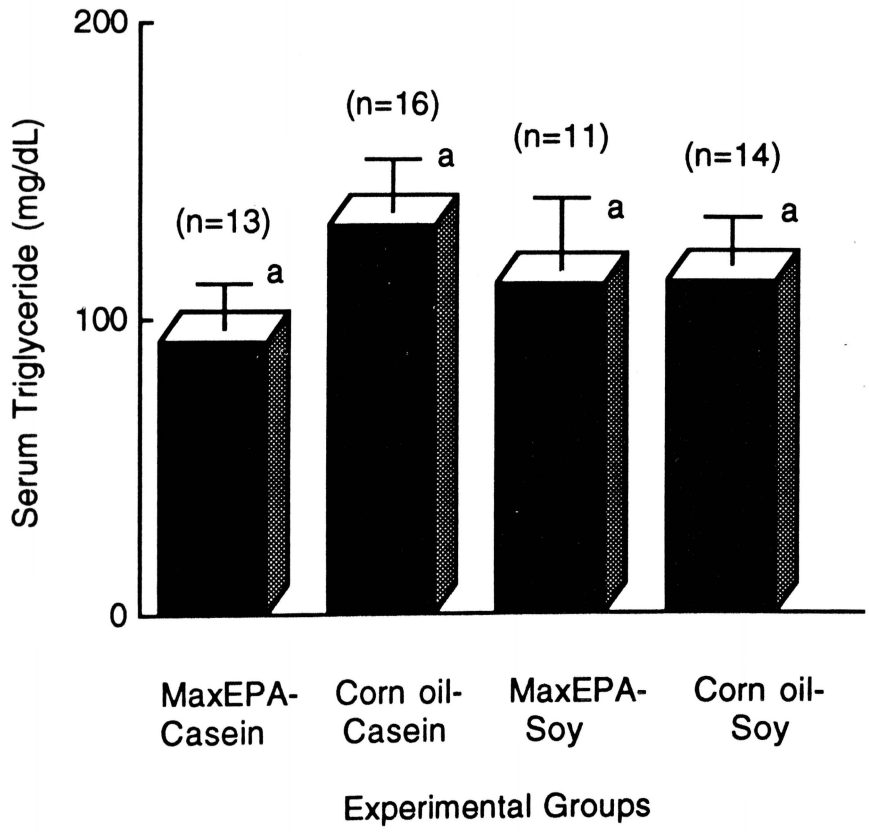

Figure 8. Effects of dietary polyunsaturated fatty acids and proteins on serum triglyceride concentrations*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences at $p<0.05$

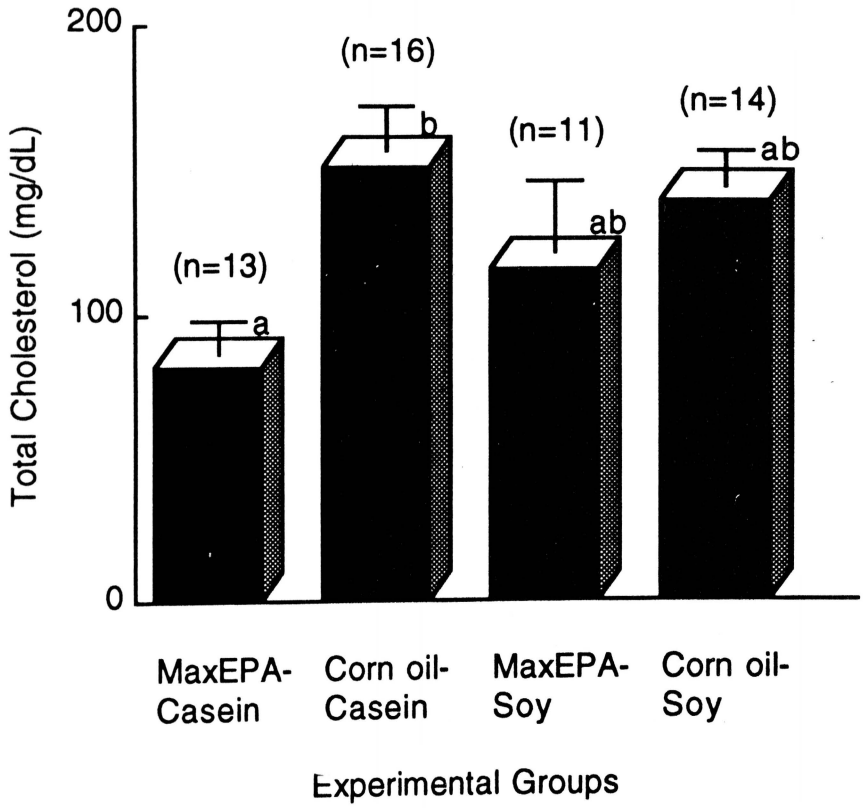

Figure 9. Effects of dietary polyunsaturated fatty acids and proteins on serum total cholesterol concentrations*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts denote significant differences at $p<0.05$

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum HDL-Cholesterol Concentrations

Serum HDL cholesterol concentrations of rats fed MaxEPA oil diets were lower than those fed corn oil diets. The COCAS diet contributed to a significantly higher serum HDL concentration when compared to the MOCAS diet (102.4 ± 9.2 mg/dL vs 49.4 ± 8.3 mg/dL). Soy protein exerted an HDL-lowering effect when fed concomitant with MaxEPA oil (MOSOY vs COSOY: 76.3 ± 16.1 mg/dL and 98.8 ± 7.1 mg/dL, respectively) (Figure 10).

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum LDL Cholesterol Concentrations

Evaluation of serum LDL-cholesterol concentrations indicated that no significant differences between the four experimental groups. Rats fed the COCAS diet exhibited a markedly higher concentration of serum LDL cholesterol (30.0 ± 8.4 mg/dL) when compared to the other three groups. Feeding MOCAS diet yielded the lowest serum LDL cholesterol values (12.8 ± 3.7 mg/dL), followed by the MOSOY group (17.4 ± 5.8 mg/dL) and the COSOY group (18.0 ± 4.4 mg/dL) (Figure 11).

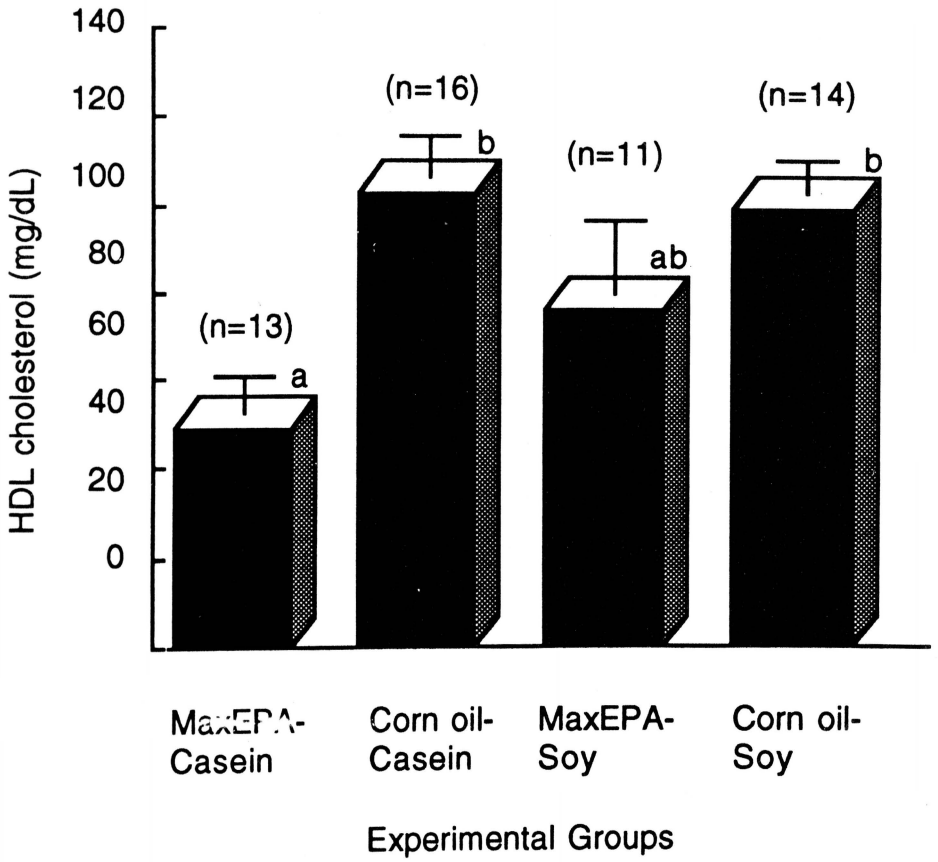

Figure 10. Effects of dietary polyunsaturated fatty acids and proteins on serum HDL cholesterol concentrations*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences at $p<0.05$

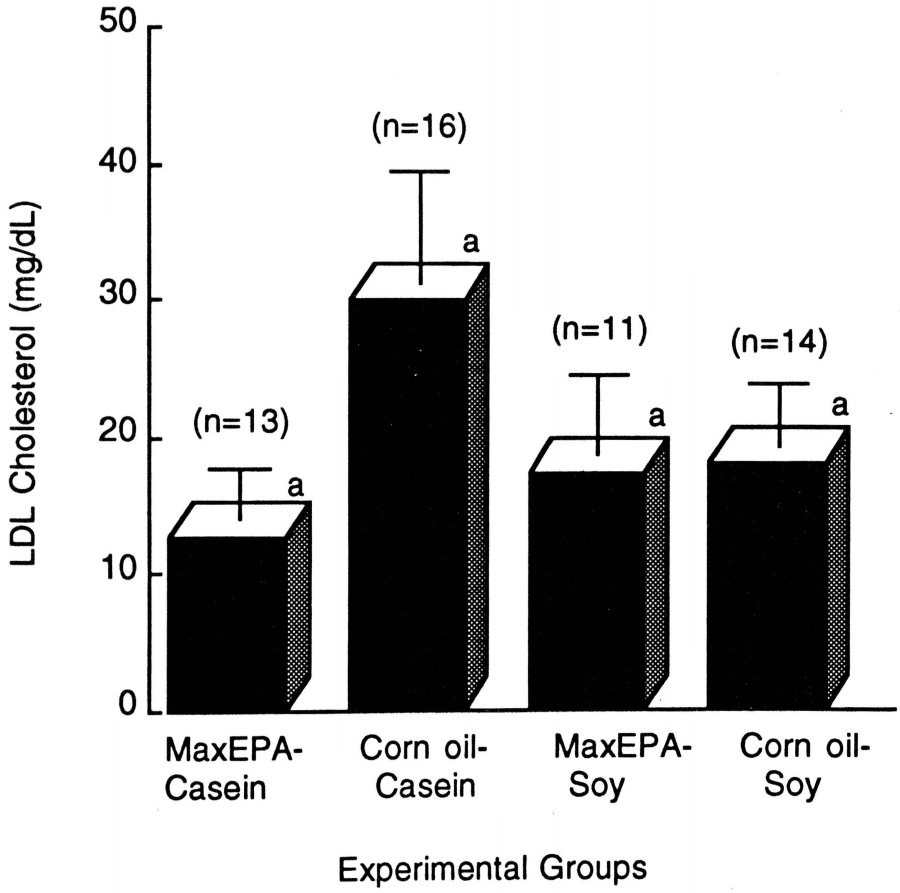

Figure 11. Effects of dietary polyunsaturated fatty acids and proteins on serum LDL cholesterol concentrations*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences at $p<0.05$

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Amino Acid Profiles

Serum concentrations of threonine, serine, alanine, leucine and proline were significantly higher ($p<0.05$) in casein-fed animals than in soy-protein fed ones (Table 4). The serum concentration of arginine, on the other hand, was higher ($p<0.05$) in the soy-fed groups than the casein-fed groups. The serum concentrations of the remaining amino acids were not significantly different among groups (Table 4).

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Fatty Acid Profiles

Determination of serum eicosapentanoic acid (EPA) concentrations showed that MaxEPA oil-feeding contributed to significantly higher ($p<0.05$) concentrations of EPA when compared to corn oil-feeding (Table 5).

Docosahexanoic acid (DHA) and myristic acid had similar retention times when analyzed using HPLC. For that reason, the concentrations of these two fatty acids were reported together. Serum DHA+myristic acid concentrations were significantly higher ($p<0.05$) in the MaxEPA-fed animals than in those fed corn oil (Table 5).

TABLE 4

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Amino Acid Profiles of Nephrectomized Rats[1,2]

Amino Acid (μmol/ml)	Diet Groups			
	MaxEPA-Casein	Corn oil-Casein	MaxEPA-Soy	Corn oil-Soy
Taurine	0.45±0.04^{a}	0.46±0.05^{a}	0.64±0.19^{a}	0.45±0.03^{a}
Aspartic	0.04±0.00^{a}	0.05±0.01^{ab}	0.07±0.01^{b}	0.06±0.00^{ab}
Threonine	0.41±0.02^{a}	0.46±0.02^{a}	0.32±0.03^{b}	0.33±0.01^{b}
Serine	0.43±0.02^{a}	0.44±0.02^{a}	0.32±0.03^{b}	0.32±0.01^{b}
Glutamic	0.31±0.02^{a}	0.30±0.03^{a}	0.22±0.04^{ab}	0.19±0.01^{b}
Glycine	0.58±0.03^{a}	0.54±0.02^{ab}	0.47±0.05^{ab}	0.45±0.01^{b}
Alanine	0.89±0.04^{a}	0.89±0.03^{a}	0.66±0.05^{b}	0.65±0.02^{b}
Valine	0.23±0.01^{a}	0.24±0.01^{a}	0.24±0.05^{a}	0.19±0.01^{a}
Cystine	0.01±0.00^{a}	0.01±0.00^{a}	0.05±0.04^{a}	0.02±0.00^{a}
Methionine	0.09±0.01^{ab}	0.09±0.00^{a}	0.07±0.01^{b}	0.08±0.00^{ab}
Isoleucine	0.16±0.01^{ab}	0.18±0.01^{a}	0.14±0.01^{b}	0.14±0.01^{b}
Leucine	0.25±0.01^{a}	0.27±0.01^{a}	0.18±0.02^{b}	0.18±0.01^{b}
Tyrosine	0.10±0.01^{a}	0.09±0.01^{a}	0.09±0.02^{a}	0.07±0.00^{a}
Phenylalanine	0.13±0.00^{a}	0.14±0.00^{a}	0.11±0.01^{a}	0.09±0.00^{a}
Lysine	0.53±0.02^{ab}	0.65±0.02^{a}	0.50±0.06^{b}	0.55±0.04^{ab}
Histidine	0.07±0.01^{a}	0.08±0.01^{a}	0.11±0.04^{a}	0.11±0.04^{a}
Arginine	0.09±0.02^{a}	0.09±0.03^{a}	0.14±0.03^{ab}	0.18±0.01^{b}
Hydroxyproline	0.06±0.00^{a}	0.07±0.00^{a}	0.05±0.00^{a}	0.06±0.01^{a}
Proline	0.25±0.01^{a}	0.28±0.01^{a}	0.17±0.02^{b}	0.21±0.01^{b}
Citrulline	0.09±0.00^{a}	0.11±0.01^{a}	0.09±0.01^{a}	0.10±0.01^{a}

[1]Analysis performed by Genetic Screening and Counselling Services, Denton, Texas

[2]Values are means ± SE. Values with different letter superscripts across the row indicate significant differences ($p<0.05$)

TABLE 5

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Serum Fatty Acid Profiles of Nephrectomized Rats[1]

Diet	Fatty Acids[2]				
	EPA (nmol/ml)	DHA/Myr (nmol/ml)	LNA (nmol/ml)	LA (nmol/ml)	AA (nmol/ml)
MaxEPA-Casein	604.65 ± 89.26[a]	912.47 ± 153.46[a]	<0.01[a]	266.62 ± 48.01[a]	462.68 ± 64.74[a]
Corn oil-Casein	<0.01[b]	248.96 ± 21.93[b]	87.49 ± 12.96[b]	1952.65 ± 238.28[b]	2290.14 ± 253.22[b]
MaxEPA-Soy	650.74 ± 103.43[a]	1399.25 ± 297.41[a]	<0.01[a]	867.71 ± 170.86[a]	610.08 ± 74.78[a]
Corn oil-Soy	<0.01[b]	250.34 ± 22.46[b]	130.54 ± 45.65[b]	1738.23 ± 180.32[b]	2033.90 ± 204.25[b]

1 Values are means ± SE. Values with different letter superscripts across the column indicate significant differences at $p<0.05$.

2 EPA = eicosapentanoic acid; DHA = docosahexanoic acid; LA = linoleic acid; Myr = myristic; LNA = linolenic acid; AA = arachidonic acid

Serum linolenic acid concentrations were significantly higher ($p<0.05$) in rats fed corn oil when compared to those fed MaxEPA oil (Table 5).

The animals that were fed corn oil had also significantly higher ($p<0.05$) concentrations of linoleic acid when they were compared to MaxEPA oil-fed rats (Table 5).

As with serum linoleic acid, the mean of serum arachidonic acid concentrations were significantly higher ($p<0.05$) in corn oil-fed animals than MaxEPA-fed groups. Both corn oil-fed groups had four times higher serum arachidonic acid concentrations when compared to MaxEPA-fed rats (Table 5).

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Remnant Kidneys

Histological examination of remnant kidneys were performed to determine the extent of renal tissue damage. A maximum of 100 glomeruli were examined in each specimen. Glomerulosclerosis was defined as the dissappearance of cellular elements from the tuft, the collapse of capillary lumens, and/or the folding of the glomerular basement membrane with an entrapment of hyaline amorphous materials.

Focal and global glomerular sclerosis was determined in 100 glomeruli. The number of glomeruli that experienced

sclerosis were combined to determine the total percentage of sclerosis. No significant difference ($p<0.05$) was detected in the degree of glomerulosclerosis among the four groups of rats (Figure 12).

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Blood Pressures

Systolic and mean blood pressures (MBP) were measured at the end of the study period for 40 rats (10 rats per group). Both systolic and MBP were similar between groups. Rats fed the MaxEPA-Casein diet had a slightly higher but non-significant systolic blood pressure (158.9 ± 5.6 mmHg) when compared to the other three groups. The systolic blood pressure of the COCAS, MOSOY, and COSOY groups were 144.7 ± 4.7 mmHg, 148.9 ± 8.0 mmHg, 149.9 ± 6.2 mmHg, respectively.

Mean blood pressure values for rats fed MaxEPA oil were non-significantly higher when compared to those of rats fed the corn oil diets. The means of MBP were 134.4 ± 5.9 mmHg, 121.2 ±5.5 mmHg, 130.2 ± 8.0 mmHg, and 116.7 ± 6.4 mmHg for the MOCAS, COCAS, MOSOY, and COSOY groups, respectively (Figure 13).

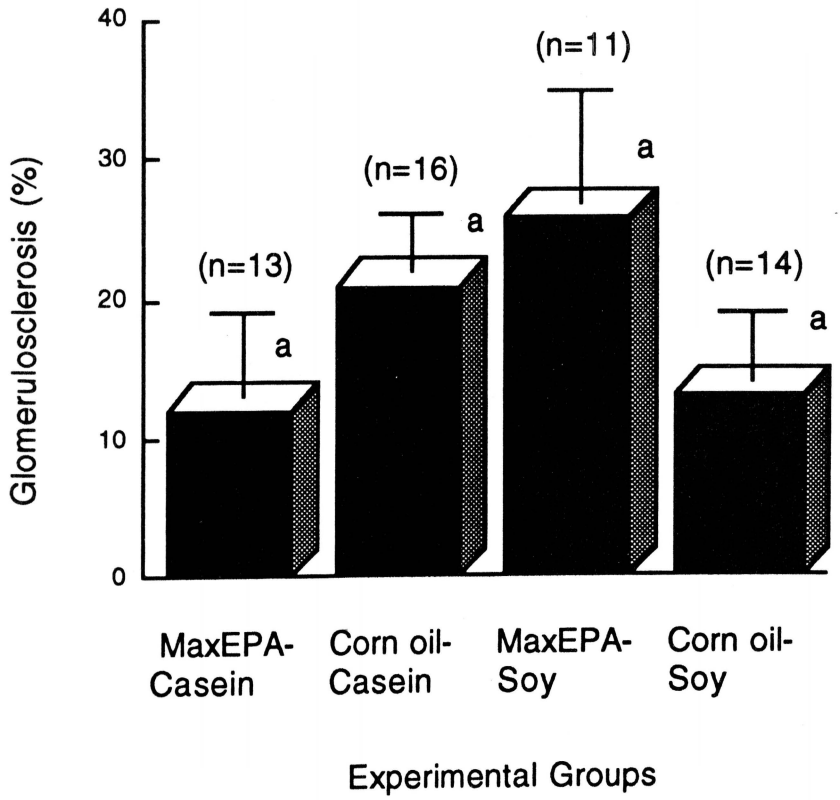

Figure 12. Effects of dietary polyunsaturated fatty acids and proteins on the extent of kidney tissue damage*,** in nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences ($p<0.05$)

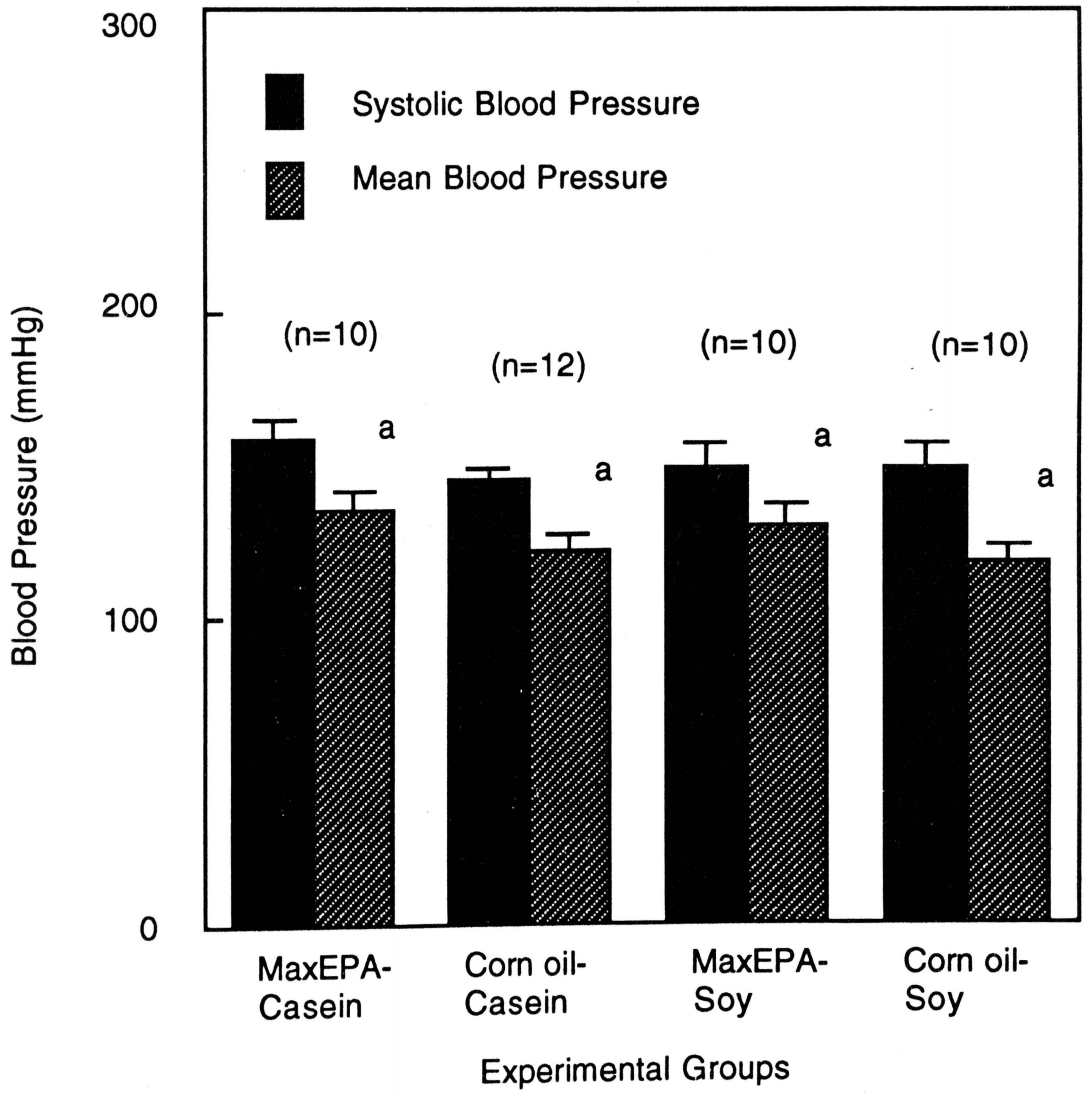

Figure 13. Effects of dietary polyunsaturated fatty acids and proteins on blood pressures*,** of nephrectomized rats.

* Values are means ± SE
**Different letter superscripts indicate significant differences at $p<0.05$

Effects of Dietary Polyunsaturated Fatty Acids and Proteins on Urinary Total Protein Excretion

There were no significant differences among the four dietary treatment groups in regards to the level of urinary total protein. However, rats that were fed the Corn oil-Soy diet exhibited the highest urinary protein excretion when compared with the other groups. Soy protein fed animals had higher levels of protein in their urine, regardless of the dietary fat source (Figure 14).

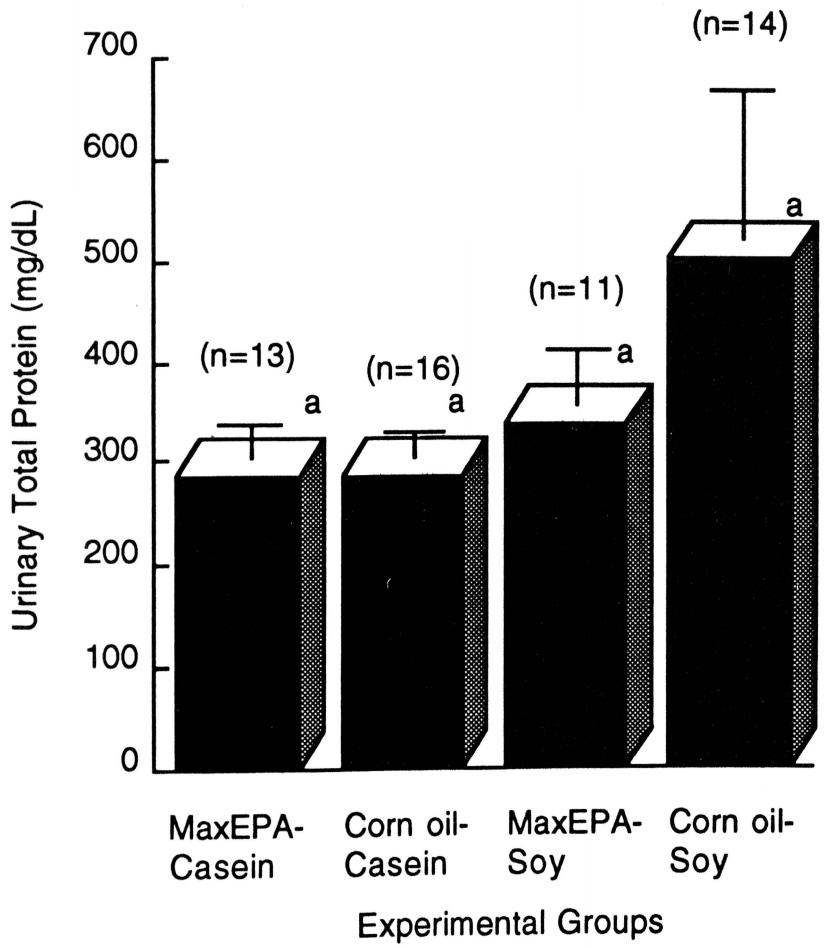

Figure 14. Effects of dietary polyunsaturated fatty acids and proteins on urinary total protein concentrations*,** of nephrectomized rats.

*Values are means ± SE
**Different letter superscripts indicate significant differences ($p<0.05$)

CHAPTER V

DISCUSSION

High blood lipid levels have been reported to occur in Chronic Renal Disease (CRD) patients and are thought to play a major role in renal function impairment and cardiovascular disease. An increase in hepatic lipoprotein synthesis and a decreased in blood lipid clearance have been reported as the possible mechanisms (Zeman, 1983) that contribute to these high blood lipid concentrations.

The promising effects of n-3 fatty acids on blood pressure and also on blood lipid profiles have been rigorously explored during this last decade (Nestel et al, 1989; Knapp and FitzGerald, 1989; Singer et al., 1985). Plant protein has also been reported to have a potential beneficial effect on serum lipid levels and also on blood pressures (Williams et al., 1987; Kontessis et al., 1990; Margetts et al., 1985). This study examined the potential benefit of omega-3 PUFAs and plant protein on the serum lipid profiles as well as on the blood pressures of nephrectomized rats.

Body Weight and Food Intake

A reduction in food intake and an impairment of weight gain in uremic animals fed different types of diets has been reviewed by Laori et al. (1990). Haines et al. (1989), on the other hand, observed normal food intake and weight gain in uremic rat models. Williams et al. (1987) found that rats fed soya protein had lower body weights when compared to casein-fed animals, suggesting a poor intestinal absorption with the soy diets.

In the present study, all groups of rats exhibited a linear weight gain whereas food intake revealed a more irregular pattern. Mean body weights and food consumption of all groups, however, were not statistically different ($p<0.05$). The achievement of these comparable results in weight gain and food intake might therefore be assumed to be non-confounding factors regarding the clinical parameters being examined in this research.

Serum Total Protein and Albumin

Abnormalities in the metabolism of serum proteins and amino acids usually occur in renal failure patients. Grossman et al. (1977) found that serum albumin concentrations were low whereas intracellular albumin concentrations in the liver were high in rats with renal failure. Lower concentrations of serum protein and albumin have been observed in uremic rats

when they were compared to their respective controls (Zoja et al, 1988). Savin et al (1989) observed significantly diminished serum protein levels in nephrectomized rats that were fed protein restricted diets (6% protein) compared with those fed normal protein diets (24% protein) and their sham-operated controls.

In the present study, both serum total protein and albumin concentrations were similar in all four groups, however, a non-significantly higher concentration of serum albumin was observed in rats fed MaxEPA-casein diet.

Serum Creatinine

Serum creatinine and blood urea nitrogen (BUN) are traditionally the most widely used screening tests to determine renal function in all species. Creatinine is a product of muscle metabolism, and serum concentrations tend to stay relatively constant, however, they may be influenced by diet or by changes in nitrogen balance (Breiler and Schedl, 1962).

In their study, Kontessis et al (1990) did not find significant differences in plasma creatinine levels in healthy volunteers after they were fed either animal or vegetable proteins. Protection of renal function, as evidenced by lower serum creatinine levels during fish oil

feeding of nephrotic rats, was observed by Ito et al., (1988).

In the present study, serum creatinine concentrations were non-significantly higher in animals maintained on MaxEPA-soy protein diets as compared to the other three experimental groups. The combination of corn oil and soy protein showed the lowest serum creatinine level.

Serum Triglyceride

The principal effect n-3 fatty acids have on blood lipids is via the lowering of triglyceride concentrations (Leaf and Weber, 1988). These favorable effects of n-3 fatty acids seem to be dose related and are more marked in individuals with hypertriglyceridemia (Herold and Kinsella, 1986). Serum triglyceride levels have also been observed to decrease in hyperlipidemic hemodialysis patients after fish oil concentrate consumption (Hamizaki et al., 1984). The role of plant protein in lowering triglyceride concentrations has also been suggested by Williams et al. (1988) for their nephrectomized rat models.

In the present study, no significant differences were found in serum triglyceride concentrations. The favorable effect normally noted for soy protein was not apparent. MaxEPA-fed groups showed a non-significantly lower triglyceride concentration when they were compared to corn

oil-fed rats. The triglyceride-lowering effects of fish oil have been suggested to be mediated mainly through inhibition of hepatic formation of VLDL. Wong et al.(1984) reported a decreased in fatty acid synthesis but an increased in fatty oxidation which together greatly reduced the availability of fatty acids for esterification to glycerides in livers of rats fed fish oil. When fed concomitantly with MaxEPA oil, casein had a non-significantly greater effect in lowering the serum triglycerides than did soy protein. However, the reverse was true when the proteins were fed concomitantly with corn oil. Based on the present findings, the types of fat might override the triglyceride-lowering effects of soy protein.

Serum Total Cholesterol

Disorders of lipid metabolism are known to be common indicators of impaired renal function. A series of studies involving consumption of polyunsaturated fatty acids on serum total cholesterol concentrations have been done quite extensively in the past few years.

Ito et al. (1987) reported that feeding higher concentrations of fish oil (14%) appeared to significantly reduce serum total cholesterol in adriamycin induced nephrotic syndrome rats. Williams et al. (1987) found that the serum cholesterol concentrations of rats fed soya diets

were lower than concentrations in rats fed casein. This pattern occurred for both subtotally nephrectomized rats and normal control rats.

The results of the present study did not totally support the findings of the above published data. Serum total cholesterol concentrations of rats fed MaxEPA oil were lower when compared to levels in rats fed corn oil diets. In contrast to the results of the above studies, rats fed a casein diet exhibited the lowest rise in serum total cholesterol level when fed concomitantly with MaxEPA oil. Diets containing a combination of corn oil and casein contributed to a significantly higher concentration in serum total cholesterol when compared to the rats that were fed the MaxEPA-casein diets. Data from the present study are in agreement with other studies as to MaxEPA and corn oil effects on total cholesterol concentrations.

Serum Lipoproteins

Dietary omega-3 polyunsaturated fatty acids have been reported to favorably influence serum lipid profiles. The most consistently reported effect of fish oil is its triglyceride-lowering effect (Nestel et al., 1989; Packard et al., 1984). It is thought that this triglyceride-lowering effect to be the results of an inhibition of hepatic triglyceride synthesis and secretion of smaller VLDL

particles which are known to be more readily converted to LDL than larger, triglyceride-rich particles.

Results from research regarding MaxEPA oil's effect on HDL-cholesterol levels are inconsistent. Some studies have indicated that fish oil therapy can significantly increase total HDL- cholesterol (Flaten et al, 1990; Harris et al., 1983, Rolf et al., 1990), whereas other studies have reported a significant reduction in HDL-cholesterol after n-3 fatty acid consumption (Nestel et al., 1984). Based on the results of the present study, feeding fish oil contributed to lower HDL-cholesterol when compared to corn oil feeding. The effect of fish oil on HDL-cholesterol needs to be explored extensively since the findings are inconsistent.

The effect of dietary fish oil on LDL-cholesterol levels have also received particular attention since this LDL-cholesterol when in an oxidized form, has been reported to play an important role in the progression of renal tissue damage (Wight et al., 1989; Steinberg et al., 1989, Wheeler et al., 1990). Most studies have found that fish oil consumption causes either no change in LDL cholesterol (Blonk et al., 1990; Flaten et al, 1990; Hughes et al., 1990; Harris et al., 1988) or increases LDL cholesterol concentration (Fumeron et al., 1991). In variance with the above findings, the present study found that MaxEPA oil feeding led to non-signifantly lower LDL cholesterol concentrations when

compared to corn oil feeding. From the present data, it would appear that fish oil consumption may be of benefit in regards to LDL cholesterol.

Serum Amino Acid Profile

The diseased kidney directly affects protein and amino acid metabolism. Some of the reasons for this involve the malfunctioning kidney's decreased capability to synthesize or catabolize certain hormones and amino acids, to degrade peptides and small proteins, and to produce or use certain amino acids.

The normal kidney plays an important role in the production and utilization of certain amino acids. The abnormally low ratio of plasma serine:glycine in renal failure patients may reflect an impaired synthesis of serine from glycine by the kidney (Pitts et al., 1970). The kidneys may also contribute to the body homeostasis of histidine, alanine, glutamic acid, asparagine and glycine as well as other amino acids.

In the present study, serum amino acid concentrations were determined to assess whether the amino acids that were predominantly present in plant (soy protein) and animal (casein) proteins would affect the blood concentrations of those specific amino acids. The analysis of serum amino acid concentrations showed that the serum amino acid arginine was

significantly higher in rats consuming soy protein diets. The serum concentrations of threonine, serine, alanine, leucine, and proline, on the other hand, were significantly higher in the casein-containing groups. Interestingly, serum amino acid concentrations were found to be positively correlated with dietary amino acids.

Serum Fatty Acid Profile

The effects of dietary polyunsaturated fatty acids on serum or plasma fatty acid profiles have received particular attention because of the important roles that the precursors of PUFAs play in regards to eicosanoid activity. Eicosapentanoic acid (20:5n-3) and DHA (22:6n-3) are the predominant fatty acids in fish oil.

Consumption of fish oil has been shown to increase the plasma or serum concentrations of EPA and DHA (Schaap et al., 1987; Kasiske et al., 1989). The findings of the present study are in agreement with results from these studies. The serum concentrations of EPA and DHA were significantly increased in the MaxEPA oil-fed groups. The mean serum linoleic, linolenic, and AA concentrations were significantly higher in corn oil-fed groups compared to MaxEPA-fed groups.

Blood Pressures

William and Walls (1987) reported that the mean blood pressure of subtotally nephrectomized rats fed either 24% casein or soya was significantly higher than control values but the blood pressure of animals ingesting a 24% casein diet did not differ from those consuming a 24% soya diet.

Administration of fish oil has also been reported to lower blood pressure (Singer et al., 1985; Knapp and FitzGerald, 1989). Mortensen et al (1983) found that systolic blood pressure fell significantly when MaxEPA oil administered to healthy volunteers.

In contrast to the above findings, the results of the present study did not support the blood pressure lowering effect of either MaxEPA oil or soy protein. The blood-lowering effects of MaxEPA oil and soy protein were not apparent in this study.

Dietary Manipulations and Progression of Renal Tissue Damage

In rats subjected to renal ablation, it has been proposed by Hostetter et al (1981) that glomerular hyperfiltration is associated with augmented macromolecular flux into the mesangium. Michael et al (1980) have postulated that the glomerular mesangium is a frequent site for the localization of macromolecular proteins in both experimental and clinical glomerular disease. Both alterations in

glomerular hemodynamics and/or increased mesangial traffic with the consequent entrapment of macromolecules, may ultimately lead to mesangial expansion and eventual glomerulosclerosis.

In the present study, the extent of renal tissue damage, as determined by examining the focal and global sclerosis of glomeruli, was not significantly different among all dietary treatment groups. The diets which contained both MaxEPA and soy protein exhibited the highest percentage of glomerular sclerosis and this percentage was twice as high as the sclerosis percentage in the MaxEPA-casein and corn oil-soy fed rats. Very limited data is available on the effect of n-3 fatty acids on the degree of glomerular sclerosis.

Urinary Total Protein Excretion

High protein diets have commonly been associated with high degree of proteinuria (Bertani et al., 1989). They reported that high protein diets (35% protein) significantly increased the degree of proteinuria whereas low protein diets were associated with mild proteinuria. The quality of protein has been reported to have a favorable effect on the degree of proteinuria. Williams and Walls (1989) reported that subtotally nephrectomized rats when fed a 24% casein diet, sustained significantly greater renal damage as assessed by greater values of proteinuria when compared to rats fed a 24%

soya diet. In their previous study, Williams et al. (1987) also observed a significantly higher progressive rise in urinary protein excretion in experimental animals fed casein diets in comparison with those fed soya diets.

The effects of fish oil on renal function have been extensively studied. It has been reported that fish oil may have beneficial or toxic effects in the rodent remnant nephron model (Barcelli and Pollak, 1989; Scharschmidt et al., 1987). Scharschmidt et al. (1987) found that fish oil provided no benefit regarding the degree of proteinuria in the 5/6 nephrectomized rats. By contrast, Barcelli and Pollak (1989) observed that fish oil reduced proteinuria compared to nephrectomized rats that were fed safflower oil or a control diet. In the present study, all groups exhibited a high degree of proteinuria, but no significant differences were detected between groups ($p<0.05$). Corn oil-Soy diet contributed to the highest degree of proteinuria with values that were almost twice as high as in the other groups. The beneficial effects of MaxEPA oil and soy protein on urinary protein excretion were not apparent in this study.

CHAPTER VI

SUMMARY AND CONCLUSION

Abnormalities in lipid and protein metabolism are the common findings in chronic renal disease patients. Dietary manipulations have been found to be able to minimize the risk of the death due to cardiovascular impairment. Epidemiological studies have shown that consumption of omega-3 fatty acids from marine origin reduce mortality from cardiovascular disease. This potential favorable effect of omega-3 fatty acids has triggered the interest of many researchers.

Food protein quality has been shown to also impact on the progression of vascular lesion development in humans and in animal models. Dietary plant proteins have been shown to lower the degree of tissue damage and proteinuria in renal disease subjects. There are limited data with regard to the effect of dietary plant proteins on serum lipid and amino acid profiles. Therefore, this study was designed to determine the effect of plant protein in combination with different types of fats on kidney metabolism and health.

The primary objective of this study was to examine the effects of dietary polyunsaturated fatty acids and proteins on the renal function and serum lipid levels of uremic rats.

No significant differences were found in the progression of renal lesions in nephrectomized rats fed different types of diets. The degree of proteinuria, although very high compared to published data, was not significantly different between groups. No significant differences in serum total protein, albumin, and creatinine concentrations were found between groups. Serum triglyceride concentrations were non-significantly lower in rats fed the MaxEPA oil diets. Serum total cholesterol and HDL-cholesterol were significantly higher in the corn oil fed groups when compared to MaxEPA fed groups. Serum LDL cholesterol was markedly higher in corn oil-casein group but no significant difference were detected ($p<0.05$) between groups. Blood pressures were not significantly different among groups. Significantly higher serum EPA and DHA and significantly lower serum linoleic and AA concentrations were observed in the MaxEPA-fed groups. Serum arginine concentrations were higher in soy-fed animals, whereas concentrations of serum threonine, serine, alanine, leucine, and proline were significantly higher in casein-fed rats.

The following conclusions can be drawn from this study:

1. The designed experimental diets did not affect the body weight and food intakes of nephrectomized rats.
2. No significant difference in serum total protein, albumin, and creatinine concentrations were noticeable between groups.
3. Rats fed MaxEPA oil diets had non-signifiantly lower serum triglyceride concentrations when compared to corn oil-fed groups of rats.
4. MaxEPA-fed rats had lower serum cholesterol than corn-oil fed rats. A combination of dietary casein and corn oil gave the highest serum total cholesterol level and the values were significantly different from those on the MaxEPA-Casein fed rats.
5. The serum HDL cholesterol concentrations of rats given MaxEPA oil diets were lower compared to corn oil-fed groups. Both casein and soy protein exerted and HDL-raising effect when fed concomitantly with corn oil.
6. Serum concentrations of threonine, serine, alanine, leucine, and proline were significantly higher in the casein-fed groups than in the soy-fed groups. On the other hand, serum arginine concentration was significantly higher in the soy-fed groups.

7. Feeding MaxEPA oil contributed to significantly higher concentrations of serum EPA and DHA+myristic concentrations compared to corn oil feeding.
8. Serum linolenic, linoleic, and AA concentrations were significantly lower in MaxEPA-fed rats as compared to corn oil-fed rats.
9. Blood pressures were not significantly different among groups.
10. No significant differences in urinary protein excretion were found between groups. All nephrectomized rats exhibited high degree of proteinuria, however, the Corn oil-Soy fed animals were the worst. The urinary total protein concentration in the COSOY group was twice as high as in the other groups.

Based on the present findings, the beneficial effects of both MaxEPA and soy protein were not apparent. The combination of those two components in normal protein level (24%) and low fat level (5%) did not give the best results in term of the serum lipid profiles, degree of proteinuria, and blood pressures. However, MaxEPA oil may have beneficial effect by lowering the serum total cholesterol, triglycerides, and LDL cholesterol, which in turn may reduce the progression of vascular lesions in nephrectomized rats.

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APPENDIX A

Research Approval

Form 1988A

Animal Project No. 1992-09 (For Office Use Only)

USE OF VERTEBRATE ANIMALS
TWU ANIMAL RESEARCH FACILITY

Project Title: The Effects of Certain Combinations of Different Types of Dietary Fats and Dietary Proteins on Renal Histopathology and Serum Lipid and Amino Acid Profiles of Uremic Rats

Investigators/Instructors (indicate Principal Investigator/Instructor with an asterisk)
George Liepa*, Viera Wenan, Jessie Ashby, Larry Forman

Department Nutrition and Food Sciences **Phone Ext.** 2636

Proposed Duration of Project: From March 2, 1992 **To** October 1, 1992

Funding Source or Proposed Funding Source
NIH Area Grant

Project Classification (check)
A. **Grant Proposal (external source)** _____
New Proposal of Pilot Project __X__ Modification of Ongoing Grant _____
Competitive Renewal _____ Grant Supplement _____
Noncompetitive Continuation (Indicate significant changes only) _____
B. **Local Research** ____X____ **funding source** __TFFC____
C. **Thesis/Dissertation Project** ___X___
D. Course _____

By whom was (will) peer review accomplished? __NIH and Ron Smith, M.D. (SW Dialysis Center; (214) 358-2300

Previously assigned Animal Project No. if application is other than a New Proposal or Pilot Project _____
Date Received by ACUC: 2-28-92
Review Board Action: Date _____
Approved ___**Approved Contingent** __**Disapproved** ___**Returned for Revision** _____
Remarks:

Additional Review Required? NO ___ **YES** _____
Safety_____**Radiation**_____**Biohazard** _____

Signature of ACUC Representative Linda Upshaw

Date Received by Safety/Radiation/Biohazard Commitee: _____

Review Board Action: Date:_____
Approved ___Approved Contingent ___Disapproved ___Returned for Revision____

Remarks:

Signature of Safety/Radiation/Biohazard Representative _____

APPENDIX B

Surgical Procedure for a 5/6 Nephrectomy in Rats

Surgical Procedure for 5/6 Nephrectomy in Rats

Instruments and Supplies:

Animal Clippers (#40 blade)

Anesthetic agent: Methoxyflurane (Metofane) inhalant

Sterile surgical gloves

Surgical face mask

Headband magnifying loops (#5 or #7)

Cotton or disposable surgical gowns

Surgical board

Saline solution

Virosan antibacterial solution

Isopropyl alcohol

Surgical blades

Surgical blade handle (Bard Parker #3)

Tuberculin syringes (1 ml)

Penicillin Procaine (300,000 units/ml)

Cotton applicators (6" handles)

Blunt Ethicon black braided silk suture (4-0 FS-2,cutting needle)

Suture needles (No. 1, 3/8" circle, cutting edge)

(1) Stainless steel scissors (Storz #E-3426 or Roboz #RS-5841)

(2) Hemostats (Halstead mosquito straight)

(1) Spool black braided silk (Ethicon 0-T6004C (100 yrds))

(1) Mouse-tooth (1x2) forceps (Storz #E-1422 or Roboz #RS-5232)

(1) Slightly curved forceps 4" (Storz #E-1416 or Roboz #RS-5136)

(1) Full curved forceps 4" (Roboz #RS-5137)

(1) Forceps 8" (Economy line, nickel plated, straight)

Shop towels (Steel Blue Kimtex Heavy Duty 33211 (drapes))

Prior to Surgery Preparation

All surfaces have to be washed and rinsed with a 50% alcohol soluion. Autoclave all instruments before and after each surgery session (20 minutes at 15-20 pounds pressure). The drapes, nonsterile gauze and cotton applicators need to be autoclaved as well (same as above). Cover the operation table with sterile drapes. Wash and alcohol-rinse the animal surgery board and cover it with sterile drape (repeat after each animal). Soak all intruments in 10% virosan solution during surgery.

Surgeons and surgical assisstants should wear clean surgical gowns and face masks. Before surgery, remove watches and rings and wash hands thoroughly. Wear sterile gloves and change them between each animal.

Procedures

1. Anesthetize the animals by exposing the animal to Metofane (Methoxyflurane) inhalant. The animal should be anesthetized to stage III, Surgical Anesthesia. In this state the animal is unconcious, maximal muscle relaxation should be evident as the rat does not respond to stimulation and footpads are bluish in color. Care must be taken, as the dividing line between adequate anesthesia and respiratory failure is minimal.
2. Shave the dorsal area of the animal thoroughly. Clean the area carefully using alcohol or betadione.
3. Place the anesthetized animal on its ventral surface, left to right of the surgeon.
4. Tape the tail and feet loosely to the surgical board. Keep the animal under an anesthetic condition by placing a nose cone containing the anesthetic agent over its face.
5. Make a small dorsoventral incision into the abdominal cavity, down the side of the rat near to the costal border of the thorax. Widen the cut by spreading the scissors widely apart.
6. Locate the left kidney and free the kidney from connective tissues and gently pull it out by grasping the perirenal fat. Tear the kidney capsule with two

curved forceps carefully to expose the veins and arteries in the area of the renal pelvis.

7. Isolate the middle and the upper branches of the renal artery. Clamp the arteries with forceps and look for a darkening of the kidney. If proper arteries are occluded, about 80% of this kidney should now be distinctly darker in color than the remaining section. Using a needleholder insert a tapered needle with a 2-0 silk suture behind the arteries. Pull the needle and thread through and leave about 2 inches of the thread. Tie the arteries with a "triple surgeons knot" method and trim the thread.

8. Place the kidney back into its proper place. Pass a piece of black braided silk through a 3/8" needlehole. Grasp the outer layer of the skin with mouse-tooth forceps and insert the needle halfway and grasp the inner layer of the skin with the other part of the needle. Pull the needle and thread through the skin layers and leave about 3 inches of thread. Make a "triple surgeon knot" twice. Repeat the suturing procedure in a specific sequence (from left to right) until the cut is completely closed. Secure the thread tightly. Clean the suture by wiping it with saline solution.

9. Turn the animal around so that the head will be on the right of the surgeon. Repeat step 5.
10. Expose the right kidney by squeezing it out gently with two thumbs. Remove surrounding fat layers and tissues and isolate the artery and vein. Clamp off the kidney with a hemostat. With a spool of black silk suture tie a square knot in front of the forceps. Cut the kidney away on the side of the hemostat using a scalpel (sterile blade). Let the knot slip into the space formerly occupied by the kidney. Repeat step 8 to close the incision.
11. Inject 1/10 cc of penicillin under the loose neck skin behind the rat's head and rub the surrounding area to spread out the penicillin evenly.
12. Remove the anesthetic containing cone from the animal. Hold the animal in a towel and warmed hands until it regains conciousness. Place the animal in a clean cage for about an hour.
13. Return the animal to the animal room. Water and a small amount of food should be provided. Watch the animal closely for any discomfort and possible suture tears everyday. The wound should be healed in about a week. The animal should resume eating at least on the second day after surgery.

APPENDIX C

Blood Pressure Measurements

A Tail Cuff Method

Blood Pressure Measurement

A Tail Cuff Method

Equipments

Flat bed chart recorder

Graph paper #10

#12 pens (upper pens)

#13 pens (lower pens)

B-63-3/4" photoelectric sensor

Cuff liner 2B

O-rings 3/4"

80M holders

Amplifier Mod.59

Manual Scanner Mod. 65-12

Cuff pump Mod. 20 NW

Heating pad or table lamp

Procedures

A. Calibrating the Recorder

1. Place the amplifier on the "Band Pass" position. The "Lo/Band Pass" switch allows the selection of two types of filters to filter the amplified tail pulses after amplification.
2. Plug the sensor in and set the scanner on that sensor.
3. Place the amplifier intensity on "10". This intensity control knob allows the setting of the light source's brightness in the photoelectric sensor.
4. With the lower and upper recorder pens on 2 or 5 volts, use the zero dial to place the lower pen in the middle and the upper pen on the "0" mark of the chart paper.
5. Change the amplifier into the "Lo Pass" position.
6. Set the amplifier "pulse gain" to 9. The "pulse gain" switch controls the amplitude of the amplified tail pulses.
7. If the pen moves, use the "offset" on the amplifier to adjust the lower pen to the center of the chart recorder paper.
8. Leave all settings "as is" except return the amplifier to "Band Pass" position. The actual testing should be done in the "Band Pass" position.
9. Set the chart recorder speed to 5 mm/sec.

B. Calibrating the Pressure

1. Calibrate with the amplifier filter off.
2. Place the tubing from the sphygmamanometer into the "Air In" outlet located on the front side of the amplifier.
3. Inflate the sphygmamanometer to 300 mmHg.
4. Turn the "Pressure Adjust" knob so that the upper pen on the recorder reads 300.
5. Release air slowly, and watch the meter versus the recorder readings. Each division of the chart paper should correspond to 3 mmHg of pressure. Use the "Pressure Adjust" knob to correct if needed.
6. After calibrating, turn the amplifier filter on.

C. Taking the Blood Pressures

1. Place the rat in the holder. It is advisable to train the animal by placing it in the warmed holder for a few hours daily prior to the test.
2. Detach the tail plate from the holder which is held by a magnet and/or snaps.
3. Hold the rat by the tail and place it on the table so that it faces away from you.
4. Take the cylindrical body of the holder and place it with its open end facing the rat. The animals have the natural tendency to walk into the holders.

5. After the animal is in the holder, the tail plate should be snapped back into place.
6. Take the tail, put it through the cuff and mount the sensor on the tail plate with nylon handscrews.
7. Turn the knob on the side of the amplifier to the corresponding number where the sensor is plugged in. Make sure that the light of the sensor is on.
8. Turn the pump pressure on.
9. Inflate the cuff to the maximum cuff pressure and the cuff liner should fit snugly to the tail. Do this step a few times.
10. When one feels comfortable with the procedure and is ready to take the blood pressure, turn the "record" switch to "on" position and push the "pen" knobs on the recorder down ("down" position) so that both pens touch the chart paper. Now, the pulse is ready to be recorded.
11. Get three or more readings. Watch the animal; it has to stand still for good results. One can adjust the amplitude of the graph by adjusting the "Pulse Gain" knob for desirable width. The common amplitude width is about 20 to 25 mm.
12. Push the "pen" knobs on the recorder once more so that both pens are in the "up" position after good results are obtained. Cover pens when they are not in use so that the ink will not dry out.

D. Calculating the Blood Pressure from the Recording

1. A good recording will have a bell-shaped curve.
2. After occlusion a relatively slow deflation is made during which time the reappearance of the tail pulses is noted. The pressure at the onset of the tail pulses is the systolic blood pressure point (SBP). The first maximum amplitude is taken as the mean blood pressure (MBP).
3. Assume that the pressure channel is calibrated for 300 mmHg maximum pressure and there are 100 divisions on the chart per channel. In this case each division is equal to 3 mmHg. If the pulse reappeared when the pressure pen was at the 50^{th} divisions on the chart then the SBP would be 150 mmHg (50 x 3 = 150).

APPENDIX D

Serum Fatty Acid Profile

Determination

Serum Fatty Acid Profile Determination

Principle

A mixture of chloroform-methanol (2:1, v/v) is used to extract fatty acids from the serum. Fatty acid separation is accomplished by placing them into 2-nitrophenylhydrazine hydrochloride (2-NPH.HCl) in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbomide hydrochloride (1-EDC.HCl) and analyzing them using high performance liquid chromatography.

Reagents and Materials

1. Acetonitrile, water [HPLC grade (85:15 v/v)]
2. Absolute ethanol
3. Hexane (HPLC grade)
4. Standard fatty acid solutions:

   Weigh 0.0108 g of margaric acid and dissolve in 1 ml of ethanol. Transfer to a 10 ml volumetric flask. Use ethanol to bring up to volume. Label "4000 nmol margaric acid/ml".

Make standard solutions of the other fatty acids exactly the same way except multiply molecular weight of each fatty acid by 0.00004 to know the exact amount to be dissolved in 10 ml of ethanol. Label each screw-cap tube with the name of the acid and "4000 nmol/ml". Make dilutions as in the case

of margaric acid.

5. Chloroform:Methanol (2:1 v/v):

   Measure 100 ml chloroform and mix it with 500 ml of methanol.

6. Pyridine solution (3%):

   Measure 3 ml pyridine and transfer into 100 ml volumetric flask, then bring to volume with absolute ethanol.

7. 1-EDC.HCl solution (0.25 M):

   Weigh 0.4793 g 1-EDC.HCl and transfer into a 10 ml volumetric flask. Use ethyl alcohol to bring up to volume.

8. 1-EDC.HCl working solution:

   Measure 10 ml of 1-EDC.HCl solution and mix with 10 ml with pyridine solution (3%).

9. 2-NPH.HCl (0.02 M):

   Aqueous: weigh 0.3790 g 2-NPH.HCl. Transfer into a 100 ml volumetric flask. Bring up to volume with deionized water.

   Acidic: weigh 0.3790 g 2-NPH.HCl. Add to a 100 ml volumetric flask. Bring up to volume with 0.25M HCl. (to make 0.25M HCL, take 2.075 ml HCl and bring to 100 ml with water).

10. KOH (Derivatization) solution (15%, W/V):

    Prepare 100 ml of methanol-deionized water (80:20).

Weigh 15 g KOH and transfer quantitatively to a 100 ml volumetric flask using methanol-water solution up to volume. Label KOH Derivatization.

11. KOH (Extraction) solution [2.5 m/L KOH/ethanol (2:8 (v/v)]

Weigh 14.025 g of KOH and transfer quantitatively to a 100 ml volumetric flask. Bring up to volume with deionized water. Take 20 ml of KOH-water solution and add 80 ml of ethanol. Label 2.5 m/L KOH-ethanol (Extraction).

12. Phosphate Buffer:

Weigh 2.2455 g KH_2PO_4. Transfer to 500 ml volumetric flask and bring up to volume with deionized water.

Weigh 3.7658 g K_2HPO_4. Transfer it to a 500 ml volumetric flask and bring up to volume with deionized water.

Adjust pH of first solution with the second to 6.4.

Measure 380 ml of 6.4 pH buffer and mix with 40 ml of 0.5 m/L HCl. To make 0.5 m/L HCl, mix 1 ml of concentrated HCl with 23 ml of deionized water.

14. <u>Solvent:</u>

1. Mix 100 ml acetonitrile with 830 ml concentrated HCl (work under the hood).

   Pour into brown bottle and label as: Acetonitrile.

2. Make 4 liters of acetonitrile-water solution (85:15) adjust to pH 4.5 using solution (1).
3. Deaerate the acetonitrile-water by bubbling with helium for 10 minutes.

Procedure

A. Standard Acids

1. Transfer 5 µl of fatty acid solution to a 5 ml test tube.
2. Add 100 µl of 2-NPH.HCl (aqueous) solution.
3. Add 200 µl working 1-EDC.HCl solution.
4. Heat at 60°C for 20 minutes.
5. Add 50 µl of 2.5 mol/l KOH-methanol (Derivatization) solution. Vortex (the color should be brown at this point).
6. Heat at 60°C for 15 minutes then cool it under running water.
7. Add 2 ml of phosphate buffer (pH 6.4) (color should turn yellow).
8. Add 1.5 ml of n-Hexane.
9. Vortex for 1 minute.
10. Centrifuge at 1500 x g for 5 minutes.
11. Transfer 1 ml of solution from hexane layer into a small test tube and evaporate at room temperature under a stream of nitrogen or

vacuum.

12. Reconstitute the sample with 100 µl of acetonitrile (yellow color).
13. Inject 5 µl of the solution into the HPLC

B. Serum Sample

1. Transfer 10 µl of serum into a 10 ml test tube.
2. Transfer 2 ml of chloroform-methanol (2:1, v/v) containing 10 µmol of margaric acid/L into the test tube.
3. Shake or mix the test tube vigorously for 10 minutes and let it settle for 3 minutes or centrifuge for 5 minutes.
4. Take out 1.5 ml of the choloroform layer and transfer it into a 10 ml test tube.
5. Evaporate the chloroform at room temperature under a nitrogen stream or vacuum.
6. Add 50 µl of 2.5 mol/L KOH.ethanol (Extraction). Vortex.
7. Heat at 90°C for 10 minutes.
8. Cool to room temperature.
9. Add 100 µl of 2-NPH.HCl (Acidic) solution (the color should turn yellow).
10. Add 200 µl working 1-EDC.HCl solution (bright rust in color).

11. Heat the test tube at 60°C for 20 minutes.
12. Add 50 µl KOH solution (Derivatization).
13. Heat at 60°C for 15 minutes. Cool under running water.
14. Add 2 ml phosphate buffer (pH 6.4) (yellow).
15. Add 1.5 ml of n-hexane.
16. Vortex for 1 minute.
17. Centrifuge at 1500 x g for 5 minutes.
18. Remove 1 ml of hexane layer (top layer) and transfer it into a small test tube.
19. Evaporate hexane at room temperature under stream of nitrogen or vacuum.
20. Redissolve the residue with 50 µl of acetonitrile.
21. Inject 5 µl into HPLC.

Calculation

1. Calculate R.F.* = amount/area
   (Use amount and area from standard)
2. Calculate Concentration
   Conc. = R.F. x area(sample) = (pmol/inj)
3. Correct for Internal Standard (I.S.) by:
   a. Find concentration for I.S. (step 2)
   b. Calculate 1000/conc for I.S. (step 3a) = factor
   c. Conc. of each fatty acid x factor
      = corrected of adjusted conc. (pmol/inj)

4. Corrected conc. x dilution factor** = nmol/ml

* R.F. = Retention Factor

** In this experiment the dilution factor was 1.5