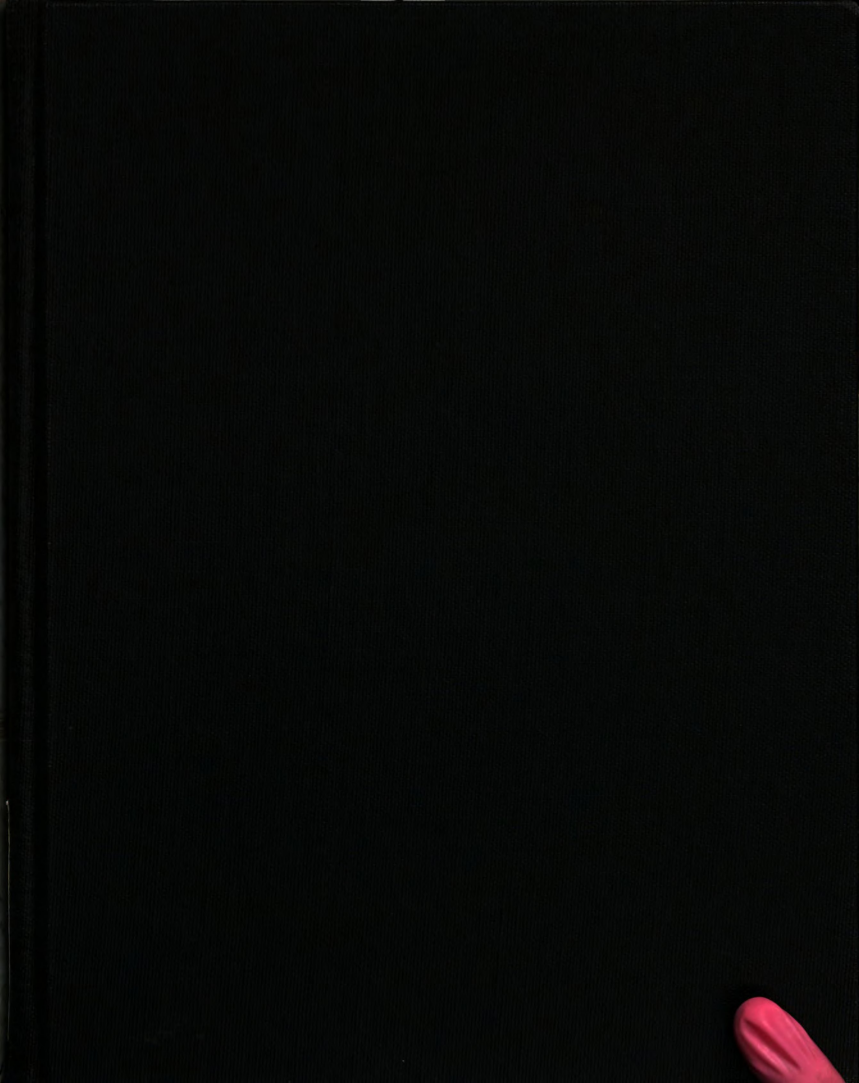

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PLASMA RETINOL AND RETINYL ESTER CONCENTRATION AND TRANSPORT IN CHRONIC RENAL FAILURE PATIENTS RECEIVING HEMODIALYSIS THERAPY

presented by

Shirley Ann Mellen

has been accepted towards fulfillment
of the requirements for

M.S. degree in Nutrition

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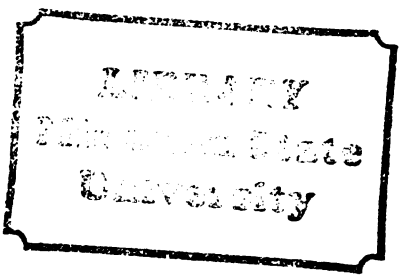
Major professor

Date August 3, 1982



PLASMA RETINOL AND RETINYL ESTER CONCENTRATION AND TRANSPORT IN CHRONIC RENAL FAILURE PATIENTS RECEIVING HEMODIALYSIS THERAPY

By

Shirley Ann Mellen

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1982

ABSTRACT

PLASMA RETINOL AND RETINYL ESTER
CONCENTRATION AND TRANSPORT
IN CHRONIC RENAL FAILURE PATIENTS
RECEIVING HEMODIALYSIS THERAPY

By

Shirley Ann Mellen

To evaluate the nature and risks of elevated plasma vitamin A observed in chronic renal failure (CRF) plasma concentrations of different forms of vitamin A and retinol-binding protein (RBP) were determined in 26 CRF patients undergoing hemodialysis therapy. Compared to results in 13 controls, CRF patients showed elevated total vitamin A, retinol, and RBP. Retinyl palmitate concentrations were elevated as compared with controls only in patients showing elevated triglycerides. Thus it appears that increased retinol with a concurrent elevation of RBP was primarily responsible for the increased total vitamin A. Plasma vitamin A in CRF patients did not correlate with time since initial dialysis treatment. Dietary assessment showed lower vitamin A intake in CRF patients than controls. Because toxicity symptoms are usually caused by excessive vitamin A intake and are associated with elevation of retinyl esters and higher total vitamin A concentrations than seen in these patients, a risk of vitamin A toxicity in chronic renal failure appears unlikely.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation and gratitude to Dr. Wanda Chenoweth for her expertise, assistance, and encouragement; to Dr. Hi Sung Park for his support of this study; to Drs. Jenny Bond, Maurice Bennick and Robert Rosemblaum for their insight and assistance. I would like to extend special thanks to Sue Cuppett for her patience and technical assistance and to my husband, Gene, for his understanding and constant support.

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Abnormal vitamin A metabolism in chronic renal failure has been documented by many studies. Serum vitamin A is typically elevated in chronic renal failure and dialysis treatment does not produce noticeable improvement. The causes of elevated serum vitamin A are still being explored. Concern over toxicity of elevated serum vitamin A levels in chronic renal failure has prompted additional research. Common symptoms of toxicity hypervitaminosis A resemble the typical symptoms seen in chronic renal failure. However, little documentation of vitamin A toxicity in chronic renal failure has been reported.

Research indicates the form of vitamin A, retinol or retinyl esters, is important in the appearance of toxic symptoms of hypervitaminosis A. Apparently this is due to the mode in which the different forms are transported in the plasma. Retinol is transported in complex with a carrier protein, retinol binding protein (RBP), which serves to direct the vitamin A to the target tissues. Retinyl esters are transported in lipoprotein complexes.

It has been suggested that the lack of vitamin A toxicity symptoms in chronic renal failure is due primarily to the form of vitamin A present in the plasma. Research

has documented elevation of the retinol form of vitamin A. Retinyl palmitate concentration in plasma is reported to be undetectable. Retinyl esters were also undetectable in results of other research with normal subjects.

However, at least one case of vitamin A toxicity has been reported in a patient on hemodialysis treatment of chronic renal failure. The plasma levels of retinol and retinyl esters were not measured in this subject; only total vitamin A was measured and the serum level was not greater than reported levels of total vitamin A in other groups of dialysis patients who did not show symptoms of toxicity from hypervitaminosis A. Also this case did not report the levels of the vitamin A transport protein, retinol binding protein (RBP), or levels of lipoproteins in the plasma.

In most previously reported studies of vitamin A status in chronic renal failure (CRF) patients only one or two parameters of vitamin A status were measured. This study was designed to measure the plasma level of retinol, retinyl esters, RBP, and triglycerides as well as dietary intake of vitamin A, in chronic renal failure patients. The concentrations and relative proportions of different forms of vitamin A in the plasma were compared and correlated with plasma levels of RBP and triglycerides. The relationship of these parameters to dietary intake of vitamin A was also evaluated. Comparison of the results

in chronic renal failure patients with those in age and sex matched controls was used to more fully clarify the nature and risks of elevated plasma vitamin A in CRF patients undergoing hemodialysis treatment. In addition the relation of lipid abnormalities and time since initial dialysis treatment to abnormalities of vitamin A metabolism were evaluated.

REVIEW OF LITERATURE

The complexity of renal disease and the many facets of vitamin A metabolism which can be altered to cause a change in plasma transport of this vitamin have necessitated that this introductory material be divided into three major sections. First vitamin A metabolism, as it is presently understood, is presented; second the characteristics of chronic renal failure are described; and finally the knowledge of the alterations in metabolism of vitamin A and its transporting compound in chronic renal failure is outlined.

Vitamin A Metabolism

Absorption

Vitamin A is present in the diet mainly as preformed retinyl esters or as one of several carotinoid precursers. The retinyl esters are hydrolyzed prior to absorption by hydrolases either from the pancreas or the brush border of the intestinal mucosa (1). It is then retinol, the alcohol form of vitamin A, which is absorbed into the intestinal cell. The transport process is accelerated by ATP or glucose (2), while dinitrophenol inhibits the transport (3), indicating that retinol transport is an active process.

During protein malnutrition the level of retinyl ester hydrolase activity is depressed, and the absorption of retinyl palmitate is reduced, while the absorption of retinol is unchanged (1). Vitamin A precursors, mainly beta-carotene, are absorbed by two pathways. They can either be absorbed and transported intact in the chylomicrons or they can be absorbed and converted to retinol in the mucosa (4). This conversion involves cleavage of beta-carotene to two molecules of retinal, the aldehyde form of vitamin A, by 15,15' - dioxygenase, an enzyme found in the mucosal cells and in the liver (5). This cleavage is followed by conversion of the retinal to retinol by a non-specific aldehyde reductase (1). Within the mucosal cell, the retinol is reesterified, mainly with the fatty acid palmitate, and introduced into the circulatory system in the chylomicrons (6). Evidence from studies with labeled retinyl palmitate indicate some other unspecified routes may exist for the absorption of retinyl esters (7).

Storage

Vitamin A is taken up from the plasma by the liver and stored as retinyl esters, mostly retinyl palmitate (8). The liver is the main storage site for vitamin A in the body, although the kidney may contain large amounts of retinyl esters. The precise pathway of uptake and storage in the liver is as yet unclear. There is some evidence to indicate that there may be transfer between cells within

the liver. The Kupffer cells may be the site of initial uptake from the plasma, with subsequent transfer of the vitamin A to the parenchymal cells for long-term storage (5). To study this hypothesis, Linder and coworkers (9) utilized a technique of preferentially digesting the parenchymal cells leaving the Kupffer cells intact. They found in rats fed a normal diet (13 IU of vitamin A per gram of diet) that the whole liver concentration of vitamin A was 116 IU per gram, the Kupffer cell concentration of vitamin A was 5 IU per gram and the hepatocyte concentration by difference, was 111 IU per gram. When rats were fed a diet high in vitamin A (1000 IU per gram of diet) the whole liver concentration was predictably higher than in the rats fed a normal diet. The vitamin A concentration of 5037 IU per gram of liver represented a greater than 40-fold increase. The Kupffer cells of these animals showed only a five-fold increase to 26 IU per gram, whereas the parenchymal cells showed an increase of 45-fold to 5014 IU per gram (9). Thus it is possible that the Kupffer cells have a role in the transfer of vitamin A between plasma and the liver storage. However, Linder et al. were unable to support this hypothesis when they gave a pulse of labelled vitamin A to rats who were depleted of vitamin A. In liver samples taken one hour after injection of the labelled vitamin A, the liver cells showed a distribution of labelled vitamin A compounds very similar to the distribution in a normal

state, in which 4 percent of liver vitamin A is found in the Kupffer cells and 96 percent is in the parenchymal cells (9).

Regarding intracellular distribution of stored vitamin A, studies by Berman and coworkers using homogenates of rat liver have shown that the majority of the vitamin A (59.5 percent) is stored in the cytosol of the liver cells (10). The microsomal fraction of these cells contained 18.1 percent of the total liver vitamin A. The remaining vitamin A in the liver homogenate was distributed between the unbroken cells and debris (6.5 percent), and a heavy particle fraction (15.9 percent).

The study by Berman (10) also found that retinol represented 5 percent of the total liver vitamin A and retinyl esters represented 95 percent. In all of the intracellular fractions except the cytosol the proportion of retinol to retinyl esters was approximately 5 to 95. In the cytosol the proportion was 10 to 90, retinol to retinyl esters, respectively. After ultracentrifugation of the cytosol two fractions were identified. There was a floating lipid layer, which contained considerable amounts of neutral lipids in the form of lipoproteins. The other fraction, the infranatant, contained a larger amount of protein, 71 mg/g tissue, compared with the floating lipid layer, 0.36 mg/g tissue. The proportion of retinol to retinyl esters was 5 percent to 95 percent and 16 percent

to 84 percent in the floating lipid layer and the infranatant, respectively.

The intracellular distribution of liver vitamin A may be more representative of the storage functions of the liver than the difference in storage between the Kupffer cells and parenchymal cells. Electron microscopy studies by Linder and coworkers (9), as well as by other research teams (11, 12), have identified lipid inclusion droplets with high concentrations of vitamin A. These droplets were found in the cytosol of both Kupffer and parenchymal cells. These lipid droplets fluctuate in number and size according to the vitamin A status of the animal (11). These lipid droplets may correspond to the floating lipid layer described by Berman et al. (10).

Heller (13) describes a cytosol retinyl ester lipoprotein complex (CRLP) in the rat liver. This complex contained 3 percent by weight retinyl compounds. Retinyl esters were about 96 percent of these compounds and retinol was about 4 percent. The CRLP appears to be the main storage form of the highly hydrophobic retinyl esters in rat liver. Thus it seems clear that the cytosol is the major storage site, but Berman and coworkers (10) postulate that the smaller microsomal fraction is a rapidly turning over fraction with an as yet unclear relation to the larger cytosol fraction. The function of the infranatant fraction within the cytosol may be to serve as a connection between the cytosol and microsomal fraction.

Release from Storage and Transport

The exact mechanism of vitamin A release from the liver is still not clear. Upon examining the membrane fractions of hepatocytes, the Golgi apparatus contains the highest concentration of retinyl esters and this concentration varies with the vitamin A status of the animal (14). Starvation was shown to increase the Golgi apparatus vitamin A concentration two-fold (14). This fraction associated with the Golgi apparatus may be the fraction about to be secreted from the liver. In view of the general secretory function associated with the Golgi apparatus, the secretion of vitamin A might reasonably occur in the Golgi apparatus. The vitamin A released from the liver storage is mainly in the form of retinol. In addition, a small amount is normally released as retinyl esters, mostly retinyl palmitate (15).

Retinol in the plasma is transported complexed with a small molecular weight protein, retinol binding protein (RBP) (16). The observation of lowered serum vitamin A in protein malnutrition (1, 17), may be due in part to a lack of RBP carrier for retinol. RBP is synthesized in the liver and has a molecular weight of approximately 21,000. There is a single binding site for a retinol molecule on each RBP molecule (18, 19). The affinity of retinol for RBP is very high since almost no free retinol exists in the plasma. The affinity of retinol for RBP is enhanced by the

further complexing of RBP to prealbumin (PA) in the plasma. The RBP:PA complex is in a 1:1 molar ratio and the in vitro extraction of retinol from the RBP:PA complex using organic solvents is more difficult than the extraction of retinol from uncomplexed RBP (20). It has been postulated that PA and RBP are complexed in such a way that the retinol molecule is shielded from contact with the external environment.

RBP levels in plasma are normally 40-50 ug/ml. The metabolism of RBP may be very closely associated with the control of the serum vitamin A levels. Studies in the rat have shown that RBP release from the liver can be altered with changes in vitamin A status. Studies of vitamin A depletion show a decreased serum RBP with a decreased vitamin A concentration in plasma (21, 22). In one study the plasma vitamin A levels decreased from 45 ug/ml to less than 2 ug/ml. The plasma RBP decreased from 50 ug/ml to 12-15 ug/ml. This decrease in plasma RBP followed a pattern similar to the decrease in plasma vitamin A, with RBP decline showing a three day lag (21). Smith and coworkers (22) showed the importance of this association by giving vitamin A depleted rats an injection of chylomicrons containing various levels of vitamin A. The very low plasma levels of vitamin A and RBP rose rapidly. The peak plasma concentration of RBP and vitamin A which was attained showed a correlation with the level of vitamin A in the injected chylomicrons. At the opposite extreme, when rats

are fed a diet very high in vitamin A, their plasma levels of RBP decrease to a level similar to that seen in the deficient animals (23). At present an explanation for this apparently similar effect of the two extremes of vitamin A status is not clear.

In studies of human patients with varying types of liver disease differing responses of serum RBP to vitamin A supplementation were seen. One study found little response to supplements of vitamin A (23). In the other study, in which liver damage was due to alcoholic cirrhosis, vitamin A supplements produced a rise in serum RBP (24).

As previously discussed, retinyl esters, primarily retinyl palmitate, form the main storage compound of vitamin A in the liver. Release from the liver involves esterase activity to convert the retinyl esters to retinol. Observations have shown that the RBP - vitamin A complex contains only all trans retinol (25), and the RBP in plasma which is free of retinol does not bind retinyl palmitate (26). Thus the retinyl esters are hydrolyzed prior to complexing with RBP.

Research by Chen and Heller has indicated the presence of esterase activity in the CRLP (27). This activity appears to have an optimal pH of 7.8 and an optimal temperature of 30° C (27). In vitro activity is inhibited by compounds known to inhibit serine esterases and by sulfhydryl reagents, but is altered very little by

heparin (27). In vitro studies have also shown that the presence of either bovine or human serum albumin increases the esterase activity 10- to 15-fold (27). Under optimal conditions of incubation more than 30 percent of the retinyl esters were hydrolyzed to retinol by six hours (27). This esterase activity within the cytosol has previously been difficult to detect and verify, as indicated by conflicting reports of several investigators (28, 29, 30, 31). Chen and Heller (27) have demonstrated that differences in experimental conditions such as presence of detergents and lack of serum albumin could be the major factor contributing to the confusing and contradictory results.

Further investigation of CRLP by Chen and coworkers (32) has suggested a function for bovine serum albumin (BSA). When CRLP is incubated with BSA, the retinol produced by esterase activity is found mainly with the BSA (32). In addition, when the BSA-retinol complex is incubated with RBP not containing a retinol molecule (apo-RBP), the retinol is transferred to the apo-RBP (32). When CRLP is incubated with apo-RBP some transfer of retinol occurs, but this is greatly enhanced by BSA (32). Thus BSA appears to function as a transfer protein for the retinol produced in the CRLP which must be transferred to RBP. It is possible that a similar transfer protein exists in vivo; since other systems have shown the same model of intracellular transport.

As previously indicated, a small amount of serum vitamin A is normally present as retinyl esters. Mallia and coworkers (33) have demonstrated an association of retinyl esters with lipoprotein complexes within the plasma. Results of several studies have indicated that the proportion of retinyl esters in serum may increase when vitamin A intakes are very high. In the study by Mallia and coworkers (33), rats fed very high doses of vitamin A showed elevated serum levels of retinyl esters. The rise in retinyl esters accounted for the entire rise seen in serum vitamin A. The additional retinyl esters were transported by lipoprotein complexes. Research by Smith and Goodman (34) also showed elevation of the plasma retinyl esters in three cases of hypervitaminosis A in human patients. In two of the cases the serum vitamin A levels shortly after stopping excess vitamin A intake were 291 ug/dl and 382 ug/dl with the retinyl esters representing 67 percent and 65 percent of the total serum vitamin A, respectively. With the cessation of the excess intake the serum levels of vitamin A fell to 49 ug/dl and 105 ug/dl. In addition, the percentage of the serum vitamin A as retinyl esters dropped to 31 percent and 48 percent, respectively (34).

Degradation of Vitamin A

Although vitamin A is in the form of retinol when released from the liver, the final active form of vitamin A at the level of target tissues appears to include several

metabolites of retinol including retinoic acid and aldehyde forms of vitamin A (35). The conversion of retinol to retinoic acid also appears to be an important step in the degradation of retinol because the reaction is irreversible (35). The kidney is a major site of metabolism of retinol to retinoic acid (36). It appears that retinoic acid is not stored within the body but is converted to metabolites which are excreted in the urine or in the feces via bile (2, 37).

Degradation of RBP

The degradation of RBP also has been examined. Evidence suggests that the kidney plays a role in the removal of RBP from the plasma and its subsequent degradation (19, 22, 38, 39, 40, 41). Most RBP in the plasma is in complex with PA (42). However, there is a small uncomplexed fraction. In normal subjects this represents a very small amount of RBP, but it is this fraction which is thought to be filtered at the glomerulous of the kidney (38, 43). There is much evidence for glomerular filtration of other small molecular weight proteins, such as insulin, Bence Jones proteins, and growth hormone (44, 45, 46, 47). These proteins are first filtered and then reabsorbed by the proximal tubule. Within the tubular cells they are metabolized to amino acids which are either recycled or further metabolized. The molecular weight of these proteins is equal to or greater than that of RBP, so it seems

feasible that the kidney could filter RBP as well. However, it would have to be the free fraction, since the RBP-PA complex forms a unit too large to be filtered.

At present the process of releasing RBP in complex with PA to free RBP in the plasma is unclear. Some research has indicated that RBP looses its affinity for PA after the retinol ligand is delivered to target tissues (25, 48, 49). This may be due to conformational changes in RBP without retinol (48, 49). Other investigations of the free fraction of RBP have shown that this RBP is not identical to that found in complex with PA (50, 51). The largest portion of free RBP is without retinol or the normal carboxy terminal amino acid (50). This altered free RBP was shown to be unable to bind to PA (51). However, there exists an additional small amount of free RBP which contains the normal carboxy terminal amino acids. This free RBP is in equilibrium with RBP complexed with PA (50). The concentration of altered RBP found in the plasma of normal subjects is 4-5 micrograms/ml (42). In studies of the turnover of I^{125} RBP it has been shown that the half life of the altered free RBP is 3.4 to 3.5 hours which results in a degradation of 150 mg/day/M^2 (42). In contrast the half life of the free RBP in equilibrium with PA is 11-15 hours (42). These results are in agreement with the hypothesis that one of the functions of the RBP-PA complex is to slow the degradation of RBP.

Recent research has conflicted with the earlier findings of an altered RBP. Fex and Hansen (52) have examined RBP isolated from urine and serum. They were unable to identify a difference in the two and actually find only one compound which migrated differently under electrophoresis due primarily to the presence or absence of the retinol ligand (52).

These conflicting studies still leave the nature of the mechanism releasing RBP from the RBP-PA complex unclear. However the evidence indicates that the free RBP in plasma is filtered by the kidney glomeruli, and subsequently reabsorbed and metabolized by the tubular cells.

The subcellular localization of RBP in the kidney has been determined by centrifugation to separate subcellular fractions (40). RBP within the kidney cells appears to be mainly in the supernatent fraction. This is consistant with hypothesis that RBP within the kidney undergoes cytosolic degradation. Further evidence for the renal degradation of RBP is seen in the results of urine analysis for RBP in renal patients with mainly tubular disorders (38, 53). Peterson and Berggard have analyzed urine for RBP in patients with tubular disorders due to cadmium poisoning (53). The 24-hour excretion of RBP was 20-150 mg, whereas normal subjects showed only 0.04-0.22 mg of RBP in 24-hour urine samples. These results indicate that normally the RBP filtered at the glomerulous is reabsorbed and

metabolized by the tubular cells. Further evidence for renal degradation of RBP comes from Scarpioni and coworkers (38), who compared urinary RBP as a percentage of urinary albumin in renal patients with either mainly tubular or mainly glomerular lesions. In the tubular lesions the RBP excretion was 9.61 percent of albumin excretion and in glomerular diseases the RBP was only 0.53 percent of albumin excretion. These results further support the hypothesis that the tubular cell is important in renal degradation of RBP.

Chronic Renal Failure

Etiology

Chronic Renal Failure (CRF) results from a progressive decrease in the number of nephrons functioning in the kidney. The decline in function can be attributed to many disorders (54). Some of these disorders are local in that they are primarily renal diseases. Others are disorders of the lower urinary tract or systemic diseases (55). In renal disorders, renal failure is usually a presenting feature, while in lower urinary tract disorders bladder dysfunction is the predominant presenting feature and renal failure is detected later (55). Some systemic diseases such as malignant essential hypertension, lupus erythematosus, analgesic overconsumption, and lead or cadmium poisoning frequently have renal failure as a presenting or early symptom. Other systemic diseases show renal failure late

in the disease development or other symptoms overshadow renal failure. Such disorders include atheroma, diabetes, cirrhosis, gout, and heart failure (55).

Physiological and Biochemical Abnormalities

The brief summary which follows describes some of the important dysfunctions which are associated with CRF.

Early in renal failure the urine flow is increased up to three times the normal flow, and the concentration is decreased as low as a specific gravity of 1.01 (54). These changes are the result of a compensatory action by the remaining functional nephrons. In order to handle body fluids the glomeruli increase their filtered volume. The tubular reabsorptive capacities are overwhelmed with a resulting polyuria and dilute urine (54). The peak of this urine flow is reached when glomerular filtration rate (GFR) is in the range of 5-25 ml per min (55). After this time the urine flow decreases with the declining GFR and decreasing number of intact nephrons.

The decreased number of functioning nephrons ultimately results in disturbances in the metabolism of sodium potassium, calcium, and phosphorous. A non-respiratory acidosis also developes as the number of functioning nephrons decreases (56). It is usually pronounced after the GFR falls to less than 20 ml/min (55).

In addition to symptoms caused by electrolyte and water imbalances, it has been postulated that a toxic substance or substances accumulate with progressive renal failure (57). Various substances have been considered, including guanidines, amines, and phenolic acids (55, 59).

Clinical Characteristics

The biochemical and physiological abnormalities previously discussed are associated with many clinical characteristics of CRF. Some of these characteristics are similar to toxicity symptoms in hypervitaminosis A. A brief description of the clinical characteristics important to this thesis is included below.

Skin and GI Tract Features

In CRF the skin may be itchy, dry and flaky, and rashes occur frequently. There is a darkening of the skin and there is sometimes a "uremic frost" due to urea crystals forming on the skin (55). The mouth is usually dry and has a metallic taste and a uremic odor (55). The patient usually experiences anorexia, nausea, vomiting, and diarrhea. Bleeding from the gastrointestinal tract is not uncommon (55).

Growth and Nutrition

Persons who have developed CRF early in life showed a retarded growth which is only partly due to a reduced food intake (58). Even in adults there is a hazard of

inadequate energy intake with restricted diets. A low energy intake can result in wasting of body muscles and fat stores (55, 56).

Hypertension

Quite commonly CRF patients show some degree of hypertension. The cause can usually be traced to abnormalities in the sodium and water balance in the presence of a normal plasma renin level. Hypertension interfers with efforts at conserving remaining renal function in CRF (55).

Edema

Edema in CRF can be due to sodium and water excretion problems; however, the primary cause may be the nephrotic syndrome or heart failure (55).

Anemia and Bleeding Tendancy

Nearly all CRF patients show some degree of anemia. The Red Blood Cells (RBC) appear normally formed with normal or slightly lowered hemoglobin content (61). There is a decrease in renal production of erythropoietin and an increase in antagonists to erythropoietin causing a hypoplasia of the bone marrow (58). Often a mild folic acid deficiency is seen in CRF; however, the RBC concentration shows little response to folic acid supplementation (54, 55). In general a decreased life span of the RBC is seen (61, 62). Uremia may be important in the etiology of this anemia in as much as hemodialysis results in improved

RBC production and decreased BUN increases RBC life span (55). CRF patients have an apparent decline in platelet function which is roughly porportional to the increase in BUN. The ability of the platelets to liberate factor III in clotting is decreased (62). The prothrombin times are normal and plasma fibrinogen is elevated (55). These platelet dysfunctions seem to respond to hemodialysis therapy (55).

Neuropathy

Peripheral nerve conduction is decreased with CRF and this symptom is improved with hemodialysis (65). Muscle cramping occurs at night and is due to water and salt loses on hemodialysis. Many other nervous and muscular symptoms accompany CRF including muscle twitches, persistant hiccups, restlessness of the limbs, insomnia, tiredness and mental fatigue (65). Convulsions sometimes occur (55).

Hemodialysis Treatment of Renal Failure

Most commonly hemodialysis is used as an intermediary treatment for renal transplant candidates. Based on the high cost of hemodialysis and the limited benefit from such treatment (55) dialysis appears to be primarily a short-term treatment; however long-term dialysis treatment is being used more frequently.

In hemodialysis the patient's blood flows through a dialyzer from a shunt or fistula. The shunt or fistula

connects an artery and a vein; thus the dialyzed blood is taken from the artery and returned to the venous system.

The basic principle of hemodialysis is that the patient's blood spreads over a thin membrane, usually cellophane, which is in contact with a balanced salt solution. The components from either fluid compartment which can permeate the membrane will exchange until an equilibrium is reached. Many of the nitrogenous waste products and ions that accumulate in CRF are removable with dialysis (63). The rate of diffusion across the membrane is determined by the surface area of the membrane, the permeability of the component, and the concentration gradient between the two compartments. Since the concentration gradient will vary with the progressive accumulation of waste products from the blood into the dialysis salt solution; the clearance rate of a substance from the blood will vary during a single dialysis session (64). Dialysance is a term used to describe the removal rate of a substance without interference from changing concentration gradients. The definition of dialysance is the rate of removal of a substance from the blood per unit difference in concentration between blood and dialysis solution. Relative dialysance is determined by comparison of a substance's dialysance with that of urea. For example, chloride and urea have a dialysance that is higher than that of amino acids and glucose (64). Water is not removed from the

patients body by diffusion but rather by ultrafiltration. This process is controlled by varying the inflow and outflow pressure of the blood (64).

There are three basic types of dialysis machines: The coil-type, the flat plate type, and the hollow-fiber (63). The Kolff-twin coil dialyzer is a commonly used coil type. It has two cellophane coils around a central core cavity. The patient's blood flows through the coils and the dialysis fluid is in the core (55). The Kiil dialyzer is a common example of the flat plate type dialyzer. It contains a stack of three polypropylene boards with two sets of two cuprophane membranes sandwiched between a pair of the boards. The patient's blood flows between the membranes, and the dialysis fluid is between the boards and the membranes (55).

Hemodialysis has the following beneficial effects. It lowers extracellular fluid volume and plasma concentrations of BUN, potassium, sulphate and phosphate, creatinine and uric acid. Some of the beneficial effects have as yet an unknown biochemical basis. They include a decrease in the incidence of restlessness, muscular fibrilation, vomiting, confusion, and coma (63, 64).

In comparison with peritoneal dialysis, hemodialysis is more effective and has less chance of infection (55). When hemodialysis is not possible due to medical or psychosocial reasons, peritoneal dialysis has been used

successfully (63). Hemodialysis equipment is very expensive and must be carefully maintained. Thus hemodialysis is an improvement over peritoneal dialysis, but transplantation is still preferable for the patients who are able to successfully receive a donor kidney (55).

Vitamin A Metabolism in Chronic Renal Failure

Vitamin A Status

Many studies have shown that chronic renal failure is associated with altered vitamin A metabolism (20, 22, 32-35, 39, 41, 65, 66, 67, 68). A marked elevation of the serum total vitamin A level is seen in patients with chronic renal failure. In one study of patients with advanced chronic renal failure the serum vitamin A level was 60.9 ug/dl in patients not on dialysis and 85.37 ug/ml in patients on regular hemodialysis treatment. The control population had a mean concentration of 27.8 ug/dl (41). A study by Smith and Goodman (39) showed plasma vitamin A to be 50.1 ug/dl in normal subjects and 98.2 ug/dl in patients with chronic renal failure. In this study the renal patient group included patients receiving hemodialysis treatment.

Pediatric dialysis patients also show elevated plasma vitamin A (69). The values for pre- and post-dialysis were 126.4 ug/dl and 146.1 ug/dl, respectively. Control

subjects had levels of 48.3 ug/dl. Vitamin A supplements had been discontinued prior to the study.

Supplemental vitamin A for dialysis patients has been investigated in several studies. In a previously mentioned study showing elevated plasma vitamin A (41), supplemental vitamin A had been discontinued 4 months prior to the study. Another study (70) compared serum vitamin A in dialysis patients receiving vitamin A supplements of 5000 IU per day and patients receiving no vitamin A supplement. Both groups had serum vitamin A significantly greater than control subjects. In addition, patients receiving supplemental vitamin A had serum levels higher than patients not receiving supplements. The serum levels of controls, non-supplemented, and supplemented patients were 40.98 ug/dl, 60.89 ug/dl, and 108.25 ug/dl respectively (70). In a study which investigated the effects of withdrawing supplemental vitamin A (5000 IU/day), Ellis and coworkers (71) did not see a decrease in serum vitamin A over an average of 16.3 months.

Not all studies of vitamin A in renal patients have shown an elevation of vitamin A in all types of renal disease. An early study by Popper and coworkers (68) found high serum vitamin A concentrations in nephritic patients, where nephritic is characterized as azotemia and hypertension. However, the same study did not show an elevation of serum vitamin A in nephrotic patients. Conflicting evidence

was found in a study of children with the nephrotic syndrome, who showed elevated serum vitamin A (65). In this study the results showed that higher fasting serum vitamin A levels were associated with higher plasma lipids although no direct correlation was seen (65).

Other early work done in the 1940's dealt with the significance of the ester and alcohol fractions of plasma vitamin A in renal disease (72). For the nephritic patient with elevated total plasma vitamin A, the amount of esters rose only slightly and the percentage of total vitamin A as esters was lower than normal. The increase in total vitamin A appeared to be due mainly to the rise in the alcohol fraction of vitamin A (72). In the nephrotic syndrome the total vitamin A concentration rose very little, while the esters rose greatly in concentration and as a percentage of the total serum vitamin A. The amount of alcohol present was actually lower than normal (72). Interpretation of these early results is not easy due to use of analytical methods which may not be sensitive or accurate enough to produce reliable results.

RBP Metabolism in CRF

RBP has been implicated in the altered vitamin A status of CRF patients. The work of Smith and Goodman (39) examining RBP in several disease states showed marked elevations of plasma RBP in chronic renal failure, 116 ug/ml as compared to 46.2 ug/ml in the normal population. They

also calculated the molar ratios of RBP to the other components of the RBP:PA complex. The RBP:PA ratio was 1.06 for renal patients and 0.4 for the normal population. The RBP:vitamin A ratio was 1.92 for renal patients and 1.22 for the normal population (39). When plasma from renal patients was chromatographed on a Sephadex G-100 column, the RBP eluted to two peaks; one was associated with PA and the other appeared to represent free RBP. In comparing the elution pattern with that of normal plasma, the concentration of RBP eluting with the free RBP peak was greatly increased in renal failure (39). When the molar ratios of RBP:vitamin A in the two peaks were compared, the peak containing free RBP had a consistantly higher ratio than did the peak associated with PA, suggesting a greater fraction of the RBP without a vitamin A ligand in the free fraction (39). This result is in agreement with the previously cited findings of Peterson and coworkers (50, 51) showing a large fraction of free RBP which differed from that in complex with PA in terms of retinol content, amino acid structure, and binding affinity for PA. Smith and Goodman (39), however, did further work with this elevated free RBP peak in renal patients. In their study (39) they added PA to plasma from a renal patient in the quantity required to return the RBP:PA molar ratio to normal. Upon chromatography the percentage of the RBP and the vitamin A eluting with the PA peak was nearly 100

percent. In an aliquot of plasma from the same patient without added PA less than 50 percent of the RBP or vitamin A eluted with PA (39), which indicates that the free RBP in this patient probably had not been altered in the manner described by Peterson.

A reduced binding of apo-RBP (RBP without a retinol ligand) with PA as compared to the binding of holo-RBP (RBP with a retinol ligand) to PA has been demonstrated by Raz and coworkers (48). Their study used apo-RBP formed by extraction of retinol from holo-RBP. They compared the percent binding of the two forms to PA and found 60 percent binding with apo-RBP and 100 percent binding with holo-RBP. Thus it seems apo-RBP has a reduced affinity for PA: however, they caution that apo-RBP isolated in this manner may have resulted in a portion of the RBP being altered in such a way that it could not form a complex with PA. Rask and coworkers also have examined free RBP in renal patients (50). Their work indicates a large elevation of the free RBP fraction in these patients, 55-100 ug/ml, as compared to normal subjects, 4.0-5.0 ug/ml, which agrees with the work of Smith and Goodman (39). However their work also identified that this elevated free fraction was mainly altered RBP which would not bind with PA. Thus there seems to exist a contradiction in experimental results concerning the nature of the increased free RBP fraction in chronic renal failure.

The results of several studies have suggested that the levels of RBP in plasma of CRF patients increase with time of receiving hemodialysis treatment. In one study the uremic patients were followed for 24 months of hemodialysis treatment. The mean serum RBP level before beginning treatment was 18.5 mg/dl compared with 25.2 mg/dl after 24 months of dialysis. The mean value for normal subjects was 4.7 mg/dl (38).

Lipoprotein Metabolism in CRF

As previously indicated, plasma vitamin A esters can be carried in complexes with lipoproteins. It has been reported that lipoprotein metabolism in CRF is altered (73-79). The alteration appears to take the form of an increased incidence of elevated serum triglycerides, which is characteristic of type IV hyperlipoproteinemia (73, 78, 79). Ibels and coworkers (74) found elevated serum triglycerides in approximately 70 percent of nondialyzed and long-term dialyzed renal patients and in 42 percent of short-term dialyzed and renal allograft patients. Bagdade and coworkers (75) reported fasting serum triglyceride levels of 164 mg and 276 mg/dl in undialyzed and dialyzed patients, respectively. Normal subjects had a mean value of 68 mg/dl. The results of Gutman and coworkers (76) failed to show any differences between the undialyzed and dialyzed patients, although the triglyceride concentrations in serum were appreciably higher than the normal values.

Research by Frank and coworkers (79) showed a rise in plasma triglycerides with decreased renal function as measured by serum creatinine and creatinine clearance. It was also noted that triglycerides were lower in patients having received treatment for longer than five years. The investigators suggested that the reason for this difference may be related to a beneficial effect of dialysis or to some degree of natural selection whereby lower levels of lipids are associated with greater longevity in dialysis patients (79).

Research by Bolzano and coworkers (77) confirmed an elevation of triglycerides in uremic patients on hemodialysis, whereas serum cholesterol concentrations were found to be normal. When specific lipoprotein fractions were examined, they found the elevated triglyceride levels were due to increased very low density lipoproteins (VLDL), and low density lipoproteins (LDL) with density between 1.006 and 1.019 g/ml (LDL_1). The concentration of low density lipoproteins with density between 1.019 and 1.063 g/ml (LDL_2) was decreased, but the ratio of triglycerides to cholesterol in this fraction was increased (77). Other research (78) has shown an elevation of triglyceride to cholesterol in LDL and HDL (high density lipoproteins), while the ratio in VLDL is slightly less than in controls. Thus the elevated triglycerides in CRF patients may be due

to altered lipoprotein structures in addition to increased triglycerides due to the elevated VLDL.

Research on the cause of the elevated lipids in chronic renal failure has included estimates of the hepatic and extra-hepatic lipoprotein lipase activity. In research by Bolzano and coworkers (77) hepatic lipase activity was depressed, but extra-hepatic activity was normal. Other research (80) has shown depression in activities of both of these enzymes. Norbeck and coworkers (78) have postulated that the elevated VLDL and the slightly elevated cholesterol concentration in VLDL could be caused by depression of one or both of these enzymes.

Dietary manipulations of hyperlipemia in hemodialysis patients have shown significant reductions of triglycerides. Reduction and modification of fat intake (81, 82) and reduction of carbohydrate intake (82, 83, 84, 85) have been successful in decreasing plasma triglycerides in patients on hemodialysis. In the research by Gokal and coworkers (81), patients reduced their triglyceride levels by decreasing their fat intake and increasing their intake of polyunsaturated fats. When subjects returned to their pretreatment diets, their serum lipid levels rose to approximately pretreatment levels.

Dietary vitamin A has also been associated with alterations of serum triglycerides. A study in rats with normal kidney function demonstrated an increase in serum

triglycerides in response to high intakes of retinoic acid and retinyl acetate (86). The investigators concluded that the level of vitamin A accumulated in the liver was not responsible for the increased serum triglycerides. However, the actual mechanism for the increased triglycerides was not clear.

Studies of patients on hemodialysis have shown some associations of abnormal serum lipids with serum vitamin A levels. Some research has identified a correlation between serum triglycerides and serum vitamin A (70, 87), while others have not (71). In one study the serum cholesterol and serum vitamin A were correlated ($r = 0.47$) (70). Subjects who received supplemental vitamin A (5000 IU/day) had significantly elevated cholesterol levels in comparison to subjects not receiving supplemental vitamin A (70). Serum triglycerides were weakly correlated with the serum vitamin A ($r = 0.37$), and vitamin supplements did not appear to affect serum triglycerides. The results of another study of patients not supplemented with vitamin A did not show a correlation of serum vitamin A and cholesterol (87). However, there was a stronger correlation of serum vitamin A and triglycerides ($r = 0.65$) (87).

In CRF abnormalities in vitamin A metabolism include an increased level of plasma vitamin A and alterations in both plasma carriers of vitamin A, RBP and lipoproteins. This research has been undertaken to examine the forms of

vitamin A in plasma of patients undergoing regular hemodialysis treatment and any associations of plasma vitamin A and the plasma carriers levels measured in hemodialysis patients. In addition the contribution of dietary intake of vitamin A and length of time since initial dialysis treatment to the elevation of plasma vitamin A will be assessed.

METHODS

Subjects

Twenty-six patients receiving intermittent hemodialysis treatment for chronic renal failure were included in this study. These patients were being treated at the West Michigan Nephrology Center in either Coldwater or Kalamazoo. Patients received dialysis 3 or 4 times weekly for 4 to 8 hours per session. Patients with known diabetes or liver disorders were excluded from the study. Medications being consumed were reviewed and patients receiving medications interfering with vitamin A metabolism were excluded. The purpose and procedures of the study were explained verbally and in written form (Appendix A), and a consent form was signed by all subjects (Appendix B). The project was approved by the Michigan State University Committee on Research Involving Human Subjects.

Thirteen healthy control subjects from faculty and students of Michigan State University were selected to provide an age and sex distribution similar to that of the patient sample.

Dietary Intake Information

Dietary intake information for all subjects was obtained during an interview in which a 24 hour recall of

food intake and a food frequency checklist of foods rich in vitamin A and protein were completed by the investigator (Appendix C). The weekly intake and the daily intake were estimated from food composition tables (88) and the labeled values of specific brand name products. Vitamin A and protein intakes were compared with the Recommended Dietary Allowances (89).

Collection and Preparation of Blood Samples

Five to seven ml of venous blood were collected in heparinized vacutainers from the subjects after an overnight fast (minimum 8 hours). For dialysis subjects the samples were taken prior to a regular dialysis session from a dialysis shunt without requiring an additional venapuncture. Blood samples were protected from light exposure by either wrapping the tubes in aluminum foil or storing them in a lightproof container until further processing. As soon as possible, but no longer than 10 hours after collection, samples were centrifuged at 4° C and plasma removed. Each plasma sample was split into two aliquots. The aliquots were flushed with nitrogen gas and frozen until analyses were performed. All of the procedure was carried out under lowered lighting and the stored samples were wrapped in aluminum foil.

Vitamin A Assay

Plasma vitamin A was determined using high pressure liquid chromatography (HPLC). Retinol and retinyl palmitate were determined separately using different operating conditions. These differences are detailed below.

HPLC Apparatus: A Waters Associates Model ALC/GPC 204 with M6000A pump, U6K septumless injector and M440 absorbance detector fitted with a 280 nm and a 313 nm filter was used for the analyses. The column was a uPorasil 4mm id x 30 cm column (Waters Associates). Linear Instruments Model 285 E1 double pen recorder was used to record the detector signals.

Sample Preparation: Samples were assayed in duplicate. Aliquots of 0.5 ml were mixed with 0.5 ml absolute ethanol and vortexed 30 sec. To this mixture was added 0.5 ml hexane. (U/v grade hexane distilled in glass was used throughout the assay.) The mixture was then vortexed for two minutes in a stoppered tube. To facilitate separation of the hexane layer, 0.5 ml of water was added and the samples were centrifuged at 2000 RPM in a clinical centrifuge for ten minutes without refrigeration. This extraction procedure is similar to that used by Thompson and coworkers (90) in the assay of retinyl palmitate in milk and margarine. The hexane layer was aspirated carefully into a leur-lock type syringe (Precision Sampling Corp.), which was fitted with a Sweeney syringe filter

holder containing a 0.45 um fluoropore filter. The hexane layer was then filtered into a clean stoppered glass tube.

Retinol Assay: Plasma retinol was determined by injecting 50 ul of the hexane layer into the HPLC. The retinol was eluted with a mobile phase of 60 percent hexane and 40 percent chloroform, containing 1 percent ethanol, at a flow rate of 2.0 ml/min. The concentration of retinol was calculated based on a standard curve determined in the following manner. A retinol standard was prepared using retinyl acetate. Approximately 10 mg of retinyl acetate was saponified in 50 ml of 95 percent ethanol and 5 ml of 50 percent potassium hydroxide solution at 80° C for 45 minutes. After addition of deionized water and 30 ml of hexane, the mixture was transferred to a separatory funnel. The saponification flask was washed with an additional 30 ml of hexane which was added to the separatory flask. The aqueous layer was drained off and rinsed twice more with hexane. The combined hexane rinses were filtered over anhydrous sodium sulfate and evaporated to dryness. The standard was reconstituted with 25 ml of hexane and diluted to give an absorbance of approximately A = 0.100 at 325 nm. This preparation technique produced a standard with a single elution peak in the system used for assay. Injections of 5 ul to 100 ul were used to generate a standard curve, which was derived using linear regression. This method is similar to that described by Widicus and Kirk (91).

Retinyl Palmitate Assay: Retinyl palmitate was determined by injecting 50 ul to 100 ul of the hexane layer previously extracted from plasma into the HPLC. The retinyl palmitate was eluted at a flow rate of 1.5 ml/min with a mobile phase of 85 percent hexane and 15 percent chloroform. These conditions are similar to those described by Widicus and Kirk (91) in their assay of retinyl palmitate in cereal products. The concentration of the retinol was determined by comparison with a standard curve prepared as follows.

A solution of retinyl palmitate standard in hexane was diluted to give an absorbance of approximately A = 0.030 at 325 nm. Injections of 5 ul to 100 ul were used to generate the standard curve, which was derived using linear regression. This method is similar to that described by Widicus and Kirk (91).

Total Vitamin A: Total vitamin A was calculated as the sum of the retinol and retinyl palmitate as assayed above.

Retinol Binding Protein Assay

RBP was assayed by radial immunodifusion using commercially available plates (Behring Diagnostics, Sommerville, New Jersey). Five microliter aliquots of plasma samples from control subjects were applied directly to the plates. Plasma from the patients was diluted 1:10 with normal saline to bring it into the assay range of the

system. Five microliter aliquots of the diluted patient samples were applied to the plates. After a minimum of 48 hours diffusion time, the plates were stained using the method of Mancini and coworkers (92). The diameters of diffusion area were measured to the nearest 0.1 mm. The concentration was estimated by comparison with a standard curve and correction for dilution in the case of the patient samples. The standard curve was prepared by diluting the standard human serum (RBP 6.0 ug/ml) provided with the diffusion plates using normal saline and assaying the standard by the same procedure used for the samples. The standard curve was then calculated by linear regression; sample concentrations were estimated using this curve.

Triglyceride Assay

Plasma triglycerides were determined in duplicate by the colorimetric of Biggs and coworkers (93). This method uses the Hantzsch reaction described in the methods of Nash (94).

Statistical Analyses

Sample means were compared statistically using a Student t test. Linear correlations were determined and the significance of these correlations was determined using a t statistic (95).

RESULTS

Dietary Intake of Vitamin A and Protein

Daily intakes of protein were calculated from the 24 hour recalls of food intake. Male patients had a mean daily protein intake of 66.4±8.0 grams; mean intake of female patients was 35.2±10.5 grams of protein per day (Table I). In control subjects the mean protein intakes were 100±25.5 and 63.4±3.7 grams for males and females, respectively (Table I). The intakes of control subjects were significantly higher than those of the patients ($P<0.05$). For all subjects except female patients protein intakes were equal to or greater than the Recommended Dietary Allowances (RDA) of 56 and 46 grams for males and females, respectively (89). The low mean intakes of the female patients reflects the exceptionally low intakes of three subjects (<25 grams/day). These subjects reported feeling ill at the time of the dietary interview. Estimates of protein intake from the food frequency checklists indicated the usual intakes of these subjects were higher than those reported in the 24 hour recalls. The food frequency checklists supported the differences between control and patient intakes seen in the 24 hour recall data.

TABLE I

Mean Ages and Dietary Intake of Subjects[1,2]

	n	Age (yr)	Protein (g/day)	Vitamin A (RE/day)[3]
Patients				
Male	13	56.4±8.6	66.4±8.0[a]	584±168[a]
Female	13	48.3±10.7	35.2±10.5[b]	384±43[b]
Total	26	51.9±10.1	45.6±12.2[c]	495±161[c]
Controls				
Male	6	55.3±8.9	100±25.5[a]	1219±150[a]
Female	7	46.4±12.1	63.4±3.7[b]	6841±10669 (682±40.0[b])[4]
Total	13	50.5±11.3	81.6±19.2[c]	4030±7425 (1004±184[c])[4]

[1]Mean ± standard deviation.

[2]Within each column numbers with the same superscript are statistically different ($P<0.05$).

[3]Retinol equivalents shown are the sum of the IU of vitamin A from animal foods, vitamin supplements, or fortified foods multiplied by 0.3 and the IU of vitamin A from unfortified vegetable food products multiplied by 0.1 (89).

[4]Dietary intake of one female control was greatly elevated (20,074 retinol equivalents) due to the consumption of chicken livers during the study period. Mean and standard deviation in parentheses and calculated omitting data for this subject.

Mean daily intakes of vitamin A in patients were 751±370 and 558±150 retinol equivalents per day for males and females, respectively (Table I). These intakes were significantly less than those of control subjects ($P<0.05$). Dietary intakes of vitamin A in male and female control subjects were 2608±492 and 894±96 retinol equivalents respectively (Table I). The Recommended Dietary Allowances (89) for vitamin A are 1000 and 800 retinol equivalents per day for males and females, respectively. Seasonal differences in intake may have contributed to the large difference between controls and patients noted in the current research. Control subjects were interviewed during July and patients were interviewed between November and February. Control subject intakes reflect a higher consumption of fruits and vegetables with vitamin A precursers which may be due to seasonal availability of these foods.

Plasma Retinol, Retinyl Palmitate, Total Vitamin A, RBP, and Triglycerides

Total plasma vitamin A levels in patients and control subjects were 194.3±63.7 and 69.6±10.6 ug/dl respectively (Table II). Normal values reported for serum vitamin A are 30-60 ug/dl (96). Plasma retinol levels were 189.8±62.8 and 66.2±10.1 ug/dl in patients and controls respectively (Table II). The differences between patients and controls were significant ($P<0.05$). Plasma retinyl palmitate was slightly elevated in patients (4.5±2.0 ug/dl)

TABLE II

Plasma Concentrations of Vitamin A, Retinol Binding Protein, and Triglycerides[1,2]

	Total Vitamin A	Retinol	Retinyl Palmitate	Retinyl Palmitate	RBP	Triglycerides
	mg/dl	mg/dl	mg/dl	% of Total A	mg/dl	mg/dl
Patients						
Male	197.7±76.2[a]	193.4±75.1[a]	4.3±2.2	2.2±1.0	27.8±6.8[a]	160.6±63.2
Female	190.9±51.2[b]	186.1±50.6[b]	4.7±2.0	2.6±1.1	25.3±7.0[b]	161.2±51.9
Total	194.3±63.7[c]	189.8±62.8[c]	4.5±2.0	2.4±1.0	27.3±6.0[c]	160.9±56.7
Controls						
Male	72.5±12.0[a]	68.8±11.2[a]	3.7±2.5	4.7±2.2	7.0±1.2[a]	74.0±42.9
Female	67.2±9.4[b]	64.1±9.3[b]	3.1±1.6	5.0±3.0	7.5±1.4[b]	64.8±25.8
Total	69.6±10.6[c]	66.2±10.1[c]	3.4±2.0	4.8±2.5	7.3±1.3[c]	69.0±33.5

[1]Mean ± standard deviation.

[2]Within each column numbers with the same superscript are statistically different ($P<0.05$).

as compared to controls (3.4±2.0 ug/dl), but the difference was not statistically significant (Table II). The retinyl palmitate represented 2.4 percent of total vitamin A in patients and 4.8 percent in control subjects. These percentages were not significantly different.

Plasma RBP levels in patients were 27.3±5.9 mg/dl and levels in controls were 7.3±1.3 mg/dl (Table II). The patient levels were significantly higher than control subjects ($P<0.05$). Normal RBP values reported with this assay method range from 3-8 mg/dl (97).

Plasma triglycerides were 160.9±56.7 mg/dl in patients and 69.0±33.5 in controls (Table II). While plasma triglycerides in patients were significantly ($P<0.05$) higher compared with controls, mean values for both groups were within the normal range for plasma triglycerides (10-190 mg/dl) (96). The range of plasma triglycerides in control subjects was 28.7 to 154.2 mg/dl. In patients, plasma triglycerides ranged from 71.9 to 292.6 mg/dl and 8 of the 26 subjects had triglyceride levels greater than 190 mg/dl.

When patients subjects were divided into subjects with elevated triglycerides (>190 mg/dl) and subjects with normal triglycerides (<190 mg/dl), the mean levels of retinyl palmitate in the two groups were 6.0±1.6 and 3.9±1.9 ug/dl, respectively (Table III). The level in the group with elevated triglycerides was significantly greater than the 3.4±2.0 ug/dl seen in control subjects ($P<0.05$).

TABLE III

Plasma Vitamin A, RBP, and Triglycerides in Controls and Patients Grouped by Triglyceride Concentrations[1,2]

	n	Retinyl Palmitate (ug/dl)	Retinyl Palmitate (% of Total Vitamin A)	Retinol (ug/dl)	Triglycerides (mg/dl)	RBP (mg/dl)
Control	13	3.4±2.0[a]	4.8±2.5	66.2±10.1[abc]	69.0±33.5[ab]	7.2±1.3[abc]
Total Patients	26	4.5±2.0	2.4±1.0	189.8±62.8[a]	160.9±56.7[a]	27.3±5.9[a]
Patients Triglycerides <190 mg/dl	18	3.9±1.9	2.2±1.1[a]	177.0±59.8[b]	133.5±38.8	27.7±6.5[b]
Patients Triglyceride >190 mg/dl	8	6.0±1.6[a]	2.8±0.7[a]	216.8±64.8[c]	222.5±39.6[b]	26.4±4.4[c]

[1]Mean ± standard deviation

[2]Within each column numbers with the same superscripts are statistically different ($P<0.05$).

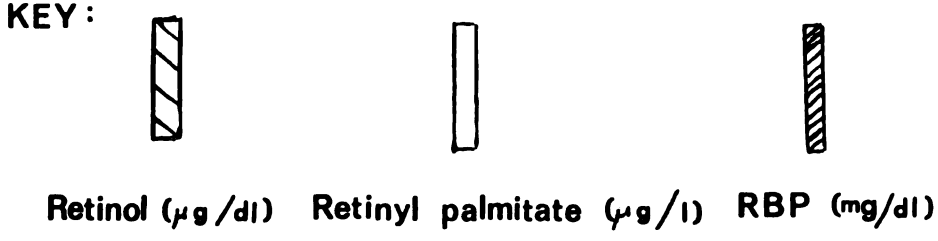
The retinyl palmitate levels were high in the patients with elevated triglycerides than in other patients, but the difference was significant only at $P<0.10$. Retinol and RBP levels in the patients were significantly higher than control subjects ($P<0.05$), but were not different than levels seen in other patients with normal triglyceride levels (Table III).

Relationship of Plasma Retinol, Retinyl Palmitate, RBP, and Triglycerides with Time Since Initial Dialysis Treatment

When dialysis patients were grouped by time since initial dialysis treatment, a pattern of increasing levels of retinol with increasing length of time on dialysis appeared (Figure 1). RBP shows a similar pattern except in the group receiving dialysis longer than 48 months where levels were lower than in other groups (Figure 1). The differences between groups of dialysis patients, however, were not statistically significant (Table IV), and plasma retinol and RBP showed no correlation with months since initial dialysis treatment. Plasma retinyl palmitate and triglycerides did not show a pattern of rising levels with increased times on dialysis (Table IV and Figure 1) and no correlation of plasma retinyl palmitate and triglycerides with months since initial dialysis was seen.

FIGURE 1

PLASMA VITAMIN A AND RBP IN DIALYSIS SUBJECTS GROUPED BY MONTHS SINCE INITIAL DIALYSIS TREATMENT



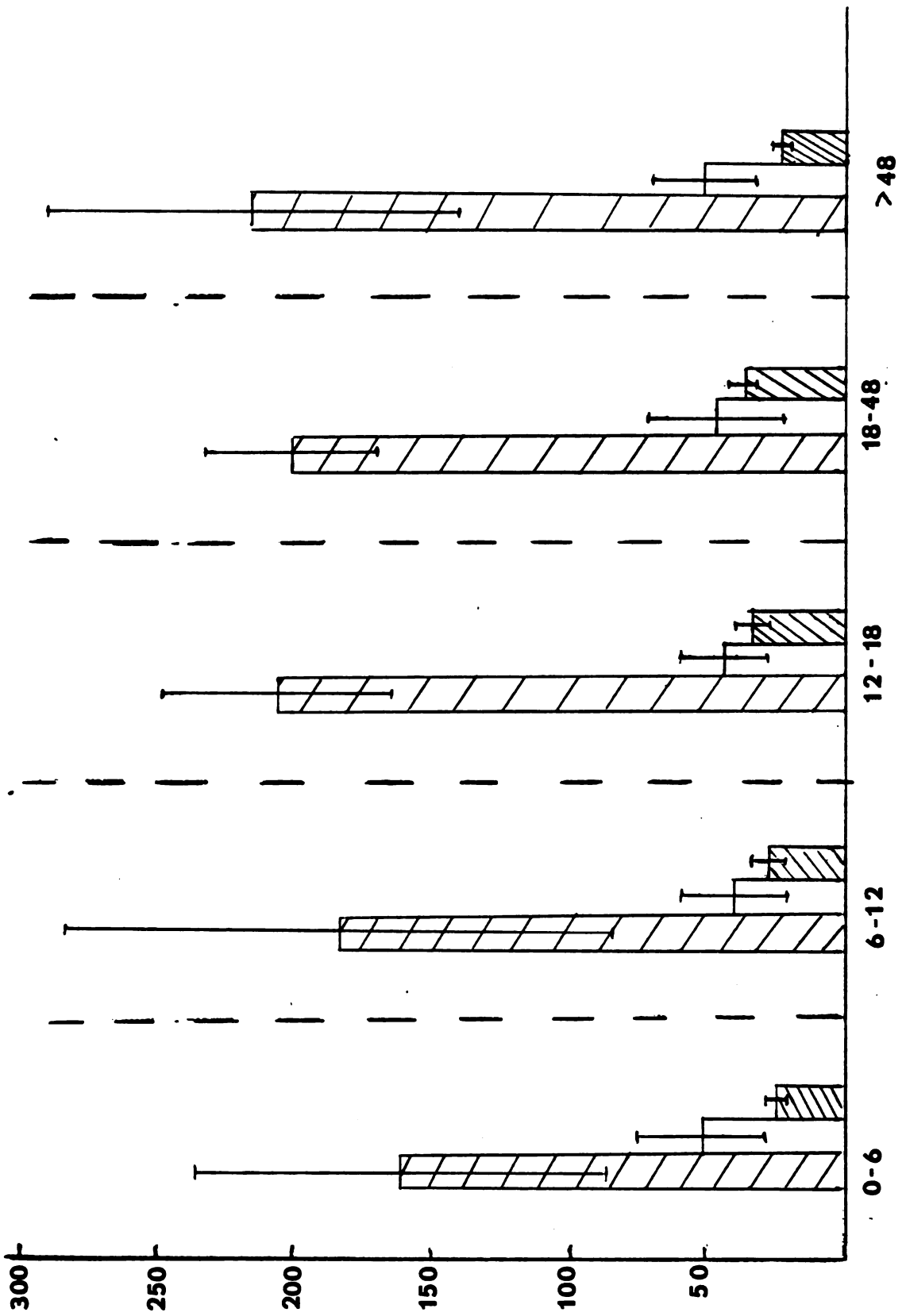

300
250
200
150
100
50
0-6
6-12
12-18
18-48
>48

TABLE IV

Plasma Vitamin A, RBP, and Triglycerides in Dialysis Patients Grouped By Treatment Time[1]

Time On Dialysis	N	Total Vitamin A	Retinol	Retinyl Palmitate	Retinyl Palmitate	RBP	Triglycerides
Months		ug/dl	ug/dl	ug/dl	% of Total A	mg/dl	mg/dl
0-6	4	160.8±74.8	155.8±72.5	5.2±2.3	3.3±0.7	23.1±6.5	176.7±41.4
6-12	6	189.2±95.0	185.6±94.7	3.6±1.9	2.0±1.3	26.9±6.7	127.2±53.3
12-18	5	204.8±41.4	200.7±40.6	4.2±1.5	2.0±0.8	29.9±6.7	145.0±38.5
18-48	6	200.3±30.7	195.6±30.4	4.5±2.6	2.3±1.3	30.2±5.3	136.5±40.0
>48	5	215.2±75.7	210.0±74.0	5.2±2.1	2.4±0.6	25.8±1.4	213.0±73.0

[1]Mean ± standard deviation.

Relationship of Plasma Retinol and Retinyl Palmitate to Plasma RBP and Triglycerides

Plasma retinol and RBP showed a significant correlation ($r = 0.66$ and $r = 0.76$) in patients and controls, respectively ($P<0.01$). Retinyl palmitate and triglycerides in patients and controls had correlation coefficients of $r = 0.56$ and $r = 0.42$ respectively, however these correlation coefficients were not statistically significant ($P>0.05$). Total vitamin A was not correlated with triglycerides in either patient or control subjects. Retinyl palmitate was not significantly correlated with RBP in patient or control subjects.

To compare the relative rise in RBP and retinol in dialysis patients, the molar ratio of RBP to retinol was calculated. The ratio was 2.1 ± 0.47 for patients and 1.5 ± 0.17 for control subjects (Appendix D). This difference was highly significant ($P<0.001$).

DISCUSSION

The total plasma vitamin A concentration in renal dialysis patients showed greater than a two-fold elevation over values for control subjects. The elevation could not be accounted for by high intakes of vitamin A from supplements or food stuffs. Dietary data indicated that the intake of total retinol equivalents in patient subjects was lower than in control subjects. In addition supplements consumed by patients did not contain vitamin A.

The use of a 24 hour recall for estimation of dietary intakes vitamin A and protein has many shortcomings; however, in the present study a food frequency checklist was used to minimize these problems. The results of the 24 hour recalls of intake in this study were very similar to the estimates of intake based on food frequency checklists. When estimating the intake of only two nutrients; i.e., protein and vitamin A, the food frequency checklist appears to be a useful tool, which is less demanding of subjects than an extended food record. Further confirmation of dietary results in this study comes from comparison with results of other research. In a study examining two day food records (98) dietary results also indicated lower intakes of vitamin A in dialysis subjects compared with

control subjects. A large part of the differences seen in these surveys of intake in dialysis patients can be attributed to restrictions of protein and electrolytes prescribed as a part of treatment during dialysis and the generally lower food intakes resulting from the anorexia associated with CRF.

The Recommended Dietary Allowances are designed to represent the nutrient needs of the healthy population. While actual vitamin A requirements of patients with chronic renal failure may differ from the healthy population no more specific recommendations are presently available.

Since dietary excesses cannot account for the elevated plasma vitamin A, an alteration in vitamin A metabolism in CRF may be responsible for the high concentrations. Most likely the alteration in vitamin A metabolism occurs as part of a more general disturbance, such as the alterations in kidney or liver function, or abnormalities in lipid metabolism. In evaluating potential causes for elevated plasma vitamin A a wide variability in the reported plasma levels of vitamin A makes comparisons of results from the different studies difficult. The reported levels of vitamin A have ranged from 60.89 ug/dl (70) to 212 ug/dl (71). One consistant finding has been that the patients had serum levels significantly greater than control subjects.

The large variability in mean vitamin A levels seen by different researchers may in part be due to differences in methods or subjects used in the assay of vitamin A. Table V shows some of the results and methods used by researchers previously mentioned in this thesis. The serum vitamin A assay method of Bessey and coworkers (99) and modifications of that method (100) tend to show higher values than the trifluoroacetic acid (TFA) methods of Neeld and Pearson (101), or fluorometric methods of Thompson and coworkers (102) and the variations of this method (103, 104). However, the results of Ellis and coworkers (71) show very high levels of vitamin A using the method of Neeld and Pearson (101). The effect of supplemental vitamin A on mean serum levels is seen in a study by Werb and coworkers (70), which showed a significant elevation in serum vitamin A of patients receiving vitamin A supplements as compared to patients not receiving supplements. However, Ellis and coworkers (71) were unable to show any decrease in serum vitamin A in patients not receiving supplements for a period of 16.3 months after receiving supplements of 5000 IU of vitamin A daily.

Assays previously mentioned do not differentiate between plasma forms of vitamin A. In this study the assays of the retinol and retinyl esters in plasma indicate significant elevations of the retinol form but only slight elevations of the retinyl ester. These results support

TABLE V

Comparison of Methodology and Results of Studies to Evaluate Vitamin A Status in Patients with Chronic Renal Failure

Research	Concentration of vitamin A* (ug/dl)	Sample (plasma or serum)	Subjects		Method of Vitamin A Assay
			Supplemental vitamin A	Treatment	
Ellis and coworkers (71)	212±19	serum	5000 IU/day	dialysis	Neeld and Pearson (101)
	205±10	serum	no supplement for 16.3 months	dialysis	
Werb and coworkers (70)	108.25±22.64	serum	5000 IU/day	dialysis	Neeld and Pearson (101)
	60.89±18.97	serum	none	dialysis	
Casey and coworkers (69)	126.4±36.5	plasma pre-dialysis	none	dialysis	O'Brien and coworkers (100)
	146.1±50.6	plasma post-dialysis	none	dialysis	
Mydlik and coworkers (87)	187.9±15.8	serum	none	not on dialysis	Bessey and coworkers (99)
	213.36±19.9	serum	none	dialysis	
Yatzidis and coworkers (41)	60.93±21.0	serum	none	not on dialysis	Neeld and Pearson (101)
	85.37±20.49	serum	none	dialysis	
Smith and Goodman (39)	116±9.3	plasma	not indicated	both on dialysis and not on dialysis	Fluorometric methods Thompson and coworkers (102) and others (103, 104)

*Mean - standard deviation

the theory that altered kidney degradation or altered liver release of retinol is the primary mechanism of elevated vitamin A, particularly the retinol form in plasma of dialysis subjects. Other research (105) examining serum forms of vitamin A have also found the retinol form elevated. Retinyl esters were reported as undetectable (105), which was consistant with the results of a study of normal subjects (106).

In the present study the significant elevation of RBP in hemodialysis patients further supports the importance of kidney and liver functions. Since RBP specifically binds and transports retinol in the plasma, the increase in RBP would expect to be accompanied by a rise in retinol. Mechanisms for elevated plasma vitamin A in dialysis patients may relate to the metabolism of RBP. It is feasible that altered RBP releases from the liver or changes in kidney degradation of vitamin A may represent important mechanisms for elevation of plasma vitamin A in CRF. The molar ratios of RBP:retinol calculated in this study indicate that the rise in plasma RBP was greater than the rise in retinol. Since research indicated that RBP released from the liver is regulated by vitamin A levels in plasma (33, 40, 41), the rise in RBP would not be expected to exceed the rise in retinol unless degradation of RBP is impaired. Since the kidney degradation of RBP in patients on dialysis is greatly reduced, this dysfunction may account for the

large rise in RBP. Some research also indicates that the decreased RBP degradation may be the underlying mechanism causing increased plasma retinol levels.

While plasma retinol levels were significantly elevated, the plasma retinyl ester levels in dialysis patients were only slightly increased and the difference from controls was not significant. Since retinyl esters are transported in lipoprotein complexes, a possible relationship between plasma retinyl esters and triglyceride was evaluated. However, no significant correlation was found. Although altered lipoprotein metabolism has been reported in dialysis patients, the incidence has been reported at only 30 to 50 percent of the patient populations studied. In this study when only the patients with elevated triglycerides were compared with the control subjects, plasma retinyl esters were significantly elevated. The levels of retinyl esters in these patients with elevated triglycerides were not as high as those reported in the study of hypervitaminosis A by Smith and Goodman (44), but the difference may represent an altered metabolism of retinyl esters in CRF.

When plasma retinol, retinyl esters, RBP and triglyceride levels in patients grouped by time since initial dialysis treatment were compared, the differences between groups were not statistically significant. However, a slight trend of increasing retinol and RBP with increased

time on dialysis was seen. Thus, the elevated plasma vitamin A of dialysis patients seems to be one which is not improved by dialysis. Dietary intake of vitamin A is not in excess of the RDA; therefore decreasing intake might not be expected to greatly affect plasma levels of vitamin A. Post renal transplant plasma vitamin A levels have shown improvement (41); however, many patients are being maintained on dialysis treatment for periods greater than four years. The potential risks of elevated plasma vitamin A levels are of interest for the long-term treatment of CRF by hemodialysis.

In this and other research on the vitamin A status of dialysis patients it has been found that the serum vitamin A levels, while they are elevated, generally do not reach the levels seen in documented cases of hypervitaminosis A. The serum vitamin A levels reported in cases with toxic symptoms of hypervitaminosis A range from 58.7 ug/dl (107) to 543.6 ug/dl (108). While the plasma levels of vitamin A in dialysis subjects rarely reach 500 ug/dl or more, the plasma vitamin A in this study and many other exceeds 60 ug/dl. The overlap in the range of plasma vitamin A levels seen in CRF and hypervitaminosis A and the similarities of symptoms seen in these two conditions have prompted researchers to explore the effects of elevated vitamin A levels in hemodialysis patients (17, 109). Some of the similarities in symptoms are listed in Table VI. As in

TABLE VI

Features Seen in Hypervitaminosis A and/or Chronic Renal Failure

Chronic Renal Failure	Hypervitaminosis A
Hypertension (54)	Hypertension (107)
Edema (63)	Edema - facial and extremity (108)
	Increased intracranial pressure (108, 109)
	Fever (108)
Fatigue (54)	Fatigue (108)
Anorexia Nausea (57, 62, 64) Vomiting	Anorexia Nausea (107, 108, 109) Vomiting
Bleeding (59, 62)	Bleeding (109)
Skin dryness and itching (58, 60, 62)	Skin dryness and itching (107, 108, 109, 110)
Pigmentation (58) Rashes (58, 60)	Pigmentation (108) Rashes (108) Cheilosis (107) Gingivitus (107) Brittle nails (107) Hair loss (107, 108, 109)
Normal or decreased serum calcium (54, 55) Elevated parathyroid hormone (55) Bone abnormalities (54)	Elevated serum calcium (108, 109) Normal parathyroid hormone (110) Bone resorption (110)
Muscle twitches (55) Peripheral paresthsia (59)	Muscle fasciculation (109) Peripheral paresthesia (109)

many other complex conditions, patients with either CRF or hypervitaminosis A rarely exhibit all the symptoms that have been associated with the specific condition. However, patients generally exhibit several of the symptoms. In the situation of trying to distinguish between CRF and hypervitaminosis A, a large number of symptoms common to both conditions may cause hypervitaminosis A to be overlooked in assessing health status of the dialysis patient. However, there are some symptoms commonly seen in cases of hypervitaminosis A that distinguish it from chronic renal failure. Serum calcium, in hypervitaminosis A, is usually elevated, even in the presence of normal parathyroid hormone levels (108, 109, 110). This is in direct contrast to the normal or decreased serum calcium seen in chronic renal failure, even when parathyroid hormone levels are elevated (55, 62, 64). A symptom diagnostic of hypervitaminosis A, which may be more easily recognized than serum calcium and parathyroid hormone levels, is the loss of hair from scalp and eyebrows. In chronic renal failure, hair loss has been documented only in cases where cytotoxic agents have been used to prevent renal homograft rejection (109). In assessing the risks associated with elevated serum vitamin A in hemodialysis patients hair loss may be an easily recognized symptom.

Hypervitaminosis A usually accompanies vitamin A intakes greatly exceeding recommended intakes. The RDA

for vitamin A is 800 R.E. (4000 IU) for women and 1000 R.E. (5000 IU) for men per day (89). Multivitamin supplements generally contain approximately 1000 R.E. (5000 IU) of vitamin A, although supplements containing up to 5000 R.E. (25,000 IU) are available without prescription. Reported cases of hypervitaminosis A have documented intakes of vitamin A ranging from 41,000 (107) to 250,000 IU (108) of vitamin A per day. With very high dose supplementation, symptoms of fever, nausea, vomiting, rash, and other skin symptoms appear quickly, often within 3 days, and patients seek attention quickly. However with the less high levels of excess intake the symptoms may appear more slowly and the patient may not seek immediate medical attention. Some patients have taken high doses of vitamin A over several months. It is important to note that the dietary source of vitamin A in most of the cases of hypervitaminosis A in adults is a vitamin supplement containing preformed vitamin A. High dietary intakes of the carotenoid precursers of vitamin A will not produce the symptoms of hypervitaminosis A because the body mechanisms for converting carotenes to vitamin A do not function rapidly enough to produce sufficient retinol to be toxic. Excess intake of carotenes usually results in only a yellowish discoloration of the skin which disappears once intake returns to normal. When levels of preformed vitamin A are ingested, the excesses are stored in the liver. Because of the large

storage of vitamin A in the liver during hypervitaminosis A, cessation of intake results in a gradual decline to normal serum values. Cases studied by Smith and Goodman (34) showed a return to normal serum levels over a period of 24 to 77 days in different patients.

Hypervitaminosis A research (44) has indicated that the concentrations of retinyl esters are generally elevated, while retinol levels are normal or only slightly elevated. Additionally, RBP levels are lower than normal (44). The results of this study show a significant elevation of retinol in dialysis patients while the retinyl esters are only slightly elevated, except in patients with elevated triglycerides where the retinyl esters are significantly elevated over control subjects. RBP levels in dialysis patients were also significantly elevated compared with controls.

Toxicity symptoms from elevated plasma retinol have not been reported. It may be the transport complex of RBP and PA, which is important in reducing toxicity of retinol. Goodman (18) postulates that one function of this protein-vitamin complex is to deliver vitamin A to specific target tissues. In addition he suggests that the complexing of retinol in plasma may protect non-targeted tissues from the membrane-altering actions of vitamin A. In view of the effect of RBP-PA in complexing retinol, it may be possible to have elevated plasma levels of total vitamin A without

symptoms of hypervitaminosis A. This might be expected if only target tissues having a high demand for vitamin A are exposed to the high levels of the vitamin. This limited exposure would occur in cases where retinol and RBP are increased either equally or RBP is increased to a greater extent than retinol.

From the observations in the present study and other research reviewed from literature, it is possible to conclude that the elevated plasma vitamin A in hemodialysis patients is an elevation of retinol with a concurrent elevation of RBP. While some research (43, 50, 51) indicates that a large proportion of the RBP may be incapable of forming a complex with retinol, more recent research (52) does not support the hypothesis of an altered RBP without binding ability. In view of such results it is questionable whether hemodialysis patients would suffer any ill effects from the elevations of plasma vitamin A seen routinely in this group.

A review of literature shows only one case, reported by Shmunes (109), describing hypervitaminosis A symptoms in a patient on hemodialysis treatment. The serum vitamin A level was 140 ug/dl and for one month the patient had been receiving supplements of 4,000 IU of vitamin A daily; during the previous two years the patient had been receiving 5,000 IU of supplemental vitamin A per day. The patient presented with complaints of itching scalp and hair loss. One month following the cessation of supplemental vitamin A

the symptoms had cleared and new hair growth at the margins of the scalp could be seen. A second serum vitamin A determination was not made due to the relocation and abrupt death of the patient from the complications of a kidney transplant operation. This study indicates a possibility that toxic symptoms of hypervitaminosis A may occur in dialysis patients with levels of plasma vitamin A frequently seen in CRF and with supplementation at normal levels. However, due to limited followup, lack of information regarding dietary intake of vitamin A in addition to supplements, plasma forms of vitamin A, and abnormalities of lipoprotein metabolism, the causes and implications of this case are difficult to assess.

CONCLUSIONS

Elevations of plasma vitamin A found in chronic renal failure patients undergoing hemodialysis treatment corresponds to an increase in the retinol form of vitamin A. Dietary intakes of vitamin A were lower than controls and frequently less than the Recommended Dietary Allowance, suggesting that excess dietary intake was not a contributing factor in elevated plasma vitamin A levels.

Because RGP and retinol were both elevated and remained in approximately the same molar ratio as seen in normal subjects, the increased plasma retinol does not appear to represent a risk for development of toxic symptoms of hypervitaminosis A.

In hemodialysis patients with abnormalities of lipoprotein metabolism, evidenced by elevated triglycerides, an increase in plasma retinyl palmitate was seen and may be associated with abnormalities in the metabolism of retinyl esters.

A significant rise in plasma vitamin A, RBP or triglycerides with increasing time since initial dialysis treatment was not found, although it appeared that there may be a slight trend for retinol and RBP to increase with

length of treatment. This trend may be important for patients on long-term dialysis treatment.

While this research indicates that dialysis patients, who are not routinely supplemented with vitamin A do not appear to be at risk for toxic symptoms of hypervitaminosis A, it is not possible to conclude that risks from elevated plasma vitamin A in dialysis patients do not exist.

In further studies of the vitamin A status of hemodialysis patients it may be possible to clarify any risks from elevated plasma vitamin A by evaluating several factors previously receiving little attention. These factors include: plasma levels of retinyl esters; lipid metabolism, particularly lipoprotein complexes which transport retinyl esters; dietary contributions of preformed vitamin A from supplements, as well as animal products and fortified foodstuffs; and clinical symptoms which differentiate chronic renal failure and hypervitaminosis A.

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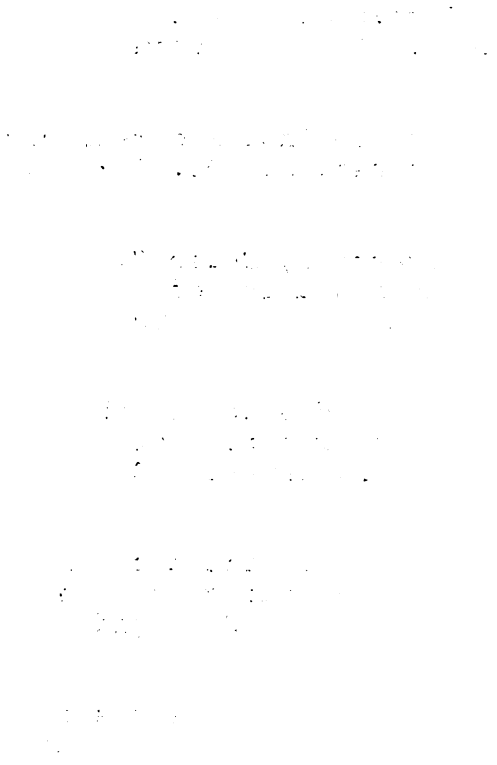

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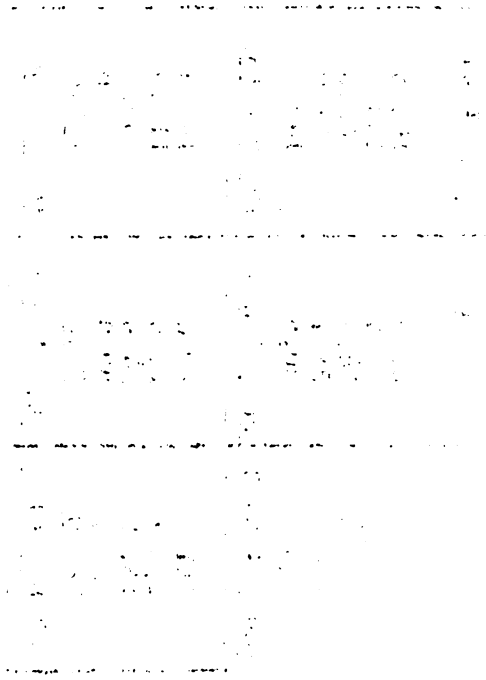
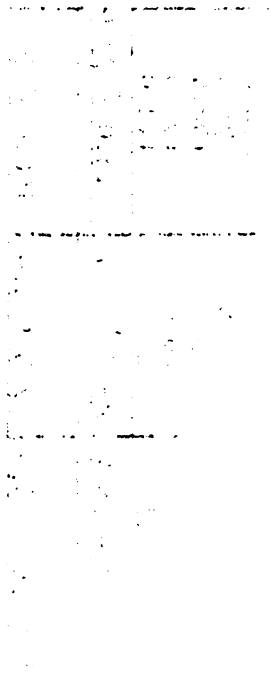
APPENDICES



APPENDIX A

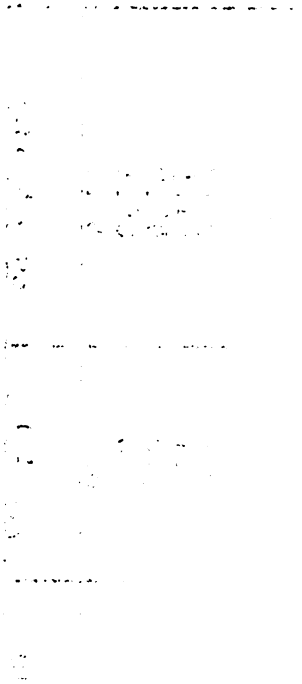
Concentrations of Plasma Vitamin A, RBP, and Triglycerides in Patients Grouped by Length of Treatment by Hemodialysis

Subject (Months on dialysis)	Retinol (ug/dl)	Retinyl Palmitate (ug/dl)	Total Vitamin A (ug/dl)	RBP (mg/dl)	Triglycerides (mg/dl)
0- 6 months on dialysis					
A 06 (5)	135.4	6.2	141.5	19.1	181.5
A 07 (6)	95.9	2.6	97.5	19.1	119.6
A 08 (4)	179.0	4.9	183.8	29.1	153.7
A 09 (2)	98.3	3.6	101.9	17.2	224.1
A 12 (2)	270.6	8.6	279.1	31.2	204.8
mean ± s.d.	155.8 ± 72.5	5.2 ± 2.3	160.8 ± 74.8	23.1 ± 6.5	176.7 ± 41.4
6-12 months on dialysis					
A 10 (12)	106.6	1.4	108.0	21.0	145.5
A 11 (11)	164.4	6.6	170.9	33.4	188.3
B 05 (11)	350.2	3.4	353.6	34.7	62.8
B 07 (8)	157.3	2.7	160.0	25.1	71.9
B 08 (10)	149.7	3.8	153.5	20.5	142.5
mean ± s.d.	185.6 ± 94.7	3.6 ± 1.9	189.2 ± 94.4	26.9 ± 6.7	122.2 ± 53.3
12-18 months on dialysis					
B 02 (18)	246.0	5.4	251.4	29.6	198.3
B 03 (13)	170.4	1.7	172.1	22.9	116.0
B 09 (18)	147.0	4.8	151.8	24.9	100.0
B 12 (18)	220.4	5.3	225.7	40.0	153.8
B 16 (14)	219.3	3.6	222.9	32.0	156.8
mean ± s.d.	200.7 ± 40.6	4.2 ± 1.5	204.8 ± 41.3	29.9 ± 6.7	145.0 ± 38.5



APPENDIX A (cont.)

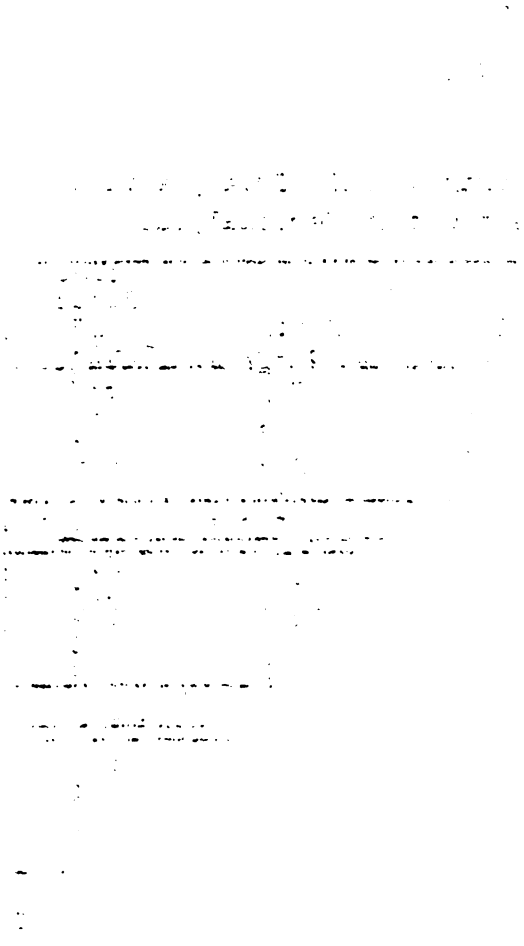
Subject (Months on dialysis)	Retinol (ug/dl)	Retinyl Palmitate (ug/dl)	Total Vitamin A (ug/dl)	RBP (mg/dl)	Triglycerides (mg/dl)
18-48 months on dialysis					
A 13 (35)	173.3	4.7	178.0	27.9	119.4
B 01 (20)	175.8	6.5	182.3	29.1	199.3
B 10 (43)	165.1	1.3	167.3	21.8	74.1
B 11 (40)	216.2	2.6	218.8	31.3	184.2
B 13 (35)	198.9	8.4	207.2	34.9	156.4
B 14 (30)	244.4	3.6	247.9	36.4	165.3
mean ± s.d.	195.6 ± 30.4	4.5 ± 2.6	200.3 ± 30.2	30.2 ± 5.3	136.5 ± 40.0
> 48 months on dialysis					
A 01 (72)	180.8	5.2	186.0	24.8	292.6
A 02 (67)	209.0	6.9	215.9	24.8	275.6
A 03 (49)	251.1	4.7	255.7	26.4	192.4
B 06 (57)	302.9	7.1	310.0	28.0	192.6
B 15 (65)	106.3	1.9	108.1	24.9	112.0
mean ± s.d.	210.0 ± 74.0	5.2 ± 2.1	215.2 ± 75.7	25.8 ± 1.4	213.0 ± 73.0
Total Patient Group mean ± s.d.	189.8 ± 62.8	4.5 ± 2.0	183.5 ± 66.9	27.3 ± 5.9	160.9 ± 56.7



APPENDIX B

Concentrations of Plasma Vitamin A, RBP, and Triglycerides in Control Subjects

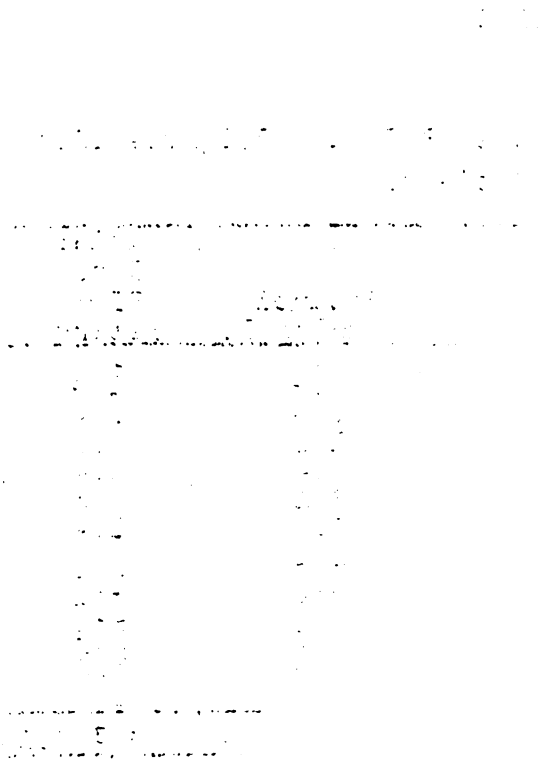
Subject	Retinol ug/dl	Retinyl Palmitate ug/dl	Total Vitamin A ug/dl	RBP mg/dl	Triglycerides mg/dl
C 01	81.4	2.1	83.5	9.8	28.7
C 02	70.6	2.9	73.5	7.2	70.4
C 03	53.5	2.9	56.3	5.4	45.8
C 04	51.0	2.2	53.2	5.8	99.7
C 05	69.2	1.8	70.9	7.3	154.2
C 06	87.7	4.3	92.0	8.9	42.8
C 07	60.1	2.2	62.3	6.0	68.3
C 08	59.0	3.7	62.1	7.7	72.0
C 09	64.8	6.4	71.2	8.1	91.3
C 10	64.9	4.0	68.8	8.1	41.5
C 11	63.0	2.0	65.0	7.0	44.9
C 12	68.8	8.4	77.3	6.0	86.1
C 13	67.1	2.0	69.1	6.8	51.8
mean $\pm$ s.d.	66.2 $\pm$ 10.1	3.4 $\pm$ 2.0	69.6 $\pm$ 10.6	7.2 $\pm$ 1.3	69.0 $\pm$ 33.5



APPENDIX C

Relationships of Plasma Vitamin A, RBP, Triglycerides Grouped by Length of Treatment by Hemodialysis

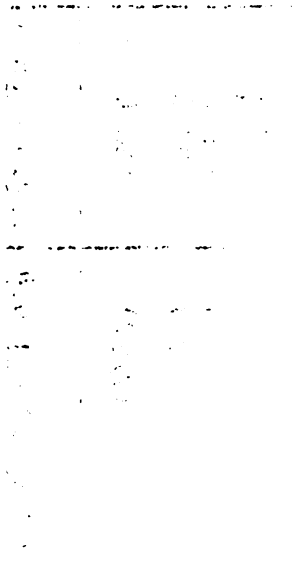
Subject (Time on Dialysis)	Retinyl Palmitate % of Total Vitamin A	RBP u moles/l.	Retinol u moles/l.	Molar Ratio RBP: Retinol
A 06	4.4	9.1	4.7	1.9
A 07	2.7	9.1	3.4	2.7
A 08	2.6	13.8	6.3	2.2
A 09	3.6	8.2	3.4	2.4
A 12	3.1	14.9	9.5	1.6
mean ± s.d.	3.3 ± 0.7	11.0 ± 3.1	5.4 ± 2.5	2.2 ± 0.4
A 10	1.3	10.0	3.7	2.7
A 11	3.8	15.9	5.8	2.8
B 05	0.5	16.5	12.2	1.4
B 07	1.7	11.9	5.5	2.2
B 08	2.5	9.8	5.2	1.9
mean ± s.d.	2.0 ± 1.3	12.8 ± 3.2	6.5 ± 3.3	2.2 ± 0.6
B 02	2.1	14.1	8.6	1.6
B 03	1.0	10.9	6.0	1.8
B 09	3.2	11.9	5.1	2.3
B 12	2.4	19.0	7.7	2.5
B 16	1.6	15.2	7.7	2.0
mean ± s.d.	2.0 ± 0.8	14.2 ± 3.2	7.0 ± 1.4	2.0 ± 0.3
A 13	2.6	13.0	6.1	2.2
B 01	3.6	13.9	6.2	2.3
B 10	0.8	10.4	5.8	1.8
B 11	1.2	14.9	7.6	2.0
B 13	4.0	16.6	7.0	2.4
B 14	1.6	17.3	8.5	2.0
mean ± s.d.	2.3 ± 1.3	14.4 ± 2.5	6.8 ± 1.1	2.1 ± 0.2
A 01	2.8	11.8	6.3	1.9
A 02	3.2	11.8	7.3	1.6
A 03	1.8	12.6	8.8	1.4
B 06	2.3	13.4	10.6	1.3
B 15	1.7	11.9	3.7	3.2
mean ± s.d.	2.4 ± 0.6	12.3 ± 0.7	7.3 ± 2.6	1.9 ± 0.8
Total Patient Group mean ± s.d.	2.4 ± 1.0	13.0 ± 2.8	6.6 ± 2.2	2.1 ± 0.5



APPENDIX D

Relationships of Plasma Vitamin A, RBP, and Triglycerides in Control Subjects

Subject	Retinyl Palmitate % of Total Vitamin A	RBP u moles/l.	Retinol u moles/l.	Molar Ratio RBP: Retinol
C 01	2.5	4.6	2.8	1.6
C 02	4.0	3.4	2.5	1.4
C 03	5.1	2.6	1.9	1.4
C 04	4.2	2.8	1.8	1.6
C 05	2.5	3.5	2.4	1.4
C 06	4.7	4.2	3.1	1.6
C 07	3.5	2.9	2.1	1.4
C 08	4.9	3.7	2.1	1.8
C 09	9.0	3.9	2.3	1.7
C 10	5.8	3.9	2.3	1.7
C 11	3.1	3.4	2.2	1.5
C 12	10.9	2.9	2.4	1.2
C 13	2.9	3.2	2.4	1.4
mean ± s.d.	4.8 ± 2.5	3.4 ± 0.6	2.3 ± 0.4	1.5 ± 0.2



APPENDIX E

Comparison of Vitamin A and Triglyceride Concentrations as Measured by Borgess Hospital and This Study

Subject	Vitamin A ug/dl		T.G. mg/dl	
	Borgess Hospital[1]	This Study[2]	Borgess Hospital[1]	This Study[2]
A 01	162 (5/77)	186.0	232 (11/77)	292.6
02	379 (9/77)	208.9	516 (6/77)	275.6
07			180 (10/77)	119.6
10	198 (8/77)	106.6	102 (8/77)	145.5
13	154 (8/77)	173.3	252 (8/77)	119.4
B 02			250 (7/77)	198.3
03	122 (1/78)	170.4	223 (1/78)	116.0
09	100 (1/78)	147.0	168 (1/78)	192.6
10	132 (5/77)	165.1		
13			137 (7/77)	156.4
15	149 (4/77)	106.3		
mean - s.d.	174.5 - 87.6	157.9 - 36.4	228.9 - 119.4	179.5 - 66.6

[1]Date of test in parenthesis
[2]Sampled 11/77.

APPENDIX F

EXPERIMENTAL EXPLANATION

In people with several types of kidney disease the level of vitamin A in the blood is higher than usually found in individuals without kidney disease. In this study the concentration of total vitamin A, the proportion of different forms of vitamin A, the two blood components which transport vitamin A, (retinol binding protein and lipoprotein complexes) will be measured in the plasma of hemodialysis patients and a group of subjects without kidney disease.

The results of these measurements will be compared to determine the differences between hemodialysis patients and people without kidney disease. Also the results will be examined to see if any relationship exists between the concentrations of these components and the length of time since the initial hemodialysis treatment.

As a subject in the control group you will be asked to give one blood sample. The sample will be taken either at Borgess Hospital when your routine Australian antigen test blood samples are drawn, or at Olin Health Center on the Michigan State University campus at a prearranged time. Because prior food intake may influence the results of the tests for vitamin A and lipids, you will be asked to fast for twelve hours before coming to give a blood sample.

In addition to providing a blood sample you will be asked to participate in a dietary interview at the time of the blood sampling. In this interview you will be asked what foods you ate in the past twenty-four hours and how frequently you eat certain foods listed on a check sheet as well as the size serving of these foods you usually eat.

Risks involved in the project are those associated with blood sampling and the protection of confidentiality of information obtained in the dietary interview. A qualified person will draw a blood sample of 5 to 7 ml, which is not in excess of the amount taken for routine blood studies. All information obtained from you in the dietary interview will be kept strictly confidential. The reports of the results will not include your name in any way. You are free to withdraw from the study at any time without penalty.

The intent of this study is to obtain information which will be beneficial to all kidney patients receiving hemodialysis. There are no guaranteed benefits to you as an individual subject. You are free to withdraw from the study at any time without penalty.

If any of the laboratory results are abnormal we will report these to your physician. In order to do this you will be requested to furnish his name and address. You may request a report of the study which will not include any individual results but will summarize results by experimental groups.

APPENDIX G

Experimental Explanation

In people with several types of kidney disease the level of vitamin A in the blood is higher than usually found in individuals without kidney disease. In this study the concentration of total vitamin A, the proportion of different forms of vitamin A, and two blood components which transport vitamin A, (retinol binding protein and lipoprotein complexes) will be measured in the plasma of hemodialysis patients and a group of subjects without kidney disease.

The results of these measurements will be compared to determine the differences between hemodialysis patients and people without kidney disease. Also the results will be examined to see if any relationship exists between the concentrations of these components and the length of time since the initial hemodialysis treatment.

As a subject in the dialysis patient group, blood samples will be taken immediately before one dialysis session. Because prior food intake may influence the results of the tests for vitamin A and lipids, you will be asked to fast for twelve hours before coming to dialysis.

In addition to providing blood samples you will be asked to participate in a dietary interview at the time of the blood sampling. In this interview you will be asked what foods you ate in the past twenty-four hours and how frequently you eat certain foods listed on a check sheet as well as the size serving of these foods you usually eat. Additional information concerning results of laboratory tests, body weight, and medications will be obtained from your medical records and will be used to clarify the results of the blood analyses.

Risks involved in the project are those associated with blood sampling and the protection of confidentiality of information obtained in the dietary interview or from your medical records. Qualified persons will draw blood samples of 5 to 7 ml, which is not in excess of the amount often taken for routine blood studies. Samples will be drawn from the dialysis tube or shunt thus removing the need for an additional vena puncture. All information obtained from you in the dietary interview or from your medical records will be kept strictly confidential. The reports of the results will not include your name in any way. You are free to withdraw from the study at any time without penalty or jeopardy to your continued medical treatment.

APPENDIX G (cont.)

The intent of this study is to obtain information which will be beneficial to all kidney patients receiving hemodialysis. There are no guaranteed benefits to you as an individual subject. You may request a report of the study which will not include any individual results but will summarize results by experimental groups.

APPENDIX H

CONSENT FORM CONTROL GROUP

DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION
MICHIGAN STATE UNIVERSITY
EAST LANSING, MICHIGAN

I, _____, agree to participate as a control subject in a study of plasma levels of vitamin A in renal patients on hemodialysis and the effects of the length of time on dialysis on the plasma vitamin A levels. The purpose of the study and the procedures have been explained to me by _____, and I have had an opportunity to ask questions. I agree to participate in a dietary interview and to have a blood sample taken for analysis. I recognize the potential risks and benefits of the procedure and I understand there are no guaranteed benefits to me as an individual. I know I may withdraw from the study at any time without penalty. I understand that all information gathered in this study from me or assays of my blood will be kept in strict confidence and that I will remain anonymous in all reports of the results. A summary of the results will be provided to me at my request.

Signed: _____

Date: _____

APPENDIX I

CONSENT FORM PATIENT GROUP

DEPARTMENT OF FOOD SCIENCE AND HUMAN NUTRITION
MICHIGAN STATE UNIVERSITY
EAST LANSING, MICHIGAN

I, _____, agree to participate in a study of plasma levels of vitamin A in renal patients on hemodialysis and the effects of the length of time on dialysis on the plasma vitamin A levels. The purpose of the study and the procedures have been explained to me by _____, and I have had an opportunity to ask questions. I agree to participate in a dietary interview and to have blood samples drawn for analysis. I recognize the potential risks and benefits of the procedures and I understand there are no guaranteed benefits to me as an individual. I give my consent to have my medical records released to the investigator to obtain information concerning results of laboratory tests, body weight, and medications. I know that I may withdraw from this study at any time without penalty or jeopardy to my medical treatment. I understand that all information gathered in this study from me, my medical records, or assays of my blood will be kept in strict confidence and that I will remain anonymous in all reports of the results. A summary of the results will be provided to me at my request.

Signed: _____

Date: _____