EFFECTS OF ENTERAL SUPPLEMENT ON FREE PLASMA CARNITINE AND NUTRIENT INTAKE OF PATIENTS WITH END-STAGE RENAL DISEASE RECEIVING HEMODIALYSIS

by

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ABSTRACT

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Effects of enteral supplementation on free plasma carnitine and nutrient intake of
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patients with end-stage renal disease receiving hemodialysis

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Carnitine is a low molecular weight amino acid that is derived from methionine and lysine with vitamin C, vitamin B_6 and iron as cofactors. Carnitine is required for transporting large chain fatty acids across the mitochondrial membrane and is important in muscle function. Low plasma levels of carnitine may indicate malnutrition in patients receiving hemodialysis. Deficiencies in carnitine may induce hypoglycemia. Levocarnitine supplementation has improved the patient's plasma lipid profile, exercise tolerance, and hematocrit.

The purpose of this study was to determine if oral supplementation with Nepro®, which is enriched with carnitine, has any effect on the plasma free-carnitine level of patients receiving maintenance hemodialysis. A consequence of dialysis is depletion of muscle carnitine. Depletion of muscle carnitine causes weakness, fatigue, and confusion.

Persons on maintenance hemodialysis are unable to replete their carnitine stores due to their diet, aversions, and continuous dialysis removal. Nepro® is enriched with carnitine; it also provides calories and protein.

The participants for this study were recruited from two dialysis centers in East Central Wisconsin. All participants signed approved consent and permission forms. The participants had been on hemodialysis for at least six months. The recruitment and data collection began July 2003.

The participants in the Treatment group were asked to drink the Nepro® at assigned times, so consumption of two cans of Nepro® would be 12 hours and 2 hours prior to the blood draws. The Non-Treatment group participants not consuming Nepro® were asked not to consume foods high in carnitine prior to their blood draw. These foods would include meat and dairy products. The blood draws were obtained before dialysis started from the participant's access, either their fistula or port.

This study also examined the participants' diets. A 24-Hour Recall was obtained from each of the participants. A food frequency had been attempted, but there wasn't a great variety in the diet of the participants. Each participant's weight, height, and 24-Hour Recall were entered into the ESHA Food Processor Program. This program generated the participants BMI, calorie intake and needs, and selected macronutrient and micronutrient intake.

The data from the blood draws and the Food Processor Program were entered into SPSS. SPSS is a computerized statistical program that was used to generate means and to run independent and dependent t-tests.

The results of this study indicated that there was no significant correlation with the consumption of Nepro® and the plasma free-carnitine concentration. However, the participants in the Treatment group did have higher calorie, protein, and other micronutrient intake than the subjects in the Non-treatment group. This appears to be a benefit of drinking Nepro®.

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CHAPTER I

Introduction

In the United States there are 20 million people with chronic kidney disease. In 2000, end-stage renal disease (ESRD) affected 458,113 people in the United States. There were 96,192 new cases of ESRD that year, and it cost $19.35 billion to treat these patients (Kidney and urologic disease statistics US, 2000).

End-stage renal disease is primarily caused by the progression of diabetes and hypertension. The human kidneys work very efficiently, and patients are often unaware they have renal disease until it is advanced. The normal glomerular filtration rate (GFR) ranges from 80 to 120 mL per minute. Patients may not have symptoms until their GFR decreases to 10 mL per minute (Zeman and Ney, 1996). At this time the kidney is not filtering the blood of the patient, urine output has decreased, and accumulating electrolytes are affecting the acidity of the blood.

Hemodialysis is utilized to remove toxins from the blood until a transplant kidney is available for the patient. Although, some patients are not able to receive a donor organ due to other medical complications, they routinely receive peritoneal or hemodialysis to remove waste from their body.

There have been many advances in the field of nephrology over the years that include, injecting patients with Epogen to promote the production of red blood cells, decreasing the prevalence of anemia, and the need for blood transfusions. The use of phosphate binders was introduced to decrease the amount of circulating phosphorus, which affects the acid base balance of the blood. Re-use of dialyzers is a common practice in many dialysis units, primarily to cut down on cost. Another practice has been

to inject patients with carnitine after their dialysis treatments. These methods have all helped dialysis patients to lead more normal lives.

The incidence of kidney disease is expected to continually increase due to the increased prevalence of diabetes and hypertension in the United States. Recent research has been looking at plasma homocysteine levels and its role in atherosclerosis. Other research has been concentrating on the role that carnitine plays in the metabolism of fatty acids. Ongoing research is being conducted to improve the prognosis of patients that have kidney diseases.

Diet also plays an important role in the health status of the patient prior to receiving dialysis and during treatment. Protein and calories are adjusted to meet the needs of the individuals. Ongoing dietary instruction is communicated to the patients to promote compliance of potassium, sodium, phosphorus, and fluid restrictions. Suggestions are given to the patients on what to eat to increase their calorie and protein intake. Enteral supplements are also encouraged when patients are unable to consume adequate nutrition.

In this study eighteen participants receiving routine hemodialysis were interviewed to obtain a 24-hour diet recall, and blood samples were drawn to determine the participant's plasma carnitine levels. The eight participants in the Treatment Group routinely consumed the enriched enteral supplement Nepro®, while the other 10 participants in the Non-Treatment Group did not consume Nepro®. None of the participants in this study used any other form of carnitine supplementation.

The results of the 24-hour recall, and the plasma carnitine levels were assessed to determine if supplementation had an effect on the carnitine values of patients receiving maintenance hemodialysis in east central Wisconsin.

Statement of Problem

The purpose of this study is to determine if compliance of a renal diet affects carnitine values, and if Nepro® supplementation has any effects on plasma carnitine levels in patients with end-stage renal disease receiving routine hemodialysis.

The subjects for the study had been receiving hemodialysis treatments at Dialysis Care and Ministry Dialysis Centers in central and eastern Wisconsin for at least six months prior to the gathering of data. Plasma blood samples and a 24-hour dietary recall were obtained from the subjects during July and August of 2003.

Research Questions

There are four questions this study wished to answer. They are:

1. To identify sources of carnitine participants are consuming and to determine if dietary intake of folate, vitamin B_{12}, and vitamin B_6 were adequate.
2. To determine if compliance of a renal diet effects carnitine values.
3. To determine if dietary intake correlates with carnitine levels.
4. To identify if enteral supplementation of Nepro®, which contains 62 milligrams of carnitine, is effective in increasing plasma carnitine levels.

Assumptions

There are several assumptions made regarding the research questions. The first two questions focused on compliance to a strict diet and the resulting carnitine value of the patient. The foods restricted in the renal diet tend to be ones that are excellent

sources of carnitine. It is assumed that the participants will have low normal plasma carnitine levels.

The third question addressed whether the participants diet history reflected the plasma carnitine level. It is assumed that the participant's oral carnitine intake will be reflective of the plasma level.

The last question addresses supplementation with a carnitine enriched enteral drink and how it affected plasma carnitine levels. It was assumed that Nepro® would have little effect on increasing the carnitine levels of the participants due to the routine removal of carnitine during dialysis treatments and diminished ability of the patient with ESRD to utilize oral supplementation of carnitine.

CHAPTER II

Review of Literature

Introduction: The review of the literature is divided into sections that will elaborate on renal disease and the nutrient carnitine. The first section will pertain to the physiology and function of the kidneys. The second section will cover renal disease and methods of treatment. Diet modifications and carnitine supplementation will the focus of the last section of the review of literature.

Human Kidney

The two kidneys are dark red, bean-shaped organs, which are about 10 centimeters long. They are located at the back of the body, behind the stomach and liver on either side of the spinal column (Curtis and Barnes, 1989). Each kidney is comprised of an outer portion, the cortex, and the inner medulla.

The nephrons are the structural and functional units of the kidney. Within each kidney there are approximately one million nephrons that work to filter waste (urea and creatinine) and water from the blood. The nephrons produce urine and reabsorb water in the body (Klahr, 1996).

Each nephron consists of a glomerulus enclosed within the Bowman's capsule, a proximal convoluted tubule, the Loop of Henle; the distal convoluted tubule, and branched collecting ducts (Montgomery, 1996). The glomerulus, Bowman's capsule, proximal convoluted tubules, and distal convoluted tubules are located in the cortex of the kidney. The Loop of Henle and collecting ducts descend into the medulla portion of

the kidney. From the collecting ducts urine is passed into the renal pelvis, to the ureter, and into the bladder. From the bladder urine is then excreted out of the body via the urethra.

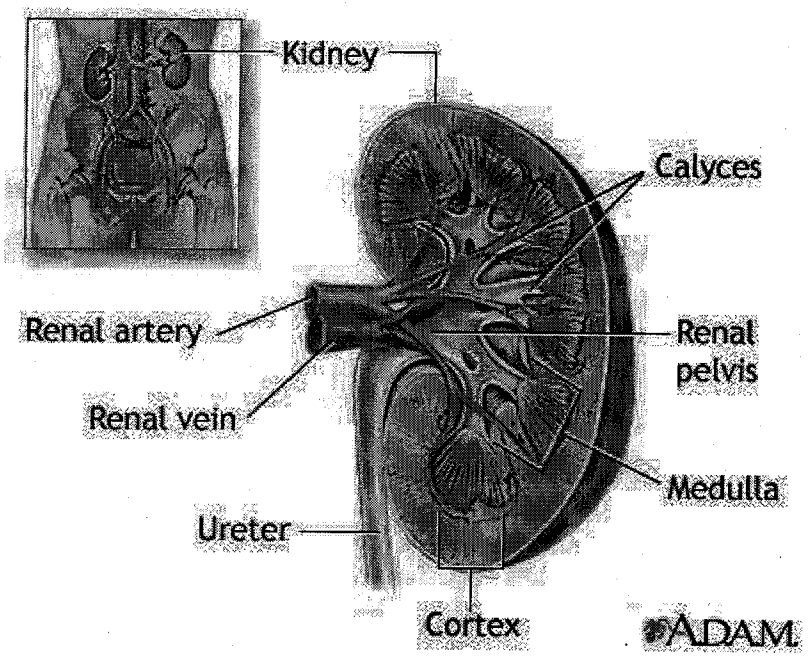


Figure 1. The Human Kidney
Source: https://MEDLINEplus%20Medical%20Encyclopedia%20Kidney%20anatomy.EML/1_multipart_xF8FF_2_MEDLINEplus%20Medical%20Encyclopedia%20Kidney%20anatomy.jpg

Functions of the Kidney

The kidney performs four basic functions: filtration, secretion, reabsorption, and excretion. The kidney acts as a filter for the circulating blood in the body. In the healthy kidney, large molecules, such as protein, cannot cross the capillary wall (Curtis and Barnes, 1989). Protein excreted in the urine is an indicator that some component of the kidney is not absorbing and/or filtering correctly.

The kidney functions include the excretion of metabolic waste products, such as urea, uric acid, and creatinine. It maintains the volume and ionic composition of body fluids, and works to eliminate and detoxify drugs and toxins in the body. The kidney regulates the systemic blood pressure, and produces erythropoietin (Masud and Mitch, 2001). This organ works to degrade and break down peptide hormones such as insulin, glucagon, parathyroid hormone, and small molecular weight proteins. The kidney aids in the regulation of metabolism, including gluconeogenesis and lipid metabolism (Klahr, 1996).

The kidneys play an essential role in vitamin D metabolism. Cholecalciferol is hydroxylated in the liver to 25-hydroxycholecalciferol. It is then converted to 1, 25-dihydroxycholecalciferol (1, 25-dihydroxyvitamin D) in the kidney, the form of vitamin D used in the body to aid in calcium absorption (Kopple, 1999).

The enzyme renin is synthesized and stored in the juxtaglomerular apparatus, which is adjacent to the afferent arteriole of the kidney (McDonald, Miller, and Guggenheim, 1977). The renin-angiotensin system (RAS) regulates blood pressure and electrolyte balance. Renin is a vasoconstrictive substance stimulated by renal ischemia and other renal diseases (Kopple, 1999). Renin stimulates the conversion of angiotensin I to angiotensin II, and causes a rise in blood pressure. Renin acts as the rate-limiting step in the synthesis of angiotensin II (Sigmund, 2002). Angiotensin II stimulates the release of aldosterone from the adrenal cortex and stimulates vasoconstriction of smooth muscle (McDonald, Miller, and Guggenheim, 1977).

Adrenal Glands

Located in the abdominal cavity above each kidney is an adrenal gland. The adrenal glands produce hormones essential for the proper functioning of the body. In the cortex of the adrenal gland glucocorticoids; such as cortisol, mineralocorticoids; such as aldosterone, androgens, estrogens, and progestins are produced (Thomas, 1997).

The medulla of the adrenal gland manufactures adrenaline and noradrenaline (Curtis and Barnes, 1989), and dopamine. The medulla and cortex of the adrenal glands function independently of each other; however, their actions are similar (Gard, 1998).

The hormone aldosterone affects sodium and water balance. Aldosterone acts on the distal segment of the renal tubule to enhance sodium reabsorption and stimulates the secretion of potassium and hydrogen ions (McDonald, Miller, and Guggenheim, 1977).

Carbohydrate, protein, and lipid metabolism are affected by glucocorticoids. Adrenaline and noradrenaline, also known as epinephrine and norepinephrine, increase blood sugar, dilate or constrict specific blood vessels, and increase the rate and strength of heartbeats (Curtis and Barnes, 1989).

The major controlling factor in the secretion of aldosterone is the renin-angiotensin system (Gard, 1998). The enzyme renin is produced by the kidney in response to a decrease in systemic blood pressure, fluid loss, or hemorrhage.

Circulating norepinephrine increases renin activity. Elevated activity of the renin-angiotensin-alderosterone system leads to increased angiotensin and aldosterone production intensifying vasoconstriction and fluid retention, respectively (Hughes and Kostka, 1999). Angiotensin II regulates a number of genes that participate in the progression of chronic renal disease (Klahr, 1999).

Secondary Hyperparathyroidism

The parathyroid consists of four endocrine glands that are located close to the thyroid gland (Thomas, 1997). The parathyroid gland releases parathyroid hormone (PTH) in response to hyperphosphatemia (elevated serum levels of phosphorus) and hypocalcemia (low serum calcium) resulting from renal insufficiency, intestinal malabsorption, and osteomalacia or rickets due to vitamin D deficiency among other causes (Garner, 1996). According to Garner (1996), "hyperphosphatemia, decreased hydroxylation of 1-25-hydroxyvitamin D, reduced intestinal absorption of calcium, and peripheral resistance to parathyroid hormone may all contribute to PTH hypersecretion in renal failure."

Parathyroid hormone has been implicated as a uremic toxin, which adversely affects many organs and tissues (Kopple, 1999). Studies on rats have shown that chronic renal failure was associated with myocardial energy production, transfer and utilization; and that a parathyroidectomy corrected these abnormalities (Smogorzewski et al., 1988).

Parathyroid hormone also inhibits the oxidation of long and short chain fatty acid oxidation by heart mitochondria. Data from a rat study conducted by Perna, Smogorzewski and Massry (1988) found that "alterations in long chain fatty acids (LCFA) in chronic renal failure or after PTH treatment are related to the ionophoric action of the hormone, and could be reversed by a calcium channel blocker." The calcium channel blocker used in the study was verapamil.

Hyperparathyroidism causes a rise in blood calcium and in blood phosphorus. Calcium is removed from the bones. Renal osteodystrophy is the result of uncontrolled

hyperparathyroidism. The bones are weakened by a lack of active vitamin D to aid in the reabsorption of calcium.

Renal Disease

Renal failure is a complex disorder; it changes the excretory, endocrine, and metabolic functions of the kidney (Kopple, 1999). The total number of end-stage renal patients in the United States increases by approximately 6% every year (Masud and Mitch, 2001). The primary causes of end stage renal disease are hypertension and diabetes. Diabetic nephropathy accounts for 40% of the new cases of ESRD in the United States, according to the American Diabetes Association (2003). Diabetic nephropathy accounts for one-third of all cases of end-stage renal disease in the United States. End Stage Renal Disease (ESRD) develops in 50% of patients with Type 1 diabetes (Molitch et al., 2001.)

Albumin in the urine, referred to as proteinuria, is the earliest clinical evidence of nephropathy. According to Molitch et al.(2001), "a urine albumin level of ≥30 mg per day or 20 *u*g per minute is considered abnormal, and intervention should be initiated." Serum creatinine is also a predictor of the progression of kidney failure. Progression of renal disease is reduced when proteinuria is decreased (Ruggenenti, Schieppati, and Remuzzi, 2001).

The glomerular filtration rate (GFR) usually declines by fifty percent or more before clinically significant increases in the serum creatinine and blood urea nitrogen (BUN) concentrations are observed (Metheny, 2000). The decline in GFR may be due to a decrease in single-nephron filtration rate, a decrease in the number of functional

nephrons, or a combination of the two (Klahr, 1996). When the GFR falls, urea and creatinine accumulate in the plasma and body fluids. In the Modification of Diet in Renal Disease (MDRD) study, there was a correlational analysis that showed the GFR decline was significantly faster in patients with higher blood pressure (Klahr, 1999).

The accumulation of nitrogenous waste in the blood is referred to as azotemia. Uremia is present when a patient has azotemia with signs and symptoms of advanced renal failure. "Weakness, ill health, insomnia, fatigue, loss of appetite, nausea, vomiting, diarrhea, itching, muscle cramps, hiccups, twitching or jerking of the extremities, fasculations, tremors, emotional irritability, and decreased mental concentration and comprehension are many of the symptoms of uremia." (Kopple, 1999).

Impaired synthesis of 1, 25-dihydroxyvitamin D in renal failure contributes to vitamin D deficiency. 1, 25-dihydroxycalciferol is the active metabolite of vitamin D (calcitriol) (Masud and Mitch, 2001). When vitamin D is deficient in the body, impaired intestinal calcium absorption occurs and results in hyperparathyroidism; bones are then resistant to the actions of parathyroid hormone and renal osteodystrophy develops (Kopple, 1999).

Other alterations in nutritional balance occur with end-stage renal disease. The injured kidneys are no longer able to excrete large amounts of sodium, water, potassium, calcium, magnesium, phosphorus, and other trace elements, such as aluminum. Patients with renal disease have a greater risk for developing other vitamin deficiencies, in addition to vitamin D deficiency. Vitamin B_6, vitamin C, and folic acid deficiencies are the most prevalent in persons with end-stage renal disease (Kopple, 1999). Dietary intervention is essential to prevent the accumulation of electrolytes and other substances

that are nephrotoxic. "An individual with ESRD may be doing everything a physician and dietitian recommends, and still feels ill, deteriorate, or have unacceptable biochemical levels due to the nature of the disease and the limitations of the treatment regimen." (Rushe and McGee, 1998).

Treatment of Renal Disease

In the first stages of kidney disease, protein and phosphorus are limited in the patient's diet. The combination of diet and ionic (phosphate) binders are used to assist the kidneys in filtering urea and other waste products that accumulate in the blood.

Angiotensin-converting enzyme (ACE) inhibitors are a recommended form of treatment for diabetic and non-diabetic patients with chronic renal disease (Jafar et al., 2001). These drugs help to decrease blood pressure by inhibiting the RAS pathway, and decrease the urinary excretion of protein (Sigmund, 2002). ACE inhibitors have been found to be renoprotective. The definition of renoprotection is "a strategy that aims to interrupt or reverse the progression of renal failure" (Ruggenenti, Schieppati, and Remuzzi, 2001).

Unfortunately kidney failure progresses rapidly when the glomerular filtration rate (GFR) is reduced. When the GFR has decreased to fifteen milliliters per minute for patients with diabetes and a GRF of ten milliliters per minute for non-diabetic patients with renal insufficiency, the options for the patient include kidney transplant, hemodialysis, or peritoneal dialysis.

A person with end-stage renal disease will be started on dialysis until a kidney transplant becomes available. The type of treatment used is determined by the amount of kidney function, the patient's body mass, and the patient's ability to assist in self-care.

Patients live for many years with essentially no renal function while on maintenance hemodialysis or peritoneal dialysis (Kopple, 1999). The dose of the dialysis prescription must be adequate to remove the waste products that cannot be filtered out of the body by the kidney. To determine if the dialysis dose is adequate a urea reduction ratio (URR) is done. A URR compares the blood urea nitrogen (BUN) level before and after dialysis. The dialysis dose is adequate if the URR is sixty-five percent or higher. A URR value of greater than sixty-five percent increases the life expectancy of persons receiving dialysis (Mitch and Klahr, 1998).

Another measure that is used to determine the adequacy of the dialysis prescription is the Kt/V (K T over V). The Kt/V is quantified using a computer program. The "K" is how much urea the artificial kidney removes, the "T" is the amount of time on dialysis, and the "V" is the volume of urea in the body from blood, urine, and body fluids. The suggested goal for Kt/V is greater than 1.2, to prevent under-dialysis and to reduce its consequences (Ahmad, 1999b). Patients that are dialyzed via peritoneal processes have different Kt/V depending on the type of peritoneal dialysis; the weekly Kt/V should be 2.0 or greater (Ahmad, 1999b). Mitch and Klahr (1998) reviewed a four-year prospective, observational study of 130 chronic hemodialysis (CHD) patients when the dose of dialysis was increased to 1.33 (measured by delivered Kt/V), the standardized mortality rates of the patients declined from 22.8% to 9.1%. Inadequate clearance of

uremic substances, due to under-dialysis, causes progressive anorexia at all stages of renal failure (Mitch and Klahr, 1998).

Hemodialysis

Hemodialysis is a method that uses an artificial kidney to cleanse the blood. Hemodialysis improves electrolyte imbalances that may cause acidosis in the patients. During hemodialysis excess fluid and waste products are filtered through a semipermeable membrane into the dialysate (Renal Practice Group-ADA, 1993). Dialysis works on the principles of osmosis and diffusion. There is a higher concentration of waste products in the patient's blood; waste passes through the semipermeable membrane into the dialysate bath (Renal Practice Group-ADA, 1994). The dialysate bath contains bicarbonate, which acts as a buffer. The dialysate also contains potassium, calcium, sodium, magnesium, and it may also contain glucose.

Patients have a vascular access created to maximize the amount of blood cleansed during hemodialysis. An arteriovenous (AV) fistula, an arteriovenous graft, or a venous catheter, are options for access. The AV fistula is created by surgically connecting an artery to a vein usually in the forearm. An AV graft is made if the patient's veins are too small to form a fistula. A synthetic tube is implanted under the skin in a patient's arm (Vascular Access for Hemodialysis-NIH, 2003). The other option of venous access is a venous catheter. A catheter is placed in the chest, and the access may be internal jugular (IJ), or subclavian, depending on which vein is accessed. The femoral vein is also used. This form of access is used temporarily until a fistula or graft can be used for routine dialysis (Ahmad, 1999b).

A dry weight is used to determine the amount of excess fluid that needs to be removed from the patient during hemodialysis. The dry weight is the body weight of a patient when he/she is free from edema and fluid retention. A weight gain of more than 0.5 to 1 kilogram per day represents fluid retention (Romano, 1997). Patients are weighed pre and post dialysis to monitor fluctuations in weight. "Dry weight should be checked periodically, as true weight loss can be concealed by chronic overhydration." (Lysen, 1997). Over-estimation of dry weight is more common than under-estimation, but either can affect the intradialytic morbidity of the patient (Ahmad, 1999b).

Hemodialysis requires three to five hours of blood exchange per treatment depending on the patient's body size, residual renal function, and diet management. This procedure is performed two to three times per week.

Nutrients lost during a dialysis treatment are an important component of dialysis related catabolism (Mitch and Klahr, 1998). According to Tremblay et al. (2000), hemodialysis patients can be supplemented more aggressively because they are at a greater risk for hydro-soluble vitamin deficiency.

Peritoneal Dialysis

Peritoneal dialysis utilizes the peritoneal membrane as the filter for removing waste products from the blood. To fill and drain the peritoneum takes approximately thirty to forty minutes. There are two forms of peritoneal dialysis that are used, continuous ambulatory peritoneal dialysis (CAPD) and continuous cycling peritoneal dialysis (CCPD).

Continuous ambulatory peritoneal dialysis (CAPD) is the most common form of peritoneal dialysis. This form of dialysis uses gravity, and does not require a machine. A catheter is placed in the abdomen, which replaces and drains the dialysate from the peritoneal cavity. There are 4 or 5 exchanges performed daily (Ahmad, 1999b). One to three liters of dialysate are infused into the peritoneal cavity and then drained after four to six hours while the patient is awake.

Continuous cycler-assisted peritoneal dialysis (CCPD) uses a machine to perform the frequent dialysate exchanges (three to five) during sleeping hours. Two liters of solution are left in the abdomen during the day. Another form of CCPD is nocturnal intermittent peritoneal dialysis (NIPD). While the patient is sleeping six or more dialysate exchanges are made, and there are none performed during the day. With this method, the solution is not left in the abdomen during the day (Ahmad, 1999b).

Diet Modification

The dietary goal for patients with chronic renal failure is to diminish the accumulation of nitrogenous wastes and uremia, prevent malnutrition, and slow the progression of renal failure (Masud and Mitch, 2001). In a study conducted by Thomas et al. (2001), they found that participants reported that the benefits of being compliant to a special diet were having fewer health problems and less risk of developing serious health problems.

When a modality of treatment for end-stage renal disease is determined, usually dialysis, the therapeutic diet of the patient is determined and adjusted as needed in accordance to monthly laboratory values. Protein and calorie needs are altered to meet

the patient's needs. Restrictions of electrolytes are imposed on the patient to prevent complications related to azotemia and uremia. It has been found that adjustments in the diet improve uremic symptoms and enhance renal function (Zarazaga et al., 2001).

The role of a low-protein diet consisting of 0.2 to 0.4 grams of protein per pound of ideal body weight to slow the progression of renal failure is controversial (Berkow et al., 1997). According to the DODE (Diet or Dialysis in the Elderly) study, it appears that a very-low-protein diet supplemented with essential amino acids, keto-analogs of amino acids, and adequate calories, can slow the progression of chronic renal failure (Maiorca et al., 2000). When protein is limited in the diet, the catabolism of existing muscle in the hemodialyzed patient occurs. The delay of dialysis with low protein diets has positive results, but may ultimately contribute to poor nutritional status, which is often seen in patients that comply with this diet (Ruggenenti, Schieppati, and Remuzzi, 2001).

According to Zarazaga et al. (2001), a hypercatabolic state occurs with hemodialysis, 25 grams of protein and 15 amino acids per 40 liters of fluid exchange are lost in the process. Protein is needed for maintenance of cell integrity. The daily nutrient recommendation of protein for a patient receiving hemodialysis is 1.2 to 1.3 grams per kilogram ideal body weight (Renal Practice Group-ADA, 1994). Patients receiving maintenance hemodialysis may consume enteral supplements. The supplements provide calories and protein needed by the patient to maintain musculature and weight.
The recommended daily nutrient requirement for calories is 30 to 35 calories per kilogram of ideal body weight per day (Kidney and urologic disease statistics for the United States, 2000). Consumption of adequate calories can be difficult for patients due to diet restriction, food aversions, and decreased appetite. Fluctuations in weight occur

due to fluid shifts between dialysis sessions. Sodium is an electrolyte that is restricted in patients with ESRD. The restriction may be due to the hypertensive condition of the patient, but is primarily recommended due to the kidneys inability to excrete a large amount of sodium (Kopple, 1999).

Sodium, phosphorus, potassium, and fluid restrictions can contribute to the low calorie intake, and restriction of foods that are needed to maintain the individual's weight and health status. Nutrition plays an important role in the health of the person on hemodialysis. Patients are more likely to be compliant to a therapeutic diet if they believe in the benefits of following the prescribed diet, if they have a supportive environment, and if they are knowledgeable about the end-stage renal disease (ESRD) diet (Thomas et al., 2001).

Table 1. Recommended Daily Nutrition Requirements for Patients on Hemodialysis

Nutrient	**Amount**
Protein	1.1-1.4 g/kg ideal body weight
Calories	30-35 kcal/kg ideal body weight/d (maintenance)
Fat	30%-40%, polyunsaturated to saturated fatty acid ratio 1:1
Carbohydrates	Rest of non-protein calories
Vitamins	
A	No additional
K	None
Folic Acid	0.8-1 mg
Pyroxidine hydrochloride	10 mg

Riboflavin	1.8-2.0 mg
Niacin	20 mg
Ascorbic acid	60 mg
Thiamin	1.5-2.0 mg
Pantothenic acid	10 mg
B_{12}	3-6 *ug*
Biotin	200-300 *ug*
E	10 IU
Minerals	
Sodium	2-3 g
Potassium	1.5-3 g
Calcium	1400-1600 mg
Phosphorus	12-17 mg/kg
Zinc	15 mg
Iron	Approximately 100 mg (elemental)
Fluid	700-1000 mL + urine output in 24 hours
Fiber	20-25 g (promotes bowel regularity)

Source: Renal Practice Group of the American Dietetic Association. 1994. A Clinical Guide to Nutrition Care in End-Stage Renal Disease 2nd Ed. Chicago, IL: American Dietetics Association.

Sodium

Sodium is an electrolyte that is needed in the diet for normal muscle function, acid-base balance, cellular permeability, and to maintain the osmotic pressure of body

fluids (Renal Practice Group-ADA, 1994). Sodium restriction is required of patients receiving hemodialysis. Patients may be on a sodium-restricted diet to prevent hypertension and volume overload (Mitch and Klahr, 1998). Sodium is also restricted to aid in compliance of fluid restrictions. In uremic patients, there is a defect in the sodium transport system that occurs. According to Bellinghieri et al. (2003), intravenous L-carnitine supplementation improves this system.

Potassium

Potassium ion (K^+) balance is important for the health of patients with kidney disease. Blood levels are monitored monthly, or intakes can be estimated by a 24-hour urine collection, when the patient does not have oliguric renal failure (Renal Practice Group-ADA, 1994).

Hyperkalemia, elevated serum potassium, may be caused by noncompliance of diet, from catabolism, acidosis, and medications (ACE inhibitors or K^+ sparing diuretics) (Mitch and Klahr, 1998).

Hypokalemia, low serum potassium, in patients with ESRD, can be caused by decreased nutritional intakes, vomiting, diarrhea, and/or excessive use of potassium binding agents (Renal Practice Group-ADA, 1994). "Hypokalemia or hyperkalemia may induce muscle weakness, and cardiac arrhythmias and hyperkalemia can cause cardiac arrest." (Renal Practice Group-ADA, 1994).

Calcium

Most of the calcium in the body is found in the bones and teeth. The majority of patients receiving hemodialysis require calcium supplementation due to low intakes of calcium rich foods, the lack of vitamin D, (Renal Practice Group-ADA, 1993) and the inability to excrete excess phosphate (Renal Practice Group-ADA, 1994).

The symptoms of low serum calcium levels (hypocalcemia) include tingling fingers, abdominal cramps, tetany, seizures, and can lead to respiratory or cardiac arrest (Renal Practice Group-ADA, 1994). Hyperparathyroidism and renal osteodystrophy are the consequences of low calcium absorption and availability.

Hypercalcemia, elevated serum calcium, may occur with supplementation of calcium and 1, 25-dihydroxyvitamin D (Renal Practice Group-ADA, 1994). This combination is prescribed to enhance the absorption of calcium and decrease the level of PTH (Mitch and Klahr, 1998). Serum calcium and phosphorus levels need to be monitored carefully with this form of treatment.

Phosphorus

According to Kopple (1999), "the recommended phosphorus intake for patients undergoing maintenance hemodialysis or CPD is about 17 mg/kg/day or less." A high dietary phosphorus intake can lead to high plasma phosphorus (hyperphosphatemia) in patients with chronic renal failure due to the inability of the kidney to excrete phosphorus. The results from a study conducted by Fukagawa et al. (1996) showed that "mild dietary phosphorus restriction can prevent PTH hypersynthesis and parathyroid hyperplasia".

Some patients with renal failure take phosphate binders. Many of theses binders contain calcium. Calcium binder doses that provide more than 2 grams of calcium daily may cause accumulation of calcium in soft tissues (Kopple, 1999).

Vitamin C

It is recommended that patients on dialysis take no more than 60 milligrams (mg) of vitamin C per day, too much vitamin C can be metabolized to oxalate, increasing the plasma and tissue levels of oxalate (Renal Practice Group-ADA, 1994).

Malnutrition

The potential causes for malnutrition of persons with ESRD according to a study conducted by Chazot et al. (2001) were macronutrient deficiencies and inadequate energy intake. Uremic malnutrition is characterized by loss of lean body mass or serum creatinine and reflected by low serum albumin or serum prealbumin. Approximately 20 to 50 percent of patients on dialysis have this form of malnutrition (Caglar, Hakim, and Ikizler, 2002). The fact that low plasma levels of carnitine may indicate malnutrition in hemodialysis patients was one of the conclusions reached by Chazot et al. (2003). In this same study, which reviewed the nutritional effects of carnitine supplementation in hemodialysis patients, it was found that serum albumin concentrations decreased during the study, indicating poor nutritional status in these participants (Chazot et al., 2003). However, in a commentary written by William E. Mitch (2002), he disputes the diagnosis of malnutrition in hemodialysis patients. He feels that this term is misleading and that the term indicates, "The abnormalities can be overcome simply by supplying more food or altering the composition of the diet."

Carnitine

In 1905 carnitine was discovered in beef muscle, and in 1927 its chemical structure was firmly established (Frenkel and McGarry, 1980). Carnitine is a required cofactor for fatty acid translocation and muscle function (Hongu and Sachan, 2003). Carnitine is derived from the essential amino acids, methionine and lysine. The biosynthesis of carnitine occurs in the kidneys, liver, and the brain (Krause, 2000 and Vlassopoulos et al., 2002). Carnitine is synthesized from lysine, which has been methylated, using methyl groups from methionine. Along with this process iron, vitamin B6, vitamin C, and niacin aid in carnitine synthesis (Groff, Gropper, and Hunt, 1995). Homeostasis of carnitine is maintained in the body due to reabsorption by the kidneys and carnitine synthesis (Rebouche, 1999). In the absence of the functioning kidney, the liver becomes the main source of endogenous carnitine (Bellinghieri et al., 2003).

Carnitine is concentrated in most tissues of the body, primarily the liver and skeletal muscle. Ninety percent of all carnitine in the body is in the skeletal muscle. Carnitine is also present in body fluids in two forms, free and esterified (Guarnieri, Situlin, and Biolo, 2001). The esterified form of carnitine is acetyl carnitine, which is the most abundant naturally occurring ester (Fornasini and Evans, 2003).

The L isomer of carnitine, levocarnitine, is the biologically active form. Levocarnitine is involved in a series of reversible transesterification reactions, in which, beta-oxidation occurs (Vlassopoulos et al., 2002). Beta-oxidation is the process that removes acetyl CoA from the fatty acid and energy is produced (Coffee, 1998).

Dietary carnitine is primarily found in meat products, due to the ability of animal cells to synthesize carnitine. Dairy foods provide some carnitine in the diet, while

vegetables contain very little of this nutrient. A deficiency in carnitine rarely occurs, although due to a lack of certain enzymes, the body may not be able to synthesis adequate amounts. For a non-vegetarian adult, the normal intake of carnitine is 50 to 600 umol per day (Rebouche, 1991).

A consequence of dialysis is depletion of muscle carnitine. The amount of depletion appears to be related to time on dialysis. During each dialysis session serum concentrations of all carnitine fractions rapidly decrease, causing compensatory carnitine release from muscle stores (Guarnieri, Situlin, and Biolo, 2001). The normal serum values for men are: free carnitine 30.9 – 61.1, total carnitine 46.2-102.2, and for women: free carnitine 23.1-52.9, total carnitine 40.2-85.8 (Rosenthal, 2002). Of concern is the diminished synthesis in end-stage renal disease due to nonfunctioning kidneys.

Carnitine Metabolism

Dietary carnitine is absorbed from the intestinal lumen across the mucosal membrane and released into the bloodstream (Guarnieri, Situlin, and Biolo, 2001). The heart and skeletal muscles do not have the enzymes required for biosynthesis of carnitine, and depend on circulating free carnitine in the body to maintain normal metabolism. According to studies conducted by Rebouche, Lombard, and Chenard (1993), "the amount of carnitine absorbed from the diet is ≈ 54-87%, and is dependent on the amount of carnitine ingested."

Carnitine is an essential cofactor for the transport of long-chain fatty acids into the mitochondria for oxidation. The oxidation of fatty acids requires carnitine to cross the inner mitochondrial membrane, a process called the Carnitine Shuttle (Coffee, 1998).

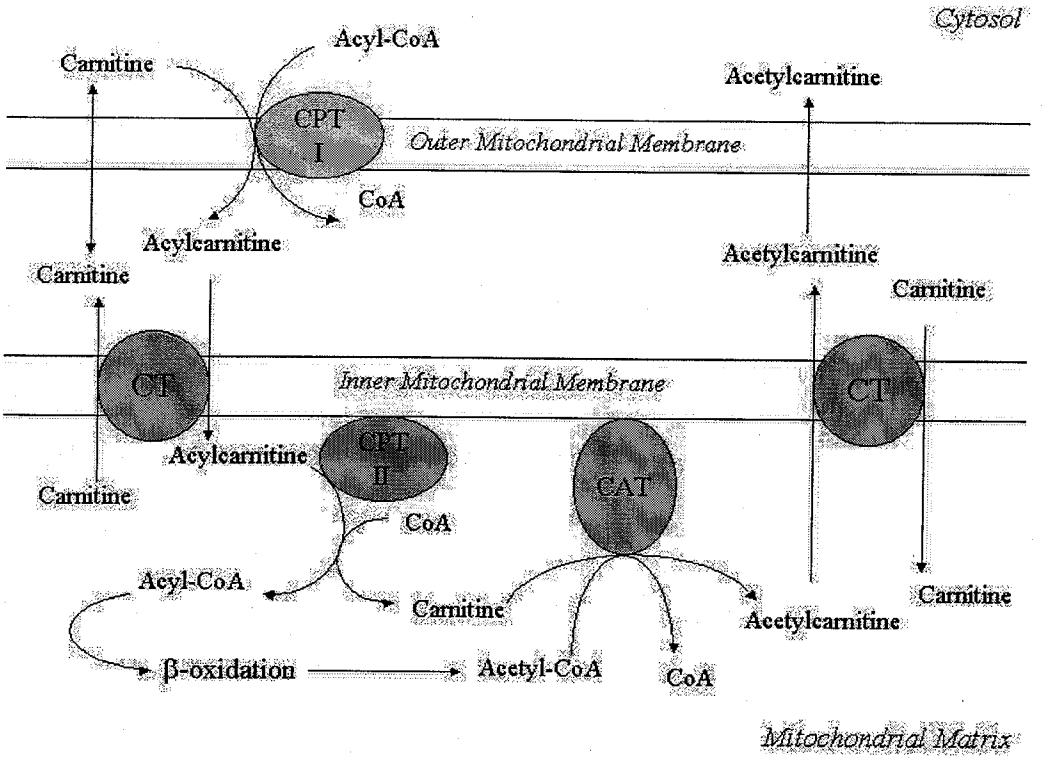
The "shuttle of long-chain fatty acids into the mitochondria is controlled by at least 3 different proteins: carnitine palmitoyltransferase I (CPT-I), acylcarnitine translocase (CT), and carnitine palmitoyltransferase II (CPT-II)" (Bellinghieri et al., 2003).

The acyl group is transferred from the cytosolic CoA to carnitine, forming O-acyl carnitine. The acyl carnitine is transported across the membrane into the mitochondrial matrix, where the carnitine is released and fatty acyl CoA is left to be used for energy production (Champe and Harvey, 1994).

Acyl carnitine is a combination of carnitine and waste products that are excreted by the kidney (Ahmad, 1999a). In renal failure, acyl carnitine accumulates in the blood. The kidney is not able to remove these moieties left by incomplete metabolism of fatty acids (Gunal et al., 1999).

In a study conducted by Rebouche (1991), he found that in health adults, carnitine appeared in serum within 2 to 4.5 hours of ingestion. L-carnitine is a low molecular weight compound that does not bind to plasma proteins (Evans et al., 2000). The proximal renal tubule in the healthy kidney reabsorbs more than 90% of filtered carnitine at normal plasma concentrations (Bellinghieri et al., 2003).

Figure 2: Carnitine Shuttle



Source: http://lpi.oregonstate.edu/infocenter/othernuts/carnitine/transport.jpg. Accessed on June 30, 2003.

Carnitine Deficiency

Deficiencies in carnitine are seen in premature infants and patients with kidney disease. Hypoglycemia due to impaired gluconeogenesis, ketogenesis, impaired fatty acid oxidation, and elevated plasma fatty acid levels are the effects of carnitine deficiency (Coffee, 1998).

Carnitine is a non-essential amino acid that is derived from dietary sources and formed endogenously by lysine and methionine. However, under certain circumstances carnitine may become conditionally essential. Iron, vitamin C, and vitamin B6 are required for the formation of carnitine. Specifically iron is needed for two enzymes

necessary for carnitine synthesis. Carnitine is synthesized in both the kidney and liver (Brass, 2000). Carnitine functions as an essential co-factor for the transport of long-chain fatty acids into the mitochondria for oxidation.

The removal of carnitine during dialysis, impaired carnitine synthesis in the liver, reduced absorption from the gut, and consumption of a carnitine poor diet are thought to be the causes of abnormal levocarnitine metabolism in patients with end-stage renal disease (Goral, 2001).

Persons receiving hemodialysis have circulating carnitine lost across the dialysis membrane due to carnitine's low molecular weight. However, plasma values are usually restored within 48 hours, due to tissue carnitine depletion (Rogerson et al., 1989). The carnitine that is needed is released from muscle, attributing not only to essential deficiency but also to muscle weakness. Carnitine deficiency may cause muscle aches, as well as fatigue, and confusion (Beers et al., 1999). Carnitine deficiency in dialysis patients causes metabolic abnormalities such as asthenia, malaise, muscle weakness, intradialytic cramps and hypotension, cardiomyopathy, EPO resistant anemia, and decreased quality of life (Guarnieri, Situlin, and Biolo, 2001).

When carnitine is deficient, there is a lack of CoA, which is needed to perform metabolic tasks in the body. Low concentrations of carnitine result in an inability to use long-chain fatty acids for metabolic fuel causing weakness (Champe and Harvey, 1994). Decreased beta-oxidation, increased lipolysis, or increased fatty acid intake contributes to an excess acyl groups present (Guarnieri, Situlin, and Biolo, 2001).

The estimated weekly loss of L-carnitine in a patient receiving approximately 12 hours of hemodialysis is 1,000 umol (Evans et al., 2000). Once hemodialysis is

complete, the plasma L-carnitine slowly return to their predialysis level, this occurs with repletion from liver and muscle stores, and through food intake and biosynthesis (Evans, 2003).

Carnitine Supplementation

The approval of intravenous levocarnitine supplementation was granted by the United States Food and Drug Administration (US FDA) in December of 1999 (Schreiber, 2000). The IV supplementation of levocarnitine for patients with end-stage renal disease who are undergoing dialysis treatment was recommended to prevent and treat carnitine deficiency (Bellinghieri et al., 2003).

On January 1, 2003, the Centers for Medicare and Medicaid Services (CMS) granted a national coverage determination for intravenous levocarnitine to treat patients with ESRD. (Feiertag, 2003). Patients eligible for reimbursement: 1) must have received dialysis for at least 3 months, 2) must have documented carnitine deficiency, and 3) must either have EPO-resistant anemia or interdialytic hypotension that precludes delivery of the intended dialysis therapy (Department of Health and Human Services, 2004).

Iron is required for the production of carnitine. The enzymes trimethyl lysine dioxygenase and γ-butyrobetaine dioxygenase are iron containing enzymes that convert lysine and methionine into carnitine.

Carnitine can be supplemented orally or intravenously. The oral formulation of levocarnitine is not recommended in patients with ESRD on dialysis due to the major metabolites formed. The damaged kidneys cannot efficiently remove the metabolites and the accumulation in the body could be teratogenic and toxic.

A study conducted by Rebouche (1991) to determine the absorption and degradation of carnitine in healthy adults found that "absorption of carnitine is inversely proportional to the level of intake, and conversely, the degree to which carnitine is degraded in the intestinal tract is directly proportional to the quantity ingested."

A study conducted by Gunal et al. (1999), they found that L-carnitine might improve insulin resistance in patients with chronic renal failure. This conclusion was based on the decrease in the insulin concentration and the increase in the rate of plasma glucose disappearance.

Choline along with carnitine supplementation has been found to decrease the excretion of carnitine, due to the shift of carnitine into the tissues (Hongu and Sachan, 2003). Evans et al. (2000) found that L-carnitine and acetyl-L-carnitine slowly accumulate into a compartment that is in slow equilibrium with plasma, this compartment is skeletal muscle.

According to Guarnieri, Situlin, and Biolo (2001), carnitine supplementation may contribute to the regeneration of sequestrated free CoA and to the maintenance of normal metabolic processes. Administration of levocarnitine following dialysis has been shown to replace the carnitine that is removed, increasing both the free carnitine and acyl-carnitine fractions. The addition of L-carnitine was found to completely prevent the fall of plasma carnitine during hemodialysis in a trial conducted by Wanner et al. (1986).

The potential clinical benefits of levocarnitine supplementation include improvement of protein metabolism and insulin resistance. It has been found that carnitine supplementation in hemodialysis patients could improve the patients plasma lipid profile; improve exercise tolerance by increasing the aerobic capacity and decrease

cramp occurrence; improve hematocrit or reduce erythropoietin (EPO) dosage (Hurot et al., 2002); and improve the overall "well-being" of the patient (Guarnieri, Situlin, and Biolo, 2001). Bellinghieri et al. (2003) concluded their review of carnitine and uremia with this suggestion, that carnitine supplementation for persons on dialysis would lead to a reduction in dialytic costs and increased quality of life.

Nepro®

Nepro® is an enteral nutritional supplement made by Ross Pharmaceuticals. It is formulated for persons that have kidney disease. Patients that are on hemodialysis consume Nepro® to increase protein and calories in their diet. Nepro® provides essential nutrients without excess phosphorus and potassium, two nutrients that are limited in the diet of a person with ESRD. One can (237 milliliters) of Nepro® provides approximately 475 calories, 16.7 grams of protein, 250 mg of potassium, and 165 mg of phosphorus.

One can of Nepro® also provides 62 mg of L-carnitine, 25 mg vitamin C, 4.5 mg iron, 1245 mg lysine, 481.4 mg methionine, and many other vitamins, minerals, and amino acids (Medical nutrition product item Nepro®, 2003). These nutrients are of importance because they are involved in the endogenous synthesis of carnitine in the kidney and liver.

However, oral supplementation of carnitine is not an effective method for replacement of carnitine for persons with kidney disease. According to studies on levocarnitine pharmacology, Evans (2003), found that intravenously administered L-carnitine is fully bioavailable, while orally administered L-carnitine is poorly absorbed

and possibly acetylated during the absorption process. Nepro is beneficial for supplying calories and protein, instead of carnitine.

Summary

It has been found that almost all patients with renal failure have nutritional, metabolic, and/or cardiovascular problems. Hypertension, hyperinsulinemia, and glucose intolerance are a few of the risk factors that are the major causes of death in long-term hemodialysis patients (Klahr, 1996). A consequence of dialysis is depletion of muscle carnitine. The amount of depletion appears to be related to time on dialysis. During each dialysis session serum concentrations of all carnitine fractions rapidly decrease, causing compensatory carnitine release from muscle stores (Guarnieri, Situlin, and Biolo, 2001). Of concern is the diminished synthesis in end-stage renal disease due to nonfunctioning kidneys.

It has been found that intravenous carnitine supplementation in hemodialysis patients could improve the patient's plasma lipid profile and improve exercise tolerance. Carnitine deficiency in dialysis patients causes metabolic abnormalities such as asthenia, malaise, muscle weakness, intradialytic cramps and hypotension, cardiomyopathy, EPO resistant anemia, and decreased quality of life. Levocarnitine supplementation would be an effective method of treatment for patients with end-stage renal disease. It has been found to be effective in relieving physical symptoms related to extensive hemodialysis treatments. Supplementation with carnitine intravenously would be beneficial to persons with renal disease that are deficient in this compound.

CHAPTER III

Methodology

The purposes of this research were 1) to identify sources of carnitine the participants were consuming, 2) to evaluate if patients receiving hemodialysis were consuming adequate calories and other nutrients especially folate, vitamin B_{12} and vitamin B_6 from foods as well as to identify if participants were compliant to the renal diet, 3) to determine if dietary intake of carnitine was reflective of plasma free-carnitine values and 4) to assess if supplementation of patients with enteral intake of Nepro® would increase free plasma carnitine levels. The plasma free-carnitine values and a 24-hour recall were used to correlate the data. Included in this chapter are recruitment, procedures, data collection and analysis of the dietary histories.

Participants

Dr. Brian Schreiber, Chief Medical Director of Dialysis Care for Northeast Wisconsin, and Frank Gedney, Medical Director of Ministry Dialysis Centers, granted approval for the patients of their dialysis centers to participate in this study. Judy Lubenow RD, the chief clinical dietitian at St. Michael's Hospital in Stevens Point, recruited participants from the Ministry Dialysis Centers in central Wisconsin for this study. The other participants were recruited the week of July 28, 2003.

The participants recruited for this study were 18 years of age or older. The participants had been receiving hemodialysis for at least 6 months continuously. None of the eighteen participants were currently receiving levocarnitine supplementation (Carnitor). Eight of the subjects that participated in this study consumed Nepro®, an

enriched enteral supplement, routinely. These participants were designated to Group A, the Treatment group. The other ten participants did not consume Nepro® were identified as Group B, the Non-Treatment group.

The eighteen participants signed a permission form (Appendix A), which allowed the researcher to conduct the dietary interviews. A food frequency was the original form that was initially to be used for the diet assessment. During the interview process, the primary interviewer switched to a 24-Hour Recall to get a more accurate assessment of what the patients were orally consuming.

Patient Information sheets were given to participants in both groups. Group A and Group B received the same basic information, but the information sheet for Group A (Appendix B), included directions on when to consume Nepro® during the testing period. The participants were asked to sign a consent form (Appendix C) prior to joining the study. The participants were given an identification code number for confidentiality purposes.

Instrumentation

In this study a 24-hour recall was used to obtain the calorie intake of the participants. The blood samples of the participants were analyzed by Tandem Mass Spectrometry to obtain the amount of carnitine present in the participants' plasma pre-dialysis. Sigma Tau Pharmaceuticals conducted all laboratory testing at their own cost. Sigma Tau Pharmaceuticals is the company that manufactures intravenous Levocarnitine.

24-Hour Recall

A 24-hour recall is a method used to estimate an individual's usual calorie consumption and nutrient intake. This method is useful in clinical settings. The interviewer conducting the 24-hour recall, helps the patient remember what they consumed by cueing and asking the portion sizes. The form of cueing used may be related to activities that the patient had been doing the day before, asking the brand name of a product, or asking if they had more than one of a food item (Lee and Nieman, 1996).

The strengths of using this form of a food record are that it is easy to administer and can be done in less than 20 minutes. A 24-hour recall can be used to estimate the nutrient intake of groups, and it is more objective than a diet history. This method is also inexpensive and of low burden to the patient (Lee and Nieman. 1996).

Inaccuracy in the estimation of food item consumption, either underreporting or over reporting is a major limitation to the 24-hour recall. The intake of the patient within the last 24 hours may not be their typical intake, due to Birthday parties, vacation, or hospitalization. The 24-hour recall relies on the memory of the patient, and in certain populations this may be a limiting factor to estimating their energy intake (Zeman and Ney, 1996).

Tandem Mass Spectrometry

The plasma carnitine in this study was analyzed using Tandem Mass Spectrometry to determine the total carnitine level circulating in the participant's blood. The tandem mass spectrometer has two mass spectrometers connected by a chamber that can break a molecule into pieces. A sample is "sorted" and "weighed" in the first mass

spectrometer, then broken into pieces in the collision cell, and a piece or pieces sorted and weighed in the second mass spectrometer.

The data results produced by the mass spectrometer are displayed as vertical lines distributed across a horizontal axis, called a mass spectrum. Where the vertical line occurs in the spectrum identifies a compound's mass while the height of the line represents how much of the compound is present.

Tandem mass spectrometers are complex instruments. The methods used to prepare the samples for analysis by mass spectrometry require specialize reagents. Tandem mass spectrometry is often called a simple blood test, but it is not a simple method. Tandem mass spectrometry requires several expert scientists to perform the analyses and medical experts to interpret the large amount of clinical data produced from the analysis of a blood sample (Chance, 2003).

Procedures

Eighteen participants were designated into two groups. Group A, the Treatment Group, consisted of eight participants that consumed Nepro®. The amount of Nepro® varied according to the physician's orders for that patient. Group B, the Non-Treatment Group, contained the other ten participants that did not consume Nepro®. The Non-Treatment Group would be considered non-equivalent comparison groups as the participants were assigned to treatment or non-treatment groups. Plasma blood samples of 5 mL (milliliter) were obtained from the eighteen participants to determine their plasma carnitine level.

All of the participants in Group A were on a Monday, Wednesday, Friday dialysis routine. The participants in Group A were notified of the dates of when to alter their normal consumption of Nepro®, which coincided with blood draw dates. The participants in Group A were instructed to drink 8 ounces of Nepro® before 6 PM on Tuesday, August 5th due to the plasma blood draw that occurred the next day predialysis on August 6, 2003. This group was also instructed to drink 8 ounces of Nepro® two hours before they arrived for their dialysis treatment on Friday August 8, 2003. A second plasma blood sample was drawn predialysis that day.

The ten participants in Group B were not consuming the enriched enteral supplement Nepro®. A 5 mL plasma blood sample was obtained from each of the participants. They were instructed not to eat foods that were higher in carnitine the morning of the blood draw, foods like beef or pork or milk. The blood samples were obtained from these participants on August 6 and August 7, 2003.

A 24-hour recall was conducted with the eighteen participants that consented to the study. The dietary interviews were conducted while the participants were receiving their hemodialysis treatments. Interviews were conducted the week of July 28 through August 1, 2003 at the participating dialysis centers.

Data Collection

Dietary information was collected using a 24-hour recall from the eighteen participants that were enrolled in the study. The dietary interviews were conducted during the participant's dialysis session during the week of July 28, 2003. The participants were asked what they had eaten the day before. Many of the participants

needed prompting due to poor memory. During the dietary interviews the participants were asked to identify quantity and size of food items. The diet histories were recorded and entered into Food Processor, a dietary analysis software program.

Blood Draw

Blood was obtained from the participants to determine the amount of carnitine present. The 5 mL plasma blood draws were obtained prior to the dialysis treatment via the participant's vascular access. Blood draws were conducted by the nurses at the dialysis centers. The blood samples were collected and spun for 20 minutes to separate the serum from the red blood cells. The specimens were stored at –20 degrees Celsius until analysis. The plasma was analyzed using Tandem Mass Spectrometry to determine the subject's level of free carnitine.

Data Analysis

The dietary information was entered into the ESHA Food Processor Program at the University of Wisconsin-Stout. The computer software generated a subject profile, which estimated the calorie needs, and BMI (body mass index) of the participants. The entered food records generated spreadsheets, bar graphs, a food pyramid, and the estimated calories, protein, and fat that was consumed by each participant.

The 24-hour diet recall identified the consumption of foods that reflect non-compliance of the prescribed renal diet. The carnitine content of the diet was evaluated by comparing recorded food items with a table that contained the carnitine content of food items produced by Rebouche and Engel (1984) (Appendix D).

The post-2 hour and post-12 hour plasma free carnitine values of Group A were compared using a paired t-test to identify if the Nepro® did cause any change in the free carnitine concentration of the participant. The carnitine values for the participants in Group B were compared with the Group A post 12-hour consumption blood draw using an independent t-test. The BMI, calorie needs and intake, and various nutrient levels were compared for significance between the Non-Treatment group (Group B) and the Treatment group (Group A) using independent t-tests.

CHAPTER IV

Results

This research project looked at the effect of enteral supplementation with Nepro®, a carnitine enriched product, had on the plasma free carnitine content as well as nutrient intake of patients with end-stage renal disease. None of the participants in this study were receiving other forms of carnitine supplementation.

The 24-hour dietary recall information was entered into the ESHA Food Processor Software, along with each participant's age, gender, height, weight, and activity level. This program generated the participant's Body Mass Index (BMI); each participant's calorie, macronutrient, and micronutrient needs; and the participant's calorie, macronutrient, and micronutrient intake. Seven of the participants were female; the other 11 participants were male. The age of the participants ranged from 40 to 81 years of age; the average age of the participants was 68.5 years.

The weights of persons receiving dialysis are monitored for gain of fluid and progressive losses. The participants in Group A were consuming Nepro® according to their physician's orders to supplement their diets. The 24-hour recall only reflects what the participants had eaten the day before they were interviewed, which happened to be a non-dialysis day. However, many patients on hemodialysis have repetitive eating habits due to the dietary restrictions of persons on dialysis.

Half of the participants in Group A had low plasma free carnitine levels, the normal value is 40 *u*mol/L, refer to Table 2. It was assumed that the plasma free carnitine concentration levels would be higher after 2 hours consumption of Nepro®, however, some of the 12 hour post Nepro® consumption free carnitine values were

greater. The positive effect of Nepro® supplementation is that it provides calories and protein.

Table 2. Free Carnitine level of Participants in the Treatment Group

Code #	Gender	2 hours post (*u*mol/L)	12 hours post (*u*mol/L)
A2	Female	54	19
A3	Male	16	17
A4	Male	46	37
A5	Female	8.5	9.2
A6	Male	11	19
A8	Female	16	21

The plasma free carnitine levels of the majority of the participants in the non-treatment group were also less than the normal range of 40 *u*mol/L (Table 3). The participants in this group may benefit from carnitine supplementation.

Table 3. Free Carnitine level of Participants in the Non-Treatment Group

Code #	Gender	Free Carnitine *u*mol/L
B2	Male	17
B3	Male	42
B4	Male	19
B5	Female	24
B6	Male	23
B7	Male	18
B8	Female	18
B9	Female	17
B10	Female	16

SPSS, a computerized statistical program was used to compare the information that was generated by Food Processor and the laboratory results of the participant's plasma free-carnitine concentrations. The participants in this study were divided into

Treatment and Non-Treatment Groups for the analysis. The participant's information was compared using an independent t-test.

The participants in Group A, the Treatment group, tended to have BMI values that were desirable, (BMI between 18.5 and 24.9); however, two of the participants were underweight (BMI < 18.5) causing the mean BMI to be 22, as shown in Table 4. In Group B, the non-treatment group, only three out of the ten subjects had a BMI value in the desirable classification, the other seven were overweight (BMI of 25-29.9) or obese (BMI ≥ 30) with a mean BMI of 32. The BMI was significantly different between the treatment and non-treatment groups (see Table 5). BMI classification was based on information proved in the second edition of the textbook, Nutritional Assessment. (Lee, and Nieman, 1996).

Table 4. Body Mass Index (BMI) of Participants in the Treatment and Non-Treatment Groups

Group	BMI
Treatment (A)	24.6
Treatment (A)	17.94
Treatment (A)	17.54
Treatment (A)	23.21
Treatment (A)	25.53
Treatment (A)	21.9
Treatment (A)	22.3
Treatment (A)	22.79
Non-Treatment (B)	24.75
Non-Treatment (B)	30.28
Non-Treatment (B)	40.96
Non-Treatment (B)	25.8
Non-Treatment (B)	31.64
Non-Treatment (B)	34.61
Non-Treatment (B)	39.44

Non-Treatment (B)	22.49
Non-Treatment (B)	41.97
Non-Treatment (B)	29.18

The results of the independent t-test revealed that the calorie needs of the non-treatment and treatment groups were significantly different (P= 0.05) with a mean of 2,194 and 1,752 Kcal needed for the non-treatment and treatment groups respectively (see Table 5). The calorie intake was also significantly different. It was greater for the treatment group than the non-treatment group, with a mean of 1,891, and 1,422 calories respectively. The total fat intake of the groups was also significantly different (P= 0.05), with the treatment group having a greater mean than the non-treatment group of 77 and 51 grams of fat respectively. The total protein intake of the groups was also significantly different (P= 0.001), with the treatment group having a greater mean than the non-treatment group of 76 and 42 grams of protein, respectively.

Table 5. BMI, Calorie Needs and Intake, Total Fat and Protein Means of all the Participants in the Treatment and Non-Treatment Groups

	Group	Number	Mean	Std Error of the Mean	t	Significance
BMI	Non-Treatment	10	32	2	4.188	0.001
	Treatment	8	22	1		
Kcal Needs	Non-Treatment	10	2194	144	2.594	0.05
	Treatment	8	1752	59		
Kcal Intake	Non-Treatment	10	1422	146	-2.243	0.05
	Treatment	8	1891	145		
Fat grams	Non-Treatment	10	51	10	-2.119	0.05
	Treatment	8	77	7		
Protein grams	Non-Treatment	10	42	6	-3.824	0.001
	Treatment	8	76	7		

The amounts of vitamins required for persons on dialysis differ from the RDA (Recommended Dietary Allowances). This is due to the body's limited ability to excrete metabolites and fluids in persons with end-stage renal disease. Table 1, in Chapter II, lists the amount of vitamins and minerals recommended for patients with end-stage renal disease to consume, according to the American Dietetic Association (1994).

The independent t-test revealed that the mean of vitamin C intake of the participants in the non-treatment and treatment groups were not significantly different, see Table 6. However, the vitamin B_6 means were significantly different (P = 0.01), with means of 3.74 mg, and 0.97 mg for the treatment and the non-treatment group, respectively. The vitamin B_{12} means for the two groups were significantly different (P = 0.05) with a mean of 5.47 ug and 2.31 ug for the treatment and non-treatment groups, respectively. The t-test revealed that the folate intake of the non-treatment and treatment groups were also significantly different (P = .001) with a mean of 586 mg and 257 mg for the treatment and non-treatment groups, respectively.

Table 6. Vitamin C, Vitamin B_6, Vitamin B_{12}, and Folate Means for the Treatment and Non-Treatment Groups

	Group	Number	Mean	Std Error of the Mean	t	Significance
Vitamin C	Non-Treatment	10	67	14	0.359	none
	Treatment	8	61	10		
Vitamin B-6	Non-Treatment	10	0.97	0.2	-3.994	0.01
	Treatment	8	3.74	0.7		
Vitamin B-12	Non-Treatment	10	2.31	0.9	-2.355	0.05
	Treatment	8	5.47	1		
Folate	Non-Treatment	10	0.257	34	-4.115	0.001
	Treatment	8	0.568	74		

The recommended daily nutrient requirement for hemodialysis patients is 10 milligrams of vitamin B_6, 3-6 micrograms (ug) of vitamin B_{12}, and 0.8-1 milligrams of folic acid (Kidney and urologic disease statistics for the United States, 2000). The average intake of the treatment group was within range for vitamin C and vitamin B_{12}, with 61 mg and 5.5 ug respectively, as shown in Table 6. Individual values are depicted in Appendix E. Research is emerging regarding supplementing dialysis patients with high doses of vitamin B_6, vitamin B_{12} and folic acid aid in reducing the body's accumulation of homocysteine, which can cause cardiovascular problems. The non-treatment group had overall lower intake of all the vitamins except vitamin C. The vitamin averages are based on food and fluid intake, which included the nutrients contributed by Nepro® supplementation. Dialysis vitamins were not taken into consideration in this study.

The mean intake of calcium tended to be significantly different ($P = 0.07$) with a mean of 579 mg and 1,027 mg for the non-treatment and treatment groups, respectively. The independent t-test also revealed that there was no significance between the non-treatment and treatment groups in their intake of potassium. The results of the independent t-test revealed that the mean intake of phosphorus was significantly different ($P = 0.01$) with a mean of 1,045 mg and 522 mg for the treatment and non-treatment groups, respectively. (Refer to Table 7).

Table 7. Calcium, Potassium, and Phosphorus Means for the Treatment and Non-Treatment Groups

	Group	Number	Mean	Std Error of the Mean	t	Significance
Calcium	Non-Treatment	10	579	148	-1.949	0.07
	Treatment	8	1027	178		
Potassium	Non-Treatment	10	1372	162	-1.666	none
	Treatment	8	1783	187		
Phosphorus	Non-Treatment	10	522	85	-3.282	0.01
	Treatment	8	1045	143		

Calcium, potassium and phosphorus are nutrients that are monitored monthly on all hemodialysis patients to prevent accumulation or excessive losses. Changes in these nutrients affect bone health, thyroid activity, and cardiac status of hemodialysis patients.

There were eighteen food records that were entered into Food Processor, only 3 participants in the treatment group and 2 participants in the non-treatment group obtained the recommended daily allowance of calcium (Appendix F).

Dietary potassium and phosphorus tend to be limited in the renal diet. Too much potassium can cause heart arrhythmias leading to cardiac arrest. Phosphorus is not completely dialyzed, patients with higher levels of this mineral ingest binders with their meals, such as TUMS or PhosLo, so the excess phosphorus can be excreted. Too much phosphorus can lead to renal osteodystrophy or metabolic bone disease, which includes osteomalacia, and osteitis fibrosa cystica (calcification of soft tissues). All of the participants in the study had adhered to their potassium and phosphorus restrictions as evidenced by their intake over the past 24-hours. (See Appendix F.

Evidence

Issues this study addressed were the patient compliance to a renal diet and how it affected carnitine values as well as sources of carnitine the participants were consuming. When looking at the diets of the participants in Group A, the Treatment group, all participants consumed at least one 8-ounce can of Nepro® daily, which contains 62 mg of carnitine. Summarized in the dietary recall were the foods that they had consumed in the last 24 hours. The carnitine containing foods that the treatment group participants had eaten included beef, hamburgers, pork, ham, sausage, cod, chicken, cheese, buttermilk, milk, white bread, eggs, green beans, apples, and applesauce; with beef containing the greatest amount of carnitine and applesauce containing the least of the foods listed. Food composition tables in books and articles were used to compare the foods (Rebouche and Engel, 1984 and Wardlaw and Insel, 1993).

The carnitine containing foods the participants in the Non-Treatment group, Group B, consumed included hamburgers, pork, bacon, chicken, bologna, Braunschweiger, turkey, cheese, milk, white bread, eggs, green beans, applesauce, tomato slices; with hamburger containing the greatest amount of carnitine and tomato slices the least of the foods listed.

When evaluating the renal diet prescriptions of the participants, many patients were restricted to 1 or 2 grams of phosphorus and 2 to 3 grams of sodium daily. Consumption of meats and dairy products, the primary sources of carnitine, are also limited in the diet due to aversions and limited phosphorus intake, respectively. Chicken, turkey, and fish, are protein foods that are commonly consumed. These foods do not

have as high of carnitine content as dairy and meat products. Adhering strictly to the renal diet would provide food sources that are lower in carnitine.

Another issue this study reviewed was the dietary intake of the participants and their carnitine level. The nine participants in Group B had free carnitine levels that ranged from 16 *u*mol/L to 42 *u*mol/L, with the normal free carnitine value of 40 *u*mol/L (refer to Table 3). Eight of the nine participants in Group B, the non-Nepro® consuming/Non-Treatment group, had fasting free carnitine levels below the normal value. The participants in Group B are not consuming adequate amounts of carnitine rich foods in their diet, or they may be adhering strictly to the renal diet.

The third question addressed if dietary intake of folate, vitamins B_{12} and B_6 were adequate. The participants in the treatment and non-treatment groups did not consume the recommended amount of folate, or vitamin B_6. The treatment group did consume the recommended daily amount of vitamin B_{12}, while the non-treatment group did not.

The fourth hypothesis question focused on whether the supplementation with Nepro®, a carnitine enriched enteral drink had an effect on plasma free carnitine concentration, which it did not. A paired t-test was run with the two sets of plasma free-carnitine blood draw results. The data was not significant.

Unanticipated Findings

The participant's profile created by the Food Processor software program calculated the body mass index (BMI) from the height and weight measurements that were entered. The BMI values of the participants in Group B were greater than the participants in Group A. The higher BMI values in Group B were expected, due to the

participants not needing to have their diet supplemented. However, the BMI of seven participants in Group B were high, reflecting obesity.

When looking at the amount of calories consumed compared to the intake of fat, many of the calories in the diets of these individuals is obtained from fat. Although, the fat intake of the participants was not excessive, if the body is deficient in carnitine the fat cannot be transported into the mitochondria and utilized for energy in turn causing excess fatty acids to be stored.

Summary of Findings

The findings suggest that the research questions of this study were answered. Compliance of a hemodialysis patient to a renal diet, which is lower in phosphorus content, does affect the amount of carnitine that is consumed. This is due to the limited quantity of foods that are good sources of carnitine, such as milk and red meats. Also the dysfunction of the kidney affects the amount of carnitine in the body since carnitine cannot be endogenously created from lysine, methionine, and vitamin C and B vitamins (folate, B_{12} and B_6) that act as coenzymes.

As reviewed in Chapter II, the patient on hemodialysis does not utilize orally supplemented carnitine as efficiently as a person with good overall health. From the data gathered in the 24-hour recall, many of the participants were not consuming adequate amounts of foods that contain carnitine on a daily basis, unless they are drinking 1 or more cans of Nepro® a day. The normal plasma free carnitine level is 40 $u$mol/L. Looking at the free-carnitine plasma levels of the participants in this study, only 3 participants out of 15 had a free carnitine level that was in the normal range. Nepro®

which is enriched with 62 milligrams of carnitine did not have an effect on the plasma free-carnitine content of the participants in the treatment group.

In summary, it appears that carnitine is a conditionally essential nutrient in persons that have impaired kidney and liver function. The process of hemodialysis depletes the blood of carnitine. The enteral supplement Nepro® is not an adequate source of carnitine, due to the inability of the person with diseased kidneys to utilize orally supplemented carnitine. However, the supplementation of Nepro® is encouraged for patients that are not able to orally consume adequate calories and protein to benefit their nutrition.

CHAPTER V

Discussion

Summary

Chronic kidney disease affects 20 million people in the United States. End-stage renal disease is primarily caused by the progression of diabetes and hypertension. The incidence of kidney disease is expected to continually increase due to the escalating prevalence of diabetes and hypertension in the United States secondary to the obesity epidemic.

Persons with kidney disease have their renal function monitored to alert them to changes in their ability to filter their blood and produce urine. When the kidneys are no longer able to filter urea and water out of the body they are started on dialysis. Dialysis, a method of filtering electrolytes and urea out of the blood, is used to maintain the health of persons with end-stage renal disease, along with adherence to diet restrictions that may include limiting potassium, phosphorus, sodium, and/or fluids. Advances in dialysis techniques and technology have increased the survival rate of persons with end-stage renal disease.

Carnitine is found in foods, primarily in dairy products and red meat. Carnitine is a component of plasma and muscle. Carnitine is a small, low molecular weight molecule, and it is dialyzed out of the blood. When carnitine is routinely removed from the blood, stored carnitine in the liver and muscle are utilized to replace it. In this study, the enteral supplement Nepro® was consumed at scheduled times to see if oral supplementation had an effect on their free carnitine plasma level.

This study also looked at the dietary intake of the participants, and the foods they consumed that contained carnitine. Many of the foods that are good sources of carnitine were limited in the participants' diet due to the phosphorus content, aversions to meat, vitamins, and lack of variety and quantity of food consumed by the participants.

The mean calorie intake of the treatment group, the Nepro® consuming group, was greater than the non-treatment group, and this may be due to the 475 calories provided by the Nepro® supplement. However, Nepro® supplementation did not have an effect on the plasma free-carnitine values of the participants in this study. Oral supplementation of carnitine in the amounts found in Nepro® is not an effective means of replacing carnitine.

Limitations

One of the limitations to this study was the smaller than anticipated sample size. There were originally 10 participants in each group; however, one of the participants from the treatment group was hospitalized on the morning of the initial blood draw. Another participant, from the treatment group declined to participate due to recommendations from his family; they were worried that the dietary interview would be too stressful for him. And two blood samples, both from different participants in the treatment group, were lost, so the actual number of free plasma carnitine samples for the treatment group totaled six.

Another limitation to the study was the dietary interviews. Originally a 9-page food frequency had been designed as the tool that would be used in this study. After the first dietary interview, it was evident that these participants did not eat a variety of foods.

Instead the interviewer switched to using a 24-hour diet recall. The limitations with using this dietary evaluation tool is the participants need to be cognitively able to recall what they had eaten in the last 24 hours. The participants were able to recall; some needed some prompting, but it was a more effective means of getting an average of their daily nutrient intake.

Conclusions

The purpose of this study was to determine if compliance to a renal diet, which may restrict phosphorus, potassium, and sodium, affected the free-carnitine values, which it does have an effect due to the limiting of certain foods that are sources of carnitine. The quantity of milk is less due to the high amount of phosphorus present, and some patients have an aversion to red meat, which also provides a greater amount of carnitine than pork or poultry.

In the absence of the functioning kidney, the liver becomes the main source of endogenous carnitine (Bellinghieri et al., 2003). The limited carnitine synthesis in the kidney along with the restriction of foods that are high in phosphorus that are good sources of carnitine, as well as reduced intake of food due to food aversions decreases plasma carnitine.

This study also looked at oral Nepro® supplementation, and if consumption of this product had any effect on plasma carnitine levels in patients with end-stage renal disease receiving routine hemodialysis. Previous studies have revealed that oral supplementation (pill form) of levocarnitine was not an effective method of carnitine replacement. This is due to the metabolites that result from the breakdown of these

medications. (Schreiber, 2000). The present study also confirmed that an enteral supplement, such as Nepro®, which provides 62 mg of L-carnitine per can, is not an effective method of carnitine replacement.

Implications

Patients that require added calories and protein to maintain their health would benefit from Nepro® supplementation, although it does not alter the carnitine status of the patient on hemodialysis.

Intravenous supplementation of levocarnitine may not benefit all patients receiving hemodialysis. The criterion for reimbursement that was determined by the CMS (Centers for Medicare and Medicaid Services) limits the use of IV levocarnitine. The cost of intravenous levocarnitine is most likely the determining factor for the limited availability of usage. Many studies have promoted the use of levocarnitine in the hemodialysis population to relieve fatigue and cramping, muscle weakness, decrease Epogen dosages, improve cardiac function, and to preserve exercise capacity. The dosage recommended is 1 mg/kg lean body weight. Recommending IV levocarnitine therapy for patients that meet the criteria may prove to be helpful and improve the quality of their life. The criteria set by CMS may need to be reviewed, because at present the use of IV levocarnitine is limited. Greater utilization of the IV Levocarnitine could improve health outcomes and quality of life of these participants.

Recommendations for Study

1) A study assessing how hemodialysis patients that are not presently being supplemented, due to being overweight categorized by having a BMI >25, may improve with enteral supplementation, using laboratory values, as indicators of improvement would be desirable.

2) A longitudinal study of hemodialysis patients could also provide information on the benefits of supplemental carnitine. Gathering and assessing anthropometrical, biochemical, clinical, and diet information with new hemodialysis patients receiving IV supplementation of levocarnitine, and monitoring their progress until they received a kidney transplant, would provide needed information to determine if routine IV supplementation prevented carnitine depletion, and its effects on muscle weakness and fatigue.

3) A study to determine what level of carnitine is needed for supplementation to maintain and establish a baseline value in patients receiving maintenance hemodialysis.

Recommendations for Practice

1) Have an assessment to see if patients qualify for IV supplementation of levocarnitine.

2) Work with Medicare to have routine patient testing for levels of free plasma carnitine

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APPENDIX A

Permission Form

I am requesting permission to talk with you to obtain a diet history. I am gathering dietary data for my graduate thesis. I am assessing the dietary intakes of patients receiving hemodialysis and comparing carnitine levels. The reason that I am collecting dietary data is to estimate the amount of carnitine, along with protein and calories that patients with renal disease are consuming.

Sarah A. Nowinsky, the primary researcher for this thesis, will conduct the dietary interviews. A food frequency questionnaire will be used to obtain the diet history. The dietary interviews will be conducted during the dialysis treatment session, and will take approximately 45 minutes. I would greatly appreciate your permission for this interview portion of my thesis.

I voluntarily agree to participate in this study.

_____ _____

Signature of Subject Date

APPENDIX B

Patient Information (Group A)

With your signed consent to participate in this study there are procedures that will be followed to substantiate the reliability of this study.

The supplemented subjects will consume Nepro® as requested on the assigned days. Nepro® will be consumed two hours before the second fasting blood draw.

No adverse side effects are anticipated due to the common use of the supplement by dialysis patients to increase calories and protein in their diet.

Blood will be drawn before the dialysis treatments. The blood draws will be conducted according to the subject's dialysis schedule. The level of free carnitine will be determined.

Carnitine is made in the body by two amino acids, methionine and lysine. Carnitine is also present in meats and dairy products. A dietary interview will be conducted to estimate carnitine intake.

Carnitine's main function in the body is to transport fatty acids to produce energy.

During dialysis carnitine is removed from the blood in the dialysate. Carnitine is then pulled from the muscles into the blood, causing deficiency and muscle weakness. By replacing the carnitine lost in the dialysis process, less muscle fatigue and cramping will occur.

Questions or concerns about participation in the research or subsequent complaints should be addressed first to the co-investigator, Sarah Nowinsky or research advisor, Dr. Carol Seaborn PhD RD, Food and Nutrition, College of Human Development, UW-Stout, Menomonie, WI 54751, phone (715) 232-2216; and second to Sue Foxwell, Human Protection Administrator, 11 HH, UW-Stout, Menomonie, WI, 54751, phone (715) 232-2477.

Thank you for your participation.

APPENDIX C

Consent Form

I understand that my participation in this study is strictly voluntary and I may discontinue my participation at any time without prejudice.

I understand that the purpose of this study is to investigate the problem, of whether diet intake or supplemental carnitine correlates with plasma carnitine levels.

I further understand that any information about me that is collected during this study will be held in the strictest of confidence. I understand that in order for this research to be effective and valuable certain personal identifiers need to be collected. I also understand that the strictest of confidence will be maintained throughout this study and that only the researchers will have access to the confidential information. I understand that at the conclusion of this study all records, which identify individual participants, will be destroyed. I am aware that I have not and am not waiving any legal or human rights by agreeing to this participation.

By signing below I verify that I am 18 years of age or older, in good mental condition, and that I agree to and understand the conditions listed above.

Signature_____Date_____

APPENDIX D

Table of Carnitine Content of Foods

Food Item	Carnitine content (milligrams)
Beef Steak	592±260
Ground Beef	582±32
Pork	172±32
Bacon	145±24
Fish (cod)	34.6±11.7
Chicken Breast	24.3±8.0
Eggs	0.075
Peanut butter	0.5162
Whole Milk	20.4
American Cheese	23.2
Ice cream	23.0
Butter	3.07
Cottage Cheese	6.96
White bread	0.912
Whole-wheat bread	2.26
Rice (cooked)	0.090
Macaroni	0.780
Corn flakes	0.078
Broccoli (cooked) (fresh)	0.0111 0.0228
Carrots (cooked) (fresh)	0.0393 0.0408
Green beans (cooked)	0.0189
Green peas (cooked)	0.0369

Devised from an article in the Journal of Clinical Investigation (Rebouche, and Engel, 1984).

APPENDIX E

Nutrient Quantities of the Participant"s Dietary Intake

Vitamin C, Vitamin B_6, Vitamin B_{12}, and Folate content of the Participants 24-Hour Recall

Code #	Vitamin C (mg)	Vitamin B_6 (mg)	Vitamin B_{12} (*ug*)	Folate (*ug*)
A2	53.88	4.19	4.2	613.43
A3	34.37	1.32	1.82	315.04
A4	75.06	5.75	10.34	789.69
A5	48.91	2.15	5.14	456.08
A6	118.85	7	9.13	915.3
A7	67.92	3.8	5.64	615.79
A8	51.32	3.13	3.17	491.82
A10	36.83	2.58	4.31	349.2
B1	137.32	1.83	6.58	431.38
B2	75.28	0.62	0.16	366.11
B3	64.61	0.68	0.43	188.82
B4	91.28	1.63	7.68	326.27
B5	105.92	0.97	0.16	84.9
B6	23.6	0.73	1.27	346.35
B7	116.08	1.79	1.21	224.17
B8	32.42	0.91	3.83	177.57
B9	11.11	0.25	0.32	238.57
B10	16.57	0.32	1.46	189.19

APPENDIX F

Nutrient Quantities of the Participant's Dietary Intake

Calcium, Potassium, and Phosphorus content of the Participants 24-Hour Recall

Code #	Calcium (mg)	Potassium (mg)	Phosphorus (mg)
A2	944.15	1972.35	1081.53
A3	611.2	796.08	508.21
A4	1741.27	2242.45	1397.68
A5	654.11	1574.3	824.82
A6	1524.62	2094.25	1495.98
A7	1578.6	2475.9	1579.62
A8	604.61	1462.71	669.12
A10	559.34	1642.75	804.45
B1	805.46	1600.85	765.66
B2	296.43	1434	429.56
B3	359.86	1196.8	473.47
B4	566.65	2073.82	1104.1
B5	73.04	1157.41	131.73
B6	382.41	1195.39	639.61
B7	1552.29	2337.18	435.79
B8	308.59	1011.29	568.51
B9	254.92	626.53	361.12
B10	1193.53	1086.77	311.97